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ORIGINAL PAPER

Real-time in-line detection in subcritical water extraction of betacyanins and phenolic compounds from beet (*Beta vulgaris* L.)

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Abstract

This study employed a Central Composite Rotational Design (CCRD), temperature and pH, to optimize the extraction of beet compounds (betacyanins and phenolic compounds) using subcritical water extraction (SWE). An innovative approach of this study was to incorporate an in-line detector to monitor the extraction process in real-time, providing instant data on the compounds being extracted and optimizing the process. The work complies with the principles of green chemistry, using clean technology with a sustainable solvent. The results indicate that the extraction of biocompounds in the treatments with lower temperatures (33.43 °C and 50.00 °C) occurred quickly after 25 min. Extractions with higher temperatures (130.00 °C and 146.56 °C) showed greater efficiency in obtaining betacyanins (extract with red-violet color) and phenolic compounds accumulated under 146.56 °C and pH 5.50. The treatment with a temperature of 130.00 °C and pH 7.00 showed a higher oxidizing response at 45.19 µmol TEAC g^{-1}. The highest concentration of phenolic compounds (20.49 mg GAE g^{-1}) was obtained in the treatment with a temperature of 130.00 °C and pH 4.0. The highest concentrations of glucose and fructose were obtained at 130.00 °C and pH 7.00 with 1.76 mg g^{-1} and 1.11 mg g^{-1}, respectively. The optimal conditions were observed at the temperature of 146.56 °C, in-line detection wavelength at 535 nm, and pH 7.62, recovering 13.02 mg g^{-1} of betacyanin, i.e., recovery of approximately 80% in up to 20 min of extraction. This arouses interest for future industrial applications in the development of new products.

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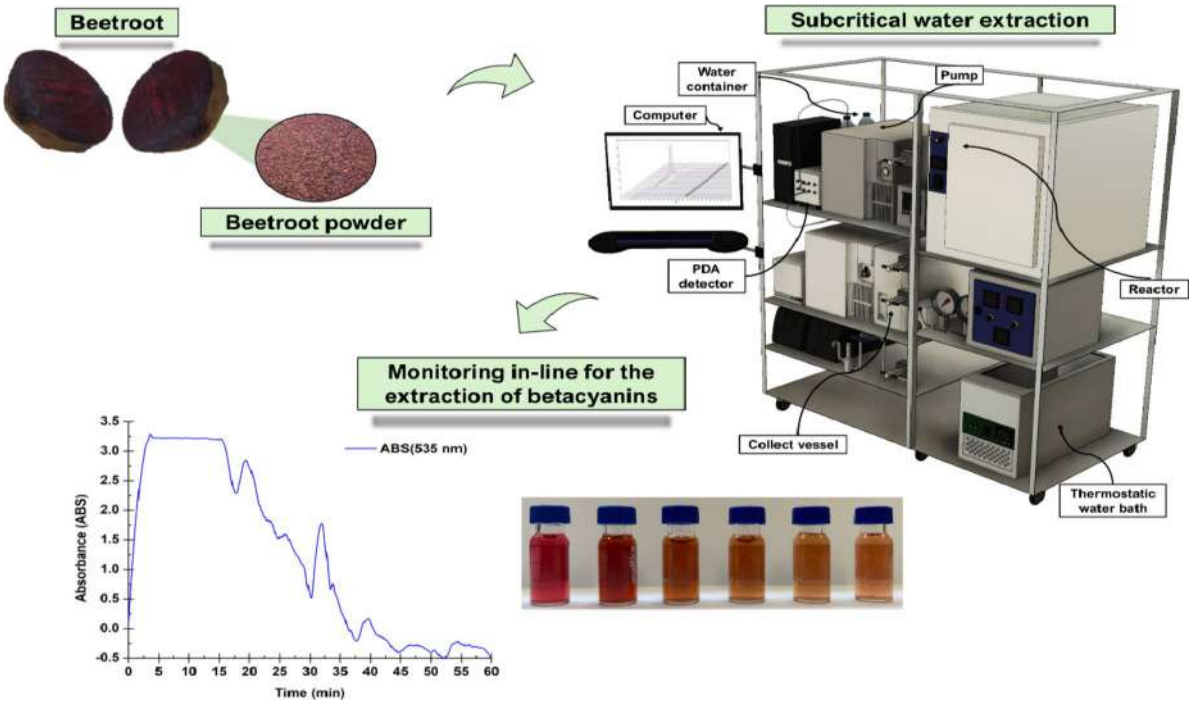
Graphical Abstract



Keywords Automated analysis · Green extraction · Betalains · Bioactive compounds

Introduction

The extraction of plant compounds is an ancient practice that originated in religious and cultural traditions, with early methods such as teas and infusions [1]. Over time, and with the progression of modern science, this practice has evolved, making plant extracts increasingly valuable across various industries. For instance, these extracts hold significant potential for use in medicine and the cosmetic and food sectors due to the diverse compounds found in plant materials [2]. These bioactive compounds, which can be sourced from different plant parts, including fruits, peels, seeds, and leaves, include polyphenols, betalains, and flavonoids-three classes notably found in beet (*Beta vulgaris L.*) [3].

Beet is a tuberous vegetable offering its leaves and roots as edible sources. Alongside the aforementioned bioactive compounds, beet is rich in minerals and vitamins, such as calcium, potassium, vitamin C, and vitamin A. It can be consumed in various forms, including raw, cooked, commercially canned, or as part of minimally processed salads. Beet extract is particularly valued in medicinal applications, as a food colorant, and in cosmetic products, thanks to its well-documented antioxidant and anti-inflammatory properties [4].

The methods used to obtain beetroot extracts range from traditional methods such as maceration and Soxhlet extraction to modern techniques such as microwave, ultrasound, pulsed electric field, membrane processing, and pressurized liquid extraction [3]. However, traditional methods have limitations, including high use of organic solvents that increase downstream waste treatment costs, prolonged processing times, or high temperatures, which can reduce yields due to the simultaneous degradation of heat-labile compounds [5]. In this sense, the methods are classified as green depending on their application and development.

Green extraction is linked with 12 principles of green chemistry (prevention, atom economy, less hazardous chemical syntheses, designing safer chemicals, safer solvents and auxiliaries, design for energy efficiency, use of renewable feedstocks, reducing derivatives, catalysis, design for degradation, real-time analysis for pollution prevention, inherently safer chemistry for accident prevention), promoting more efficient extraction processes with lower energy and solvent consumption [6]. It is worth noting that the use of water as a solvent meets the requirements of green chemistry principles. Additionally, it prioritizes obtaining compounds free of organic solvents.

Among the approaches to green extractions, extraction with pressurized liquids (PLE) has been explored in the literature for some time [7]. The extractive conditions of

pressurized liquids increase the solubility of compounds and the mass transfer rate [8]. Sanches et al. (2022) [9] pointed out that the use of PLE applied to orange by-products obtained a good yield in the recovery of phenolic compounds, which may be an effect of the reduction in surface tension, influencing the penetration of the solvent into the solid pores. Another benefit of the PLE technique is that it can be coupled with other techniques in just one step, such as PLE coupled with a UV-vis detector (PLE-SPE-UV), with chromatography (PLE-HPLC) and with extractions (PLE-SPE-HPLC) [10, 11].

Among the PLE, subcritical water extraction (SWE) is a new alternative for obtaining biotactive compounds from plants using sustainable solvents at high pressures and temperatures. In addition, SWE is an advantageous method due to its low solvent usage, high extraction rate and short process time [12]. Xu et al. (2021) [13] obtained a considerable yield of phenolic compounds when applying SWE to distillery waste.

Most of the extraction methods currently used operate offline, i.e. the extraction stage is carried out separately from the detection stage of the desired compounds. The offline system has some disadvantages, such as process time. This is because the process steps are separate, so the extraction needs to be carried out over a long period, and only then analyzed for the compounds present. Another problem with the process being carried out separately is the increased inference of errors. In addition, the process slows down if there are many samplesecause the injections are done manually [14].

The in-line process is a fully automated method and can provide more injections while the process is still ongoing, enabling real-time feedback. The use of SWE coupled with the in-line detector is a promising technology, as it allows SWE to be carried out more accurately and in less time and labor [15]. The PDA (photodiode array detector) is a fundamental tool for providing fast and accurate monitoring of compounds at the time of extraction, as it makes it possible to determine the wavelength spectra of these compounds in real time. From this perspective, the potential application of SWE coupled with PDA (SWE-PDA) could make it possible to monitor extraction in real time [14].

Therefore, the study aims to evaluate the efficiency of beet compound extraction using SWE through an experimental design varying temperature and pH and monitor the in-line detection on process optimization. However, monitoring the extraction process is crucial due to the natural variation in the concentration of the compounds present in beet. Our proposal uses an in-line photodiode array (PDA) detector to monitor the extraction process in real-time, providing instantaneous data on the extracted compounds. The real-time monitoring allows for a faster response to identify the end of the extraction process and raw material degradation.

Materials and methods

Sample

The beet sample, purchased from CEASA (Campinas, SP, Brazil), was dried at 60 °C for 24 h. Next, it was ground in a mill and sieved to obtain 1 mm-sized particles. The dried sample was packaged and stored at (−18 °C) to maintain its integrity for subsequent physicochemical characterization and extraction procedures. The analysis included total solids, volatile solids, moisture, ash, protein, and nitrogen [16].

Chemical materials

The standards used in liquid chromatography determinations and spectrophotometric analyses (glucose, fructose,5-hydroxymethylfurfural, 6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-Tris(2-pyridyl)−1,3,5-triazine (TPTZ) and gallic acid) were provided by Sigma Aldrich (St. Louis, MO, USA). Folinciocalteau, Somogyi-Nelson reagent, ethanol (99.5%), cloridric acid (HCL), sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), and sodium carbonate (Na_2CO_3) were purchased from Dinâmica (São Paulo, Brazil). Acetonitrile (C_2H_3N), sulfuric acid (H_2SO_4), and acetic acid (CH_3COOH) (HPLC-grade) was supplied from J.T. BAKER® (Goiânia, Brazil). The ultrapure water was supplied by a Direct-Q® 3UV purification system (Waters Corporation, Massachusetts-USA).

Subcritical water extraction (SWE) coupled in-line with photodiode array detection (PDA)

The variables and operating conditions were based on previous works [14, 17, 18]. The best result obtained using the offline system was selected for monitoring in an in-line system (SWE-PDA). The system is presented in Fig. 1 (patent BR 1320210187220). It consists in a deionized water reservoir; a high-pressure liquid pump with a double piston (Model 36-USA), responsible for feeding and pressuring the entire system; two K-type thermocouples, used to control the extraction and hydrolysis temperatures inside and outside the reactor; HPLC pumps, degasser, photodiode array (PDA) detector, high-pressure binary pump (Waters, 1525, Milford, MA, USA); oven for extraction cell (Memmert GmbH, UF55, Buechenbach, Germany); valves for selection, depressurization, automatic pressurization, samples collection (Waters, Milford, MA, USA); PDA detector

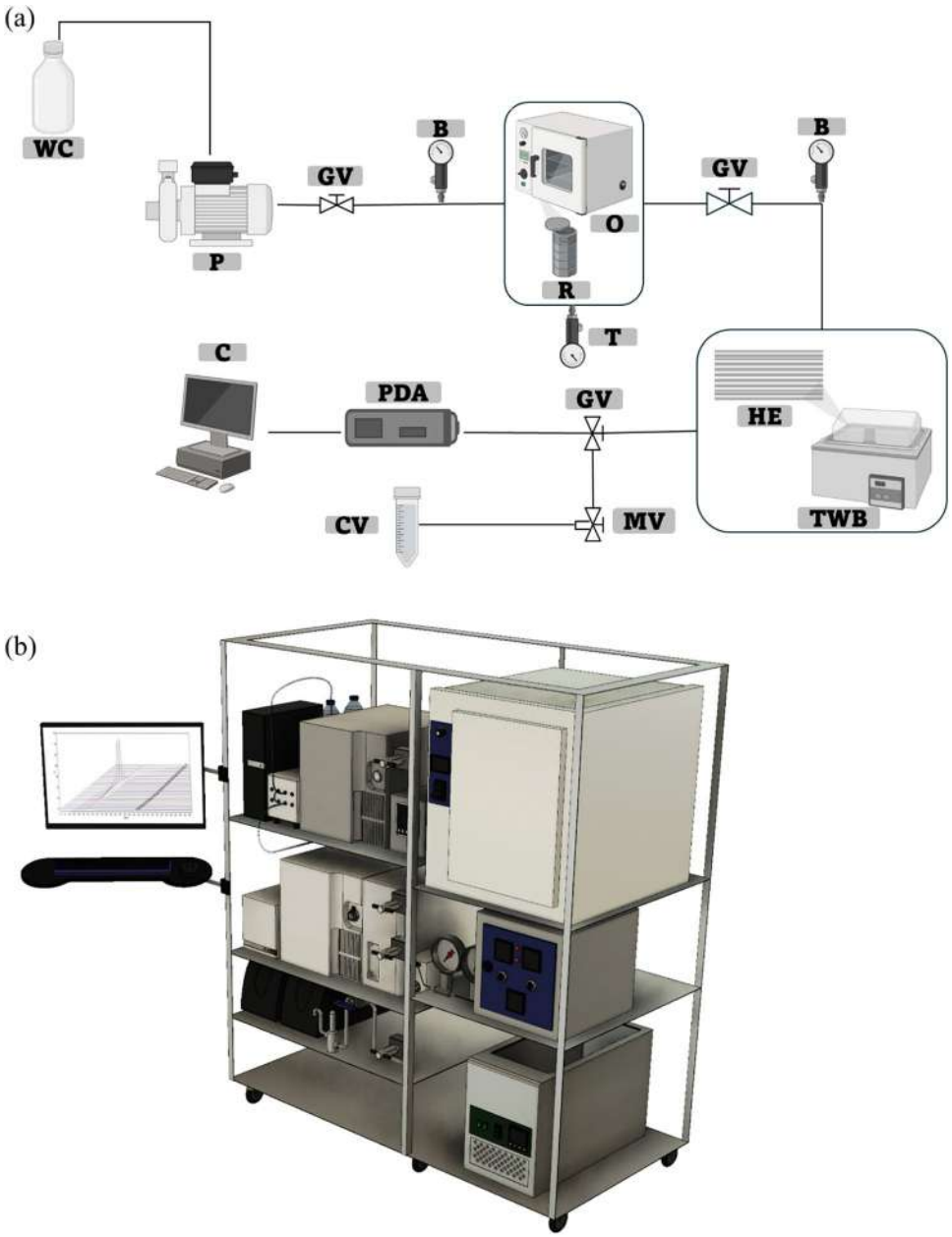


Fig. 1 An experimental unit is used to extract beet. **a** schematic diagram; **b** 3D representation. Label: WC, Water container; P, Pump; GV, Gate valve; B, Barometer; O, Oven; R, Reactor; T, Thermostat; TWB, Thermostatic water bath; HE, Heat exchanger; PDA, Detector; C, Computer; BPR, Micrometric valve; CV, Collect vessel

(Waters, Milford, MA, USA), a PLE stainless-steel extraction cell (15 mm × 100 mm, Waters Corporation, Milford, MA, USA) and a SPE column (4.6 mm × 50 mm I.D.).

To carry out the extraction, the stainless-steel extraction cell was filled with a layer of glass wool (approximately 1 g), a layer of sample, and another layer of glass wool, and the remaining volume of the cell was filled with glass beads. A coil immersed in a thermal bath (Marconi, model MA-184, São Paulo, SP, Brazil) using water at a temperature of 10 °C was used to cool the liquid after the extract. The system's pressure was regulated using a micrometric valve and measured using pressure gauges (0–50 MPa).

The pH of pure water, used as extraction solvent, was adjusted using phosphoric acid. The reactor was fed with 1.5 g of powdered beet. The extraction was operated semi-continuously at a flow of 2 $mL.min^{-1}$ and 15 MPa for 60 min, with the extract collected every 5 min. After extraction, the samples were centrifuged (3000 rpm; 10 min; 25 °C), and the supernatant obtained was stored at −18 °C for later analysis. The process conditions for obtaining beet extracts employed a Central Composite Rotational Design (CCRD) with two factors (2^2). Temperature (X_1) and pH (X_2) were used as operational variables (Table 1). 11 experiments (4 linear points, 4 axial points, and 3 repetitions in the central points) were performed. The responsive variable used

Table 1 Operational conditions of subcritical water extraction

Treatments	Non-codified variables		Codified variables	
	Temperature (°C)	pH	Temperature °C)	pH
SB1	130.00	4.00	+1	–1
SB2	33.43	5.50	–1.41	0
SB3	130.00	7.00	+1	+1
SB4	90.00	5.50	0	0
SB5	90.00	5.50	0	0
SB6	90.00	5.50	0	0
SB7	50.00	7.00	–1	+1
SB8	146.56	5.50	+1.41	0
SB9	50.00	4.00	–1	–1
SB10	90.00	3.38	0	–1.41
SB11	90.00	7.62	0	+1.41

*SB: subcritical beet

to guide the experiments was the yield of betacyanin, FRAP (Ferric Reducing Antioxidant Power), and TPC (Total phenolic compounds). However, only the optimum condition for betacyanin was considered for the extraction with in-line detection, in other words, the absorbances of the betacyanins were obtained simultaneously with the extraction process using the PDA.

Extract evaluation

Betacyanin evaluation

The absorbance of the beet extracts was determined based on the method of Ferreira et al. (2023) [18] (adpated) using a spectrophotometer (Hach, model DR 4000U, São Paulo, SP, Brazil) at a wavelength of 535.5 nm [19]. Equation 1 was used to calculate the total betacyanin content.

$$Betacyanin\ \left(mg\ g^{-1}\right) = \frac{A \times\ MM \times\ DF \times\ 1000}{\epsilon\ \times\ l} \quad (1)$$

where A is the extract's absorbance; MM is the molecular mass of betacyanin (550 g.mol^{-1}); DF is the dilution factor used for measuring the absorbance; Ɛ is the molar extinction coefficient (60,000 L mol^{-1} cm^{-1}) of betacyanin; and l is the optical path length of the cuvette (1 cm).

Total phenolic compounds

Total phenolic compounds (TPC) were determined according to the Folin-Ciocalteu method, with some modifications [20]. The reaction included the addition of 60 µL of extract, 300 µL of Folin-Ciocalteu reagent, and 3 mL of distilled water. After homogenizing the reaction, it was kept for 3 min in a dark room. Then 0.9 mL of sodium carbonate (15%) and 1.74 mL of distilled water were added. After 2 h, the solution absorbance was determined using a wavelength of 765 nm in a UV-Vis spectrophotometer (Model UV-M51, Bell Photonics). The results were calculated from a calibration curve of 5 points (0–1000 mg.L^{-1}) using gallic acid as the standard solution. The results were presented in mg of gallic acid equivalent of beet (mg GAE g^{-1}).

Antioxidant activity by FRAP assay

Antioxidant capacity by FRAP assay was carried out according to the model of Benzie et al. (1996) [21], with adjustments. The test included the addition of 200 µL of sample, 200 µL of 3 mM ferric chloride solution, and 3.6 mL of TPTZ (2,4,6-Tris(2-pyridyl) -S-Triazine). The solution was kept at 37 °C for 30 min. Subsequently, it was stored for 10 min in a dark room. After this, the absorbance was measured at a wavelength of 620 nm using a UV-Vis spectrophotometer (model UV-M51, Bell Photonics). A Trolox calibration curve (100–1000 µm) was used to quantify the antioxidant capacity. The results were expressed in triplicate and presented as µmol of Trolox equivalent antioxidant capacity (TEAC) per gram of beet powder (µmol.g^{-1}).

Color parameters

A UV-Vis spectrophotometer (Model UV-M51, Bell Photonics) was used to measure the transmittance values between 340 and 830 nm every 5 nm to determine the colorimetric parameters of the beet extracts. The colorimetric parameters defined were L* (luminosity), a* (red/green value), and b* (blue/yellow value) from the CIElab system [22, 23]. Equation 2 shows the calculus for the hue angle (h°).

$$h^{\circ}\ = tan^{-1}\left(\frac{b*}{a*}\right) \quad (2)$$

pH

A digital pH meter (IonLab, model THS-3E, USA), adequately calibrated with pH 4 and 7 buffer solutions, was used to measure the pH values of the beet extracts.

Complementary analysis

Due to the composition of the matrix of the raw material chosen, the analyses for the detection and quantification of other compounds were carried out complementary to the work, allowing other routes of exploitation of the extracts obtained. Sugars and furanic aldehydes were analyzed. The latter can be seen as fermentation inhibitors [24] or important matrix compounds for the synthesis of various compounds [25], such as biofuels [26] and polymers [27].

The sugars (glucose and fructose) and inhibitor 5-hydroxymethylfurfural (5-HMF) in the beet extract were quantified using an HPLC (High-Performance Liquid Chromatography) coupled with a Refractive Index Detector (RID). The analysis was developed according to the method carried out by Barroso et al. (2022) [28]. The analysis took place over 48 min. The glucose, fructose, and 5-HMF concentrations were calculated according to the calibration curves of the respective standards. The results were obtained in triplicate and presented in mg.g^{-1} of beet powder. The Limit of Detection (LOD) and Limit of Quantification (LOQ) are crucial for validating the method's sensitivity. LOD is the lowest concentration of 5-HMF detectable under the specified conditions and LOQ is the minimum concentration of 5-HMF quantifiable with acceptable precision and accuracy [29]. The method's sensitivity was validated with an LOD of 0.02 g. L^{-1} and an LOQ of 0.1 g. L^{-1} for 5-HMF, determined by a signal-to-noise ratio of 3:1 and 10:1, respectively. It is important to note that the amount of 5-HMF found in the sample was 0.65 g. L^{-1}.

Table 2 Composition of beet powder

Parameters	Beet powder
Total solids (%)	89.56 ± 0.07
Volatile solids (%)	81.52 ± 0.13
Moisture (%)	10.44 ± 0.07
Total protein (%)	12.62 ± 0.20
Total nitrogen (%)	2.02 ± 0.03
Elementary analysis	
Ashes (%)	8.04 ± 0.15
Nitrogen (%)	2.33
Carbon (%)	37.83
Hydrogen (%)	5.96
Sulfur (%)	0.00
Oxygen (%)	45.84

The results are presented as the mean ± standard deviation. The analysis was performed in triplicate ($n = 3$)

The Somogyi-Nelson method determined the concentration of reducing sugars and total reducing sugars in the samples [30]. The extracts were subjected to acid hydrolysis (HCl, 2 mol.L^{-1}) for 1 h at 95 °C. The samples were then neutralized with NaOH (2 mol.L^{-1}). Next, the reaction occurred by adding Somogyi-Nelson reagent, with subsequent heating and cooling. A ten-point calibration curve (50–500 mg.L^{-1}) using glucose was used to calculate the sugar content in the sample. The results were obtained in triplicate and presented in mg of glucose per g of beet powder (mg. g^{-1}).

Subcritical water extraction yield

Equation 3 was used to calculate the yield of the samples obtained from the extraction beet. The volume of each sample, the total mass of beet used in the reactor, and the solvent/feed ratio (S/F) in ($g_{water}.g_{beet}^{-1}$) used throughout the kinetics were considered for the calculation.

$$Yield\ \left(mg\ g^{-1}\right) = \frac{compound\ produced}{initial\ mass\ beet} \times \frac{S}{F} \tag{3}$$

Statistical analysis

The results were obtained in triplicate ($n = 3$) and presented as mean and standard deviation. The experimental data obtained was statistically verified for significance and its relationship between the response variables through a statistical analysis using an analysis of variance (ANOVA). The data on betacyanins, phenolic compounds, antioxidant activity, reductants, and total sugars were assessed using a Response Surface Methodology (RSM). Minitab software (version v. 19) was used for statistical analyses.

Results and discussion

Sample characterization

The characterization of the beet powder is shown in Table 2. The moisture content determined in the beet powder was 10.44%, a value equivalent (10.44%) to that found in work by Crocetti et al. (2016) [31] and like others, Kaur et al. (2021) [32] (9.12%), Ozai et al. (2021) [33] (8.68%), Lucky *e al.* (2020) [34] (7.20%) and Dhawan e al. (2019) [35] (6.30%). The amount of potein found in the sample (12.62%) was similar to te work of Ozaki et al. (2021) [33] (12.72%), higher than Itrat et al. (2022) [36] (2.18%), Dhawan et al. (2019) [35] (0.61%) and lower than that of Luckyet al. (2020) [34] (13.01%), Crocetti et al. (2016) [31] (16.92). The ash content obtained (8.04%) ws considerably

similar to studies uch as Dhawan et al. (2019) [35] (7.89%), Ozaki et al. (2021) [33] (9.12%) and highe than Itrat et al. (2022) [36] (5.06% and Lucky et al. (2020) [34] (3.57%). Some differenes observed in the physicochemical coposition of beet powder may be related to independent factors applied to the raw material, such as climate and harvesting. Elemental analysis revealed that beet powder is mainly composed of oxygen (45.84%) and carbon (37.83%), and to a lesser extent, hydrogn (5.96%) and nitroen (2.33%).

Betacyanin

The betacyanin recovered from beet using extraction with subcritical water is illustrated in Fig. 2, in which it is noticeable that the treatments SB8 (14.45 mg. g^{-1}), SB3 (7.87 mg.g^{-1}), SB6 (7.77 mg.g^{-1}) and SB1 (7.12 mg. g^{-1}) showed greater extraction efficiency, all of them using temperatures above 90 °C.

The response surface of betacyanin was further analyzed, as depicted in Fig. 3a, revealing the direct influence of temperature on the pigment's concentration. The assessment of betacyanin yield within the experimental design led to deriving the following model (Eq. 4). Higher temperatures resulted in increased betacyanin levels. Work such as Zin et al.. (2023) [37] corroborates this information, in which, among the temperatures studied, the highest temperature obtained the best results for extracting betacyanins from beet peel (*Beta vulgaris L.*) using microwaves (MAE). In the work by Sharma et al. (2020) [38], they tested the extraction of betacyanin from beet peel using ultrasound and microwaves and obtained the highest yield at higher temperatures.

$$\begin{aligned} Betacyanin =& 6.47 - 0.1057 \cdot (Temperature) + 0.04 \cdot (pH) \\ &+ 0.000819 \cdot (Temperature^2) \\ &- 0.03 \cdot (pH^2) + 0{,}00513 \cdot (Temperature * pH), (R^2 = 0.8733) \end{aligned} \quad (4)$$

Moreover, the Pareto diagram depicted in Fig. 3b enables visualization of the factors influencing the response in descending order of significance. It indicates that temperature is the sole statistically significant factor in this case, with a confidence level of 95%. The study by Zin et al. (2022) [39] obtained similar results, in which the statistical analysis indicated that temperature was the parameter that most influenced the extraction of betacyanins from the crown of beet (*Beta vulgaris L.*). Kumar et al. (2023) [40]

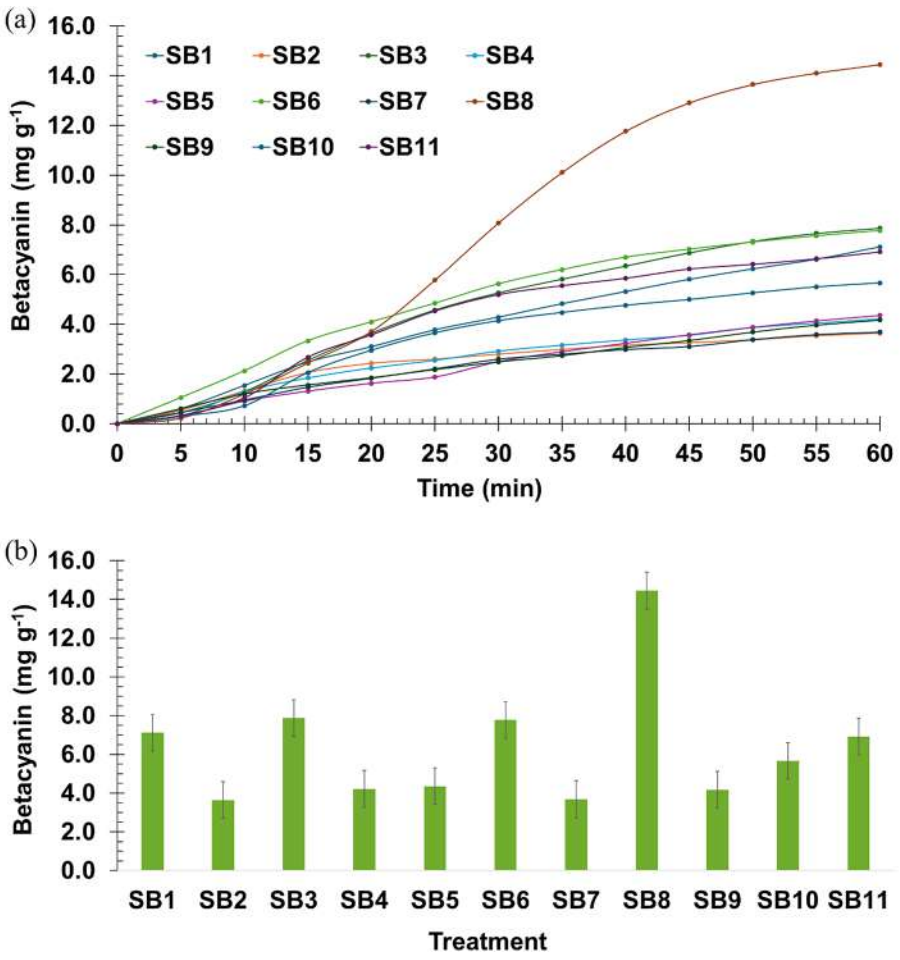


Fig. 2 Betacyanin recovered from beet powder through the subcritical water extraction process. **a** Betacyanin; **b** accumulated betacyanin recovered

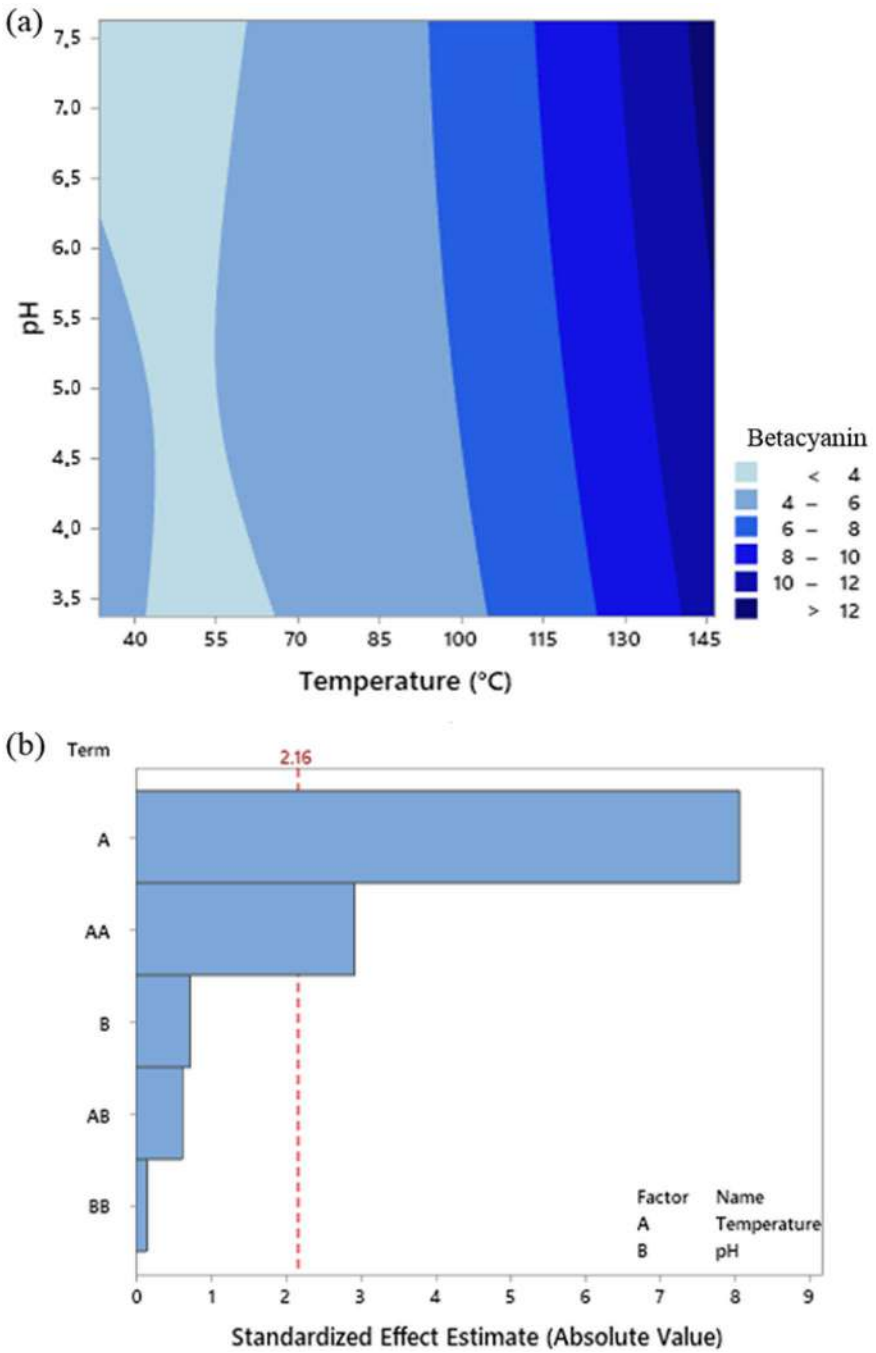


Fig. 3 The influence of temperature and pH on the antioxidant capacity of beet powder extracts. **a** Betacyanin contour curve; **b** Betacyanin pareto chart

also showed that the temperature directly influenced the concentration of betacyanin during extraction using ethanol in the beet powder, in which the highest temperature during short time intervals proved very efficient. While this parameter significantly affects the stability of the pigment and may even inhibit its synthesis [41], the use of subcritical water at high temperatures facilitated the extraction of betacyanins in the beet. This reaffirms the effectiveness of this extraction method for obtaining betacyanins, as Ferreira et al. (2023) [18] suggested.

Potential degradation mechanisms and their impact on extraction efficiency and product quality were analyzed. Potential degradation mechanisms during subcritical water extraction include hydrolysis, thermal decomposition, and oxidation, which can significantly impact extraction efficiency and product quality. At elevated temperatures and pressures, sensitive compounds like betacyanins and phenolic compounds may degrade into less bioactive forms, reducing their yield and functional properties. For example, betacyanins are prone to thermal degradation, leading to a loss of color and antioxidant activity, while phenolic

compounds can undergo oxidation, forming unwanted by-products. The only treatment that produced furfural (a substance that generates degradation) was SB8. However, only a small amount was produced, which did not affect the results.

Total phenolic compounds

The results regarding the cumulative values of TPC and the kinetics of the extractions from the experiment are presented in Fig. 4. In the analysis of cumulative values (Fig. 4b**)**, treatment SB8 stands out, showing the highest recovery of phenolic compounds compared to the other treatments (20.49 mg GAE g^{-1}). In contrast, treatment SB9 yielded the lowest (2.47 mg GAE g^{-1}). Treatment SB8 stands out due to its high temperature, constituting an axial point in the experimental design. Previous studies have highlighted the significant influence of temperature on the recovery of phenolic compounds due to the intensification of hydrolytic processes, favoring the breakdown of the lignocellulosic chain and the release of associated phenolic compounds [17].

Additionally, other research has emphasized the crucial role of extraction temperatures in obtaining high TPC contents. For example, in the study by Alibekov et al. (2023) [42], when extracting TPC from jackfruit seeds using subcritical water, the optimal extraction temperature was identified as 210 °C, resulting in a recovery of 1.84 mg GAE 100 g^{-1}. However, at 240 °C, a reduction in the recovery of compounds was observed, reaching 71.11% o extractable TPC at 35 min. This suggests that high temperatures may result in the extraction of undesirable and the degradation of thermolabile compounds. High temperatures can lead to the formation of degradation compounds and sugar degradation (such as furfurals) during the extraction time, which can influence the simultaneous reading ofhe phenolic compounds in the medium [17]. However, in this study, only

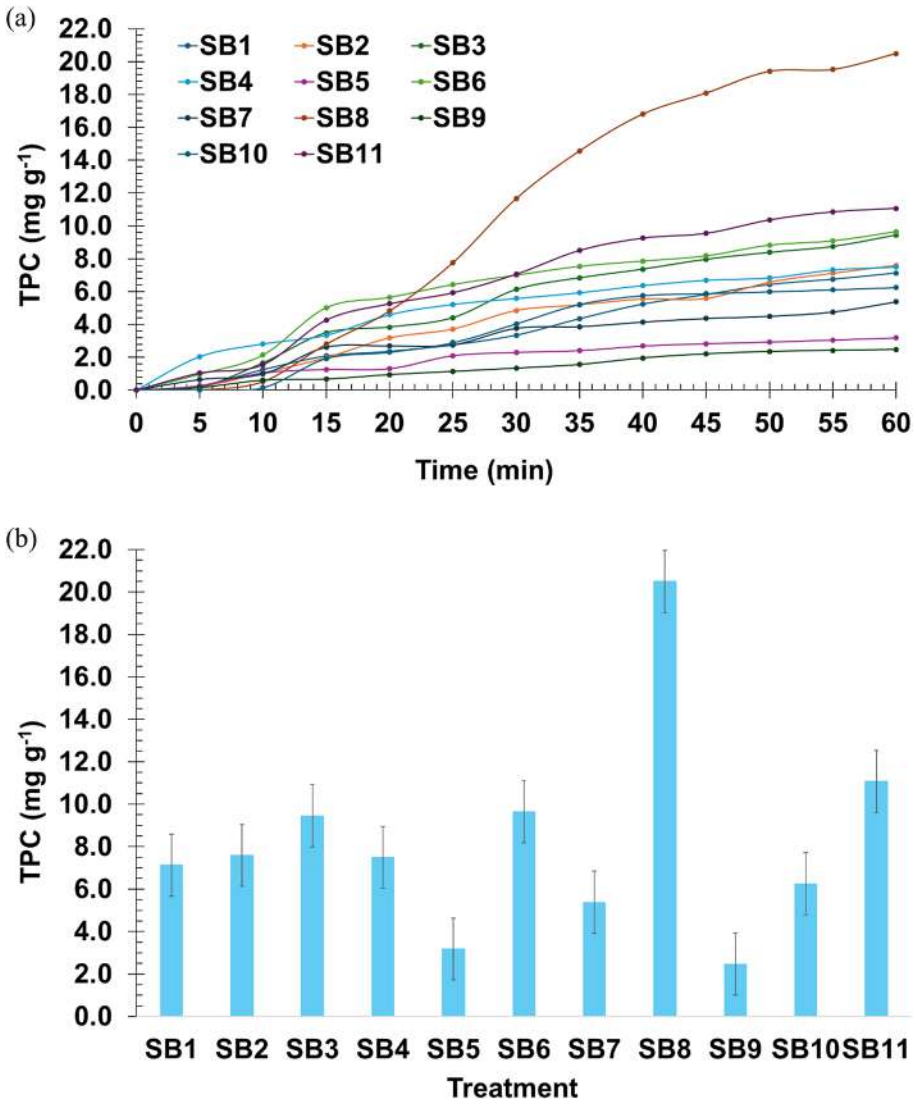


Fig. 4 Total phenolic compounds (TPC) of beet powder obtained from subcritical water extraction. **a** TPC; **b** accumulated TPC

treatment SB8 showed the presence of 5-HMF in significantly reduced amounts (0.0517 mg g^{-1}).

Guine et al. (2018) [43] applied maceration extraction using ethanol and acetone as solvents to dehydrated beets and found values of 1.45 and 2.70 mg GAE g^{-1} for ethanol and acetone, respectively, considerably lower than those found in this study. This highlights the efficiency of extraction with subcritical water, which demonstrated a greater capacity to extract the TPC with higher yields. However, in a study by Maravić et al. [44], which used the same technique (SWE) to extract TPC from beetroot residues, leaves, and stems, only 9.83 mg GAE g^{-1} was obtained. This is attributed to the lower concentration of TPC in these parts of the plants. Thus, while subcritical water exhibits high extraction capacity, it is essential to consider the variation in phenolic compound concentration in different parts of the plant when interpreting the results.

The kinetic analysis (Fig. 4a) reveals essential information about the diffusive release rate of TPC over the extraction time. The first five minutes in all assays are characterized by a progressive increase in TPC recovery, with recovery peaks observed at different times depending on the treatment. For example, in treatment SB8, a significant recovery is observed at 30 min, reaching 71.11% of extractable TPC at 35 min. Considering the energetic aspects, this indication may guide decisions regarding the need to conduct the extraction process until the 60-minute mark.

Figure 5 presents the contour curve and the pareto chart of the study. The Pareto chart demonstrates the influence of temperature in its linear and quadratic factors on TPC responses and the pH in its linear factor, all with $p<0.05$. The contour plot shows that the highest TPC recovery was achieved at higher temperatures in the study and noticed at less acidic pH and neutrality. The assessment of TPC yield within the experimental design led to the derivation of the following model (Eq. 5).

$$\begin{aligned} TPC = &-0.8 - 0.163 \cdot (Temperature) \\ &+ 3.04 \cdot (pH)\ 0.001449 \cdot (Temperature^2) \\ &- 0.165 \cdot (pH^2)\ 0.0025 \cdot (Temperature * pH)\,, (R^2 = 0.8381) \end{aligned} \quad (5)$$

Antioxidant activity by FRAP assay

Antioxidant capacity by FRAP assay, measures the capacity to reduce ferric iron (Fe^{3+}) [45]. The results of the kinetic extractions are depicted in Fig. 6. It is noteworthy that the SB1 exhibited the highest antioxidant response, with 45.19 µmol TEAC g^{-1}, closely followed by SB3, which recorded 43.83 µmol TEAC g^{-1}. Both treatments share the exact temperature of 130 °C. Conversely, the highest temperature treatment, SB8, did not yield an extract with more significant antioxidant capacity. This result suggests that the higher temperatures employed in this extraction may have led to the degradation of compounds with antioxidant potential. It is important to emphasize that the antioxidant capacity is not directly related to the total phenolic compounds (TPC) obtained. As in the work by Ferreira et al.. 2023 [18], in the present study, the treatment that used a higher temperature obtained a greater amount of TPC, and the treatments with slightly lower temperatures obtained greater antioxidant activity by the FRAP method.

When evaluating the extraction kinetics, the behavior depending on the treatment. However, all treatments exhibit an initial increase in the levels of extracted antioxidant compounds. This is followed by a reduction in recovery due to raw material depletion. This observation can be verified by forming a plateau in the final stages of extraction. For instance, in the treatment with the highest recovery of compounds with antioxidant activity, SB1, it was observed that approximately 67% of the total compounds were extracted within 40 min. This leads to a consideration of the technical-economic feasibility of extending the extraction process to 60 min. Studies on extraction times in other research support this analysis. For example, Ramirez-Melo et al. (2023) [46], using ultrasound in the treatment of beet juice, found that, with the amplitude set at 80%, increasing the extraction time from 7 to 10 min resulted in only a 2% (w/v) increase in yield. Furthermore, after 14 min of extraction, a degradation of about 11% (w/v) was observed compared to the yield obtained at 7 min. These data indicate that extending the extraction time beyond a certain point may not be advantageous, as the marginal gain in compound recovery is minimal and may be accompanied by significant degradation of the compounds with antioxidant capacity, especially in batch processes.

Figure 7 presents the contour plot and the Pareto chart of the experimental design. As observed in the Pareto chart, only temperature had a significant response in the recovery of compounds with antioxidant activity ($p<0.05$). This is also evident in the contour plot, where higher temperatures yield higher yields. A regression equation was generated to predict the yield of compounds with antioxidant activity (Eq. 6).

$$\begin{aligned} FRAP = &33.8 - 0.091 \cdot (Temperature) \\ &- 8.4 \cdot (pH) + 0.00206 \cdot (Temperature^2) + 0.675 \cdot (pH^2) \\ &+ 0.0067 \cdot (Temperature * pH)\,,\ (R^2 = 0.8538) \end{aligned} \quad (6)$$

Colors parameters

Figure 8 shows the color of the extracts obtained in the 11 treatments using the subcritical water extraction process, in which this parameter can show the time of highest recovery of betacyanin through a given color. The extraction

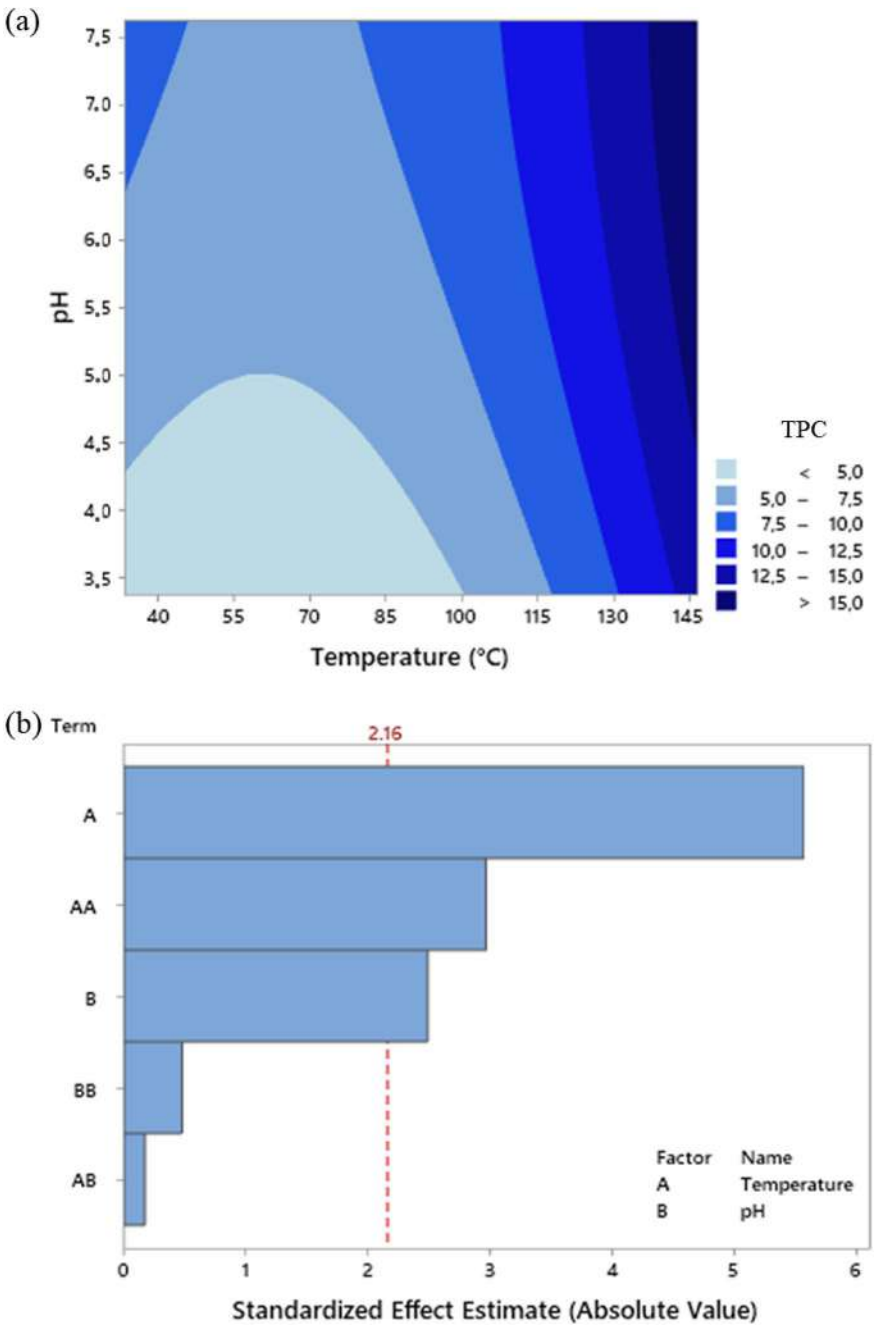


Fig. 5 The influence of temperature and pH on the antioxidant capacity of beet powder extracts. **a** TPC contour curve; **b** TPC pareto chart

of biocompounds in the treatments with lower temperatures (SB2, SB7, and SB9) occurred on average in the first 25 min, in which the presence of phenolic compounds is evidenced by the pink color of the extracts [47]. Treatments SB8, SB3, SB6, and SB1, which used temperatures above 90 °C showed greater performance in obtaining betacyanins and an intense color (darker). This is indicative of the existence of a relationship between saturated coloration and more efficient recovery of betacyanins, as observed in the work by Ferreira et al.. (2023) [18]. The treatments show a yellowish color transition, which may indicate the existence of compounds related to hydrolysis [28]. After 35 min, the extracts showed a reduction in color intensity, which may indicate that the extraction process had ended, as seen in works such as Maciel-Silva et al. (2022) [14].

The SB8 treatment showed a change in color between the 25 and 35 min times, exhibiting a brown color. This coloration may be related to Maillard reaction products, which are associated with processes that use high temperatures [48, 49]. Similar results were found in studies such as Maciel-Silva et al. (2022) and Jimenez Moreno et al. (2024) [47, 50]. They observed that treatments with stronger colors

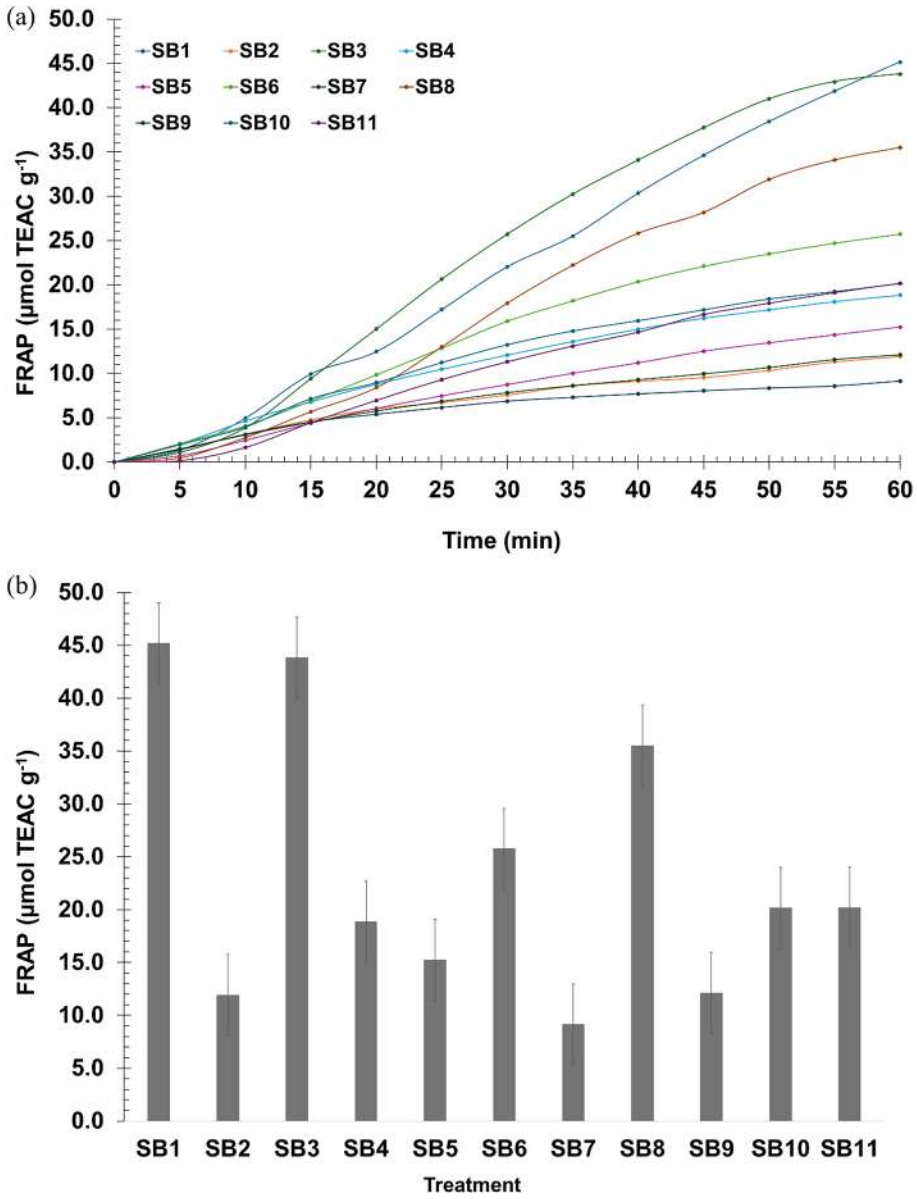


Fig. 6 Antioxidant activity of samples obtained from beet powder by the FRAP method using subcritical water extraction. **a** FRAP; **b** FRAP accumulated

showed a production of reducing sugars. Treatment SB1 and SB8 showed the highest reducing sugar production, mainly around the 35 min mark. Similar results were found for beer waste [51], jabuticaba peel extracts [17], and pitaya peel [18].

The CIELab color parameters (h°, b*, L*, a*) of the extracts obtained through hydrothermal treatment are shown in Table 3. The low-temperature treatments, SB2, SB7, and SB9, showed luminosity values (L*) ranging from 50.98 to 87.78 after 15 min. According to the work of Lee Kwan Yee et al. (2023) [52], these values may indicate that the color of the extracts using lower temperature treatments is closer to white. The treatments carried out at higher temperatures, SB1, SB3, and SB8 showed a high variation (between 14.60 and 80.23). This indicates a relationship between the rise in temperature and the fluctuation in luminosity. In addition, the treatment obtained using the highest temperature, SB8, showed a variation in luminosity between 18.15 and 38.45 between minutes 10 and 40. This indicates a relationship between the high temperature and luminosity closer to black.

The highest values found for the a* parameter (green (-) to red (+)) were during the first 15 min of all the treatments, showing a generally redder color during this period. Furthermore, in general, during the first 15 min of the treatments, he highest yield of betacyanins was obtained, which may indicate a relationship between these aspects [53]. The highest peak in the a* color parameter (64.72) was in the SB1 treatment, demonstrating a relationship between high temperatures and an increase in the parameter. In addition,

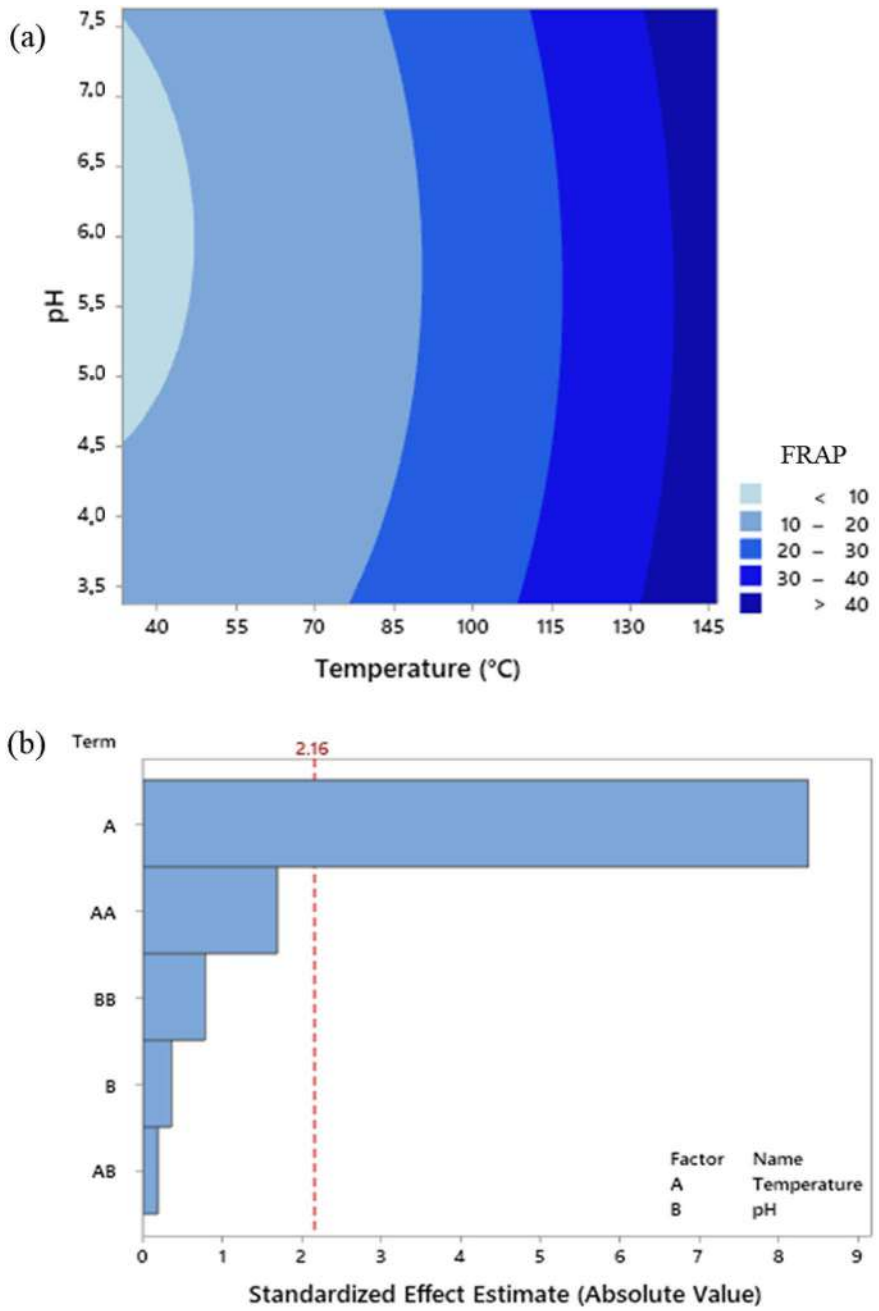


Fig. 7 The influence of temperature and pH on the antioxidant capacity of beet powder extracts. **a** FRAP contour curve; **b** FRAP pareto chart

this increase in the a* parameter at high temperatures may be indicative of Maillard reaction or phenolic compounds oxidation in the extract [54].

The b* parameter is characterized by a variation in color between blue (-) and yellow (+). When evaluating the b* parameter, it was found that the treatment with the lowest temperature (SB1) showed the lowest peak of this parameter (2.75) among the treatments. This indicates a relationship between low temperatures and a decrease in this parameter. It was observed that the highest peak of the b* parameter (73.76) occurred in the treatment with the highest temperature (SB8), indicating that as the temperature increased, there was a change in the color of the extract, from reddish to yellowish, in line with what was observed in Fig. 8 [55].

The hue angle (h°) corresponds to a coloration that varies between red (0°), yellow (90°), green (180°) and blue (360°). For low temperatures, such as the SB2 treatment, the h° angles varied between 6.59 and 34.94. This suggests that the extract is reddish, as shown in Fig. 8. The three treatments in which high temperatures were applied, SB1, SB3, and SB8, showed a variation in the h° angle between 28.09 and 77.11, 26.48 and 83.67, and 20.38 and 77.56, respectively. This result indicates that the samples color varies between red and yellow, which is evident in Fig. 8.

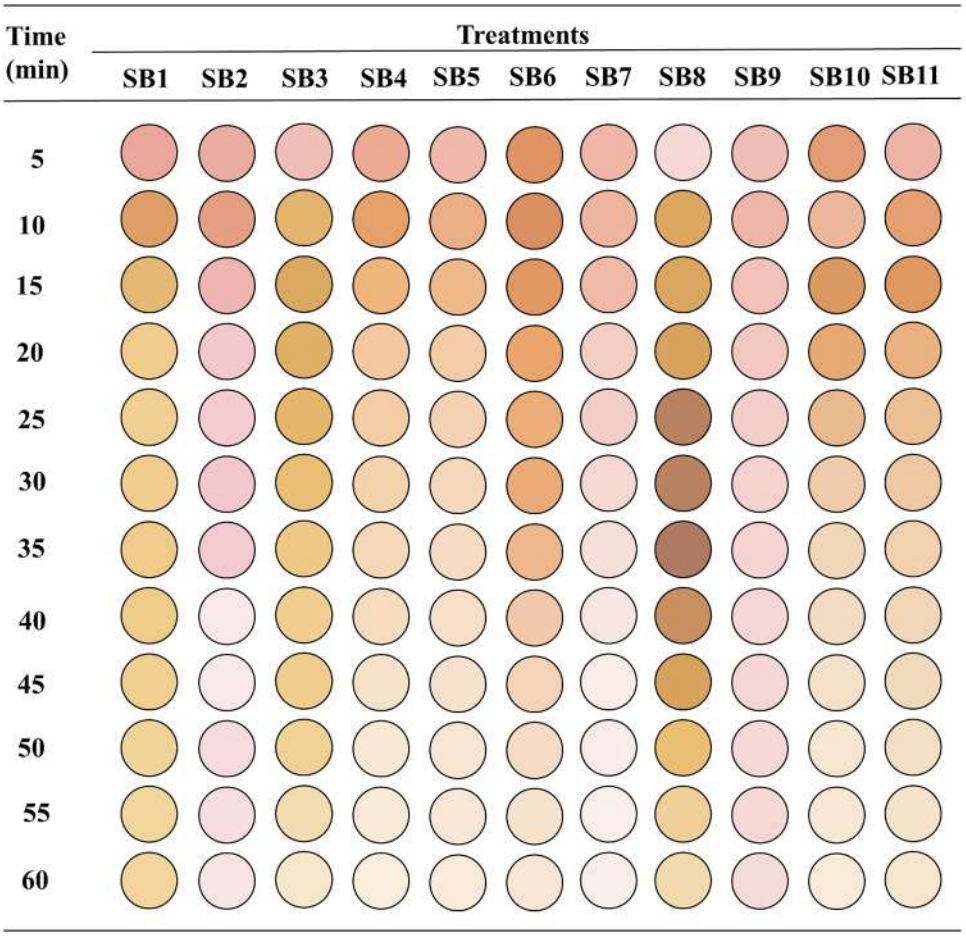


Fig. 8 Reproduction of the colors of the respective extracts obtained from beet powder

pH

Based on the observations outlined in Fig. 9, it can be inferred that the extracts pH generally remains stable throughout the kinetics, with some exceptions observed in treatments SB3 and SB8. Specifically, for the SB3, there is a noticeable alkaline shift in the pH of the extract after 50 min of extraction, reaching a value of 9.74 by the 60-minute mark. Conversely, in the case of treatment SB8, the pH initially tends towards acidity (5.50) during the first 30 min of extraction, which may be attributed to the extraction of various acids at elevated temperatures [56], such as phenolic acids [57]. Additionally, the rise in temperature is likely to lead to increases in the ionic product of water (Kw), which could contribute to the initial decrease in pH observed within the first 30 min of extraction [58]. Conversely, starting from minute 35, there is a notable increase in pH, ultimately resulting in alkalinity (pH 9.51) by the end of the extraction process. This phenomenon could be attributed to Maillard reaction product which are induced by both the elevated temperature and prolonged extraction times [18]. Similar pH behavioralso observed in extracts of *Sargassum thunbergii* [56] and pitaya peel [18].

Sugars and 5-hydroxymethylfurfural (5-HMF)

Figure 10 shows the kinetics and accumulation of glucose and fructose, the sugars that showed the best results in the beet extract. Figure 10a-b shows that the best results for glucose and fructose were obtained through the SB3 treatment, with 1.76 mg g^{-1} and 1.11 mg g^{-1}, respectively. The work of Amo-Mateos et al.. (2023) [59] with beet extract using microwaves obtained similar values for glucose (1.90 mg g^{-1}) using similar temperature conditions (160 °C). These values were higher than those found for some beet varieties, such as Redval (1.26 mg g^{-1} for glucose and 0.58 mg g^{-1} for fructose) [60]. The work of Duyar et al. (2024) [61] to produce beet juice, both treatments obtained lower glucose peaks (0.25 mg g^{-1} and 0.07 mg g^{-1}) than the present study and similar fructose peaks (3.50 mg g^{-1} and 3.80 mg g^{-1}). Furthermore, as with Wruss et al. (2015) [60], the glucose values were higher than the fructose values, as shown in Fig. 10c.

5-Hydroxymethylfurfural (5-HMF) is characterized by being a compound that inhibits fermentation, its origin is directly linked to high temperatures, such as the degradation of sugars (mainly glucose and fructose) [62]. These

Table 3 Color parameters of extracts obtained from beet powder through the subcritical water extraction process

Parameters	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11
*L**											
5 min	44.17	46.03	56.30	46.50	53.04	33.07	51.51	72.53	56.45	37.10	49.98
10 min	36.38	38.27	45.21	39.24	46.23	29.31	50.98	38.45	52.21	50.29	38.61
15 min	47.76	51.22	38.49	49.58	52.27	33.65	54.07	37.20	57.49	32.72	33.88
20 min	61.18	62.73	42.23	60.44	62.75	41.42	64.45	35.00	62.46	41.18	46.40
25 min	63.22	65.35	46.84	63.79	67.30	45.26	65.93	18.15	66.18	51.18	54.29
30 min	61.47	63.00	52.01	67.07	70.36	44.61	71.56	18.34	68.05	61.48	59.42
35 min	59.60	63.92	58.92	70.73	72.92	50.31	76.45	25.82	69.97	67.50	64.21
40 min	60.11	83.99	62.15	73.07	75.14	61.16	81.56	35.12	71.05	72.17	67.95
45 min	62.66	83.80	61.27	77.00	77.34	67.91	86.45	51.82	71.08	75.73	70.20
50 min	65.66	74.13	64.22	81.65	79.55	73.04	85.74	62.43	72.51	79.35	74.57
55 min	67.89	76.35	72.15	83.53	81.50	77.58	87.78	70.41	71.93	81.00	76.43
60 min	67.06	79.83	80.23	85.24	83.31	80.51	86.70	14.60	73.83	83.46	79.15
*a**											
5 min	64.72	60.63	54.09	63.61	56.24	60.63	58.43	36.79	54.12	61.14	57.02
10 min	47.64	61.47	29.50	56.33	54.28	55.99	56.78	37.91	58.06	47.79	55.93
15 min	27.50	61.99	32.20	46.13	44.84	56.84	53.70	35.55	52.24	45.93	51.11
20 min	20.31	51.35	29.62	36.46	35.09	55.36	39.78	35.68	48.47	46.08	43.05
25 min	18.82	49.45	28.65	30.49	30.40	53.03	40.21	33.93	44.33	38.05	37.51
30 min	20.79	51.82	25.86	27.04	27.23	51.93	35.35	34.63	42.66	30.12	31.37
35 min	21.90	50.58	21.51	23.42	24.45	48.57	28.00	36.80	40.30	24.93	26.14
40 min	21.29	23.38	19.28	20.77	21.56	37.79	20.73	36.16	38.90	20.02	21.76
45 min	19.12	23.54	20.33	16.88	19.01	29.32	16.28	27.36	37.32	16.52	19.00
50 min	17.03	37.38	18.04	12.42	16.64	22.96	16.90	18.53	36.14	13.34	16.29
55 min	15.33	34.96	10.91	10.42	14.64	18.21	14.63	12.41	34.51	11.18	13.81
60 min	16.41	29.62	4.70	8.63	12.65	14.81	14.95	32.24	31.26	9.13	11.95
*b**											
5 min	34.54	33.24	26.95	41.97	31.95	52.86	35.24	13.67	27.66	50.24	30.71
10 min	56.92	42.95	66.04	60.15	52.12	48.84	38.89	63.05	33.41	42.90	53.82
15 min	67.98	24.64	62.90	64.11	58.76	55.49	35.64	62.06	27.68	54.03	55.98
20 min	69.73	13.17	66.85	54.70	50.64	62.50	25.25	58.66	22.37	59.00	57.70
25 min	69.10	9.84	71.09	55.73	45.99	60.52	22.24	30.68	18.68	53.71	54.02
30 min	70.08	11.06	73.31	50.85	41.62	58.85	18.24	31.01	16.14	46.82	49.37
35 min	71.99	10.29	72.00	46.62	38.49	53.63	15.92	43.73	14.52	41.87	46.20
40 min	72.69	2.76	69.85	42.79	35.98	45.97	11.57	58.86	13.58	37.89	41.82
45 min	70.92	2.75	70.77	37.96	33.62	40.34	9.37	73.76	13.92	34.87	40.41
50 min	69.68	5.55	68.41	31.65	30.90	35.31	8.70	64.85	13.01	31.76	36.83
55 min	66.98	4.71	56.45	29.08	28.20	30.96	7.65	56.27	16.24	29.78	35.50
60 min	66.84	3.42	42.35	26.57	25.86	27.96	8.16	24.91	13.32	27.61	32.77
h°											
5 min	28.09	28.73	26.48	33.42	29.60	41.08	31.09	20.38	27.07	39.41	28.31
10 min	50.07	34.94	65.93	46.88	43.84	41.10	34.41	58.98	29.92	41.91	43.90
15 min	67.98	21.68	62.89	54.26	52.65	44.31	33.57	60.19	27.92	49.63	47.60
20 min	73.76	14.38	66.10	56.31	55.28	48.47	32.40	58.69	24.77	52.01	53.27
25 min	74.76	11.25	68.05	61.32	56.53	48.77	28.95	42.12	22.85	54.68	55.22
30 min	73.48	12.05	70.57	62.00	56.81	48.57	27.29	41.84	20.72	57.25	57.57
35 min	73.08	11.50	73.37	63.33	57.58	47.83	29.62	49.92	19.81	59.23	60.50
40 min	73.68	6.73	74.57	64.11	59.07	50.58	29.17	58.44	19.24	62.15	62.51
45 min	74.91	6.66	73.97	66.03	60.51	53.99	29.92	69.65	20.46	64.65	64.82
50 min	76,27	8.45	75.23	68.57	61.70	56.97	27.24	74.05	19.80	67.22	66.14
55 min	77.11	7.67	79.06	70.29	62.56	59.54	26.60	77.56	25.20	69.42	68.74
60 min	76.21	6.59	83.67	72.01	63.93	62.09	28.63	37.69	23.08	71.70	69.96

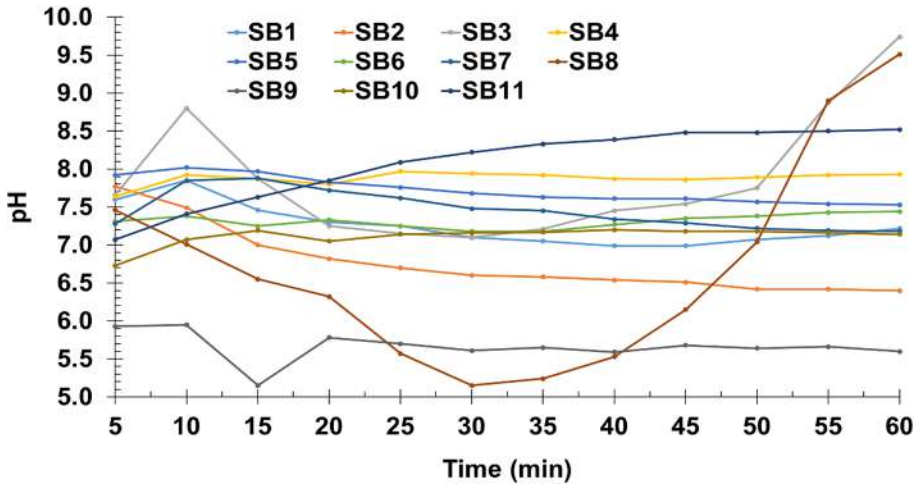


Fig. 9 pH of extracts obtained from the subcritical water extraction process of beet powder

characteristics are evidenced by the fact that 5-HMF was only found in the treatment with the highest temperature SB8, equivalent to work of Abdilla-Santes et al. 2019 [63], in which 5-HMF was obtained from beet extract using a two-phase reactor at high temperatures (150 °C). In addition to temperature, the formation of 5-HMF is related to time, which may explain why the highest values are found in the final extraction times, similar to work of Rocha et al. 2021 [64], where the highest yield of 5-HMF from beet occurred at high temperatures (170 °C) and over prolonged times using microwave heating and subcritical water conditions. In addition to temperature, the formation of 5-HMF is related to time, which may explain why the highest values are found in the final extraction times. 5-HMF also has antioxidant activity [65], which may be related to SB8 being one of the treatments with the highest antioxidant activity. In addition, 5-HMF is related to foods rich in sugar [66]. This is evidenced by the amount of sugars identified in the SB8 treatment. The formation of 5-HMF is related to brown pigments [62]. This is shown in Fig. 8, specifically at 35 min when the 5-HMF peak is obtained in SB8 (Fig. 11) and shows a brown pigment. SB8 was extracted at a higher temperature (146.56 °C) compared to SB1 and SB3 (130 °C). At such elevated temperatures, reducing sugars like glucose and fructose undergo degradation through pathways such as the Maillard reaction or caramelization, leading to the formation of compounds like 5-HMF. This explains why SB8 shows lower levels of glucose, fructose, and total reducing sugars despite being processed for the same duration. The sugars are consumed in reactions to produce 5-HMF and other intermediates.

Figure 12a illustrates the quantification of reducing sugars during the extraction kinetics of the treatments. Treatments SB1 and SB8 presented the highest values of reducing sugars, (172.97 mg g^{-1}) and (172.58 mg g^{-1}), respectively. Furthermore, it was observed that an extraction temperature of 50 °C yielded a lower response, indicating that higher extraction temperatures have a beneficial effect on sugar extraction when using subcritical water, as suggested by Rambabu et al. (2022) [67]. This is attributed to the fact that an increase in temperature decreases water's surface tension and viscosity while increasing its diffusivity. These factors collectively lead to greater mass transfer, resulting in improved extraction of the sugars present in the sample [68].

Additionally, Fig. 12b illustrates the total reducing sugars during the extraction kinetics. It was observed that the highest values were identified in treatments SB1 and SB3, with 339.25 mg g^{-1} and 316.14 mg g^{-1}, respectively. Consequently, it can be concluded that treatment SB9 does not facilitate the release of reducing sugars or total reducing sugars. Furthermore, Fig. 12c depicts the accumulated values of reducing sugars and total reducing sugars. Once again, it is evident that treatments with higher temperatures promoted the release of sugars in the beet pulp.

In-line system

Figure 13 shows the inline process monitoring using the optimum conditions (146.56 °C; pH 7.62) to extract betacyanins using subcritical water. Using the photodiode array (PDA) detector, it was possible to obtain the absorbances of the betacyanins in real time at the time of extraction, and then, using Eq. 1, the content of betacyanins present was obtained. The betacyanin yield obtained using the optimum conditions (13.02 mg g^{-1}) was similar to the theoretical value (12.90 mg g^{-1}). Figure 13 shows that the absorbance peak at 535 nm occurred approximately 15 min after the start of the extraction process and that after 20 min, the values dropped, showing that the betacyanin concentration reduced. This indicates that approximately 80% of the betacyanins have already been extracted. Even if it is possible to extract certain quantities of betacyanins, it is not worth

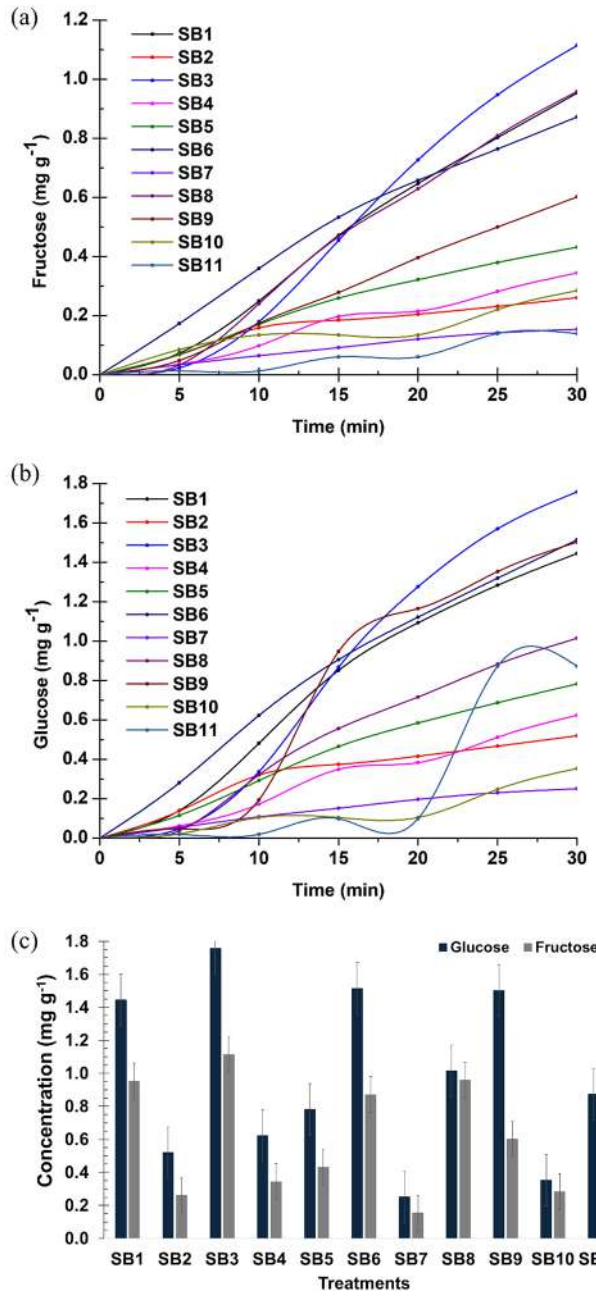


Fig. 10 Production of monosaccharides of beet powder obtained from high-pressure hydrothermal processing. **a** Fructose; **b** glucose; **c** accumulated glucose and fructose

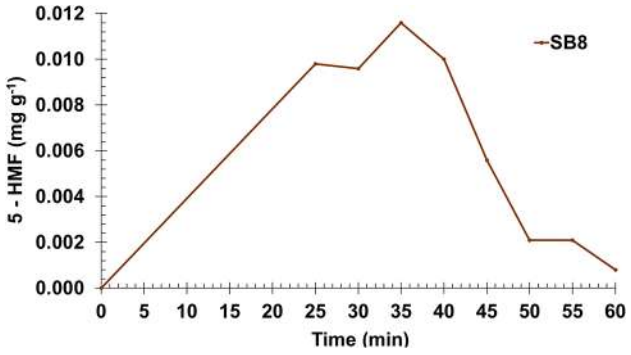


Fig. 11 5-HMF obtained from beet powder using subcritical water extraction

continuing the process after this time. This is because the amount of betacyanin extracted after this period does not cover the operating costs.

This corresponds to the phases of an extraction kinetic curve: constant extraction rate phase (CER), falling extraction rate phase (FER), and diffusion-controlled phase (DC). During the constant extraction rate phase (CER), the most accessible solute is extracted at an almost constant rate. FER is characterized by a period in which the extraction rate decreases considerably due to the reduction of the mass transfer surface, and diffusion begins to be relevant to the process. During the diffusion-controlled phase (DC), the most accessible solute range is consumed, thus characterizing a slow stage of the extraction period [69].

The work of Sanches et al.. (2024) [70] to obtain hesperidin from an industrial citrus lime residue using HIUS-assisted PLE coupled in-line with SPE (HIUS-PLE-SPE) and on-line with HPLC-UV portrayed a similar process. CER is observed from the start of the process until approximately 40 min. FER characterizes the period in which the extraction of hesperidin by diffusion began and is observed from 40 min to approximately 90 min. DC, diffusion extraction, is observed from 90 to 120 min. In the study carried out by Strieder et al.. (2024) [71] using PLE coupled in-line with SPE and on-line with HPLC-PDA (PLE-SPE × HPLC-PDA) to obtain bioactive compounds from a coffee coproduct, the phases of the extraction kinetic curve were observed. There was the CER period of up to approximately 20 min, where the compounds are extracted by convection at an almost constant rate. The FER phase takes up to 45 min, characterized by the period in which the extraction rate decreases rapidly due to the reduction in the mass transfer area and the start of diffusion. Finally, the DC period, portrayed by diffusion extraction, is seen in the final curve. As in the work of Bhagya Raj et al. (2020) [72], which used ultrasound-assisted extraction (UAE) on dragon fruit peel to obtain phytocompounds. The decrease in polyphenol yield after 20 min was related to a reduction in the diffusion rate. This was due to two factors: an increase in the diffusion distance and a reduction in the diffusion area, corresponding to a longer exposure time. In the work of [73], this observation is supported by the fact that after 45 min of extracting phenolic compounds from millet fermented with *Aspergillus awamori*, no significant increase was noted. This can be substantiated by Fick's second law of diffusion, which states that "the final equilibrium will be reached between the solute concentrations in the solid matrix (plant matrix)

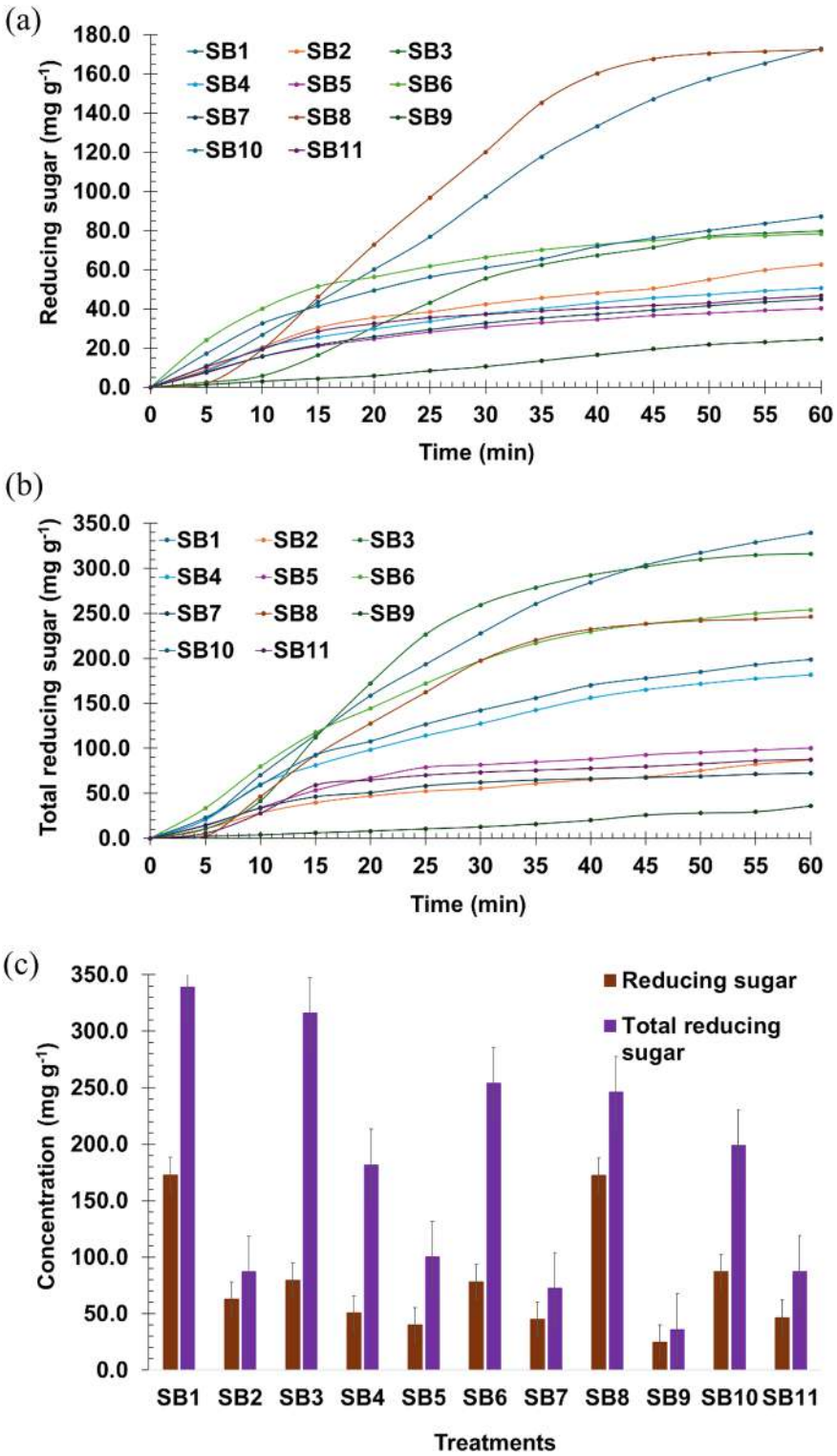


Fig. 12 Production of reducing and total sugars from beet powder obtained from subcritical water extraction. **a** Reducing sugars; **b** total reducing sugars; **c** accumulated reducing sugars and total reducing sugars

and in the bulk solution (solvent) after a period". In other words, longer extraction times are irrelevant for extracting more compounds.

In addition, it is worth noting that because the inline system is automated, it makes it possible to monitor the process simultaneously, collect simple data, and reduce possible errors related to the offline method [15].

Table 4 compares phenolics and antioxidant activity, as well as the content of 5-HMF and sugars. The results showed that SB8 had the highest phenolic content (20.49 mg GAE g^{-1}) but moderate antioxidant activity (35.51 µmol TEAC

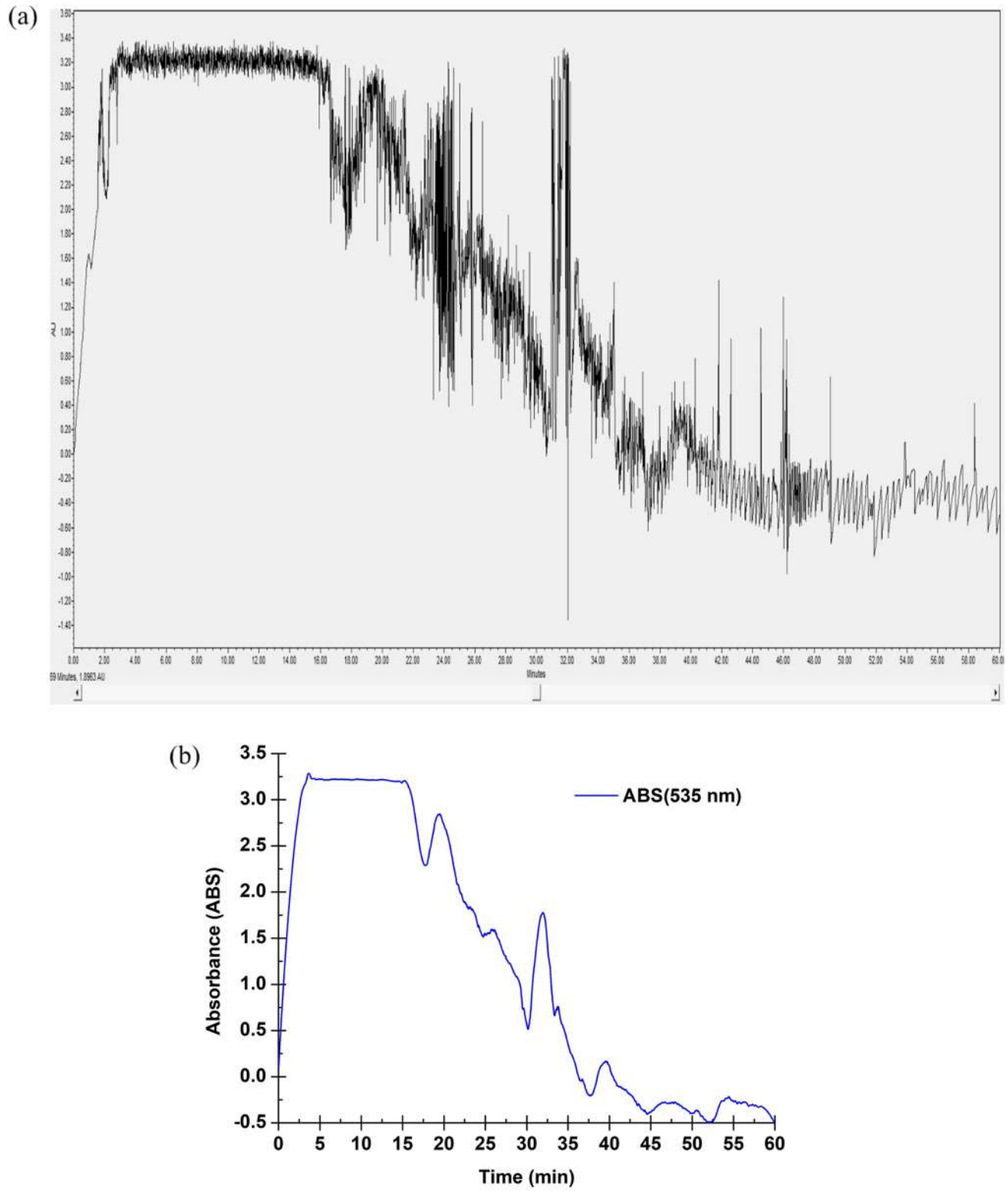


Fig. 13 In-line process monitoring using the best conditions for recovering betacyanins

Table 4 Values of phenolics, antioxidant activity, and also the content of 5-HMF and sugars in different Raw materials

Parameter	Beet study (SB1, SB3, SB8)	Study Jabuticaba fruit [29] (better condition)	Study Pitaya fruit [48] (better condition)	Study Cambuci fruit [50] (better condition)	Comparison/notes
Phenolic Content (mg GAE g^{-1})	SB1: 7.13 SB3: 9.45 SB8: 20.49	0.75	41.71	46.40	SB8 shows comparable values; slightly lower than Jabuticaba fruit
Antioxidant Activity (µmol TEAC g^{-1})	SB1: 45.19 SB3: 43.83 SB8: 35.51	10.25	46.00	118.07	SB1 shows superior antioxidant activity compared to Jabuticaba fruit.
Reducing Sugars (mg g^{-1})	SB1: 172.97 SB3: 79.59 SB8: 172.58	369.44	18.09	132.54	Higher sugar retention at 130 °C than Pitaya and Cambuci fruit; SB8 shows sugar degradation.
5-HMF Content (mg g^{-1})	SB1: ND SB3: ND SB8: 0.05	37.06	-	0.87	SB8's higher 5-HMF correlates with elevated extraction temperature.

g^{-1}), suggesting degradation of thermolabile compounds at 146.56 °C. In contrast, SB1 and SB3, extracted at 130 °C, retained higher reducing sugar content (172.97 and 79.59 mg g^{-1}, respectively) and showed superior antioxidant activity (45.19 and 43.83 µmol TEAC g^{-1}). The formation of 5-HMF in SB8 (0.0517 mg g^{-1}) underscores the impact of elevated temperatures on sugar degradation, consistent with findings by Alibekov et al. (2023) [42] and Choudhary et al. (2021) [66]. These results highlight the trade-off between sugar retention and phenolic recovery, emphasizing the importance of optimizing extraction conditions based on the desired application.

The in-line monitoring capability enables real-time process optimization and control, facilitating the transition from laboratory scale to industrial-scale production. This scalable approach holds significant promise for the cost-effective and sustainable production of high-quality beet extracts for various applications. The compound stability is emphasizes the importance of lower extraction temperatures to minimize thermal degradation and maintain the stability of the extracted compounds. About scalability the study mentions the in-line monitoring capability as a key factor in facilitating the transition from lab-scale to industrial-scale production.

Conclusion

In conclusion, this study demonstrated the exploratory novelty of incorporating an in-line detection system to monitor the extraction process in real time, providing instant data on the extracted compounds. The research evaluated the efficiency of extracting compounds from beet using subcritical water extraction (SWE) through an experimental design varying temperature and pH and the impact of in-line detection on process optimization. The results indicated that the extraction of biocomposites in treatments with lower temperatures (33.43 °C and 50.00 °C) occurred rapidly after 25 min.

Extractions with higher temperatures (130.00 °C and 146.56 °C) showed greater efficiency in obtaining betacyanins (red-violet extract) and phenolic compounds accumulated under the conditions of 146.56 °C and pH 5.50. The highest yield of TPC (20.49 mg GAE g^{-1}) was obtained at 146.56 °C and pH 5.50. Treatment at 130.00 °C and pH 7.00 revealed a superior oxidant response in the 45.19 µmol TEAC g-1 value. The highest concentrations of glucose and fructose were obtained at 130.00 °C and pH 7.00, with 1.76 mg g^{-1} and 1.11 mg g^{-1}, respectively.

It is concluded that the in-line process monitoring, using the optimal conditions of 146.56 °C, absorbance at 535 nm, and pH 7.62, allowed for a high recovery of betacyanins (13.02 mg g^{-1}), in which, among the 60 min evaluated, 20 min were sufficient to recover approximately 80% of betacyanin. In this way, SWE incorporated into online process monitoring using the PDA proved to be a promising and sustainable resource for obtaining high value-added compounds, as it showed a reduction in process time and raw material degradation. Therefore, complementary analyses, such as mass spectrometry for compound quantification and stability and toxicity assessments of betacyanins and phenolic compounds, could be performed to provide significant information that allows a technical-economic analysis to be carried out to know its potential applicability in the pharmaceutical and food industries since a short extraction period saves time and money.

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Author contributions L.F.M.: Methodology, Data curation, Formal Analysis, Writing - Original draft preparation. J.A.J.M: Formal Analysis, Writing - Reviewing and Editing. T.L.C.T.B.: Formal Analysis, Writing - Reviewing and Editing. L.S.C.: Formal Analysis, Writing

- Reviewing and Editing. M.A.R.: Supervision. T.F.C.: Conceptualization, Methodology, Writing - Reviewing and Editing, Supervision.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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