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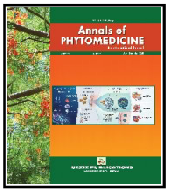

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Optimizing aqueous extraction of pigment from blue butterfly pea (*Clitoria ternatea* L.) using ultrasonication by response surface methodology

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Abstract

Blue butterfly pea (*Clitoria ternatea* L.) is a simple ornamental plant species, renowned for its therapeutic benefits. Its edible blossoms contain a significant level of "ternatins," a group of anthocyanins that contribute not only to its vibrant blue colour, but also to its antioxidant and medicinal properties. The blue colour anthocyanin pigment from blue butterfly pea is pH-dependent, and the colour stability of the pigment is highly influenced by the extraction method. The colour changes profoundly with a change in pH, making it a natural choice for embedding in halochromic smart packaging films. The study aimed to optimize process conditions for maximizing anthocyanin pigment extraction from blue butterfly pea using ultrasonication as pre-treatment by the statistical tool response surface methodology (RSM). For optimizing extraction in order to maximize yield, the composite central design (CCD) has been used with 20 replications. Here, the response surface model was created to correlate three factors that affect extraction yield: temperature, residence time, and solid-to-liquid ratio. Three factors which have been evaluated were extraction time (25-45 min), extraction temperature (40-60^{o}C), and solid-to-liquid ratio with extraction yield produced (%) as the dependent factor. Based on the experiment, the optimized aqueous extraction conditions were the extraction time of 37 min at 55^{o}C, with a solid-to-liquid ratio of 1:33 to get an aqueous extraction yield of 46.83%. The colour stability test of blue butterfly extract was carried out at pH 1 during a period of 28 days.

1. Introduction

Natural food colourants produced from plants have drawn attention in recent days owing to growing consumer awareness of health, safety, and environmental concerns (Melo Miranda *et al*., 2025). Among plant-based pigments, anthocyanins are particularly valued for their vivid red, violet, and blue hues. Antioxidant activity and associated health advantages, such as lowering the chance of stroke, cardiovascular disease, arthritis, and cancer (Kungsuwan *et al*., 2014).

C. ternatea habitually known as Blue butterfly pea, is a perennial climber plant of the Fabaceae family that is distributed in tropical and subtropical climate regions in abundance, including India (Hariadi *et al*., 2024). This plant is often farmed for its decorative appeal, but it also has significant phytopharmaceutical potential. Its edible blossoms, particularly the dark blue variety, are high in anthocyanins and have long been employed in herbal beverages and are much preferred for are natural colour for culinary purposes. The blue butterfly pea blossom has sparked interest owing to its vibrant blue colour and abundance of bioactive chemicals with medicinal qualities, including antioxidant, anti-inflammatory, antidiabetic and antibacterial effects (Suarna and Wijaya, 2021).

The distinctive anthocyanin's present in *C. ternatea* are referred to as ternatins, a group of polyacylated derivatives that are responsible for the strong blue colouration of the petals. Ternatins are structurally classified as polyacylated delphinidin 3, 3, and 5 triglucoside derivatives. Their colour expression is pH dependent, resulting in bright colours ranging from violet to blue and green. These compounds are water-soluble flavonoid pigments that provide a promising natural alternative to manufactured colourants (Handayani *et al*., 2024b). The global market for natural pigments is expanding, with blue colourants posing special challenges due to the scarcity and volatility of blue pigments such as anthocyanin (Vidana Gamage *et al*., 2021). Accordingly, there is a significant industrial need for stable, organically generated blue dyes.

Colour influences consumer perception and food quality. Natural colourants such as anthocyanins have gained in popularity as consumers become more health-conscious and demand clean-label, safe, and natural food additives (Neves *et al*., 2021). The market for

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natural pigments is expanding globally, with blue colourants posing special challenges due to the scarcity and volatility of blue pigments such as anthocyanin (Buecker *et al*., 2025). As a result, there is a significant industrial need for stable, organically generated blue dyes.

Traditional extraction procedures, such as aqueous extraction, are easy and inexpensive, but are sometimes limited by prolonged extraction times and the possible destruction of heat-sensitive chemicals. Aqueous solvents are particularly appealing for food applications because of their safety and biocompatibility (Queffelec *et al*., 2024). The response surface methodology tool has developed as an effective statistical approach for increasing extraction efficiency, particularly when water is used as a solvent. RSM allows concurrent optimization of several parameters, decreases the experimental runs, and shows variable interaction, making it perfect for improving plant pigment extraction methods (Baskaran *et al*., 2019).

In this experimental study, the current work attempts to improve the process conditions for aqueous extraction of anthocyanins since aqueous extraction removes solvent residues and offers a safe, food-grade, and environmentally friendly approach, it was given priority for use in food and nutraceutical applications from *C. ternatea* flowers utilizing RSM to determine the optimal conditions for maximising pigment yield, while keeping the bioactive integrity of the blue dye. The finding is expected to contribute to a sustainable, food-safe, and commercially feasible approach for extracting natural blue colourants from Blue butterfly pea blossoms.

2. Materials and Methods

2.1 Plant material preparation and drying

Fresh flowers of *C. ternatea* were sourced from the Agricultural College and Research Institute, TNAU, Madurai, India. *C. ternatea* Herbarium, which is part of the Department of Agriculture's Plant Varieties Protection Division in Bangkok, Thailand. The Voucher Specimen has the number BKU066793 on file. The flowers were hand-picked, thoroughly cleaned with distilled water to get rid any dust or debris, and the petals were uniformly spread in single layers on stainless steel trays and dried in a cabinet dryer at 55°C for 10-12 h until a constant weight was achieved. This controlled drying process aimed to minimize pigment degradation while ensuring adequate moisture removal.

2.2 Grinding process and sample storage

The dried blue butterfly pea flowers were crushed using a fine-grinding electrical blender. The resulting powdered samples were sieved to achieve a consistent particle size using a standard mesh (250 µm) screen, which helps in consistent pigment extraction. To prevent moisture absorption from the humid surroundings, the powdered samples were immediately transferred to airtight containers and kept in a desiccator at room temperature for further use (Handayani *et al*., 2024a). The prepared powdered flower samples were systematically labeled as No. 1 to No. 20 according to the 20 trial responses of the experimental design generated by the statistical tool response surface methodology (RSM).

2.3 Ultrasound-assisted aqueous extraction procedure

Dried petals of *C. ternatea* were finely powdered to maximize surface area. Anthocyanin pigments were then extracted *via* maceration (water-based) followed by ultrasound assisted procedure, optimizing three parameters using response surface methodology (RSM): temperature between (40-60°C), solid-to-liquid ratio between (1:20-1:60 w/v)and, residence time between (25-45 min), Powdered samples were mixed with distilled water according to the specified ratio, heated to the set temperature, and agitated for the designated time. For each experimental run, precisely 5 g of dried aqueous extracted *C. ternatea* flower powder was weighed and placed into a 50 ml glass beaker. Distilled water, according to the specified liquid-to-solid ratio for that particular trial run, was added to the beaker. The beaker was then placed in the ultrasonicator (Saiut *et al*., 2024), ensuring the water level in the bath was consistent with the solvent level in the beaker for efficient sonication. The ultrasonic power and extraction time were set according to the design matrix for each run (Ultrasonic frequency-48 kHz; Ultrasonic power effective-500 W; Capacity 24 liters; Tank 500 × 300 × 200 mm). The extraction temperature was maintained at 30°C throughout the experiments by circulating water in the ultrasonicator. After the sonication time, the blend was immediately filtered to separate the solid scum from the aqueous extract using Whatman No. 1 filter paper. The obtained extract was then centrifuged at 3000 rpm for 10 min to get rid of any last bits of debris. The supernatant was collected and kept for storage in amber glass vials at 4°C before analysis, typically within one day of hours. This study uses water as a green solvent in conjunction with ultrasonication to address the dearth of sustainable, food-grade extraction techniques. In contrast to deep eutectic solvent, microwave-assisted, or ethanol techniques, our process minimizes thermal degradation, avoids hazardous residues, and uses less energy.

2.4 Analytical methods

2.4.1 Total anthocyanin content (TAC)

The pH differential approach, as outlined by Giusti and Wrolstad (2001) was used to ascertain the total anthocyanin concentration 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) were used to create two dilutions of each extract. Each dilution absorbance was measured with a UV-V is Spectrophotometer at 510 and 700 nm (Riniati *et al*., 2024). Equation (1)'s total anthocyanin content was determined using the formula below and represented as mg of Cyanidin-3-Glucoside Equivalent (mg CGE/g DW) per gram of dry weight.

$$TAC = \frac{A \times MW \times DF \times 1000}{€ \times L}$$

In this case, A= [A510-A700 (10^{-9})] pH 1.0 [A510-A700 (10^{-9})] pH 4.5; DF (dilution factor) = the dilution factor of the extract MW (molecular weight) = 449.2 gram/mol for Cyanidin-3-Glucoside; õ (molar extinction coefficient) = 26900 L/mol.cm for cyanidin-3-glucoside; 1000 = Factor for converting g to mg; L (Path length of cuvette) = 1 cm

2.4.2 Colour intensity (CI)

The aqueous extract colour intensity was ascertained by measuring the absorbance at the maximum visible wavelength (λ_{max}) for *C. ternatea* anthocyanins, which typically falls within the scale of 600-620 nm, by means of the UV-V Spectrophotometer (Marpaung *et al*., 2023). Specifically, the absorbance at 605 nm was recorded for the

aqueous dilution. Higher absorbance values indicate greater colour intensity. Every measurement was carried out in triplicate.

2.5 Statistical analysis and optimization

Design-expert software (version 13, Stat-Ease Inc., Minneapolis, USA) was used to evaluate the experimental data. Analysis of variance (ANOVA) was used to assess the statistical significance of the independent variables and their interactions on the measured responses. Based on the coefficient of determination (R^2), adjusted R^2, predicted R^2, and adequacy precision, the fitness of the quadratic models was evaluated. Response surface plots and contour plots were generated in three dimensions to visualize the relationships between the independent variables and the responses, and to identify the optimal region for extraction. The ideal combination of ultrasonic power, extraction time, and liquid-to-solid ratio was then found through numerical optimization using design-expert desirability function, which maximized both total anthocyanin content and colour intensity. Verification experiments were conducted at the predicted optimal conditions to validate the accuracy of the developed models. All statistical analyses were performed at a 95% confidence level ($p<0.05$).

3. Results

This portion systematically extends and interprets the experimental data achieved from the ultrasonication-assisted aqueous extraction of anthocyanin pigment from blue butterfly *pea* petals, optimized through the statistical tool response surface methodology (RSM). The discussion integrates these findings with existing scientific literature, highlighting the significance of the optimized conditions and the stability of the extracted pigment.

3.1 Design of experiments and model fitting

A central composite design (CCD) comprising of 20 experimental runs was strategically employed to investigate the individual and combined effects of three critical independent variables on pigment extraction yield: ultrasonication time (A: 25-45 mins), temperature (B: 40-60°C), and solid-to-liquid ratio [C: (1:A-1:B)], representing the range around 1:20-1:60 w/v) Specifically, we used a standardized format of grams of solid per milliliter of solvent (g/ml). For example, instead of "1:A-1: B", we will explicitly state the range as 1:20 to 1:60 g/ml. The observed pigment extraction yield (%), which was the dependent factor, for each experimental run, is presented in Table 1.

Table 1: Experimental design matrix (coded and actual values) and corresponding pigment yield (%) for the ultrasonication-assisted aqueous extraction of *C. ternatea* pigment

Run	Independent variables			Responses
	Time (min) A	Temperature (°C) B	Solid-to-liquid ratio (g/ml) C	Pigment extraction yield (%)
1.	50	25	40	46.62
2.	55	35	40	49.54
3.	50	35	40	47.81
4.	55	30	30	47.14
5.	40	30	50	31.07
6.	45	40	30	40.19
7.	55	40	30	46.62
8.	50	35	60	39.18
9.	55	35	20	38.91
10.	50	35	40	46.87
11.	50	35	40	47.29
12.	55	40	50	43.54
13.	40	30	30	38.11
14.	50	35	40	47.79
15.	45	35	40	38.45
16.	45	40	50	39.89
17.	40	35	40	36.07
18.	50	45	40	47.65
19.	60	25	40	45.12
20.	55	30	50	42.57

Table 2: ANOVA for the pigment extraction yield (%) from quadratic model

Source	Sum of squares	df	Mean square	F-value	*p*-value	
Model	428.86	9	47.65	10.10	0.0006	Significant
	83.85	1	83.85	17.78	0.0018	Significant
	0.0747	1	4.72	0.0158	0.9023	Significant
	10.65	1	10.65	2.26	0.1637	Significant
	1.79	1	1.79	0.3789	0.5519	Significant
	3.06	1	3.06	0.6493	0.4391	Significant
	6.52	1	6.52	1.38	0.2668	Significant
	63.81	1	63.81	13.53	0.0043	Significant
	0.1621	1	0.1621	0.0345	0.8564	Significant
	89.37	1	89.37	18.95	0.0014	Significant
Residual	47.16	10	4.72			
Lack of fit	46.56	7	6.65	32.88	0.0048	Not-Significant
Pure error	0.6068	3	0.2023			
Total	476.02	19				

The experimental data for pigment yield were subsequently fitted to a quadratic polynomial model, a standard approach for analyzing CCD outcomes. The statistical adequacy and significance of this model were rigorously evaluated through analysis of variance (ANOVA), with the detailed results summarized in Table 2.

The ANOVA results indicated a statistically significant model (*e.g.,* F-value =10.10, $p<0.0001$) for predicting the pigment extraction yield. This high significance confirms that the independent variables collectively accounted for a substantial part of the variability observed in the response. The coefficient of determination (R^2) for the pigment yield model was found to be R^2 value 0.90, signifying that 95% of the difference in pigment yield could be reliably explained by the developed model. Furthermore, the non-significant lack of fit ($p>0.05$) provided strong evidence that the model adequately represented the experimental data and possessed strong predictive capabilities within the studied range.

3.2 Effect of independent variables on pigment yield

The individual and combined effects of the independent variables to that of pigment yield are visualized through 3D response surface plots (Figures 1-3) and their corresponding 2D contour plots, which offer a more detailed view of the optimal regions.

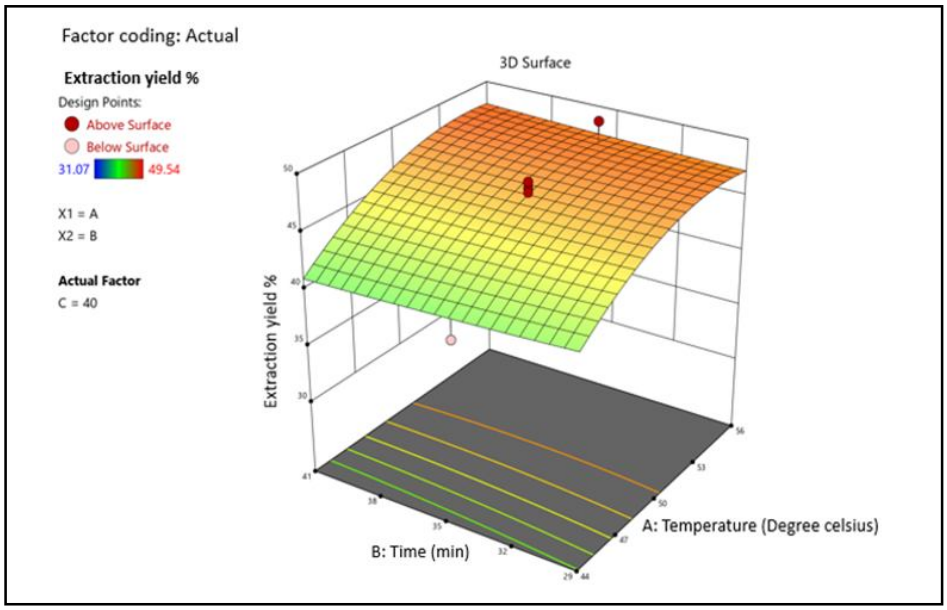

Figure 1: 3D response surface plot showing the interactive effect of extraction time. (A) temperature, (B) on pigment yield with solid-to-liquid ratio, and (C) held constant at its central level.

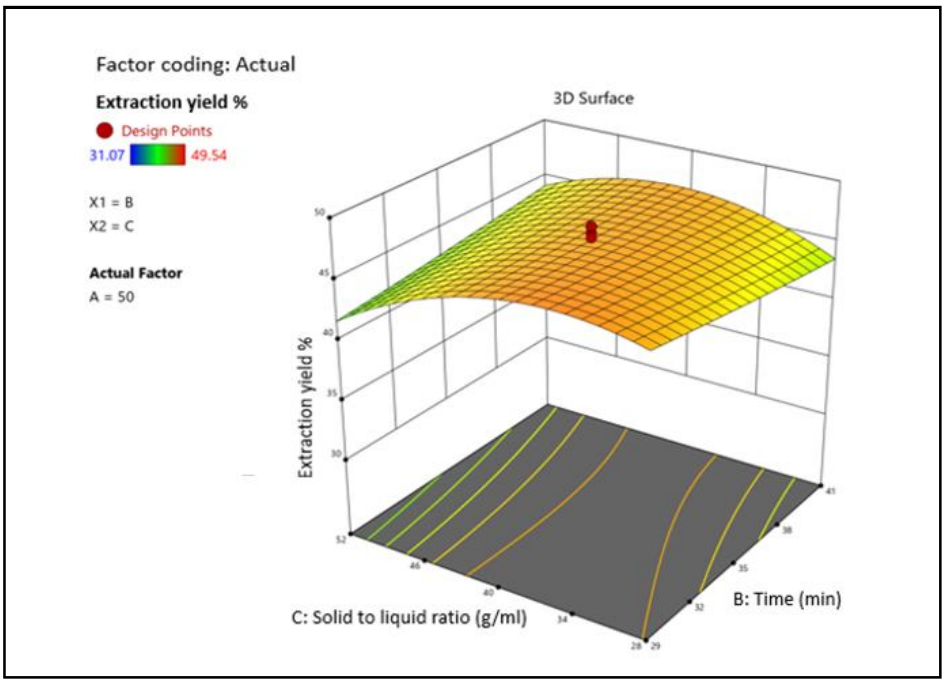

Figure 2: 3D response surface plot showing the interactive effect of extraction time. (A) solid-to-liquid ratio, (C) on pigment yield with temperature, and (B) held constant at its central level.

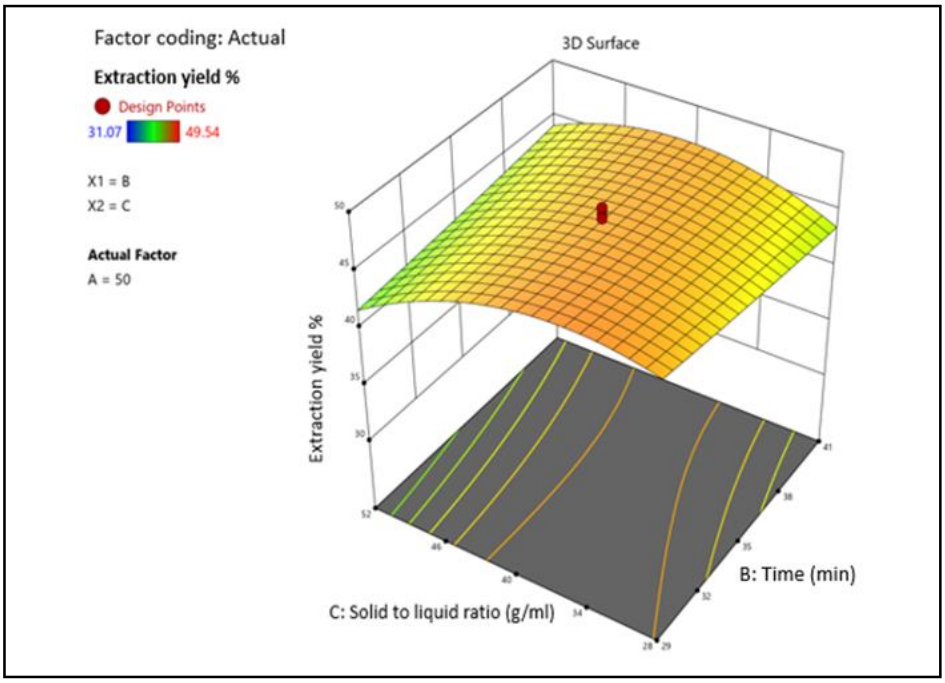

Figure 3: 3D response surface plot showing the interactive effect of temperature. (B) solid-to-liquid ratio, (C) on pigment yield with extraction time, and (A) held constant at its central level.

Analysis of these response surface plots, coupled with the ANOVA results (Table 2), revealed that all three independent variables (temperature, ultrasonication time, and solid-to-liquid ratio) exerted a significant influence on the pigment yield. The significance of the quadratic terms (A^2, B^2, C^2) further underscores a non-linear association between the variables and the extraction yield, indicating the presence of optimal points within the experimental domain. Specifically, pigment extraction yields initially exhibited a robust increase with extraction time up to the optimal 33 min, beyond which a decline was observed. Similarly, increasing extraction

temperature enhanced pigment release, but an optimum was observed at 55°C, with higher temperatures leading to a decline in yield percentage. The solid-to-liquid ratio also showed an optimal range around 1:37; increasing the solvent volume relative to the solid initially improved yield, but excessive dilution offered no further significant benefit. Significant interaction effects were identified between 55°C and 33 min, suggesting that the optimal level of one variable is dependent on the level of another.

3.3 Optimization of extraction conditions and validation

The application of statistical tool response surface methodology (RSM) proved highly effective in optimizing the complex interplay of ultrasonication parameters for pigment extraction from *C. ternatea*. The high R^2 values and significant F-values obtained from the ANOVA (Table 2) confirm the statistical robustness of the developed models. Based on the established response surface model and numerical optimization, the optimized extraction conditions for maximizing pigment yield were precisely determined to be: temperature 55°C, extraction time 33 min, and solid-to-liquid ratio (1:37). Under these specific conditions, the model robustly predicted a maximum extraction yield of 45.51%. To rigorously validate the accuracy and reliability of the RSM model, verification experiments were conducted in triplicate at these predicted optimal conditions. The experimentally obtained pigment yield was 45.51%. This result showed excellent agreement with the predicted value, thereby unequivocally confirming the robustness and predictive capability of the developed RSM model for optimizing the ultrasonication-assisted extraction of *C. ternatea* pigment. This approach allowed for the efficient identification of optimal conditions with a reduced number of experiments compared to one-factor-at-a-time methods, highlighting the benefits of RSM in process optimization, consistent with findings in similar studies on plant extract optimization (Baskaran *et al*., 2019; Izza and Tristantini, 2021 and Maia *et al*., 2025).

3.4 Impact of ultrasonication parameters on pigment extraction

The results demonstrate the significant influence of ultrasonication time (A: 25-45 min), temperature (B: 40-60°C), and solid-to-liquid ratio [C: (1:A-1: B)], representing the range around 1:20-1:60 w/v) on the pigment yield and quality.

3.4.1 Ultrasonication time

The pigment yield initially increased with ultrasonication time, reaching a peak before declining (Figures 1-3). This can be attributed to the acoustic cavitation phenomenon characteristic of ultrasonication. Initial cavitation promotes cell wall disruption and increases the release of intracellular pigments within the solvent (Liu *et al*., 2022). However, prolonged ultrasonication may lead to the degradation of heat-sensitive colour pigments, such as anthocyanins (the primary pigments in *C. ternatea*), due to excessive cavitation and localized heating (Moses *et al*., 2017). This observed optimum time is consistent with previous studies on ultrasound-assisted extraction of anthocyanins, where prolonged exposure led to degradation (Li *et al*., 2022).

3.4.2 Temperature

Temperature also exhibited a significant effect, with an optimal range observed for pigment extraction. Increased temperature generally enhances the solubility and dispersal rate of compounds from the plant matrix within the solvent (Antony and Farid, 2022). The higher kinetic energy at elevated temperatures facilitates pigment release. However, similar to extended sonication time, excessive temperatures can cause thermal degradation of anthocyanins, which are known to be sensitive to heat, leading to a reduction in pigment content and colour stability (de Almeida *et al*., 2025). The optimal temperature range identified in this study balances enhanced extraction efficiency with minimal pigment degradation.

3.4.3 Liquid-to-solid ratio

The liquid-to-solid ratio also played a crucial role in maximizing pigment yield. An increase in the solvent volume relative to the solid material generally improves the concentration gradient between the plant material and the solvent, facilitating better diffusion of the pigments (Predescu *et al*., 2016). Beyond a certain point, however, a higher liquid-to-solid ratio might lead to increased solvent consumption without a proportional increase in extractable pigment, thus reducing the efficiency of the process. The optimal ratio identified suggests a balance between sufficient solvent for complete extraction and economic viability.

3.4.4 Interactive effects

The contour and 3D response surface plots (Figures 1-3) highlighted significant interactive effects between the variables. For instance, the interaction between ultrasonication time and temperature suggests that at higher temperatures, a shorter ultrasonication time might be sufficient to achieve optimal extraction, mitigating the risk of pigment degradation. This synergistic effect, where ultrasonication facilitates rapid mass transfer and temperature enhances solubility, is a common advantage of ultrasound-assisted extraction (Mazumder *et al*., 2023).

3.5 Analytical methods

The stability of the optimized *C. ternatea* extract was comprehensively evaluated over a period of 28 days under two distinct storage conditions: 4°C (refrigerated) and room temperature (RT). The stability tests were conducted at a consistent pH of 1, a critical condition where the anthocyanin pigment predominantly exists in its flavylium cation form, known for its enhanced stability in aqueous environments.

3.5.1 Total anthocyanin content

Total anthocyanin content (TAC) of the optimized blue butterfly pea extract was also quantified over the 28-day storage period to complement the colour retention data. Initially, both samples of the optimized extract exhibited a total anthocyanin content of 35.78 mg CGE/g DW. Over the 28-day storage period, distinct trends in TAC degradation were observed depending on the storage temperature. For the extract stored under refrigerated conditions (4°C), the TAC demonstrated remarkable stability. After 7 days, the content slightly decreased to 35.42 mg CGE/g DW. This minor reduction continued, reaching 35.06 mg CGE/g DW by Day 14 and remaining consistent up to Day 21. By the end of the 28-day period, the TAC was 34.70 mg CGE/g DW. This indicates an approximate retention of 97.0% of the initial anthocyanin content at 4°C, signifying minimal degradation and excellent stability under cold storage. In contrast, the extract stored at room temperature experienced a more significant and continuous decline in TAC. From an initial 35.78 mg CGE/g DW, the content dropped to 32.92 mg CGE/g DW by Day 7. This degradation continued steadily, with TAC values of 31.49 mg CGE/g DW at Day 14 and 30.77 mg CGE/g DW at Day 21. By Day 28, the total

anthocyanin content had reduced to 29.70 mg CGE/g DW, representing an approximate retention of 83.0% of the initial content. This demonstrates a more substantial reduction in anthocyanin concentration at ambient temperatures.

Figure 4 graphically illustrates these changes in total anthocyanin content over time for both storage conditions. The distinct separation of the curves, with the 4°C line showing a much flatter trajectory, visually confirms that the degradation of anthocyanin content directly mirrors the observed decrease in colour intensity, with significantly better preservation achieved at lower temperatures (Jaafar *et al*., 2020). The quantitative reduction in TAC at room temperature, relative to the minimal loss at 4°C, strongly suggests that temperature is a primary driver of anthocyanin degradation, even at an optimal pH. The observed superior stability at 4°C and pH 1 for both colour and total anthocyanin content underscores the importance of controlled storage conditions for preserving the quality of *C. ternatea* extracts. The stability of the flavylium cation at pH 1 significantly contributes to the overall stability, as this form is less susceptible to hydration and ring opening, which are common degradation pathways for anthocyanins (Yilmaz and Türker, 2024). However, even at this stable pH, the influence of temperature is evident, with higher temperatures accelerating the degradation processes, likely through oxidation, enzymatic activity, or other chemical reactions. These findings suggest that for applications requiring prolonged storage and colour integrity, refrigeration at acidic pH is crucial.

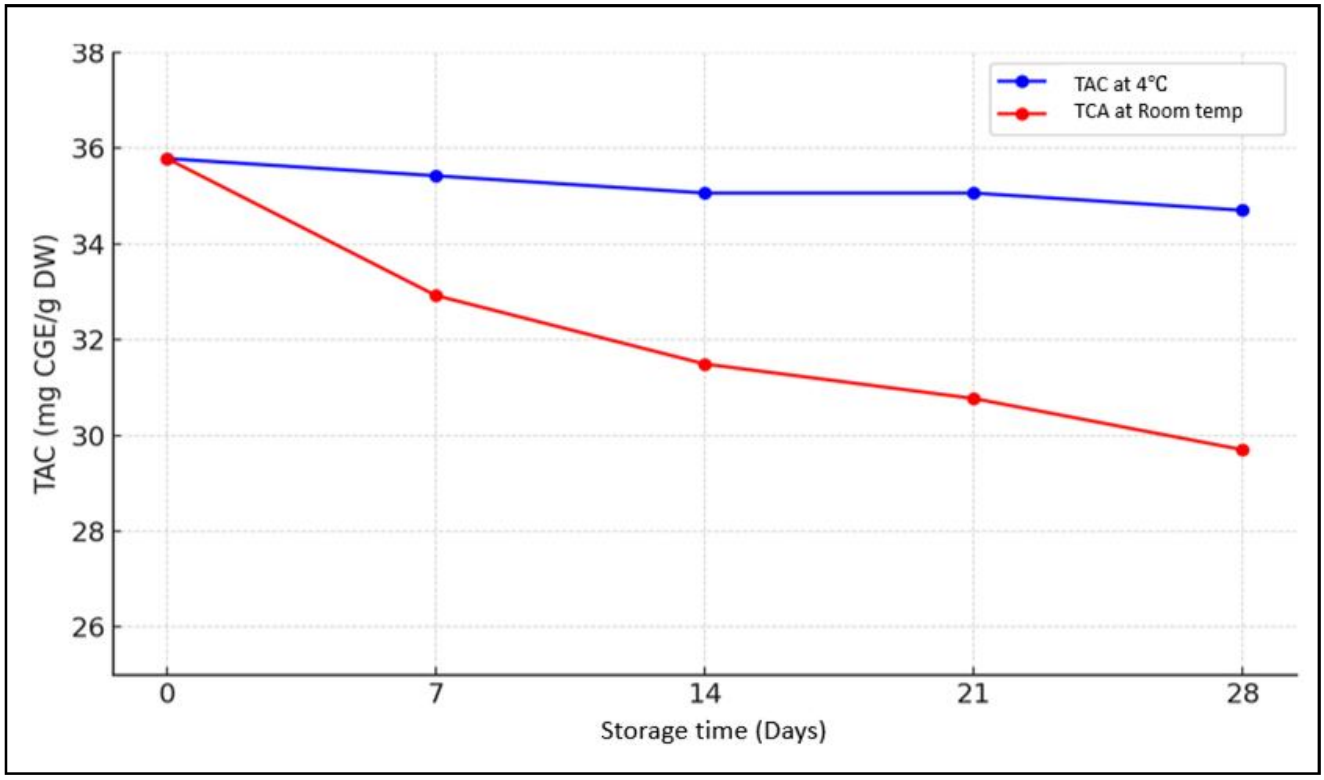

Figure 4: Changes in total anthocyanin content of the optimized *C. ternatea* extract over time.

3.5.2 Colour intensity

The colour stability of the optimized *C. ternatea* extract was monitored by measuring the percentage of anthocyanin retained (based on absorbance at 548 nm) over 28 days. Initially, at Day 1, the extract exhibited 100% anthocyanin retention under both 4°C and room temperature storage conditions. After 7 days, the extract stored at 4°C maintained excellent stability, showing 99% retention, while the room temperature sample experienced a more noticeable decline to 92% retention. By Day 14, the refrigerated extract retained 98% of its initial colour, whereas the room temperature sample further decreased to 88 %. This trend continued to Day 21, with 98% retention at 4°C and 86% at room temperature. By the end of the 28 days study, the anthocyanin pigment extracted by the aqueous method exhibited superior colour retention when stored at 4°C, maintaining 97% of its initial absorbance at 548 nm. In contrast, the extract stored at room temperature showed a more pronounced decline in colour, retaining 83% of its initial absorbance over the same period. This differential degradation highlights the significant impact of storage temperature on the chromophore's integrity (Otto *et al*., 2024). Figure 5 visually represents this trend, clearly demonstrating the consistently higher percentage of anthocyanin retention at 4°C compared to at room temperature. The gradual decrease in absorbance at both temperatures, with a more pronounced decline at room temperature, suggests a thermally accelerated degradation pathway for the anthocyanin chromophore (Fu *et al*., 2021).

3.6 Comparison with conventional methods and previous studies

This study offers several advantages for the ultrasonication-assisted aqueous extraction method over conventional extraction techniques (*e.g.,* maceration, hot water extraction). Ultrasonication has been widely reported to ease extraction time, decrease solvent consumption, and increase extraction yields of various bioactive compounds,

including pigments (Shen *et al*., 2023). The high pigment yield achieved under the optimized conditions in this study compares favorably with or even surpasses those reported for traditional aqueous extraction methods of blue butterfly anthocyanin pigment (Handayani *et al*., 2024a). While some studies have explored the extraction of anthocyanin's from *C. ternatea* using different solvent (ethanol) or method (microwave-assisted extraction) (Gomez *et al*., 2022; Nurhayati *et al*., 2024), this study specifically focused on aqueous extraction combined with ultrasonication, which is advantageous for food-grade applications due to the non-toxic nature of the solvent. These research findings on optimal parameters align with the general principles of efficient ultrasound-assisted extraction of natural pigments, emphasizing the need for carefully controlled conditions to prevent degradation.

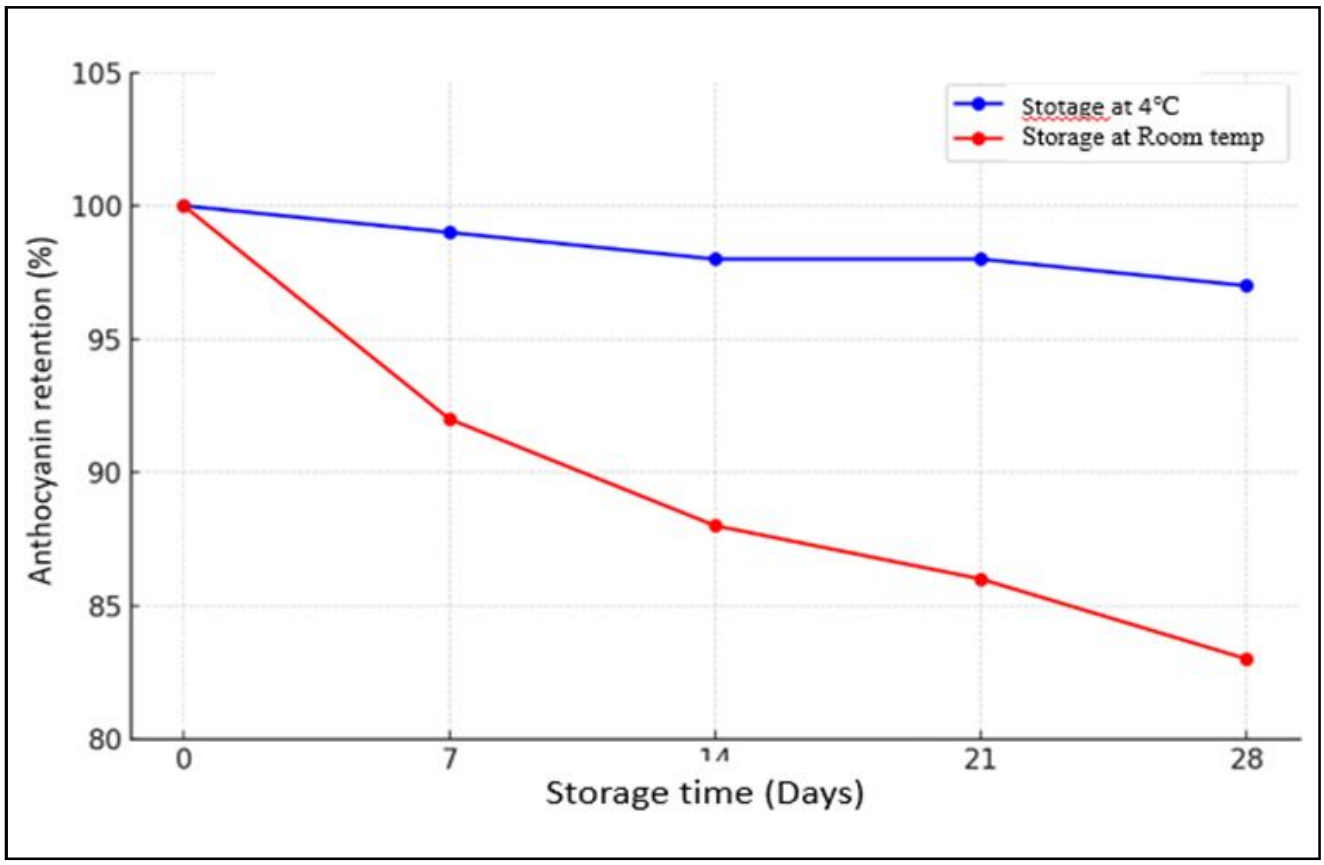

Figure 5: Colour stability of the optimized *C. ternatea* extract at 4°C compared to room temperature.

3.7 Limitations and future perspectives

While this study successfully optimized the aqueous pigment extraction from *C. ternatea* using ultrasonication, certain limitations warrant consideration for future research. The primary focus was on maximizing the overall pigment yield, often quantified spectrophotometrically based on a single wavelength absorbance. To provide a more comprehensive understanding, future studies should include a detailed qualitative and quantitative characterization of the extracted anthocyanin profile using advanced analytical techniques such as high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS). This would allow for the identification and quantification of specific anthocyanin derivatives (acylated ternatins) present in the extract under optimized conditions, providing deeper insights into its specific composition and purity.

Furthermore, beyond the pH 1 stability test, the stability of the extracted pigment needs to be thoroughly investigated across a broader range of environmental stressors, including varying pH values (pH 3, 5, 7, relevant for different food matrices), different light exposures (dark, ambient, UV), and diverse storage temperatures (refrigeration, room temperature, accelerated conditions). Such comprehensive stability studies are crucial for accurately determining its shelf-life and suitability for diverse commercial applications in the food sector, cosmetic, and pharmaceutical industries. Lastly, while the abstract highlights the antioxidant and medicinal properties of *C. ternatea*, this study primarily focused on pigment extraction. Future research should also explore and quantify the bioactivity (antioxidant activity, anti-inflammatory properties) of the optimized extract, linking back to the therapeutic benefits mentioned, to fully leverage the potential of this valuable plant. Finally, scaling up the optimized laboratory-scale process to pilot or industrial scale would also necessitate further techno-economic feasibility studies to assess commercial viability and energy consumption.

4. Discussion

The current results clearly demonstrate how temperature, duration, and solvent ratio work in concert to maximize pigment yield during ultrasonication-assisted extraction. The ideal circumstances that produced a 45.51% yield demonstrate how important ultrasonication is in breaking down the plant cell matrix at 33 minutes and exposing

more surface area for mass transfer. The solvent enters the cells more effectively than with traditional soaking thanks to the sonic cavitation process, which creates micro streaming and shear stresses. As a result, pigment diffusion is sped up, supporting earlier claims that ultrasonication provides better kinetics than passive diffusion techniques.

However, temperature turns out to be a key element in striking a balance between structural stability and pigment recovery. In the current investigation, a solvent ratio of 1:37 and a moderate temperature of 55°C worked well. Excessive heat can cause pigment degradation, especially in sensitive ternatin molecules, but it can also promote solubility and dispersion by reducing solvent viscosity. The findings imply that a modest heat input avoids over-processing, which otherwise cause would pigment disintegration to cancel out the advantageous cavitation effects. This supports the idea that heat serves as a kinetic activator rather than a solubilizing force in such an optimized system, guaranteeing quick extraction before degradation events become thermodynamically beneficial. As a result, optimization strikes a careful balance between maintaining molecular integrity and increasing yield.

The stability of the extract under storage conditions is another benefit of the developed technique. When kept in acidic and chilled settings (pH 1, 4°C), the pigments maintained their distinctive acylated flavylium cation structure, indicating that the extraction process successfully maintained their inherent stability-a crucial factor for commercial use. According to Yilmaz and Türker (2024), the acylation of anthocyanin's is crucial for enhancing pigment durability against pH and temperature changes. This structural preservation guarantees a high potential for utilization in commercial food and medicinal formulations. When taken as a whole, these results lend credence to the idea that ultrasonication, when done under properly monitored conditions, is very effective low-impact pigment extraction method that provides both increased yields and functional stability.

5. Conclusion

This study effectively optimized the ultrasonication-assisted aqueous extraction of anthocyanin pigment (ternatins) from *C. ternatea* blossoms. By employing the statistical tool response surface methodology, the research identified optimal extraction conditions at 55°C, an ultrasonication time of 33 min, and a 1:37 solid-to-liquid ratio, resulting in aqueous, ultrasound-assisted extraction method for *C. ternatea* anthocyanins presented in this work achieves a remarkably high yield (45.51%) without the use of organic solvents. This specific set of parameters represents a crucial balance, ensuring that the temperature provides sufficient energy for cell wall disruption and pigment release while simultaneously preventing thermal degradation that maximizes pigment release while maintaining anthocyanin stability, demonstrating a more environmentally friendly and energy-efficient option than traditional ethanol-based, microwave, or deep eutectic solvent methods. The optimized sonication time and solid-to-liquid ratio further contributed to efficient and comprehensive pigment recovery. Additionally, the extracted pigment demonstrated good colour stability when tested at pH 1 for 28 days, a key indicator of its potential robustness in acidic applications where the stable flavylium cation form predominates. Overall, this research delivers a promising, efficient, and environmentally friendly method for obtaining a high-quality natural blue pigment, underscoring *C. ternatea* significant potential as a sustainable and versatile source in various industries. This work contributes to the rising demand for natural compounds, providing a clear pathway for the practical application of this vibrant botanical extract. These results address the rising need for clean-label, safe, and sustainable blue colourants and provide prospects for direct use in drinks, dairy goods, confections, and pH-responsive smart packaging films. This study establishes a new standard for environmentally friendly, scalable pigment manufacturing from botanical sources by fusing sophisticated process optimization with practical functional performance.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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