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Process optimization of ultrasonic assisted extraction of betalains from red beet, *Beta vulgaris* L. waste stalks

Shikhangi Singh[1]*, P.K. Omre[1], Khan Chand[2], Anil Kumar[3] & Pratima Awasthi[4]

[1]Department of Post-Harvest Process & Food Engineering, College of Technology, GBPUA&T, Pantnagar, Uttrakhand, India

[2]Department of Agricultural Engineering, School of Agricultural Sciences and Rural Development, Central University of Nagaland, Medziphema, Dimapur, Nagaland, India

[3]Department of Food Technology, College of Agriculture, GBPUA&T, Pantnagar, Uttrakhand, India

[4]Department of Human Nutrition, College of Home Science, GBPUA&T, Pantnagar, Uttrakhand, India

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Beetroot stalks are regarded as agroindustrial waste, despite their nutritious and colourant characteristics. The pigments found in beetroot waste stalks were extracted using an ultrasonication approach in this investigation. Multiple regression analysis was used to investigate and assess the individual and interaction effects of power intensity, solid-solvent ratio, and sonication period on the betalains. Under optimal conditions (power intensity of 79.801 W/cm^2, solid-solvent ratio of 22.4 g/ml, and sonication time of 26.7 min), the maximum betacyanin (3 mg/g) and betaxanthin (4.36 mg/g) were achieved. All responses of betalains extracted from beetroot waste stalks showed a significant (P <0.05) influence of process factors. It was also revealed in the study that ultrasound assisted extraction had actual yield of betalains (betacyanin, 2.92 mg/g and betaxanthin 4.27 mg/g), total phenolic content (69.74 mg GAE/g), as well as superior antioxidant activity (76.32%), than traditional techniques, under optimal conditions of independent process variables.

Keywords: Agroindustrial waste, Beetroot stalks, Betacyanin, Betaxanthin, Box-Behken design, Pigments, Response Surface Methodology (RSM), Ultrasound assisted extraction

The role of colour in evaluating the consistency of many materials is widely recognised. The abundance of coloured bioactive ingredients from natural sources, such as fruits and vegetables, is influenced not only by their variety, but also by the extraction method used. Because of the toxicity of certain synthetic colourants, interest in using natural pigments in food product production has grown, and so has the recent trend in food industry to replace synthetic colours with natural colours derived from natural materials. Finding new natural colourants from natural sources, especially waste plant material, is thus not only a way to minimise emissions but also a way to manufacture higher-value, healthier goods at a lower cost [1-3].

Betalains are natural pigments that are becoming increasingly common in the food industry as colourants[4]. The two main forms of betalains are betacyanins and betaxanthins. Betacyanins are soluble in polar solvents including water, ethanol, and methanol, as well as mixtures of these solvents[5]. The use of betalains is gaining popularity because they are extremely bioactive and provide health benefits due to their properties as antiradicals, antioxidants, and anti-inflammatories, among other aspects[6; 7]. Betalains are used to colour a variety of foods, including ice cream, wine, jelly, and yoghurt. Furthermore, betalains are stable at pH 3–7 and regenerate quickly after heat treatment[8].

The red beet, *Beta vulgaris L.*, is a root vegetable with a leafy top that grows aboveground and is one of the known sources of betalains. Beets are listed in top ten most potent antioxidant vegetable[9]. The stalks of red beet were discarded as waste materials after processing, and they may pollute the atmosphere. However, the stalks contain a significant amount of pigments that are thought to have both strong colouring ability and favourable physiological effects for human wellbeing[10-11].

Traditional methods for recovering natural pigments from plant materials include homogenization and solvent extraction[12]. Many

*Correspondence:
Phone: +91 9410514757 (Mob.)
E-Mail: shikhangisingh16@gmail.com

factors affect extraction efficiency, including solvent type and composition, extraction time, temperature, solvent to solid ratio, power intensity, and extraction pressure[13-14]. Advanced extraction technologies such as ultrasound[15], microwave[16], or pulsed electric fields[17] have recently been developed as alternatives to conventional approaches for obtaining betalains. Their advantages involve the reduction of extraction time, solvent consumption, increase efficiency and the generation of waste-water during post treatment. Ultrasound is widely used because it causes cavitation, which breaks the solid matrix's cell wall, allowing solvent penetration and mass transfer, resulting in higher extraction speeds[18]. During their passage in a medium, ultrasonic waves undergo numerous modifications in their properties (velocity, attenuation, and frequency spectrum). Their applicability in food processing, analysis, and quality control is determined by their frequency range. Ultrasounds are divided into two types: low-energy and high-energy ultrasounds[19]. Ultrasound for food processing has several advantages, including more efficient mixing and micro-mixing, faster energy and mass transfer, reduced thermal and concentration gradients, lower temperatures, selective extraction, use of smaller equipment, faster response to process extraction control, faster start-up, increased production, and elimination of process steps[20]. Ultrasound techniques are recognised for their low cost, simplicity, and capacity to save energy, and as a consequence, they have quickly become a popular tool for investigating and modifying food products[21]. However, the use of ultrasonic assisted extraction (UAE) in the extraction of betalains from red beet stalks has been documented in one study only[22], but the influence of power intensity and different solvent concentrations was not investigated, since then no study has done on betalains extraction from waste beetroot stalks. As a result, the aim of this research was to use the UAE method to extract natural pigments (betacyanin and betaxanthin) from waste red beet stalks. This research used a three-level Box-Behnken reaction surface configuration with three factors (power intensity, solid–liquid ratio, and sonication time) to analyse and optimise the effect of process variables on pigment extraction yield. The extracted betalains (at optimized values) and bioactive compounds (total phenolic content and antioxidant activity) were differentiated at optimized condition with traditional methods.

Materials and Methods

Materials and Reagents

Beetroot waste stalks were purchased from the local market of Pantnagar. The process is done in an aqueous medium by taking distilled water. Green leafy portion were removed from the stalks and then cleaned under running water. Hot water blanching was done as a pre-treatment for 1 minute in 90°C distilled water heated by electric heaters, which inhibits enzyme activity while keeping the colour. The stalks were dried in the tray drier at 40ºC for i020 h. The dried stalks were grinded in Willy mill grinder (Specification: MAC, MSW 342) and sieved into fine powder through 250 µ sieve of 60 mesh screen having square shaped openings. The dried powder was stored in dark in a refrigeration temperature at 0-4ºC in plastic zip bag prior to experiments.

Ultrasound assisted extraction of betalains.

Ten grams of dried beetroot stalk powder was weighed and mixed with the solvent (distilled water) in the solid solvent ratio (1: 10, 1:20 and1:30 g/mL) in the beaker. After that, the mixture solution was mounted under the probe (20 mm diameter) of an ultrasonication apparatus (Electronic Industries model—EI—250, 230 VAC Single step, 250 watts ultrasonic control) at different ultrasound power intensities (48, 64, and 80 W/cm^2) and sonication time (20, 25 and 30 min) for extraction of betalains given in Table 1. After the extraction time was over, the suspension was drained into muslin cloth and centrifuged (8000 rpm for 15 min). The supernatant was collected, stored in sealed vials in the refrigerator and evaluated same day as extraction.

Analytical methods

Quantification of Betalains

Quantification was used to determine the amount of betalain pigment found in the derived colourant and were determined according to the method given by Maran and Priya[22]. The amount of betacyanin (BC) and betaxanthin (BX) pigments present in each gram of beetroot stalk powder in extracted solution, which are responsible for the red-violet and red-yellow colours, respectively, is measured. In the test tubes,

Table 1 — Independent variables and their levels for betalains extraction

Variables	Unit	Levels		
		–1	0	1
Power Intensity (X_1)	W/cm^2	48	64	80
Solid-Liquid ratio(X_2)	g/mL	10	20	30
Sonication time (X_3)	min	20	25	30

one ml of sample was mixed with 2 mL of purified water. A spectrophotometer (Scientific instruments industries, Make-Lasany) was used to measure absorbance at 600 nm for both betacyanin and betaxanthin[22]. The following equation to calculate the pigment content was used to compute the values for betacyanin (BC) and betaxanthin (BX) content of the extracts as indicaxanthin and betanin equivalents, as given below:

$$\text{BS (mg/g)} = \frac{\text{A} \times \text{DF} \times \text{MW}}{\varepsilon \times \text{L}}$$

where, BS = betacyanin or betaxanthin, A = absorption value at 600 nm, L= path length of the cuvette (1 cm), DF = dilution factor, MW represents molecular weight (betaxanthin= 308 g $mol^{-1}$and betacyanin= 550 g mol^{-1}), ε = molar extinction coefficient (betaxanthin = 48000 L mol^{-1} cm^{-1} and betacyanin= 60000 L mol^{-1} cm^{-1}).

Total phenolic content (*TPC*)

The Folin-Ciocalteu (FC) approach is used in the phenolic quantification assay. Phosphomolybdic/phosphotungstic acid complexes are present in the FC reagent. A spectrophotometer can detect the reduced Folin-Ciocalteu reagent at 760 nm. The reaction temperature (37°C) was used to shorten the time it took to achieve the desired colour.

TPC was calculated using extracts obtained under optimal conditions by UAE and traditional methods. The methodology followed from **23** for analysis of TPC with some modifications. About 1.0 mL of aliquot and 2 mL of Folin-Ciocalteu's reagent (10%) was combined and shaken. After 5 min, 2.5 mL sodium carbonate (7.5%) was added. It was left to incubate at room temperature (30±2ºC) for 2 h. A deep blue colour was developed. After incubation, absorbance was measured using a spectrophotometer at 760 nm.Gallic acid was used to create a calibration curve, which was expressed as gallic acid equivalent (GAE) in mg/g of sample.

Antioxidant activity

The antioxidant activity was calculated using the same extracts that were used to determine TPC. At 4°C, a 0.1 mM DPPH solution was stirred overnight. As a result, the purple coloured DPPH free radical solution was prepared and stored at –20°C for later use. By serial dilution of the stock solution (10 mg/mL) of each extract, three separate concentrations (5, 10 and 15 g/mL) of methanolic solutions of each extract were prepared. 2.5 mL 0.1 mM DPPH solution added to each 0.5 mL extract sample.

A control was prepared by mixing 0.5 mL distilled water and 2.5 mL 0.1 mM DPPH solution. These samples were shaken well and kept in dark for 30 min at room temperature. The absorbance was measured at 517 nm against the blank solution consisting of 2.5 mL MeOH and 0.5 mL distilled water. The radical scavenging activity was expressed as the radical scavenging percentage using the following equation[23,24],

$$\%\ \text{scavenging} = 1 - \frac{\text{A}_\text{s}}{\text{A}_\text{c}} \times 10$$

where, A_S = absorbance of sample solution, and A_C = absorbance of control

Statistical analysis

The Box-Behnken design of response surface methodology was used to investigate and optimise the impact of independent variables (Power intensity, Solid-Liquid ratio and Sonication time) on the betalains yield, as shown in Table 1. Design Expert software version 11 was used to create a total of 17 tests with 5 centre points. The information gathered was put into regression analysis to see if there was a relationship between the independent and dependent variables[25]. A mathematical equation that correlates the response surfaces was used to describe each response. After that, the response was reported as a second-order polynomial equation.

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^n + \sum_{i=1}^{n} \sum_{j=i=1}^{n} \beta_{ii} X_i X_j$$

Y = response, β_0, β_i, β_{ii} are regression coefficients and n = number of independent variables (n = 3). X_i and X_j = independent variables (where, i = 1, 2, …n and j = 1, 2, …n).

Model validation

The developed models for describing the effects of power intensity, solid-solvent ratio, and sonication time on the responses (betacyanin and betaxanthin) were confirmed using the optimal conditions predicted by design expert software. The error percentages were used to estimate the model's "fit" from the equation given below:

$$\text{Error (\%)} = \frac{1}{\text{n}_\text{c}} \sum_{\text{i=1}}^{\text{n}} \frac{\text{V}_\text{P} - \text{V}_\text{E}}{\text{V}_\text{P}} \times 100$$

where V_P and V_E are predicted and experimental values respectively.

Results and Discussion

Analysis of Betacyanin and Betaxanthin

The Box-Behnken design was used to optimise the variables of the ultrasound-assisted extraction process (UAE) and analyse their effects on the betalains, as seen in Table 2. The results indicated that the betacyanin and betaxanthin was in range of 0.04-2.94 mg/g and 0.23-4.31 mg/g respectively. The maximum yield of betalains was obtained under experimental condition of power intensity at 80 W/cm^2, solid-solvent ratio of 20 g/mL and sonication time of 30 min. The maximum betalains yield might be due to the high level of power intensity and higher sonication time. UAE increases cell wall matrix breakup at sufficiently high ultrasound power intensities, resulting in stronger interactions between solvent and material, as an ultrasonic wave passes through a liquid medium, it creates a violent shock wave and a high-speed jet, which causes the surfaces to swell and the pores in the materials to expand and thus improved betalains yield[26]. Also, compounds may not be extracted sufficiently or may be degraded if the extraction time is too short or too long. Longer extraction time only improved betalains extraction efficiency because the cavitation effect of ultrasonic waves enhanced swelling and hydration of plant material. According to[27], longer extraction periods (45 min) resulted in lower yields of betacyanins and betaxanthins from Bougainvillea glabra flowers. However, the minimum betalains were found under experimental conditions of power intensity at 64 W/cm^2, solid-solvent ratio of 10 g/ml and sonication time of 20 min. This might be due to the lower sonication time and solid-solvent ratio which fails to enable the contact area between the plant material and the solvent for dissolving the pigments in the solvent. Also, the high frequency, low power ultrasound had a negative impact on anthocyanins and phenolics in extracts, as well as antioxidant activity in berry extracts[28]. This is due to the fact that high ultrasonic frequencies produce a large number of free radicals, which breakdown phenols and decrease their antioxidant capabilities.

Table 2 — Experimental values obtained for betacyanin and betaxanthin to check the effect of operating variables using Box-Behnken Design

Exp No.	Power Intensity (X_1, W/cm^2)	Solid-Liquid ratio (X_2, g/mL)	Sonication time (X_3, min)	Betalains	
				Beta-cyanins	Beta-xanthin
1	48	10	25	0.93	1.29
2	64	20	25	2.18	3.06
3	64	20	25	2.06	2.98
4	48	30	25	1.43	2.04
5	80	30	25	2.72	3.98
6	80	20	20	1.4	2.99
7	64	20	25	2.06	2.56
8	48	20	20	0.8	1.15
9	64	10	30	2.29	2.12
10	64	10	20	0.04	0.23
11	64	20	25	2.12	2.7
12	64	30	20	1.36	1.94
13	64	30	30	1.29	1.83
14	80	10	25	2.68	2.92
15	80	20	30	2.94	4.31
16	64	20	25	2.24	2.89
17	48	20	30	1.26	1.78

Statistical analysis of Betacyanin and Betaxanthin

ANOVA was used to determine the statistical significance of the proposed quadratic model for Betacyanin and betaxanthin, and the results are described in Table 3. In this analysis, the F-value of model for betacyanin and betaxanthin was 70.41 and 46.63 respectively, which was associated with high *p* value of <0.0001 in all the responses. The high F-value and low p value showed that both of the suggested models are highly significant, and the extraction variables had a significant impact on the responses. Moreover, the lack of fit value for regression models was not found to be significant, indicating that the model equation was sufficient to explain betacyanin and betaxanthin. Furthermore, the R^2 values for betacyanin and betaxanthin were 0.9891 and 0.9836, respectively, implying that the model could account for 98.91 and 98.36 percent of the results. The difference between the expected and modified coefficient of determination should be less than 0.2, the appropriate precision should be greater than 4, and the coefficient of variance should not exceed 10% for the model to be more suitable. In this case, the "Pred R^2" of 0.8605 and 0.8847 for betacyanin and betaxanthin, respectively, was in

Table 3 — Analysis of variance (ANOVA) table for the fit of study data to the response surface model

Factors	Product response	
	Betacyanin (mg/g)	Betaxanthin (mg/g)
Model F-value	70.41***	46.63***
Lack of fit	4.62^{ns}	0.8122^{ns}
CV (%)	6.98	8.21
R^2	0.9891	0.9836
Adjusted R^2	0.9750	0.9625
Predicted R^2	0.8605	0.8847
Model adequate precision	32.2084	26.6916

[*** significant at *P* <0.0001, ns non-significant]

direct correlation with the "Adj R^2" of 0.9750 and 0.9625. For betacyanin and betaxanthin, adequate precision values of 32.208 and 26.691, as well as coefficients of variance of 6.98 and 8.21%, were determined, confirming the model's accuracy suitability. The coefficient of determination (R^2) and modified determination coefficient (Adj R^2) were both close to 1, meaning that the measured and expected values were highly correlated. The coefficient of determination (R^2) and modified determination coefficient (Adj R^2) were both close to 1, meaning that the measured and expected values were highly correlated.

RSM was used to create second-order models for predicting betacyanin and betaxanthin. In Table 4, the model coefficients (in coded form) are shown. Individual parameters for UAE of beetroot waste stalks, as well as model features (linear, quadratic, and interaction factors) were also calculated using Analysis of Variance (ANOVA). It was observed that on linear and quadratic level, positive coefficient was obtained for power intensity (X_1, X_1^2) signified the increases in betalains content with increase in power intensity, whereas negative coefficient was obtained for solid-liquid ratio (X_2, X_2^2) and sonication time (X_3, X_3^2), signifying the negative effect on betalains. However, the larger coefficient of power intensity (X_1) showed the higher effect on all the responses in comparison with other independent variables. The negative coefficient of combined effect of power intensity and solid solvent ratio (X_1X_2) and solid-solvent ratio and sonication time (X_2X_3) on the betacyanin suggested that yield content decreases when the high level of power intensity was applied with high solid solvent ratio and when high solid-solvent ratio was used with high sonication time, respectively. However, the positive coefficient effect of power intensity and sonication time (X_1X_3) implies that betacyanin content increases with increase in power intensity along with high sonication time. Similarly, on interactive level, the positive coefficient effect of power intensity and solid solvent ratio (X_1X_2) and power intensity and sonication time (X_1X_3) on the betaxanthin suggested that yield content increases when the high level of power intensity was applied with high solid solvent ratio and when high power intensity was used with high sonication time, respectively. However, negative coefficient effect of solid-solvent ratio and sonication time (X_2X_3) on the betaxanthin suggested that yield content decreases when the high level of power intensity was given.

Table 4 — Coefficient of regression analysis for each process parameter and product response

Process parameter	Product responses	
Fitted model-quadratic	Betacyanin (mg/g)	Betaxanthin (mg/g)
Intercept	1.26	1.01
Power Intensity (X_1, W/cm^2)	0.4313***	1.13***
Solid-Liquid ratio (X_2, g/mL)	–0.9728**	–2.21**
Sonication time (X_3, min)	–0.6453**	–0.5371*
X_1X_2	–0.1144	0.0771
X_1X_3	0.2703**	0.1716
X_2X_3	–0.5817***	–0.5000**
X_1^2	0.0817	0.3698**
X_2^2	–0.2708**	–0.6540***
X_3^2	–0.6142***	–0.6540***

[*** significant at P <0.001, ** at P <0.01 and * at P <0.05]

Analysis of 3D graphs of betacyanin and betaxanthin

For better understanding, the effect of combined processing parameters (power intensity, solid-solvent ratio, and sonication time) on the product response (betacyanin and betaxanthin was evaluated and illustrated by three dimensional plots (3D) in Fig. 1. Because the maximal betacyanin content was observed at greater levels of ultrasound power intensity with increased sonication duration, ultrasound power intensity was one of the effective elements in ultrasound-assisted extraction methodology (Fig. 1A). This rise in betacyanin content might be due to the breakdown of the cell wall and subsequent release of cellular material when the ultrasonic power intensity is sufficiently high, when an ultrasonic wave passes through a liquid medium, it creates a powerful shock wave and a high-speed jet, which causes the materials to swell and the pores in the materials to enlarge[29]. Fig. 1 B and C revealed that increasing the solid-solvent ratio and sonication time enhanced betalains (betacyanin and betaxanthin) level. This rise might be due to the passage of time, which provides more chances for reactivity with the solvent. Longer contact time between the extractor and the plant materials allows more mass to be transferred from the solid particles to the solution[30]. Microjets were also linked to asymmetric collapse of micro-bubbles at surfaces, which might induce disruption and excellent solvent penetration into the matrix by diffusion, improving the washing out of pigment content from plant material to surrounding solvents and improving extraction efficacy[31]. Furthermore, a larger solid-liquid ratio reduced the concentration and viscosity of

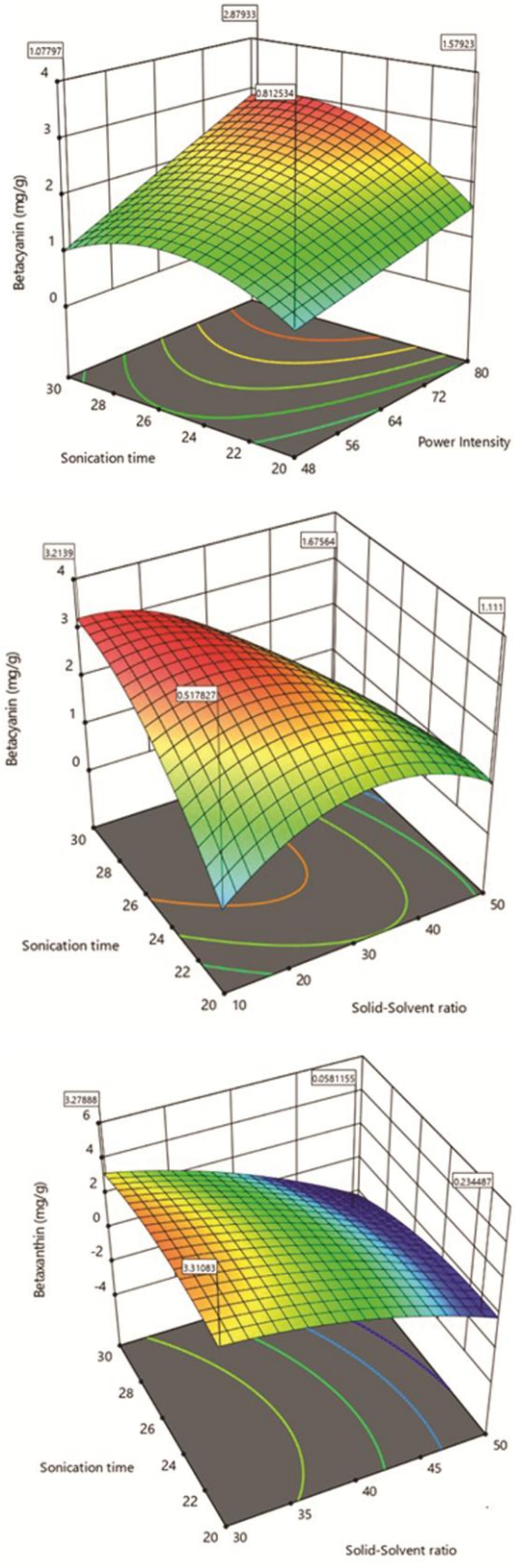

Fig. 1 — Interactive effect of sonication time and (A) power intensity; (B) solid- solvent ratio; and (C) solid liquid ratio on Betaxanthin

the extraction solvent, allowing the betalains to dissolve in the solvent and increasing the pigment extraction yield. Because cavitation demands negative pressure in the rarefaction zone of the wave function, the natural cohesive forces are overcome, lowering the extraction yield[22].

Optimization and Validation of variables

Betalains extraction variables were optimized using the RSM software to obtain the maximum yield in terms of the selected variables. The main standard for optimization was the betacyanin and betaxanthin which were maximized. As per the statistical analysis of data, process variables that yielded the highest betalains content were power intensity 79.8 W/cm^2, solid-solvent ratio 22.4 g/ml and sonication time 26.7 min. Table 5 shows the comparison between predicted and experimental values of process variables and revealed a non-significant difference between the predicted and experimental values of the extracted betalains with a low deviation or error percentage between the means. The optimized values obtained for betacyanin and betaxanthin were 3 mg/g and 4.36 mg/g respectively.

Comparison of methods for betalains extraction

Betalains were extracted from beetroot waste stalks by conventional solid-liquid extraction methods, such as Maceration and Soxhlet. Based on the Fig 2, the

Table 5 — Predicted and experimental values of responses

Particulars	Optimum value	Desirability of model	Error (%)
Power intensity (W/cm^2)	79.80		
Solid-solvent ratio (g/mL)	22.4	1.00	
Sonication time (min)	26.7		
	Predicted values	Exp. values	
Betacyanin (mg/g)	3.00	2.92	2.66
Betaxanthin (mg/g)	4.36	4.27	2.06

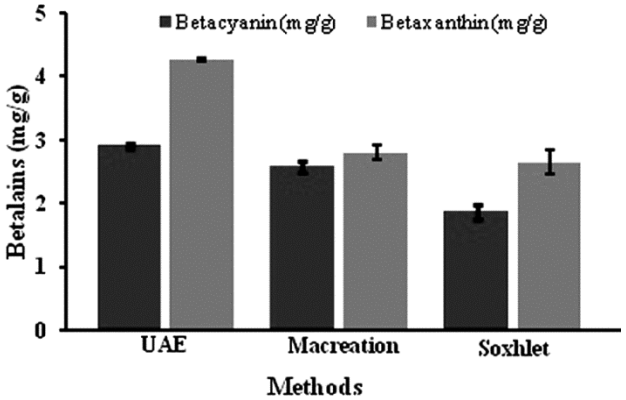

Fig. 2 — Comparison between different methods for betalains extraction

content of betacyanin and betaxanthin obtained from ultrasound assisted extraction were significantly higher than those obtained with maceration and Soxhlet method. The extraction of betalains from pitaya fruit, reported that maceration had a greater extraction yield (95.25%) than ultrasound-assisted extraction (47.07%)[32]. Another study was conducted on beetroot pomace for betalains extraction by Soxhlet and reported betacyanin 19.36 and 17.40 mg/L of betaxanthin[33]. But in present study, higher betalains were obtained from ultrasound assisted extraction might be basically for two reasons: (i) Greater concentrations of methanol or ethanol were less effective than aqueous extraction, resulting in higher pigment concentrations in the extracts; and (ii) Ultrasound's acoustic cavitation aids solvent penetration through the plant matrix's cellular walls, allowing for greater release of chemicals of interest into the extraction medium.

TPC and Antioxidant activity from different methods

TPC content and antioxidant activity were found to be greater when employing UAE with the optimal extraction conditions for betacyanins and betaxanthins than when using traditional extraction methods (Macreation and Soxhlet) Fig. 3. TPC obtained in this study was higher than the values obtained by[29], who used UAE with red beet root (65. 62 mg GAE/g), and by that employed conventional methods to extract betalains from beet stems (5.36 mg GAE/g). In one of the studies, obtained total phenolic content from the red beet extract with β-cyclodextrin-enhanced ultrasound-assisted extraction reported was 79.4 mg GAE/g[34].

As pigments with greater TPC concentration had greater antioxidant activity percentages, whereas pigments with lower TPC concentration had lower antioxidant activity percentages, it is proved that the antioxidant activity was clearly linked to TPC[35]. Several investigations have found that betalains, a kind of dietary cationized anhydride, have a significant radical scavenging activity. After thermal destruction, betalains have a unique regeneration capability in the presence of antioxidants, establishing the link between betalain content and antioxidant activity[36]. However, higher antioxidant activity was found in present study with maceration (59.89%±3.84) as compared to reported antioxidant activity of beetroot stalks (43%±1) using maceration technique[37]. Due to the difference in extraction processes, ultrasonic waves penetrate the bioactive

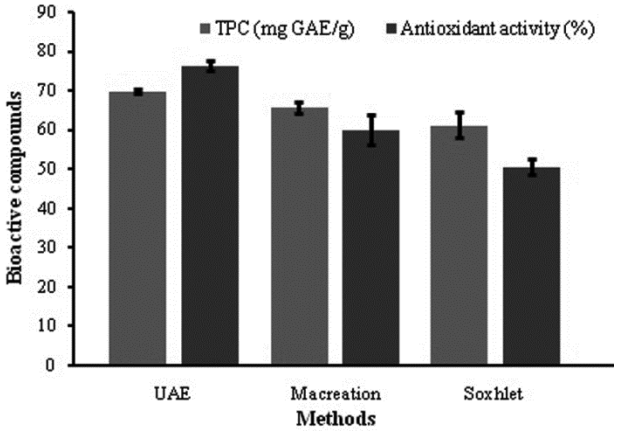

Fig. 3 — Total phenolic compounds & antioxidant activity by different methods

substances from the plant cells more efficiently than traditional methods, and therefore, high antioxidant activity discovered in this study for the stalks[38]. The high amount of total phenolic content in the pigment might possibly explain the strong antioxidant activity in pigments. Food antioxidant are significant because they can minimise oxidation interactions with lipids and proteins, among other molecules, which can have a detrimental impact on sensory and nutritional quality.

The ultrasound assisted aqueous extraction of betalains therefore, proven to be a better alternative to conventional extraction methods by offering maximum betalains yield, shorten extraction period, lesser use of distilled water and improving the quality of extracts in terms of its antioxidant activity and total phenolic content.

Conclusion

According to the current situation, beetroot is commercially cultivated for the production of juice and other processed products such as jams and jellies, and the majority of the stalk is discarded as waste without knowledge of the extraction of pigments (betalains) and antioxidants from the waste. Power intensity of 79.8 W/cm2, solid solvent ratio of 22.4 g/mL, and sonication time of 26.7 min were found to be the most optimal conditions. The maximum betalains achieved under these conditions were 3 mg/g betacyanin and 4.36 mg/g betaxanthin, respectively. The current work shows that betalains were extracted from beetroot waste stalks using an ultrasound-assisted extraction methodology, which has been shown to be an efficient extraction method due to its reduced extraction time, lesser solvent

usage, less power consumption, and better extraction yield. Ultrasound had more betalains and total phenolic content, as well as better antioxidant activity, than traditional methods, especially when betacyanins and betaxanthins were examined under optimal conditions. As comprehensive studies have been done only on red beets betalains, various other sources of betalains (mainly from bio-wastes) needs to be explored. Apart from the maximizing the pigment yield, its stability, applications, and the factors behind decomposition such as light, temperature, pH, oxygen, water activity and metal ions should also be focused for future work. In general, the colour degradation of betalains is due to temperature, thus more study on low thermal extraction techniques is in need.

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Conflicts of Interest

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

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