BÁRBARA AVANCINI TEIXEIRA

ULTRASOUND-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM PLANT SOURCES: OPTIMIZATION, KINETIC, AND MODELING STUDY

Thesis submitted to the Food Science and Technology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Paulo Cesar Stringheta

Co-adviser: Márcia Cristina T. R. Vidigal

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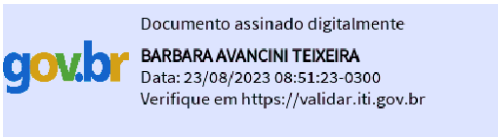

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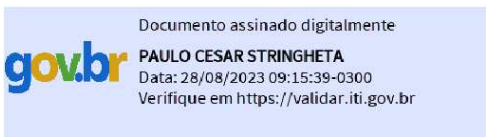

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First, I would like to thank a greater force and conscience, the cosmic algorithm, which some call God, which allowed me to get this far, giving me strength day after day.

To my whole family, especially my mother, for my base, where I draw my strength. If I managed to go far, it was because my roots were strong.

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“The roots of education are bitter, but the fruit is sweet”. (Aristotle, 384 – 322 a. C.)

ABSTRACT

TEIXEIRA, Bárbara Avancini, D.Sc., Universidade Federal de Viçosa, June, 2023. **Ultrasound-assisted extraction of bioactive compounds from plant sources: optimization, kinetic, and modeling study**. Adviser: Paulo Cesar Stringheta. Co-adviser: Márcia Cristina Teixeira Ribeiro Vidigal.

In recent years, emerging technologies, such as ultrasound, have been implemented in the area of bioactive compound extraction because they present a series of advantages when compared to conventional technologies. Such advantages can range from a decrease in process time, energy consumption, and use of solvents, as well as an increase in the recovery of target compounds, efficiency, and productivity of the process. In this context, the objective of this study was to optimize the ultrasound-assisted extraction of bioactive compounds (anthocyanins and total phenolics) from three raw materials from plant sources: raspberries (raw material rich in simple carbohydrates such as sucrose and fructose), purple tomato (vegetable that contains fibers and other bioactive compounds like lycopene), and purple sweet potato (vegetable rich in carbohydrates like starch). For the optimization of the extraction process of bioactive compounds using ultrasound, three process variables were studied: time (min), temperature (°C) and solid:liquid ratio (m/v), using the Box-Behnken Design. From the data generated by the experimental design, it was possible to generate second-order regression equations, Pareto charts, and desirability profiles. The desirability profile was the tool that enabled the optimization of the processes, through the simultaneous combination of the studied variables, in order to achieve the highest recovery of the bioactive target compounds. The data predicted by the desirability profile were experimentally validated in the laboratory with satisfactory precision, for the three raw materials under study. In sequence, the optimized ultrasound-assisted extraction process was compared to the conventional extraction process, by maceration extraction with solvents. In all cases, the use of ultrasound significantly increased the recovery of the bioactive target compounds. The kinetic and modeling studies were only developed for raspberry and purple sweet potato. For raspberry, only a mathematical model was validated. While for the purple sweet potato, three mathematical models were validated. Furthermore, the three raw materials studied were analyzed under the lens of scanning electron microscopy, in order to observe the structural changes attributed to the action of ultrasound on the raw

materials. In summary, the studies indicated that the ultrasound-assisted extraction process is an efficient technology for extracting bioactive compounds from plant sources.

Keywords: Ultrasound. Extraction. Bioactive compounds. Optimization. Kinetics. Modeling.

RESUMO

TEIXEIRA, Bárbara Avancini, D.Sc., Universidade Federal de Viçosa, junho de 2023. **Extração assistida por ultrassom de compostos bioativos de fontes naturais: estudo de otimização, cinética e modelagem**. Orientador: Paulo Cesar Stringheta. Coorientadora: Cristina Teixeira Ribeiro Vidigal.

Nos últimos anos, tecnologias emergentes, como o ultrassom, têm sido implementadas na área de extração de compostos bioativos por apresentarem uma série de vantagens, quando comparadas as tecnologias convencionais. Tais vantagens podem incluir desde a diminuição do tempo do processo, do consumo de energia e do uso de solventes, bem como o aumento da recuperação dos compostos de interesse, da eficiência e da produtividade do processo. Neste contexto, o objetivo deste estudo foi otimizar a extração assistida por ultrassom de compostos bioativos (antocianinas e fenólicos totais) de três matérias-primas de origem vegetal: framboesas (matéria-prima rica em carboidratos simples como sacarose e frutose), tomate roxo (vegetal que contém fibras e outros compostos bioativos como licopeno), e batata-doce roxa (vegetal rico em carboidratos como amido). Para a otimização do processo de extração de compostos bioativos utilizando ultrassom foram estudadas três variáveis de processo: tempo (min), temperatura (°C) e razão sólido:líquido (m/v), utilizando o Delineamento Box-Behnken. A partir dos dados gerados pelo desenho experimental foi possível gerar equações de regressão de segunda ordem, gráficos de Pareto, e perfil desejabilidade. O perfil desejabilidade foi a ferramenta que possibilitou a otimização dos processos, por meio da combinação simultânea das variáveis estudadas, para se atingir a maior recuperação dos compostos bioativos de interesse. Os dados previstos pelo perfil desejabilidade foram validados experimentalmente em laboratório com uma precisão satisfatória, para as três matérias-primas estudadas. Na sequência, o processo de extração assistido por ultrassom otimizado, foi comparado ao processo de extração convencional, por extração por maceração com solventes. Em todos os casos, o uso do ultrassom aumentou significativamente a recuperação dos compostos bioativos de interesse. Já o estudo cinético e de modelagem só foram desenvolvidos apenas para a framboesa e para a batata-doce roxa. Para a framboesa, somente um modelo matemático foi validado. Enquanto que para a batata-doce roxa, três modelos matemáticos foram validados. Ademais, as três matérias-primas estudadas foram analisadas sob as

lentes da microscopia eletrônica de varredura, a fim de observar as mudanças estruturais atribuídas a ação do ultrassom nas matérias-primas. Resumidamente, os estudos indicam que o processo de extração assistida por ultrassom é uma tecnologia eficiente para extração de compostos bioativos de fontes vegetais.

Palavras-chave: Ultrassom. Extração. Compostos bioativos. Otimização. Cinética. Modelagem.

SUMMARY

1. GENERAL INTRODUCTION

Nowadays, the population is increasingly concerned about the positive and/or negative impacts of food on their health. As a result, the consumption and incorporation of bioactive molecules from natural sources, with potential beneficial effects on health, in commercial foods have gained prominence. Although some bioactive compounds are already produced on a commercial scale, the transition from laboratory-scale research to industrial production of some of these metabolites still remains a challenge. To transform a bioactive compound into a functional ingredient, several steps are necessary, which include: extraction of bioactive compounds from the plant matrix, purification of the active ingredient/separation of contaminants, characterization of physical and chemical properties, toxicity studies, evaluation of bioactivity in vitro, in vivo and studies with humans - simulation of digestion, bioaccessibility and bioavailability -, and finally, studies that aim to apply these bioactive compounds in a stable and biologically viable form in different food matrices (OKOLIE et al., 2019).

In recent years, classical extraction techniques such as maceration, due to their limitations - such as long process times, low yields and high consumption of toxic solvents – these techniques have been supplemented with emerging “assisted” extraction techniques in which additional physical phenomena are used to intensify the process. Such physical phenomena range from the use of pressure, microwaves, ultrasound, to electric fields, etc (RENARD, 2018). In this context, ultrasound-assisted extraction (UAE) has emerged as a “green” and economically viable alternative technique when compared to conventional extraction techniques for bioactive compounds. The main benefits of this technique include an increase in yield, efficiency, and productivity of the process, a decrease in extraction time, energy consumption, and toxic solvents. In addition, the use of ultrasound has shown great potential for applications in processes on an industrial scale (CHEMAT et al., 2017).

However, several studies show that different plant matrices require different combinations of variables involved in the extraction process for a better yield in the extraction rates of the target compound (BARAN; GOUD; DAS, 2017; DRANCA; OROIAN, 2016; ESPADA-BELLIDO et al., 2017; GOULA et al., 2017; HE et al., 2016; ROCHA et al., 2017). Thus, the variables used in the process, such as the type and concentration of the extracting solvent, temperature, time, frequency, power, amplitude of the ultrasonic power, etc. can have a significant influence on the extraction process.

Consequently, it is of paramount importance to determine the best combination of the variables involved in the process, simultaneously, to achieve the best yield of the extraction of the compound of interest from various natural sources (BOATENG, 2023; ESPADA-BELLIDO et al., 2017).

Currently, the UAE of bioactive compounds from various natural sources has been intensively investigated with good results. Generally, the increase in the UAE yield of bioactive compounds is attributed to the cavitation phenomenon, which is caused by ultrasonic waves. However, studies that permeate the mechanisms in which cavitation causes increased extraction yield are still scarce in the literature (CHEMAT et al., 2017; RENARD, 2018). Furthermore, it is important that the development of the UAE technique is accompanied by the development and improvement of phenomenological mathematical models, as these models make it possible to generalize the experimental results obtained, which allow the prediction of results in systems different from those studied, as well as studies viability for scaling up the process (VELIČKOVIĆ et al., 2006, 2008).

In this context, three vegetable raw materials of different physicochemical constitutions were studied, namely: raspberry (red fruit rich in simple carbohydrates such as sucrose, fructose and glucose), purple tomato (vegetable with complex carbohydrates such as fibers and other bioactive compounds such as lycopene), and purple sweet potato (tuberous root with carbohydrates such as starch). The objective of this research was to evaluate how the physical phenomenon of ultrasound can influence the process of extracting bioactive compounds (anthocyanins and phenolic compounds) from these three different plant sources. For raspberry **(Paper 1),** optimization studies were conducted – of the controlled variables involved in the process – time, temperature and solid:liquid ratio, mathematical modeling studies, and morphology studies. For the purple tomato **(Paper 2),** optimization studies were carried out – of the controlled variables involved in the process – time, temperature and solid:liquid ratio, and morphology studies. And for the purple sweet potato **(Paper 3),** optimization studies were conducted – of the controlled variables involved in the process – time, temperature and solid:liquid ratio, mathematical modeling studies, and morphology studies.

** The papers presented are in the format requested by the different journals in which the works were published.

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2. PAPER 1

Teixeira, B.A. *et al.* Optimization, Kinetic and Phenomenological Modeling of Ultrasound-Assisted Extraction Process of Bioactive Compounds from Raspberries (*Rubus idaeus* L.). **Food Anal. Methods, 2023.** https://doi.org/10.1007/s12161-023-02462-z

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Optimization, Kinetic and Phenomenological Modeling of Ultrasound-Assisted Extraction Process of Bioactive Compounds from Raspberries *(Rubus idaeus* L.*)*

Bárbara Avancini Teixeira[1] · Márcia Cristina Teixeira Ribeiro Vidigal[1] · Bruno de Castro Leite Júnior[1] · Érica Nascif Rufino Vieira[1] · Eliane Maurício Furtado Martins[2] · Paulo Cesar Stringheta[1]

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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. 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 and for TPC occurs in 57.5 min predicting the content of 156.30 mg GAE/100 g. On validation of the optimized conditions, less than a 5% difference was found between the predicted and experimental values (24.131 mg/100 g for TA, and 149.226 mg GAE/100 g 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 24 h in conventional extraction to less than 1 h in optimized UAE stands out. Furthermore, the Film Theory model tested fitted well to the extraction process under study. Thus, the study indicates that the UAE process is an efficient green methodology for recovering bioactive compounds from raspberries.

Keywords Ultrasonic extraction · Green technology · Kinetic and phenomenological modeling · Anthocyanins · Phenolic compounds

Introduction

Raspberries *(Rubus idaeus* L.*)* are fruits that stand out for their exotic taste, pleasant appearance, and high content of health-beneficial compounds (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). Although the use of synthetic colorants are 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

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Keywords: ultrasonic extraction · green technology · kinetic and phenomenological modeling · anthocyanins · phenolic compounds

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Introduction

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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). Although the use of synthetic colorants are 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. A morphological study, using scanning electron microscopy, was carried out, and 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.

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, Manaus, AM, Brazil). From this, a system composed of 5 g of mashed plant matrix plus the volume of solvent ethanol:H_2O 70:30 (v/v) was formed. The system solvent 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). The choice of solvent has a significant effect on extraction process efficiency. Usually, methanol, acetone, and ethanol are the most commonly used solvents, but methanol and acetone are considered as more toxic and hazardous to handle than ethanol. Ethanol is more environmentally friendly, its use is allowed in food, and also recovers bioactive compounds with good quality characteristics (Baran 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:

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

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)	Temperature X_2 (°C)	Solid:liquid ratio X_3 (g/mL)	TA (mg/ 100g)	TPC (mg GAE/100 g)
1	5 (-1)	30 (-1)	1:10 (0)	18.927	96.362
2	75 (+1)	30 (-1)	1:10 (0)	20.709	125.87
3	5 (-1)	70 (+1)	1:10 (0)	22.558	125.03
4	75 (+1)	70 (+1)	1:10 (0)	17.745	150.27
5	5 (-1)	50 (0)	1:5 (-1)	17.832	98.719
6	75 (+1)	50 (0)	1:5 (-1)	19.285	133.33
7	5 (-1)	50 (0)	1:15 (+1)	19.131	77.602
8	75 (+1)	50 (0)	1:15(+1)	14.607	126.72
9	40 (0)	30 (-1)	1:5(-1)	20.343	121.93
10	40 (0)	70 (+1)	1:5 (-1)	19.857	136.99
11	40 (0)	30 (-1)	1:15 (+1)	20.915	138.25
12	40 (0)	70 (+1)	1:15 (+1)	21.326	151.93
13	40 (0)	50 (0)	1:10 (0)	18.921	135.57
14	40 (0)	50 (0)	1:10 (0)	20.242	132.28
15	40 (0)	50 (0)	1:10 (0)	20.096	137.91
16	40 (0)	50 (0)	1:10 (0)	20.915	127.73
17	**40 (0)**	**50 (0)**	**1:10 (0)**	**21.756**	**137.47**

Ultrasound-Assisted Extraction

An 800 W ultrasonic bath (Elmasonic TI-H-10, Elma, Singen, Germany), with internal dimensions (width/depth/height) of 30/24/15 (cm) and 8.6 L of tank operating capacity was used. Crude phenolic extracts were processed at a constant frequency of 25 kHz, and at an ultrasonic power amplitude of 50%, totaling an output power of 400 W. The equipment has temperature adjustment control. 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, Monza, Italy). The calculation of TA content in the extract was given directly by Eq. 2 and expressed in mg per 100 g of plant material.

$$C = \frac{Abs \times MM}{\varepsilon \times b} \qquad (2)$$

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, Monza, Italy). The quantification of TPC was obtained through the standard curve of gallic acid (R^2 = 0.9959) 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 replications.

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).

$$\left(1 - \frac{c}{c_{seq}}\right) = (1 - b)e^{-kt} \qquad (3)$$

$$ln\left(1 - \frac{c}{c_{seq}}\right) = ln(1 - b) - kt \qquad (4)$$

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).

Scanning Electron Microscopy (SEM)

To better assess differences related to extraction process, the micro-structures of untreated raspberries and after conventional and UAE were analyzed unig a scannig electron microscope (JSM-6010 LA, Jeol, Tokyo, Japan). First, samples, fixed by a carbon tape on a metal support (stubs), were covered by a thin layer of gold by means of sputtering apparatus (Quorum Q150R, Quorum Technologies Ltd, East Sussex, UK). Then, samples were scanned with SEM under partial vacuum at 10 kV.

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

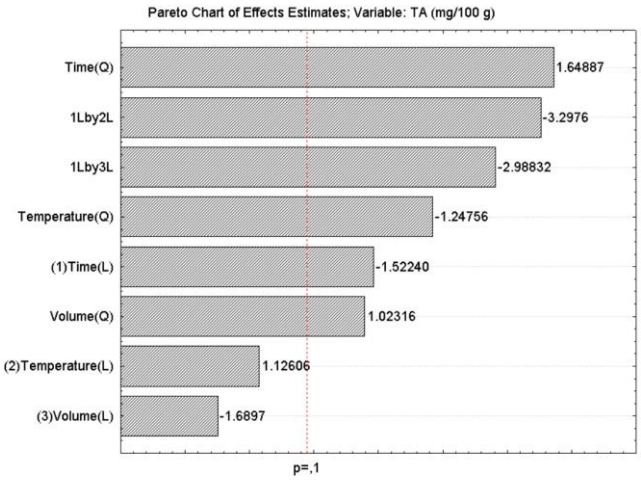
Optimization of Ultrasound-Assisted Extraction

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. 1 a), 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. 1 b), 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.

a



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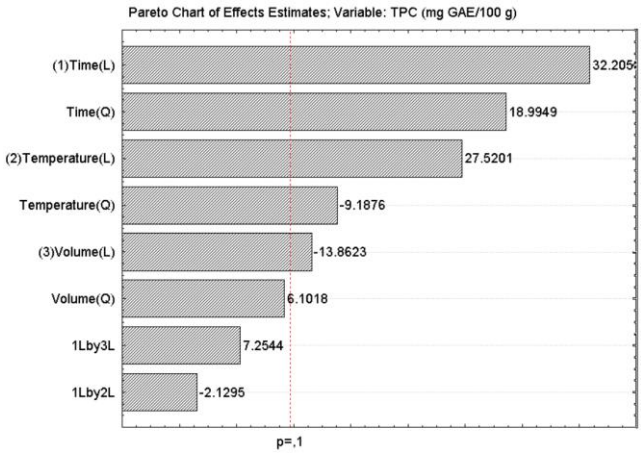


Fig. 1 Pareto Chart of the significance of the effects of the independent variables studied for the response variable (a) TA and (b) TPC; ($p < 0.10$) of raspberries.

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	19.436	<0.0001***	123.58	<0.0001***
X_1	-1.5224	0.049045**	32.206	0.00072200***
X_2	1.1261	0.16673	27.520	0.0025930**
X_3	-1.6897	0.10220	-13.862	0.088239*
X_1^2	1.6489	0.0083660**	18.995	0.0012400**
X_2^2	-1.2476	0.0266230**	-9.1876	0.032616*
X_3^2	1.0232	0.053661*	6.1018	0.11591
X_1X_2	-3.2976	0.0093800**	-2.1295	0.76533
X_1X_3	-2.9883	0.014338*	7.2544	0.32820

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.

$$Y_{TA} = 19.436 - 1.5224X_1 + 1.6489X_1^2 - 1.2476X_2^2 + 1.0232X_3^2 - 3.2976X_1X_2 - 2.98832X_1X_3 \quad (5)$$

$$Y_{TPC} = 123.58 + 32.206X_1 + 27.520X_2 - 13.862X_3 + 18.995X_1^2 - 9.1876X_2^2 \quad (6)$$

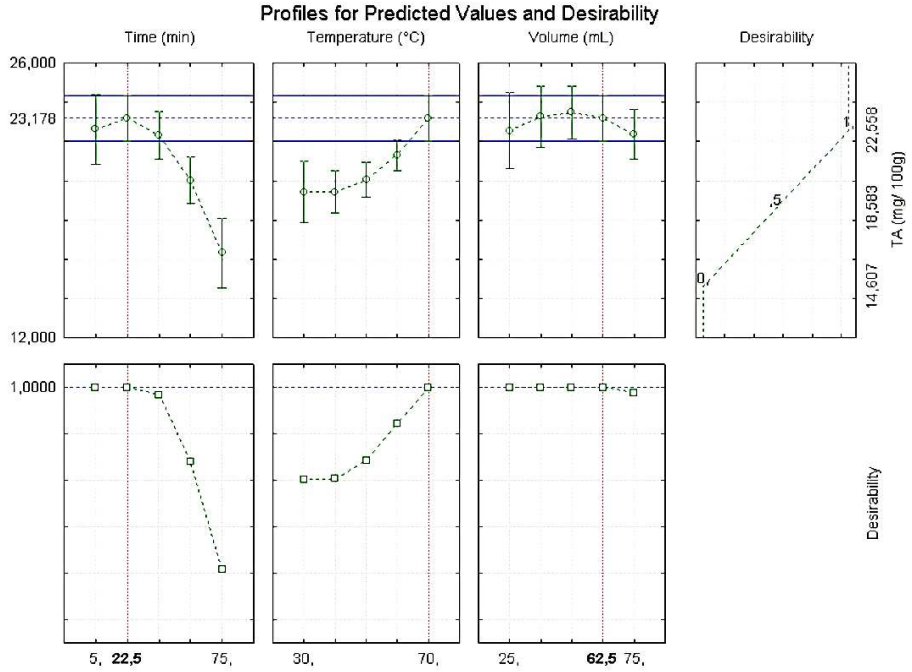
Desirability profiles (D = 1.0 on a scale of 0-1) were used to optimize the three studied variables (time, temperature, and solid: liquid ratio) simultaneously to obtain the highest contents of TA and TPC. Thus, for the response variable TA (Fig. 2 a), the optimal combination of the studied variables 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. 2 a 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.

a



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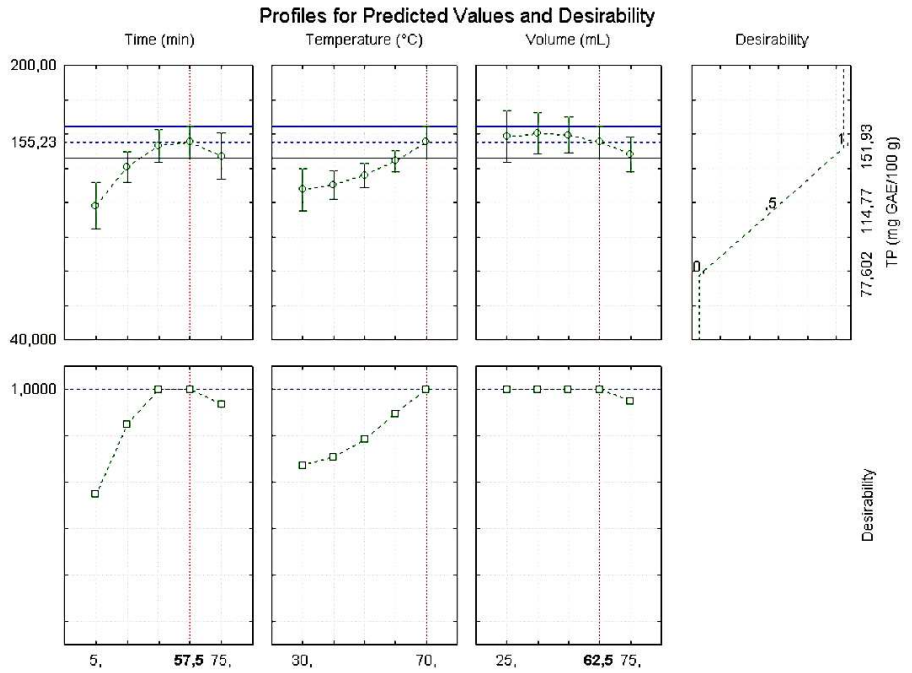


Fig. 2 Desirability profile of **(a)** TA (mg/ 100 g) and **(b)** TPC (mg GAE/ 100 g).

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. 2 a 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. 2 a 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).

In the last decade, “green” extraction techniques, such as ultrasound-assisted extraction, have gained attention considering that they encourage the elimination of toxic substances and volatile organic solvents. Although many engineering and process management approaches seek to minimize waste or achieve greater energy efficiency, green chemistry looks for more deep goals, optimizing each step of a process, to ensure human and environmental health. Two of the twelve principles proposed by green chemistry concern the use of solvents and safer reaction conditions, and waste prevention. Therefore, ultrasound emerges as a key technology to achieve the sustainable goals of green chemistry, as it allows the extraction of several compounds from different plant matrices, with high reproducibility, in reduced times, using less energy and toxic solvents (Khadhraoui et al. 2018; da Silva et al. 2022).

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.

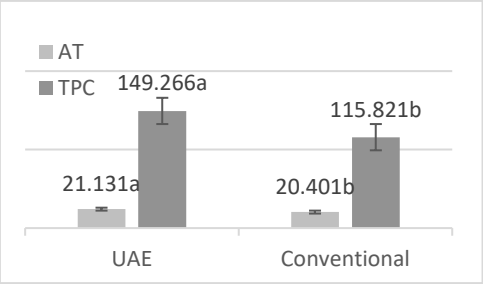


Fig. 3 Comparison of UAE and conventional extraction on the TA (mg/100 g) and TPC (mg GAE/100 g), tests performed in triplicates. Different letters indicate that the samples differ significantly from each other.

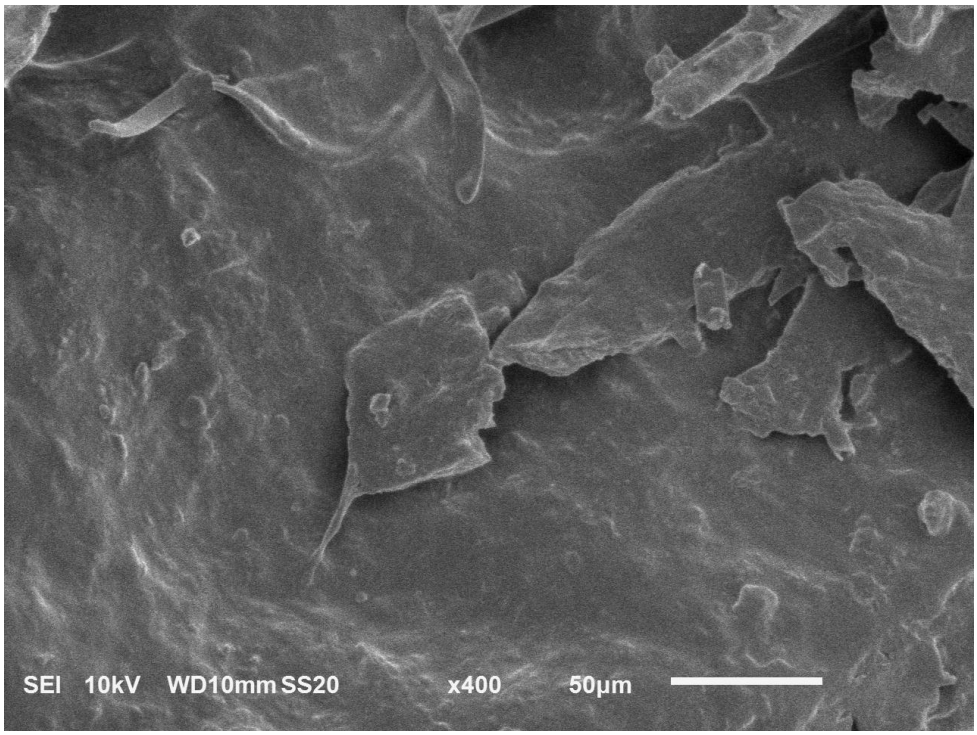
When compared to conventional extraction – which is done at a temperature of 5 ± 1 °C; in solid: liquid ratio of 1:10 m/v; and time of 24 hours, it becomes evident that the UAE contributed positively to the increase in the extraction rate of the studied bioactive compounds. The main highlight is the 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 (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 as a viable alternative for the extraction of anthocyanins and phenolic compounds from varied plant sources (Das and Eun 2018).

Thus, currently, mechanisms behind the positive effects caused by the use of ultrasound in the extraction of bioactive compounds have been studied and are beginning to be elucidated. Chemat et al. (2017) reviewed the mechanism of UAE for food and natural products, which may include: fragmentation, erosion, capillarity, detexturation and sonoporation. Therefore, the single or combined effects of these mechanisms may increase ultrasonic efficiency through cell disruption and promoting mass transfer. In order to observe the action of these mechanisms in the raspberry plant matrix, a scanning electron microscopy study was carried out. As can be seen, the untreated plant matrix has a mostly intact surface (Fig. 4 a). After conventional extraction (24 h under refrigeration at pH ~ 2), the plant matrix shows a high fragmentation (Fig. 4 b), which may be due to the long time exposed in acidic medium. When compared to the 22.5 min/ 70 °C ultrasonic extraction (Fig. 4 c), it is possible to observe a lower fragmentation, which may be due to the shorter extraction time, however, greater erosion, sonoporation and detexturization of the surface of the plant material, which becomes more intense as the extraction time increases to 57.5 min/70 °C (Fig. 4 d). In this way, these different mechanisms contributed to maximize yields

of extraction by increasing the surface area contact between solvent and plant matrix components (Khadhraoui et al. 2018; Yusoff et al. 2022).

a



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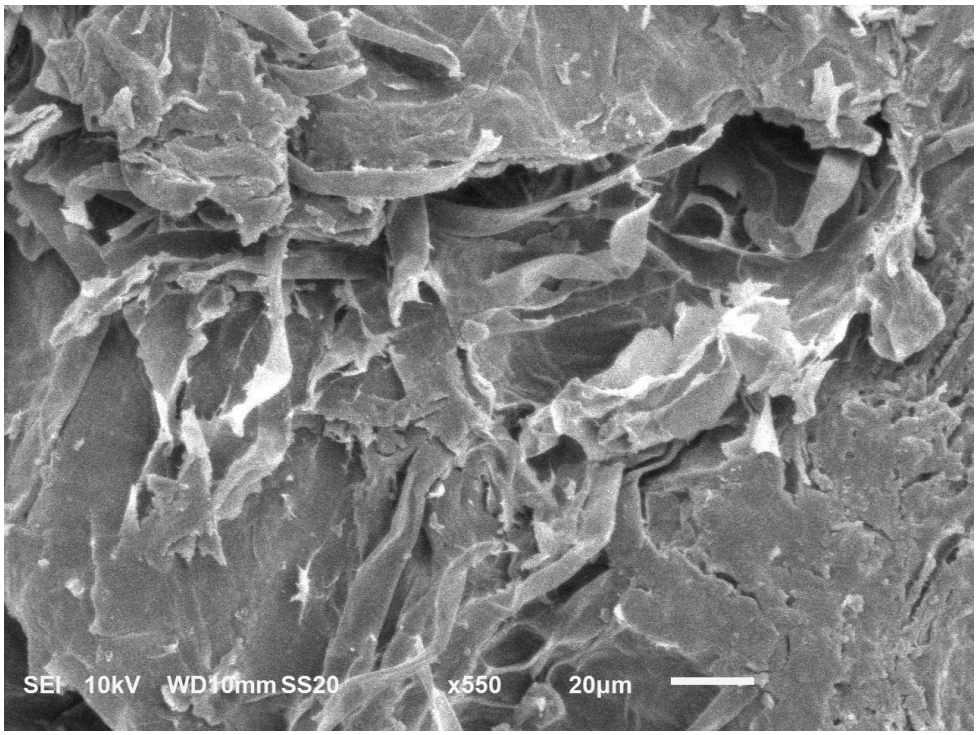

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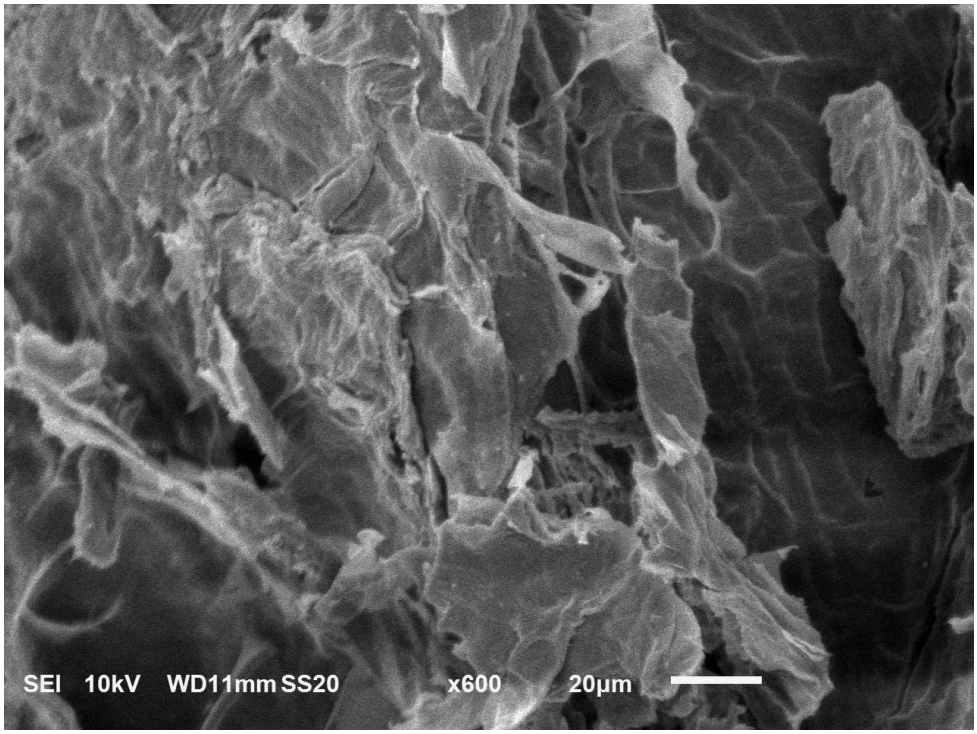

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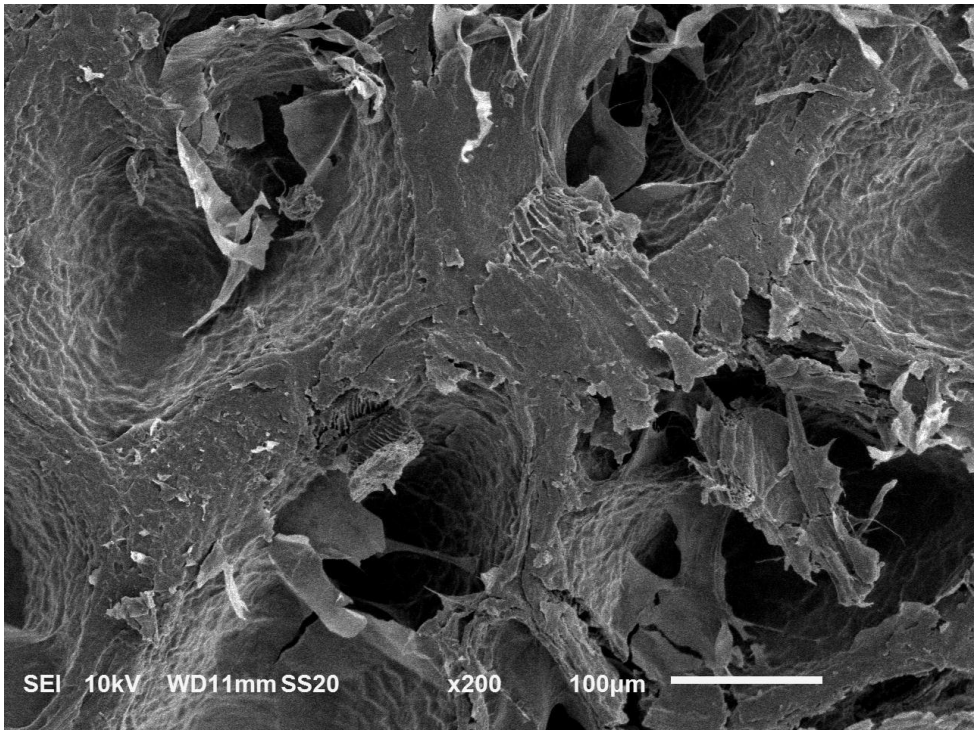


Fig. 4 Scannig electron micrograph of **(a)** untreated raspberry **(b)** conventional extraction **(c)** UAE after 22.5 min and **(d)** UAE after 57.5 min.

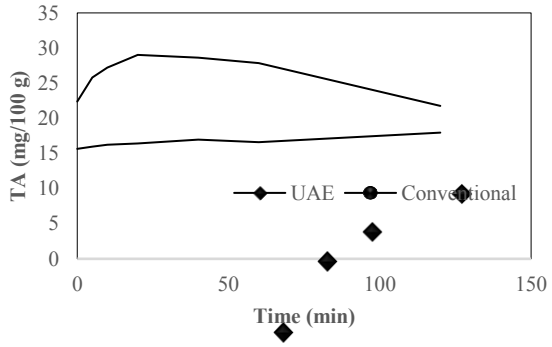
When observing the extraction yield of TA (mg/100 g), as a function of time (min), comparing the UAE with the conventional extraction (Fig. 5 a), it is evident a greater recovery of the target compound in the UAE. Furthermore, the curve for the UAE presents a better extraction efficiency in the first 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. 5 b). 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. 5 a 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).

a



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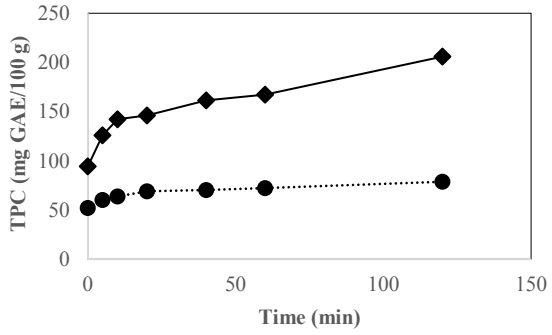


Fig. 5 Kinetics of extraction of (a) TA (mg/100 g) and (b) TPC (mg GAE/100 g) from raspberries as a function of time (min).

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 ($R^2 < 0.90$). Therefore, a longer analysis time would be recommended.

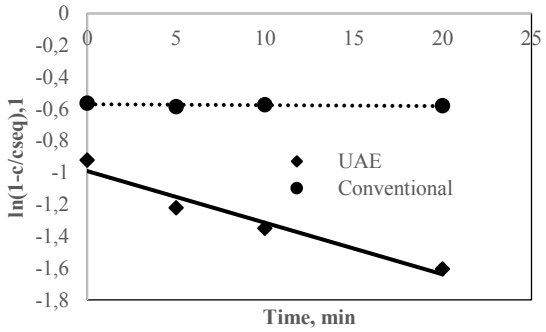
The parameters of the kinetic models were calculated from the experimental data using the linear regression method, using the linearized form of the proposed kinetic equation (Table 3, Fig. 6 a and b). In general, as expected, the washing coefficient (b) has values higher than the slow diffusion coefficient (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	0.0740	0.9269
	Conventional	0.2222	0.0008	0.7758

a



b

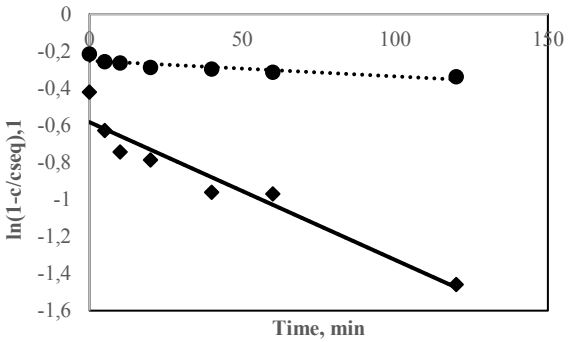


Fig. 6 Linearized form of the kinetic equation based on film theory for extraction of (a) TA and (b) TPC.

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 green methodology, being an alternative for the industrial production of natural colorants from these fruits.

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

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

Declarations

Conflict of Interest The authors declare no conflict of interest.

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3. PAPER 2

Paper submitted to the journal Ciência Rural and awaiting approval.

Optimization of ultrasound-assisted extraction of anthocyanins from purple tomatoes

Otimização da extração assistida por ultrassom de antocianinas de tomates roxos

ABSTRACT

This study aimed to optimize the ultrasound-assisted extraction (UAE) of total anthocyanins from two stages of ripening purple tomatoes (low and high) and to compare the optimized extraction with the conventional one. In the optimization of UAE, the studied variables were time (5 to 75 min), temperature (30 to 70 °C), and solid: liquid ratio (1:5 to 1:15 m/v). The optimal condition of the UAE process, for low-ripened purple tomatoes, occurs at a time of 75 min, temperature of 40 °C, and solid: liquid ratio of 1:15 m/v, predicting the content of 12.487 mg/100g. For high-ripened purple tomatoes, the optimal condition occurs at a time of 40 min, temperature of 50 °C, and solid: liquid ratio of 1:15 m/v, predicting the content of 8.802 mg/100 g. On validation of these optimized conditions, less than a 3% difference was found between the predicted and experimental values (12.267 mg/100 g for low-ripened, and 8.894 mg/100 g for high-ripened purple tomatoes). When comparing the optimized UAE with the conventional extraction, it was observed that UAE increased ($p < 0.05$) the extraction of total anthocyanins content by 73% for low-ripened and by 54% for high-ripened purple tomatoes. Thus, the study indicates that the UAE is an efficient technology for recovering bioactive compounds from purple tomatoes.

Key words: ultrasonic extraction · optimization · anthocyanins · purple tomatoes

RESUMO

Este estudo teve como objetivo otimizar a extração assistida por ultrassom (EAU) de antocianinas totais de dois estágios de maturação de tomates roxos (baixo e alto) e comparar a extração otimizada com a convencional. Na otimização da EAU, as variáveis estudadas foram tempo (5 a 75 min), temperatura (30 a 70 °C) e razão sólido:líquido (1:5 a 1:15 m/v). A condição otimizada da EAU, para tomates roxos em baixa maturação, ocorreu no tempo de 75 min, temperatura de 40 °C e razão

sólido:líquido de 1:15 m/v, prevendo o teor de 12,487 mg/100 g. Para tomates roxos em alta maturação, a condição ideal ocorreu no tempo de 40 min, temperatura de 50 °C e razão sólido:líquido de 1:15 m/v, prevendo o teor de 8,802 mg/100 g. Na validação das condições otimizadas, foi encontrada uma diferença inferior a 3% entre os valores previstos e experimentais (12,267 mg/100g para baixa-maturação e 8,894 mg/100 g para alta-maturação). Ao comparar a EAU otimizada com a convencional, observou-se que a EAU aumentou ($p < 0,05$) a extração de antocianinas totais em 73% para tomates em baixa-maturação e 54% para tomates roxos em alta-maturação. Assim, o estudo indica que os EAU é uma tecnologia eficiente para a recuperação de antocianinas de tomates roxos.

Palavras-chave: extração ultrassônica otimização antocianinas tomates roxos

INTRODUCTION

The color of flowers and fruits is caused by the presence of different kinds of pigment belonging to the phenylpropanoid and terpenoid classes, whose three major groups are chlorophylls, carotenoids, and anthocyanins (GONZALI; MAZZUCATO; PERATA, 2009). Anthocyanins are a group of naturally occurring water-soluble pigments belonging to the flavonoid family and responsible for the blue, purple, red, and orange color of plant organs. Anthocyanins have important functions in plants, including defense against pathogens and protection against ultraviolet irradiation (MAZZUCATO et al., 2013).

Tomatoes (*Solanum lycopersicum*) are among the most widely consumed crops. This fruit is consumed both *in natura* and processed in a wide range of products (CAMPESTRINI et al., 2019). Usually, the main bioactive compounds present in tomatoes are carotenoid pigments (mostly lycopene), polyphenols, and ascorbic acid. Moreover, in the cultivated tomato species, anthocyanins are not produced in the fruit due to the lack of expression of the chalcone isomerase (CH1) gene in the flavonoid biosynthetic pathway in the peel of tomato fruit (HAZRA; LONGJAM; CHATTOPADHYAY, 2018).

Recently, genetic engineering in search to induce and enhance the biosynthesis of anthocyanins in plants has developed a line of transgenic tomatoes with high levels of anthocyanins (ČERMÁK et al., 2015; SU et al., 2016). Purple tomatoes contain anthocyanins as well as other phytochemicals (carotenoids and polyphenols) commonly found in conventional red tomatoes. The genetically modified purple tomato

was found to have additional health-promoting effects by prolonging the life of cancer-susceptible mice than conventional tomatoes, which may be due to additional bioactivities promoted by the presence of anthocyanins, in addition to the usual carotenoids, such as lycopene (LI, H. et al., 2014).

In the last decade, ultrasound-assisted extraction (UAE) emerges as a key technology to achieve the sustainable goals of the green chemistry approach, as it allows the extraction of several compounds from different plant matrices, with high reproducibility, in reduced times, using less energy and toxic solvents, when compared to conventional forms, as solvent extraction (KHADHRAOUI et al., 2018; SILVA, R. F. DA et al., 2022). The ultrasonic enhancement of the extraction is attributed to cell destruction, capillary effects, better solvent penetration, and mass transfer intensification (CHEMAT, Farid et al., 2017; CHEMAT, Farid; ZILL-E-HUMA; KHAN, 2011; O'DONNELL et al., 2010).

Furthermore, several studies show that different plant matrices require different combinations of variables involved in the process - such as extraction time, temperature, pH, solvent solid: liquid ratio, frequency, power, etc - for better yields in the extraction rates of the target compound (HE et al., 2016; DRANCA; OROIAN, 2016; BARAN; GOUD; DAS, C., 2017; ROCHA et al., 2017; ESPADA-BELLIDO et al., 2017).

In this context, this study aimed to optimize the UAE for the highest recovery of anthocyanins in purple tomatoes, in two stages of ripening (low and high), by studying the effect of the three variables involved in the UAE process, namely: time (min), temperature (°C) and solid: liquid ratio (m/v). The optimized UAE process was compared to the conventional solvent extraction. In addition, a morphological study, using scanning electron microscopy, was also performed.

MATERIAL AND METHODS

Chemicals and reagents

Folin-Ciocalteu phenol reagent was 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.

Plant material preparation

Purple tomatoes (*Solanum lycopersicum*), cultivate M82, were provided by Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa – MG, Brazil. The tomatoes were harvested in October 2021, had a rounded shape, measured about 2.5 ± 0.5 cm in diameter and weighed about 3.0 ± 1.0 g. Fruits were selected at two stages of ripening, low (purple color) and high (purple-red color), as shown in Figure 1. They were sanitized, frozen, packaged, and stored in a conventional freezer at -20 °C until the moment of analysis.

Design of experiments

Box-Behnken Design (BBD) was selected to optimize the UAE conditions for the highest recovery of total anthocyanins from purple tomatoes (JIANG, Z. M. et al., 2019; SANG, Jun et al., 2017; ZHOU et al., 2022). 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. Total anthocyanins yield was used as the response variable. The experimental data obtained were fitted to a quadratic polynomial model (Equation 1) and the regression coefficients were generated from multiple linear regression:

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

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; GOUD; DAS, C., 2017; JIANG, L. et al., 2019).

The best simultaneous combination of the studied independent variables (time, temperature, and solid: liquid ratio) to provide the maximum extraction of anthocyanins from purple tomatoes was obtained through the desirability profile (D = 1, on a scale from 0 to 1). From this optimal combination, the content of total anthocyanins was experimentally obtained for validation of the method (BOATENG, 2023).

Ultrasound-assisted extraction

The purple tomatoes 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, in a 250 mL glass Erlenmeyer. The volume of solvent 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 - in an acid medium anthocyanins are found in the most stable form of the flavylium cation (ROCHA et al., 2017). The phenolic extracts obtained from the UAE were vacuum filtered with Whatman filter paper n° 1 and their volume was adjusted to a standard volume with 70% ethanol (v/v). The extracts were stored at -20 °C until subsequent analysis (TEIXEIRA et al., 2023).

An 800 W ultrasonic bath (Elmasonic TI-H-10, Elma, Germany), with internal dimensions (width/depth/height) of 30/24/15 (cm) and 8.6 L of tank operating capacity was used. Crude phenolic extracts were processed at a constant frequency of 25 kHz, and at an ultrasonic power amplitude of 50%. 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 min, 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). For validation of the optimized conditions, the extracts were obtained in triplicates.

Conventional extraction and comparative study of extraction efficiency

The conventional extraction process was carried out according to the methodology of ROCHA et al. (2017), in which the proportion of mashed tomatoes: solvent (70% ethanol (v/v)) is 1:10 (m/v). From this, a system composed of 5 g of mashed plant matrix plus 50 mL of solvent was formed. 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 n° 1 and their volume was adjusted to a standard volume with 70% ethanol (v/v). The extracts were obtained in triplicates, and stored at -20 °C until subsequent analysis.

This conventional extraction process was compared with the UAE process under optimized conditions to evaluate the efficiency of using ultrasound in the recovery of anthocyanins from purple tomatoes.

Analysis of total anthocyanins

The total anthocyanin content was determined by the methodology described by FULEKI & FRANCIS (1968) with some modifications. An aliquot of the sample was diluted in 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 total anthocyanins content in the extract was given by Equation 2 and expressed in mg per 100 g of tomatoes.

$$C = \frac{Abs \times MM}{\varepsilon \times b} \qquad (2)$$

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

Total carotenoid content

The total carotenoid content was determined using the methodology described by RODRIGUEZ-AMAYA (2001), with some modifications. First, 5 g of mashed purple tomatoes were weighed, and 60 mL of cooled acetone was added. The obtained extract was vacuum filtered and transferred to a separatory funnel containing 50 ml of cooled petroleum ether. The extract was washed with ultrapure water until the acetone was completely removed. The final extract was recovered and its final volume was adjusted with petroleum ether. Absorbance was measured at 470 nm using a UV-Vis spectrophotometer (Bel Engineering, Model UV-51, Italy). The calculation of total carotenoid content in the extract was given by Equation 3 and expressed in mg per 100 g of tomatoes.

$$C = \frac{Abs \times y \times 10^3}{\varepsilon \times 100} \qquad (3)$$

where C is the concentration of lycopene in mg; Abs is the absorbance read at 470 nm; y is the volume of the extract in mL; and ε is the molar absorptivity coefficient of petroleum ether (in this case. 2592).

Scanning electron microscopy (SEM)

To better assess differences related to the extraction process, the micro-structures of untreated high-ripened purple tomatoes and after conventional and UAE processes were analyzed using a scanning electron microscope (JSM-6010 LA, Jeol, Tokyo, Japan). First, samples, fixed by carbon tape on metal support (stubs), were covered by a thin layer of gold using sputtering apparatus (Quorum Q150R, Quorum Technologies Ltd, East Sussex, UK). Then, samples were scanned with SEM under a partial vacuum at 10 kV.

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 ($p < 0.05$) and optimize the UAE process conditions through the desirability profile.

The results of the contents of total anthocyanin and total carotenoids 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 $p < 0.05$. All analysis were performed in triplicates.

RESULTS AND DISCUSSION

Optimization of ultrasound-assisted extraction

In this study, the content of total anthocyanin ranged from 8.805 to 12.458 mg/100 g for low-ripening purple tomatoes, and from 6.201 to 8.497 mg/100 g for high-ripening purple tomatoes (Table 1). BBD was used to determine the linear, quadratic, and interaction effects of the studied variables on the considered response variable (BOATENG, 2023). The regression analysis coefficients and *p*-values for total anthocyanins responses, for low and high-ripened purple tomatoes, are shown in Table 2.

In the regression analysis, for low-ripened purple tomatoes, it is observed that the linear volume (X_3) was the most significant effect on the model ($p < 0.05$), followed by the effect of the quadratic temperature (X_2^2), the interaction of linear time with the volume of linear solid: liquid ratio (X_1X_3), the interaction of linear time with linear temperature (X_1X_2), and the quadratic time (X_1^2), the other effects did not contribute significantly to the proposed polynomial model. In the regression analysis for high-ripened purple tomatoes, quadratic temperature (X_2^2) was the most significant effect on the model ($p < 0.05$), followed by the effect of the quadratic time (X_1^2), and linear volume (X_3), the other effects did not contribute significantly to the proposed polynomial model.

From the regression analysis, the studied factors were fitted to the second-order polynomial regression model (Equation 1), showing significant F test values, non-significant lack of fit, and R^2 values equal to 0.958 and 0.928 for TA from low and high-ripened purple tomatoes, respectively. The high values of the coefficient of determination ($R^2 > 0.90$) show that the regression equations adjusted for low (Equation 4) and high-ripened purple tomatoes (Equation 5) fit the experimental data. It is noteworthy that although the empirical models of Equations 4 and 5 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 during the process and in predicting these contents.

$$Y_{Low} = 11.449 + 1.967X_3 + 0.799X_1^2 + 0.937X_2^2 - 1.656X_1 + 1.657X_1X_3 \quad (4)$$

$$Y_{HIGH} = 7.113 + 1.085X_3 + 0.749X_1^2 + 0.948X_2^2 \quad (5)$$

Desirability profiles were used to optimize the three studied variables (time, temperature, and solid: liquid ratio) simultaneously to obtain the highest total anthocyanins contents (BOATENG, 2023; GOULA et al., 2017). Thus, for low-ripened purple tomatoes (Figure 2 a), the optimal combination of the studied variables was at a time of 75 min, the temperature of 40 °C, and a volume of solid: liquid ratio of 1:15 m /v (which corresponds to 75 mL of 70% ethanol solvent (v/v), for 5 g of mashed sample). This combination predicts a total anthocyanin content of 12.487 mg/100 g. On the validation of this optimized condition, the experimental value of 12.267 ± 0.18 mg of total anthocyanins per 100 g was observed. This value is only 2% lower than predicted by the mathematical model.

Similarly, for high-ripened purple tomatoes (Figure 2 b), the optimal combination of the input variables studied was in the time of 40 min, the temperature of 50 °C, and volume of solid: liquid ratio of 1:15 m/v (corresponding to 75 mL of 70% ethanol solvent (v/v), for 5 g of mashed sample). This combination predicts a total anthocyanin content of 8.802 mg /100 g. On the validation of this optimized condition, the experimental value of 8.894 ± 0.01 mg /100 g was observed for total anthocyanins, a value only 1% higher than that predicted by the model.

The efficiency of ultrasound-assisted extraction has been extensively reported to increase extraction rates of target compounds with reduced extraction time (BARAN; GOUD; DAS, C., 2017). In this work, the extraction process was carried out by varying the time between 5 to 75 minutes. From desirability profiles (Fig. 2 a and b), it is observed that the effect of time was important for the recovery of anthocyanins from low and high-ripened purple tomatoes. Note that for tomatoes with a lower level of ripening, a longer extraction time (75 min) was necessary, while for tomatoes with a higher level of ripening, a shorter time was sufficient (40 min). This is due to the ripening process promotes changes in the cell wall, induced by the increase in the solubility of pectic polysaccharides, contributing to the softening of the fruit, which facilitates the action of ultrasonic waves on the cell matrix, requiring a shorter extraction time (HUBER; KARAKURT; JEONG, 2001).

It was also observed that for the extraction of anthocyanins from low and high-ripened purple tomatoes, a temperature close to 40 – 50 °C was the most suitable condition for the highest efficiency of the processes (Fig. 2 a and b). Generally. in a solid-liquid extraction. the increase in temperature leads to greater recoveries of bioactive compounds (DRANCA; 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, the solid: liquid ratio m/v proved to be an essential factor for increasing the recovery of anthocyanins. In both cases, for low and high-ripened purple tomatoes, the best yield occurred at the solid: liquid ratio of 1:15 m/v, values on the highest level under study (Fig. 2 a 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).

In this study, it becomes evident that the stage of maturation influences the content of bioactive compounds present in purple tomatoes. It is noted that for this tomato cultivar, as in normal cultivars, the content of phenolics strongly depends on the ripening stage of tomato fruits, as they are more abundant in low and intermediate ripening stages, decreasing in full ripened tomatoes. In addition, during ripening, the level of carotenoids increases due to lycopene accumulation, because of the increased expression of genes involved in isoprenoid biosynthesis. Consequently, the red color starts to appear as the purple color of anthocyanins becomes less evident (GIUDICE, DEL et al., 2015).

Thus, there was a significant difference ($p < 0.05$) in the content of bioactive compounds, between the two ripening stages of studied tomatoes. The purple tomato at the beginning of the ripening showed a total carotenoid content (lycopene) of 0.463 ± 0.06 mg/100 g that increased to 2.108 ± 0.09 mg/100 g in a more advanced stage of ripening, which corresponds to an increase of approximately 350%. Moreover, as expected, the total anthocyanin content had an opposite response, as its initial content of 12.267 ± 0.18 mg /100 g decreased to 8.894 ± 0.01 mg /100 g as the ripening stage advanced, which corresponds to a decrease of approximately 28%. Furthermore, although the total contents of anthocyanins and carotenoids give us good indications of the content of these components in purple tomatoes, studies that properly identify and quantify these bioactive compounds are needed and will be conducted in the future.

Comparative study of extraction processes

Over the past few years, UAE has been widely used as an alternative to overcome the limitations of conventional solvent extraction. In this sense, the UAE optimized in this study was compared with the conventional extraction of anthocyanins from purple tomatoes. In the UAE optimized for the highest recovery of total anthocyanins, the optimal simultaneous combination of the studied variables occurs in a time of 75 min, at a temperature of 40 °C, and in a solid: liquid ratio of 1:15 m/v, for low-ripened stage purple tomatoes. It was observed that UAE significantly increased ($p < 0.05$) the extraction yield of total anthocyanins with values of 12.267 ± 0.18 mg/100 g when compared to 7.099 ± 0.31 mg/100 g obtained by conventional solvent 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.

On the other hand, for high-ripened stage purple tomato, the optimal simultaneous combination of the studied variables occurs at a time of 40 min, at a temperature of 50 °C, and a solid: liquid ratio of 1:15 m/v. It was observed that the UAE significantly increased ($p < 0.05$) the extraction yield of total anthocyanins with values of 8.894 ± 0.01 mg/100 g when compared with 5.790 ± 0.09 mg/100 g obtained by conventional solvent extraction. Thus. the use of UAE proved to be efficient in overcoming limitations of the conventional technique, since UAE increased the recovery yield of total anthocyanins by approximately 73% and 54% for purple tomatoes in low and high stages of ripening, respectively, when compared to conventional extraction. Besides, when comparing these two processes, the shorter extraction time of about 1h in the optimized UAE compared to the 24h required for conventional extraction, plus the greater recovery of the target compound in the UAE, stands out. The reduction in process time is important because it reduces energy consumption. in addition to enabling greater processing capacity for a sort of raw materials (CHEMAT, S. 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, even as allows the scale-up to industrial levels, and requires low initial investments, the UAE has been standing out, in recent years, as a viable green alternative for the extraction of anthocyanins from varied plant sources, including purple tomatoes(DAS, P. R.; EUN, 2018).

Scanning electron microscopy (SEM)

Nowadays, mechanisms behind the positive effects caused by the use of ultrasound in the extraction of bioactive compounds have been studied and are beginning to be elucidated. CHEMAT et al. (2017) reviewed the mechanism of the UAE for food and natural products, which may include: fragmentation, erosion, capillarity, detexturation, and sonoporation. This means that the single or combined effects of these mechanisms are what can increase ultrasonic efficiency through cell disruption and promote mass transfer in the extraction process.

To observe the action of these mechanisms on a high-ripened purple tomato plant matrix, a scanning electron microscopy study was carried out. As can be seen, the untreated plant matrix has a mostly intact surface (Fig. 3 a). After conventional extraction (24 h under refrigeration at pH ~ 2), the plant matrix shows some level of fragmentation and erosion (Fig. 3 b), which may be due to a long time of exposure to an acidic medium. When compared to the ultrasonic extraction (40 min at 50 °C) it is possible to observe high levels of fragmentation, erosion, sonoporation, and detexturization of the surface of the plant material (Fig 3 c). In this way, these different ultrasound mechanisms, along with the heating, contributed to maximizing the yields of extraction by increasing the surface area contact between solvent and plant matrix components (KHADHRAOUI et al., 2018; YUSOFF et al., 2022).

CONCLUSION

BBD together with desirability profiles was successfully employed in the optimization of the studied variables for the extraction of total anthocyanins from purple tomatoes. Under the optimized conditions, the yields predicted by the model and experimentally observed for total anthocyanins for low and high stages of ripening differed by less than 3%.

When compared UAE optimized with conventional extraction, the use of ultrasonic treatment promoted an increase of 73% and 54% in the recovery of total anthocyanins from purple tomatoes with a low and high stage of ripening. respectively. In addition, highlights the great difference in processing time from 24 hours in the conventional extraction to less than 1 hour in the optimized conditions of the UAE. Furthermore, mechanisms behind the positive effects caused by the use of ultrasound in the extraction of anthocyanins could be observed by scanning electron microscopy.

Thus, this study indicates that the use of ultrasound-assisted extraction of anthocyanins from purple tomatoes is an efficient and fast green technology, with relative low initial cost, being an alternative for extract anthocyanins from purple tomatoes. As future considerations, individual identification and quantification of bioactive compounds present in purple tomatoes are still necessary.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

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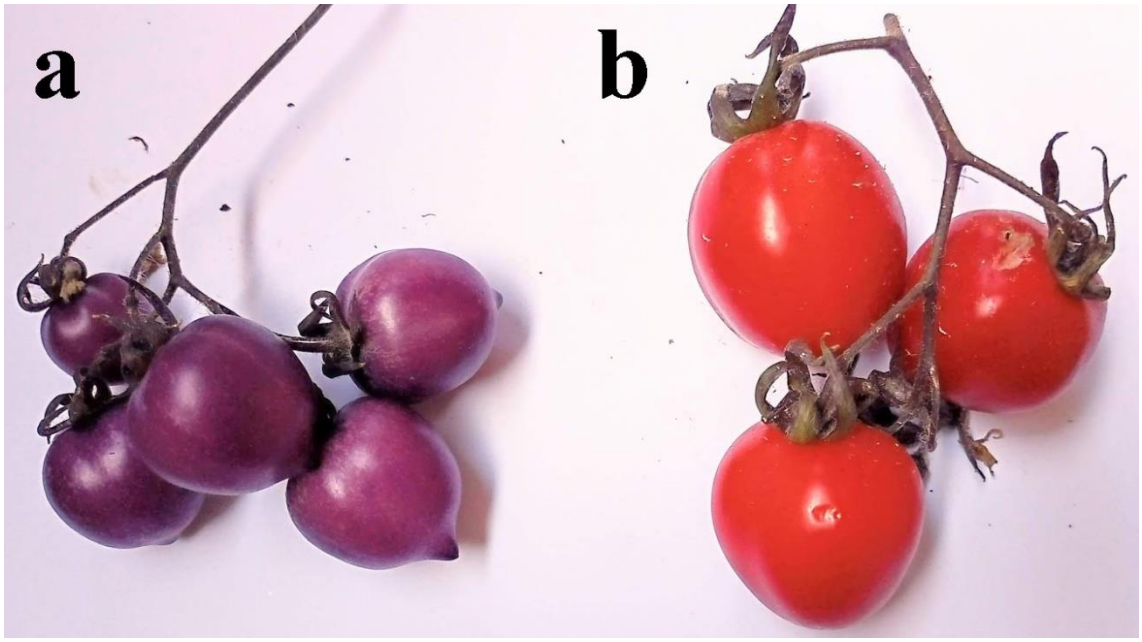
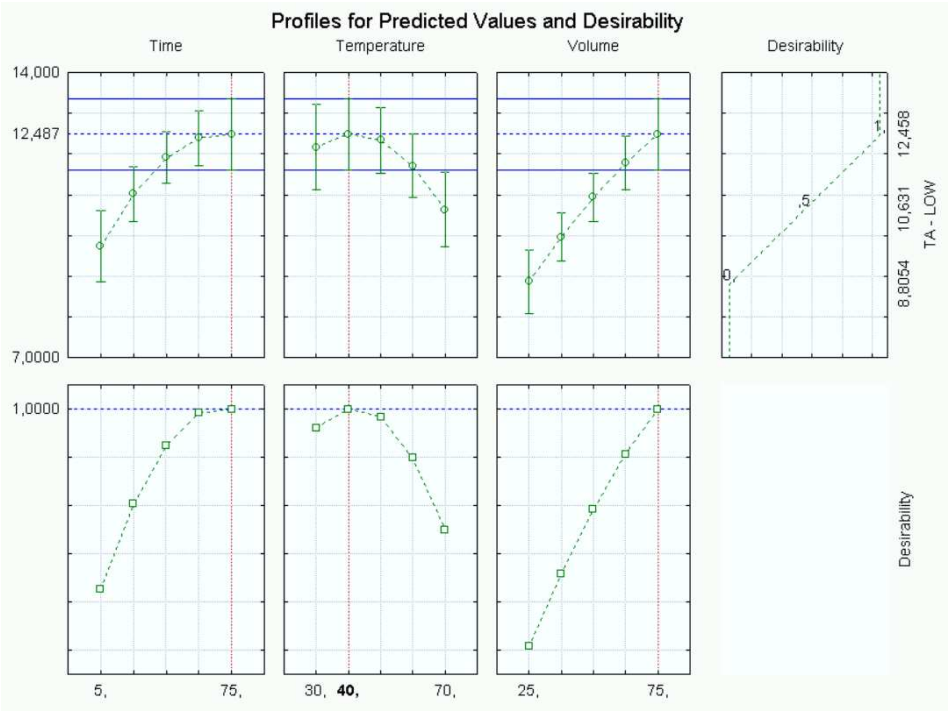


Figure 1 – Stages of ripening of tomatoes (a) low-ripened (purple color) and (b) high-ripened (purple-red color)

a



b

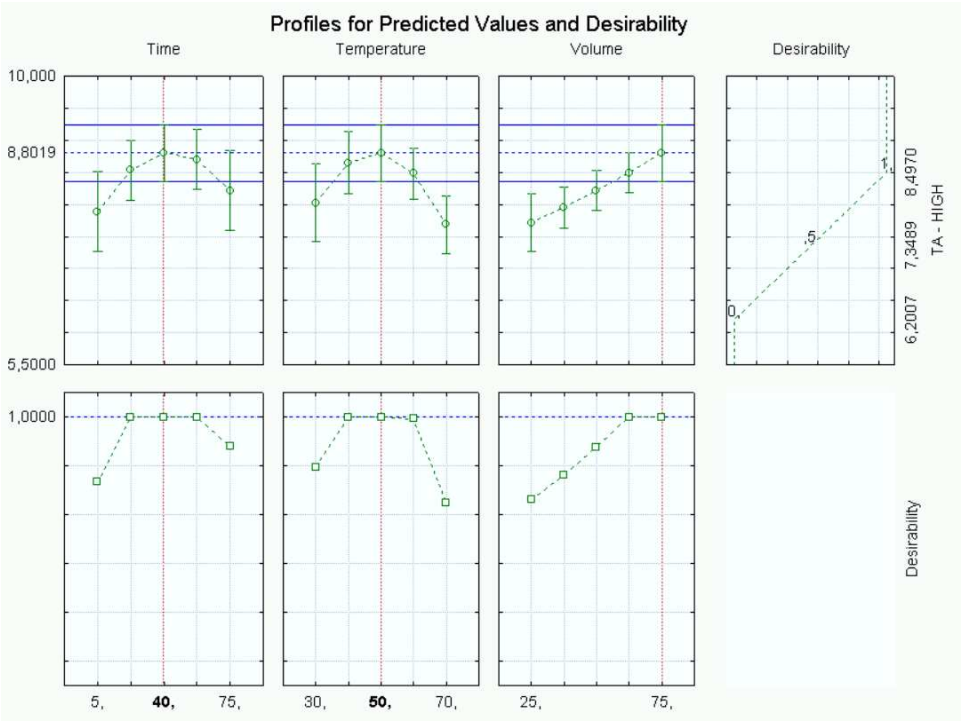


Figure 2 – Desirability profiles of total anthocyanins (TA) content (mg/ 100g) for (a) low-ripened and (b) high-ripened purple tomatoes.

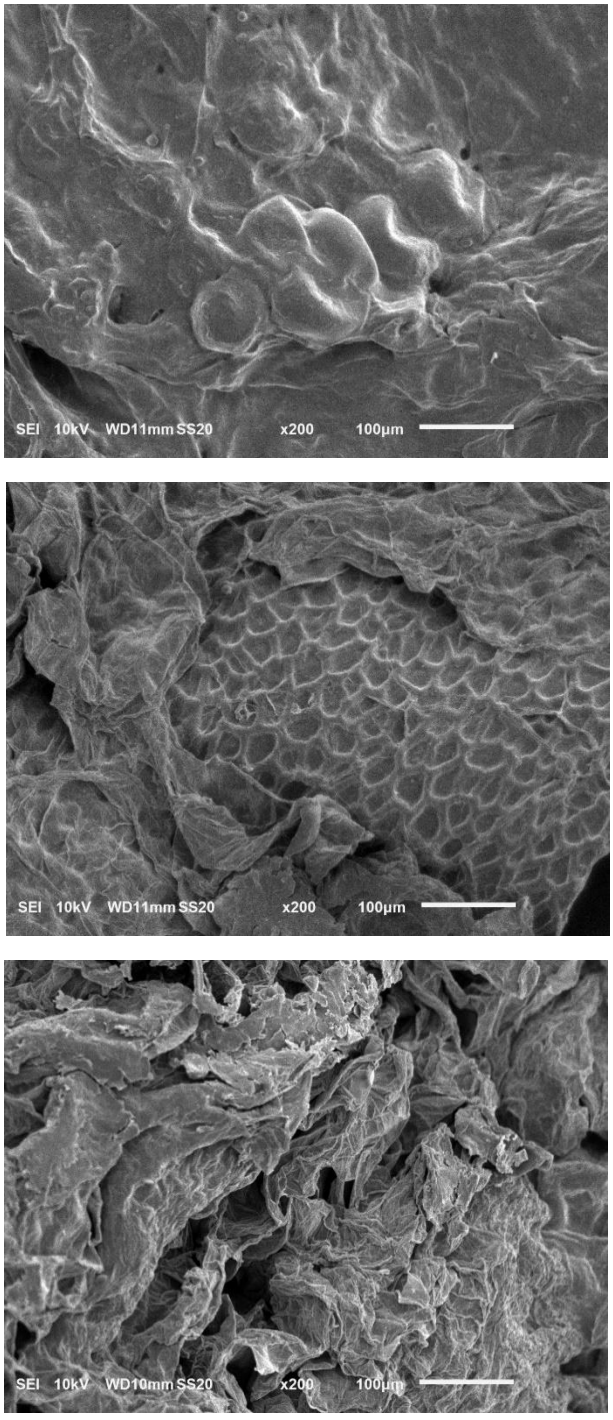


Figure 3 - Scanning electron micrographs of (a) untreated high-ripened purple tomato, after (b) conventional extraction, and (c) UAE (40 min at 50 °C)

Table 1 - BBD and the values of the response for total anthocyanins (mg/100 g of tomatoes) content in low and high-ripened purple tomatoes

Run	Time X_1 (min)	Temperature X_2 (°C)	Solid:liquid ratio X_3 (g/mL)	Low	High
1	5 (-1)	30 (-1)	1:10 (0)	8.805	6.916
2	75 (+1)	30 (-1)	1:10 (0)	10.499	6.573
3	5 (-1)	70 (+1)	1:10 (0)	10.583	6.372
4	75 (+1)	70 (+1)	1:10 (0)	8.964	6.201
5	5 (-1)	50 (0)	1:5 (-1)	9.973	7.069
6	75 (+1)	50 (0)	1:5 (-1)	8.825	6.985
7	5 (-1)	50 (0)	1:15 (+1)	10.290	7.701
8	75 (+1)	50 (0)	1:15(+1)	12.458	8.285
9	40 (0)	30 (-1)	1:5(-1)	9.122	7.020
10	40 (0)	70 (+1)	1:5 (-1)	9.306	6.740
11	40 (0)	30 (-1)	1:15 (+1)	11.620	7.950
12	40 (0)	70 (+1)	1:15 (+1)	10.951	7.538
13	40 (0)	50 (0)	1:10 (0)	11.138	8.200
14	40 (0)	50 (0)	1:10 (0)	11.205	8.497
15	40 (0)	50 (0)	1:10 (0)	11.972	8.176
16	40 (0)	50 (0)	1:10 (0)	11.736	7.741
17	40 (0)	50 (0)	1:10 (0)	11.195	8.448

Table 2 - BBD regression analysis for total anthocyanins (TA) for low and high-ripened purple tomatoes.

	TA - LOW		TA - HIGH	
Variable	**Coefficient**	***p*-value**	**Coefficient**	***p*-value**
Constant	11.449	<0.0001**	7.113	<0.0001**
X_1	0.273	0.335	-0.004	0.985
X_2	0.113	0.720	-0.339	0.194
X_3	1.967	0.001*	1.085	0.003*
X_1^2	0.799	0.004*	0.749	0.002*
X_2^2	0.937	0.002*	0.948	0.001*
X_3^2	0.263	0.194	-0.047	0.745
X_1X_2	-1.656	0.004*	0.086	0.773
X_1X_3	1.657	0.004*	0.334	0.284

X_1 – time (min); X_2 – temperature (°C); X_3 – volume of solid:liquid ratio (mL); * $p < 0.05$; ** $p < 0.001$.

4. PAPER 3

Ultrasound-assisted extraction of bioactive compounds from purple-fleshed sweet potatoes: optimization, kinetic, and modeling study

Abstract

This study aimed to optimize and modeling the ultrasound-assisted extraction process (UAE) of total anthocyanins (TA) and total phenolic content (TPC) from purple-fleshed sweet potato (PFSP), 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. The optimal condition of the UAE process occurs at 75 min, 70 °C, and 1:15 m/v, for both response variables. This optimal condition predicts a content of 18.372 mg/100 g for TA and 6.2237 mg GAE/100 g for TPC. On validation of the optimized conditions, were funded 18.822 mg/100 g for TA and 6.656 mg GAE/100 g for TPC, which corresponds to about a 7% difference between the predicted and experimental values. When comparing the optimized UAE with the conventional extraction, it was observed that UAE significantly increased ($p < 0.05$) the extraction of TA content by 81% and TPC by 91%. The three kinetic models tested fitted well to the extraction process under study ($R^2 > 0.90$). Thus, the study indicates that the UAE process is an efficient methodology for recovering bioactive compounds from PFSP.

Keywords: ultrasonic extraction; optimization; kinetic and modeling; anthocyanins; phenolic compounds

Introduction

Sweet potato (*Ipomea batatas* L.), a member of the *Convolvulaceae* family, is one of the most important root crops worldwide and is highly easy to manage and cultivate. In recent years, sweet potato has become a research focus due to its unique nutritional and functional properties. This tuberous root is rich in carbohydrates such as starch, as well as crude protein, dietary fiber, vitamins, minerals, and bioactive compounds, such as polyphenols and pigments. Based on the color of the peel and flesh, sweet potatoes can be classified into white, yellow, orange, and purple ones. Whereas purple-fleshed sweet potato (PFSP), a special cultivar of sweet potato, contains abundant anthocyanins and starch (C. Chen, Lin, Chen, & Chiang, 2019; Yong et al., 2018).

Anthocyanins are a class of naturally presenting polyphenols compounds, a pigment widely distributed in fruits, beans, cereals, and vegetables, that can act as natural colorants in pharmaceuticals, cosmetics, food; and beverages, in addition to being a potent antioxidant agent against oxidative stress which contribute to the protection of various chronic diseases, such as cardiovascular disorders, degenerative diseases, and cancer (J. yu Chen, Du, Li, & Li, 2020; Fang, 2015; He et al., 2016; Teng et al., 2017). Although synthetic colorants are still prevalent in the 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, such as anthocyanins, are emerging as a viable alternative to replacing them (Lv et al., 2022; Sharma, Gupta, Singh, Bansal, & Singh, 2016; Strieder, Silva, & Meireles, 2019). As a negative point, anthocyanins can be easily degraded into colorless or brown-colored compounds during processing and storage, which limits their use as a commercial colorant. The stability of anthocyanins depends on temperature, light, enzymes, metal ions, sugars, ascorbic acid, oxygen, and the presence of other phenolic compounds. The anthocyanins present in PFSP are recognized as more stable than the pigments of strawberries, blueberries, cranberries, red cabbage, perilla, and other plants, for showing high pH stability, thermostability, and antioxidant activity. Thus, PFSP has been regarded as an alternative and promising source of stable anthocyanins as a colorant (Han et al., 2021; Hwang, Choi, Choi, Chung, & Jeong, 2011).

To produce a natural colorant, one of the first steps is to extract them from the vegetable matrix where it is found. But to produce it on large scale, understand the influence of the extraction process on the functional properties of these bioactive ingredients guarantees an adequate final product both quantitatively and qualitatively (Okolie, Akanbi, Mason, Udenigwe, & Aryee, 2019). For having certain limitations, such as long extraction times, low yield, and high toxic solvent consumption, that do not suit a world that needs environmentally sustainable processes, classical extraction techniques, such as maceration, have been supplemented with "assisted" extraction techniques in which additional physical phenomena are used to intensify the extraction process (Renard, 2018).

In the last decade, ultrasound-assisted extraction (UAE) emerges as a key technology to achieve the sustainable goals of the green chemistry approach, as it allows the extraction of several compounds from different plant matrices, with high

reproducibility, in reduced times, using less energy and toxic solvents. Since while many engineering and process management approaches seek to minimize waste or achieve greater energy efficiency, green chemistry looks for more deep goals, optimizing each step of a process, to ensure human and environmental health (da Silva et al., 2022; Khadhraoui et al., 2018). Therefore, UAE is an economically viable alternative technique for natural colorants, since it overcomes the limitations of conventional extraction, following green chemistry recommendations. The ultrasonic enhancement of the extraction is attributed to cell destruction, capillary effects, better solvent penetration, and mass transfer intensification. Furthermore, the use of ultrasonic waves has shown great potential for applications in industrial processes (F. Chemat et al., 2017; F. Chemat, Zill-E-Huma, & Khan, 2011; O'Donnell, Tiwari, Bourke, & Cullen, 2010). 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 target compounds (Baran, Goud, & Das, 2017; Dranca & Oroian, 2016; Espada-bellido et al., 2017; He et al., 2016; Rocha et al., 2017).

In addition, the development, design, and optimization of the extraction process involve several steps, as variables used 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. 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 compounds from the plant matrix. Furthermore, the development of the extraction technique must be accompanied by the development and improvement of mathematical models to understand their mechanisms and kinetics and enable the generalization of the experimental results for further scale-up of the process. The most frequently used simplified models are based on the concept of film theory, unsteady-state diffusion through plant material, and Ponomaryov's equation (Kitanović, Milenović, & Veljković, 2008).

In this context, the objective of this study was to optimize and model the extraction process of bioactive compounds from PFSP (anthocyanins and phenolics contents) by studying the effect of three variables involved in the green ultrasound-assisted extraction, namely: time (min), temperature (°C) and solid: liquid ratio (m/v). The natural extract obtained may be a viable stable alternative for replacing synthetic colorants. A morphological study, using scanning electron microscopy, was carried out,

and the phenomenological kinetic modeling of the UAE and conventional extraction were also performed, using three different empirical equations.

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.

Plant material

Purple-fleshed sweet potatoes cultivated in Ribeirão Corrente, SP, Brazil (latitude: 20º27'25" south, longitude 47º35'25" west) were used. Tuberous roots were acquired previously selected, sanitized, frozen, packaged, and stored in a conventional freezer at -20 °C until the moment of analysis.

Design of experiments

Box-Behnken Design (BBD) was selected to optimize the UAE conditions for recovery of TA and TPC from PFSP. 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 yields 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:

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

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) (Baran et al., 2017; Ding, Zheng, Xia, Ren, & Kan, 2016; He et al., 2016).

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)	6.765	3.382
2	75 (+1)	30 (-1)	1:10 (0)	11.77	4.404
3	5 (-1)	70 (+1)	1:10 (0)	8.202	3.552
4	75 (+1)	70 (+1)	1:10 (0)	14.56	5.211
5	5 (-1)	50 (0)	1:5 (-1)	9.917	2.194
6	75 (+1)	50 (0)	1:5 (-1)	11.33	2.973
7	5 (-1)	50 (0)	1:15 (+1)	6.750	3.403
8	75 (+1)	50 (0)	1:15(+1)	16.42	5.387
9	40 (0)	30 (-1)	1:5(-1)	9.979	3.037
10	40 (0)	70 (+1)	1:5 (-1)	11.02	3.601
11	40 (0)	30 (-1)	1:15 (+1)	12.40	5.181
12	40 (0)	70 (+1)	1:15 (+1)	13.83	5.411
13	40 (0)	50 (0)	1:10 (0)	9.583	3.847
14	40 (0)	50 (0)	1:10 (0)	9.124	3.769
15	40 (0)	50 (0)	1:10 (0)	10.27	3.932
16	40 (0)	50 (0)	1:10 (0)	9.589	3.798
17	40 (0)	50 (0)	1:10 (0)	9.842	3.923

2.4. Green ultrasound-assisted extraction

The purple-fleshed sweet potato was ground in a conventional mixer (PMX600, Philco, Manaus, AM, 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).

An 800 W ultrasonic bath (Elmasonic TI-H-10, Elma, Singen, Germany), with internal dimensions (width/depth/height) of 30/24/15 (cm) and 8.6 L of tank operating capacity was used. Crude phenolic extracts were processed at a constant frequency

of 25 kHz, and at an ultrasonic power amplitude of 50%. The equipment has temperature adjustment control. 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).

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, 25, 50, 75, 100, 150, 200 e 250 min. Similarly, a study was conducted under conventional extraction conditions – to compare the kinetics of the two processes. After the extraction, the crude phenolic extracts obtained were filtered on Whatman no. 1 and the volume was adjusted to a standard volume with 70% v/v ethanol. Extracts were stored at -20 °C until further analysis.

The concentrations of the target compounds obtained in the kinetic curve were used to model the phenomenological kinetics of the extraction process. Modeling was based on film theory, the theory of unsteady diffusion through plant material, and

Ponomaryov's empirical equation (Veličković, Milenović, Ristić, & Veljković, 2006). The models used are shown in Table 2.

Table 2
Kinect models of bioactive substances extracted from plant material by solvent

Model	Kinetic equation	Linear transformation
Film theory	$\left(1-\frac{c}{c_{seq}}\right)=(1-b)e^{-kt}$	$\ln\left(1-\frac{c}{c_{seq}}\right)=\ln(1-b)-kt$
Theory of unsteady diffusion through plant material	$\frac{q}{q_0}=(1-b')e^{-k't}$	$\ln\left(\frac{q}{q_0}\right)=\ln(1-b')-k't$
Empirical equation of Ponomaryov	$1-\frac{q}{q_0}=b"+k"t$	

Notation: b, b' and b''- washing coefficient according to the film model, the unsteady diffusion model, and the empirical model of Ponomaryov, respectively, 1; c—concentration of TA or TPC in liquid extract during the extraction, mg/L; c_{seq}—maximum concentration of TA or TPC in saturated liquid extract at equilibrium, mg/L; k and k'—slow extraction coefficient according to the film model and the unsteady diffusion model, min^{-1}; k''—specific rate of slow extraction according to the empirical model of Ponomaryov, i.e., slope of the dependence of $(1 - q/q_0)$ versus time, min^{-1}; q—content of TA or TPC in plant material during the extraction, mg/100 g or mgGAE/100g, respectively; q_0— initial content of TA or TPC present in plant material, mg/100 g or gGAE/100g, respectively; and t—time, min.

The determination of the concentration of the target compound in the saturated liquid extract at equilibrium (c_{seq}) was based on the work of Veličković et al. (2008), with some modifications. First, 5 g of plant material in 75 mL of 70% v/v ethanol underwent an extraction cycle in the ultrasonic bath, under the optimized conditions of UAE. The liquid portion of the extract was obtained by vacuum filtration, and a new portion of plant material (5 g) was added, for a new extraction cycle to be run. A total of three extraction/filtration cycles were conducted for the content of the target compound to reach equilibrium. The c_{seq} values obtained for TA and TPC were 8.923 ±0.3678 and 10.151 ± 0.0271 mg/L, respectively.

The determination of the initial TA and TPC content in plant material (q_0) was also based on the work of Veličković et al. (2008), with some modifications. In this case, first, 5 g of plant material in 75 mL of 70% v/v ethanol underwent an extraction cycle in the ultrasonic bath, under the optimized conditions of UAE. The liquid portion of the extract was separated by vacuum filtration, and 50 mL of fresh solvent (2/3 of

the previous value) was added to the plant residue obtained, then a new extraction cycle could be run. A total of three extraction/filtration cycles were conducted for the initial content of the compound of interest to reach equilibrium. The q_0 values obtained for TA and TPC were 54.333 ±3.4318 and 12.547 ±0.1619 mg/100 g, respectively.

Analysis of total anthocyanins (TA)

The total anthocyanin content of purple-fleshed sweet potato extracts was determined by the methodology described by Fuleki & 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, Monza, Italy). The calculation of TA content in the extract was given directly by Eq. 2 and expressed in mg per 100 g of plant material (mg/100 g).

$$C = \frac{Abs \times MM}{\varepsilon \times b} \qquad (2)$$

where C is the concentration of anthocyanins in g/L; Abs is the absorbance read at 535 nm; MM is the molar mass in g/mol – in this case, cyanidin-3-glycoside MM 449.2 g/mol was used; ε is the molar absorptivity in a 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 &Rossi (1965). 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, Monza, Italy). The quantification of TPC was obtained through the standard curve of gallic acid (R^2 = 0.9944) and the results were expressed in mg of gallic acid equivalent per 100 g of sample (mg GAE / 100 g).

Total starch content

The total starch content, in the raw material, was determined according to method 76-13 (AACC, 2009), using the total starch assay kit (Megazyme International, Wicklow, Ireland). The total starch content found in PFSP was 10.872 ± 0.149 g/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 purple-fleshed sweet potato 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 addition, 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.

Scanning electron microscopy (SEM)

To better assess differences related to the extraction process, the micro-structures of untreated purple-fleshed sweet potato and after conventional and UAE were analyzed using a scanning electron microscope (JSM-6010 LA, Jeol, Tokyo, Japan). First, samples, fixed by carbon tape on metal support (stubs), were covered by a thin layer of gold using sputtering apparatus (Quorum Q150R, Quorum Technologies Ltd, East Sussex, UK). Then, samples were scanned with SEM under a partial vacuum at 10 kV.

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 ($p < 0.05$), and optimize the UAE process conditions through the desirability profile. All the results were expressed as mean ± standard deviation, and the significant differences between different conditions were obtained using analysis of variance (ANOVA) with $p < 0.05$.

Results and discussion

Optimization of green ultrasound-assisted extraction

Purple-fleshed sweet potato 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 6.750 to 16.424 mg/100 g, while the total phenolic content ranged from 2.194 to 5.411 mg GAE/100 g (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 3.

In the regression analysis for the TA response variable, with the aid of the Pareto chart (Table 3, Fig. 1 a), it was observed that the linear time (X_1) was the most significant effect on the model ($p < 0.01$), followed by the effect of the interaction of linear time with the linear volume of solid: liquid ratio (X_1X_3), the negative effect of quadratic volume of solid: liquid ratio (X_3^2), and the effect of linear temperature (X_2), both with $p < 0.05$. In addition, for the TPC response variable (Table 3, Fig. 1 b), the effect of the linear volume of solid: liquid ratio (X_3) and the effect of linear time (X_1) were the most significant for the model ($p < 0.01$); followed by the negative effect of quadratic temperature (X_2^2), the effect of the interaction of linear time with the linear volume of solid: liquid ratio (X_1X_3), the effect of quadratic time (X_1^2), and the effect of linear temperature (X_2), both with $p < 0.05$.

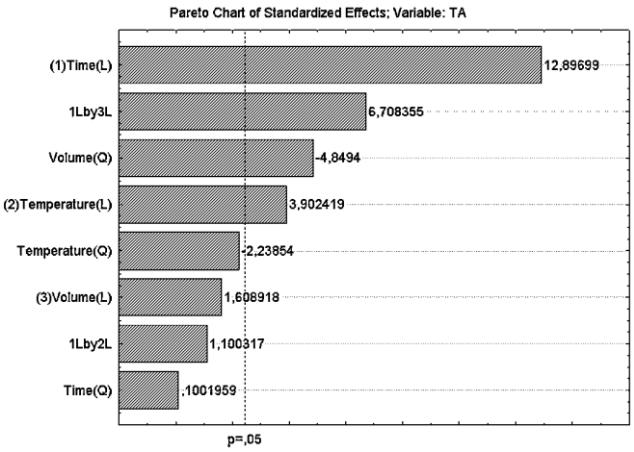
Table 3

BBD regression analysis for TA and TPC of purple-fleshed sweet potato

	TA (mg/ 100 g)		TPC (mg GAE/100 g)	
Variable	**Coefficient**	**p-value**	**Coefficient**	**p-value**
Constant	11.079	<0.0001*	3.9781	<0.0001*
X_1	5.6126	<0.0001*	1.3612	<0.0001*
X_2	1.9610	0.0079620*	0.38042	0.045752 *
X_3	0.80850	0.15876	1.7044	<0.0001*
X_1^2	0.03005	0.92345	0.26750	0.025284*
X_2^2	-0.67141	0.066481	-0.55085	0.00088800*
X_3^2	-1.4545	0.0028530 *	0.096971	0.32452
X_1X_2	0.67718	0.31336	0.31841	0.13678
X_1X_3	4.1286	0.00053300*	0.60264	0.017472*

X_1 – time (min); X_2 – temperature (°C); X_3 – volume of solid:liquid ratio (mL); *$p < 0.05$

a



b

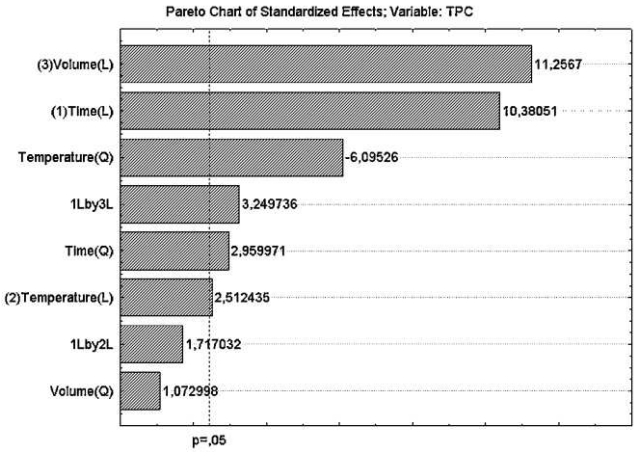


Fig. 1. Pareto Chart of the significance of the effects of the independent variables studied for the response variable (a) TA and (b) TPC; ($p < 0.05$) of purple-fleshed sweet potato

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 0.9779 and 0.9819 for TA and TPC variables, respectively. The high values of the coefficient of determination ($R^2 > 0.90$) show that the regression equations used for TA (Eq. 5) and TPC (Eq. 6) fitted 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 from PFSP and in predicting these contents.

$$Y_{TA} = 11.079 + 5.6126X_1 + 1.9610X_2 - 1.4545X_3^2 + 4.1286X_1X_3 \quad (5)$$

$$Y_{TPC} = 3.9781 + 1.3612X_1 + 0.380417X_2 + 1.7044X_3 + 0.26750X_1^2 - 0.55085X_2^2 + 0.60264X_1X_3 \quad (6)$$

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 both response variables TA and TPC (Fig. 2), the optimal combination of the studied variables was at a time of 75 min, at a temperature of 70 °C, and at the volume of solid: liquid ratio 1:15 m /v (which corresponds to 75 mL of 70% ethanol solvent (v/v), for 5 g of mashed PFSP). This combination predicts a TA content of 18.372 mg/100 g and a TPC content of 6.2237 mg GAE/100 g. On the validation of these optimized conditions, the experimental value of 18.822 ± 1.59 mg/100 g and 6.656 ± 0.50 mgGAE/ 100 g, for TA and TPC, respectively, were observed. These values are only approximately 3% higher for TA and 7% higher for TPC than those predicted by the desirability profile (Eq. 5 and 6).

The study shows that the extraction of TA and TPC increased as the three factors studied also increased. 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 (Fig. 2). Generally, in a solid-liquid extraction, the increase in temperature leads to greater recoveries of bioactive compounds (Dranca & Oroian, 2016). This effect can be attributed to the fact that, when the temperature is raised, the solubility and diffusion coefficients of the compounds that will be extracted from the plant matrix increase, as the solvent viscosity decreases, facilitating the mass transfer of the system (Goula, Ververi, Adamopoulou, & Kaderides, 2017).

The solid: liquid ratio m/v also proved to be an essential factor for increasing the recovery of both TA and TPC. In both cases, the best yield occurred at the highest level evaluated solid: liquid ratio, which was 1:15 m/v (Fig. 2). Increase in solvent volume can improve the efficiency 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).

Besides, the optimization of the extraction process was carried out by varying the time between 5 to 75 minutes. From desirability profiles (Fig. 2), it is observed that the effect of time was highly significant for the recovery of TA and TPC, both with the best extraction rate efficiency at 75 min. In the last decade, the efficiency of green ultrasound-assisted extraction has been extensively reported to increase extraction

rates of target compounds and decrease extraction time (Das & Eun, 2018; Dranca & Oroian, 2016; He et al., 2016; Rocha et al., 2017; Sang, Sang, Ma, Hou, & Li, 2017).

Usually, anthocyanins are highly sensitive to factors such as temperature, pH, oxygen, and water activity, which often limits their yield in the extraction process (He et al., 2016). Besides, when compared with conventional extraction, the cavitation and mechanical effects produced by ultrasound may destroy the structure of anthocyanins in UAE (Tan et al., 2022). Some studies have already investigated the greater stability of PFSP anthocyanins when compared to other sources such as strawberries, blueberries, cranberries, red cabbage, perilla, and others, and attributed it to several factors like the presence of: highly acylated anthocyanins such as cyanidin 3-sophoroside-5-glucoside and peonidin 3-sophoroside-5-glucoside (Torres, Aguilar-Osorio, Camacho, Basurto, & Navarro-Ocana, 2021), others polyphenols such as cinnamoyl quinic acids (Chamorro, Cueva-Mestanza, & de Pascual-Teresa, 2021), protein-bound anthocyanin compounds (Jiang et al., 2020) and copigmentation (Lv et al., 2022).

Another hypothesis for the greater stability of anthocyanins present in PFSP when compared to other sources of anthocyanins, may be due to the starch content (10.872 ± 0.149 g/100 g) present in this raw material. The starch present in vegetables is found in granular structures, as can be seen in Fig. 3, and formed by amylose and/or amylopectin molecules. The UAE of anthocyanins and phenolic compounds took place in a liquid and acidic medium (pH 2), at 70 °C, for 75 min. Under these conditions, the starch granules underwent gelatinization (irreversible swelling) with consequent disruption of some of these structures, which can release amylose and amylopectin molecules into the liquid medium. Under acidic conditions, starch molecules can undergo depolymerization, forming, for example, dextrins, which can be complex with anthocyanins and phenolic compounds, increasing their stability (Audilakshmi & Swarnalatha, 2018; Qin et al., 2021; Tutunchi, Roufegarinejad, Hamishehkar, & Alizadeh, 2019). Despite the need for more future in-depth studies on these mechanisms, due to their high stability, mainly thermal, the anthocyanins of PFSP are shown to be a great alternative for the production of natural colorants.

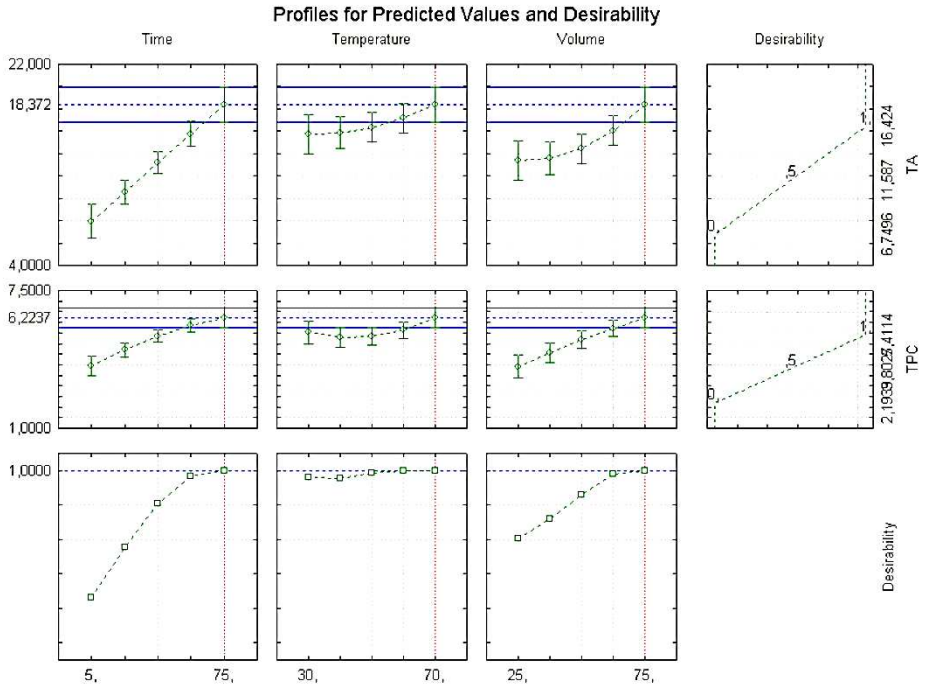


Fig. 2. Desirability profile of **(a)** TA (mg/ 100 g) and **(b)** TPC (mg GAE/ 100 g).

Optimized extraction compared to conventional

In the optimization of the UAE for both response variables TA and TPC, the optimal simultaneous combination of the studied variables occurs in a time of 75 min, at a temperature of 70 °C, and in a solid: liquid ratio of 1:15 m/v, while conventional extraction occurs at a temperature of 5 ± 1 °C; in solid: liquid ratio of 1:10 m/v; and time of 24 hours. The study shows that UAE significantly increased ($p < 0.05$) the extraction yield of TA with values of 18.822 ± 1.59 mg/100 g when compared to 10.396 ± 0.05 mg/100 g obtained by conventional extraction and extraction yield of TPC with values of 6.656 ± 0.50 mg GAE/100 g when compared with 3.481 ± 0.25 mg GAE/100 g obtained by conventional extraction. Thus, the UAE has proven to be an green efficient technology as it overcomes the limitations of the conventional technique, since UAE increased the recovery yield of TA by approximately 81% and TPC by approximately 91%, which corresponds to almost double when compared to the conventional extraction, highlighting the reduction in the processing time, from 24 hours in conventional extraction, to just 75 min in the UAE. The reduction in process time is important because it reduces energy consumption, in addition to enabling greater processing capacity for vegetable raw materials (S. Chemat, Lagha, AitAmar, Bartels, & Chemat, 2004).

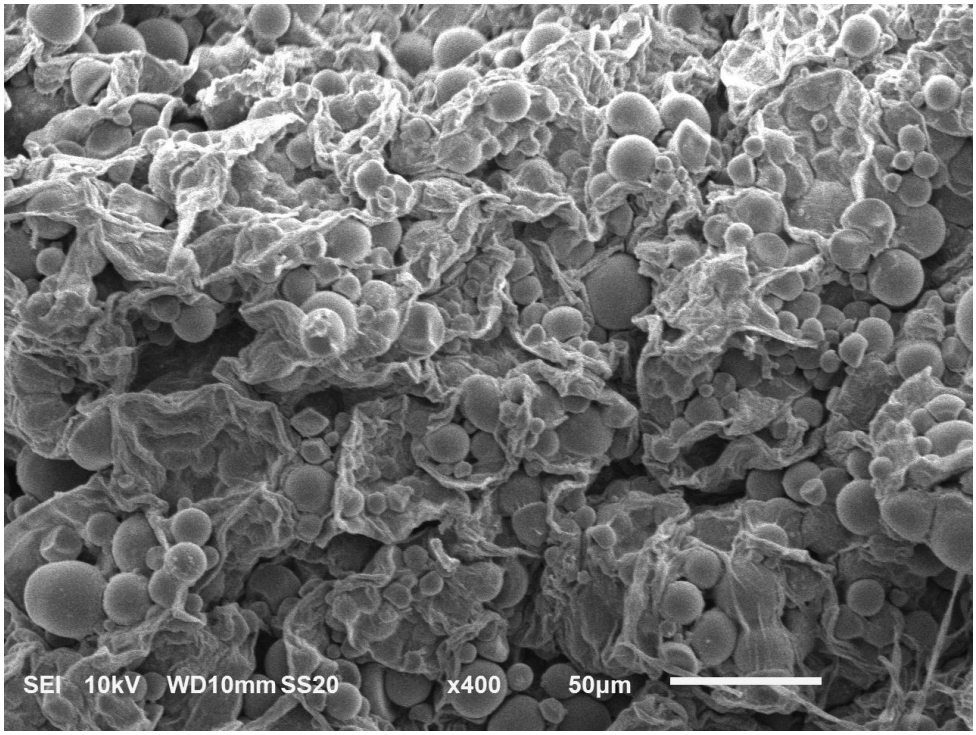
When compared to conventional extraction, the better performance of UAE can be attributed to the ultrasonic waves, by promoting the phenomenon of cavitation, contributing to better penetration of the solvent in the plant matrix, and increasing the mass transfer rates of the target compounds in the extracting solvent. Beyond that, by breaking the cell walls of the plant matrix, the ultrasonic power can increase the movement of the target compounds to the extracting solvent, and consequently, increases the yield of extraction of target compounds. Given the advantages of using ultrasound technology, and because it allows the scale-up to industrial levels, requires low initial investments, and adapts to green chemistry recommendations, the UAE has been standing out as a viable alternative for the extraction of anthocyanins and phenolic compounds from varied plant sources, including PFSP (Das & Eun, 2018; He et al., 2016; Yusoff, Mat Taher, Rahmat, & Chua, 2022).

Comparative study of scanning electron microscopy

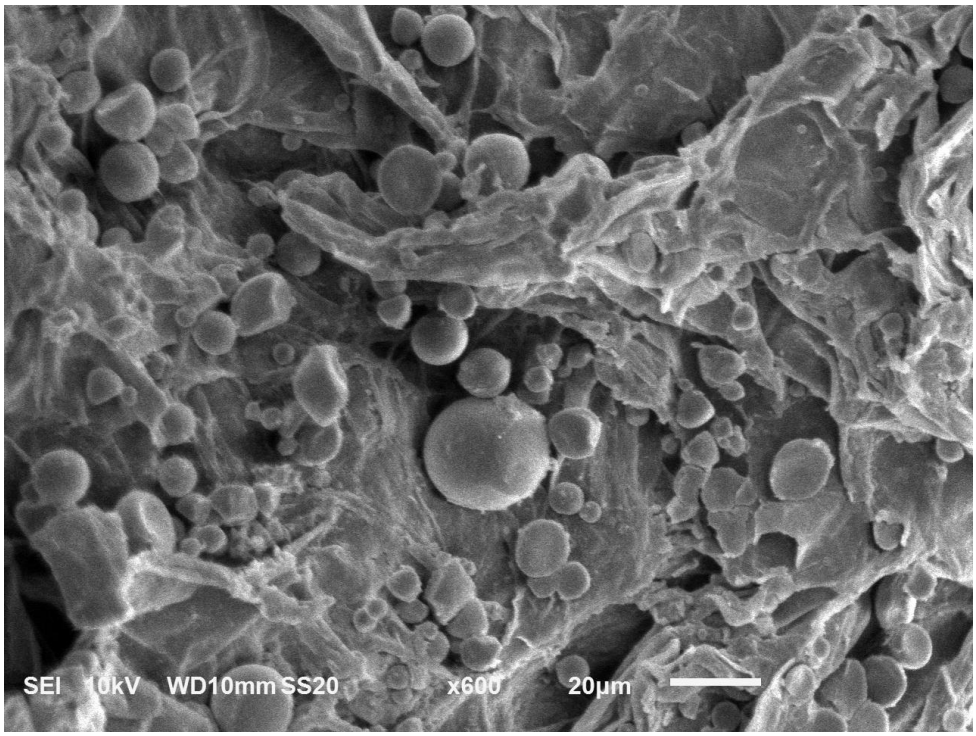
Nowadays, mechanisms behind the positive effects caused by the use of ultrasound in the extraction of bioactive compounds have been studied and are beginning to be elucidated. Chemat et al. (2017) reviewed the mechanisms of the UAE for natural products, which may include: fragmentation, erosion, capillarity, detexturation, and sonoporation. This means the single or combined effects of these mechanisms are what can increase ultrasonic efficiency through cell disruption and promote mass transfer in the extraction process. To observe the action of these mechanisms on the PFSP plant matrix, a scanning electron microscopy study was carried out. As can be seen, the untreated plant matrix has a mostly intact surface (Fig. 3 a), in addition, it is possible to observe the presence of a high amount of spherical starch granules (Yong et al., 2018). After conventional extraction (24 h under refrigeration at pH ~ 2), the plant matrix shows a high fragmentation (Fig. 3 b), which may be due to the long time exposed in an acidic medium, and a visible decrease in the number of starch granules. When compared to the ultrasonic extraction (Fig. 3 c), it is possible to observe a lower fragmentation, which may be due to the shorter extraction time, however, greater erosion, sonoporation, and detexturization of the surface of the plant material, and a great decrease in the presence of starch granules, which may have been broken after gelatinization (75 min at 70 °C). In this way, these different ultrasound mechanisms, along with the heating and acidic medium, contributed to maximizing the yields of extraction by increasing the surface area

contact between solvent and plant matrix components (Khadhraoui et al., 2018; Yusoff et al., 2022).

a



b



c

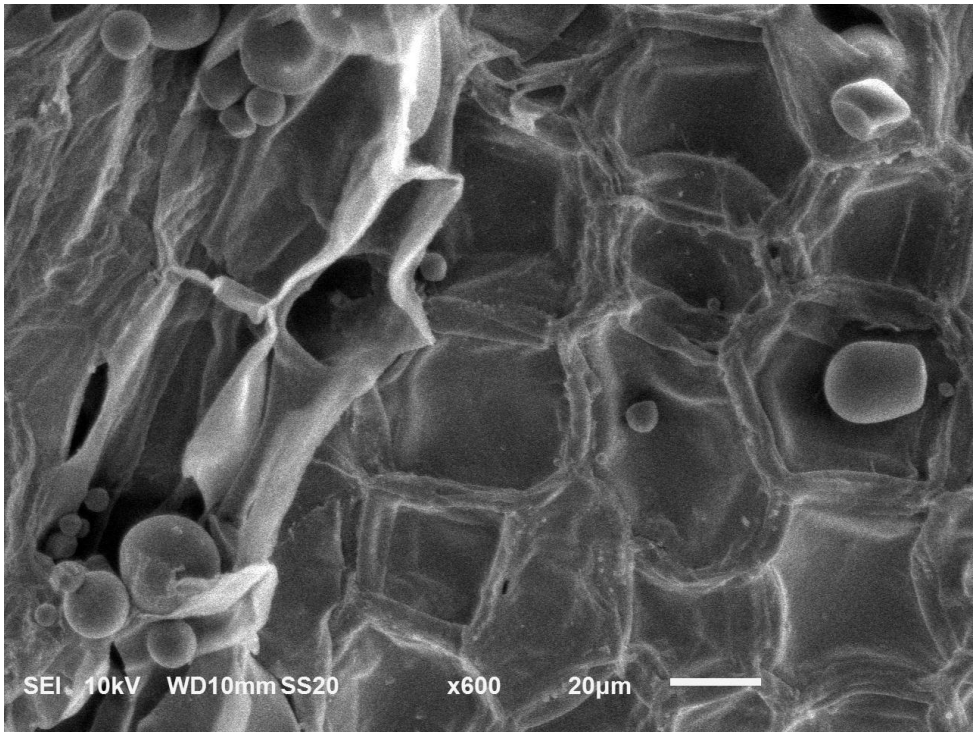


Fig. 3. Scanning electron micrograph of **(a)** untreated purple-fleshed sweet potato **(b)** conventional extraction **(c)** UAE.

Comparative study of processes kinetics

When observing the extraction yield of TA and TPC, as a function of time (min), comparing the UAE with the conventional extraction (Fig. 4), it is evident that the UAE method was more efficient than the conventional one in terms of both shortened time and increased yield. Furthermore, the curve for the UAE, for the TPC response variable, presents a better extraction efficiency near the first 75 minutes of the process, which corroborates the optimal time obtained in the optimization study for this compound. After this period, it observes certain linearity in the kinetic curve of TPC extraction. Regarding the extraction yield of TA as a function of time (min), an increase in TA extraction can be observed after 75 minutes of extraction, which may be due to the stabilization of anthocyanins promoted, among other hypotheses, by the presence of starch in the plant matrix. Besides, the greater slope of the curves at the beginning of the extraction process (Fig. 4), especially in the UAE ones, can be attributed to the fact that this process has two main stages: the first one, which is characterized by a fast mass transfer rate, 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(Goula et al., 2017; Veličković et al., 2006).

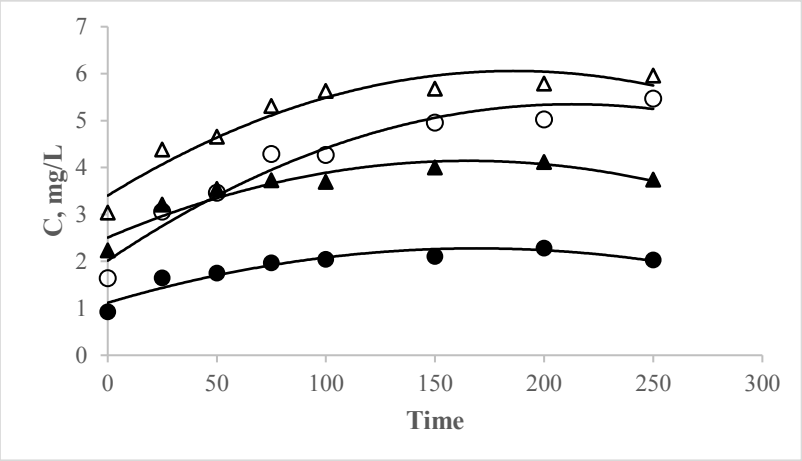


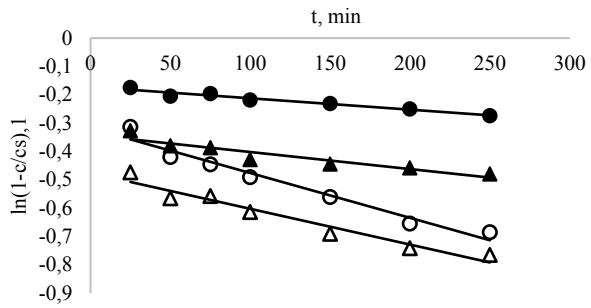
Fig. 4. Kinetics of extraction of TA (mg/100 g) and TPC (mg GAE/100 g) from purple-fleshed sweet potato as a function of time (min). TA: circles, TPC: triangles, UAE: open symbols, and conventional extraction: black symbols.

In addition to the kinetic study, the development of the UAE technique must be accompanied by the development of mathematical models, that describes the extraction process, through mathematical approximation. Veličković et al. (2006) modeled the extraction process of bioactive substances using the film theory, the unsteady diffusion through plant material, and the empirical equation of Ponomaryov (Table 2), in which the derivation of model equations can be found in Veljković & Milenović (2002). These models are two-parametric and all based on the two-stage extraction mechanism. One of these parameters characterizes the washing stage (b, washing coefficient) and the second one characterizes the slow extraction (k, slow extraction coefficient). It should be noted that 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 (Goula et al., 2017).

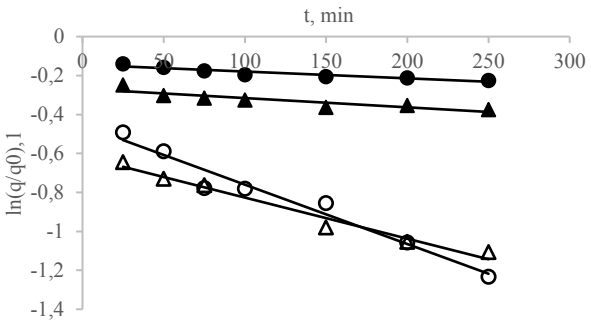
Fig. 5 illustrates the linearized forms of the corresponding basic kinetic equations given in Table 2. The parameters of the kinetic models were calculated from the experimental data using the linear regression method (Table 4, Fig. 5 a, b, and c), applying the linearized form of the proposed kinetic equations from Table 2. The criteria used to evaluate how well models represent the experimental data was the coefficient of determination (R^2), that in all cases, was higher than 0.90, which shows that the experimental data fit well with the proposed models. Values of kinetic parameters

depend on both the kinetic models, as expected, the type of bioactive compound, and the extraction process used, although the relationships cannot easily be seen as being too complex. When compared, the coefficients (b and k) obtained for UAE were superior to those obtained for conventional extraction. In general, the washing coefficient (b) has higher values than the slow diffusion coefficient (k). That’s because the first stage of extraction occurs the rapid transfer of soluble constituents, through washing, followed by the second stage, the slow diffusion one, in which the residual soluble constituents migrate to the extracting solvent slowly.

a



b



c

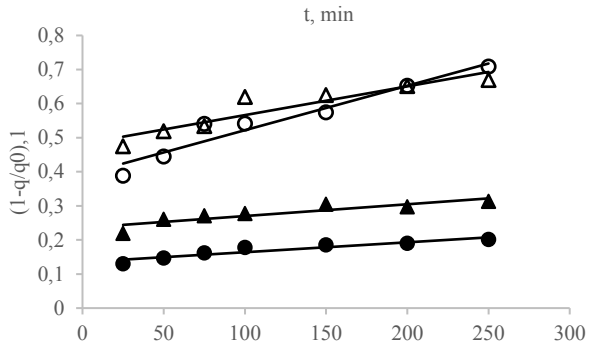


Fig. 5. Linearized form of the kinetic equations based on (a) film theory, (b) unsteady diffusion through plant material, and (c) Ponomaryov's empirical equation. TA: circles, TPC: triangles, UAE: open symbols, and conventional extraction: black symbols.

Table 4
Values of kinetic parameters

Model	Process	TA			TPC		
		R^2	k x 10^3 (min^{-1})	b (1)	R^2	k x 10^3 (min^{-1})	b (1)
Film theory	UAE	0.9619	1.5820	0.2723	0.9496	1.2690	0.3772
	Conventional	0.9540	0.4006	0.1583	0.9304	0.6186	0.2848
Unsteady diffusion	UAE	0.9624	3.0510	0.3656	0.9742	2.1100	0.4597
	Conventional	0.9410	0.3594	0.1312	0.9814	0.3412	0.2502
Ponomaryov's equation	UAE	0.9365	1.3040	0.3916	0.9599	0.8762	0.4691
	Conventional	0.9361	0.2989	0.1316	0.9878	0.2431	0.2209

Conclusion

BBD together with desirability profiles was successfully employed in the optimization of the studied variables for the extraction of TA and TPC from PFSP. Optimal values of time, temperature, and solid:liquid were combined simultaneously to predict the highest extraction yield of TA and TPC. Under optimized conditions, the yields predicted by the model and experimentally observed for TA and TPC differed by about 7%. The use of ultrasonic treatment promoted an increase of 81% and 91 % in the recovery of TA and TPC, respectively, compared to conventional extraction. Highlights the reduction of the processing time from 24 hours in the conventional extraction to 75 min under optimized conditions of the UAE.

Furthermore, mechanisms behind the positive effects caused by the use of ultrasound in the extraction of bioactive compounds could be observed by scanning electron microscopy, and three empirical models of solid-liquid extraction were well evaluated using the experimental data. All models tested were shown to be suitable to model the extraction of TA and TPC from PFSP under the operating conditions employed, which was proved by a high linear correlation coefficient ($R^2 > 0.90$). Thus, the study indicates that the use of green ultrasound-assisted extraction of bioactive compounds from PFSP is a promising, efficient and fast green technology, with low cost, being an alternative for the industrial production of natural colorants from this source.

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Conflict of interests

The authors declare that they have no conflict of interest.

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5. GENERAL CONCLUSIONS AND PERSPECTIVES

In the optimization of ultrasound-assisted extraction of bioactive compounds from raspberries (Paper 1), the best process temperature was 70 °C, in the solid:liquid ratio of 1:12.5 m/v, both for anthocyanins and for phenolic compounds. While the best extraction time was 22.5 °C for anthocyanins, and 57.5 °C for phenolic compounds. In validation of these optimized conditions, less than 5% difference was found between the predicted values and the experimental values. In this study, the ultrasonic treatment, under the optimized conditions, increased the recovery of anthocyanins by 18.28% and of phenolic compounds by 28.88%, when compared to conventional extraction. Furthermore, from the kinetic curves of the optimized UAE and the conventional extraction, it was possible to adjust the mathematical model of Film Theory. In this first study, the experimental methodology to adjust the mathematical models began to be developed and adapted in the laboratory, and at this point in the research it was not possible to adjust the other mathematical models proposed in the research project.

In the optimization of the ultrasound-assisted extraction of bioactive compounds from purple tomato (Paper 2), only the recovery of anthocyanins was studied, as the content of phenolic compounds in this raw material was very low. However, two stages of purple tomato maturation (low-ripened and high-ripened) were studied. The best simultaneous condition of the variables studied in the process, for low-ripened purple tomatoes was at a time of 75 min, at a temperature of 40 °C and solid:liquid ratio of 1:15 m/v, for high-ripened purple tomatoes the ideal condition occurred at a time of 40 min, temperature of 50 °C and solid:liquid ratio of 1:15 m/v. In validation of these optimized conditions, less than 3% difference was found between the predicted values and the experimental values. Furthermore, when comparing optimized UAE with conventional extraction, an increase in anthocyanin recovery of 73% for low-ripened purple tomatoes and 54% for high-ripened purple tomatoes was observed. In this second study, because the raw material was obtained in small quantities: 462 g for low-ripened purple tomatoes (~94% moisture) and 838 g for high-ripened purple tomatoes (~93% moisture), it was not possible to continue the studies of kinetics and mathematical modeling.

In the study of the optimization of the ultrasound-assisted extraction of bioactive compounds from purple sweet potato (Paper 3), the optimal condition for the extraction

for both anthocyanins and phenolic compounds occurs in a time of 75 min, at a temperature of 70 °C, and in solid:liquid ratio 1:15 m/v. In validation of these optimized conditions, only about 7% difference was observed between the predicted values and those obtained experimentally. When comparing optimized UAE with conventional extraction, ultrasonic treatment increased the recovery of anthocyanins by 81% and phenolic compounds by 91%. In this work, through a better understanding and adaptation of experimental methodologies for the mathematical modeling of the process, both in a laboratory bench and in experience with the equations, it was possible, through the kinetic curves of the processes, to adjust the three proposed mathematical models. The purple sweet potato, among the three raw materials studied, was the one with the highest thermal stability over the process time. This data should be used for further research in the field of extraction of natural colorants, since high stability is an essential property to apply a compound to another food matrix.

In view of these three studies, it becomes evident that ultrasound-assisted extraction is a promising, efficient and fast green technology, with relatively low initial cost, being an alternative for the industrial extraction of bioactive compounds from different plant sources. As future considerations, the best adaptation of ultrasound-assisted extraction of bioactive compounds in pilot and industrial scale is still necessary.