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Ultrasound-assisted pressurized liquid extraction of anthocyanins from *Aronia melanocarpa* pomace

Thalles Allan Andrade [a], Fabiane Hamerski [b], Damian Estuardo López Fetzer [b], Maria Cinta Roda-Serrat [a], Marcos Lúcio Corazza [b], Birgir Norddahl [a], Massimiliano Errico [a,*]

[a] Department of Green Technology, Faculty of Engineering, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark
[b] Department of Chemical Engineering, Federal University of Paraná, Francisco H. Santos Av. 100, 81531-990 Curitiba, PR, Brazil

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ABSTRACT

This work aimed to investigate the optimum conditions of pressure, temperature, ultrasound intensity, solvent concentration and solvent flowrate of ultrasound-assisted pressurized liquid extraction of anthocyanins from *Aronia melanocarpa* pomace. The novel setup operates with an aqueous solution of citric acid as extraction solvent. The optimization was performed in order to maximize the total anthocyanins content extracted from the pomace. The optimized conditions of 70 °C, 180 bar, a solvent concentration of 1.5 % wt. citric acid, in a 200 W ultrasound bath were obtained. These conditions resulted in 88.0 % wt. extraction of anthocyanins in 45 min. A kinetic study was performed to study the influence of the temperature on the total anthocyanin yield obtained at the optimal extraction conditions.

Moreover, the component stability was assessed to prove that the system could successfully operate at 80 °C. Compared to classical batch extraction using the same solvent concentration and feedstock, the total anthocyanin content of the extract was increased by 19%.

1. Introduction

Aronia melanocarpa berries, common name black chokeberries, are originally from the United States and Canada. Black chokeberry has been introduced and cultivated in different parts of Europe [1] and has been recently expanded to Asia [2].

Aronia melanocarpa is well-known among health-conscious shoppers. However, it is rarely consumed as fresh fruit due to its bitter and astringent taste. Conversely, it reaches the market in different forms like juice, jam, herbal tea, puree, soft spreads, food colorants, or ornamental plants [3–5]. This berry has been appreciated by consumers primarily for its nutritional benefits such as high content of dietary fiber, vitamins, and essential minerals. Furthermore, many studies have demonstrated that chokeberry contains, high levels of bioactive phenolic compounds, like anthocyanins, phenolic acids, flavonols, and procyanidins [6,7].

Anthocyanins are important pigments of vascular plants and are responsible for the shiny orange, pink, red, and blue colors in some flowers and fruits [8]. Additionally, their antioxidant activity plays an important role in the prevention of some diseases such as diabetes, neuronal cancer, cardiovascular illnesses, inflammatory and immunomodulatory effects [5,9,10].

As anthocyanin sources, chokeberry extracts are, therefore, attractive candidates to food producers and consumers, contributing to the creation of a superfood market globally quantified in 137 USD billion in 2018 [11].

Solid-liquid extraction or leaching is by far the most common technique in the recovery of plant-derived chemicals [12]. Extracts can be produced directly from the berries or wastes like stems, leaves, and pomace [13–15]. Oszmianski and Wojdylo [16] compared the content of phenolics in berries, juice, and pomace while Vagiri and Jensen [17] studied the influence of blanching, freezing, maceration temperatures, and enzyme treatments before juice pressing on the yield and anthocyanin composition of juice and pomace. In both studies, it was highlighted that chokeberry wastes have a high total phenolic content and antioxidant activity. In the present study, in order to develop an integrated process, oriented to waste valorization, chokeberry pomace was used as feedstock. This approach aims at promoting process diversification allowing juice producers to reach the market with different formulations and increase their competitiveness. Once the feedstock is characterized, the selection of the extraction method, solvent, and

* Corresponding author.
E-mail address: maer@igt.sdu.dk (M. Errico).

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operating conditions emerges as a fundamental step in defining an economically feasible process.

Ideally, the extraction method should:

- be based on non-toxic solvents with low vapor pressure and low production cost
- use inert solvents concerning the target components
- assure a high extraction yield
- operates at a high feedstock-to-solvent ratio

In this study, anthocyanins have been selected as target components due to their possible application as natural colors with health-promoting properties that can replace synthetic colorants [17]. Due to their polarity, anthocyanins are expected to show higher extraction efficiencies in polar solvents [18]. Moreover, because of their high reactivity, the choice of the solvent appears crucial in obtaining a stable form.

In aqueous solutions at a pH lower than 3, the anthocyanins are red and more stable, while the colorless form dominates at a pH greater than 4 [19]. Taking into account polarity and stability, methanol, ethanol, acetonitrile, and in general organic solvents containing hydrochloric, phosphoric, formic, or citric acid have been considered [18]. Aqueous citric acid was proved to be an effective solvent for the extraction of anthocyanins from chokeberry pomace [20]. Citric acid solutions in water are a green and cheap alternative to organic solvents. The recovery of citric acid from the final product also appears to be less critical than other solvents or acidity modifiers since citric acid is a safe ingredient that occurs in the metabolism of living organisms [21]. For these reasons, the citric acid aqueous solution was selected as a solvent in this study. Solvent extraction surely represents a simple operation that can be easily extended to an industrial level. However, this simplicity comes with the necessity to operate with high amounts of solvents and the high cost of the subsequent separation step. For this reason, non-conventional methods are starting to emerge as alternatives to compensate or partially reduce the drawbacks of conventional solvent extraction [22].

Different studies have been focusing on environmentally friendly alternatives that can enhance the performance of conventional extraction methods. This is the case of microwave-assisted [23], ultrasound-assisted extraction [24–29], enzyme-assisted extraction [30]. Another approach is to use alternative methods based on supercritical fluid extraction [31], or subcritical water extraction [32]. In this case, the use of lower temperatures allows the preservation of the target compounds. Wilkes et al. [33] reported that when chokeberry juice was subjected to temperatures about 90 °C, 93% of the anthocyanins were degraded. An intermediate case concerning supercritical extraction is the use of high-pressure solvents. Pressurized liquid extraction (PLE) is classified as an environmentally friendly technique since it generates small volumes of waste and it can reduce costs and time [34]. The methodology is based on solvents below their critical point in order to keep the liquid phase during the extraction. The conditions of pressure and temperature are chosen to enhance the mass transfer rates by decreasing the solvent surface tension and viscosity and increasing the solubility of the components. This allows the solvent to penetrate easier and deeper into the solid matrix being extracted [35].

In the present study, a novel continuous extraction system for the recovery of anthocyanins from *Aronia melanocarpa* pomace is proposed. Ultrasound extraction was selected as a complementary method for PLE. It is expected that the use of ultrasounds promotes cell wall disruption allowing an easier release of the anthocyanins [36].

Temperature, pressure, solvent flow, and concentration, ultrasound power output, were optimized concerning the anthocyanins extraction yield and the results compared to the classical batch extraction procedure.

2. Materials and methods

2.1. Materials

The reagents used in the study are citric acid 99.5% purity (Dinâmica - Indaiatuba, Brazil), hydrochloric acid 37% purity (Vetec – São Paulo, Brazil), potassium chloride 99.5% purity (Vetec – Duque de Caxias, Brazil), sodium acetate trihydrate 99.0% purity (Neon – Suzano, Brazil), methanol 99.9% purity (Panreac – Barcelona, Spain), acetonitrile 99.9% purity (VWR Prolabo - Søborg, Denmark), trifluoroacetic acid 99.8% purity (Sigma Aldrich - Søborg, Denmark).

2.2. Sample preparation

Aronia melanocarpa (black chokeberry) pomace was provided by the juice producer Elkærholm (Egtved, Denmark) in November 2017 and it was initially stored at −20 °C. The pomace was freeze-dried (Christ Alpha 1 2 LDplus, Buch & Holm, Denmark). The ice condenser temperature at the freeze dryer was −55 °C and the chamber pressure was reduced to 0.043 bar. Dry matter content was determined by drying the freeze-dried chokeberry pomace in an oven at 105 °C for 24 h. The freeze-dried pomace was milled, and the average particle size was estimated using Tyler test sieves in a vertical vibratory sieve shaker (Bertel, Indústria Metalúrgica Ltd., São Paulo, Brazil) for 15 min. The sieves used in the vibratory shaker had the following opening sizes: 2.38, 1.19, 0.85, 0.50, and 0.42 mm. The mean particle diameter of the collected fractions was determined by the methodology of Gomide [37]. The particles retained in the sieves were afterward mixed for homogeneity, whereas all particles larger or finer than the sieves used were discarded.

2.3. Exhaustive anthocyanin extraction

To determine the total monomeric anthocyanin content that can be extracted from the dried *Aronia melanocarpa* pomace, samples were submitted to exhaustive extraction in triplicate, following the procedure proposed by Vagiri and Jensen [17] with some modifications. Chokeberry pomace (0.25 g) was first extracted with 7.5 mL of methanol/ water/ hydrochloric acid solution (75:25:1; v/v) and sonicated for 10 min. The extract was separated by centrifugation (2000 rpm, 5 min) at room temperature, and the residue was submitted to five new extraction procedures. All extracted solutions were pooled into a volumetric flask of 50 mL, and distilled water was used to make up the volume. The total anthocyanin content (TAC) of the solution was taken as a reference to compare the TAC obtained in the proposed extraction method.

2.4. Experimental design of the pressurized liquid extraction (PLE)

The schematic experimental design is illustrated in Fig. 1. Pressurized liquid extraction was carried out in an AISI 316 stainless steel column (200 × 10 mm) with a 15.7 mL internal volume. 3.5 g of milled chokeberry pomace was loaded to the column. Stainless steel filters were placed in the extractor inlet and outlet to retain the pomace inside the column and prevent the system from clogging. The extraction column was horizontally fixed, 5 cm above the sonotrode, in a thermostatic water bath with ultrasound generation (Q5.9/37A Eco-Sonics, Brazil). The ultrasound bath, with 5.9 L of volume capacity, was operated at a frequency of 37 Hz and tolerates a maximum temperature of 80 °C, a maximum power output of 200 W.

PLE took place with the pomace fixated inside the column and with a continuous flux of solvent through it. The feed tank contained the citric acid solution used as an extraction solvent. A Solvent Delivery Unit (LC 20AT, Shimadzu, United States) was used to pump the feed solvent to the extraction column at a constant flow rate. A heating coil immersed in the thermostatic bath ensured the solvent reached the temperature of the system. A back-pressure regulator valve (KPB 1S0A425P20000,

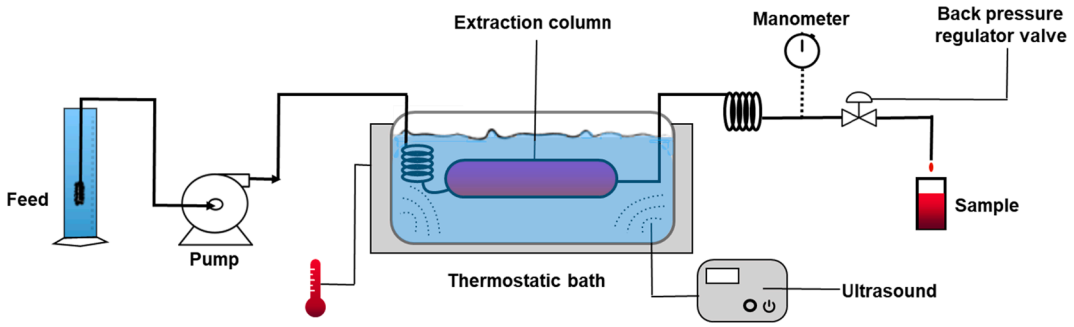

Fig. 1. Schematic design of the experimental set-up used for the ultrasound-assisted pressurized liquid extraction.

Swagelok) was used to control the pressure of the system. The extracted solution was cooled down to room temperature. Samples were collected at 2.5, 5, 7.5, 10, 20, 30, and 45 min.

Pressurized liquid extraction was initially assessed at a solvent flow rate of 5.0 mL min^{-1} that resulted in an average residence time of 77 s. The citric acid concentration was 1.5 wt%, according to the extraction optimization previously evaluated by Roda-Serrat et al. [20]. Extractions were carried out at pressures of 20, 100, and 180 bar, extraction temperatures of 30, 50, and 70 °C, and ultrasound power outputs of 0, 100, and 200 W. Furthermore, the solvent flow rate and citric acid concentration were evaluated separately to obtain a maximum anthocyanin extraction from the black chokeberry pomace.

2.5. Determination of the total anthocyanin content (TAC) and anthocyanin profile

The extract obtained was analyzed for TAC determination by the pH differential method based on the procedures described by Giusti and Wrolstad [38] and Lee et al. [39]. The extracts were diluted in buffers of potassium chloride (0.025 mol L^{-1}, pH 1.0) and sodium acetate (0.4 mol L^{-1}, pH 4.5). The dilutions were prepared respecting the maximum volume of 20% of the sample to 80% of the buffer solution. The accuracy of the results can be affected by the accuracy of the measurements in volume-basis. Therefore, to minimize possible analytical uncertainties, all anthocyanin analysis were performed in an acclimatized laboratory at 25 °C, using glassware and calibrated pipettes.

The absorbance (A) was measured in a spectrophotometer (GTA97, Global Analyzer, Brazil) at 520 and 700 nm, 15 min after the solution preparation. The anthocyanin pigment concentration was expressed as milligrams of cyanidin-3-glucoside equivalents (CGE) per liter of extract (mg L^{-1}) as shown in Equation (1).

$$Anthocyanin\ pigment\ (\text{mg L}^{-1}) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad (1)$$

where $A = (A_{520nm} - A_{700nm})_{\text{pH 1.0}} - (A_{520nm} - A_{700nm})_{\text{pH 4.5}}$; MW (molecular weight) = 449.2 g mol^{-1}; DF = dilution factor; 1000 = factor for conversion from g to mg; ε = molar extinction coefficient: 26,900 mol $L^{-1}cm^{-1}$; and 1 = optical path of cuvette (cm).

The total anthocyanin content (%), also referred to as extraction yield, is calculated as the ratio between the TAC at a specific time and conditions and the maximum TAC obtained from the exhaustive extraction.

The different anthocyanins in the extracts were identified by high-performance liquid chromatography (HP1200, Agilent Technologies Aps, Nærum, Denmark). The column was a reverse-phase Gemini 5µ C18 110A, 250 × 4.6 mm i.d. (Phenomenex Aps – Værlose, Denmark), and the eluent was a gradient of 0.05% trifluoroacetic acid in water (solvent A) and 0.05% trifluoroacetic acid in acetonitrile (solvent B). The flow rate was 1 mL/min and the composition varied as follows: 0–1 min, 1–10 %B; 1–20 min, 10–20 %B; 20–24 min, 20–24 %B, 24–30 min, 24–100 %B, 30–32 min, 100 %B; 32–33 min, 100–1 %B; 33–35 min, 1 % B. Detection was performed by a photodiode array detector recording at a wavelength of 520 nm.

2.6. Kinetic extraction of anthocyanins

The influence of the temperature on the total anthocyanin content extracted was evaluated using the kinetic study proposed by Peleg [40] for the description of sorption processes. This method has been used to evaluate the solid–liquid extraction of total polyphenols from grape seeds [41], chokeberry pomace [42], and chicory grounds, assisted by ultrasound [43]. In the present study, pressurized liquid extraction was carried out for 45 min at different temperatures using the optimal extraction conditions. The extraction temperatures were 30, 40, 50, 60, 70, and 80 °C.

According to the modified Peleg's model, when the initial concentration of anthocyanins in the solvent is equal to zero, the extraction yield of anthocyanins is given by:

$$TAC(t) = \frac{t}{\frac{1}{K_1} + \frac{1}{K_2}t} \quad (2)$$

where $TAC(t)$ is expressed as mg g^{-1}DW (dry weight), t is the extraction time, given in min, K_1 is Peleg's extraction rate constant (mg g^{-1}DW min^{-1}) and K_2 is the Peleg's extraction capacity constant (mg g^{-1}DW).

From the kinetic parameters obtained at different temperatures, the temperature dependence of the extraction rate constants was established employing the Arrhenius equation, given by:

$$K_i = A\exp - \frac{E_a}{RT} \quad (3)$$

where E_a (kJ mol^{-1}) is the activation energy, R is the universal gas constant, A (mg g^{-1}DW min^{-1}) is the pre-exponential factor and T is the temperature of the extraction.

2.7. Statistical method

Statistica v.13.5 (TIBCO Software Inc.) was used for the data analysis. Response surface methodology was applied to evaluate the effect of the temperature, pressure, and ultrasound power on the total anthocyanin content. The Central Composite Design method was used for the experimental design, consisted of three factors at two different levels with a central point.

The parameters K_1 and K_2were fitted using the supplement Solver in Microsoft Excel. The parameters determination was based on the experimental data by using nonlinear regression and minimizing the root-mean-square deviation of the calculated total anthocyanin content from the experimental data.

3. Results and discussion

3.1. Pomace characterization

The freeze-drying step removed water from the black chokeberry pomace corresponding to 57% of its initial weight. After this step, it was found that the dry matter content in the pomace was about 94.4%. Granulometry and homogenization of the material resulted in pomace particles with an average diameter of 1.73 ± 0.03 mm.

The exhaustive extraction results showed that the total monomeric anthocyanin content in the black chokeberry pomace was 48.6 ± 0.4 mg CGE g^{-1} DW. This value is higher than the TAC present in the pomaces of cherry (0.065 mg CGE g^{-1} DW) [44], *Myrciaria cauliflora* (3.92 mg CGE g^{-1} DW) [45], red grape (3.94 – 11.22 mg malvidin-3-glucoside g^{-1} DW) [46] and blackberry (8.43 – 17.3 mg CGE g^{-1} DW) [47]. The significantly higher content of anthocyanins in chokeberries compared to other fruits justifies the investigation of this bio-waste for the extraction of natural colorants [48,49]).

3.2. Effect of the ultrasound-assisted PLE parameters on the anthocyanin extraction yield

The influence of the extraction parameters temperature, pressure, ultrasound power output, citric acid concentration, and solvent flow rate were investigated to maximize the TAC extracted from the chokeberry pomace.

The TAC (%) measured during 45 min extraction at constant citric acid solution concentration and flow rate is shown in Fig. 2. Extractions at 70 °C resulted in higher anthocyanin yields compared to 30 °C due to enhanced diffusion and mass transfer. Besides that, extractions at 30 °C (Fig. 2a) could not reach the maximum extraction in 45 min. Conversely, using an extraction temperature of 70 °C (Fig. 2b) resulted in a higher extraction yield due to the increase in the diffusion coefficient and, accordingly, in the diffusion rate of anthocyanins to the solvent. Besides, the maximum extraction was reached before 45 min. While an extraction yield of around 83–86% was achieved after 30 min extraction at 70 °C, a small and linear increase of a maximum of 2% was observed for all these extractions at 45 min. It is also evident that the ultrasound power output has a more pronounced effect at the lowest temperature. This result is in agreement with Galvan ĎAlessandtro et al. [28] that studied ultrasound-assisted extraction of *Aronia* pomace in classical batch solvent extraction using a mixture of ethanol and water as a solvent [42].

In that way, extractions at high temperatures would also require lower extraction time, which can also result in a decrease in the energy consumed by the ultrasound system during the extraction.

Response surface plots reporting the effect of the temperature, pressure and ultrasound power output on the TAC (%) are shown in Fig. 3. The plots were built using the fitted quadratic polynomial equations obtained from the regression analysis. This regression includes the linear, quadratic, and interaction effects of the parameters in the TAC. The terms that presented significant influence ($p < 0.05$) in the extraction yield were the linear and quadratic effects of temperature, the linear effect of the power, and the interaction effect temperature-power output. The influence of the extraction pressure was less significant than the other conditions on the TAC. Additional information regarding the statistical analysis is reported in the Supplementary Material.

Similar to the observations with temperature, higher extraction yields were also obtained at a higher pressure and power output. While the effect of the pressure was more prominent at lower temperatures, higher extraction pressure ensures the wetting of the solid material and minimizes solvent evaporation.

Sonication had a positive effect on the anthocyanin extraction by facilitating and enhancing the mass transfer of anthocyanins from the pomace to the solvent [42,50]. Also, the use of ultrasounds improved the fluid dynamic along the extraction column. As the effect of the pressure, the effect of sonication was more pronounced at low temperatures. A similar positive effect of ultrasound-assisted extraction was observed for the extraction of anthocyanins from red cabbage [51], grape skin [52], and blueberry [50].

Based on this optimization, pressurized liquid extraction at 70 °C, 180 bar, in an ultrasound bath with a power of 200 W resulted in the highest anthocyanin extraction from *Aronia melanocarpa* pomace, extracting around 88.0% anthocyanins, in weight, from the pomace in 45 min.

The optimization was further improved by exploring the influence of the solvent flowrate and the citric acid concentration.

3.2.1. Effect of the solvent flowrate on the anthocyanin extraction yield

When the system is operated at a flow rate of 5 mL min^{-1}, a total volume of 225 mL of solvent passes through the extraction column, resulting in a solvent-to-pomace ratio of about 64 g/g DW. Taking this into consideration, the effect of the solvent flow rate on the TAC extracted was evaluated. Fig. 4 shows the extraction yield obtained when the flow rate was varied from 3 to 4, 5, 6, 7 mL min^{-1} while keeping the same extraction time and solvent-to-pomace ratio. The time-based TAC refers to total anthocyanin content extracted after 45 min. Meanwhile, volume-based TAC indicates the total anthocyanin content extracted after a volume of 225 mL of citric acid solution passes through the column. This comparison was included because, at different flow rates, different amounts of the solvent will run into the pomace.

The increase in the solvent flow rate from 3 to 5 mL min^{-1} resulted in a higher percentage of anthocyanins extracted. The higher solvent flow rate enhanced the driving force for the mass transfer, leading to a higher extraction rate and extraction yield. Similar behavior was observed in the extraction of anthocyanins in red onions in continuous flow mode

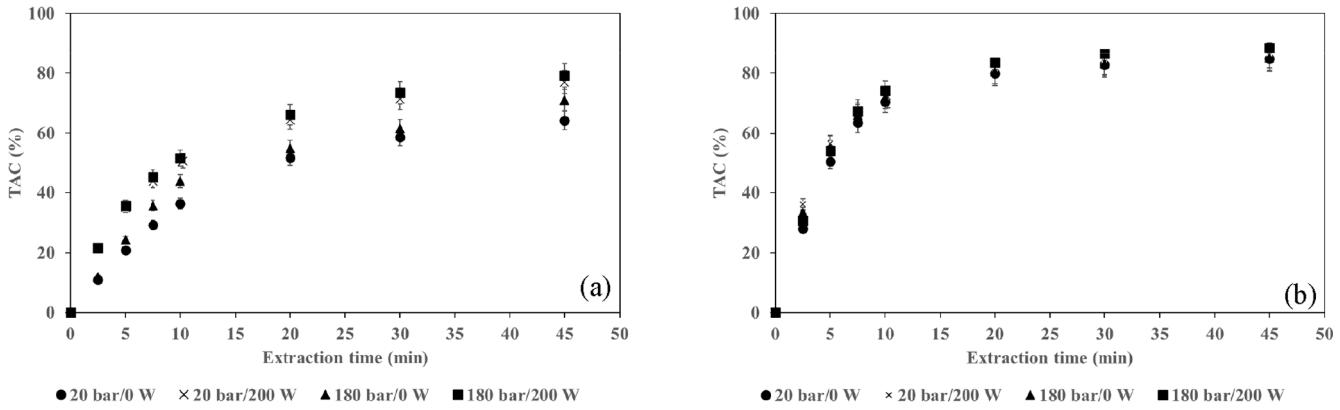

Fig. 2. Pressurized liquid extraction of anthocyanins with citric acid solution 1.5 wt% at 5.0 mL min^{-1} at (a) 30 °C and (b) 70 °C.

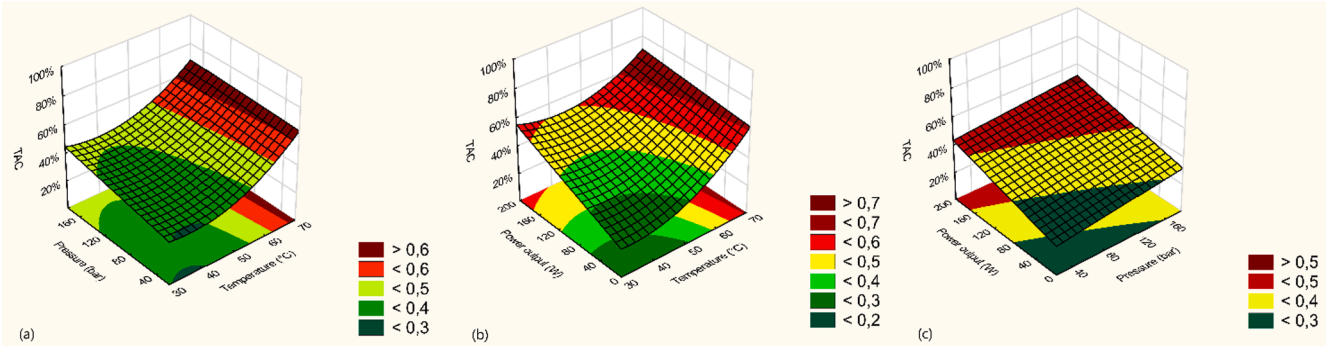

Fig. 3. Response surface plots of the effect of the pressure, ultrasound power output, and extraction temperature on the total anthocyanin content extracted with citric acid solution 1.5 wt% at a flow rate of 5.0 mL min^{-1} for 45 min, at fixed (a) power output – 100 W, (b) pressure – 100 bar, and (c) temperature – 50 °C.

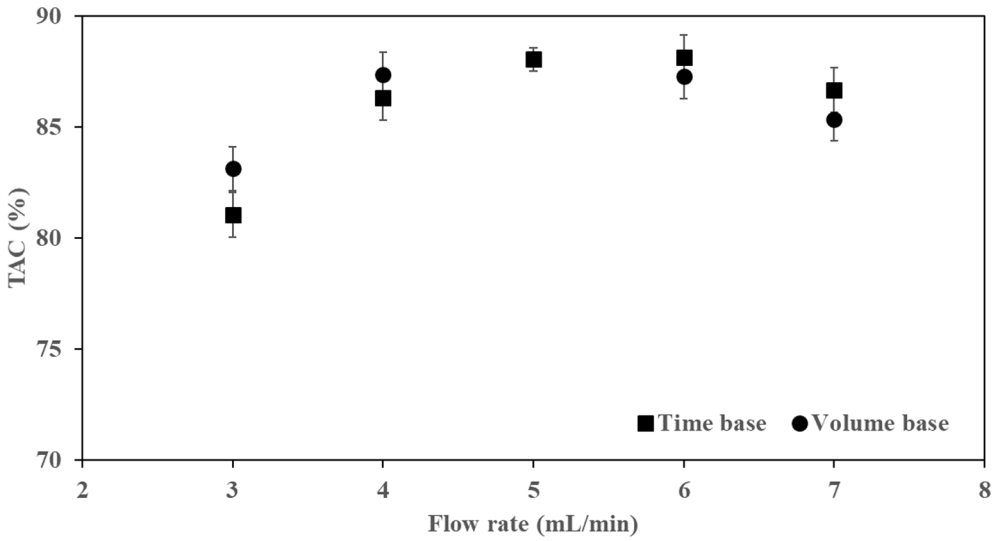

Fig. 4. Effect of the flow rate on the total anthocyanin content extracted from black chokeberry pomace at 70 °C, 180 bar, 200 W, and 1.5 wt% citric acid solution.

[53].

The TAC remained nearly constant when the flow rate was increased from 5 to 6 mL min^{-1}. However, a further increase in the flow rate resulted in decreased extraction yield. The use of higher flow rates shortens the residence time of the solvent in the extraction column, reducing the chances of interaction between the solvent and the matrix, hence leading to a lower extraction yield. The same effect was observed when the flow rate was increased in the semi-continuous extraction of anthocyanins from grape pomace [54].

Given the fact that a similar extraction yield was obtained at 5 and 6 mL min^{-1}, the lower flow rate is recommended to reduce solvent consumption. Besides that, a lower flow rate avoids exhaustion of the pump and reduces the further dilution of the extract [53].

Table 1 presents the extraction yields obtained at different flow rates after different extraction times. The yields reported in bold indicate the time necessary to reach a solvent-to-pomace ratio of around 64 g/g DW (total volume of 225 mL of solvent). At this ratio, the TAC was approximately constant at 5 and 6 mL min^{-1}. However, a lower flow rate would require a longer extraction time. At all different conditions, increases of less than 3% in the TAC were observed when extending the extraction from 30 to 45 min. Therefore, performing a 30 min extraction could achieve a high yield while reducing the solvent-to-pomace ratio to approximately 43 g/g. This lower ratio can ease not only the extraction step but also the recovery and purification of anthocyanins in the downstream process.

Table 1
TAC extracted from black chokeberry pomace at different extraction times.

Flow rate (mL min^{-1})	20 min	30 min	32 min	37.5 min	45 min	56.3 min	75 min
3	73.5 ± 2.2	78.4 ± 2.4	–	–	81.1 ± 2.4	–	**83.1** ± 2.5
4	80.4 ± 1.6	84.2 ± 1.7	–	–	86.3 ± 1.6	**87.4** ± 1.7	–
5	82.1 ± 1.6	85.7 ± 1.7	–	–	**88.0** ± 1.8	–	–
6	82.7 ± 3.3	86.0 ± 3.4	–	**87.3** ± 2.9	88.1 ± 3.1	–	–
7	82.5 ± 2.6	85.0 ± 2.6	**85.4** ± 2.6	–	86.7 ± 2.6	–	–

3.2.2. Effect of the citric acid concentration on the anthocyanin extraction yield

The effect of citric acid concentration (CA, wt%) in the solvent on the anthocyanin extraction yield can be observed in Fig. 5. A generally increasing trend in the anthocyanin extraction yield was obtained when the concentration of citric acid increased from 0 wt% (TAC = 73.9%) to 1.5 wt% (TAC = 85.7%) after 30-min extraction (Fig. 5a). The anthocyanin extraction yield is commonly associated with the stability of anthocyanins that depends on the pH of the solution. Anthocyanins are generally more stable in media at a pH lower than 3, where the predominant form is the relatively stable flavylium cation, also

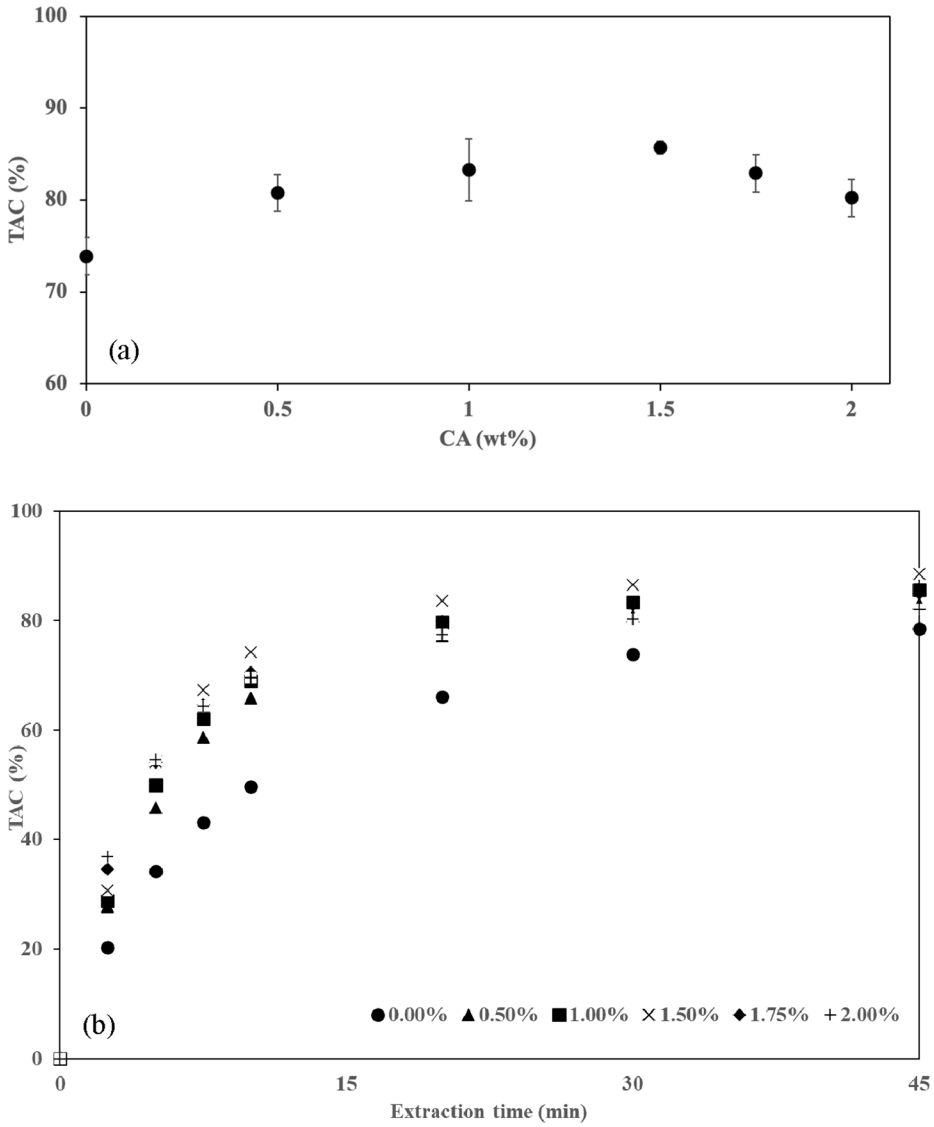

Fig. 5. Effect of the citric acid concentration (in weight) on the anthocyanin content from black chokeberry pomace at 70 °C, 180 bar, 200 W, and 5 mL min^{-1} (a) at 30-min extraction and (b) extraction profile for 45 min.

characterized by red color [55,19]. The results obtained in the present work are aligned with this principle. The lowest anthocyanins yield was obtained in the extraction without CA where the extract reached a pH of 3.52. The extracts with different CA concentrations showed pH close to 2, respectively, 2.29, 2.05, 1.91, 1.88, and 1.79 for extractions from 0.5 up to 2 wt% of CA. Although the extraction yields with CA were similar, an increase in the citric acid concentration above 1.5 wt% had a slightly negative effect on the TAC. The excess of acid could have led to the slow hydrolysis of the anthocyanins during the extractions [52,56], decreasing the total anthocyanin content to 80%.

Apart from the extraction without the addition of citric acid, all other extractions reached equilibrium after ~20 min (Fig. 5b).

3.2.3. *Effect of extraction temperature on the anthocyanin profile*

As can be observed in Fig. 3, a continuous increase in temperature, pressure, and ultrasound power resulted in increased TAC extraction in the range of conditions tested. However, when high temperatures are considered, the effect of pressure and power output on the TAC appears limited thus a further increase of their values would not be relevant.

Further increase in temperature, on the other hand, shows the potential for greater TAC recovery.

This peculiar behavior differentiates the ultrasound-assisted PLE continuous extraction system from the batch counterpart. Roda-Serrat et al. [20] reviewed the operative conditions used for batch extraction of chokeberry pomace, highlighting that rarely the optimal extraction temperature reached 70 °C. Galvan ÓAlessandro et al. [42] observed a decrease in the TAC at 70 °C for extraction performed with pure water or a solution of water–ethanol 50% vol, with or without sonication. In their study, the authors concluded that insignificant degradation was observed at 40 °C. A more detailed description of the anthocyanins' thermal degradation mechanism was reported by Sui [55].

In this study, it is hypothesized that exposure to high temperatures during short residence times of the extract in the extraction unit does not result in significant anthocyanin degradation. However, whereas the extracts leave the system after a short residence time (approximated 77 s when the flow rate is 5 mL min^{-1}), the pomace is exposed to the given extraction temperature throughout the entire process (up to 45 min).

In order to assess that the characteristics of the extract do not change with extraction temperature and time exposure, the profile of individual anthocyanins was evaluated after 5 min and 30 min extraction time for the temperatures of 40, 50, 60, 70, and 80 °C for extractions at 180 bar, 200 W and 1.5 wt% citric acid solution.

All extracts showed an anthocyanin profile consisting of four cyanidin glycosides very well known for chokeberry: cyanidin-3-O-

galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, and cyanidin-3-O-xyloside. The same anthocyanins were identified by Esatbeyoglu et al. [57] in the fractionation and isolation of polyphenols from *Aronia melanocarpa* pomace.

The individual composition of anthocyanins in black chokeberry pomace extracts obtained at four different extraction temperatures and two sampling times is shown in Fig. 6. In all cases cyanidin-3-O-galactoside was the dominant anthocyanin followed by cyanidin-3-O-arabinoside, cyanidin-3-O-xyloside, and cyanidin-3-O-glucoside, in agreement with the results of Grunovaité et al. [58]. The extraction temperature did not have a significant influence on the anthocyanin profiles ($p > 0.05$). However, the extraction time did show an effect ($p < 0.05$). The samples collected after 5 min extraction showed identical anthocyanin profiles (63.2 ± 0.1%, 2.5 ± 0.0%, 31.2 ± 0.1%, and 3.1 ± 0.1% absorbance, respectively), and so did the samples collected after 30 min (60.7 ± 0.3%, 2.2 ± 0.0%, 33.9 ± 0.3%, and 3.2 ± 0.0% absorbance, respectively). The differences in the anthocyanin profiles associated with the extraction time, even statistically significant, were very minor (<3%) and are not considered to affect the properties nor the quality of the extract.

With the ultrasound-assisted continuous extraction method proposed in the present work, the TAC reached the value of 94.30% by allowing the process temperature to reach 80 °C without observing selective component degradation.

The potential of the system proposed can be fully understood when compared with the corresponding extraction process performed in batch. In a previous study, Roda-Serrat et al. [20] considered the same pomace for extraction with homogenization in batch mode using citric acid aqueous solutions. The extraction parameters were optimized obtaining 1.5 wt% as the concentration of citric acid and 45 °C as optimal temperature. For a ratio of 64 g of solvent per g of pomace, the authors obtained a TAC of 79.19%. The proposed system is capable of achieving a TAC that is 19% higher than the optimal value obtained in the batch mode.

3.3. Kinetic extraction of anthocyanins from black chokeberry pomace

Table 2 presents the kinetic parameters K_1 and K_2 for the anthocyanin extraction from black chokeberry at different temperatures for extractions at 180 bar, 200 W, 5 mL min^{-1} solvent flow rate, and 1.5 wt % citric acid solution. The regression coefficients of determination R^2 obtained suggest a good fit for the kinetic modeling. The root-mean-square deviation (RMSD) between the experimental and calculated total anthocyanin content indicates that TAC can be estimated from the kinetic parameters obtained. In addition, according to both R^2 and RMSD, better kinetic modeling fit was achieved at lower extraction temperatures. Higher values of both kinetic parameters were observed with increasing extraction temperature, clearly accounting for the higher extraction rate and yields measured at different extraction temperatures.

The calculated activation energies for the kinetic parameters K_1 and K_2 were 16.35 and 1.87 kJ mol^{-1}, respectively. This suggests that the extraction rate is more temperature-sensitive than the maximum extraction yield. The pre-exponential factors of those parameters were found to be respectively 7313.67 and 198.66 mg g^{-1}DW min^{-1}.

The calculated and experimental anthocyanin extraction yields at different temperatures are shown in Fig. 7. As described in the previous section, due to the short residence time of the extract in the extraction column, the increased temperature did not result in selective anthocyanin degradation. This can be further confirmed based on the continuous increase in the extraction rate and the highest extraction yield reported at 80 °C when 92.6% anthocyanin yield was obtained after 30 min extraction.

Operation at higher temperatures, however, demands higher energy consumption, and accordingly, there is a rise in the extraction costs. Therefore, to select the optimal extraction conditions, a full economic assessment should be developed.

4. Conclusion

Ultrasound-assisted extraction was combined with pressurized liquid extraction to improve the yield of anthocyanin extraction from black chokeberry pomace. The effects of temperature, pressure, citric acid concentration, solvent flow rate, and ultrasound action were evaluated. This work proves that the effects of pressure and sonication were more pronounced at low temperatures and that using citric acid with a concentration above 1.5 wt% had a negative effect on the TAC. The solvent flow rate was the variable that influences the TAC most. In the range 5–6 mL min^{-1} at 70 °C and 180 bar a TAC of about 88% was reached in 45 min. Furthermore, in order to optimize the extraction yield, the temperature was allowed to reach 80 °C proving that the ultrasound-assisted continuous extraction method proposed can reach over 94% TAC without compromising the stability of the components. This result is 19% higher than the same extraction performed in batch mode.

The study was completed with a kinetic analysis using a modified Peleǵs model. The kinetic parameters were evaluated as a function of the temperature and they represent a useful tool for process design.

Pressurized liquid ultrasound-assisted extraction shows clear advantages reaching a high total extraction yield. However, an economic assessment is necessary to evaluate the influence of the capital investment on the process profitability.

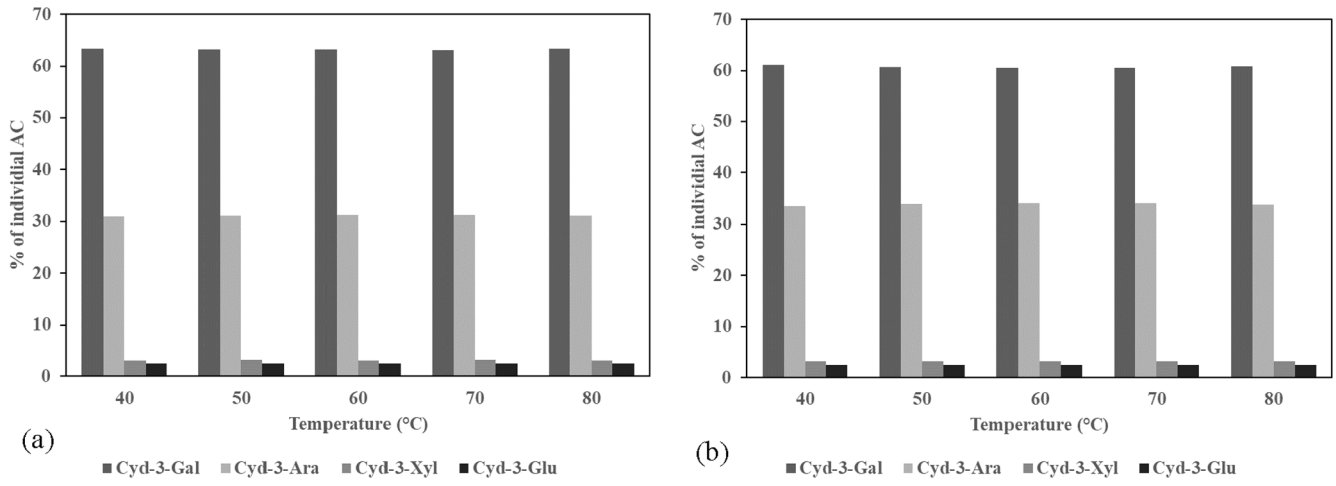

Fig. 6. Individual composition of extracted anthocyanins at different temperatures after (a) 5 min and (b) 30 min of extraction.

Table 2
Kinetic parameters obtained for the anthocyanin extraction at different temperatures.

	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C
$K_1\,[mgg^{-1}DWmin^{-1}]$	11.48	13.33	16.45	20.44	22.45	29.15
$K_2\,[mgg^{-1}DW]$	93.43	97.58	99.87	101.44	103.14	104.22
R^2	0.999	0.999	0.994	0.989	0.968	0.990
RMSD	0.23	0.06	1.71	2.21	3.29	1.50

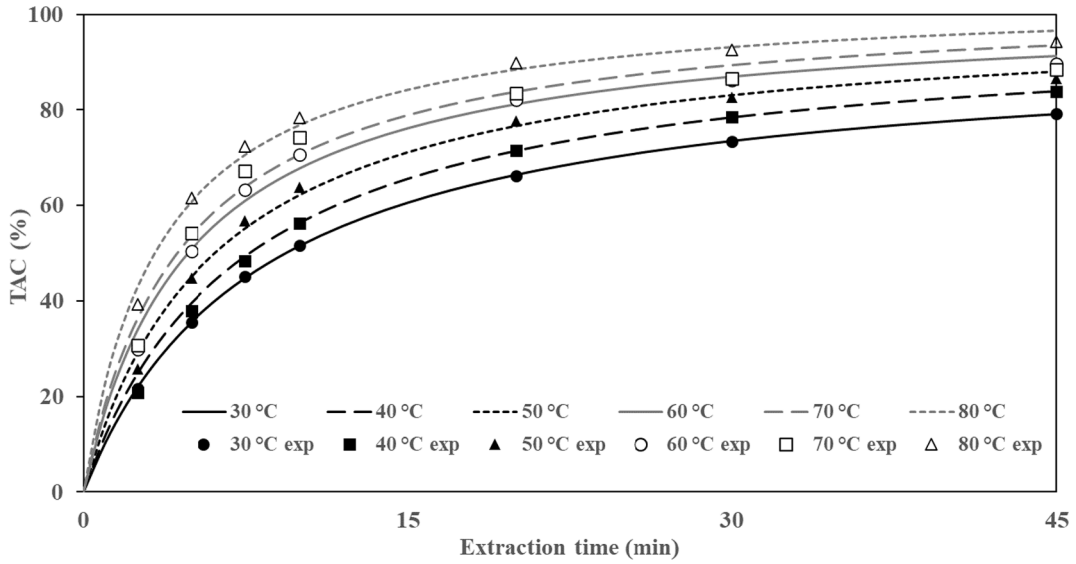

Fig. 7. Profile of the calculated (lines) and experimental (dots) anthocyanin extraction yields obtained at different temperatures.

CRediT authorship contribution statement

Thalles A. Andrade: Conceptualization, Writing–original draft, Writing–review & editing, Formal analysis, Data curation. **Fabiane Hamerski:** Conceptualization, Writing–original draft, Writing–review & editing, Formal analysis. **Damian E. Lopez-Fetzer:** Conceptualization, Writing–original draft, Writing–review & editing, Formal analysis. **Maria Cinta Roda-Serrat:** Conceptualization, Writing–original draft, Writing–review & editing, Formal analysis. **Marcos L. Corazza:** Conceptualization, Writing–original draft, Writing–review & editing, Formal analysis, Funding acquisition, Project administration. **Birgir Norddah:** Conceptualization, Funding acquisition, Project administration. **Massimiliano Errico:** Conceptualization, Writing–original draft, Writing–review & editing, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2021.119290.

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