Optimization, kinetic and phenomenological modeling of ultrasound- assisted extraction process of bioactive compounds from raspberries (Rubus idaeus L.)

Bárbara Avancini Teixeira (barbara.avancini@gmail.com)
Universidade Federal de Viçosa
Paulo César Stringheta
Universidade Federal de Viçosa
Márcia Cristina Teixeira Ribeiro Vidigal
Universidade Federal de Viçosa
Bruno de Castro Leite Júnior
Universidade Federal de Viçosa
Érica Nascif Rufino Vieira
Universidade Federal de Viçosa
Eliane Maurício Furtado Martins
Instituto Federal de Educação, Ciência e Tecnologia do Sudeste de Minas Rio Pomba

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Abstract

Bioactive compounds are of considerable interest due to their antioxidant properties and potential beneficial health effects. Thus, this study aimed to optimize and model the ultrasound-assisted extraction process (UAE) of response variables total anthocyanins (TA) and total phenolic content (TPC) from raspberries, and to compare the optimized extraction process with the conventional one. The variables used were time (5 to 75 min), temperature (30 to 70°C), and solid: liquid ratio (1:5 to 1:15 m/v), applied to the Box-Behnken Design (BBD). The optimal condition of the UAE process occurs at a temperature of 70°C and a solid: liquid ratio of 1:12.5 m/v, for both response variables. The optimal time for extraction of TA occurs in 22.5 min predicting the content of 23.181 mg/100 g of the fruit and for TPC occurs in 57.5 min predicting the content of 156.30 mg GAE/100 g of the fruit. On validation of the optimized conditions, less than a 5% difference ware found between the predicted and experimental values (24,131 mg/100 g of fruit for TA, and 149.226 mg GAE/100 g of fruit for TPC). When comparing the optimized UAE with the conventional extraction, it wares observed that UAE increased ($p < 0.05$) the extraction of TA content by 18.28% and TPC by 28.88%. The process time reduction from 24h in conventional extraction to less than 1h in optimized UAE stands out. Thus, the study indicates that the UAE process is an efficient methodology for recovering bioactive compounds from raspberries.

Introduction

Raspberries *(Rubus idaeus L.)* are fruits that stand out for their exotic taste, pleasant appearance, and high content of health-beneficial compounds (J. Yu Chen et al. 2020). Although this fruit comes from regions with a cold climate - such as Northern Europe and North America - it has been gaining ground in the production and market in Brazil (Barbieri and Vizzotto 2012). The increased demand for these fruits occurs because the raspberry is considered an important source of bioactive compounds, such as phenolic compounds. These compounds are considered natural antioxidants and have aroused scientific interest due to the numerous positive health effects they can cause (Das and Eun 2018).

Among the phenolic compounds present in raspberries, anthocyanins stand out, mainly cyanidin-3-sophoroside and cyanidin-3-glycoside, which in addition to being potent antioxidant agents against oxidative stress - which contribute to the protection of various chronic diseases, such as cardiovascular disorders, degenerative diseases, and cancer - can also act as natural colorants in foods and beverages (Fang 2015; He et al. 2016; Teng et al. 2017, Chen et al. 2020). Currently, although the use of synthetic colorants is still prevalent in the food industry, they are associated with several health problems, including allergies and intolerances, especially in children. Consequently, synthetic colorants are being progressively banned from the market, and colorants obtained from natural sources are emerging as a viable alternative to replacing them (Sharma et al. 2016; Strieder et al. 2019).

To produce a natural colorant, first, it is necessary to extract them from the vegetable matrix where it is found. When one wants to produce them on large scale, understanding the influence of the extraction process on the functional properties of these bioactive ingredients guarantees an adequate final product both quantitatively and qualitatively (Okolie et al. 2019). Aiming for large-scale production, classical extraction techniques such as maceration - for having certain limitations, such as long extraction times, high solvent consumption, and low yield - have been supplemented with "assisted" extraction techniques ” in which additional physical phenomena are used to intensify the process (Renard 2018).

Recently, ultrasound-assisted extraction (UAE) has emerged as a “green” and economically viable alternative technique when compared to conventional extraction techniques for natural colorants. The main benefits of this technique include reduced extraction and processing time, energy consumption, unit operations, and CO_2 emission. Furthermore, the use of ultrasonic waves has shown great potential for applications in industrial processes (O’Donnell et al. 2010; Chemat et al. 2011, 2017). However, several studies show that different plant matrices require different combinations of variables involved in the process for better yields in the extraction rates of the target compound (Dranca and Oroian 2016; He et al. 2016; Baran et al. 2017; Espada-bellido et al. 2017; Rocha et al. 2017).

Variables used in the extraction process such as type and concentration of extracting solvent, temperature, time, etc; and when it comes to UAE, variables such as frequency, power, and the amplitude of the ultrasonic power; can have a significant influence on the extraction process. Thus, it is of paramount importance to determine the best combination of variables involved in the process, simultaneously, to achieve the best yield of extraction rates of the target compound from the plant matrix. Furthermore, the development of the UAE technique must be accompanied by the development and improvement of mathematical models, that enable the generalization of the experimental results obtained and allows to understand of a possible scale-up of the process.

In the literature, there are some works involving the extraction of bioactive compounds from raspberries, as well as studies on extraction optimization using different technologies (Suthanthangjai et al. 2005; F. Chen et al. 2007; Sun et al. 2007; Mihailović et al. 2019; Wang et al. 2019). However, it is important to emphasize that the content of bioactive compounds present in raspberries can be influenced by several factors, mainly genetic and environmental (Bowen-Forbes et al. 2010; Çekiç and Özgen 2010; Bobinait et al. 2012; Yang et al. 2020). Furthermore, comprehensive studies involving optimization of the UAE of anthocyanins and phenolic compounds together with phenomenological kinetic modeling are still scarce in the literature.

In this context, the objective of this research was to optimize and model the extraction of anthocyanin and phenolic compounds contents of raspberry by studying the effect of the variables involved in the ultrasound-assisted extraction process, namely: time (min), temperature (°C) and solid: liquid ratio (m/v). The natural extract obtained from this process is an alternative for replacing synthetic food colorants. The phenomenological kinetic modeling of the UAE process and conventional extraction using the Film Theory Model were also performed.

Material And Methods

Chemicals and Reagents

Folin-Ciocalteu phenol reagents were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). The phenolic standard of gallic acid was purchased from Carlo Erba (Milano, Italy). All other chemicals and reagents were analytical-reagent grade and all solutions were prepared using ultrapure water (Model OS10LXE, Gehaka, Brazil).

Plant Material Preparation

Raspberries (*Rubus idaeus L.*) cultivated in Barbacena – MG, Brazil (latitude: 21º13'33" south, longitude 43º46'25" west) were used. Fruits were acquired previously selected, sanitized, frozen, and packaged, and were stored in a conventional freezer at -20°C until the moment of analysis.

The raspberries were ground in a conventional mixer (PMX600, Philco, Brazil). From this, a system composed of 5 g of mashed plant matrix plus the volume of aqueous solvent ethanol 70% (v/v) was formed. The volume varied between 25, 50, or 75 mL, according to the solid: liquid ratio generated by the experimental design (Table 1). The system was acidified to pH 2.00 ± 0.10 with concentrated HCl to ensure greater stability of anthocyanins present in the plant matrix during the extraction process (Rocha et al. 2017).

Design of Experiments

Box-Behnken Design (BBD) was selected to optimize the UAE conditions of TA and TPC of raspberries. Preliminary studies (data not shown) were conducted to select the high and low values of the levels of the three independent variables studied, which include: time, temperature, and solid: liquid ratio. The selected variables were evaluated at three levels, coded as -1, 0, and + 1 corresponding to low, medium, or high values, respectively. Thus, 17 combinations were generated, including five repetitions of the central point, as described in Table 1. The orders of the experimental tests were random. TA and TPC were used as response variables. The experimental data obtained were fitted to a quadratic polynomial model (Eq. 1) and the regression coefficients were generated from multiple linear regression:

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

1

where Yi and Xi are the dependent and independent variables, respectively. β0, βi, βii and βij are the regression coefficients for constant (intercept), linear effect, quadratic effect and interaction effects, respectively. Xi and Xj are the levels of the independent variables studied (i ≠ j) (Ding et al. 2016; He et al. 2016; Baran et al. 2017; Jiang et al. 2019).

Desirability profiles (D = 1, on a scale from 0 to 1) were used to select the maximum values of the independent variables studied, simultaneously, to obtain the highest content of TA and TPC. In this way, the optimal condition of the UAE was determined. From this optimal combination, predicted values were generated for extracting TA and TPC that were tested for validation of the method.

Table 1
BBD and the responses values for total anthocyanins (TA) and total phenolic content (TPC)

Run	Time X_1 (min)	TemperatureX_2 (°C)	Solid:liquid ratioX_3 (g/mL)	TA (mg/ 100g)	TPC (mg GAE/100 g)
1	5 (-1)	30 (-1)	1:10 (0)	18,92706	96,36197
2	75 (+ 1)	30 (-1)	1:10 (0)	20,7086	125,8672
3	5 (-1)	70 (+ 1)	1:10 (0)	22,55829	125,0275
4	75 (+ 1)	70 (+ 1)	1:10 (0)	17,74462	150,2738
5	5 (-1)	50 (0)	1:5 (-1)	17,83214	98,71865
6	75 (+ 1)	50 (0)	1:5 (-1)	19,2854	133,3301
7	5 (-1)	50 (0)	1:15 (+ 1)	19,13076	77,60201
8	75 (+ 1)	50 (0)	1:15(+ 1)	14,60737	126,7222
9	40 (0)	30 (-1)	1:5(-1)	20,34257	121,9329
10	40 (0)	70 (+ 1)	1:5 (-1)	19,8569	136,9927
11	40 (0)	30 (-1)	1:15 (+ 1)	20,91544	138,2485
12	40 (0)	70 (+ 1)	1:15 (+ 1)	21,32648	151,9289
13	40 (0)	50 (0)	1:10 (0)	18,92052	135,5662
14	40 (0)	50 (0)	1:10 (0)	20,24235	132,282
15	40 (0)	50 (0)	1:10 (0)	20,09596	137,9062
16	40 (0)	50 (0)	1:10 (0)	20,91535	127,7273
17	40 (0)	50 (0)	1:10 (0)	21,75559	137,4682

Ultrasound-assisted extraction

Crude phenolic extracts were obtained using ultrasound-assisted extraction, using an 800 W bath sonicator (Elmasonic TI-H-10, Elma, Germany), operating at 50% amplitude and 25 kHz frequency. UAE was performed under the controlled conditions of the equipment, according to the combination of variables generated by the experimental design (Table 1). The plant matrix: solvent systems were subjected to the respective variables: X_1 - extraction time, ranging from 5 to 75 minutes, X_2 - temperature, ranging from 30 to 70°C, and X_3 - solid: liquid ratio, ranging from 1:5 to 1:15 g/mL (Table 1). The phenolic extracts obtained from the UAE were vacuum filtered on Whatman filter paper no. 1 and its volume was adjusted to a standard volume with 70% ethanol (v/v). The extracts were stored in volumetric flasks at -20°C until subsequent analysis (Teixeira 2018).

Analysis of total anthocyanins (TA)

The total anthocyanin content of raspberry extracts was determined by the methodology described by Fuleki and Francis (1968) with some modifications. An aliquot of the sample was diluted in a volumetric flask using 85:15 v/v ethanol: HCl as solvent. Absorbance was measured at 535 nm using a UV-Vis spectrophotometer (Bel Engineering, Model UV-51, Italy). The calculation of TA content in the extract was given by Eq. 2 and expressed in mg per 100 g of plant material.

$$C = \frac{Abs \times MM}{\epsilon \times b}$$

2

where C is the concentration of anthocyanins in g/L; Abs is the absorbance read at 535 nm; MM is the molar mass of the major anthocyanin of the raw material under study in g/mol – in the case of raspberries, cyanidin-3-glycoside MM 449.2 g/mol was used-; ε is the molar absorptivity coefficient of the major anthocyanin of the raw material understudy in the specific solvent in L/mol.cm (in this case, 26900); and b is the cuvette thickness, 1 cm.

Analysis of Total Phenolic Content (TPC)

The total phenolics content was measured according to the method described by Singleton and Rossi (1965). In which 0.6 mL of the diluted extract was mixed with 3.0 mL of Folin-Ciocalteu reagent at 10% (v/v) plus 2.4 mL of 7.5% sodium carbonate (m/v) in a volumetric flask. This mixture was kept protected from light for 1h at room temperature. Its absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Bel Engineering, Model UV-51, Italy). The quantification of TPC was obtained through the standard curve of gallic acid and the results were expressed in mg of gallic acid equivalent per 100 g of sample (mg GAE / 100 g).

Validation of optimized UAE and comparative study of extraction efficiency

The best simultaneous combination of the studied independent variables (time, temperature, and solid: liquid ratio) to provide the maximum extraction of TA and TPC from raspberries was obtained through the desirability profile. From this optimal combination, the contents of TA and TPC were experimentally obtained for validation of the method, in three replications.

A comparative study between the optimized conditions of UAE and conventional extraction was carried out to evaluate the efficiency of the ultrasound influence on the recovery of TA and TPC.

Conventional Extraction

The conventional extraction process was carried out according to the methodology of Rocha et al. (2017), in which the proportion of ground vegetable matrix: solvent (70% ethanol (v/v)) is 1:10 (m/v). This system was acidified with concentrated HCl to pH 2.00 ± 0.10 and refrigerated for 24 hours (5 ± 1°C). After this period, the crude extracts were vacuum filtered with Whatman filter paper no. 1 and stored in amber vials in a conventional freezer (-20°C) until further analysis. Conventional extraction was also performed in three repetitions.

Kinetics and Phenomenological Modeling of Extraction

To investigate the possible improvement in the efficiency of the target compounds extraction process, a comparative kinetics study between the optimized UAE and the conventional extraction process was carried out, based on the research developed by Veličković et al. (2008) and Dias et al. (2017). The samples were submitted to the optimized conditions of the UAE process (temperature and solid: liquid ratio), at times of 0, 5, 10, 20, 40, 60, and 120 min. Similarly, a study was conducted under conventional extraction conditions – to compare the kinetics of the two processes. After the extraction process, the phenolic extracts obtained were filtered on Whatman no. 1 and the volume was adjusted to a standard volume with 70% w/v ethanol. Extracts were stored at -20°C until further analysis.

Kinetic Modeling

To model the phenomenological kinetics of the extraction process, the concentrations of target compounds obtained in the kinetic curve were used. The modeling was based on the Film Theory (Veličković et al. 2006), which presents the kinetic equations (Eq. 3) and their linearized form (Eq. 4).

$$\left(1 - \frac{c}{c_{seq}}\right) = (1 - b)e^{-kt}$$

3

$$ln\left(1 - \frac{c}{c_{seq}}\right) = ln(1 - b) - kt$$

4

where c is the concentration of the target compound during the extraction process (g/L); c_{seq} is the maximum concentration of the target compound at a certain temperature (g/L); b is the washing coefficient according to the film model (1); k is the slow extraction coefficient according to Film Theory model (min^{-1}); and t is the time (min).

The determination of the concentration of the target compound in the liquid extract saturated at equilibrium (c_{seq}) was based on the study of Veličković et al. (2008), with some modifications. First, an extraction under optimized ultrasonic conditions was conducted. After the extraction process, the crude extract was separated/filtered from the plant residue. From this, in the crude extract obtained from this first stage, a new portion of fresh plant material was added and a new extraction process was conducted. These extraction/filtration cycles were conducted until the target compound content reached equilibrium, to then calculate the c_{seq} (a total of three extraction/filtration cycles were required).

Statistical Analysis

The statistical program Statistica 7.0 (StatSoft Inc. USA) was used to analyze the BBD data, generate and test the significance of the regression coefficients ($p < 0.10$) and optimize the UAE process conditions through the desirability profile.

The results of the contents of TA and TPC were expressed as mean ± standard deviation and the significant differences between the optimized condition in UAE and the conventional extraction were obtained using analysis of variance (ANOVA) with $p < 0.05$.

Results And Discussion

Optimization of ultrasound-assisted extraction

Raspberry crude phenolic extracts were obtained by ultrasonic treatment under the study of the following variables: extraction time, X_1, 5 to 75 min; ultrasonic bath temperature, X_2, 30 to 70°C; and solid: liquid ratio, X_3, 1:5 to 1:15 g/mL, totaling 17 experimental runs. Under these conditions, the content of total anthocyanins ranged from 14.6 to 22.6 mg/100 g of fruit, while the total phenolic content ranged from 77.6 to 151.9 mg GAE/100 g of fruit (Table 1). BBD was used to determine the linear, quadratic, and interaction effects of the studied variables on the considered response variable. The regression analysis coefficients and p-values for TA and TPC responses are shown in Table 2.

In the regression analysis for the TA response variable, with the aid of the Pareto chart (Table 2, Fig. 1a), it is observed that the squared time (X_1^2) was the most significant effect on the model ($p < 0, 05$), followed by the effect of the interaction of linear time with linear temperature (X_1X_2), the interaction of linear time with the volume of linear solid: liquid ratio (X_1X_3) and the quadratic temperature (X_2^2), both with $p < 0, 05$. In addition, the effects of linear time (X_1) and volume squared of solid: liquid ratio (X_3^2) also contributed significantly to the proposed polynomial model ($p < 0.10$). In the regression analysis for the TPC response variable (Table 2, Fig. 1b), the effect of the linear time variable (X_1) was the most significant for the model ($p < 0.01$); followed by the effect of squared time (X_1^2) and linear temperature (X_2) with $p < 0.05$. Furthermore, the effect of the squared temperature (X_2^2) and the volume of linear solid: liquid ratio (X_3) also contributed significantly to the proposed polynomial model.

Table 2
BBD regression analysis for TA and TPC of raspberries.

	TA (mg/ 100 g)		TPC (mg GAE/100 g)	
Variable	**Coefficient**	**p-value**	**Coefficient**	**p-value**
Constant	19,4363	< 0,0001***	123,5839	< 0,0001***
X_1	-1,5224	0,049045**	32,2058	0,000722***
X_2	1,12606	0,166727	27,5201	0,002593**
X_3	-1,6897	0,102196	-13,8623	0,0882390*
X_1^2	1,64887	0,008366**	18,9949	0,001240**
X_2^2	-1,24756	0,026623**	-9,1876	0,032616*
X_3^2	1,02316	0,053661*	6,1018	0,115914
X_1X_2	-3,2976	0,00938**	-2,1295	0,765326
X_1X_3	-2,98832	0,014338*	7,2544	0,328202
X_1 – time (min); X_2 – temperature (°C); X_3 – volume of solid:liquid ratio (mL); *p < 0,10; ** p < 0,05; *** p < 0,001.				

From the regression analysis, the studied factors were fitted to the second-order polynomial regression model (Eq. 1), showing significant F test values, non-significant lack of fit, and R^2 values equal to 0.9159 and 0 .9533 for TA and TPC variables, respectively. The high values of the coefficient of determination ($R^2 > 0.90$) show that the regression equations adjusted for TA (Eq. 5) and TPC (Eq. 6) fit the experimental data. It is noteworthy that although the empirical models of Eqs. 5 and 6 cannot describe the phenomena that govern the ultrasonic extraction process, they can be used to determine the effects of time, temperature, and solid: liquid ratio on the capacity of extraction of anthocyanins and phenolic compounds during the extraction process and in predicting these contents.

$$Y_{TA} = 19{,}4363 - 1{,}5224X_1 + 1{,}64887X_1^2 - 1{,}24756X_2^2 + 1{,}02316X_3^2 - 3{,}2976X_1X_2 - 2{,}98832X_1X_3$$

5

$$Y_{TPC} = 123{,}5839 + 32{,}2058X_1 + 27{,}5201X_2 - 13{,}8623X_3 + 18{,}9949X_1^2 - 9{,}1876X_2^2$$

6

Desirability profiles (D = 1.0 on a scale of 0–1) were used to optimize the three studied variables (time, temperature, and solid: liquid ratio) simultaneously to obtain the highest contents of TA and TPC. Thus, for the response variable TA (Fig. 2a), the optimal combination of the studied variables was at a time of 22.5 min, at a temperature of 70°C, and at the volume of solid: liquid ratio 1:12.5 m /v (which corresponds to 62.5 mL of 70% ethanol solvent (v/v), for 5 g of mashed fruit). This combination predicts a TA content of 23.178 mg/100 g of fruit. On the validation of this optimized condition, the experimental value of 24.131 ± 0.5681 mg of TA per 100 g of fruit was observed. This value is only 4.1% higher than predicted by the mathematical model (Eq. 5).

Similarly, for the response variable TPC (Fig. 2b), the optimal combination of the input variables studied was in the time of 57.5 min, the temperature of 70°C, and the volume of solid: liquid ratio of 1:12.5 m/v (corresponding to 62.5 mL of 70% ethanol solvent (v/v), for 5 g of mashed fruit). This combination predicts a TPC of 155.23 mg GAE/100 g of fruit. On the validation of this optimized condition, the experimental value of 149.226 ± 7.604 mg GAE/100 g of fruit was observed for the TPC of raspberry, a value only 4.0% lower than that predicted by the model.

The efficiency of ultrasound-assisted extraction has been extensively reported to increase extraction rates of target compounds and decrease extraction time (Dranca and Oroian 2016; He et al. 2016; Baran et al. 2017; Rocha et al. 2017; Sang et al. 2017; Das and Eun 2018). In the present research, the extraction process was carried out by varying the time between 5 to 75 minutes. From desirability profiles (Fig. 2a and b), it is observed that the effect of time was significant for the recovery of TA and TPC. It is also noted that for the response variable TA, shorter extraction times, 22.5 min, contributed to a greater recovery of target compounds. While for the recovery of TPC, a time close to 60 min presented better results.

It should be noted that from the anthocyanins present in the raspberry extract, the contents of cyanidin-3-glucoside and cyanidin-3-sophoroside stand out, the shorter time for anthocyanin extraction, which may be due to the conditions of the ultrasonic extraction over time - as the temperature, frequency, and ultrasonic amplitude - directly affect the stability of the anthocyanin structures present in the extracts, which can cause degradation and polymerization of these compounds during the extraction process. In the study developed by J. Yu Chen et al. (2020) the degradation kinetics of anthocyanins present in a model system and raspberry juice were evaluated. It was observed that over the shelf life, both cyanidin-3-glucoside and cyanidin-3-sophoroside, major anthocyanins present in raspberries, degraded giving rise to simpler phenolic compounds, such as caffeic acid and protocatechuic acid. This can explain both the decrease in the content of TA and the increase in the TPC in a longer extraction time because as the degradation and loss of anthocyanins occurs, simpler phenolic compounds are generated, which can increase their content throughout the process.
In the present study, it was observed that both for the extraction of TA and TPC, the temperature of 70°C was the most suitable condition for the highest efficiency of the processes (Fig. 2a and b). Generally, in a solid-liquid extraction, the increase in temperature leads to greater recoveries of bioactive compounds (Dranca and Oroian 2016). This effect can be attributed to the fact that, when the temperature is raised, the solubility coefficients and diffusion of the compounds that will be extracted from the food matrix will increase, as well as the solvent viscosity will decrease, facilitating the mass transfer of the system (Goula et al. 2017).

In addition to time and temperature, in this study, the solid: liquid ratio m/v proved to be an essential factor for increasing the recovery of both TA and TPC. In both cases, the best yield occurred at the solid: liquid ratio of 1:12.5 m/v, values above the average level (Fig. 2a and b). Increasing the solid: liquid ratio can improve the efficiency of the process by facilitating the access of the solute to the solvent. Furthermore, the solvent is the liquid medium in which ultrasonic acoustic cavitation occurs: a phenomenon that produces a series of mechanical effects, such as particle collision and cell disintegration. Consequently, larger volumes of solvent can help the occurrence of these phenomena (He et al. 2016).

Comparative study of the phenomenological kinetics of processes

In the optimization of the UAE for the TA response variable, the optimal simultaneous combination of the studied variables occurs in a time of 22.5 min, at a temperature of 70°C, and in a solid: liquid ratio of 1:12.5 m/v. It was observed that UAE significantly increased the extraction yield of TA ($p < 0.05$) with values of 24.131 ± 0.5681 mg / 100 g of fruit when compared to 20.401 ± 0.221 mg / 100 g of fruit obtained by conventional extraction (Fig. 3).

On the other hand, in the optimization of the UAE for the TPC response variable, the optimal simultaneous combination of the studied variables occurs at a time of 57.5 min, at a temperature of 70°C, and a solid: liquid ratio of 1:12.5 m/v. It was observed that the UAE significantly increased the extraction yield of TPC ($p < 0.05$) with values of 149.226 ± 7.604 mg GAE/100 g of fruit when compared with 115.821 ± 7.908 mg GAE/100 g of fruit obtained by conventional extraction (Fig. 3). Thus, the use of UAE proved to be efficient in overcoming limitations of the conventional technique, since UAE increased the recovery yield of TA by 18.28% and TPC by 28.88% when compared to conventional extraction.

When compared to conventional extraction – which is done at a temperature of 5 ± 1°C; in solid: liquid ratio of 1:10 m/v; and time of 24 hours, it becomes evident that the UAE contributed positively to the increase in the extraction rate of the studied bioactive compounds. The main highlight is the reduction of the processing time from 24 hours of conventional extraction to less than 1 hour under optimized UAE conditions. The reduction in process time is important because it reduces energy consumption, in addition to enabling greater processing capacity for vegetable raw material (S. Chemat et al. 2004).

The better performance of UAE compared to conventional extraction can be attributed to the ultrasonic waves that promote better penetration of the solvent in the plant cell-matrix, increasing the mass transfer rates of the target compounds in the extracting solvent. Furthermore, the ultrasonic power, by breaking the cell walls of the plant matrix, can increase the movement of the target compounds to the extracting solvent, and consequently, increase the recovery of these compounds (He et al. 2016). Because of this series of advantages, and because it allows the scale-up to industrial levels, and requires low initial investments, the UAE has been standing out, in recent years, as a viable alternative for the extraction of anthocyanins and phenolic compounds from varied plant sources (Das and Eun 2018).

When observing the extraction yield of TA (mg/100 g), as a function of time (min), comparing the UAE with the conventional extraction (Fig. 4a), it is evident a greater recovery of the target compound in the UAE. Furthermore, the curve for the UAE presents a better extraction efficiency in the first 20 minutes, which corroborates the optimal time obtained in the optimization study for this compound. After 40 min, there is a drop in the recovery of the target compound, which may be due to the degradation of anthocyanin structures due to extraction conditions. In the conventional extraction curve, however, there was no significant difference between the extractions ($p < 0.05$), in the time considered. Thus, the kinetic curve was linear with an average TA content of 16.5 ± 0.76 mg/100 g of fruit.

Regarding the extraction yield of TPC (mg GAE/100 g) as a function of time (min), comparing the UAE with the conventional extraction, it can also be observed the greater recovery of the target compound in the UAE (Fig. 4b). Furthermore, it is noteworthy that in the time of 120 min the TPC obtained by UAE was approximately 2.6 times greater than that obtained by the conventional method.

The greater slope of the curves at the beginning of the extraction process (Fig. 4a and b), especially in the UAE, can be attributed to the fact that this process has two main stages: the first, which is characterized by a fast mass transfer rate, it occurs the penetration of the solvent into the plant cell structure followed by the washing of the soluble constituents in the extractant solvent. In the second stage, there is a slow diffusion of soluble constituents through the porous structure of the residual solids to the extracting solvent, consequently, the curve becomes less steep (Veličković et al. 2006; Goula et al. 2017).
In addition to the kinetic study, the development of the ultrasound-assisted extraction technique must be accompanied by the development of mathematical models. These mathematical models aim to describe the extraction process, through the mathematical approximation of the problems involved in the process. Veljković and Milenović (2002) used the Film Theory model, according to which they predict the occurrence of a thin diffusion layer around the particles, to define the mass transfer rate of the particles in suspension during the process (Eq. 3). According to Goula et al. (2017), mechanistic models provide more than a basic understanding of a given system, as they serve as a basis for extrapolating the process to larger scales, such as pilot and industrial.

When adjusting the predicted equation for Film Theory, only the first 20 minutes of the anthocyanin extraction process were considered for the modeling, as they are compounds that present degradation. Furthermore, the value obtained for c_{seq} for TA was 0.005956 g/L and for TPC was 46.96 g/L.

It is observed that the Film Theory equation fits well in the UAE, for TA and TPC, presenting $R^2 > 0.90$ (Table 3). Although the Film Theory model was conceived to explain the conventional extraction process, it did not fit well with the obtained data, presenting $R^2 < 0.90$. It is noteworthy that in the time of just 20 min used to model the extraction of TA by the conventional method, there was no significant difference between the extractions ($p < 0.05$), which contributed to the low value of the coefficient of determination. Therefore, a longer analysis time would be recommended.

The parameters of the kinetic models were calculated from the experimental data using the linear regression method, using the linearized form of the proposed kinetic equation (Table 3, Fig. 5a and b). In general, as expected, the washing coefficient (b) has values higher than the slow diffusion coefficient (k). Since the first stage of extraction occurs the rapid transfer of soluble constituents through washing, followed by the slow diffusion stage in which the residual soluble constituents migrate to the extracting solvent.

It is observed (Table 3) that the UAE of TA had the highest washing coefficient (b), which can be explained by the maximum extraction of the anthocyanin content occurring in a shorter process time, of approximately 20 min. As for the values of the slow diffusion coefficient (k), the UAE of phenolic compounds had the highest value, since, in addition to the extraction of the TPC originally present in the sample, there is also the accumulation of phenolic compounds originating from the degradation and polymerization of anthocyanins throughout the extraction process.

Table 3
Values of kinetic parameters of equations based on film theory for extraction of TA and TPC.

Response variable	Extraction	b (1)	K (min^{-1})	R^2
TA	UAE	0,6286	0,0325	0,9508
	Conventional	0,4347	0,0006	0,2571
TPC	UAE	0,4417	0,074	0,9269
	Conventional	0,2222	0,0008	0,7758

Conclusion

The use of ultrasonic treatment promoted an increase of 18.28% and 28.88% in the recovery of TA and TPC, respectively, about conventional extraction. Highlights the reduction of the processing time from 24 hours in the conventional extraction to less than 1 hour in the optimized conditions of the UAE.

BBD together with desirability profiles was successfully employed in the optimization and modeling of the studied variables for the extraction of TA and TPC from raspberries. Optimal values of time, temperature, and solid: the liquid was combined simultaneously to predict the highest extraction yield of TA and TPC. In UAE, the optimum temperature was 70°C, and the optimum solid: the liquid ratio was 1:12.5 m/v for both response variables. The optimal time for TA was 22.5 min and for TPC it was 57.5 min. Under the optimized conditions, the yields predicted by the model and experimentally observed for TA and TPC differed by less than 5%.

Furthermore, when fitting the kinetic curves of the extraction process to the phenomenological mathematical model of film theory, the EAU presented a good fit ($R^2 > 0.90$). From the kinetic data, it can be observed that TA extraction had the highest washing coefficient, while TPC extraction had the highest slow diffusion coefficient.

Thus, the study indicates that the use of ultrasound-assisted extraction of bioactive compounds from raspberries is an efficient, low-cost, ecologically friendly, promising, and fast methodology.

Declarations

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Author Contribution BAT designed and conducted the experiments, analysed the results, drafted and revised the manuscript. PCS designed the experiment, provided laboratory support, and revised the manuscript. MCTRV analysed the results and revised the manuscript. BCLJ, ENRV, and EMFM revised the manuscript.

Data Availability All data generated or analyzed during this study are included in this article.

Conflict of Interest The authors declare no conflict of interest.

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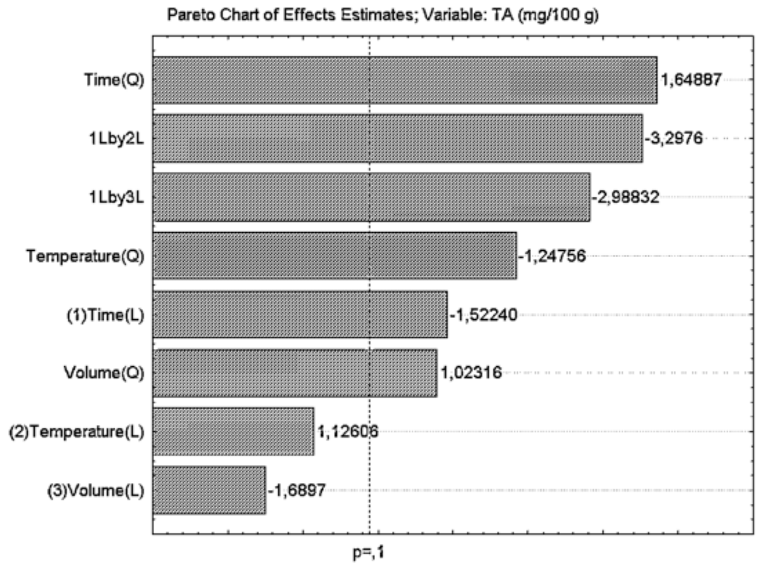
Figures

a



b

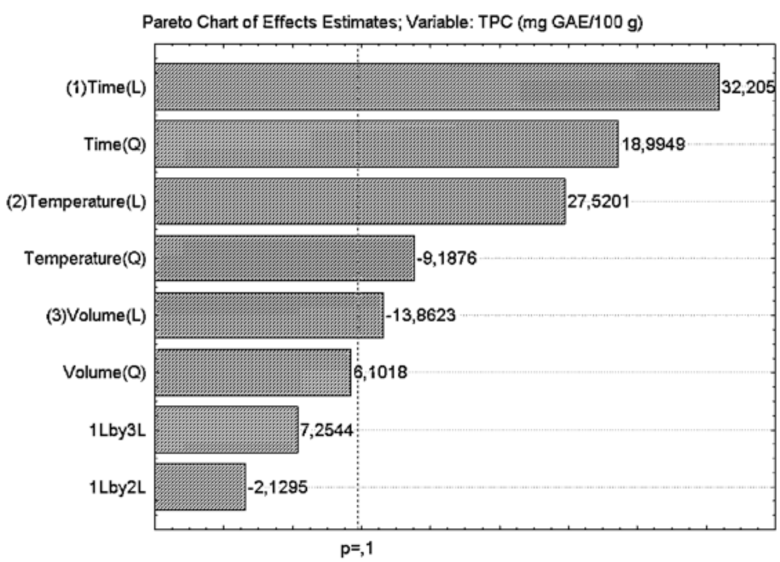


Figure 1

Pareto Chart of the significance of the effects of the independent variables studied for the response variable (a) TA and (b) TPC; ($p < 0.10$) of raspberries

Figure 2

Desirability profile of **(a)** TA (mg/ 100 g) and **(b)** TPC (mg GAE/ 100 g).

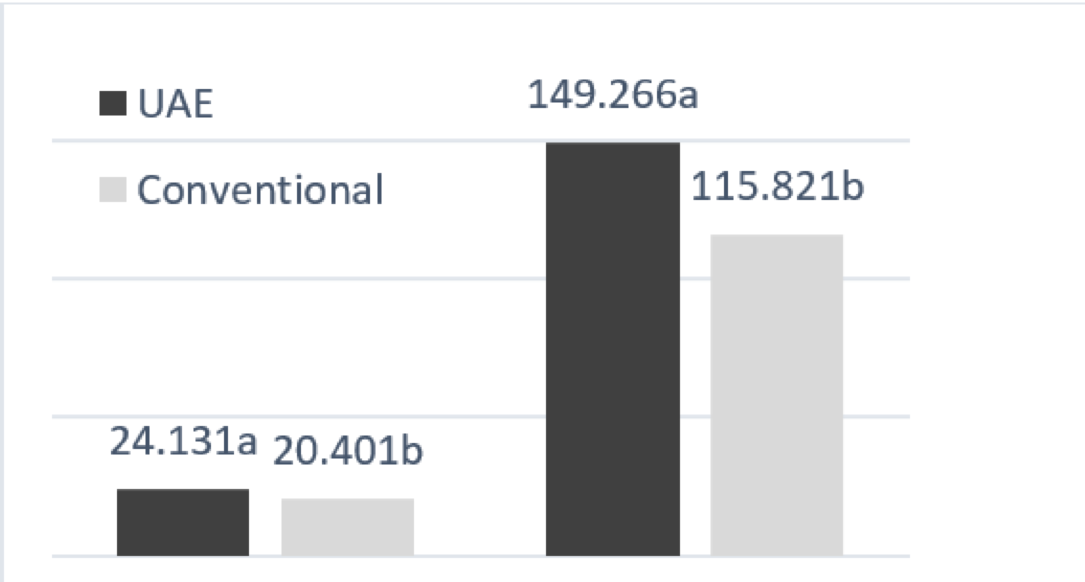
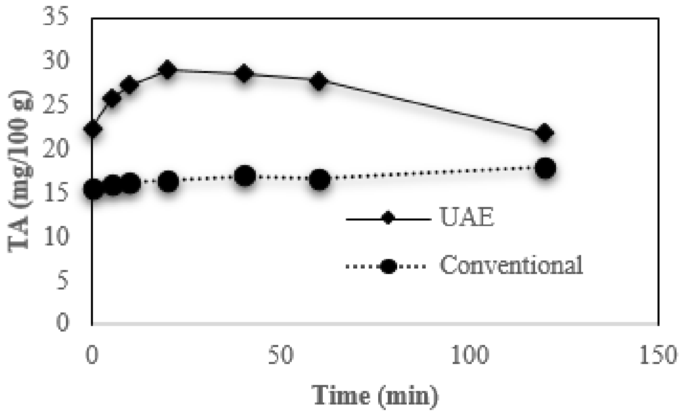


Figure 3

Comparison of UAE and conventional extraction on the TA (mg/100 g of fruit) and TPC (mg GAE/100 g of fruit). Different letters indicate that the samples differ significantly from each other.

a



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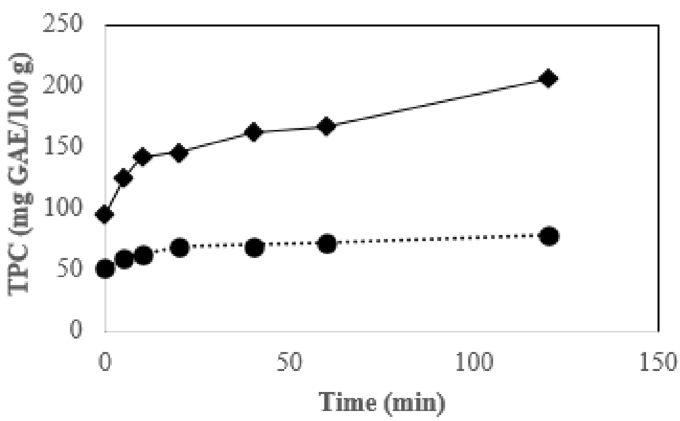
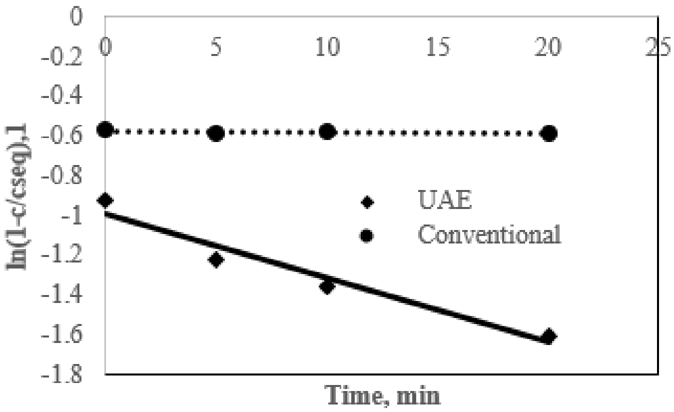


Figure 4

Kinetics of extraction of (a) TA (mg/100 g) and (b) TPC (mg GAE/100 g) from raspberries as a function of time (min).

a



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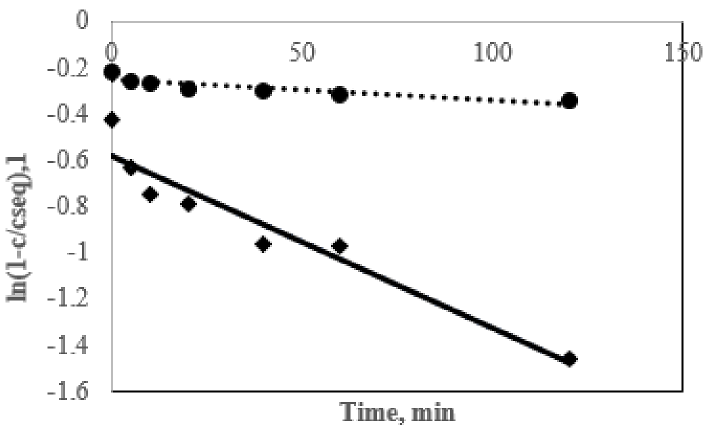

Figure 5

Linearized form of the kinetic equation based on film theory for extraction of (a) TA and (b) TPC.