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FOOD TECHNOLOGY

Optimization of ultrasound-assisted extraction of anthocyanins from purple tomatoes

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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 reported 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 indicated that the UAE is an efficient technology for recovering bioactive compounds from purple tomatoes.
Key words: ultrasonic extraction · optimization · anthocyanins · purple tomatoes.

Otimização da extração assistida por ultrassom de antocianinas de tomates roxos

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 et al., 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 et al., 2018).

Recently, genetic engineering in search to induce and enhance the biosynthesis of anthocyanins in plants has developed a line of transgenic tomatoes

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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 reported 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 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, et al., 2022). The ultrasonic enhancement of the extraction is attributed to cell destruction, capillary effects, better solvent penetration, and mass transfer intensification (CHEMAT et al., 2017; CHEMAT et al., 2011; O'DONNELL et al., 2010).

Furthermore, several studies showed 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 et al., 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.

MATERIALS 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 et al., 2019b; SANG 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 ($i \neq j$) (DING et al., 2016; HE et al., 2016; BARAN et al., 2017; JIANG et al., 2019a).

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

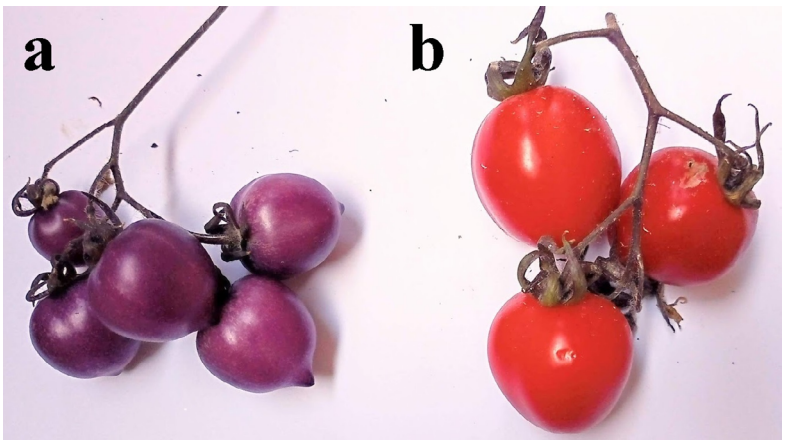

Figure 1 - Stages of ripening of tomatoes (a) low-ripened (purple color) and (b) high-ripened (purple-red color).

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 reported 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. The equipment has a

Table 1 - Box-Behnken Design 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

temperature control, the bath temperature control was validated by an external thermometer. 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.

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

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.

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

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 was 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 ($R^2 > 0.90$) showed 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 2a), 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 2b), 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 et al., 2017). In this research, the extraction process was carried out by varying the time between 5 to 75 minutes. From

Table 2 - Box-Behnken Design for 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); * $P < 0.05$; ** $P < 0.001$.

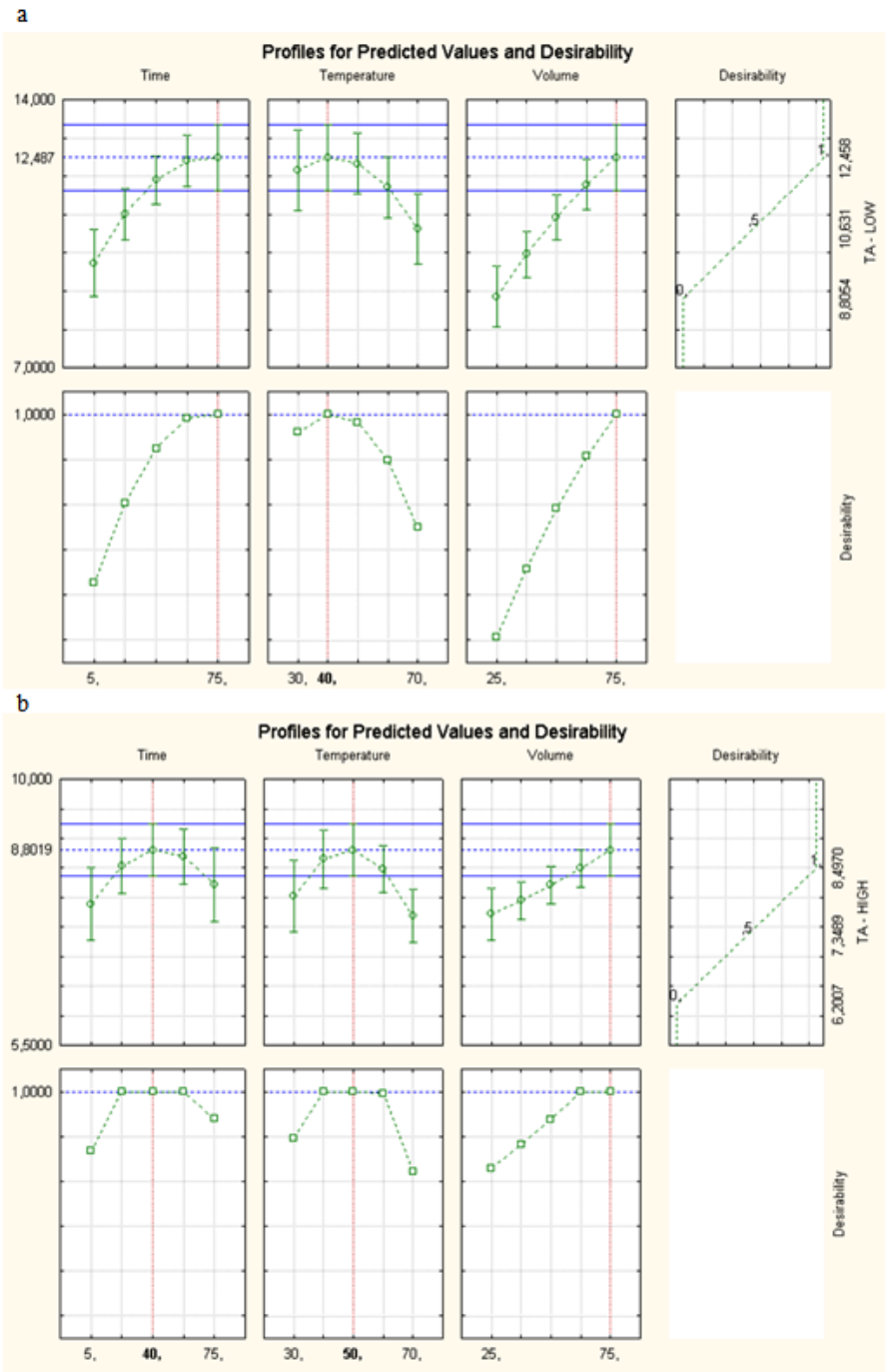

Figure 2 - Desirability profiles of total anthocyanins (TA) content (mg/ 100g) for (a) low-ripened and (b) high-ripened purple tomatoes.

desirability profiles (Figure 2a 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 et al., 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 (Figure 2a 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 (Figure 2a and b). Increasing the solid: liquid ratio can improve the efficiency of the process by facilitating the access of the solute to the solvent. Furthermore. the solvent is the liquid medium in which ultrasonic acoustic cavitation occurs: a phenomenon that produces a series of mechanical effects, such as particle collision and cell disintegration. Consequently, larger volumes of solvent can help the occurrence of these phenomena (HE et al., 2016).

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 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.

Conversely, 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 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 & 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 (Figure 3a). After conventional extraction (24 h under refrigeration at pH ~ 2), the plant matrix shows some level of fragmentation and erosion (Figure 3b), 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 (Figure 3c). 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

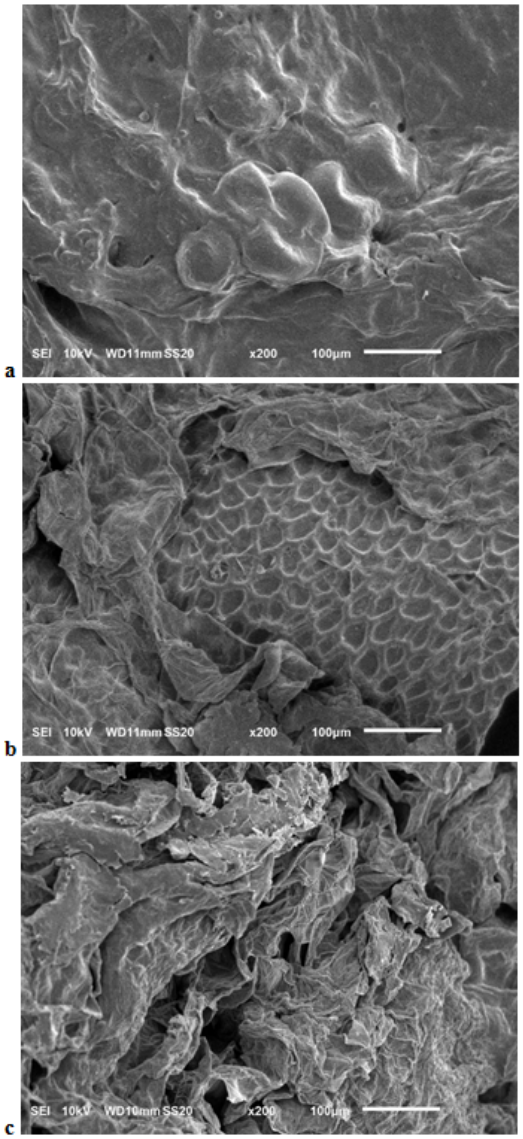

Figure 3 - Scanning electron micrographs of (a) untreated high-ripened purple tomato, after (b) conventional extraction, and (c) UAE (40 min at 50 °C).

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 indicated 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

BAT designed and conducted the experiments, analyzed the results, drafted and revised the manuscript. MCTRV analyzed the results and revised the manuscript. PCS designed the experiment, provided laboratory support, and revised the manuscript.

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