ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating (2026): 4.97
IJAFS 2026; 8(7): 290-295
www.agriculturaljournals.com
Received: 08-05-2026
Accepted: 13-06-2026

Shikhangi Singh
Department of Food Process Engineering, College of Food Technology, BUAT, Banda, Uttar Pradesh, India

Pramod Kumar More
Department of Post-Harvest Process & Food Engineering, College of Technology, GBPUA&T, Pantnagar, Uttarakhand, India

Shikha
Department of Foods and Nutrition, College of Home Science, GBPUA&T, Pantnagar, Uttarakhand, India

Corresponding Author:
Shikhangi Singh
Department of Food Process Engineering, College of Food Technology, BUAT, Banda, Uttar Pradesh, India

Process optimization of ultrasound-assisted betalain extraction from beetroot waste stalks

Shikhangi Singh, Pramod Kumar More and Shikha

DOI: https://www.doi.org/10.33545/2664844X.2026.v8.i7d.1692

Abstract
Beetroot stalks are considered as agro-industrial waste, by ignoring its nutritional properties and colorant ability. In this study, betalain pigments present in beetroot waste stalks were extracted using ultrasonication technique. The Betacyanin pigment (BC) which imparts red-violet color ranged from 1.80-6.5 mg/g and Betaxanthin pigment (BX) responsible for imparting orange-yellow color ranged from 1.26-4.56 mg/g over entire experimental conditions. For optimization of the extracted pigments, response surface methodology was adopted three factor/five level Central Composite design with pH, solid-solvent ratio (g/ml) and sonication time (min) as the main process parameters. A maximum yield of BC and BX of 6.31 and 4.43 mg/g respectively, were achieved under the optimized conditions (pH 4.7, solid-solvent ratio 50 g/ml and sonication time 30 min). It was also found that pH has no significant effect on the betalain pigments. It is evident from the study that the beetroot waste stalks contain considerable amount of pigment and can be used as a food additive in form of colorant, rather than discarding it as a residue. Thus, extraction of colorant from beetroot waste stalks could be one of the sources of betalain pigments and helps in reducing the environmental pollution.

Keywords: Betalain, response surface methodology (RSM), betacyanin (BC), betaxanthin (BX), central composite design (CCD), ultrasonication

Introduction
The rapid growth of the food processing industry has resulted in the generation of substantial quantities of agro-industrial residues, including peels, pomace, seeds, leaves, and stalks. These by-products are often discarded despite being rich sources of valuable bioactive compounds such as phenolics, dietary fibre, carotenoids, and natural pigments (Saini *et al*., 2025) [8]. Their improper disposal contributes to environmental pollution, whereas their valorization offers an opportunity to produce high-value functional ingredients in line with the principles of the circular bioeconomy and sustainable food processing.

Beetroot (*Beta vulgaris* L.) is a widely cultivated root vegetable recognized for its high nutritional value and antioxidant potential. During harvesting and processing, considerable amounts of stalks and leaves are removed and treated as waste, although they contain appreciable quantities of betalain pigments. Betalains are water-soluble, nitrogen-containing pigments classified into *betacyanins*, which impart red-violet colour, and *betaxanthins*, which produce yellow-orange colour (Azeredo, 2009) [2]. Besides their colouring properties, betalains possess antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, making them attractive natural alternatives to synthetic food colourants.

The increasing consumer preference for clean-label foods and concerns regarding the safety of synthetic dyes have accelerated research on natural pigments. Beetroot-derived betalains are already used as natural colourants in foods; however, commercial extraction mainly utilizes edible root s, increasing production costs. In contrast, beetroot waste stalks represent an inexpensive and underutilized source of these pigments, offering a sustainable approach for waste valorization while reducing environmental burden.

Extraction technology plays a crucial role in determining pigment yield and quality. Conventional extraction techniques such as maceration and Soxhlet extraction are associated with long extraction times, high solvent consumption, and possible thermal degradation of heat-sensitive compounds. In contrast, ultrasound-assisted extraction (UAE) has emerged as an efficient and environmentally friendly alternative (Chemat *et al*., 2022) [4].

The technique relies on acoustic cavitation, where the formation and collapse of microscopic bubbles disrupt plant cell walls, enhance solvent penetration, and improve mass transfer, thereby increasing extraction efficiency while reducing processing time and solvent requirements (Ahmadi *et al*., 2021; Usman *et al*., 2023) [1, 10].

Several factors, including extraction pH, solid-to-solvent ratio, and sonication time, significantly influence the recovery of betalain pigments (Khan *et al*., 2023) [5]. Optimizing these parameters through conventional one-factor-at-a-time experiments is laborious and fails to account for interactions among variables. Response Surface Methodology (RSM), particularly the Central Composite Design (CCD), is an effective statistical approach for evaluating the combined effects of multiple process variables while minimizing the number of experimental trials (Bhattacharya, 2021) [3]. RSM has been widely applied in food engineering for optimizing extraction processes and developing predictive mathematical models.

Although numerous studies have investigated betalain extraction from beetroot roots and other plant materials, limited information is available on the systematic optimization of pigment extraction from beetroot waste stalks using ultrasound-assisted extraction. Therefore, the present study aimed to optimize the extraction of betacyanin and betaxanthin pigments from beetroot waste stalks using UAE and Response Surface Methodology. The effects of pH, solid-to-solvent ratio, and sonication time on pigment yield were evaluated, and optimum extraction conditions were established using a Central Composite Design. This work demonstrates the potential of converting an underutilized agricultural residue into a value-added natural colourant for applications in the food, pharmaceutical, cosmetic, and nutraceutical industries, thereby contributing to sustainable waste management and resource utilization.

2. Materials and Methods

2.1 Raw Material Preparation

Fresh beetroot (*Beta vulgaris* L.) waste stalks were procured from the local vegetable market of Pantnagar, Uttarakhand, India. The stalks were thoroughly washed under running tap water to remove adhering dirt and foreign matter, and the green leaves were manually separated. The cleaned stalks were dried in a tray dryer at 40 °C for 20 h until a constant weight was achieved. The dried stalks were ground using a laboratory grinder and sieved through a 250 µm (60 mesh) sieve to obtain a uniform powder. The powdered sample was packed in airtight polyethylene zip-lock bags and stored under refrigerated conditions (0–4 °C) until further analysis.

2.2 Ultrasound-Assisted Extraction of Betalains

Ultrasound-assisted extraction (UAE) was employed for the recovery of betalain pigments from beetroot waste stalk powder. Five grams of dried powder was mixed with distilled water at the desired solid-to-solvent ratio according to the experimental design. The pH of the extraction medium was adjusted using dilute hydrochloric acid or sodium hydroxide solution.

The extraction was carried out using an ultrasonic processor operating at a frequency of 20 kHz, power output of 200 W, and supply voltage of 200 V. The sonication time was varied according to the Central Composite Design (CCD). During ultrasonication, acoustic cavitation generated microbubbles that collapsed violently, causing disruption of plant cell walls and enhancing the diffusion of intracellular pigments into the extraction solvent.

Following extraction, the slurry was filtered in two stages. Initially, a muslin cloth was used to remove coarse solid particles, followed by filtration through Whatman No. 46 filter paper. The filtrate obtained was collected and used for pigment analysis.

2.3 Determination of Betalain Pigments

The extracted samples were analysed spectrophotometrically for betacyanin (BC) and betaxanthin (BX) contents following the method reported by Maran *et al.* (2015) [6]. The absorbance of the extract was measured using a UV–Visible spectrophotometer, and pigment concentration was calculated using the respective molecular weight and molar extinction coefficient of each pigment. The results were expressed as mg g^{-1} dry weight of beetroot stalk powder.

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

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−1and betacyanin= 550 g mol−1),
ε = molar extinction coefficient
(betaxanthin = 48000 L mol−1 cm−1 and betacyanin= 60000 L mol−1 cm−1)

2.4 Experimental Design

Optimization of the extraction process was performed using Response Surface Methodology (RSM) based on a three-factor, five-level Central Composite Design (CCD). The independent variables selected were extraction pH (X_1), solid-to-solvent ratio (X_2), and sonication time (X_3), while betacyanin and betaxanthin yields were considered as the response variables.

The CCD consisted of 20 experimental runs, including eight factorial points, six axial points, and six centre points to estimate the experimental error and evaluate model adequacy. The coded and actual levels of the process variables are presented in Table 1.

Table 1: Coded and actual levels of independent variables for design of experiment

Sl. No.	Independent parameters	Coded value	Value of levels				
			-1.68 (α)	-1	0	1	+1.68 (α)
1.	pH	X_1	3.31	4	5	6	6.68
2.	Solid solvent ratio (g/ml)	X_2	23.19	30	40	50	56.8
3.	Sonication time (min)	X_3	16.59	20	25	30	33.40

A second-order polynomial model (Equation 1) was used to establish the relationship between the independent variables and the response variables:

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

where Y is the predicted response, β_0 is the intercept, β_i represents the linear coefficients, β_{ii} the quadratic coefficients, β_{ij} the interaction coefficients, and X_i and X_j are the coded independent variables.

2.5 Statistical Analysis

The experimental data were analysed using Design-Expert® software (Version 10.0.1, Stat-Ease Inc., USA). Analysis of variance (ANOVA) was performed to evaluate the significance of the developed models and individual regression coefficients. Model adequacy was assessed based on the coefficient of determination (R^2), adjusted R^2, predicted R^2, coefficient of variation (CV), lack-of-fit test, and adequate precision. A significance level of $p < 0.05$ was considered statistically significant.

2.6 Optimization and Model Validation

Numerical optimization was carried out using the desirability function approach to determine the optimum extraction conditions for maximum recovery of betacyanin and betaxanthin pigments. The optimized conditions predicted by the model were experimentally validated by conducting triplicate extraction experiments. The experimental pigment yields were compared with the predicted values to verify the reliability and predictive capability of the developed regression models.

3. Results and Discussion

3.1 Optimization of Ultrasound-Assisted Extraction

The extraction of betalain pigments from beetroot waste stalks was optimized using Response Surface Methodology (RSM) with a three-factor Central Composite Design (CCD). A total of 20 experimental runs were conducted to evaluate the combined effects of pH (X_1), solid-to-solvent ratio (X_2), and sonication time (X_3) on the extraction yield of betacyanin (BC) and betaxanthin (BX). The experimental responses obtained under different processing conditions are presented in Table 2. The betacyanin content varied from 1.80 to 6.50 mg g^{-1}, whereas betaxanthin ranged between 1.26 and 4.56 mg g^{-1}, indicating that the extraction parameters significantly influenced pigment recovery.

Table 2: Experimental results for ultrasound assisted extraction of betalain pigments using CCD.

Sl. No.	pH (X_1)	Solid-Liquid Ratio (X_2, g/ml)	Sonication Time (X_3, min)	Betalain yield (mg/g)	
				BX	BC
1	5	40	25	4.19	5.98
2	5	40	25	4.56	6.5
3	5	40	25	4.26	6.08
4	5	23.18	25	1.26	1.8
5	4	30	30	3.14	4.48
6	6.68	40	25	3.45	4.9
7	4	50	30	3.89	5.5
8	6	30	20	2.45	3.5
9	6	30	30	2.29	3.27
10	5	40	25	4.23	6.04
11	3.31	40	25	3.16	4.5
12	5	40	33.4	3.59	5.12
13	5	56.81	25	3.98	5.68
14	6	50	30	3.94	5.6
15	4	50	20	2.85	4.07
16	4	30	20	1.33	1.9
17	5	40	25	4.16	5.94
18	5	40	16.59	1.75	2.5
19	5	40	25	4.22	6.028
20	6	50	20	3.85	5.5

Quadratic polynomial models were developed to establish the relationship between the process variables and pigment yield. The second-order models adequately described the extraction process, confirming the suitability of RSM for optimization of ultrasound-assisted extraction.

3.2 Model Adequacy and Statistical Analysis

The adequacy of the developed regression models was evaluated using analysis of variance (ANOVA) shown in Table 3. The quadratic models exhibited high coefficients of determination (R^2 = 0.9702 for betacyanin and 0.9712 for betaxanthin), indicating that more than 97% of the variation in pigment yield could be explained by the selected process variables. The adjusted R^2 values (0.9435 and 0.9453) were in close agreement with the corresponding R^2 values, demonstrating excellent model reliability. Furthermore, the predicted R^2 values showed good agreement with the experimental observations, confirming satisfactory predictive capability of the developed models.

Table 3: ANOVA and significance of regression coefficients

Source	DF	SS	MS	F-value	p-value
Betaxanthin					
Model	9	38.97	4.33	36.23	< 0.0001**
pH (X_1)	1	0.49	0.49	4.12	0.0699
Solid-solvent ratio (X_2)	1	14.44	14.44	120.87	< 0.0001**
Sonication time (X_3)	1	5.03	5.03	42.07	< 0.0001**
X_1X_2	1	0.16	0.16	1.36	0.2707
X_1X_3	1	2.14	2.14	17.93	0.0017**
X_2X_3	1	0.084	0.084	0.70	0.4213
X_1^2	1	2.75	2.75	23.02	0.0007**
X_2^2	1	8.69	8.69	72.68	< 0.0001**
X_3^2	1	8.14	8.14	68.12	< 0.0001**
Betaxanthin					
Model	9	19.23	2.14	37.47	< 0.0001**
pH (X_1)	1	0.24	0.24	4.20	0.0677
Solid-solvent ratio (X_2)	1	7.17	7.17	125.70	< 0.0001**
Sonication time (X_3)	1	2.53	2.53	44.31	< 0.0001**
X_1X_2	1	0.076	0.076	1.33	0.2750
X_1X_3	1	1.07	1.07	18.69	0.0015**
X_2X_3	1	0.034	0.034	0.59	0.4592
X_1^2	1	1.31	1.31	22.98	0.0007**
X_2^2	1	4.26	4.26	74.71	< 0.0001**
X_3^2	1	3.99	3.99	69.93	< 0.0001**

The model F-values for betacyanin (36.23) and betaxanthin (37.47) were highly significant ($p < 0.0001$), indicating that the regression models adequately represented the experimental data. In addition, the low coefficient of variation (CV < 10%) and high adequate precision values (>30) suggested good experimental precision and an adequate signal-to-noise ratio. Therefore, the developed quadratic models were considered statistically valid for predicting betalain extraction under different experimental conditions.

3.3 Effect of Process Variables on Betalain Extraction

3.3.1 Effect of pH

Extraction pH plays an important role in determining the stability and recovery of betalain pigments. Within the investigated range (3.31–6.68), pigment recovery was highest under slightly acidic conditions. The statistical analysis indicated that pH alone had a comparatively smaller effect on pigment extraction than the other variables ($p > 0.05$). However, its interaction with sonication time significantly influenced betalain recovery ($p < 0.01$), suggesting that extraction efficiency depends on the combined influence of processing conditions rather than pH alone. Slightly acidic media help maintain betalain stability by minimizing pigment degradation during extraction.

3.3.2 Effect of Solid-to-Solvent Ratio

The solid-to-solvent ratio significantly affected both betacyanin and betaxanthin yields ($p < 0.0001$). Increasing the ratio from approximately 23 to 50 g mL^{-1} enhanced pigment extraction due to improved solvent availability and increased concentration gradients between the plant matrix and extraction medium. These conditions promoted efficient mass transfer of intracellular pigments into the solvent (Silva *et al*., 2021) [9]. However, further increases in solvent volume did not improve pigment recovery, indicating that excessive solvent usage does not proportionally enhance extraction efficiency and may increase processing costs as shown in Figure 1.

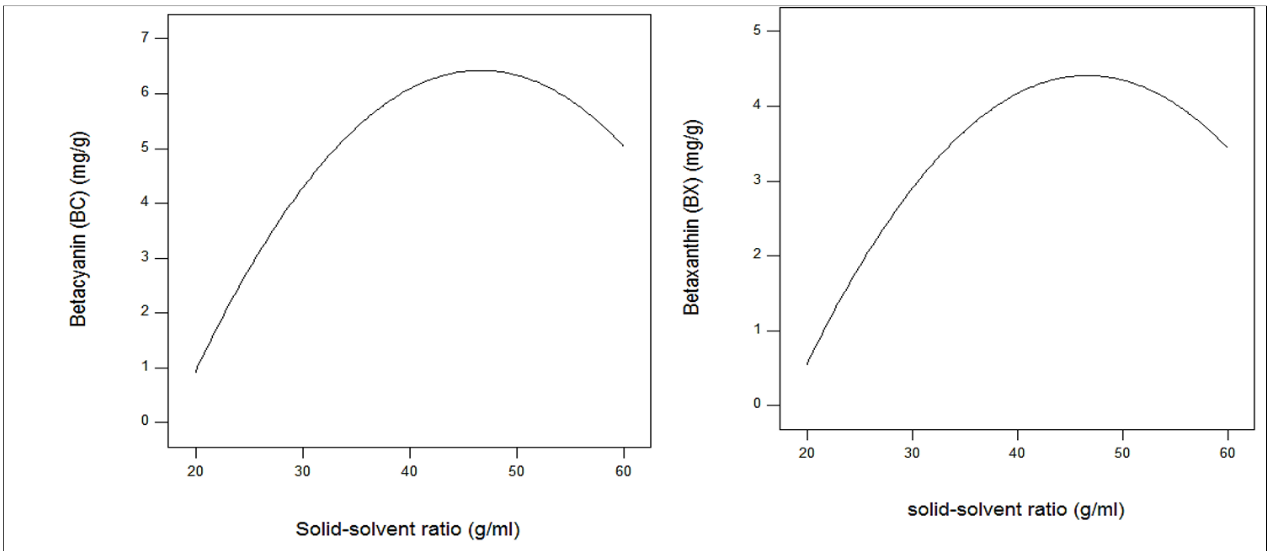

Fig 1: Effect of Solid- Solvent ratio on Betacyanin and Betaxanthin

3.3.3 Effect of Sonication Time

Sonication time exhibited a significant positive influence on pigment extraction ($p < 0.0001$). The recovery of both betacyanin and betaxanthin increased with increasing extraction time up to approximately 30 min, after which no substantial improvement was observed Figure 2. This behaviour can be attributed to ultrasonic cavitation, which progressively disrupts plant cell walls and enhances solvent penetration during the initial stages of extraction (Wang *et al*., 2024) [11]. Prolonged sonication beyond the optimum duration may expose pigments to localized heating and free radicals generated during cavitation, potentially leading to pigment degradation. Therefore, an optimum sonication time is essential to maximize extraction efficiency while preserving pigment stability.

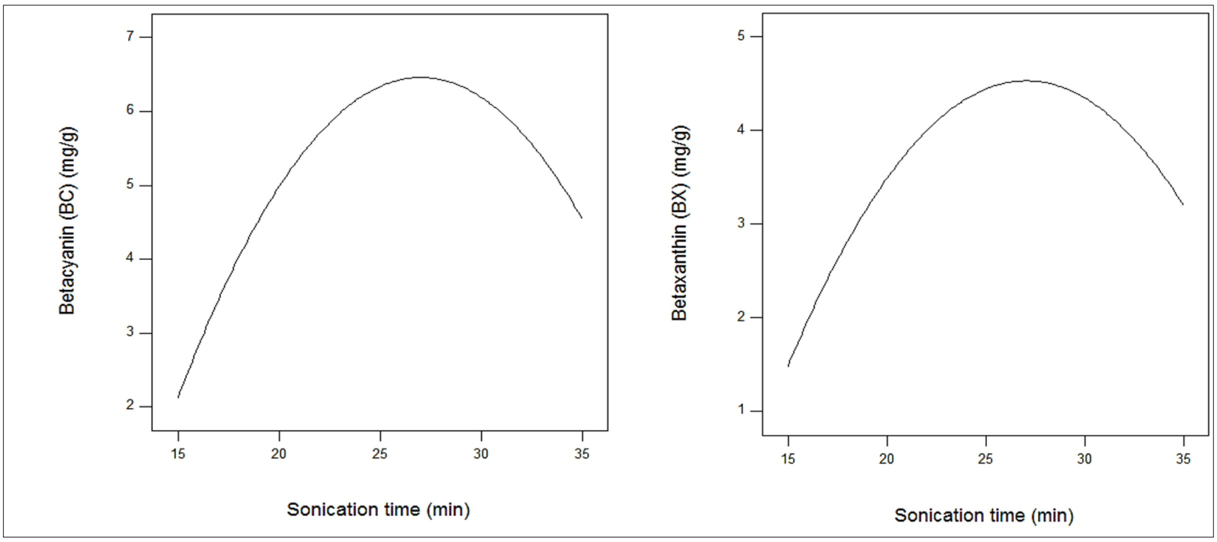

Fig 2: Effect of sonication time on Betacyanin and Betaxanthin

3.4 Interaction Effects of Process Variables

Three-dimensional response surface plots illustrated the interaction between extraction variables and their influence on pigment recovery. The interaction between pH and sonication time was statistically significant for both responses, whereas interactions between pH and solid-to-solvent ratio, and between solid-to-solvent ratio and sonication time, were comparatively less significant. The response surfaces revealed that maximum pigment recovery was achieved at intermediate pH values combined with higher solid-to-solvent ratios and longer sonication times. These observations demonstrate the importance of simultaneously optimizing process variables rather than evaluating them individually.

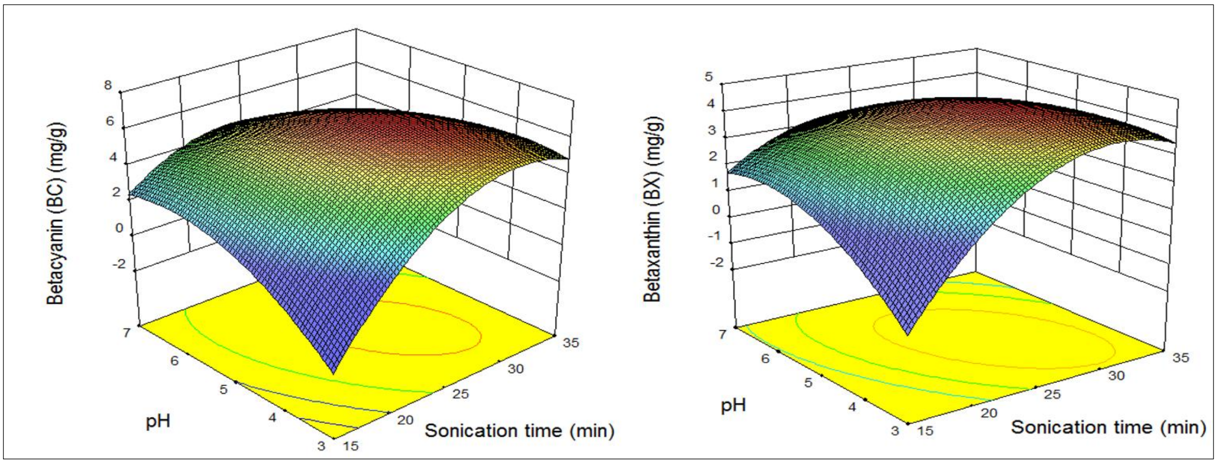

Fig 3: Interactive effect of pH and sonication time on Betacyanin and Betaxanthin

3.5 Optimization and Validation

Numerical optimization using the desirability function predicted the optimum extraction conditions as pH 4.7**,** solid-to-solvent ratio 50 g mL^{-1}**,** and sonication time 30 min. Under these conditions, the predicted betacyanin and betaxanthin yields were 6.31 mg g^{-1} and 4.43 mg g^{-1}, respectively. Experimental validation conducted under identical conditions produced values of 6.17 mg g^{-1} for betacyanin and 4.29 mg g^{-1} for betaxanthin, closely matching the predicted responses. The small deviation between predicted and observed values confirms the adequacy and reliability of the developed quadratic models for process optimization (Table 4).

Table 4: Optimal levels of variables of colorant extraction from beetroot waste stalks

Parameters	Goal	Lower limit	Upper limit	Coded value	Actual value
pH	In range	3.31	6.68	0.31	4.7
Solid-solvent ratio (g/ml)	In range	23.19	56.81	1	50
Sonication time (min)	In range	16.59	33.40	1	30
Betacyanin (mg/g)	Maximize	Predicted: 6.31		Observed: 6.17	
Betaxanthin (mg/g)	Maximize	Predicted: 4.43		Observed: 4.29	

3.6 Practical Significance

The present study demonstrates that beetroot waste stalks, which are generally discarded during vegetable processing, can serve as an economical source of natural betalain pigments. Ultrasound-assisted extraction significantly improved pigment recovery while reducing extraction time and solvent consumption compared with conventional extraction techniques. The optimized process provides a sustainable strategy for converting agricultural waste into value-added natural colourants suitable for food, pharmaceutical, cosmetic, and nutraceutical applications. Moreover, the utilization of beetroot processing residues supports waste valorization, resource efficiency, and circular bioeconomy principles, contributing to environmentally sustainable food processing practices.

4. Conclusion

The present study successfully demonstrated the potential of beetroot (*Beta vulgaris* L.) waste stalks as an economical and sustainable source of natural betalain pigments. Ultrasound-assisted extraction (UAE) proved to be an efficient green extraction technique for recovering betacyanin and betaxanthin, offering advantages such as reduced extraction time, lower solvent consumption, and enhanced pigment yield compared to conventional extraction methods. The extraction process was effectively optimized using Response Surface Methodology (RSM) based on a Central Composite Design (CCD), which enabled the evaluation of the individual and interactive effects of extraction variables.

Among the investigated factors, the solid-to-solvent ratio and sonication time significantly influenced the extraction efficiency of both pigments, whereas pH exhibited a comparatively smaller individual effect within the selected experimental range. The optimized extraction conditions of pH 4.7**,** solid-to-solvent ratio of 50 g mL^{-1}, and 30 min sonication time resulted in maximum predicted pigment yields of 6.31 mg g^{-1} betacyanin and 4.43 mg g^{-1} betaxanthin, which closely agreed with the experimentally obtained values. The close agreement between predicted and observed responses confirmed the reliability and predictive capability of the developed quadratic regression models.

The findings demonstrate that beetroot waste stalks, which are generally discarded during postharvest handling and processing, can be effectively valorized into high-value natural food colourants. The application of ultrasound-assisted extraction not only enhances process efficiency but also supports environmentally sustainable processing through reduced energy and solvent requirements. The recovered betalain pigments have considerable potential for application in the food, pharmaceutical, cosmetic, and nutraceutical industries as natural alternatives to synthetic colourants.

References

1. Ahmadi S, *et al*. Ultrasound-assisted extraction of natural pigments: Recent advances and applications. Ultrasonics Sonochemistry. 2021;76:105638.
2. Azeredo HM. Betalains: Properties, sources, applications, and stability—a review. International Journal of Food Science and Technology. 2009;44(12):2365–2376.
3. Bhattacharya S. Central composite design for response surface methodology and its application in pharmacy. In: Response Surface Methodology in Engineering Science. London: IntechOpen; 2021.
4. Chemat F, *et al*. Green extraction technologies for food bioactive compounds. Trends in Food Science & Technology. 2022;124:75–91.
5. Khan MK, *et al*. Betalains: Chemistry, extraction, stability and applications. Food Chemistry. 2023;405:134845.
6. Maran JP, Priya B, Nivetha CV. Optimization of ultrasound-assisted extraction of natural pigments from *Bougainvillea glabra* flowers. Industrial Crops and Products. 2015;63:182–189.
7. Rodriguez-Amaya DB. Natural food pigments and colorants. Comprehensive Reviews in Food Science and Food Safety. 2022;21:3510–3542.
8. Saini RK, Khan MI, Kumar V, Shang X, Lee JH, Ko EY. Bioactive compounds of agro-industrial by-products: Current trends, recovery, and possible utilization. Antioxidants. 2025;14(6):650.
9. Silva S, *et al*. Valorization of agro-industrial by-products through green extraction technologies. Journal of Cleaner Production. 2021;315:128196.
10. Usman M, Nakagawa M, Cheng S. Emerging trends in green extraction techniques for bioactive natural products. Processes. 2023;11(12):3444.
11. Wang L, *et al*. Ultrasound-assisted extraction of bioactive compounds from plant materials: Process optimization and industrial perspectives. Food and Bioproducts Processing. 2024;145:89–103.