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RESEARCH PAPER

Ultrasound treatment to enhance functional and antioxidant properties of beetroot residue

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Abstract

The valorization of agro-industrial residues through sustainable technologies is a promising strategy for enhancing the nutritional and functional quality of food ingredients. In this study, ultrasound extraction was applied to beetroot (*Beta vulgaris* L.) residue to improve its functional properties, antioxidant capacity, and *in vitro* bioaccessibility. Using a central composite design and response surface methodology (RSM), the effects of sonication amplitude (70–85%) and time (8–13 min) were optimized. The optimal conditions (77.5% amplitude, 9 min) significantly improved water retention capacity (12.20 g/g), oil holding capacity (10.21 g/g), and the extraction of bioactive compounds, including betanins (3327.08 mg BE/100 g dw), betaxanthins (2381.34 mg BE/100 g dw), and total phenolic content (496.95 mg GAE/100 g dw). Antioxidant activity assessed via ABTS, DPPH, FRAP, and chelating assays was also enhanced. Ultrasound treatment reduced microbial load and induced structural modifications, as observed by scanning electron microscopy, which contributed to increased fiber content and altered physicochemical properties. *In vitro* digestion assays revealed an improvement in the bioaccessibility of betalains, though phenolic compounds showed moderate losses, possibly due to degradation during processing. These results underscore the potential of ultrasound as an environmentally friendly and effective method for the revalorization of beetroot residue, supporting its incorporation into functional food formulations.

Keywords

Bioactive compounds, Food waste valorization, *In vitro* bioaccessibility, Response surface methodology (RSM)

Introduction

Beetroot (*Beta vulgaris* L.), a member of the Chenopodiaceae family, is a root vegetable characterized by a bulbous, thickened stem that serves as a storage organ, for sugars and starches. Typically the plant reaches an average height of one meter, and exhibits a dense texture, and exhibits a purple-red color (El-Wahab et al. 2022). Its nutritional profile includes carbohydrates (9.96 g/100 g), proteins (1.68 g/100 g), fats (0.18 g/100 g), dietary fiber, and micronutrients such as vitamins A, C, and B9, and minerals like phosphorus, potassium, calcium, sodium, copper, zinc, manganese, magnesium, and iron. Additionally, beetroot is a rich source of bioactive compounds including flavonoids, phenolic acids, carotenoids, and its natural pigments, betalains-classified into betanins (violet-red) and betaxanthins (orange-yellow) (Székely and Máté 2022). Many of these phytochemicals are closely associated with dietary fiber, which, as defined by the American Association of Cereal Chemistry Fiber Committee (2001), comprises plant-based components that resist digestion and absorption in the small intestine and undergo partial or complete fermentation in the large intestine.

The functional properties and nutritional versatility of beetroot have led to its application in various food products. Recent studies have explored its incorporation into formulations such as instant cream soups, highlighting improvements in sensory properties, nutritional value, and antioxidant capacity (Angkasa et al. 2020). Furthermore, beetroot is commonly processed into products such as pickles and juices (Chhakira et al. 2019; Akan et al. 2021). Although evidence suggests that pickling can significantly alter its nutrient content, particularly betalains and polyphenols, underscoring the importance of optimizing processing techniques to preserve its functional constituents (Srivastava and Singh 2016). In agricultural contexts, beetroot productivity has been investigated under diverse environmental conditions, including saline arid soils, which not only affect yield but may also influence the nutritional and phytochemical profile of the roots (Nadaf et al. 2000).

Despite its broad use in the food system, the potential of beetroot residue remains underutilized. These by-products, often discarded or used as natural colorants in products such as tomato paste, jams, sauces, and cereals, still retain considerable quantities of bioactive compounds including polyphenols, vitamins, carotenoids, and dietary fiber (Constantin et al. 2025; Stoica et al. 2025). To harness these compounds effectively, various extraction methods involving solvents, agitation, heat, and extended time have been developed. However, efficient recovery must balance extraction efficiency, product quality, economic feasibility, and environmental sustainability. As such, innovative and green extraction technologies have gained traction, driven by consumer preferences for eco-friendly, chemical-free processes and industry-wide goals for sustainable practices (Kumar-Jha and Sit 2022).

Among these, ultrasound extraction has emerged as a promising non-thermal technology. It operates through acoustic cavitation—rapid formation and collapse of microbubbles—that disrupts plant cell walls and facilitates the release of intracellular compounds. Ultrasoun extraction has been successfully employed to extract antioxidants from various plant-based residues. For example, high yields of phenolics and ascorbic acid have been reported in mango residues (Castañeda-Valbuena et al. 2021), and enhanced antioxidant activities (ABTS and DPPH) have been observed in residues from blackberry, pomegranate, and apple peels. Similarly, grape skins treated with ultrasound showed increased anthocyanin and phenolic content compared to conventional solvent extraction (Rifna et al. 2021).

The objective of this study was to evaluate the impact of ultrasound extraction on the microbial load, functional properties, fiber content, antioxidant activity, and *in vitro* bioaccessibility of beetroot residue. Additionally, optimal ultrasound processing parameters were identified, and the extraction efficiency was compared against conventional methods using water, ethanol, and hydroalcoholic solvents.

Materials and methods

Beetroot residue preparation

Beetroot (*Beta vulgaris* L. cv. *rubra*) was procured from a local market in Tulancingo, Hidalgo, Mexico with a maturity degree of 15° Brix. The juice was extracted using a Turmix Standard extractor (Mexico), and the remaining solid residue was collected. The residue was subsequently lyophilized, ground into a fine powder, and passed through a mesh sieve to achieve a uniform particle size of approximately 0.5 mm. The resulting powder was stored in sealed plastic bags, once the samples have been obtained, the analyses were in fresh, to the exception of antioxidants determination, these were stored at -32 °C for 2 days and subsequently the analyses were realized.

Ultrasound treatment

Beetroot powder (16 g) was suspended in 400 mL of distilled water and subjected to ultrasound treatment using an ultrasonic processor (VCX-1500, Sonics & Materials, Inc., Newtown, CT, USA) operating at 1500 W and a constant frequency of 20 kHz. The pulse sequence consisted of 4 s on and 2 s off, with an amplitude range of 70–85% and a sonication time between 8 and 13 min. An untreated sample was used as the control. Following sonication, all samples were immediately analyzed for microbial growth. The treated samples were then centrifuged, and the supernatant was utilized for the determination of antioxidants content and antioxidant activity, while the precipitate was used to assess functional properties such as water retention capacity (WRC) and oil holding capacity (OHC).

Experimental design using response surface methodology

The response surface methodology (RSM) provides some advantages over the traditional optimization designs, including experimental design, model fitting, model validation and condition optimization. The central composite design is a type of RSM experimental design that is highly convenient because it requires a smaller number of experimental runs (Chelladurai et al. 2021).

In the present study, the optimization of the ultrasound conditions was performed using a central composite rotatable design for two independent variables at five levels. The independent variables, amplitude (70–85%) and sonication time (8–13 min) were applied. The complete design consisted of thirteen combinations patterns, 4 factorial points, 4 axial points including five replicates of the center point (Table 1). Experimental data from the central composite design was analyzed using a response surface regression (JMP 7.0.2, SAS Institute Inc., 2007) fitted to a second-order polynomial model Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i} \sum_{j=i+1} \beta_{ij} X_i X_j \quad (1)$$

Where Y is the predicted response, β_0 the constant coefficient, β_i, the linear coefficient, β_{ii} the quadratic coefficient, β_{ij} is the cross product coefficient, X_i (amplitude, %) and X_j (time, min) are the independent variables and the determinations that fit the mathematical model ($R^2 \geq 0.90$). The Design Expert software (SigmaPlot 12.0, Institute Inc.) was used to obtain the three-dimensional shapes from the response surface analysis and superposition of the contour figures to show the optimal process condition.

Table 1. Nonrandiomized experimental design matrix.

Number	Pattern	Amplitude level (%) X_1	Time (min) X_2
1	a0	67	9
2	0A	77.5	15
3	+-	85	5
4	-+	70	13
5	00	77.5	9
6	00	77.5	9
7	0a	77.5	3
8	00	77.5	9
9	A0	88	9
10	++	85	13
11	00	77.5	9
12	--	70	5
13	00	77.5	9

Microbiological analysis

Serial dilutions of the samples were prepared in sterile peptone water and diluted up to 10^3. One milliliter of each dilution was transferred onto sterile Petri dishes containing the appropriate culture medium. Aerobic mesophilic bacteria were enumerated using plate count agar, incubated at 30 °C for 48 h (LSI-3016A, Labtech, Korea), while Enterobacteriaceae were determined using violet-red bile glucose (VRBG) agar, incubated at 37 °C for 24 h. The colony-forming units were counted and expressed as $\log^{10}$ CFU/mL of beetroot residue.

Water retention capacity and oil holding capacity

Water retention capacity (WRC) and oil holding capacity (OHC) were determined according to the methodologies described by Lajolo and Wensel (1998) and Robertson et al. (2000). A 250 mg sample was weighed into 15 mL centrifuge tubes, combined with 10 mL of distilled water or soybean oil, and vortexed for 10 min. The mixture was left to stand at room temperature for 24 h before centrifugation at 3400 rpm for 20 min (Hamilton Bell, New Jersey, USA). The supernatant was decanted, and the sediment was weighed. The supernatant weight was determined by difference. WRC and OHC were calculated using Eq. (2):

$$WRC \ or \ OHC \ (g/g) = Residue \ fresh \ weight - residue \ dry \ weight / residue \ dry \ weight \quad (2)$$

Betalains determination

The content of betanins and betaxanthins were determined according to Stintzing et al. (2002) with a reading at 538 nm and 480 nm respectively, using a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA). The absorbance was obtained to calculate the betalain concentration for each sample. The betalain content (BC) was calculated as Eq. (3):

$$BC \ (mg/L) = [(A \times DF \times MW \times 1000) \ / \ (e \times l)] \quad (3)$$

where A is absorbance, DF is the dilution factor, MW is molecular weight (550 g/mol for betanin and 308 g/mol for betaxanthin), e is the molar extinction coefficient (60,000 L/mol for betanin and 48,000 L/mol for betaxanthin in H_2O), and l is the path length of the cell (0.316 cm). The results were expressed as milligrams of betalain equivalents per 100 g of dry weight (mg BE/100 g dw).

Total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu method. A 100 µL sample was mixed with 500 µL of 1:10 diluted Folin-Ciocalteu reagent, followed by the addition of 400 µL of sodium carbonate (7.5%). The mixture was incubated at room temperature for 30 min, and absorbance was recorded at 765 nm using a microplate reader (Power Wave XS UV-Biotek, KC Junior software, USA). Gallic acid was used as the reference standard, and results were expressed as milligrams of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g dw) (Stintzing et al. 2005).

Chelating activity

The chelating activity of iron ions was assessed following the methodology of Gülcin et al. (2003). Briefly, 100 µL of sample were mixed with 50 µL of ferrous chloride (2 mM) solution and 450 μL of methanol were added, vortexed, and incubated for 5 min at room temperature. Subsequently, a 5 mM ferrozine solution (400 μL) was added, and the mixture was vortexed again and allowed to stand for 10 min. Ethylenediaminetetraacetic acid (EDTA, 0.1 M) served as the reference chelating agent, while deionized water was used as a control. Absorbance was measured at 562 nm using a microplate reader (PowerWave XS UV-Biotek, KC Junior software, USA). The chelating activity was calculated using Eq. (4):

$$Chelating\ activity\ (\%) = [(A_0 - A_1)\ /\ A_0] \times 100 \quad (4)$$

Where: A_0 = absorbance of the control sample. A_1 = absorbance of the sample.

Antioxidant activity assays

ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay: The ABTS radical cation (ABTS•+) was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate in the dark at room temperature for 16 h. The solution was diluted with deionized water to achieve an absorbance of 0.70 ± 0.10 at 754 nm with a microplate reader (PowerWave XS UV-Biotek, KC Junior software, USA). Then, 100 μL of sample and 900 μL of ABTS solution was added to an Eppendorf vial, vortexed and incubated at temperature room for 7 min. The extract was read to the same absorbance. Antioxidant capacity was expressed as μmol Trolox equivalents per 100 g of dry weight (μmol TE/100 g dw) (Kuskoski et al. 2005).

DPPH (1,1-diphenyl-2-picrylhydrazyl radical) assay: Radical scavenging activity was measured using an ethanolic solution (7.4 mg/100 mL) of DPPH. The sample (100 μL) plus 500 μL of DPPH solution was mixed and incubated at darkness during 1 h. The absorbance was measured at 520 nm in a microplate reader (PowerWave XS UV-Biotek, KC Junior software, USA). The results were expressed as μmol Trolox equivalents per 100 g of dry weight (μmol TE/100 g dw) (Morales et al. 2011).

FRAP (Ferric reducing antioxidant power) assay: The FRAP was conducted according to Benzie and Strain (1996). The FRAP solution (10:1:1) included acetate buffer (300 mM) at pH 3.6, TPTZ (2,4,6-tripyridyl-s-triazine) solution (10 mM) in 40 mM of HCl, and $FeCl_3 \cdot 6H_2O$ solution (20 mM) and was maintained at 37 °C during use. The sample (30 μL) was mixed with 90 μL of distilled water and 900 μL of FRAP solution, incubated at 37 °C for 10 min, and measured at 593 nm using to a microplate reader (PowerWave XS UV-Biotek, KC Junior software, USA). Results were expressed as μmol Fe(II) per 100 g dry weight (μmol Fe(II)/100 g dw).

Scanning Electron Microscopy (SEM)

Morphological changes in the beetroot residue before and after ultrasound-assisted extraction were examined using SEM. Samples were deposited on a silicon wafer and coated with a thin layer of gold (Denton Vacuum Desk V, Moorestown, NJ, USA) under a vacuum of 20 millitorr and a current of 20 mA for 4 min. Imaging was performed using a JEOL JSM-6300 scanning electron microscope (Peabody, MA, USA) at magnifications of 250× and 500×. Micrographs were captured to compare the structural integrity of the control and ultrasound-treated samples.

Total dietary fiber (TDF) determination

The TDF content, including soluble dietary fiber (SDF) and insoluble dietary fiber (IDF), was determined using the enzymatic-gravimetric method described by Horwitz and Latimer (2005). A Total Dietary Fiber Assay Kit (TDF-100A, Sigma) was used for the analysis. The sum of SDF and IDF was considered as the total dietary fiber content.

In vitro intestinal bioaccessibility

The bioaccessibility of antioxidant compounds was assessed using an *in vitro* digestion model followed by dialysis, based on the method described by Ramírez-Moreno et al. (2018). A 500 mg of lyophilized sample was homogenized in 20 mL of water and adjusted to pH 2.0 with 6 mol/L HCl. The sample was sequentially incubated with 120 μL of pepsin solution (40 mg/mL in 0.1 mol/L HCl) at 37 °C for 2 h, by 1.5 mL of by pancreatin-bile solution (5 mg/mL pancreatin and 25 mg/mL porcine bile in 0.1 mol/L $NaHCO_3$). Digestion products were placed in dialysis membranes (MWCO 12,000–14,000; width 35 mm, Sigma Aldrich, USA) and dialyzed in 250 mL of sodium bicarbonate solution (pH 7.5) for 16 h. Polyphenol content and antioxidant activity in the dialysate were analyzed to estimate bioaccessibility.

Statistical analysis

All experiments were conducted in triplicate. Data was analyzed using Student's t-test ($P < 0.05$) for comparisons between the optimal ultrasound treatment and the control sample regarding fiber content, functional properties, antioxidants and *in vitro* bioaccessibility of antioxidants. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA).

Results and discussion

Microbiological analysis

All response variables showed high correlation coefficients with the mathematical model, as indicated by adjusted R-square (R^2) values ≥ 0.91, except for microbial content. However, the results demonstrated that ultrasound treatment effectively reduced microbial load. The initial counts for aerobic mesophiles and Enterobacteriaceae in control beetroot residue were 4.13 and 4.06 log^{10} CFU/mL, respectively, while various treatments resulted in reductions of 0.83–1.28 and 0.76–1.21 log^{10} CFU/mL, respectively (Suppl. material 1). The microbial inactivation observed with ultrasonicated treatments was attributed to cavitation during ultrasound, which causes the collapse of bubbles within or around bacteria, inducing a mechanical effect that damages bacterial cells (Onyeaka et al. 2021). In addition to the above, the results of fiber and phenols content in ultrasonicated sample (see results below) probably favored the reduction of microorganisms, since by-products obtained from vegetables are viewed as potential sources of high-value compounds possessing antioxidant and/or antimicrobial activities, such as phenols and dietary fibers (O'Shea et al. 2012).

Functional properties

The WRC and OHC of ultrasonicated beetroot residue ranged from 11.58–13.06 and 9.96–12 g/g, respectively, showing an increase compared to the control sample (Suppl. material 2). WRC was significantly influenced ($P < 0.001$) by the quadratic term of amplitude (β_{11}), while OHC was significantly affected by its linear term (β_1) at $P < 0.001$ (Table 2). Both properties increased as the amplitude intensified, as seen in Fig. 1a, b. These increases can be attributed to sonication, which propagates the formation of microfractures in plant cell walls, fragmenting the fiber structure and increasing the surface area, thereby enhancing the capacity to absorb and retain both water and oil components (Vela et al. 2024).

Antioxidant content

Phenolic acids, tannins, and betalains are major bioactive compounds found in plant-based foods, contributing significantly to human health due to their antioxidant activity (Raham et al. 2023). The Suppl. material 2 presents the mean values for betanins, betaxanthins, and TPC, which the ultrasonicated samples ranged from 953.65–1049.74 mg BE/100 g dw, 682.79–758.94 mg BE/100 g dw, and 375.44–566.09 mg GAE/100 g dw, respectively. The control beetroot residue exhibited the lowest values for these compounds. Previous studies in tomato residues observed increases in TPC, following similar treatment (Mavridis et al. 2025). On the other hand, betalains have also shown an improvement after ultrasonication in dragon fruit peel (Xiaolan et al. 2022); showing that ultrasound can also be used for compounds release. The quadratic term of amplitude (β_{11}) significantly negatively affected the betanins and betaxanthins ($P < 0.0001$ and $P < 0.001$, respectively), while the interaction between amplitude and time (β_{12}) had the most substantial

Table 2. Regression coefficients and ANOVA of regression parameters of the predicted response surface quadratic models.

Coefficient	WRC	OHC	Betalains	Betaxanthins	TPC	ABTS	DPPH	FRAP	Chelating activity
β_0	12.12^a	10.42^a	1039.71^a	746.58^a	496.94^a	1804.42^a	1619.84^a	79.80^a	26.88^a
β_1	0.24^c	0.43^b	-6.11	4.76	12.02	-49.09^b	254.06^c	-5.22^a	0.79
β_2	0.11	0.26^d	2.25	-5.12	23.93^d	54.26^a	74.50	-6.41^a	1.05^d
β_{12}	0.21^d	0.49^c	35.80^b	18.135^c	71.38^b	-246.19^a	318.45^c	3.24^b	-5.13^a
β_{11}	0.30^b	0.37^c	-40.04^a	-23.36^b	-13.29	-27.11^c	139.87^d	-2.56^b	1.75^c
β_{22}	-0.16^d	-0.02	0.14	3.91	-31.37^c	-23.73^d	-347.22^b	-5.16^a	-0.61
R^2	0.92	0.92	0.96	0.94	0.93	0.99	0.93	0.99	0.94

Significance level: $^aP < 0.0001$; $^bP < 0.001$; $^cP < 0.01$; $^dP < 0.05$.

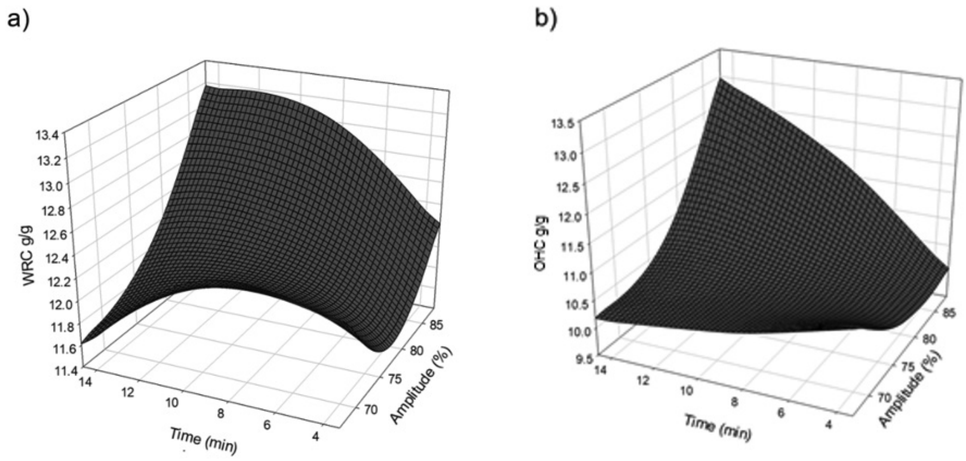

Figure 1. Effect of the ultrasound extraction on the functional properties of beetroot residue. **a**) water retention capacity; **b**) oil holding capacity.

effect ($P < 0.001$) on TPC content (Table 2). As shown in Fig. 2a, b, betalain degradation was most pronounced at the highest amplitude, while its interaction with time led to an increase in TPC (Fig. 2c). The degradation of betalains can be explained by the greater production of hydroxyl (OH) and hydrogen peroxide (H_2O_2) radicals at high amplitudes, during the cavitation induced by the ultrasound and consequently reduces their stability (Quang-Hieu et al. 2023). Also, these compounds degrade when they have high water activity due to breaking aldimine bonds into their structure (Fu et al. 2020). Let us remember that the sample is aqueous extract and therefore the water activity is high. Additionally, time plays a crucial role, Righi et al. (2018) noted that excessively short or long extraction times could prevent proper extraction or lead to compound degradation. However, in the case of TPC, high amplitude and extended time favored cell rupture, facilitating the release of additional compounds.

Antioxidant activity

Suppl. material 2 presents the antioxidant activity of ultrasonicated beetroot residue. The values for antioxidant activity by ABTS, DPPH, FRAP, and chelating activity ranged from 1507.93–2126.83 µmol TE/100 g dw, 879.02–2199.21 µmol TE/100 g dw, 60.46–87.51 µmol Fe(II)/100 g dw, and 20.67–33.28%, respectively, with the ultrasonicated residues demonstrating higher values than the untreated beetroot residue. Other studies have similarly reported that ultrasound treatment enhances the antioxidant capacity of food residues, helping maintain or increase bioactive compounds (Ramírez-Moreno et al. 2018; Perera et al. 2021). The increase in antioxidants observed with ultrasound treatment is attributed to the release of phenolic compounds, ascorbic acid, and betalains, which participate in free radical scavenging and the release of pro-oxidant metal ions during ultrasound processing. Furthermore, the antioxidant activity of flavonoids may be enhanced due to changes in the degree of hydroxylation of molecules induced by OH- radicals formed during sonication (Hu and Li 2022). The linear term of time (β_2) positively influenced antioxidant activity by ABTS at $P < 0.0001$, while its interaction with amplitude (β_{12}) also significantly affected antioxidant activity ($P < 0.0001$) (Table 2), as illustrated in Fig. 3a. The greater the ultrasound application, the more effective the ABTS radical scavenging, while reduced amplitude and time resulted in lower antioxidant activity. In the case of DPPH, the interaction between amplitude and time (β_{12}) had a positive effect at $P < 0.01$ (Table 2), suggesting that increasing both amplitude and time enhanced antioxidant activity (Fig. 3b). In blackberry residue, a similar trend was observed, with time exerting a significant influence on antioxidant activity ($P < 0.0001$) (Zafra-Rojas et al. 2016). For FRAP, the linear and quadratic terms of amplitude and time (β_1, β_2 and β_{22}) negatively affected antioxidant activity ($P < 0.0001$) (Table 2), indicating that increased amplitude and extraction time reduced antioxidant activity (Fig. 3c). A similar pattern was noted in pomegranate peel, where the linear term of time negatively affected antioxidant activity ($P < 0.05$), while the linear term of amplitude had a positive effect ($P < 0.05$) (Sharayei et al. 2019). Finally, the amplitude and time interaction decreased chelating activity (β_{12}, $P < 0.0001$) (Table 2), as shown in Fig. 3d.

Optimization by response surface superposition

The response surface model predictions, based on the determined optimal conditions for ultrasound processing, indicated that the ideal conditions for maximum antioxidant and functional properties were 77.5% amplitude for 9 min of treatment (Fig. 4). These conditions resulted in WRC of 12.20 g/g, OHC of 10.21 g/g, betanins content of 3327.08 mg BE/100 g dw, betaxanthins content of 2381.34 mg BE/100 g dw, TPC of 496.95 mg GAE/100 g dw, ABTS and DPPH antioxidant activities of 1804.42 and 1619.84 µmol TE/100 g dw, respectively, FRAP value of 79.81 µmol Fe(II)/100 g dw, and a chelating activity of 27.07%.

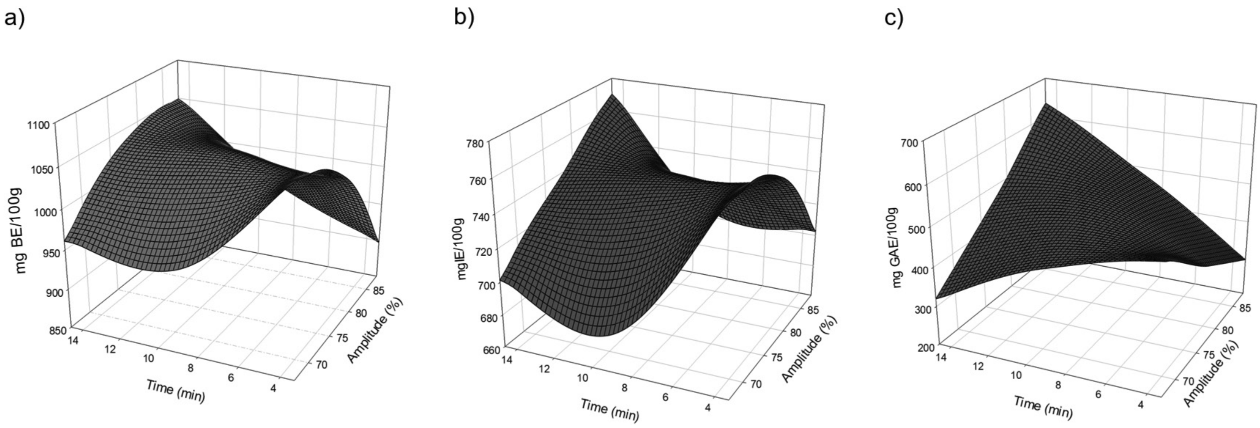

Figure 2. Effect of the ultrasound extraction on the antioxidant content of beetroot residue. **a**) betanins; **b**) betaxanthins; **c**) total phenolic content.

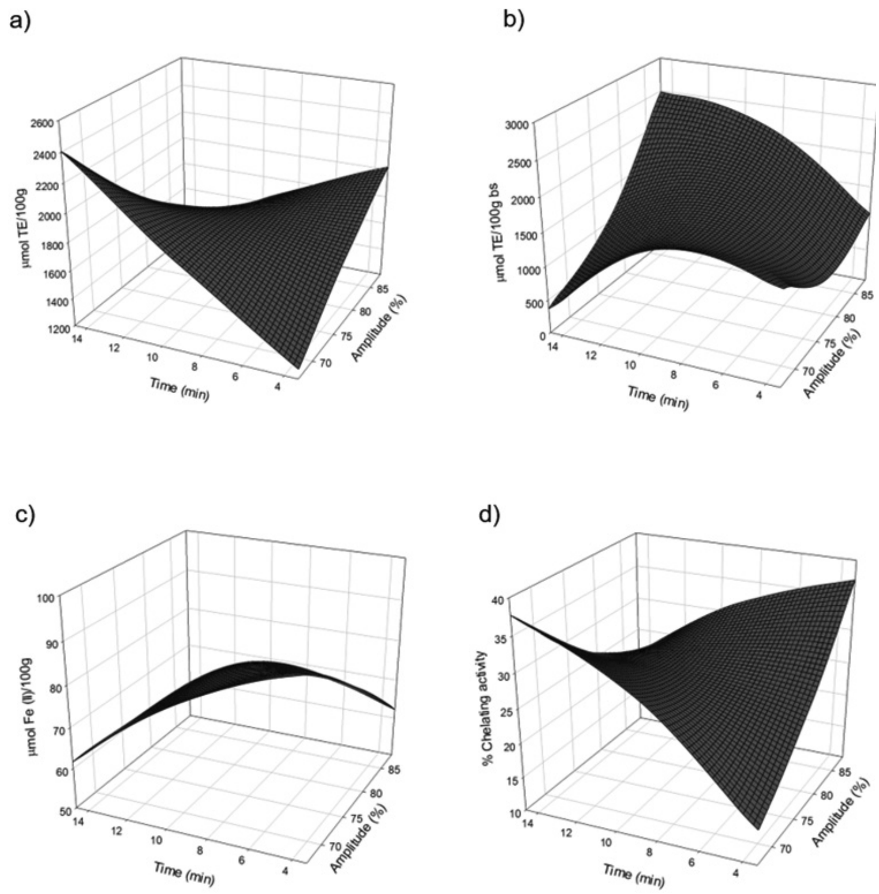

Figure 3. Effect of the ultrasound extraction on the antioxidant activity of beetroot residue. **a**) ABTS; **b**) DPPH; **c**) FRAP; **d**) chelating activity.

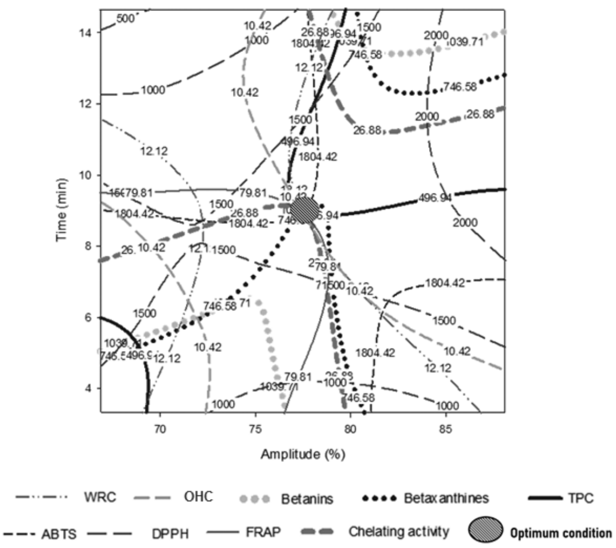

Figure 4. Optimal ultrasound extraction conditions of beetroot residue.

Comparison of optimal ultrasound conditions and untreated residue

Microstructure, fiber content (total, soluble, and insoluble), functional properties, and *in vitro* intestinal bioaccessibility of antioxidants were analyzed to compare the optimal ultrasound treatment with the control sample.

Effect of ultrasound on physical structure of beetroot residue

Scanning electron micrographs of beetroot residue powder before and after ultrasound treatment at 250× and 500× magnifications (Fig. 5) revealed an intact, irregular, and polyhedral structure in the control sample (Fig. 5a, b). After ultrasound treatment (Fig. 5c, d), cellular damage was observed, characterized by variations in particle shape and size, as well as the formation of aggregates. This behavior was attributed to tissue rupture caused by intramolecular heating and cavitation effects during ultrasound (Vela et al. 2021).

Fiber content and functional properties

The fiber content, WRC, and OHC values of beetroot residue before and after ultrasound treatment are summarized in Table 3. The ultrasonicated residue exhibited significantly ($P < 0.05$) high values across all parameters compared to the control sample. This increase in total fiber could be explained by internal heating during ultrasonication, which causes dispersion in water and promotes Maillard reaction products, leading to increased lignin content and the formation of resistant starch fractions. This results in a redistribution of soluble fiber into insoluble fiber (Wen et al. 2020). Additionally, the enhanced functional properties

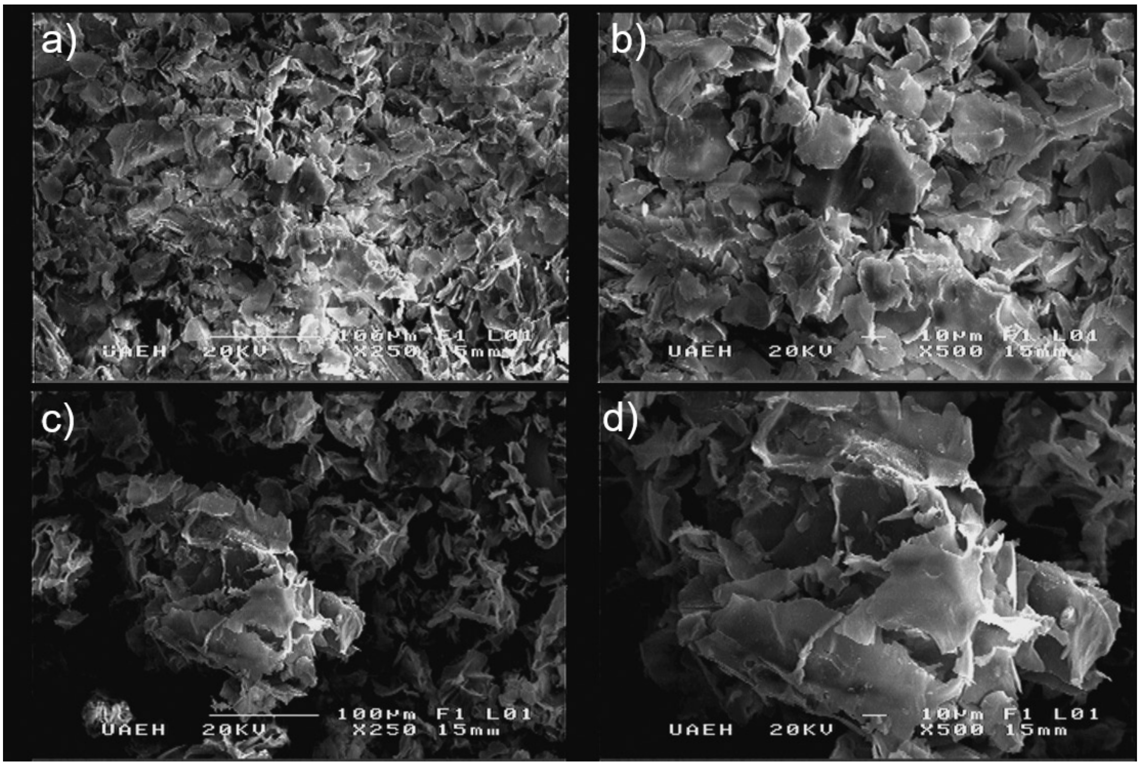

Figure 5. Scanning electron micrographs of beetroot residue powder before (**a** and **b**) and after (**c** and **d**) ultrasound treatment. Both at 250× and 500×.

Table 3. Fiber content and functional properties of beetroot residue treated by ultrasound.

Sample	Total fiber	Insoluble fiber	Soluble fiber	WRC	OHC
Control	37.47 ± 0.08	20.19 ± 0.18	17.28 ± 0.10	10.52 ± 0.44	9.36 ± 0.12
Optimum	42.25 ± 0.13*	23.82 ± 1.31*	18.43 ± 1.18*	12.77 ± 0.52*	10.98 ± 0.24*

WRC: water retention capacity; OHC: oil holding capacity. * Indicate significant differences ($P < 0.05$) between control and optimum ultrasonicated values according to the t-student test.

of the ultrasonicated residue are attributed to tissue rupture during ultrasound, which creates a more porous and bulky structure, facilitating the flow of water and/or lipids through the fiber (Martinez-Solano et al. 2021). The above can be seen in Fig. 5 on tissue rupture of ultrasonicated beetroot residue. These findings suggest potential applications in the food industry, including as a gelling agent, thickener, emulsifier, or to prevent syneresis in certain products, while also increasing viscosity (Yiming et al. 2021).

Effect of ultrasound on *in vitro* intestinal bioaccessibility of antioxidants

The ultrasonicated beetroot residue in its original samples (before *in vitro* bioaccessibility) were high ($P < 0.05$) in betanins, betaxanthins and TPC (816.11 ± 2.20 mg BE/100 g dw, 604.65 ± 15.93 mg BE/100 g dw and 566.67 ± 3.08 mg GAE/100 g dw), respectively compared with control sample (785.12 ± 8.46 mg BE/100 g dw, 535.47 ± 9.11 mg BE/100 g dw and 453.49 ± 2.25 mg GAE/100 g dw, respectively) (data not shown). As previously noted, ultrasound enhances cell rupture, thereby aiding the release of further compounds (Onyeaka et al. 2021).

The *in vitro* digestion model provided insights into the bioaccessibility of antioxidants in beetroot residue. After *in vitro* digestion (Fig. 6) the ultrasonicated residue exhibited significantly ($P < 0.05$) high concentrations of betanins (45.28 ± 2.59 mg BE/100 g dw) and betaxanthins (22.73 ± 1.62 mg BE/100 g dw) compared to the control (39.08 ± 0.67 mg BE/100 g dw and 19.46 ± 0.25 mg BE/100 g dw, respectively) (Fig. 6a, b), although bioaccessibility was low at 6% and 4% for betanins and betaxanthins, respectively. During the *in vitro* digestion process, the sample passes by different pH causing degradation of various compounds as the betalains, due to be stable between 3.5–7.0 pH values (Otálora et al. 2020). In gastric conditions a low pH exists (already 2.0) and consequently, the molecule undergoes a decarboxylation (Herbach et al. 2006). Therefore, the bioaccessibility of these compounds was low.

Besides, betanins undergo isomerization in the intestinal environment, forming isobetanins, which may be the primary metabolite absorbed after ingestion. Also, other metabolic processes, including glucosidase and amylase activities, may also limit the bioaccessibility of betanins (Vieira-Teixeira et al. 2019).

Regarding TPC, the control residue showed significantly high phenolic content (54.43 ± 0.58 GAE/100 g dw)

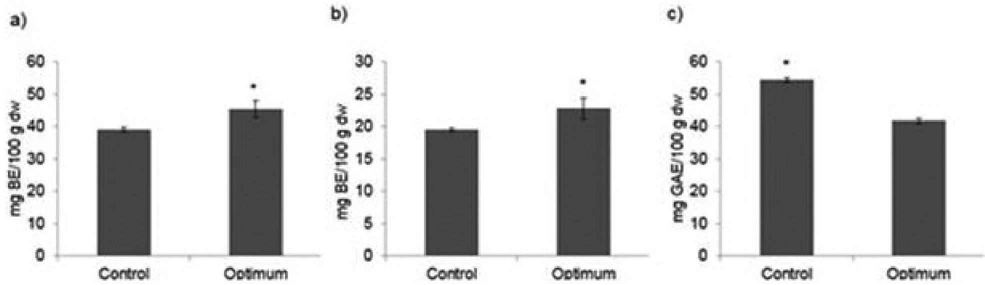

Figure 6. *In vitro* bioaccessibility of **a**) betanins, **b**) betaxanthins, and **c**) total phenolic content in control and ultrasound-optimized beetroot residue. Values represent the mean ± standard deviation (n = 3). Asterisks indicate statistically significant differences (P < 0.05) between treatments.

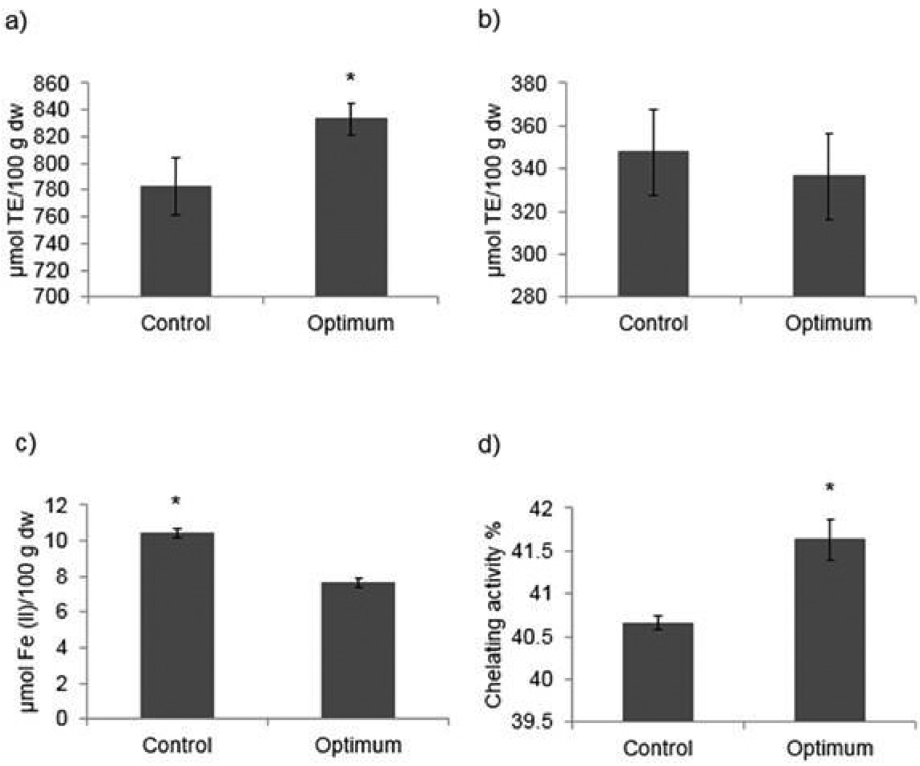

Figure 7. *In vitro* bioaccessibility of antioxidant acivity evaluated by (**a**) ABTS, (**b**) DPPH, (**c**) FRAP, and (**d**) chelating activity assays in control and ultrasound-optimized beetroot residue. Results are expressed as mean ± standard deviation (n = 3). Asterisks denote significant differences between treatments (P < 0.05).

in the bioaccessible fraction compared to the ultrasonicated residue (41.75 ± 0.90 GAE/100 g dw) (Fig. 6c), with absorption rates of 12% and 10%, respectively. This trend can be explained by the ability of ultrasound treatment to degrade phenolic acids, thus reducing the bioaccessibility of bioactive compounds. Further studies could investigate the metabolic pathways that affect antioxidant bioavailability in the gut and liver, as well as the efficacy of these compounds after systemic circulation.

Concerning antioxidant and chelating activity before *in vitro* bioaccessibility, the behaviour was similar to antioxidants content, except to chelating activity. The antioxidant activity by ABTS, DPPH and FRAP were significantly high in ultrasonicated sample (868.32 ± 17.72 µmol TE/100 g dw, 1056.57 ± 14.13 µmol TE/100 g dw and 84.45 ± 1.91 µmol Fe(II)/100 g dw, respectively) with respect to control sample (597.66 ± 42.04 µmol TE/100 g dw, 844.71 ± 31.28 µmol TE/100 g dw and 75.59 ± 2.11 µmol Fe(II)/100 g dw, respectively), while in chelating activity had not significant difference reporting 34.77 ± 0.98% to control and 33.75 ± 2.68% to ultrasonicated beetroot residue (data not shown).

Regarding *in vitro* bioaccessibility, the ultrasonicated beetroot residue exhibited significantly high (P < 0.05) ABTS and chelating activity values (833.20 ± 12.11 µmol TE/100 g dw and 41.63 ± 0.23%, respectively) (Fig. 7a, d) compared to the control residue (782.58 ± 21.72 µmol TE/100 g dw and 40.66 ± 0.08%, respectively). These values corresponded to absorption percentages of 96% and 123% for ABTS, and 131% and 117% for chelating activity, relative to their original samples. In contrast, no significant difference (P > 0.05) was observed in DPPH capacity between ultrasonicated (336.38 ± 20.12 µmol TE/100 g dw) and control residues (347.62 ± 19.8 µmol TE/100 g dw), with absorption percentages of 32% and 41%, respectively (Fig. 7b). For FRAP, the control residue exhibited significantly higher activity ($P < 0.05$) (10.41 ± 0.27 µmol Fe(II)/100 g dw) than the ultrasonicated sample (7.66 ± 0.25 µmol Fe(II)/100 g dw) (Fig. 7c), with corresponding absorption values of 14% and 9%. The limited bioaccessibility of these compounds may be attributed to their retention within the non-digestible fraction of the food matrix, preventing their release and absorption across the intestinal barrier (Di Lorenzo et al. 2021).

Conclusions

This study demonstrated that ultrasound treatment is an effective method for enhancing the functional and antioxidant properties of beetroot residue. The optimization process identified 77.5% amplitude and 9 min as the ideal conditions, leading to significant improvements in water and oil holding capacity, as well as the extraction of bioactive compounds. Furthermore, ultrasound reduced microbial contamination, altered microstructure, and increased fiber content. Despite some degradation of phenolic compounds, the bioaccessibility of betalains improved, highlighting the potential of ultrasound-treated beetroot residue as an ingredient for functional foods. These findings support the use of ultrasound as a sustainable strategy for valorizing food industry by-products, promoting their incorporation into health-oriented food formulations.

Author contributions

Conceptualization: LMRM. Data curation: LMRM. Formal analysis: JLHT, ERM. Funding acquisition: NSCC. Investigation: JAAO, NSCC, RMVE. Methodology: JLHT. Project administration: ERM, NSCC. Software: QYZR. Supervision: ERM, JAAO. Validation: RMVE. Visualization: QYZR, AAN, JAAO, RMVE. Writing – original draft: QYZR. Writing – review and editing: AAN, LMRM.

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Supplementary material

Supplementary material 1
Microbiological load of the ultrasonicated beetroot residue (.docx file)
Link: https://doi.org/10.3897/ejfa.2025.160284.suppl1

Supplementary material 2
Functional and antioxidant properties of the ultrasonicated beetroot residue (.docx file)
Link: https://doi.org/10.3897/ejfa.2025.160284.suppl2