

Article

Ultrasonic-Assisted Enzymatic Extraction: An Innovative Technique for the Obtention of Betalains and Polyphenols from Dragon Fruit Peel

Cristhel Guadalupe Puc-Santamaria ¹, Rosa Us-Camas ¹, Emanuel Hernández-Núñez ¹ , Luis Alfonso Can-Herrera ¹, Dany Alejandro Dzib-Cauich ¹, Adán Cabal-Prieto ² , Nattha Pensupa ^{3,4}  and Julio Enrique Oney-Montalvo ^{1,*} 

¹ Tecnológico Nacional de México, Instituto Tecnológico Superior de Calkiní, Av. Ah Canul S/N por Carretera Federal, Calkiní 24900, Campeche, Mexico; 7324@itescam.edu.mx (C.G.P.-S.); ryuscamas@itescam.edu.mx (R.U.-C.); ehernandez@itescam.edu.mx (E.H.-N.); lacan@itescam.edu.mx (L.A.C.-H.); dadzib@itescam.edu.mx (D.A.D.-C.)

² Tecnológico Nacional de México, Instituto Tecnológico Superior de Huatusco, Av. 25 Poniente No. 100, Colonia Reserva Territorial, Huatusco 94106, Veracruz, Mexico; acabalp@huatusco.tecnm.mx

³ Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand; natthape@nu.ac.th

⁴ Centre of Excellence in Fat and Oils, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand

* Correspondence: jeoney@itescam.edu.mx

Abstract

Dragon fruit peel is a by-product rich in bioactive compounds, such as polyphenols and betalains. In this study, ultrasound-assisted enzyme extraction (UAEE) was proposed to exploit this, combining the advantages of the enzymatic hydrolysis and ultrasound extraction. The effect of extraction time, temperature, and enzyme quantity were evaluated using a Box–Behnken design. Total betalains and polyphenol contents were determined spectrophotometrically. The results show that the extraction of total polyphenols was significantly affected ($p \leq 0.05$) by the enzyme quantity, while temperature had a significant effect ($p \leq 0.05$) on the extracted betalains. The optimal conditions for the extraction of total betalains and polyphenols were a temperature of 20 °C, an extraction time of 20 min, and an enzyme/substrate ratio of 400 mg/g. Under optimized conditions, the extraction efficiency reached $565.6 \pm 12.9 \mu\text{g/g}$ for total betalains and $14.9 \pm 2.4 \text{ mg/g}$ for total polyphenols. In addition, UAEE showed the best extraction yields compared to other methodologies, such as microwave, ultrasound, and enzymatic hydrolysis extraction ($p \leq 0.05$). This study helps us to understand how the temperature, time, and amount of enzymes affect the extraction of total polyphenols and betalains present in the peel of the dragon fruit using the UAEE technique.

Keywords: Box–Behnken design; digestive enzymes; response surface



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1. Introduction

Dragon fruit (*Hylocereus undatus*), or pitahaya, is a tropical fruit characterized by an ellipsoidal shape. The mesocarp of the fruit consists of white pulp interspersed with numerous black seeds, while the exocarp is a pink peel and is covered by triangular protruding bracts [1]. The dragon fruit peel is considered a by-product, representing between 22 and 44% of the fruit's total weight [2]. This by-product is a substantial source of antioxidant compounds, particularly betalains and polyphenols, which are associated with various health benefits [3].

Betalains are water-soluble pigments, which are classified into (a) betacyanins and (b) betaxanthins. These compounds are important in the food industry due to the fact that they are used as food additives to prevent the discoloration of foods and enhance nutritional value through their antioxidant and pharmacological properties [4]. The European Union approved their use, and they offer the advantage of presenting greater stability to changes in pH and temperature compared to anthocyanins. Therefore, betalains could be applied to a broader variety of foods, particularly those with acidic pH, where other natural pigments tend to exhibit lower stability [4].

Polyphenols are a broad group of bioactive compounds of plant origin, characterized by the presence of one or more phenolic groups in their chemical structure [5]. These compounds are considered secondary metabolites of plants and play essential roles in the defense against environmental stress, ultraviolet radiation, and attack by pathogens [5]. In pitahaya, these compounds provide a shell with antioxidant capacity, making it an attractive raw material for producing functional ingredients, with applications in the food, cosmetic, and pharmaceutical sectors [6].

Different extraction techniques have been used to obtain the compounds mentioned above [7]. Conventional methods for betalain and polyphenol extraction include solvent and solid–liquid extraction. The conventional methods require organic solvents, high temperature, and long extraction time. This may lead to compound degradation because betalains are sensitive to heat, oxygen, and pH. Green techniques, such as ultrasonic extraction, microwave extraction, and enzyme-assisted extraction, are interesting because they reduce the amount of solvent used and minimize compound degradation [8]. Ultrasound-assisted extraction is effective for extracting betalains and polyphenols, especially from dried plant material or food industry waste. Moreover, enzyme-assisted extraction employs specific enzymes to break down cell wall components, boosting the release and yield of valuable phytochemicals. This process often occurs under mild conditions that help to maintain compound stability. Therefore, ultrasound and enzymatic hydrolysis are prominent [9,10]. Both techniques offer distinct advantages in transferring these compounds from the matrix to the solvent, which could be enhanced by combining the two extraction methods, resulting in an innovative ultrasonic-assisted enzyme extraction technique [11]. The enzymes used for ultrasound-assisted enzymatic extraction of bioactive compounds from complex matrices such as plants, cellulases, xylanases, hemicellulases, and pectinases are particularly noteworthy [12]. The use of a mixture of these enzymes is recommended to break down the various bonds within the matrix and facilitate the release of phytochemicals [13]. It is important to evaluate the factors that may influence the extraction performance when implementing any technique [14]. In this case, the amount of enzymes used, as well as the temperature and extraction time, should be considered [14].

Previous studies optimized the ultrasound-assisted enzymatic extraction of betalains and total polyphenols from red beetroot (*Beta vulgaris* L.) and its associated waste materials [15,16]. However, there is no information available on the application and optimization of this technique for the simultaneous extraction of betalains and total polyphenols from dragon fruit by-products. Therefore, this study proposes ultrasonic-assisted enzymatic extraction as an innovative technique for obtaining betalains and polyphenols from dragon fruit peels. To optimize the extraction of these bioactive compounds, it is crucial to evaluate the influence of key operational parameters, including temperature, extraction time, and enzyme concentration. This knowledge could be utilized to obtain extracts rich in total betalains and polyphenols, which can be used for the formulation of functional foods, allowing for the integral use of pitahaya and increasing the added value of this fruit.

2. Materials and Methods

2.1. Raw Material

Dragon fruit (*Hylocereus undatus*) was purchased at a market in Calkiní, Campeche, Mexico. The peel was separated from the pulp and dried at 45 °C for 72 h in a convection oven. Subsequently, the dried fruit peel was ground and sieved at a size of 40 mesh (0.4 mm) to homogenize the particle size. Finally, the obtained powder was stored in Falcon tubes and refrigerated at −4 °C until it was analyzed.

Enzymes used in this study were a blend of vegetarian digestive enzymes of the brand Life Extension® (Fort Lauderdale, FL, USA). The enzymatic formulation was applied as provided by the manufacturer, with the following composition: protease SP (*Aspergillus oryzae*, *Bacillus subtilis*) 25,000 FCC, bromelain (*Aspergillus comosus*) 800,000 FCC, lactase (*Aspergillus oryzae*) 2000 FCC, cellulase (*Aspergillus niger*) 2000 FCC, lipase (*Candida rugosa*) 2500 FCC, alpha-galactosidase (*Aspergillus niger*) 150 FCC, hemicellulase (*Aspergillus niger*) 4000 FCC, pectinase (*Aspergillus niger*) 25 FCC, protease 3.0 (*Aspergillus niger*) 10 FCC, and xylanase (*Trichoderma longibrachiatum*) 500 FCC. The FCC represents Food Chemicals Codex units.

2.2. Ultrasonic-Assisted Enzymatic Extraction

An ultrasonic bath (Creworks, Ontario, CA, USA) with a 3 L capacity, 120 W sonication energy, and a frequency of 40 kHz was employed. The extraction was performed by weighing 250 mg of the sample and adding 8 mL of water as solvent. The conditions for the ultrasonic-assisted enzyme extraction were set at temperatures ranging from 20 to 50 °C, extraction times from 20 to 70 min, and an enzyme/substrate ratio from 0 to 400 mg/g.

After extraction under the abovementioned conditions, the samples were centrifuged at 4500 rpm for 10 min, and the supernatant was filtered with a nylon membrane disc filter with a pore size of 45 µm. The extracts obtained were analyzed to determine total betalains and polyphenols.

2.3. Box–Behnken Experimental Design

Table 1 shows the different levels of the factors to be evaluated: (1) temperature, (2) extraction time, and (3) enzyme/substrate ratio. A Box–Behnken design was used to analyze how the factors mentioned above and their interactions contributed to the extraction of total betalains and polyphenols from the dragon fruit peel.

Table 1. Conditions for optimizing ultrasonic-assisted enzymatic extraction of betalains and total polyphenols.

Factor	Level		
	−1	0	1
	Actual Value		
Temperature (°C)	20	35	50
Time (min)	20	45	70
Enzyme/substrate (mg/g)	0	50	100

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 (1):

$$Y_i = \beta_0 + \sum_{i=1}^3 \beta_i x_i + \sum_{i=1}^3 \beta_{ii} x_i^2 + \sum_{i \neq j=1}^3 \beta_{ij} x_i x_j \quad (1)$$

where Y_i represents the response variable, specifically the concentration of betalains, total polyphenols, and antioxidant activity. Meanwhile, β_0 , β_i , β_{ii} , and β_{ij} are the coefficients representing the regression model, while x_i and x_j 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 using STATGRAPHICS Centurion XIX (Statgraphics Technologies Inc., The Plains, VA, 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.

2.4. Determination of Total Betalains

The total betalains were determined using the method described by Shakir and Simone [17], with certain modifications. The samples were placed in cells, and absorbance measurement were conducted at 538 nm and 480 nm for betacyanins and betaxanthins, respectively. The concentrations of total betalains were calculated as the sum of the betacyanins and betaxanthins concentrations. The analysis was performed with a UV-Vis spectrometer (PerkinElmer®, Waltham, MA, USA). The absorbance values were analyzed using the following formula to determine the concentrations of betacyanins and betaxanthins in the extracts:

$$\text{Betacyanins or betaxanthins } (\mu\text{g/mL}) = \frac{(A)(DF)(MW)}{(\epsilon)(l)}. \quad (2)$$

The absorbance (A) was measured at 538 nm for betacyanins and 480 nm for betaxanthins. DF denotes the dilution factor; MW represents the molecular weight (550 g/mol for betacyanins and 308 g/mol for betaxanthins); ϵ refers to the molar extinction coefficient (60,000 L/mol·cm for betacyanins and 48,000 L/mol·cm for betaxanthins); and l is the path length of the cuvette, which was set at 1 cm.

2.5. Determination of Total Polyphenols

Total polyphenol content was quantified using the Folin-Ciocalteu colorimetric method described by Singleton et al. [18] with some modifications. A volume of 50 μL of the extract was mixed with 3 mL of distilled water and 250 μL of Folin-Ciocalteu reagent. The mixture was homogenized and allowed 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 h.

The absorbance of the extracts was measured using a UV-Vis spectrophotometer (PerkinElmer®) at 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.

2.6. Comparison with Other Extraction Techniques

The best conditions of the ultrasonic-assisted enzyme extraction obtained from the experimental design previously described were compared with three extraction techniques, which were as follows: (1) ultrasound, (2) microwave, and (3) enzymatic hydrolysis. These techniques are described in the following section.

2.6.1. Ultrasound Extraction

Ultrasound-assisted extraction was conducted based on the methodology described by Singh et al. [19], with slight modifications. To this end, 250 mg of the sample was accurately weighed and mixed with 8 mL of distilled water. The resulting suspension was subjected to ultrasonic treatment for 30 min, centrifuged at 4500 rpm for 10 min, and the supernatant was filtered with a nylon membrane with a pore size of 45 µm.

2.6.2. Microwave Extraction

Microwave extraction was performed by placing the samples in a microwave oven (Acros AM1007Q, Shanghai, China, 1050 W). For the extraction process, 500 mg of the sample was placed in a beaker with 10 mL of distilled water. The extraction was carried out within 30 s using 100% of the power of the equipment. After the microwave extraction process, the extracts were transferred to 15 mL Falcon tubes and centrifuged at 4500 rpm for 15 min. The supernatant was filtered through a syringe filter for further analysis.

2.6.3. Enzymatic Hydrolysis

The extraction procedure was performed using the methodology described by Lombardelli et al. [10]. For extraction, 250 mg of the sample and 50 mg of a digestive enzyme blend were used, followed by the addition of 8 mL of distilled water as the extraction medium. The suspension was subjected to continuous vortex agitation for 60 min to ensure adequate enzymatic interaction and compound solubilization. The samples were centrifuged at 4500 rpm for 10 min, and the supernatant was filtered through a nylon membrane with a pore size of 45 µm.

2.6.4. Statistical Analysis

The collected data were analyzed using one-way analysis of variance (ANOVA) to evaluate the statistical significance of the differences between the various extraction methods employed for obtaining betalains and total polyphenols. Subsequently, a least significant difference (LSD) test was then applied at a 95% confidence level ($p \leq 0.05$). All statistical analyses were performed using the STATGRAPHICS Centurion XIX software (Statgraphics Technologies Inc., The Plains, VA, USA).

3. Results and Discussion

3.1. Results of the Box–Behnken Experimental Design

Table 2 summarizes the results of the Box–Behnken experimental design used to evaluate the effect of factors on the extraction of total betalains and polyphenols using the ultrasonic-assisted enzyme extraction technique, showing the different combinations of temperature, time, and amount of enzymes. Experiment 5 gives the best yields for the extraction of total betalains (569.8 µg/g) at a temperature of 20 °C, with an estimated time of 45 min of extraction and an enzyme/substrate ratio of 0 mg/g of enzyme. The best extraction of total polyphenols was reported in experiment 11 (15.90 mg/g), which was a temperature of 35 °C, an extraction time of 45 min, and an enzyme/substrate ratio of 400 mg/g.

Table 2. Results obtained from the Box–Behnken experimental design for ultrasonic-assisted enzyme extraction of total betalains and polyphenols.

Exp	Temperature (°C)	Time (min)	E/S Ratio (mg/g)	Total Betalains (µg/g)	Total Polyphenols (mg/g)
1	20	20	200	556.9	9.68
2	50	20	200	368.6	5.13
3	20	70	200	512.0	9.12
4	50	70	200	313.2	8.31
5	20	45	0	569.8	4.75
6	50	45	0	250.1	5.90
7	20	45	400	500.7	10.92
8	50	45	400	299.8	10.02
9	35	20	0	435.0	4.87
10	35	70	0	442.8	6.48
11	35	20	400	559.4	15.90
12	35	70	400	456.2	12.22
13	35	45	200	517.9	9.67
14	35	45	200	395.0	7.90
15	35	45	200	412.2	8.43

3.2. Analysis of the Effect of Factors

The ANOVA (Table 3) partitions the variability in total betalain content into distinct components corresponding to each factor evaluated. It assesses the statistical significance of these factors by comparing their mean squares to an estimate of the experimental error. In this analysis, two effects exhibited p -values ≤ 0.05 , indicating a statistically significant contribution at the 95.0% confidence level. The coefficient of determination (R^2) reveals that the fitted model accounts for 95.79% of the total variability in betalain content. The adjusted R^2 , which is more appropriate for comparing models with varying numbers of predictors, is 88.23%.

Table 3. ANOVA of the effect of temperature, time, and enzyme factors on the extraction of total betalains by EEAU.

Source	Sum of Squares	Df	Mean Square	F-Ratio	p -Value
A: Temperature	102,990	1	102,990	91.89	0.0002 *
B: Time	4787.31	1	4787.31	4.27	0.0936
C: Enzyme	1752.32	1	1752.32	1.56	0.2665
AA	1399.80	1	1399.80	1.25	0.3146
AB	27.56	1	27.56	0.02	0.8815
AC	3528.36	1	3528.36	3.15	0.1362
BB	8785.50	1	8785.50	7.84	0.0380
BC	3080.25	1	3080.25	2.75	0.1583
CC	969.51	1	969.51	0.87	0.3950

Table 3. *Cont.*

Source	Sum of Squares	Df	Mean Square	F-Ratio	p-Value
Total error	5604.01	5	1120.80		
Total (Corr.)	133,302	14			

Note: (*) indicates significant differences with a p -value ≤ 0.05 .

The significant effect of temperature on the extraction of total betalains may be attributed to its direct influence on the solvent characteristics, altering its surface tension and viscosity, while enhancing the matrix porosity, facilitating the transfer of analytes from the matrix to the solvent [20]. Furthermore, temperature could also affect the betalains, potentially promoting the degradation of these compounds. Several studies reported that moderate temperatures (approximately 30–60 °C) are generally optimal for betalain extraction [9,21–23]. Higher temperatures can degrade betalains, especially betaxanthins, reducing yield and pigment quality [24]. This behavior has been observed in other pigments when subjected to sonication at high temperatures, which affects the extraction performance [25].

The ANOVA (Table 4) partitions the variability in total polyphenol content into distinct components associated with each evaluated factor. It then assesses the statistical significance of each effect by comparing the corresponding mean squares with an estimate of the experimental error. In this case, one effect exhibited a p -value ≤ 0.05 , indicating a statistically significant contribution at the 95.0% confidence level. The coefficient of determination (R^2) shows that the fitted model explains 90.46% of the total variability in polyphenol content. The adjusted R^2 , which provides a more accurate comparison between models with differing numbers of predictors, is 73.29%.

Table 4. ANOVA of the effect of temperature, time, and enzyme factors on the extraction of total polyphenols by EEAU.

Source	Sum of Squares	Df	Mean Square	F-Ratio	p-Value
A: Temperature	3.26	1	3.26	1.33	0.3004
B: Time	0.04	1	0.04	0.02	0.9059
C: Enzyme	91.53	1	91.53	37.38	0.0017 *
AA	4.64	1	4.64	1.90	0.2269
AB	3.49	1	3.49	1.43	0.2857
AC	1.05	1	1.05	0.43	0.5414
BB	2.66	1	2.66	1.09	0.3453
BC	6.99	1	6.99	2.86	0.1518
CC	1.74	1	1.74	0.71	0.4381
Total error	12.24	5	2.45		
Total (Corr.)	128.37	14			

Note: (*) indicates significant differences with a p -value ≤ 0.05 .

However, the amount of enzyme had a significant effect on the concentration of total polyphenols extracted from the matrix, as shown in Table 4. This may be due to the enzyme concentration affecting the reaction kinetics, as a higher concentration of the enzyme accelerates substrate degradation [26].

3.3. Optimization of Ultrasonic-Assisted Enzymatic Extraction

Figure 1 shows the response surface diagram of the effects of temperature, time, and enzyme quantity on the extraction of total betalains using ultrasonic-assisted enzyme extraction. Figure 1a shows the impact of the combined factors of temperature and time on betalain extraction. These findings suggest that a decrease in both temperature and extraction time leads to an increased betalain yield. The experimental design identified the optimal condition for maximizing the concentration of these compounds as an extraction temperature of 20 °C for 20 min.

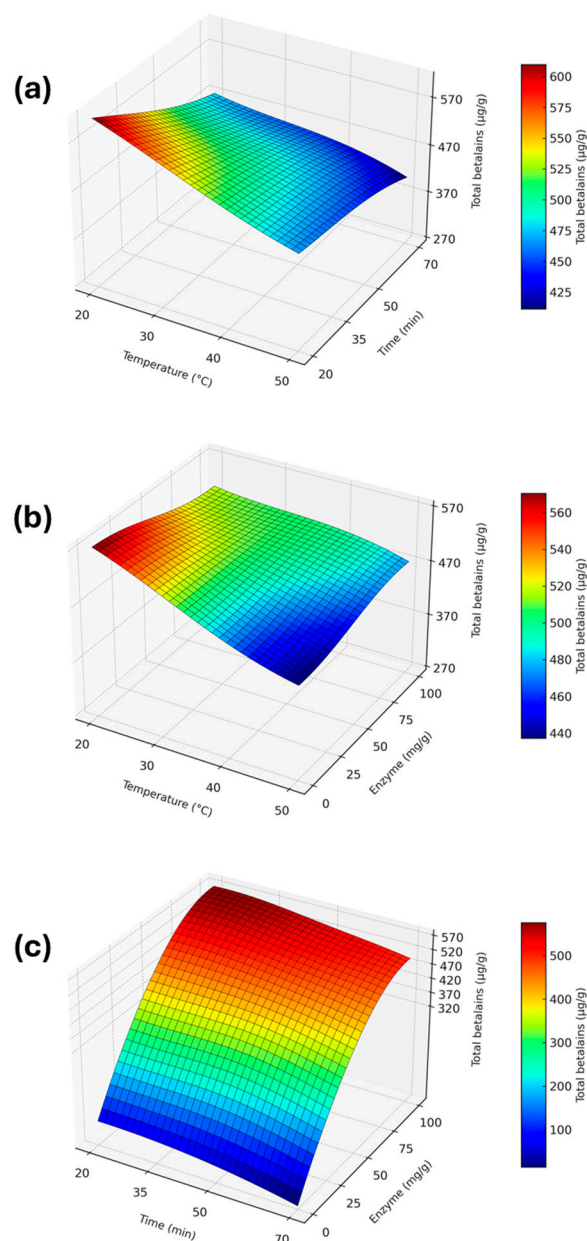


Figure 1. Response surface diagram for ultrasound-assisted enzyme extraction of total betalains. (a) Effect of temperature and time, (b) effect of temperature and enzyme quantity, and (c) effect of time and enzyme quantity.

Figure 1b shows the effect of the interaction between temperature and enzyme quantity, with the highest extraction occurring at a low temperature (20 °C) and low quantities of the enzyme/substrate ratio (0 mg). On the other hand, Figure 1c represents the effect of

the time and enzyme quantity; in this, it is possible to see that the highest extraction of the betalains happened when the extraction was realized in a short time (20 min).

The combination of levels of factors that maximized total betalains extraction in the evaluated region resulted in a theoretical extraction maximum of 601.8 µg/g. This optimum value was achieved at a temperature of 20 °C, an extraction time of 20 min, and an enzyme/substrate ratio of 400 mg. Theoretical prediction of total betalain content as a function of the evaluated factors was described by the following second-order polynomial model:

$$\text{Total betalains} = 408.4 - 113.5(A) - 24.5(B) + 14.8(C) - 19.5(A^2) - 2.6(A \times B) + 29.7(A \times C) + 48.8(B^2) - 27.8(B \times C) + 16.2(C^2).$$

According to this model, the predicted optimal value was 601.897 µg/g. These optimal conditions could contribute to better extraction and reduced betalain degradation. For example, temperatures above 40 °C degrade pigments such as betalains, especially betacyanins, which are responsible for the characteristic red-violet coloration of the pitahaya peel [27]. This is corroborated by the study by Prenhaca-Silva et al. [28], who analyzed the effects of different factors on the extraction of betalains by the ultrasound technique, and observed that low temperatures (less than 30 °C) and short extraction times (less than 30 min) were the most suitable for preventing the degradation of these compounds. In the case of the enzyme/substrate ratio, 400 mg/g was the optimal condition, which coincides with the results reported by Görgüç et al. [29]. A positive linear effect was observed on the extraction of antioxidant compounds with the amount of enzyme used in the ultrasonic-assisted enzymatic extraction.

Figure 2 shows the response surface diagram of the effects of temperature, time, and enzyme quantity on the extraction of total polyphenols by the ultrasonic-assisted enzyme extraction technique. Figure 2a shows the effect of the interaction between temperature and time on the extraction of polyphenols. The response surface diagram demonstrates that the combination of these factors does not have a significant effect on extraction, as it is a saddle point-shaped graph.

On the other hand, Figure 2b shows the interaction between temperature and enzyme quantity in the extraction of total polyphenols; in this case, the factor enzyme quantity has a higher effect, showing that when it increases, the extraction of polyphenols is better, while in comparison, the temperature does not show a change in the range. Figure 2c shows the effect of the interaction between time and enzyme. The results show that enzyme quantity had a greater influence on total polyphenol extraction than extraction time. The maximum extraction under the evaluated conditions was achieved with a short extraction time and high enzyme quantities.

The maximum total polyphenol extraction in the established working range was achieved at a temperature of 20 °C, time of 20 min, and enzyme/substrate of 400 mg/g. Under this condition, the theoretical extraction value was 15.47 mg/g. The total polyphenol content under the evaluated conditions was predicted by the following second order polynomial equation:

$$\text{Total polyphenols} = 8.33 - 0.64(A) - 0.07(B) + 3.38(C) - 1.12(A^2) - 0.94(A \times B) + 0.51(A \times C) + 0.85(B^2) - 1.32(B \times C) + 0.69(C^2)$$

The lowest temperature resulted in the highest extraction of total polyphenols. The outcome is likely due to the prevention of the thermal degradation of these compounds at this temperature, which in turn prevents the formation of new compounds known as Maillard reaction products [30].

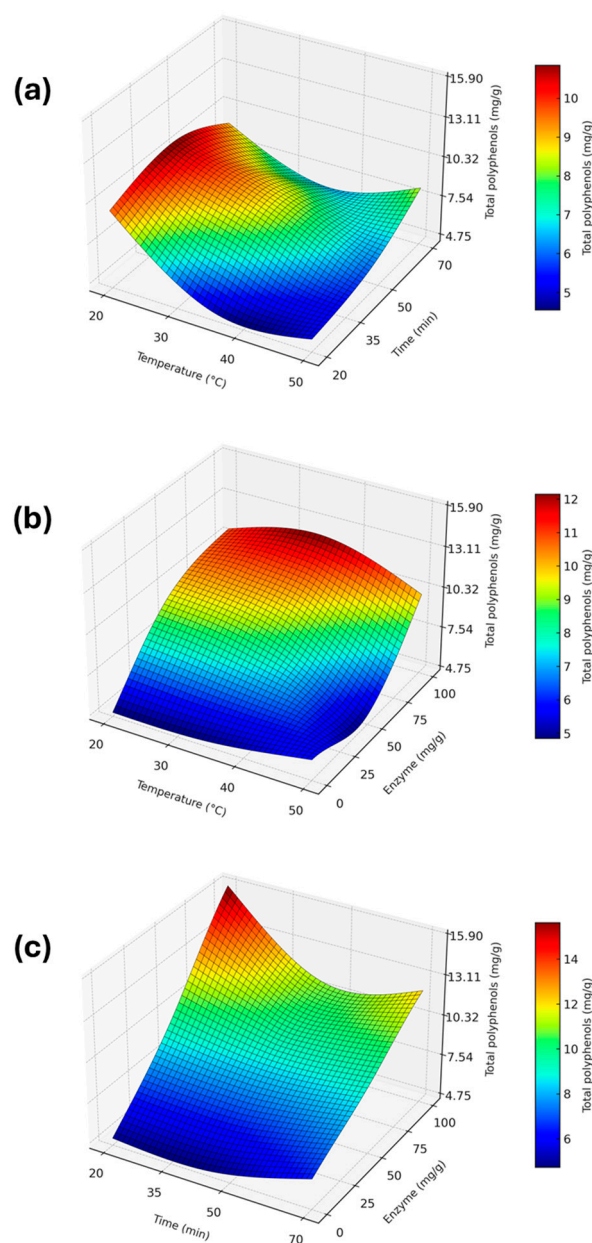


Figure 2. Response surface diagram for ultrasonic-assisted enzyme extraction of total polyphenols. (a) effect of temperature and time, (b) effect of temperature and enzyme quantity, and (c) effect of time and enzyme quantity.

Time is considered to be one of the main factors affecting the extraction of this type of compound, along with the extraction temperature [31]. Although the extraction times for polyphenols can be extended up to 10 h, this depends on the extraction method used [31]. In the case of ultrasonic extraction, extraction periods ranging from 15 to 30 min have been reported, with the degradation of these compounds occurring in times greater than 30 min [31]. The observed effect of the amount of enzymes on polyphenol extraction coincides with that reported by Winkler-Heemann et al. [32], who observed a linear increase in polyphenol extraction in green yerba mate (*Ilex paraguariensis* A. St. Hil.). This behavior is due to an increase in enzyme kinetics due to the increased enzyme/substrate ratio.

3.4. Comparison of Extraction Methods

The results of the comparison of the UAEE with other extraction methods in total betalains and total polyphenols are shown in Figures 3 and 4, respectively. Microwave extrac-

tion, ultrasound-assisted extraction, and enzyme hydrolysis are widely used eco-friendly methods for extracting various valuable compounds from plant materials. Microwave extraction (MAE) utilizes microwave energy to quickly heat water molecules within plant cells, leading to cell rupture and aiding in the release of pigments. Ultrasonic extraction (UAE) employs high-frequency sound waves to form cavitation bubbles in the solvent. The collapse of these bubbles produces localized high pressure and temperature, which disrupts plant cell walls and enhances the release of betalains into the solvent [29]. In contrast, enzyme hydrolysis involves the use of digestive enzymes (such as cellulases, hemicellulase, pectinases, and xylanase) to break down cell wall polysaccharides, loosening the plant matrix and allowing betalains to diffuse out [33]. Figure 3 presents the extraction yields of the total betalains of the four methods, where the UAEE had the highest extraction yield ($565.6 \pm 12.9 \mu\text{g/g}$), followed by enzymatic hydrolysis ($485.86 \pm 11.2 \mu\text{g/g}$) and microwave extraction ($424.8 \pm 23.3 \mu\text{g/g}$), while ultrasonic extraction had the lowest extraction yield ($373.4 \pm 30.1 \mu\text{g/g}$). The degradation of betalains may be attributed to the heat generated during microwave and ultrasonic extraction. In contrast, enzyme hydrolysis extraction yielded a higher concentration of betalains than the microwave and ultrasonic extraction methods. This is likely due to the mild conditions under which enzyme hydrolysis is conducted, which prevents betalain degradation. Ultrasonic-assisted enzyme extraction (UAEE) integrates ultrasound with enzyme hydrolysis, where ultrasound facilitates enzyme access to substrates by disrupting cell walls, and enzymes further degrade the matrix. Consequently, UAEE results in the highest yield of betalains. These results demonstrate the efficiency of UAEE in extracting total betalains compared to other conventional methods, showing a significant performance ($p \leq 0.05$). This is due to the combination of the advantages of ultrasonic extraction, mainly attributed to its mechanical effects, which greatly facilitate the transfer of mass between phases and the release of bioactive compounds by breaking down the bonds forming the matrix with the aid of digestive enzymes [34].

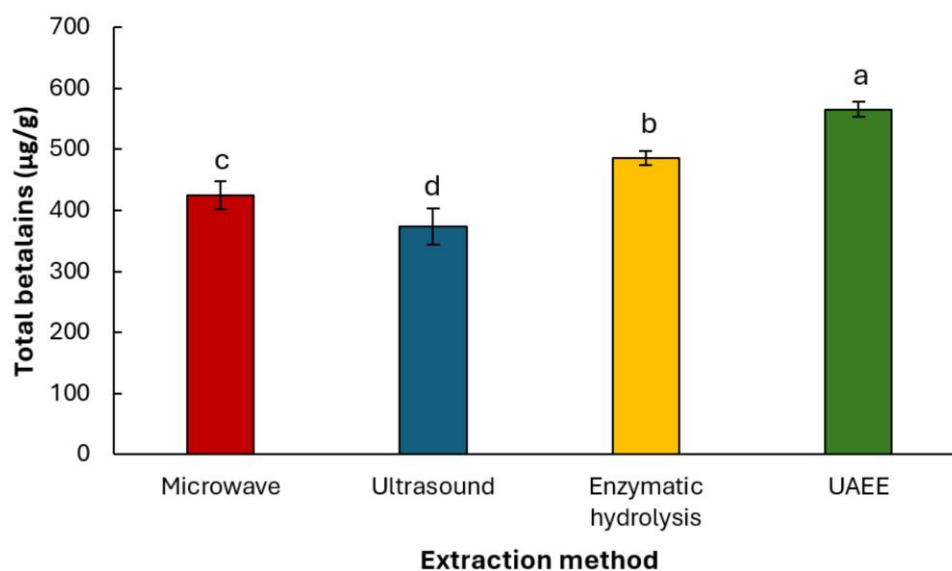


Figure 3. Comparison of the UAEE with other methods for extracting total betalains from dragon fruit peel. Superscript letters (a, b, c and d) denote statistically significant differences between means at $p \leq 0.05$.

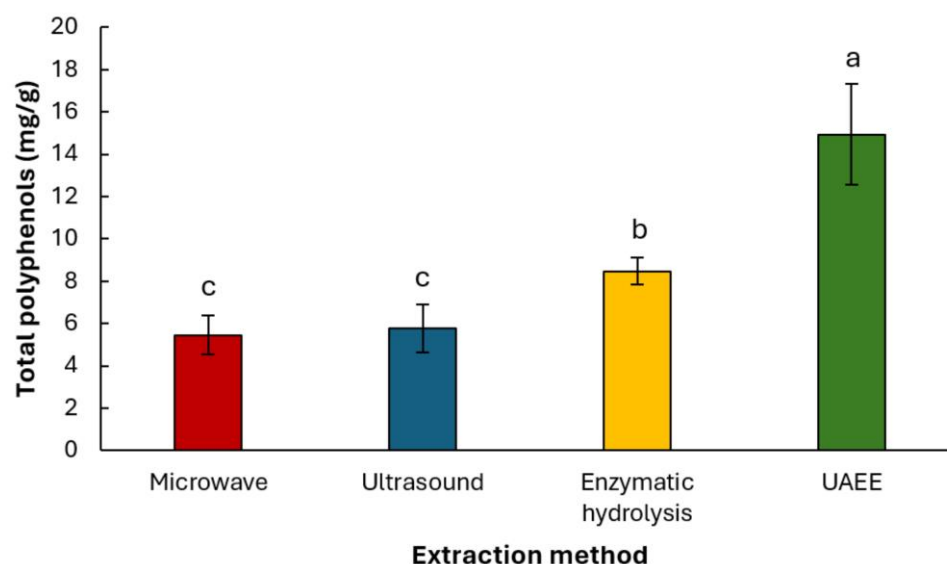


Figure 4. Comparison of the UAEE with other methods for extracting total polyphenols from dragon fruit peel. Superscript letters (a, b, and c) denote statistically significant differences between means at $p \leq 0.05$.

Figure 4 shows the extraction yields of the total polyphenols of the methods evaluated, being also the UAEE, the method that had the highest yields (14.9 ± 2.4 mg/g), followed by enzymatic extraction (8.47 ± 0.6 mg/g). Conversely, microwave and ultrasound had the lowest extraction yields. The UAEE demonstrated superior efficiency in extracting total polyphenols compared to other conventional methods, with a significant difference ($p \leq 0.05$).

4. Conclusions

In conclusion, the obtained results confirm that the ultrasonic-assisted enzyme extraction (UAEE) represents an efficient and sustainable method for the simultaneous recovery of betalains and polyphenols from dragon fruit peel, a sub-utilized agro-industrial by-product. The application of the Box–Behnken design allowed for optimization of the process conditions, identifying 20 °C, 20 min of extraction time, and an enzyme/substrate ratio of 400 mg/g as optimal parameters. Under these conditions, significant higher extraction yields were obtained (565.6 ± 12.9 µg of total betalains/g and 14.9 ± 2.4 mg of total polyphenols/g) compared with traditional methods such as enzymatic hydrolysis, microwave-assisted extraction, and conventional ultrasound. Temperature and enzyme/substrate ratio were the most influential factors affecting the extraction of betalain and polyphenols, respectively. These findings provide a solid foundation for the development of high-added-value functional ingredients, promoting the integral use of pitahaya and contributing to innovation in the design of foods with health-enhancing properties.

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