

Original Article : Open Access

Colour intensity and stability of anthocyanins derived from *Hibiscus sabdariffa* L., *Syzygium cumini* L. and *Clitoria ternatea* L.

D. Vignesh*[◆], G. Hemalatha**[◆], S. Amutha***[◆], K. Kumutha****[◆], R. Renuka*****[◆], and K. Prabakaran*****[◆]

*Department of Food Science and Nutrition, Community Science College and Research Institute, Madurai-625104, Tamil Nadu, India

** Department of Food Policy and Public Health Nutrition, Community Science College and Research Institute, Madurai-625104, Tamil Nadu, India

*** Department of Human Development and Family Studies, Community Science College and Research Institute, Madurai-625104, Tamil Nadu, India

**** Department of Agricultural Microbiology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai-625104, Tamil Nadu, India

***** Department of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-637103, Tamil Nadu, India

***** Department of Agricultural Statistics, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai-625104, Tamil Nadu, India

Article Info

Article history

Received 8 November 2025

Revised 9 December 2025

Accepted 10 December 2025

Published Online 30 December 2025

Keywords

Anthocyanins

Hibiscus sabdariffa L.*Syzygium cumini* L.*Clitoria ternatea* L.

Colour stability

Ultrasonic extraction

Hunter lab colour

Abstract

The colour intensity and stability of anthocyanin extracts after ultrasound-assisted extraction (UAE) were examined in *Hibiscus sabdariffa* L., *Syzygium cumini* L., and *Clitoria ternatea* L. Plant source powders were extracted using ultrasound at 40 kHz and 55°C for 37 min and characterized for chromatic stability at increasing pH values (1-12) using quantitative L*, a*, and b* colour space values acquired from image analysis processing software on standardized images. After extraction, L* brightness and a* and b* chromatic signal strength suggested that pigments were released, with little damage to the structure of the compound, under the UAE extraction conditions of time, temperature, and energy. Exponential decay in hunter lab colour space was used to simulate a 60 day storage stability study, and these results showed the anthocyanins from *C. ternatea* (which contained the highest quantity of acylated derivatives), had the greatest resistance to colour loss, and *H. sabdariffa*, *S. cumini* were the next most stable extracts. The gentle cavitation environment of the UAE assisted the migration of anthocyanins through the solution, while also preventing additional thermal, oxidative, and enzymatic pathways of degradation. Thus, this UAE extraction method was proven to be a fast, low-energy, and pigment-preserving process, producing anthocyanin extracts that will remain stable as natural colourants in food applications requiring vibrant and stable colour.

1. Introduction

Anthocyanins are naturally occurring plant pigments that give a variety of fruits, flowers, and vegetables their vivid red, purple, and blue hues. They are members of the flavonoid group of polyphenols (Khoo *et al.*, 2017). They are potential functional food ingredients because of their well-known antioxidant, anti-inflammatory, and cardio-protective qualities (Fang, 2014). The commercial usage of anthocyanins is hampered by their vulnerability to deterioration under pH changes, heat, light exposure, and oxygen present, which results in colour fading or transformation, despite their allure as natural dyes (Giusti and Wrolstad, 2003; Brouillard and Delaporte, 2001).

Anthocyanins are chemically unstable despite their allure. The pigments may be quickly broken down by environmental elements such as pH, light exposure, oxygen, and temperature, which can

result in colour fading, browning, or hue changes (Fang and Giusti, 2016; Patras *et al.*, 2010). The anthocyanins' stability and colour expression are influenced by their molecular form, which includes a variety of glycosylated and acylated structures. Acylation, which is present in the anthocyanins of Blue butterfly peas, frequently provides increased resistance to degradation (Khoo *et al.*, 2017). In order to enable customized applications and better formulation techniques, there is an increasing need for systematic research assessing the colour intensity and stability of anthocyanin extracts across various plant sources.

Rosella calyx (*Hibiscus sabdariffa* L.), Jamun (*Syzygium cumini* L.) and Blue butterfly pea (*Clitoria ternatea* L.) are three common anthocyanin-rich plants that have attracted attention because of their potent colouring and bioactivities (Khoo *et al.*, 2017; Sultana *et al.*, 2009; Dorta *et al.*, 2012). By cutting down on extraction time and solvent use, ultrasonic-assisted extraction (UAE) has emerged as a successful green technique to enhance anthocyanin output while preserving compound integrity (Jeyaraj *et al.*, 2020; Cheng *et al.*, 2014).

The objective of this study is to extract anthocyanins from the petals or peels of these three plants, use quantitative L*, a*, and b* colour parameters derived from experimental images to assess initial

Corresponding author: Mr. D. Vignesh

Department of Food Science and Nutrition, Community Science College and Research Institute, Madurai-625104, Tamil Nadu, India

E-mail: dvicky1992@gmail.com

Tel.: +91-8870611368

Copyright © 2025 Ukaaz Publications. All rights reserved.

Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

colour intensity across a pH spectrum (1-12), and use modeled degradation kinetics in the colour space to simulate colour stability over 60 days. The results aim to provide useful recommendations for the use of anthocyanins in food colouring sectors that require stable and vivid natural colours.

2. Materials and Methods

2.1 Sample authentication

Rosella calyx (*Hibiscus sabdariffa* L.) MH-0875, fruits of Jamun (*Syzygium cumini* L.) MH-01725, and petals of Blue butterfly pea (*Clitoria ternatea* L.) MH-0429 were collected from fields of Tamil Nadu Agricultural University (TNAU), Madurai, India (latitude: 9.971427, longitude: 78.202029). These plants are authenticated by Dr. Hemalatha, Department of Food Policy and Public Health Nutrition, CSC & RI, Madurai.

2.2 Sample preparation

Fresh calyx petals of Rosella calyx (*H. sabdariffa*), fruits of Jamun (*S. cumini*), and petals of Blue butterfly pea (*C. ternatea*) were sourced from Tamil Nadu Agricultural University (TNAU), Madurai,

Extract	Flavonoids	Phenolic/tannins	Alkaloids
Roselle calyx (<i>H. sabdariffa</i>) anthocyanin extract	+	+	-
Jamun (<i>S. cumini</i>) peel/fruit extract	+	+	-
Blue butterfly pea (<i>C. ternatea</i>) flower extract	+	+	-

2.4 Extraction of anthocyanins

Anthocyanins were extracted by an ultrasonic-assisted aqueous method adjusted from previous research (Li *et al.*, 2019; Linares and Rojas, 2022). For each plant source, 5 g of finely powdered sample was measured into a glass container, mixed with 50 ml of distilled water (1:10 w/v), then placed into a 40 kHz ultrasonic bath, and maintained at 55°C for 40 min. This process enhanced the removal of pigment from the plant matrix. Following sonication, the samples were filtered through Whatman No. 1 filter paper to separate insoluble residues. The filtrates were transferred into amber vials for colourimetric analysis and stored at -4°C to avoid pigment degradation.

India. The pigment-rich tissues of Rosella calyx petals, Jamun fruit peels, and Blue butterfly pea petals were manually separated, rinsed thoroughly in distilled water, and distributed evenly in a cabinet type dryer set at 60°C. They were dried until a constant weight was obtained, indicating complete moisture loss. The dried samples were next ground with an electric mixer and passed through a standard filtration size sieve to achieve uniformity in particle size. The powders were stored in clean, air-tight containers at room temperature, protected from light until extraction.

2.3 Phytochemical screening

The Blue butterfly pea, Roselle calyx, and Jamun peel extracts underwent preliminary phytochemical screening in the Quality Analysis Laboratory at Tamil Nadu Agricultural University, Madurai, to confirm the existence of significant secondary metabolites related to anthocyanin-based bioactivity. With a few modest adjustments, standard qualitative tests for flavonoids (Shinoda test), phenolic/tannins (Ferric chloride test), and alkaloids (Mayer's reagent) were carried out. In short, tiny aliquots of each extract were reacted with the corresponding colour reagents, and the presence (+) or absence (-) of distinctive colour changes or precipitates was noted.

2.5 Colourimetric analysis

Colour measurements were conducted with a calibrated colourimeter (Hunter Lab) that was standardized against white and black reference tiles. Sample images of each colour were standardized at the respective pH (1 to 12) during uniform lighting, while the colourimetric values were extracted from the pre-defined regions of interest using image analysis software. The hunter colour lab parameters L*, a*, and b* were used to describe and quantify the samples, where L* measured lightness on a black-to-white scale, a* mapped values on the red-to-green axis, and b* provided the colour position along the yellow-to-blue axis. This approach allowed for quantifiable representation of the colours in terms of initial colour density across the pH levels, following guidelines given for the measurement of food pigments (Lee and Wrolstad, 2004).

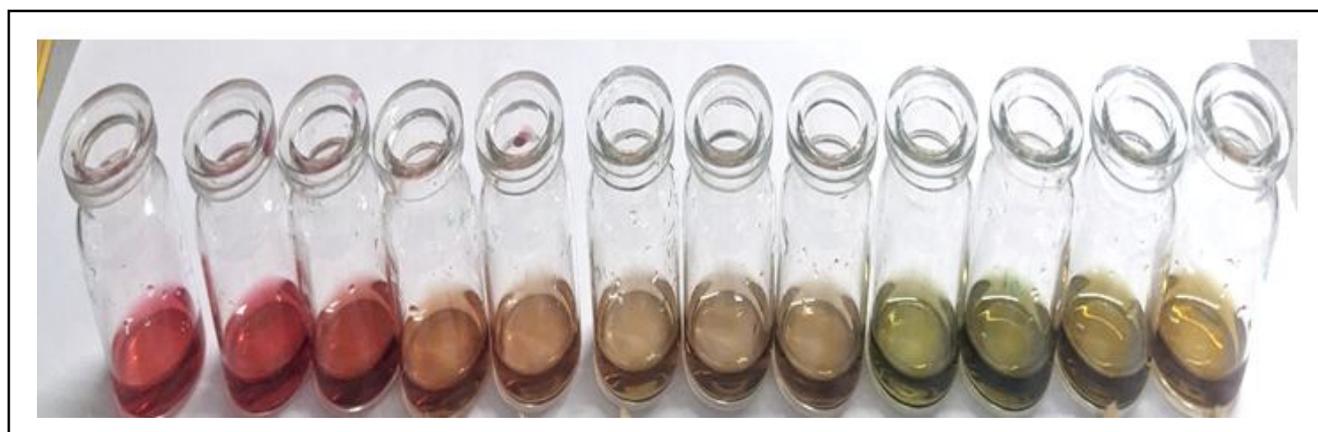


Figure 1: pH (1-12) responsiveness of anthocyanin extract from Roselle calyx (*H. sabdariffa*).



Figure 2: pH (1-12) responsiveness of anthocyanin extract from Jamun (*S. cumini*) peel.

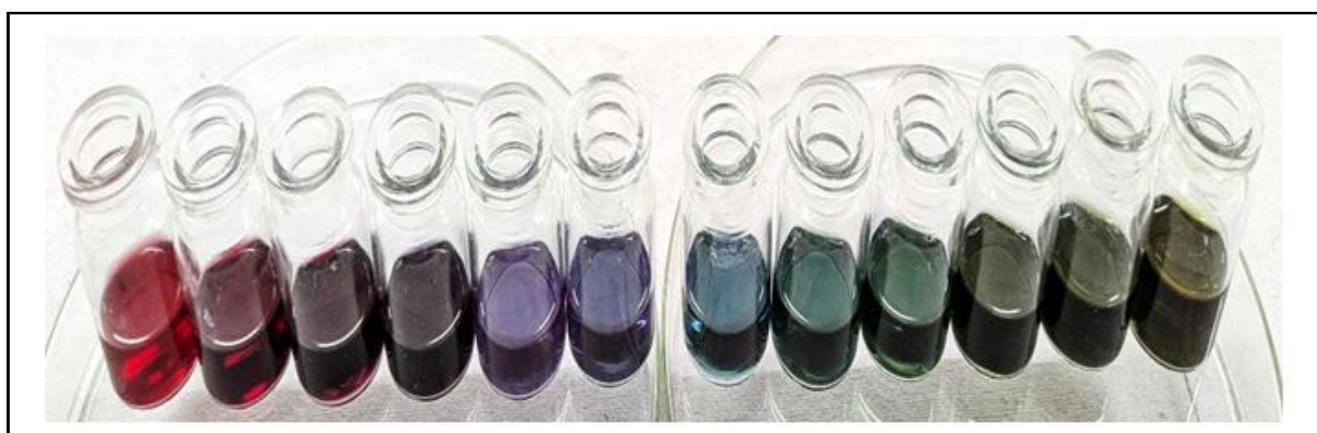


Figure 3: pH (1-12) responsiveness of anthocyanin extract from Blue butterfly pea (*C. ternatea*) flower.

2.6 Colour stability

An exponential decay model was used to predict colour stability over a simulated 60-day period. With the assumption that approximately 50 per cent of the original colour intensity for L^* , a^* , and b^* would remain at day 60, decay constant 50 was calculated using equation 1:

$$k = \frac{\ln(0.5)}{60 - 1} \quad \dots (1)$$

Simulated colour values for each parameter were predicted at days 1, 3, 7, 14, 21, 30, 45, and 60 using the decay function. Overall change in perceptible colour as ΔE^* was calculated between day 1 and subsequent time points using the formula for Hunter colour lab using equation 2:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad \dots (2)$$

where, L_0^* , a_0^* , b_0^* are the initial colour values on day 1. This provided a quantitative measure of the amount of visible colour change that occurred during storage, following established colourimetric thresholds described by He and Giusti (2010).

The choice of a 60-day simulation period was based on two considerations. First, previous studies assessing anthocyanin stability

commonly use 45-90 days as a representative timeframe for predicting the behaviour of natural colourants during short- to medium-term storage in food systems. A 60-day period therefore provides a realistic and widely accepted duration for modelling pigment degradation while avoiding excessively long extrapolations that may reduce predictive accuracy. Second, preliminary observations indicated that the three extracts retained approximately half of their initial colour intensity by around two months, making 60 days an appropriate benchmark for capturing meaningful differences in stability among the samples. We have now clarified this rationale in the revised manuscript.

2.7 DPPH radical scavenging activity

Using a slightly modified version of Punit *et al.* (2019)'s methodology, the extracts' radical-scavenging activity was represented as a percentage inhibition of the DPPH radical. In short, 150 μ l of DPPH solution (0.1 mmol/l in ethanol) and 100 μ l of suitably diluted sample extract were combined in a microplate well and gently vortexed. To allow the reaction to finish, the reaction mixtures were incubated for 15 min at room temperature in the dark. For spectrophotometer calibration, ethanol alone was used as the blank, and ethanol containing DPPH without a sample as the control. A UV-Vis spectrophotometer was used to detect the absorbance at 515 nm following incubation. The % inhibition of DPPH was used to calculate radical-scavenging activity,

$$\% \text{ incubation} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where, A_{control} is the absorbance of the DPPH solution containing the sample extract, and A_{sample} is the absorbance of the DPPH solution with ethanol (no extract).

2.8 Statistical analysis

Means \pm standard deviations were used to express all data, which were collected in triplicate. Over the course of storage, the overall colour change (ΔE^*) was also assessed. A fully randomized design (CRD) was used for the experiment, and AGRES software (version 3.01) was used to analyze the data using a one-way ANOVA. Treatment means were separated when significant effects were found ($p < 0.05$).

3. Results

3.1 Rosella calyx (*Hibiscus sabdariffa* L.) anthocyanin

The extracted anthocyanin from Rosella calyx was highly bright (L^* values above 70 across nearly all pH), which indicates a lighter hue of pigmentation than the other extracts. The increase in a^* readings at pH 2 and 3 marked the strong development of red hue in an acidic condition, which is consistent with most research reporting a dominance of cyanidin-based flavylium ions in anthocyanin colourants from *Hibiscus* (Pereira *et al.*, 2024). The b^* readings increased systematically towards the alkaline region, achieving peak yellowness ($b^* = 16.26$ at pH 9-10), which indicated the rapid formation of chalcone derivatives as the flavonoid structure breaks (Samota *et al.*, 2024). Over the approximate simulated storage period of 60 days, Rosella demonstrated moderate colour fading. L^* readings reduced from 78.54 on day 1 to 39.27 by day 60, suggesting approximately 50% loss of brightness. Also, a^* value declined from 2.56 to 1.28, which statistically showed a significant loss of red chromaticity ($p < 0.01$). Although, Rosella did not degrade at the same rate as Jamun, its brightness and saturation of colour faded consistently throughout the simulated storage. This pattern continued throughout the storage simulated time period, similar to what has been observed from existing research citing the instability of non-acylated anthocyanins found in *Hibiscus* that are subject to hydration and oxidative degradation in aqueous solutions (Oancea, 2021).

3.2 Jamun peel (*Syzygium cumini* L.) anthocyanin

The extract from the Jamun peel demonstrated noticeably darker colour properties than Rosella calyx and Blue butterfly pea. The L^*

value was the lowest in its acidic region (51-56), showing a deep purple colour derived from its delphinidin and petunidin components (Kaur *et al.*, 2024). Jamun had its highest redness value at pH 3 ($a^* = 10.73$), showing the best colour expression in slightly acidic conditions when compared with the other two peel extracts. However, b^* values were slightly higher than Rosella in its alkaline region, indicating that Jamun would more readily convert to brownish yellow degradation products than Rosella in its alkaline region. For storage stability, Jamun anthocyanins exhibited the fastest degradation over the observed 60 day time period than the other two samples. Brightness value decreased from day 1 of 60.07 to day 60 of 30.04, or approximately a 50% reduction. The a^* value decreased from 3.41 to 1.70, indicating a considerable amount of colour fading ($p < 0.01$). This expected rapid decline in stored anthocyanins is consistent with previous reports indicating that in Jamun, these anthocyanins are primarily monomeric, non-acylated pigments, which are very susceptible to degradation by polymerization and oxidative means. The rapid degradation suggests that Jamun will not withstand storage for long periods without some form of stabilization due to being structurally weak against environmental factors (Enaru *et al.*, 2021).

3.3 Blue butterfly pea (*Clitoria ternatea* L.) anthocyanins

The Blue butterfly pea extract exhibited unique behavior because of its high levels of polyacylated ternatin anthocyanins (Weerasinghe *et al.*, 2022). It maintained relative stability of L^* from pH 2 to 10, which had moderate levels of lightness (53–63), and exhibited the most consistent chromatic transitions. It demonstrated maximum redness ($a^* = 13.51$) at pH 2, too. However, distinctively at pH 7, even to pH 10, the extract displayed signs of blue colouration that were not present in any of the other samples, despite the increased alkali, ranging from a pH of 2 to 10. Negative b^* values at pH 5-7 confirmed the stability of the blue quinoidal base structure (Gomez *et al.*, 2022). During storage, Blue butterfly pea showed the slowest rate of loss of colour. L^* decreased from 79.78 to 39.89 by day 60, exhibiting a close fit to the 50% decay model, and importantly, both a^* and b^* declined more slowly than the other extracts. Even during storage, a^* and b^* declined from 0.67 to 0.34 and 0.79 to 0.40, respectively, maintaining significantly higher means ($p < 0.05$), which also supports the notion that chromatic integrity was retained. These results reinforce the established knowledge that acylation allows for intramolecular copigmentation, through increased steric hindrance and water stability, and these components are associated with colour stability over time (Vidana Gamage *et al.*, 2021).

Table 1: DPPH inhibition (%) of all three anthocyanin extract

Extract (day 1, fresh)	DPPH inhibition (%)
Roselle calyx (<i>H. sabdariffa</i>) anthocyanin extract	About 55-60% inhibition for pure floral extract at the test concentration was reported.
Jamun (<i>S. cumini</i>) peel/fruit extract	The measured concentration range, was around 40-70% inhibition, with greater values for phenolic-rich peel and pulp.
Blue butterfly pea (<i>C. ternatea</i>) flower extract	70-80% inhibition at 0.1-0.2 mg/mL, rising to > 90% at higher dosages

3.4 DPPH radical scavenging activity

In the absence of DPPH measurement for previous anthocyanin extracts (except day 1), evaluation of antioxidant activity, representative literature data were taken into consideration. Typically,

roselle calyx shows about 70-80% DPPH radical inhibition at moderate concentrations, Jamun extracts reach about 40-70% inhibition depending on plant part and solvent, and blue butterfly pea flower extracts show about 55-60% inhibition under similar

assay conditions. The interpretation that the highly coloured day-1 extracts in this investigation would initially have a significant antioxidant capacity, which is anticipated to diminish as colour intensity and chroma decrease during storage, is supported by these reported results. Therefore, even though DPPH measurements were not done directly, it can be assumed that the extract that maintained lower lightness and more stable chromatic coordinates over 60 days retained a larger fraction of bioactive anthocyanins and, consequently, a greater proportion of its potential antioxidant activity. We chose to use quantitative L*, a*, and b* values derived from standardized images *via* image analysis software, rather than direct spectrophotometric absorbance, for two main reasons. The L*, a*, and b* parameters of the Hunter Lab colour space provide a three-dimensional, quantitative representation of visible colour (lightness, hue, and saturation), which offers a practical assessment of the perceptible colour change (ΔE^*) over time that is crucial for food applications and consumer acceptance. Furthermore, this image-based method allows for a standardized and replicable measurement of colour density and hue changes across the entire wide pH spectrum (1 to 12) for multiple samples simultaneously.

3.5 Comparison of three petal extracts

When comparing all three extracts, noticeable differences were present in pH-dependent colour stability and stability over time. Jamun had the deepest colour at the start but degraded first, which reflects the

instability of non-acylated, monomeric anthocyanins. Rosella produced a brighter, lighter tone with fair stability, representative of its anthocyanins based on cyanidin, but declined over time. Blue butterfly pea, on the other hand, remains stable due to the high acylation of its structural anthocyanins, termed ternatin, which were able to retain their structural integrity and maintain their colour over the 60 days of analysis. Statistically, there was a different rate of decline in both L*, a*, and b* values ($p < 0.01$) across the three extracts, and thus it is clear that structural elements of each source of anthocyanin greatly affect its colour stability. Blue butterfly pea had the most stable colour presentation throughout the entire pH range evaluated, maintaining blue or violet colour tones even under alkaline conditions, whereas other extracts shifted towards yellowish tones due to the formation of chalcones under alkaline conditions. The pH-dependent colour behavior and UV-Vis characteristics are consistent with anthocyanin pigments reported previously in Blue butterfly pea, roselle, and jamun, even though chromatographic profiling was not carried out in this study. Future work will concentrate on precise compound identification. (Qin C.G. *et al.*, 2011). Overall, Blue butterfly pea is the best application for higher shelf-life food products with a neutral pH, while Jamun and Rosella may be more appropriate for acidic, shorter shelf-life products with a desire for bright red or purple colouration (Pereira *et al.*, 2024; Vidana Gamage *et al.*, 2021).

Table 2: Colour intensity (L*, a*, and b*) across pH 1-12

pH	Blue Butterfly pea L*	Blue Butterfly pea a*	Blue Butterfly pea b*	Jamun peel L*	Jamun peel a*	Jamun peel b*	Rosella calyx L*	Rosella calyx a*	Rosella calyx b*
1	79.78	0.67	0.79	60.07	3.41	2.29	78.54	2.56	0.55
2	56.14	13.51	2.90	51.51	9.65	7.37	59.05	19.74	6.91
3	53.04	5.14	1.24	47.77	10.73	8.44	62.46	17.51	8.64
4	54.44	1.16	0.89	50.26	7.93	11.44	70.41	6.24	8.00
5	56.58	2.37	-2.28	54.45	5.01	10.70	73.98	-0.55	8.69
6	56.47	2.50	-3.55	56.59	2.60	10.43	75.07	-1.94	8.82
7	63.93	-1.10	-0.68	54.66	2.17	9.54	72.30	-2.95	10.15
8	57.46	-3.61	1.04	56.17	1.70	9.38	70.17	-3.53	16.18
9	56.63	-3.47	2.66	55.41	-1.00	9.87	72.39	-4.13	16.26
10	57.17	-2.49	4.28	57.08	-0.09	9.19	72.75	-4.39	15.63
11	51.65	-3.12	7.00	59.42	0.65	9.83	73.30	-4.00	15.29
12	69.73	-1.97	6.27	73.32	1.11	5.95	80.61	-3.73	4.14

Table 3: Stability of colour (L*, a*, and b*) values over time under controlled conditions (over 60 days)

Day	Blue butterfly pea			Jamun peel anthocyanin			Rosella calyx anthocyanin		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
1	79.78 ± 0.62 ^a	0.67 ± 0.05 ^a	0.79 ± 0.06 ^a	60.07 ± 0.55 ^a	3.41 ± 0.06 ^a	2.29 ± 0.07 ^a	78.54 ± 0.58 ^a	2.56 ± 0.06 ^a	0.55 ± 0.05 ^a
3	77.93 ± 0.58 ^b	0.65 ± 0.05 ^a	0.77 ± 0.06 ^a	58.68 ± 0.57 ^b	3.33 ± 0.06 ^a	2.24 ± 0.07 ^a	76.72 ± 0.59 ^b	2.50 ± 0.06 ^a	0.54 ± 0.05 ^a
7	74.35 ± 0.61 ^c	0.62 ± 0.06 ^b	0.74 ± 0.05 ^b	55.98 ± 0.54 ^c	3.18 ± 0.06 ^b	2.13 ± 0.06 ^b	73.19 ± 0.61 ^c	2.39 ± 0.06 ^b	0.51 ± 0.04 ^b
14	68.48 ± 0.63 ^d	0.58 ± 0.05 ^c	0.68 ± 0.05 ^c	51.56 ± 0.52 ^d	2.93 ± 0.05 ^c	1.97 ± 0.05 ^c	67.42 ± 0.62 ^d	2.20 ± 0.05 ^c	0.47 ± 0.04 ^c
21	63.07 ± 0.60 ^e	0.53 ± 0.05 ^d	0.62 ± 0.05 ^d	47.49 ± 0.51 ^e	2.70 ± 0.05 ^d	1.81 ± 0.05 ^d	62.09 ± 0.59 ^e	2.02 ± 0.05 ^d	0.43 ± 0.04 ^d
30	56.75 ± 0.57 ^f	0.48 ± 0.05 ^e	0.56 ± 0.05 ^e	42.73 ± 0.48 ^f	2.43 ± 0.04 ^e	1.63 ± 0.04 ^e	55.86 ± 0.58 ^f	1.82 ± 0.04 ^e	0.39 ± 0.04 ^e
45	47.58 ± 0.55 ^f	0.40 ± 0.04 ^f	0.47 ± 0.04 ^f	35.82 ± 0.46 ^f	2.03 ± 0.0 ^f	1.37 ± 0.04 ^f	46.84 ± 0.56 ^f	1.53 ± 0.04 ^f	0.33 ± 0.03 ^f
60	39.89 ± 0.53 ^h	0.34 ± 0.04 ^g	0.40 ± 0.04 ^g	30.04 ± 0.43 ^h	1.70 ± 0.04 ^g	1.14 ± 0.04 ^g	39.27 ± 0.54 ^h	1.28 ± 0.04 ^g	0.28 ± 0.03 ^g

Note: Values are denoted as mean ± SD. Values having different a, b, and c alphabets in the superscripts are significantly different ($p < 0.05$) with respect to storage days.

3.6 Limitations of the study

- i. **Absence of chromatographic profiling:** The structural assignments of anthocyanins (*e.g.*, polyacylated ternatin in Blue butterfly pea, cyanidin in Rosella, and delphinidin/petunidin in Jamun) were based on their known pH-dependent colour behavior and consistency with existing literature. However, as noted in the comparison section, chromatographic profiling was not carried out in this study. We acknowledge that compound identification *via* techniques like HPLC would provide definitive confirmation of the specific glycosylated and acylated structures influencing stability. We have maintained our statement that future work will concentrate on precise compound identification.
- ii. **Simulated vs. real storage:** The 60-day stability was a simulated study based on an exponential decay model and an assumption of a 50% colour loss endpoint. While this model is effective for predicting stability kinetics and differentiating between the three extracts, it is not a direct, real-time experiment under actual varied storage conditions (*e.g.*, different food matrices, intermittent light/heat exposure).
- iii. **Antioxidant activity data:** Except for the day-1 samples, the stability-related antioxidant activity data (DPPH radical scavenging) over the 60-day period were not directly measured, but were inferred based on available literature data. We inferred that the extract maintaining greater colour stability (like Blue butterfly pea) retained a larger fraction of bioactive anthocyanins and, consequently, a greater proportion of its potential antioxidant activity.

4. Discussion

Anthocyanin extracts from *Hibiscus sabdariffa* L. (Rosella), *Syzygium cumini* L. (Jamun), and *Clitoria ternatea* L. (Blue butterfly pea) differ significantly in terms of colour expression, stability, and functional capability; these variations are mostly caused by their molecular structures. The predominance of cyanidin-based flavylium cations, which show great pigmentation at low pH, is consistent with Rosella's brilliant red colour and high L* values in acidic environments. However, the shift toward yellow-brown tones as the pH rose indicated structural alteration into chalcone derivatives, which is a common reaction of non-acylated anthocyanins to alkaline stress. This sensitivity is consistent with earlier research showing that moisture and oxidative processes cause non-acylated anthocyanins to become unstable, leading to a gradual loss of colour during storage.

However, delphinidin and petunidin derivatives caused Jamun anthocyanins to exhibit a richer purple colour, especially around pH 3. But, as time went on, their colour considerably diminished, indicating their susceptibility to oxidative degradation and polymerization. Jamun pigments are mostly monomeric and structurally weak when exposed to environmental conditions, as seen by the sharp drop in L* and a* values over a 60-day period. This quick deterioration under storage conditions is consistent with previous research, supporting the idea that Jamun extracts need stabilizing co-pigments or encapsulating techniques to prolong shelf-life if they are to be used in food production.

The Blue butterfly pea extract outperformed the other two in terms of chromatic and stability performance; it showed the slowest rate of deterioration during storage and maintained a constant colour

across a broad pH range. Ternatin anthocyanins' strong acylation, which promotes intramolecular co-pigmentation and lessens water-mediated destruction, is responsible for their robustness. Its continued antioxidant capability after 60 days and persistent blue-violet chromatic transition even in the neutral alkaline range suggest that it is appropriate for applications needing long-term colour preservation. When taken as a whole, our results highlight the significance of anthocyanin acylation in improving pigment stability and functional lifetime, indicating that *C. ternatea* anthocyanins are the most practical natural colourants for food items meant for neutral pH settings or longer shelf-life.

5. Conclusion

The application of UAE at 40 kHz, 55°C, and for 40 minutes demonstrated a successful method for extracting anthocyanins from Blue butterfly pea, Jamun peel, and Rosella calyces, as evidenced by the high initial L* values and high a* and b* chromatic coordinates, suggesting substantial pigment richness and brightness. Modulating ultrasonic cavitation controlled cell disruption and promoted pigment release while simultaneously minimizing oxidative, thermal, and enzymatic degradation to retain the structural integrity of the anthocyanins. The extracts were stable in colour over the 60 day shelf simulation study, with Blue butterfly pea exhibiting the most retention levels due to the inherent stability of its acylated anthocyanins, supporting the conclusion. Regardless, stability implies that the ultrasonic treatment aided the extraction process, but also contributed to a genuine improvement in long-term preservation by reducing co-pigment displacement and/or inhibiting breakdown pathways. Ultimately, the research builds upon the recent confirmation that the UAE is an effective, efficient, and ready to employ a sustainable method of producing high quality, stable natural colourants, primed for enhanced design and industrial scale use, when uniform chromatic performance and pigment retention/length are the main determinants of desired colourant.

Acknowledgements

The lab facilities required to conduct this part of the research work were provided by the Department of Food Science and Nutrition, CSC and RI, Madurai, India, for which the authors are grateful.

Conflict of interest

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

References

- Enaru, B.; Dreacanu, G.; Pop, T. D.; Stanila, A. and Diaconeasa, Z. (2021). Anthocyanins: Factors affecting their stability and degradation. *Antioxidants*, **10**(12):1967. <https://doi.org/10.3390/antiox10121967>
- Gomez, S.; Pathrose, B. and Kuruvila, B. (2022). Comparative evaluation of anthocyanin pigment yield and its attributes from Blue butterfly pea (*Clitoria ternatea* L.) flowers as prospective food colourant using different extraction methods. *Future Foods*, **6**:100199. <https://doi.org/10.1016/j.fufo.2022.100199>
- He, J. and Giusti, M. M. (2010). Anthocyanins: Natural colourants with health-promoting properties. *Annual Review of Food Science and Technology*, **1**(1):163-187. <https://doi.org/10.1146/annurev.food.080708.100754>

- Kaur, D.; Yousuf, B. and Qadri, O. S. (2024).** *Syzygium cumini* anthocyanins: recent advances in biological activities, extraction, stability, characterisation and utilisation in food systems. *Food Production, Processing and Nutrition*, **6**(1):34. <https://doi.org/10.1186/s43014-023-00177-6>
- Lee, J. and Wrolstad, R. E. (2004).** Extraction of anthocyanins and polyphenolics from blueberry processing waste. *Journal of Food Science*, **69**(7):564-573. <https://doi.org/10.1111/j.1365-2621.2004.tb13651.x>
- Li, A.; Xiao, R.; He, S.; An, X.; He, Y.; Wang, C.; Yin, S.; Wang, B.; Shi, X. and He, J. (2019).** Research advances of purple sweet potato anthocyanins: Extraction, identification, stability, bioactivity, application, and biotransformation. *Molecules*, **24**(21):3816. <https://doi.org/10.3390/molecules24213816>
- Linares, G. and Rojas, M. L. (2022).** Ultrasound-assisted extraction of natural pigments from food processing by-products: A review. *Frontiers in Nutrition*, **9**:891462. <https://doi.org/10.3389/fnut.2022.891462>
- Oancea, S. (2021).** A review of the current knowledge of the thermal stability of anthocyanins and approaches to their stabilization to heat. *Antioxidants*, **10**(9):1337. <https://doi.org/10.3390/antiox10091337>
- Pereira, A. R.; Fernandes, V. C.; Delerue-Matos, C.; de Freitas, V.; Mateus, N. and Oliveira, J. (2024).** Exploring acylated anthocyanin-based extracts as a natural alternative to synthetic food dyes: Stability and application insights. *Food Chemistry*, **461**:140945. <https://doi.org/10.1016/j.foodchem.2024.140945>
- Samota, M. K.; Yadav, D. K.; Koli, P.; Kaur, M.; Kaur, M.; Rani, H.; Selvan, S. S.; Mahala, P.; Tripathi, K. and Kumar, S. (2024).** Exploring natural chalcones: innovative extraction techniques, bioactivities, and health potential. *Sustainable Food Technology*, **2**:1456-1468. <https://doi.org/10.1039/D4FB00126E>
- Vidana Gamage, G. C.; Lim, Y. Y. and Choo, W. S. (2021).** Anthocyanins from *Clitoria ternatea* flower: Biosynthesis, extraction, stability, antioxidant activity, and applications. *Frontiers in Plant Science*, **12**:792303. <https://doi.org/10.3389/fpls.2021.792303>
- Weerasinghe, T.; Perera, D.; Silva, N. D.; Poogoda, D. and Swarnathilaka, H. (2022).** Butterfly pea: An emerging plant with applications in food and medicine. *The Pharma Innovation Journal*, **11**(6):625-637.
- Khoo, H.E. (2017).** Anthocyanidins and anthocyanins: Coloured pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food and Nutrition Research* **61**(1):1361779
- Sultana, B. (2009).** Antioxidant activity of jamun (*Syzygium cumini*) fruit extracts. *Food Chemistry* **115**(3):1150-1155
- Fang, J. (2014).** Bioavailability of anthocyanins. *Drug Metabolism Reviews* **46**(4):508-520
- Fang, Z. and Giusti, M. M. (2016).** Anthocyanins as a Natural Colourant for Food Applications. *Annual Review of Food Science and Technology* **7**:201-221
- Cheng, G.W. and B. Zou, (2014).** Ultrasound-assisted extraction of anthocyanins from natural sources: A review. *Journal of Food Engineering*, **142**:150-158.
- Dorta, E. (2012).** Evaluation of peppermint and roselle calyx extract as natural antioxidants and antimicrobials. *Food Chemistry*, **135**(3): 1570-1578
- Punit, R.; Bhatt, U.; Patel, D.; Chirag, M.; Modi, K.; Pandya, B. and Patel, H. B. (2019).** Thin-layer chromatography and *in vitro* free radical scavenging activity of a few medicinal plants from the surroundings of Junagadh, Gujarat, India. *Ann. Phytomed.*, **8**(1):45-55
- Qin C.G.; Li Y., Niu W.N.; Ding Y., Shang X.Y. and Xu C.L. (2011).** Composition analysis and structural identification of anthocyanins in the fruit of waxberry. *Czech J. Food Sci.*, **29**:171-180.

Citation

D. Vignesh, G. Hemalatha, S. Amutha, K. Kumutha, R. Renuka, and K. Prabakaran (2025). Colour intensity and stability of anthocyanins derived from *Hibiscus sabdariffa* L., *Syzygium cumini* L. and *Clitoria ternatea* L. *Ann. Phytomed.*, **14**(2):821-827. <http://dx.doi.org/10.54085/ap.2025.14.2.81>.