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Research Article

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Posted Date: November 9th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11423024/v1

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Additional Declarations: No competing interests reported.

Optimization of the Ultrasound-Assisted Extraction of Anthocyanins from Acerola (*Malpighia emarginata*) Using Response Surface Methodology

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Abstract

The extraction of anthocyanins from acerola (*Malpighia emarginata*) has potential for obtaining bioactive compounds, and ultrasound-assisted extraction (UAE) is an alternative to conventional methods. In this study, the kinetics and the influence of operating variables on the total anthocyanin content (TAC) obtained by UAE were investigated. Extraction was performed in an ultrasonic bath at 40 kHz, using an extraction solvent of 95% ethanol and 1.5 mol L^{-1} HCl at an 85:15 (v/v) ratio. Extraction kinetics were evaluated using Fick's Law, the Power Law, and the Peleg model, while the experimental conditions were optimized through statistical analysis of a Box-Behnken design. TAC was determined by the single pH method at 535 nm. Among the kinetic models, the Peleg model showed a satisfactory fit to the experimental data, with $R^2 > 0.99$ and RMSE=0.1975, indicating the relevance of diffusion in the mass transfer process. In the experimental design, TAC ranged from 9.32 to 25.46

mg/100 g. ANOVA indicated a significant effect of the linear term of the solid/liquid ratio ($p = 0.0012$) and of the quadratic terms of temperature ($p = 0.0065$) and time ($p = 0.0151$). The quadratic regression model showed $R^2 = 0.9403$ and a non-significant lack of fit ($p = 0.2633$). The maximum response predicted by the model was 27.45 mg/100 g, associated with a temperature of 38.8 °C, a time of 7.87 min, and a solid/liquid ratio of 0.02 g/mL. The results revealed that combining kinetic modeling and optimization is a suitable approach for understanding the ultrasound-assisted extraction of anthocyanins from acerola.

Keywords: bioactive compounds; sonication; extraction; acerola, DoE.

Introduction

Anthocyanins are natural plant pigments that are water-soluble and belong to the class of phenolic compounds known as flavonoids. They are widely found in flowers, fruits, stems, and leaves. According to Tena and Asuero (2022), anthocyanins have drawn increasing attention because of their potential health benefits, including (i) anti-inflammatory, antioxidant, antidiabetic, and anticancer properties; (ii) prevention of cardiovascular diseases, neurological disorders, obesity, and benefits for eye health; (iii) improvement of the gut microbiome; and (iv) reduction of H_2O_2-induced cell apoptosis of the human normal liver cell line. They also show low toxicity and a high degree of safety for use in foods, pharmaceuticals, cosmetics, and other fields.

The extraction of anthocyanins is a crucial step in obtaining these compounds because of their low stability, which is easily influenced by numerous factors, such as relative humidity, light, pH, temperature, sugars, vitamin C, oxygen levels, enzymes, and metal ions (Enaru et al. 2021).

Several methods are available for extracting anthocyanins, the most common being the classical techniques of maceration and organic solvent extraction, as well as ultrasound-assisted extraction (UAE). Solvent extraction is the most widely used in laboratories, but the choice of method depends on the process conditions and the purpose of the extraction. Most of these conventional approaches have limitations, such as the use of toxic solvents (health risks), high temperatures, and long extraction times (degradation of target compounds).

Previous studies have shown that bioactive compounds can be obtained using techniques such as ultrasonic bath (Zurob et al. 2020), shaker (Dutra et al. 2017), mechanical stirring (Correia et al. 2012), Soxhlet (George et al. 2015), and vortex (Paradiso et al. 2019).

Notably, UAE is a potential alternative to conventional extraction of plant metabolites. It is widely applied because it reduces the use of toxic solvents and requires lower temperatures and shorter extraction times.

Therefore, this method is one of the most efficient, inexpensive, simple, rapid, and with greater reproducibility (Rezende et al. 2017).

Acerola (*Malpighia emarginata*) is a fruit native to Central America and northern South America, with large cultivated areas in Brazil. Brazil has recently expanded its acerola cultivation areas, and the fruit is widely marketed fresh, as pulp, and as juice (Rodriguez-Amaya 2016). The demand for the fruit has grown mainly due to its rich content of ascorbic acid, carotenoids, and phenolic compounds, besides its high antioxidant activity (Rezende et al. 2017).

The red color of ripe acerola is due to the presence of anthocyanins. These pigments are water-soluble phenolic compounds belonging to the flavonoid group and are widely distributed in the plant kingdom, giving fruits, flowers, and roots shades ranging from orange to red (Lima et al. 2003).

Structurally, anthocyanins occur as glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium salts (also called the flavylium cation). Fig. 1 shows the basic skeleton of anthocyanins, in which 2-phenylbenzopyrylium is the core structure.

There are six common anthocyanins, namely, cyanidin, petunidin, peonidin, pelargonidin, delphinidin, and malvidin (Fig. 1). The different types of anthocyanins arise mainly from differences in the position of the glycosidic bond, the type and number of sugars, the number of hydroxyl groups, and the type and number of aromatic acids, among other factors (Tan et al. 2022). Anthocyanins contain unsaturated double bonds and easily oxidized groups, which makes them extremely unstable.

Owing to the ionic nature of their molecular structure, the color of anthocyanins is influenced by pH, and they can occur in four distinct chemical forms depending on the pH of the solution. Thus, in an acidic environment at pH = 1, anthocyanins are present as the flavylium cation (red color), which makes them highly soluble in water; this form is also responsible for the red and purple colors. When the pH increases to the range of 2–4, the quinoidal blue species predominates, whereas at pH 5–6 the carbinol pseudobase and a chalcone appear, both colorless. Finally, at pH above 7, anthocyanins degrade, a process that depends on the substituent groups present (Enaru et al. 2021).

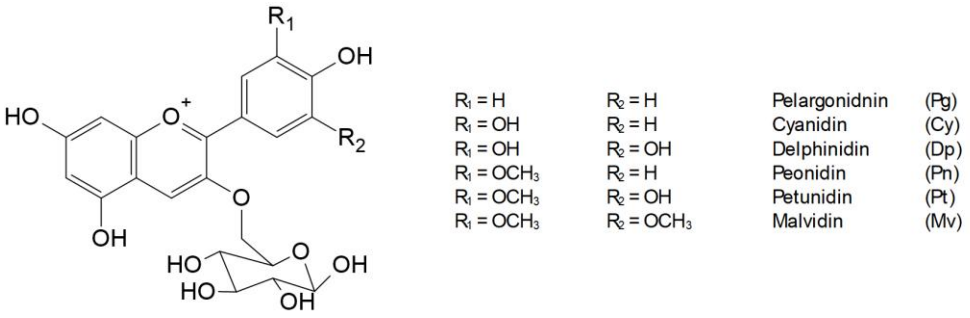


Fig. 1 Basic structure of anthocyanins

The extraction factors (solid-liquid ratio, ultrasonic power, microwave power, extraction temperature, and extraction time) must be reasonably controlled to optimize the extraction rate of anthocyanins by using response surface methodology, orthogonal test, genetic algorithm, and neural networks coupled with genetic algorithms (Tan et al. 2022).

UAE relies on high-frequency mechanical waves. The method operates through cavitation, in which the ultrasonic waves create a cycle of bubbles and cavities in the solvent that shear the plant material, thereby facilitating the extraction of bioactive compounds.

Ultrasound can make the extraction of natural anthocyanins more efficient than traditional extraction, up to a certain extent. However, the cavitation and mechanical effects produced by ultrasound may destroy the structure of anthocyanins during UAE. Therefore, the ultrasonic conditions (ultrasound power, extraction temperature, solid-liquid ratio, and extraction time) must be strictly controlled to take full advantage of ultrasonic extraction (Tan et al. 2022).

Overall, UAE has proven to be a promising alternative due to its simplicity, ease of operation, low cost, and high efficiency. Thus, this study is relevant, as it aims to optimize the extraction of bioactive compounds (anthocyanins) from acerola fruits by UAE using response surface methodology.

Materials and Methods

Sample Preparation

Acerola fruits (*Malpighia emarginata*) were collected from different plants in the region of Campina Grande, Paraíba, Brazil (7°13′50″ S, 35°52′52″ W). Fruits free of defects and at the same ripening stage were selected. They were washed under running water and air-dried at 25 °C prior to the assays. The pulp was then manually separated from the seeds and homogenized in a blender to obtain a uniform material.

Anthocyanin Extraction

Anthocyanins were extracted from acerola fruits following the methodology described by Moo-Huchin et al. (2015) and Gualberto et al. (2021). Between 1 and 9 g of the pulp obtained in the sample preparation step was mixed with 50 mL of extraction solvent consisting of ethanol (95%) and HCl (1.5 mol L^{-1}) (Dinâmica®) at an 85:15 (v/v) ratio. Extraction was carried out in an ultrasonic bath (Limatec, LT 210/6) at a frequency of 40 kHz, at different temperatures and times according to the experimental design.

Quantification of Anthocyanin Content

After extraction, the samples were filtered through Whatman No. 2 filter paper, and absorbance was measured at 535 nm using a spectrophotometer (WebLaborSP, WUV-M51). TAC was determined by the single pH method described by Moo-Huchin et al. (2015) and calculated in mg per 100 g of sample according to Eq. 1:

$$TAC = \frac{A_{535} \times DF \times V_{ext}}{E^{1\%}_{1\ cm,535} \times m} \times 100 \quad (1)$$

where *TAC* is the total anthocyanin content (mg 100 g^{-1} of pulp), A_{535} is the absorbance of the sample measured at 535 nm after extraction, *DF* is the dilution factor, V_{ext} is the volume of extraction solvent (mL), *m* is the mass of pulp used (g), and $E^{1\%}_{1\ cm,535}$ is the mean extinction coefficient (98.2 L g^{-1} cm^{-1}) for acidified ethanol, which refers to the absorbance of an anthocyanin mixture in acidified ethanol at a concentration of 1% (w/v), measured in a 1 cm cell at 535 nm.

Absorption Spectrum of Anthocyanins

Because the stability of anthocyanins is influenced by medium acidity, which may affect the efficiency of anthocyanin extraction from acerola pulp, the molecular absorption spectra of acerola anthocyanins were obtained for three ratios of ethanol (95%) to HCl (1.5 mol L^{-1}) in the extraction solvent (75:25, 85:15, and 95:5, v/v). For each ratio, 100 mL of extraction solvent containing 8.0 g of acerola pulp was sonicated at 25 °C for 15 min. The absorbance peaks of each sample were then identified.

Kinetic Modeling

Kinetic modeling was performed using TAC data collected at 1 min intervals over 12 min, in order to interpret the mass transfer process during the UAE of anthocyanins from acerola. A total of 8.0 g of acerola pulp

was extracted in 100 mL of ethanol/HCl extraction solvent (85:15, v/v) at 25 °C. Three mathematical models were evaluated: Fick's Law, the Power Law, and the Peleg model. Each model was fitted to the experimental data by nonlinear regression, and the goodness of fit was assessed using the coefficient of determination (R^2) and the root mean square error (RMSE), given by Eqs. 2 and 3, respectively:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \tag{2}$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2} \tag{3}$$

where n is the total number of experimental points, y_i are the experimental TAC values, $\bar{y}$ is the mean of the experimental values, and $\hat{y}_i$ are the TAC values predicted by the model.

According to Roslan et al. (2026), Fick's Law describes the basic mechanism of the extraction process, in which mass transfer occurs by diffusion, and it is the fundamental law used to describe extraction processes. The model can be expressed by Eq. 4:

$$C(t) = C_{eq}\,(1 - e^{-kaRt}) \tag{4}$$

where C is the concentration of solutes in the liquid phase (mg 100 mL^{-1}) at time t, t is the contact time (min), kaR is the solvent-phase mass transfer coefficient (min^{-1}), and C_{eq} is the saturation (equilibrium) concentration at the surface (mg 100 mL^{-1}).

According to Sivri (2024), the power-law model is an empirical equation used to describe solid-liquid extraction and is given by Eq. 5:

$$C(t) = Bt^n \tag{5}$$

where $C(t)$ is the solute concentration at time t, t is the extraction time, B is a phenomenological constant related to the initial rate and extraction capacity, and n is the diffusional exponent, the most critical parameter for characterizing the type of transport involved, which must be < 1 when extraction is performed from plant cells.

The Peleg model describes extraction as a two-stage process, in which the kinetics initially follow first-order behavior and then transition to zero-order behavior during the final stage. This model was developed to study the extraction kinetics of bioactive compounds from plants and can be expressed as:

$$C(t) = \frac{t}{k_1 + k_2 t} \tag{6}$$

where t is the extraction time (min), $C(t)$ is the concentration of bioactive compounds at time t (mg 100 mL^{-1}), k_1 is the Peleg rate constant (min mL 100 mg^{-1}), and k_2 is the Peleg capacity constant (mL 100 mg^{-1}). The constant k_1 relates to the extraction rate of bioactive compounds, whereas k_2 represents the maximum extraction yield.

Experimental Design

The Box-Behnken design (BBD) is a second-order multifactorial experimental approach based on three-level factorial designs. It is widely used to evaluate the effects of experimental parameters on extraction processes. The independent variables and the three levels evaluated in this study are depicted in Table 1.

Table 1 Independent variables and their levels used in the BBD runs

Factor	Levels		
	-1	0	1
Solid/liquid ratio (g mL^{-1})	0.02	0.10	0.18
Temperature (°C)	25	40	55
Time (min)	4	8	12

To evaluate the effects of the independent variables (solid/liquid (S/L) ratio, temperature, and extraction time) on the dependent variable (TAC), the experimental matrix was built using response surface methodology based on the Box-Behnken design, comprising 12 runs plus 3 replicates at the center point.

Statistical Analysis

Statistical analysis of the experimental data obtained from the BBD runs was performed in the free software R (*rsm* package), from which the response function was obtained considering a second-order model, described by Eq. 7:

$$y = \beta_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X_i^2 + \sum \sum_{i<j=2}^{k} \beta_{ij} X_i X_j \quad (7)$$

where y is the response variable (TAC), β_o is a constant (mean), β_i is the linear coefficient for factor X_i, β_{ii} is the quadratic coefficient for factor X_i^2, and β_{ij} is the interaction coefficient between X_i and X_j. These terms are defined for a number of factors $k = 3$.

The model that best fitted the experimental data was selected based on analysis of variance (ANOVA), the coefficient of determination (R^2), and the lack-of-fit test, and statistically significant terms were determined at $p < 0.05$. The normal probability plot of residuals and the residuals versus predicted values plot were then examined to verify normality and homoscedasticity.

To determine the optimum condition for anthocyanin extraction from acerola, the *optim* function in R was used, which iteratively maximizes a mathematical function based on the polynomial model generated by the ANOVA.

Results and Discussion

Kinetic Analysis

Understanding the kinetics is essential for the extraction process, as it characterizes and elucidates the mass transfer mechanism. Accordingly, the experimental data were fitted to different kinetic models, namely Fick's Law, the Power Law, and the Peleg model (Eqs. 4-6). Table 2 presents the kinetic and statistical parameters of the models evaluated.

Table 2 Kinetic and statistical parameters for the ultrasound-assisted extraction of anthocyanins from acerola

Model	Temperature (°C)	Model constants		Statistical parameters	
				R^2	RMSE
Fick's Law		C_{eq}	kaR		
		mg/100mL	min^{-1}		
	25.0	20.2111	0.2956	0.9956	0.4305
Power Law		B	n		
		mg/100mL.min	(<1)		

	25.0	7.4598	0.4161	0.9893	0.6271
Peleg model		k_1	k_2		
		min.mg/100mL	mg/100mL		
	25.0	0.1333	0.0387	0.9990	0.1975

As shown in Table 2, the three models fitted the experimental data satisfactorily, with R^2 values above 0.98 and RMSE below 0.63. The model that presented the best fit was the Peleg model, relating TAC extraction to time through two parameters (speed constant k_1 and capacity constant k_2). It described the experimental data satisfactorily, with an RMSE of 0.1975 and R^2 = 0.9990. The relatively low value indicates good agreement between the predicted and experimental values, which is corroborated by Figure 2. indicating that diffusion played a significant role in TAC extraction.

The Fick's Law provided a satisfactory empirical description of the extraction kinetics. This model indicates that diffusion is the limiting step of the process, driven by the concentration gradient between the sample and the solvent. relating. The model adequately described the experimental data, with an RMSE of 0.4305 and R^2 of 0.9956, indicating good agreement between predicted and experimental values, as corroborated by Fig. 2.

The Power Law model describes the extraction kinetics by considering the nonlinear relationship between the extracted TAC and time. It predicts that TAC increases rapidly during the initial period due to the high concentration gradient between the sample and the solvent. As extraction progresses, the TAC extraction rate decreases gradually and tends toward a plateau, as shown in Fig. 2, reflecting the reduction in the mass transfer driving force as equilibrium is approached (Yuniati et al. 2025). In this model, the coefficient *B* (mg 100 mL^{-1} min^{-1}) represents the mass transfer rate; the higher the value of *B*, the faster the mass transfer. The time-dependent nature of the model is described by a power exponent *n*, which modulates the rate of decrease. The Power Law showed a good statistical fit, with R^2 = 0.9893 and RMSE = 0.6271. The diffusional exponent *n* (0.4161) was below the characteristic value for Fickian diffusion (0.43). This indicates that TAC extraction is governed mainly by Fickian diffusion, in which mass transfer is controlled by molecular diffusion through the plant matrix. The relatively low *n* values suggest that solvent penetration and solute migration occur without a significant contribution from matrix relaxation, swelling, or other non-Fickian transport mechanisms (Roslan et al. 2026).

Fig. 2 illustrates the relationship between contact time and the efficiency of anthocyanin extraction from acerola by UAE.

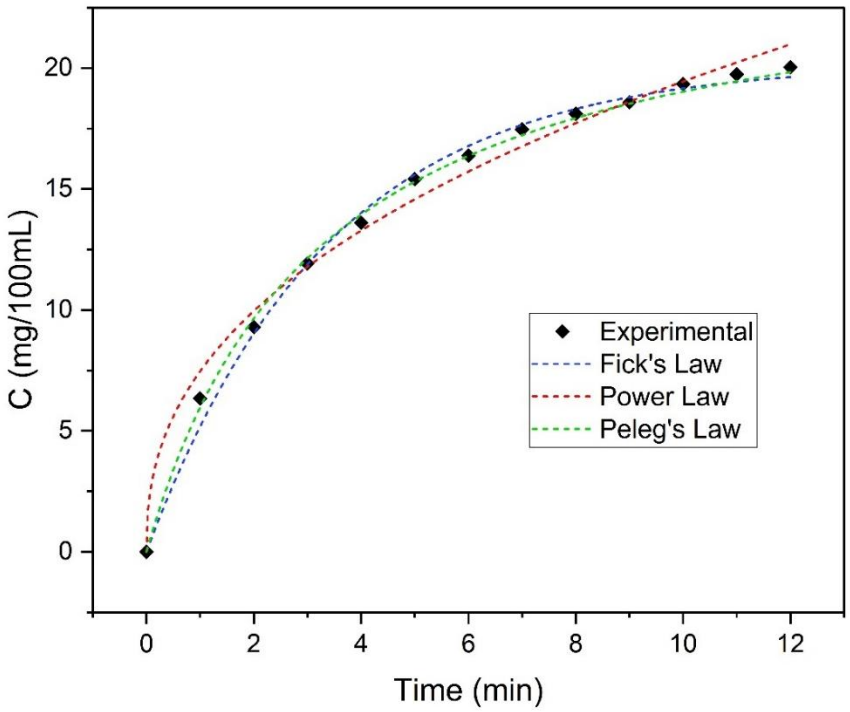


Fig. 2 Kinetics of the ultrasound-assisted extraction of anthocyanins from acerola

Maximum Absorption Spectra

Anthocyanins are known to be more stable in acidic medium, where they occur as the flavylium cation. For this reason, a mixture of ethanol and hydrochloric acid is used in the extraction solvent.

Fig. 3 shows the absorption spectra, in the 400–800 nm region, of the anthocyanin extracts obtained by ultrasound-assisted extraction using different ratios of ethanol and 1.5 mol L^{-1} hydrochloric acid. Ethanol/HCl ratios of 75:25, 85:15, and 95:5 (v/v) were evaluated.

The spectra obtained for the 75:25 and 85:15 ratios showed a similar profile, characterized by an intense absorption band at approximately 530–540 nm. The absorption maximum was observed near 535 nm, a region commonly used for the spectrophotometric determination of anthocyanins. This band is related mainly to anthocyanin species in the flavylium cation form, whose relative concentration is favored in acidic medium.

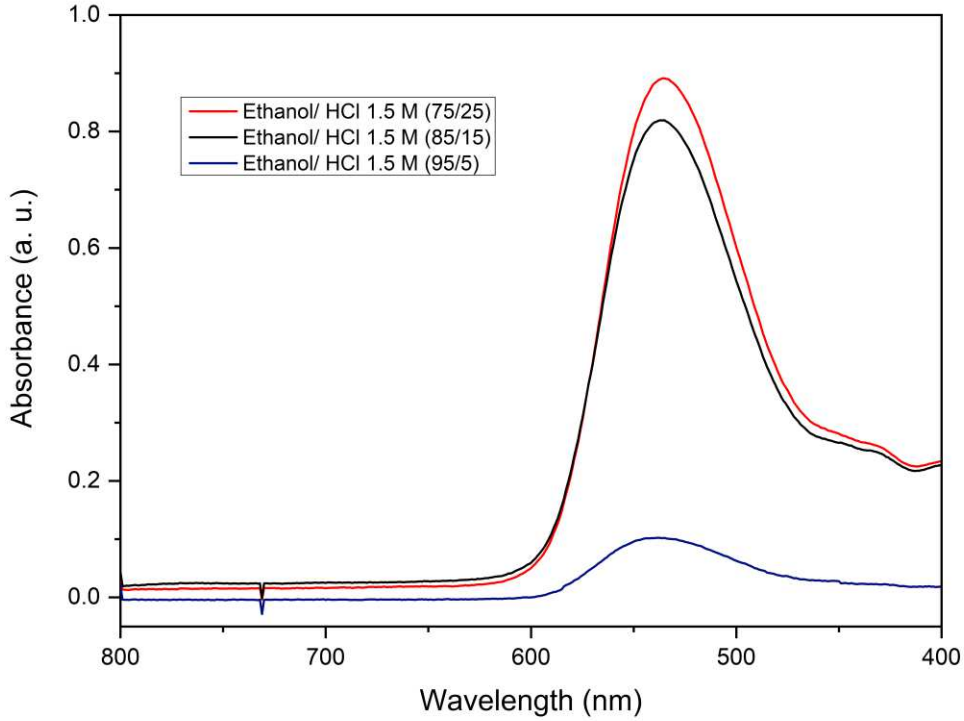


Fig. 3 Absorption spectra in the 400–800 nm region of anthocyanin extracts obtained by ultrasound-assisted extraction using different ethanol/HCl ratios

Among the conditions evaluated, the 75:25 ethanol/HCl ratio exhibited the highest absorption intensity, with a maximum absorbance of 0.8911, whereas the 85:15 ratio showed a maximum absorbance of approximately 0.8193. With the 95:5 ethanol/HCl ratio, the maximum absorbance was only 0.1022. Because no marked increase in maximum absorbance was observed between the 75:25 and 85:15 solutions, the 85:15 ratio was adopted in this study, following the criteria described in the literature and minimizing the use of strong acids. In all solutions tested, the maximum absorbance of the anthocyanins was observed at a wavelength of 535 nm.

Experimental Design

The independent variables of the ultrasound-assisted extraction of anthocyanins from acerola, namely S/L ratio (X_1), temperature (X_2), and extraction time (X_3), were statistically evaluated using a Box-Behnken design to investigate their effects on extraction efficiency, measured as total anthocyanin content (TAC, mg 100 g^{-1}). For this purpose, 15 runs were performed (12 factorial runs plus 3 center-point replicates), as described in Table 3. A

second-order polynomial model was fitted to the experimental data using the free software R. The resulting model is given in Eq. 8:

$$TAC\ (mg/100g) = 22.38 - 5.63X_1 - 0.61X_1^2 + 0.82X_2 - 5.62X_2^2 + 0.41X_3 - 4.55X_3^2 + 1.74X_1X_2 + 0.77X_1X_3 - 0.73X_2X_3 \quad (8)$$

The experimental and model-predicted results for anthocyanin extraction are listed in Table 3.

Table 3 Results of the Box-Behnken design runs for the ultrasound-assisted extraction of anthocyanins from acerola fruits

Run	S/L ratio (g mL^{-1})	Temperature (°C)	Time (min)	TAC (mg 100 g^{-1})	
	X_1	X_2	X_3	Experimental	Predicted
1	0.02 (-1)	25 (-1)	8 (0)	20.93	22.70
2	0.18 (+1)	25 (-1)	8 (0)	9.32	7.96
3	0.02 (-1)	55 (+1)	8 (0)	19.50	20.86
4	0.18 (+1)	55 (+1)	8 (0)	14.83	13.06
5	0.02 (-1)	40 (0)	4 (-1)	25.46	23.21
6	0.18 (+1)	40 (0)	4 (-1)	9.53	10.40
7	0.02 (-1)	40 (0)	12 (+1)	23.37	22.50
8	0.18 (+1)	40 (0)	12 (+1)	10.52	12.77
9	0.10 (0)	25 (-1)	4 (-1)	9.76	10.25
10	0.10 (0)	55 (+1)	4 (-1)	12.45	13.34
11	0.10 (0)	25 (-1)	8 (+1)	13.43	12.53
12	0.10 (0)	55 (+1)	8 (+1)	13.19	12.70
13	0.10 (0)	40 (0)	8 (0)	22.13	22.38
14	0.10 (0)	40 (0)	8 (0)	20.89	22.38
15	0.10 (0)	40 (0)	8 (0)	24.13	22.38

The ANOVA, shown in Table 4, was used to assess the significance and adequacy of the model. Statistical analysis of variance was performed using Fisher's *F*-test and the *p*-value.

Table 4 Analysis of variance for the Box-Behnken design for anthocyanin extraction from acerola fruits

Source of variation	Sum of squares	Degrees of freedom (df)	Mean square	*F*-value	*p*-value
S/L ratio (L) (X_1)	253.800	1	253.800	43.7535	0.0012*
S/L ratio (Q) (X_1^2)	1.387	1	1.387	0.2391	0.6455
Temp (L) (X_2)	5.330	1	5.330	0.9189	0.3818
Temp (Q) (X_2^2)	116.844	1	116.844	20.1432	0.0065*
Time (L) (X_3)	1.370	1	1.370	0.2361	0.6476
Time (Q) (X_3^2)	76.454	1	76.454	13.1802	0.0151*
X_1X_2	12.041	1	12.041	2.0758	0.2092
X_1X_3	2.372	1	2.372	0.4088	0.5507
X_2X_3	2.146	1	2.146	0.3700	0.5696
Regression	456.939	9	50.770	8.7524	0.0139
Residual	29.003	5	5.801		
Lack of Fit	23.658	3	7.886	2.9508	0.2633
Pure Error	5.345	2	2.673		
Total SS	485.932	14	34.709		

*p-value < 0.05 indicates that the model terms are significant. ** R^2 = 0.9403.

The analysis of variance of the quadratic model fitted to the data on ultrasound-assisted extraction of anthocyanins from acerola revealed that the solid/liquid ratio had a significant linear effect on the response (F = 43.75; p = 0.0012). In contrast, its quadratic term was not significant (p = 0.6455), indicating that, within the experimental range evaluated, the influence of the solid/liquid ratio was predominantly linear. For temperature, the linear effect was not significant (p = 0.3818), whereas the quadratic term was significant (F = 20.14; p = 0.0065), evidencing curvature in the response. Similar behavior was observed for extraction time, whose linear term was not significant (p = 0.6476), while its quadratic term was significant (F = 13.18; p = 0.0151). These results indicate that the effects of temperature and time on extraction cannot be described exclusively by linear relationships within the conditions studied.

The interactions between factors were not significant at the 5% level, with p = 0.2092, 0.5507, and 0.5696 for X_1X_2, X_1X_3, and X_2X_3, respectively. Therefore, there was insufficient statistical evidence that the effect of one variable depends on the level of the others. The model showed R^2 = 0.9403, indicating that approximately 94.03% of the variability observed in the response was explained by the fitted equation. The lack-of-fit test was not

significant (F = 2.95; p = 0.2633), indicating that the discrepancy between the experimental values and those estimated by the model was not statistically greater than the experimental variability given by the pure error. The absence of significant lack of fit, together with the high explanatory capacity of the model, indicates that the quadratic equation adequately represents the response under the experimental conditions evaluated.

According to Brahmi et al. (2019), a model with a high F-value and a small p-value (< 0.05) is considered statistically significant. Thus, it can be concluded that the linear coefficient for the S/L ratio (X_1) and the quadratic coefficients for temperature (X_2^2) and time (X_3^2) were significant, indicating that they significantly affect the dependent variable, in this case TAC (mg 100 g^{-1}). The Pareto chart shown in Fig. 4 corroborates these observations.

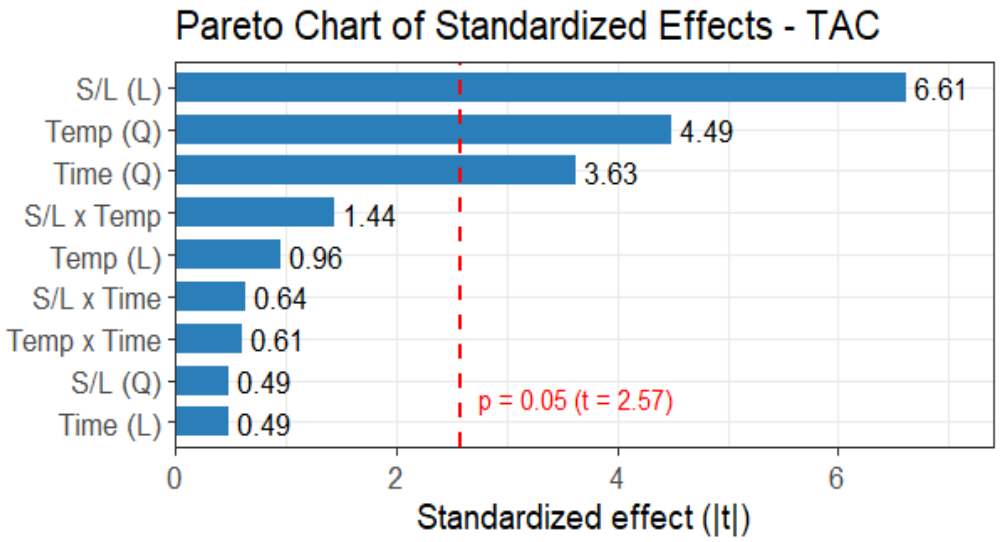


Fig. 4 Pareto chart of standardized effects for the ultrasound-assisted extraction of anthocyanins from acerola

The relationship between the experimentally observed values and those predicted by the quadratic model is shown in Fig. 5. The points are distributed relatively close to the identity line ($y = x$), indicating good agreement between the experimental responses and the values estimated by the model. The coefficient of determination calculated from the observed and predicted values was R^2 = 0.9403, indicating that approximately 94% of the variability in the response was explained by the proposed model. The root mean square error (RMSE) was approximately 1.39 mg 100 g^{-1}, showing a relatively small difference between the experimental and estimated values. The largest deviations were observed in runs 5 and 8, with residuals of approximately +2.25 and −2.25 mg 100 g^{-1}, respectively.

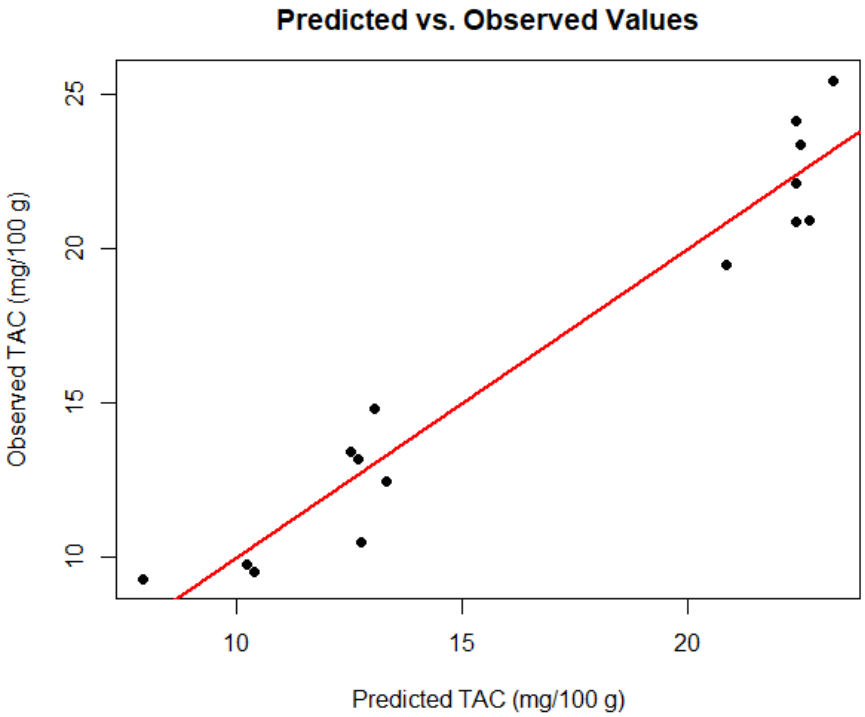


Fig. 5 Predicted versus observed values for the ultrasound-assisted extraction of anthocyanins from acerola

The center-point runs exhibited experimental values of 22.13, 20.89, and 24.13 mg 100 g^{-1}, whereas the model estimated 22.38 mg 100 g^{-1} for this condition. The dispersion among the center-point replicates represents the experimental variability of the procedure and provides an estimate of pure error, which is important for assessing model adequacy. Overall, the closeness between observed and predicted values, together with the behavior of the residuals, indicates that the quadratic model satisfactorily describes the influence of the experimental conditions on the ultrasound-assisted extraction of anthocyanins from acerola.

The normality of the residuals was assessed using the normal probability plot (Q–Q plot). As shown in Fig. 6, the residuals followed an approximately linear distribution relative to the reference line, mainly in the central region, with no visual evidence of pronounced systematic deviations. Minor deviations were observed at the tails, but they were not pronounced enough to indicate, visually, a major violation of the normality assumption. The behavior of the residuals was therefore compatible with applying analysis of variance to the fitted quadratic model.

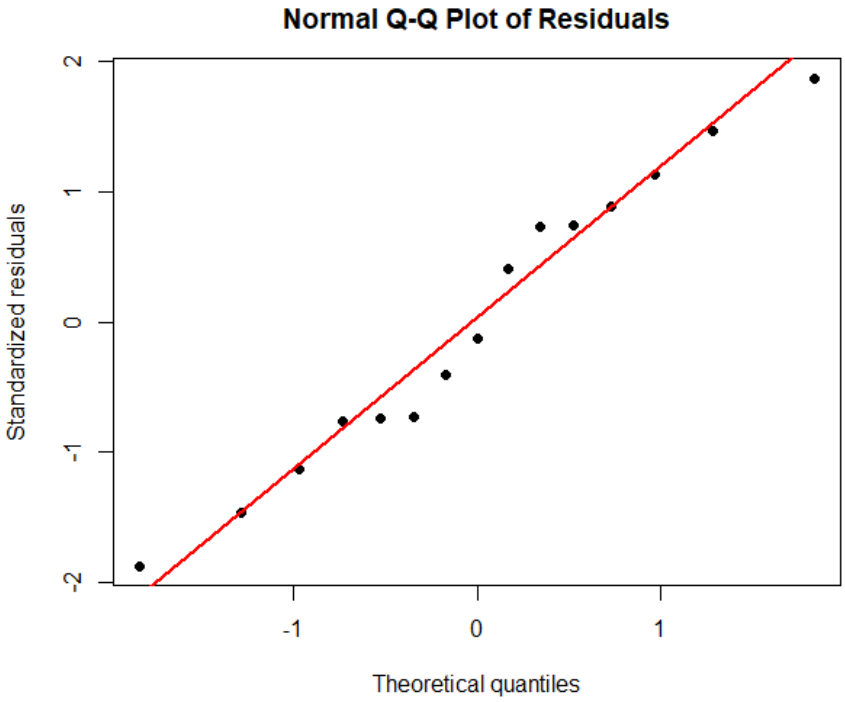


Fig. 6 Normal Q–Q plot of the residuals of the model used to describe the ultrasound-assisted extraction of anthocyanins from acerola

The regression model is presented below as response surfaces and contour plots for the operating variables within the experimental range investigated. The effects of the S/L ratio and temperature are shown in Fig. 7, which presents the response surface for anthocyanin content as a function of the solid/liquid ratio and temperature, with extraction time held at the center point of the design ($X_3 = 0$). The response decreases sharply as the solid/liquid ratio increases, consistent with the significant linear effect of this variable ($F = 43.75$; $p = 0.0012$) and with the negative linear coefficient in the model. In contrast, temperature behaved predominantly in a quadratic manner, since its linear effect was not significant ($p = 0.3818$), whereas its quadratic term was statistically significant ($F = 20.14$; $p = 0.0065$). The observed curvature indicates an intermediate temperature region associated with higher anthocyanin contents, suggesting that increasing temperature may initially favor mass transfer and pigment solubilization, whereas higher temperatures may contribute to pigment degradation.

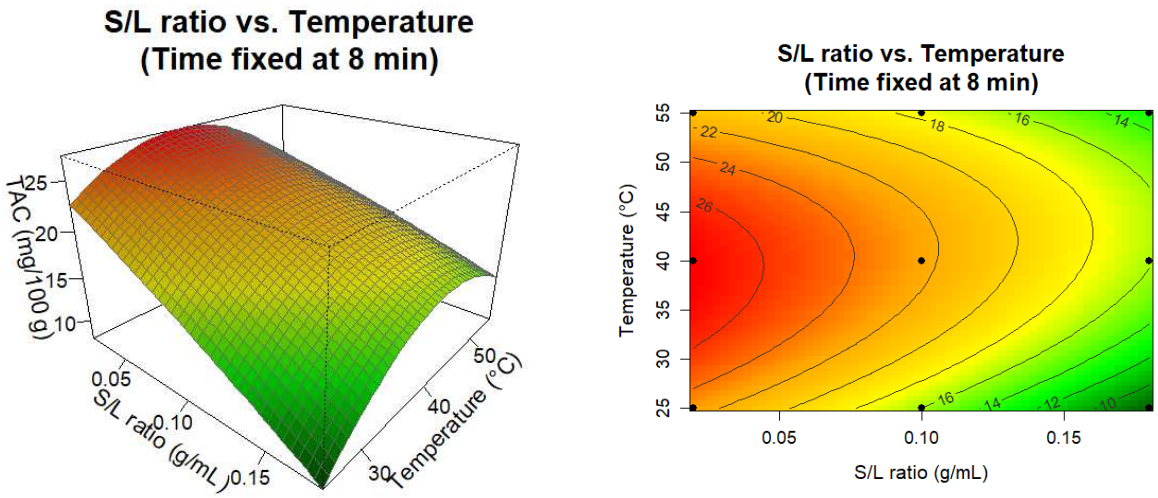


Fig. 7 Response surface (3D) and contour plot (2D) for the S/L ratio and temperature in the ultrasound-assisted extraction of anthocyanins from acerola

The region of highest response is located near the lowest S/L ratio levels and an intermediate temperature range. Although a change in the slope of the surface due to the interaction between these variables is visible, the X_1X_2 interaction was not statistically significant ($F = 2.08$; $p = 0.2092$), so there was insufficient evidence that the effect of one variable depends on the other under the conditions studied. The adequacy of the surface is supported by the high coefficient of determination ($R^2 = 0.9403$) and the absence of significant lack of fit ($F = 2.95$; $p = 0.2633$). Therefore, the surface adequately represents the trends observed for anthocyanin extraction in the experimental region investigated.

The response surface in Fig. 8 shows the joint influence of the solid/liquid ratio and extraction time on anthocyanin content, with temperature held at the center level of the design. The response decreases sharply as the solid/liquid ratio increases, whereas the effect of time is curvilinear, with higher anthocyanin contents associated with an intermediate time region.

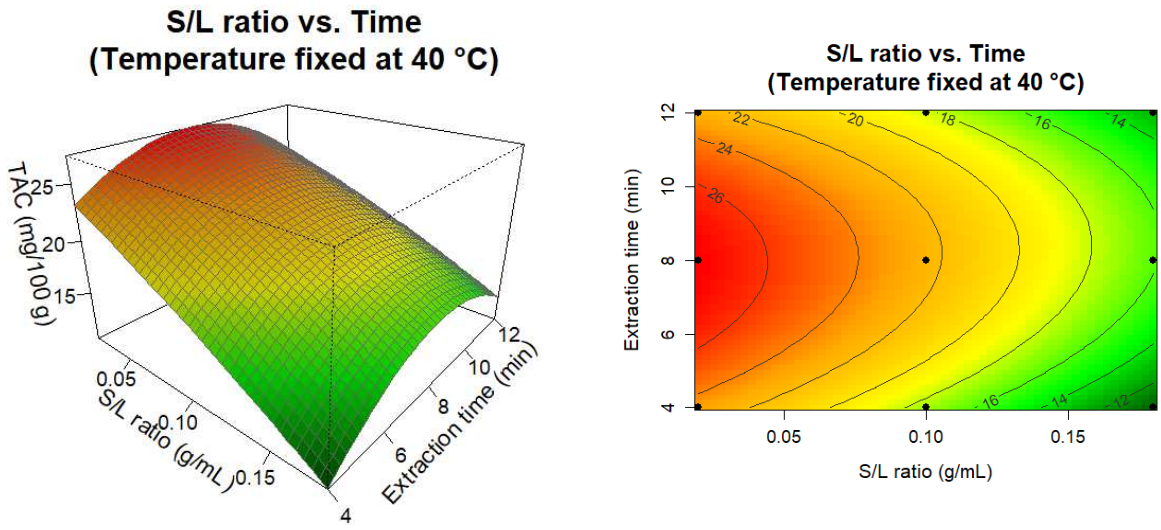


Fig. 8 Response surface (3D) and contour plot (2D) for the S/L ratio and extraction time in the ultrasound-assisted extraction of anthocyanins from acerola

This behavior is consistent with the quadratic model, in which the linear effect of the solid/liquid ratio was significant (p = 0.0012), whereas the linear effect of time was not (p = 0.6476) and its quadratic term was significant (p = 0.0151). The surface thus indicates that reducing the solid/liquid ratio has a more pronounced influence on the response, whereas time has an intermediate range that is more favorable for extraction, possibly because of a balance between increasing mass transfer and the eventual degradation of pigments at longer times.

Fig. 9 illustrates the response surface for anthocyanin content as a function of temperature and extraction time, with the solid/liquid ratio held at the center level of the experimental design. The behavior is curvilinear, with higher responses in the central region of the surface and decreasing anthocyanin content toward the extreme levels of both variables. This profile reflects the influence of the quadratic terms of temperature and time, indicating that the relationship between these factors and the response is not exclusively linear. This behavior is consistent with the analysis of variance, in which the quadratic terms of temperature (p = 0.0065) and time (p = 0.0151) were statistically significant, whereas their respective linear terms were not significant at the 5% level.

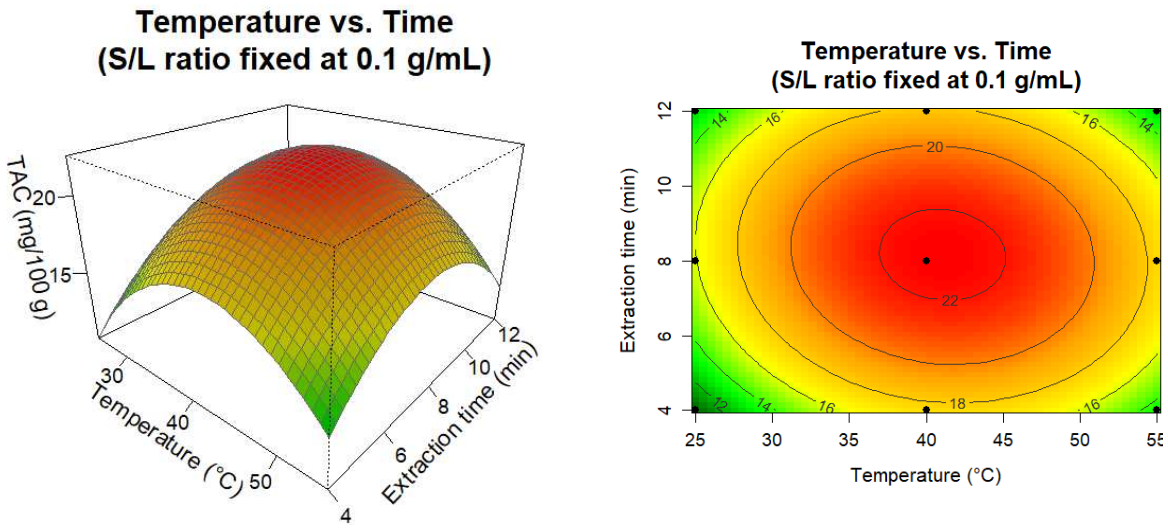


Fig. 9 Response surface (3D) and contour plot (2D) for temperature and extraction time in the ultrasound-assisted extraction of anthocyanins from acerola

The observed curvature suggests an intermediate range of temperature and time that is more favorable for anthocyanin extraction. This behavior may reflect a balance between the enhancement of mass transfer and pigment solubilization, on the one hand, and the possible degradation of these compounds under more intense or prolonged extraction conditions, on the other.

The experimental conditions were optimized using the *optim()* function in R (package *stats*), which iteratively maximizes or minimizes a mathematical function. Figure 10 shows the optimal point (blue asterisk) for temperature and extraction time variables. For the S/L ratio variable, the optimal condition is at the minimum level tested (0.02 g/mL).

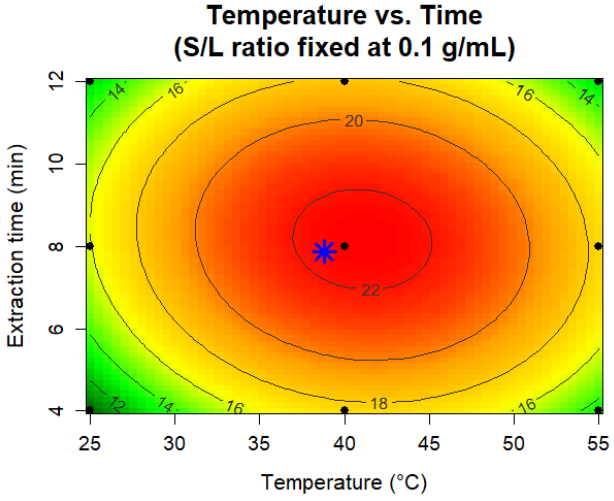


Fig. 10 Optimal condition (blue asterisk) for temperature and extraction time in the ultrasound-assisted extraction of anthocyanins from acerola

A maximum TAC of 27.45 mg 100 g^{-1} was predicted at a temperature of 38.8 °C, an extraction time of 7.87 min, and an S/L ratio of 0.02 g mL^{-1}. For comparison, Rosso et al. (2008) reported anthocyanin contents between 6.5 and 8.4 mg 100 g^{-1} in acerola obtained by conventional solvent extraction, which highlights the advantage of UAE.

Conclusion

Ultrasound-assisted extraction proved to be a suitable approach for obtaining anthocyanins from acerola (*Malpighia emarginata*), allowing the kinetic aspects and the effects of the main operating variables of the process to be evaluated simultaneously. The experimental anthocyanin contents obtained in the design runs ranged from 9.32 to 25.46 mg 100 g^{-1}, showing the influence of the extraction conditions on the response.

The kinetic analysis showed that Fick's Law, the Power Law, and the Peleg model satisfactorily represented the experimental data, with coefficients of determination above 0.98. Peleg model showed the highest R^2 (0.9990) and an RMSE of 0.1975, however, as it is an empirical model, its parameters provide information on the initial rate and the asymptotic extraction capacity, but they do not, on their own, allow for the determination of the predominant mass transfer mechanism. The Power Law also showed a high coefficient of determination (R^2 = 0.9893) and an RMSE of 0.6271, with n = 0.4161, a value compatible with a predominantly diffusive process. The Fick's Law performed equally satisfactorily (R^2 = 0.9956; RMSE = 0.4305), reinforcing the ability of the evaluated models to describe the time course of extraction, indicating that diffusion is a relevant mechanism for the transfer of anthocyanins from the plant matrix to the solvent. Taken together, the kinetic results underscore the importance of diffusion and concentration gradients in the transport of anthocyanins from the plant matrix to the solvent.

Response surface methodology made it possible to identify the individual and combined effects of the solid/liquid ratio, temperature, and extraction time. The solid/liquid ratio had a significant linear effect (p = 0.0012), whereas the quadratic terms of temperature (p = 0.0065) and time (p = 0.0151) were significant, indicating nonlinear behavior of these two variables. The interactions between factors were not statistically significant at the 5% level. The quadratic regression model showed R^2 = 0.9403 and a non-significant lack of fit (p = 0.2633), indicating that the model adequately describes TAC behavior within the experimental region investigated.

Mathematical optimization indicated a maximum predicted response of 27.45 mg 100 g^{-1} at a temperature of 38.8 °C, an extraction time of 7.87 min, and a solid/liquid ratio of 0.02 g mL^{-1}. The kinetic results and response

surface modeling show that the process is strongly influenced by mass transfer phenomena and operating conditions, particularly solvent availability and the nonlinear effects of temperature and time.

In conclusion, combining kinetic modeling with the Box-Behnken design and response surface methodology provided a more comprehensive understanding of the ultrasound-assisted extraction of anthocyanins from acerola. While the kinetic models characterized the temporal behavior of extraction and highlighted the relevance of diffusive phenomena, the statistical modeling identified the effects of the operating variables and their relationships with anthocyanin content. These analyses may strengthen the reliability of predictions and contribute to the development of more efficient and reproducible extraction conditions.

Author Contributions

J. L. G. Cavalcante: Writing – original draft, Methodology, Data acquisition. G. M. N. Santos: Writing – original draft, Methodology, Data acquisition. R. L. Cavalcante: Writing – original draft, Methodology, Data acquisition. J. F. da Silva: Writing – original draft, Methodology, Data acquisition. C. O. Rocha: Methodology, Formal analysis, and Conceptualization. Antonio J. F. Gadelha: Writing – original draft, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization, and Data curation.

Data Availability Statement

All relevant data are included in the paper or its Supplementary Information.

Statements and Declarations

Competing interests

The authors declare there is no conflict of interest.

Funding

No funding was received to assist with the preparation of this manuscript.

References

Brahmi L, Kaouah F, Boumaza S, Trari M (2019) Response surface methodology for the optimization of acid dye adsorption onto activated carbon prepared from wild date stones. Appl Water Sci 9:171. https://doi.org/10.1007/s13201-019-1053-2

Correia RTP, Borges KC, Medeiros MF, Genovese MI (2012) Bioactive compounds and phenolic-linked functionality of powdered tropical fruit residues. Food Sci Technol Int 18:539-547. https://doi.org/10.1177/1082013211433077

Dutra RLT, Dantas AM, Marques DA, Batista JDF, Meireles BRLA, Cordeiro AMTM, Magnani M, Borges GSC (2017) Bioaccessibility and antioxidant activity of phenolic compounds in frozen pulps of Brazilian exotic fruits exposed to simulated gastrointestinal conditions. Food Res Int 100:650-657. https://doi.org/10.1016/j.foodres.2017.07.047

Enaru B, Drețcanu G, Pop TD, Stănilă A, Diaconeasa Z (2021) Anthocyanins: Factors affecting their stability and degradation. Antioxidants 10:1967. https://doi.org/10.3390/antiox10121967

George VC, Kumar DRN, Suresh PK, Kumar RA (2015) Antioxidant, DNA protective efficacy and HPLC analysis of *Annona muricata* (soursop) extracts. J Food Sci Technol 52:2328-2335. https://doi.org/10.1007/s13197- 014-1289-7

Gualberto NC, Oliveira CS de, Nogueira JP, Jesus MS de, Araújo HCS, Rajan M, Leite Neta MTS, Narain N (2021) Bioactive compounds and antioxidant activities in the agro-industrial residues of acerola (*Malpighia emarginata L.*), guava (*Psidium guajava L.*), genipap (*Genipa americana L.*) and umbu (*Spondias tuberosa L.*) fruits assisted by ultrasonic or shaker extraction. Food Res Int 147:110538. https://doi.org/10.1016/j.foodres.2021.110538

Lima VLAG de, Mélo E de A, Maciel MIS, Lima DE da S (2003) Avaliação do teor de antocianinas em polpa de acerola congelada proveniente de frutos de 12 diferentes aceroleiras (*Malpighia emarginata* D.C.). Ciênc Tecnol Aliment 23:101-103. https://doi.org/10.1590/S0101-20612003000100021

Moo-Huchin VM, Moo-Huchin MI, Estrada-León RJ, Cuevas-Glory L, Estrada-Mota IA, Ortiz-Vázquez E, Betancur-Ancona D, Sauri-Duch E (2015) Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. Food Chem 166:17-22. http://doi.org/10.1016/j.foodchem.2014.05.127

Paradiso VM, Squeo G, Pasqualone A, Caponio F, Summo C (2019) An easy and green tool for olive oils labelling according to the contents of hydroxytyrosol and tyrosol derivatives: Extraction with a natural deep eutectic solvent and direct spectrophotometric analysis. Food Chem 291:1-6. https://doi.org/10.1016/j. foodchem.2019.03.139

Rezende YRRS, Nogueira JP, Narain N (2017) Comparison and optimization of conventional and ultrasound assisted extraction for bioactive compounds and antioxidant activity from agro-industrial acerola (*Malpighia emarginata* DC) residue. LWT – Food Sci Technol 85:158-169. https://doi.org/10.1016/j.lwt.2017.07.020

Rodriguez-Amaya DB (2016) Acerola juice. In: Shahidi F, Alasalvar C (ed) Handbook of functional beverages and human health. Taylor & Francis, Boca Raton, pp. 85-91.

Roslan R, Kamal N, Mansor S, Kurnia KA, Aziz AHA, Ruslan MSH (2026) Optimisation and kinetic modelling of anthocyanins extracted from *Hibiscus sabdariffa* in the ultrasonic-assisted extraction with natural deep eutectic solvents (NADES)-enhanced water. Chem Pap 12:132-149. https://doi.org/10.1007/s11696-026-05452-7

Rosso VV, Hillebrand S, Cuevas Montilla E, Bobbio FO, Winterhalter P, Mercadante AZ (2008) Determination of anthocyanins from acerola (*Malpighia emarginata* DC.) and açai (*Euterpe oleracea* Mart.) by HPLC–PDA–MS/MS. J Food Compos Anal 21:291-299. https://doi.org/10.1016/j.jfca.2008.01.001

Sivri GT (2024) Mathematical modeling of extraction kinetics from black chokeberries enhanced by cold plasma pretreatment. LWT – Food Sci Technol 202:116297. https://doi.org/10.1016/j.lwt.2024.116297

Tan J, Han Y, Han B, Qi X, Cai X, Ge S, Xue H (2022) Extraction and purification of anthocyanins: A review. J Agric Food Res 8:100306. https://doi.org/10.1016/j.jafr.2022.100306

Tena N, Asuero AG (2022) Up-to-date analysis of the extraction methods for anthocyanins: Principles of the techniques, optimization, technical progress, and industrial application. Antioxidants 11:286. https://doi.org/10.3390/antiox11020286

Yuniati Y, Jimmy J, Pramono YB, Mahfud M (2025) Enhancing decaffeination selectivity in arabica coffee via microwave-assisted extraction: A case study of trade-offs, parameter effects, and kinetic modeling. Results Eng 28:107714. https://doi.org/10.1016/j.rineng.2025.107714

Zurob E, Cabezas R, Villarroel E, Rosas N, Merlet G, Quijada-Maldonado E, Romero J, Plaza A (2020) Design of natural deep eutectic solvents for the ultrasound-assisted extraction of hydroxytyrosol from olive leaves supported by COSMO-RS. Sep Purif Technol 248:117054. https://doi.org/10.1016/j. seppur.2020.117054