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Extraction and storage stability of anthocyanin rich Ixora coccinea flower extract using carbonated water

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Research Article

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Title: Extraction and storage stability of anthocyanin rich *Ixora coccinea* flower extract using carbonated water

Running title: Ixora flower pigment extraction and stability

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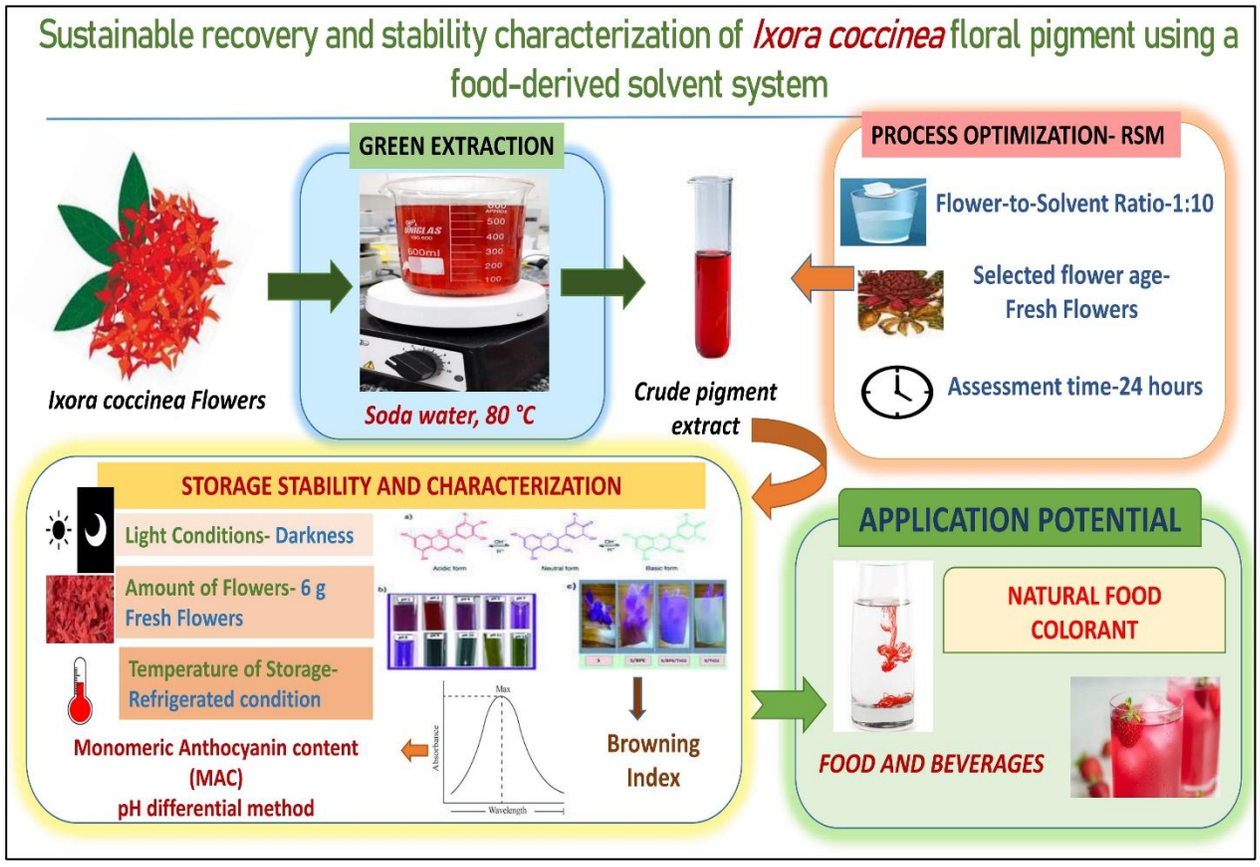
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Abstract:

Ixora coccinea flowers are a potential source of natural pigments, but the use of carbonated water for their extraction and subsequent stability of the resulting extract require further evaluation. This study investigated carbonated water as an extraction medium and assessed the resulting crude flower extract using spectrophotometric measurements. Monomeric Anthocyanin Content (MAC) was determined by the pH differential method, while the absorbance at 520 nm and browning index (BI) were used to monitor changes in color-related responses. Carbonated water, distilled water, and acidified ethanol were compared under the extraction conditions specified in the study. The effects of flower-to-solvent ratio and post-harvest withering were also examined. Storage conditions were evaluated with respect to light and temperature, and response surface methodology was applied to investigate the effects of flower mass and storage temperature on MAC and BI. Under the tested conditions, dark and refrigerated storage were associated with better retention of the measured responses. The model-derived operating conditions and their experimental validation are reported after verification of the model structure and calculations. These findings provide preliminary evidence for the use of carbonated water in extracting *Ixora coccinea* flower pigments and identify processing and storage variables for further investigation in food-colorant development.

Keywords: Browning index; Color stability; Food-grade extraction; Green extraction; Natural food colorants; pH differential method; Response surface methodology

Graphical Abstract:



1. Introduction

Anthocyanins are water-soluble polyphenolic pigments widely distributed in fruits, vegetables, and flowers and are increasingly investigated as natural alternatives to synthetic food colorants. The recovery of anthocyanins from plant materials has attracted considerable interest, particularly through extraction approaches that can improve pigment recovery while reducing reliance on conventional organic solvents. Green and food-compatible extraction strategies are therefore being explored for the development of naturally derived food colorants [1]. However, anthocyanins are inherently susceptible to structural and color changes during processing and storage, with pH, temperature, oxygen and light influencing their stability. Therefore, quantitative assessment of pigment content and color-related changes is important when evaluating extraction systems intended for food-colorant applications [2].

Ixora coccinea L, commonly known as the Rangan flower, belongs to the family of Rubiaceae and is characterized by intensely colored flowers ranging from red to orange and other bright shades. It is widely cultivated in tropical and subtropical regions and represents a potentially useful botanical source of natural pigments [3, 4]. Previous studies have reported anthocyanins and other flavonoid pigments in Ixora flowers, including cyanidin-3- rutinoside and delphinidin derivatives, which contribute to their characteristic red to purple-red coloration [4]. Interactions among co-occurring flavonoids may also influence the color and stability of the pigments [5].

The intense coloration of Ixora flowers has led to their investigation as a source of natural dyes for several applications [6, 7]. However, applications outside the food sector do not establish the suitability of the pigment as a food colorant, for which the extraction medium, pigment recovery, color stability, and processing compatibility are particularly important.

For pigment recovery, Ixora flowers have been used either directly in dried and powdered form or subjected to solvent-based extraction. Previous extraction approaches have commonly employed acidified ethanol and other organic solvent systems to facilitate pigment recovery [4, 8, 9]. Although such systems can facilitate pigment recovery, their use may introduce additional considerations related to solvent handling, recovery, residual solvent, and suitability for direct food applications [10]. Consequently, food compatible aqueous extraction systems ae of particular interest for the development of naturally derived food colorants. Green extraction approaches using water and other alternative solvent systems have therefore received increasing attention [1, 11]; however, the application of food-compatible green extraction systems to *Ixora coccinea* pigments remains insufficiently investigated.

The use of food-compatible solvents is an important consideration in the development of extraction processes for natural food colorants [10]. Carbonated water represents a potentially useful aqueous extraction medium because dissolved carbon dioxide generates a mildly acidic environment that may favor anthocyanin extraction and color expression [12]. Its food-compatible nature may also reduce the need for subsequent removal of organic solvents, thereby simplifying the extraction process. Despite the growing interest in green extraction technologies, the use of carbonated water for recovering pigments from *Ixora coccinea* flowers has not been systematically investigated.

To the best of our knowledge, the systematic evaluation of carbonated water as a food-compatible extraction medium for anthocyanin-rich pigments from *Ixora coccinea* flowers has not been reported. Accordingly, this study investigated carbonated water as an extraction medium and compared its performance with distilled water and acidified ethanol. The effects of flower-to-solvent ratio and post-harvest withering were evaluated, followed by assessment of pigment stability under different light and temperature conditions. Monomeric anthocyanin content,

absorbance at 520 nm, and browning index were used to characterize pigment behavior, while response surface methodology was applied to optimize selected processing and storage variables. The study aimed to assess the potential of carbonated water as a simple, food-compatible extraction approach for anthocyanin-rich *Ixora coccinea* pigments while maintaining pigment quality during storage.

2. Materials and methods

2.1 Chemicals and flower preparation

The chemicals used in this study were of reagent grade and were procured from Merck Chemicals, Germany. Commercial carbonated water (soda water) manufactured by PepsiCO, India was used as the aqueous extraction medium. According to the manufacturer's product label, the product contains purified water, carbon dioxide, and sodium, with a reported pH of approximately 3-4. Deep-red flowers of *Ixora coccinea* were collected from local gardens in Haldia, East Midnapur district, West Bengal, India. The flowers were manually separated from the stalks, and damaged or visibly spoiled flowers were discarded. The selected flowers were rinsed with water, and surface dried in shade for around 4 hours under ambient conditions. The dried flowers were subsequently cut into smaller pieces using sterile scissors before extraction to facilitate contact between the plant material snd extraction solvent and promote pigment release [4].

2.2 Solvent selection and extraction procedure

Four extraction conditions involving three solvent systems were evaluated to identify a suitable medium for extracting anthocyanin-rich pigments from *Ixora coccinea* flowers. A solvent-to-flower ratio of 60 mL/g (1:60 w/v) was maintained for all extraction treatments based on the procedure reported by Patil and Datar [4]. The cut flower material and extraction medium were transferred to 100-mL conical flasks, which were cotton-plugged during extraction.

The extraction conditions were as follows:

i. 70% ethanol acidified with 0.1 (M) hydrochloric acid, maintained at 28 ˚C with shaking at 150 rpm for 50 minutes (ET) [8].
ii. Distilled water maintained at 80˚C for 50 minutes (DW) [9].
iii. Carbonated water maintained at 80 ˚C for 50 minutes (SW).
iv. Carbonated water maintained at 28 ˚C with shaking at 150 rpm for 50 minutes (SWS).

For the 80˚C treatments, temperature-controlled hot plates were used, whereas an incubator shaker was employed for the 28 ˚C treatment. Carbonated water was evaluated under both heated and non-heated conditions, with the latter combined with agitation, to assess the effects of temperature and shaking on pigment extraction.

The extracted solutions were initially evaluated visually for color retention at approximately 7-day intervals for 30 days. Based on the visual screening, selected extracts were subsequently analyzed spectrophotometrically at 520 nm using a UV-Visible dual-beam spectrophotometer (Simadzu UV-1800) to monitor changes in optical absorbance during storage [13].

2.3 Optimization of solvent-to-flower ratio and post-harvest flower age

To determine an appropriate flower-to-solvent ratio for pigment extraction, the mass of flower material was varied

from 1 to 10 g while maintaining the solvent volume of 60 mL. The resulting extracts were evaluated by measuring absorbance at 520 nm using a UV-Visible spectrophotometer. The flower-to-solvent ratio was selected based on the observed extraction response together with the visual appearance of the extracts.

Total anthocyanin content of flowers may change during the post-harvest period because pigment synthesis, degradation, oxidation, and other biochemical processes can continue after harvest [14, 15]. Therefore freshly harvested *Ixora coccinea* flowers were evaluated at different post-harvest ages, corresponding to 0-4 days after harvest, to determine the effect of post-harvest ageing on monomeric anthocyanin content. Monomeric anthocyanin content (MAC) was determined using pH differential spectrophotometric method in triplicate [13], with the procedure adapted for *Ixora coccinea* based on the method reported by Patil and Datar [4]. The extract were diluted separately with potassium chloride buffer (pH 1.0) and a sodium acetate buffer (pH 4.5) at a dilution ratio of 1:4. Absorbance was measured at 520 and 700 nm after a few minute incubation at room temperature, with distilled water used as the blank. The absorbance difference (ΔA) was calculated as:

$$\Delta A = [A_{520} - A_{700}]_{\mathbf{pH1}} - [A_{520} - A_{700}]_{\mathbf{pH4.5}}$$

Mac was expressed as cyanidine-3-o-rutinoside equivalents (mg/L), using a molecular weight (MW) of 595.2 g/mole, a molar extinction coefficient (€) of 7000 L mol^{-1} cm^{-1} , the dilution factor (DF) of 1 part to 4 part, and a path length (l) of 1 cm, according to the following equation:

$$MA = \frac{\Delta A \; X \; MW \; X \; DF \; X \; 1000}{\in X \; 1} \; \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; (1)$$

2.4 Determination of post-extraction assessment time

Following extraction, the pigment extract may undergo changes in color and optical properties during holding. Therefore the post-extraction assessment period was determined by monitoring the browning index (BI) of freshly prepared carbonated-water extracts at regular time intervals. The browning index (BI) was determined spectrophotometrically using absorbance measurements at 520 and 420 nm, calculated as the ration of absorbance at 520 nm to that at 420 nm, as described below-

$$BI = \frac{A420}{A520} \; \ldots\ldots\ldots\ldots\ldots\ldots \; (2)$$

Freshly extracted carbonated-water pigment was analyzed immediately after extraction and subsequently at 3-h intervals for up to 24 h. During this assessment period, samples were maintained under ambient atmospheric conditions [16].

2.5 Optimization of storage conditions using OFAT and RSM

Following the preliminary optimization of extraction ratio, post-harvest flower age, and post-extraction assessment period, the selected conditions were kept constant during the storage-stability experiments. The effects of light exposure were initially evaluated using a one-factor-at-a-time (OFAT) approach under three conditions: ambient room light exposure, direct sunlight exposure, and complete darkness. Temperature effects were evaluated under four conditions: room temperature (30 °C), refrigerator temperature (4-6 °C), air conditioned (15-20 °C), and elevated temperature storage conditions, respectively (40 °C) [4, 17].

Based on the OFAT results, temperature and flower amount were selected as the principal variables for subsequent multivariate optimization. A central composite design (CCD) under response surface methodology (RSM) was then employed to evaluate their combined effects on monomeric anthocyanin content and browning index. The experimental data were analyzed using response surface methodology with a central composite design implemented in Design-Expert software version 7.0.0, (2021), Stat-Ease Inc. (Minneapolis, USA). Temperature and flower amount were used as the independent variables, while monomeric anthocyanin content and browning index were used as the response variables.

2.6 Statistical analysis

All experiments were performed in three independent replicates, and the results are presented as (mean ± standard deviation). Statistical differences among treatments were evaluated using one-way or two-way analysis of variance (ANOVA), as appropriate. A significance level of $p < 0.05$ was considered statistically significant. Where applicable, significant differences among treatments were further evaluated using Fisher's post hoc test. Response surface analysis and optimization were performed using Design-Expert software version 7.0.0, (2021), Stat-Ease Inc. (Minneapolis, USA).

3 Results and discussion

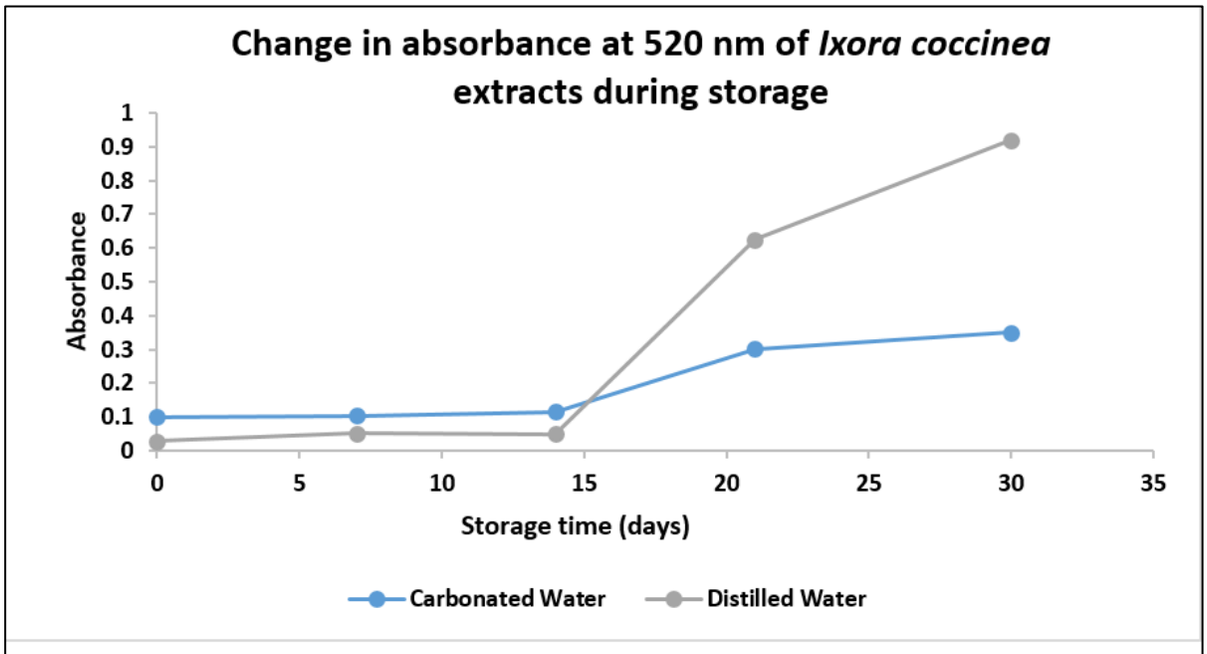
3.1 Performance of extraction solvent

The extraction behavior differed markedly among the solvent systems. SWS did not produce a visibly colored extract, whereas acidified ethanol (ET) produced an orange extract that gradually shifted toward yellow during storage. In contrast, both distilled water (DW) and Carbonated water (SW) produced red to deep-pink extracts, with comparatively better visual color retention over the 30-day observation period (Fig 1). Because anthocyanins are water-soluble flavonoid pigments whose color expression is strongly influenced by the extraction environment and subsequent storage conditions, the observed differences in extract color provide an initial indication of differences in pigment recovery and stability [2, 4].



Figure 1: Visual color retention of *Ixora coccinea* pigment extracts prepared utilizing different extraction conditions during 30 days of storage. From left to right: acidified ethanol (ET), carbonated water at 80 °C (SW), distilled water (DW), and carbonated water at 28 °C with shaking (SWS).

However, visual color alone cannot distinguish intact monomeric anthocyanins from degradation or polymerization products and was therefore followed by spectrophotometric assessment. As DW and SW demonstrated better visual color retention than the other two extraction conditions, these two extracts were selected for quantitative spectrophotometric monitoring over a 30-day period. Absorbance was monitored at 520 nm as an indicator of the color intensity associated with anthocyanin pigments, following the established spectrophotometric approach for anthocyanin-rich extracts [13]. Anthocyanins have been reported as major pigments in Ixora flowers, together with other phenolic constituents such as flavonoids [4, 18]. The absorbance profiles of the DW and SW extracts are presented in Fig 2, and the corresponding two-way ANOVA results are presented in Table 1.



Number of Days	0	7	14	21	30
Carbonated Water	0.123±0.025	0.099±0.004	0.11±0.005	0.325±0.025	0.366±0.02
Distilled Water	0.028±0.005	0.057±0.005	0.046±0.004	0.658±0.03	0.916±0.03

Figure 2: Changes in absorbance at 520 nm of *Ixora coccinea* pigment extracts prepared using distilled water (DW) and carbonated water (SW) during 30 days of storage.

Table 1: Two-way ANOVA data for selection of extraction solution with respect to time

Source of Variation	SS	df	MS	F	P-value	F crit
Solvent type	0.139537	1	0.139537	338.7922	5.24E-14	4.351244
Storage time	1.789633	4	0.447408	1086.294	4.47E-23	2.866081
Solvent x Storage time	0.503069	4	0.125767	305.3594	1.28E-17	2.866081
Error	0.008237	20	0.000412			
Total	2.440477	29				

Immediately after extraction, the Day 0 absorbance value of the DW extract was lower than that of the SW extract, indicating a greater initial color response in the carbonated-water extract. This observation was consistent with the

visual assessment of color intensity. Both extracts maintained relatively stable absorbance values up to Day 14, after which a noticeable increase was observed. The subsequent increase in absorbance was lower in the SW extract than in the DW extract, suggesting comparatively lower development of dark-colored products in the carbonated-water extract during storage. Two-way ANOVA showed that solvent type, storage time, and their interaction had significant effects on the measured absorbance ($p < 0.001$ for all three effects; Table 1). This indicates that the change in absorbance during storage depended on both the extraction medium and storage time.

The increase in absorbance observed during storage may be associated with the development of brown-colored products resulting from anthocyanin degradation and subsequent condensation or polymerization reactions. Anthocyanin stability is influenced by several factors, including pH, temperature, oxygen and light, and structural transformations during storage can alter the observed color response [2, 19]. The comparatively lower increase in absorbance observed for SW than for DW suggests lower development of dark-colored products in the carbonated-water extract under the tested storage conditions. Heating of carbonated water can cause partial loss of dissolved carbon dioxide [20]; nevertheless, the extraction medium remains mildly acidic, which may favor the colored flavylium form of anthocyanins [2, 21].Therefore, the comparatively favorable color retention observed for SW may be associated, at least in part, with the acidic aqueous extraction environment. However, individual anthocyanin transformations were not characterized in the present study, and this proposed mechanism should therefore be considered interpretative rather than directly demonstrated. Based on the combined visual and spectrophotometric observations, carbonated water was selected for subsequent optimization.

3.2 Solvent-to-flower ratio

Flower weight was varied relative to the solvent volume to determine an appropriate flower-to-solvent ratio for pigment extraction. The extracts exhibited increasing absorbance values at 520 nm with increasing flower weight, as shown in Table 2.

Table 2: Effect of flower loading on absorbance at 520 nm and flower-to-solvent ratio of carbonated-water *Ixora coccinea* extracts.

Flower weight (g)	Amount of solvent (ml)	Flower-to-solvent ratio (g: ml)	Absorbance at 520 nm
1	60	1:60	0.093[a]
2	60	1:30	0.188[a]
3	60	1:20	0.226[b]
4	60	1:15	0.472[c]
5	60	1:12	0.596[d]
6	60	1:10	0.672[e]
7	60	1:8.5	0.953[f]
8	60	1:7.5	1.034[f]
9	60	1:6.6	1.482[g]
10	60	1:6	1.758[h]

The increase in absorbance with increase in flower weight indicates a greater color response as more flower material was extracted using the same solvent volume. However, at higher flower loadings, the constant volume provided relatively less solvent per unit mass of flower material, and visible darkening of the extract was observed under the heating conditions. The characteristic color of the fresh ixora pigment was maintained up to 6 g of flower

material, whereas a marked increase in absorbance and darkening was observed at higher loadings. Therefore, 6 g of flower material in 60 mL of carbonated water, corresponding to a1:10 flower-to-solvent ratio, was selected as the working condition for subsequent experiments.

The selected 1:10 flower-to-solvent ratio can be compared with ratios reported in earlier *Ixora coccinea* studies, which have varied according to the solvent system and intended application. Reported ratios include 1:60 for anthocyanin extraction [4], 1:8 for natural color extraction [6], 1:5 for lipstick formulation [22] 1:15 for eco-dyeing [23], and other ratios for cosmetic and dye sensitized solar cell applications [11, 24, 25, 26]. These differences highlight the influence of extraction conditions and intended application on the choice of flower-to-solvent ratio. In the present study, the 1:10 ratio was selected based on the observed balance between color response and visual color quality under the tested extraction conditions.

3.3 Effect of post-harvest ageing on monomeric anthocyanin content

Post-harvest withering markedly affected the measured monomeric anthocyanin content (MAC) of the *Ixora coccinea* flower extract (Fig 3). The fresh flowers showed a MAC of 657.26 mg/L, while the value after one day of withering was 676.82 mg/L; these two values were statistically comparable, as indicated by the same superscript letter. From the second day onward, the measured MAC increased significantly, reaching 824.77,1017.36 and 1282.22 mg/L after 2, 3 and 4 days of withering, respectively. Thus, under the present experimental conditions, post-harvest withering was associated with an apparent increase in the spectrophotometrically determined MAC rather than a decrease [15].

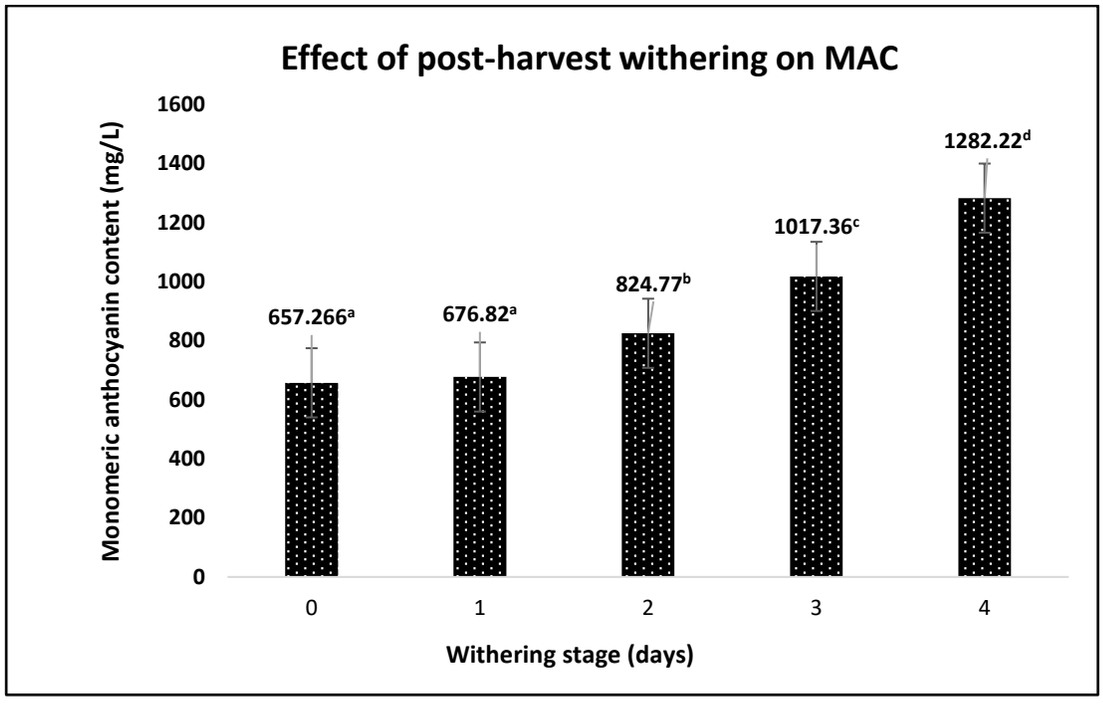


Figure 3: Effect of post-harvest withering (0-4 days) on the measured monomeric anthocyanin content (MAC) of *Ixora coccinea* extracts prepared with carbonated water.

The increase observed in the spectrophotometrically determined MAC after two or more days of withering should therefore be interpreted cautiously. Anthocyanin accumulation would not necessarily be expected to increase progressively during post-harvest senescence, because anthocyanins are susceptible to degradation and structural transformation during developmental and senescence-related processes [2, 15]. However, the pH-differential

method used in the present study estimates the monomeric, pH-responsive anthocyanin fraction and does not provide information on the individual anthocyanin profile or directly demonstrate the formation of degradation products [27]. Therefore, the increase in measured MAC cannot by itself be interpreted as evidence of de novo anthocyanin formation. Changes in pigment composition, extract matrix, tissue characteristics during withering, or the spectrophotometric response of the extract may contribute to the observed increase.

Browning index can provide complementary information on changes in anthocyanin-associated pigmentation during ageing, as changes in browning have been associated with alterations in anthocyanin content and pigment composition [16]. However, the present study did not directly quantify individual anthocyanins or polymeric pigments; therefore, the specific chemical basis of the observed increase in measured MAC cannot be established from the present data alone.

From a process-development perspective, fresh and one-day-old flowers were therefore selected for subsequent extraction experiments because they provided comparable measured MAC values without the progressive increase observed during longer withering periods. This selection minimizes the influence of post-harvest changes on the starting anthocyanin profile and provides a more consistent raw material for subsequent optimization of the pigment extraction and storage conditions. The present results also indicate that post-harvest flower age should be considered as an important pre-extraction variable when developing anthocyanin-rich extracts from *Ixora coccinea*.

3.4 Determination of the post-extraction assessment period

Determination of an appropriate post-extraction assessment period is important for establishing a consistent baseline for subsequent storage-stability experiments. In the present study, the browning index (BI) of the freshly prepared soda water extract was monitored over 24 h to capture early changes in pigment-associated optical properties [16]. The BI values showed only a relatively small change during the observation period, remaining approximately within the range of 0.5-0.67, with the values becoming more stable toward the end of the 24-h period (Fig. 4). ANOVA indicated significant variation in BI among the assessment times, while LSD post-hoc analysis showed that BI differed significantly from the initial value at 3 h and continued to show significant differences among several subsequent time points up to 15 h. From 18 to 24 h, the BI values did not differ significantly, indicating relative stabilization during the latter part of the observation period. Based on the observed stabilization within the 24-h observation window, 24 h was selected as the post-extraction assessment period for the subsequent storage experiments.

The selected 24-h period provides a standardized post-extraction reference point for subsequent storage experiments. This is particularly relevant for anthocyanin-rich extracts because pigment color and stability are influenced by factors such as pH, temperature and light exposure, and changes in browning-related optical properties can accompany anthocyanin degradation [2, 16]. Previous work on *Ixora coccinea* has also demonstrated sensitivity of its anthocyanin-rich color extract to light and temperature, supporting the need for a defined and consistent assessment point before storage studies [4]. The 24-h was therefore used as the common assessment point for evaluating changes in monomeric anthocyanin content and the browning index under the subsequent storage conditions.

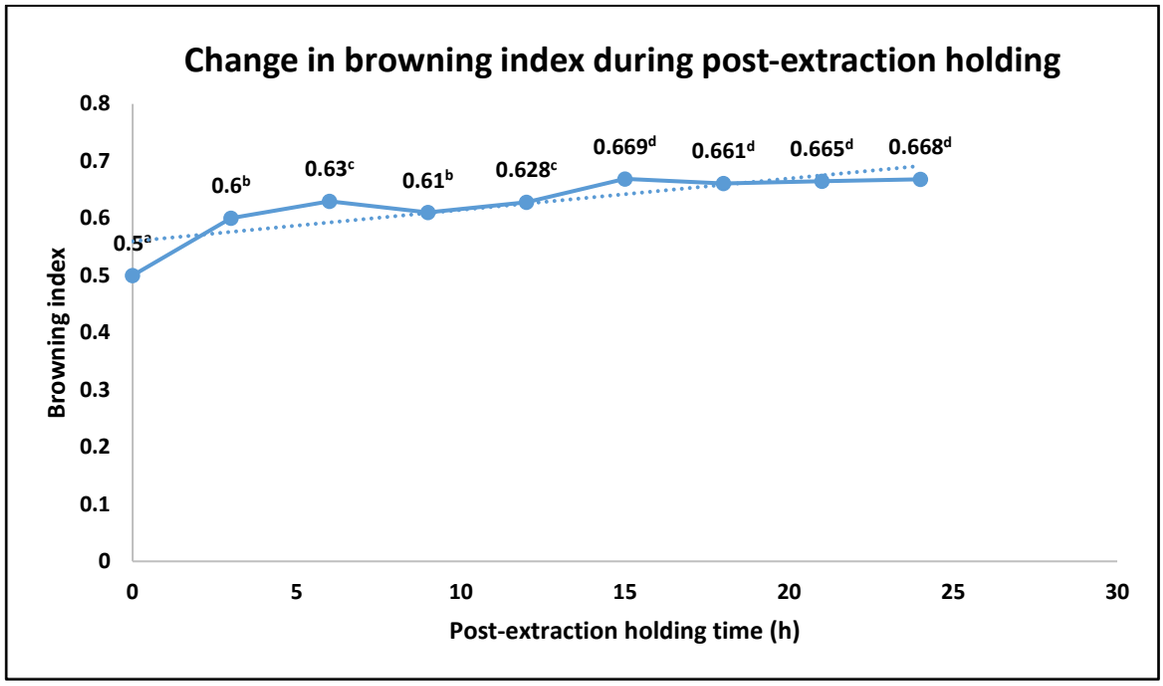


Figure 4: Changes in browning index (BI) of the carbonated-water-extracted *Ixora coccinea* pigment during the 24-h post-extraction holding period

3.5 OFAT assessment of light and temperature dependent pigment stability

Following the preliminary selection of the extraction ratio and post-extraction assessment period, fresh-cut *Ixora coccinea* flowers were extracted with carbonated water at the selected flower-to-solvent ratio. The resulting extracts were cooled and subsequently evaluated under different light and temperature conditions. The effects of light exposure on monomeric anthocyanin content (MAC) and browning index (BI) are presented in Fig. 5.

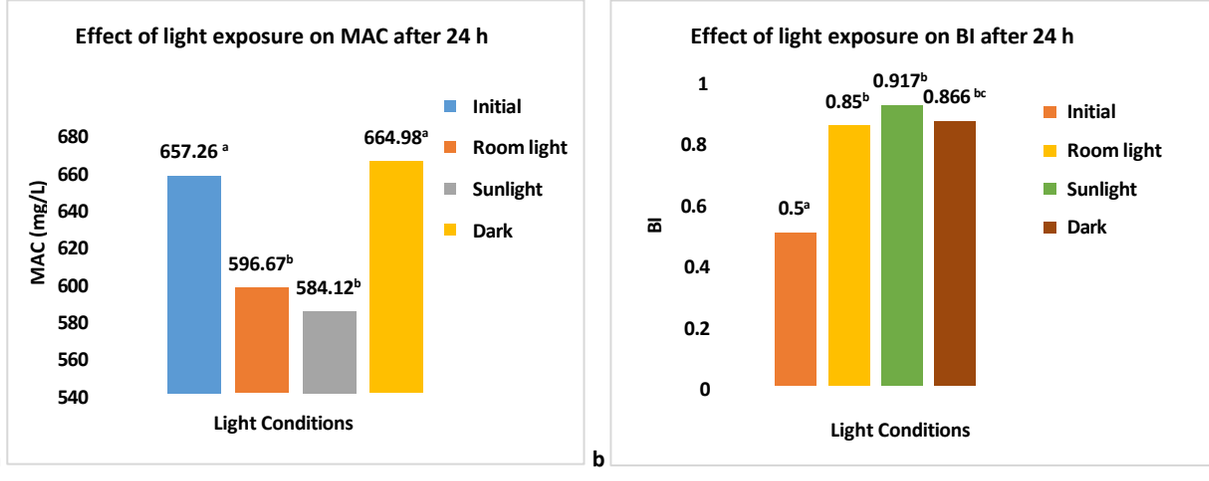


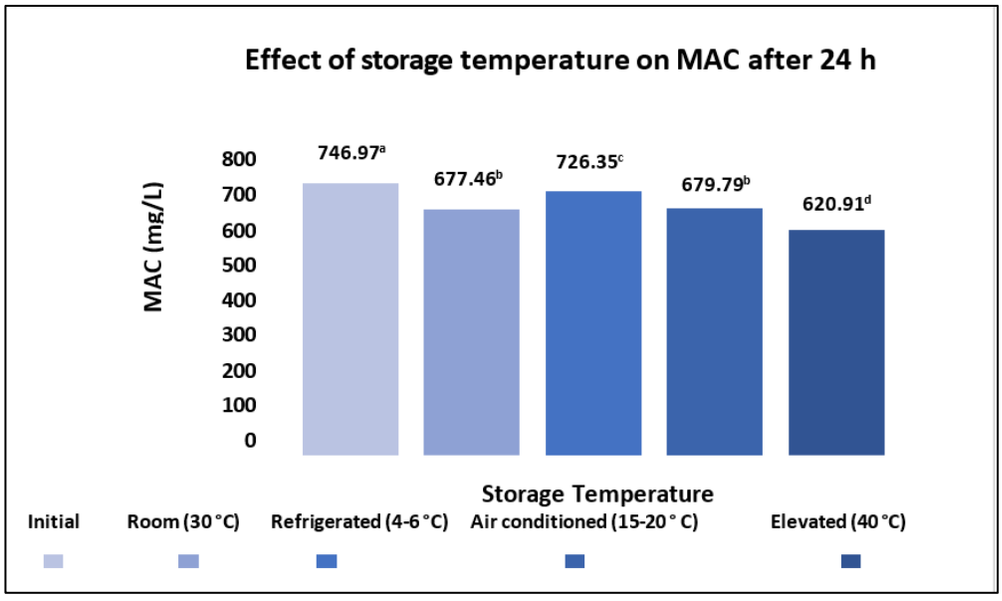
Figure 5: Changes in monomeric anthocyanin content (MAC) (a) and browning index (BI) (b) of the *Ixora coccinea* pigment extract after 24 h under different light-exposure conditions.

Under the tested light conditions, MAC showed a clear treatment-dependent response (Fig. 5a). The initial MAC was 657.26 mg/L, whereas the values decreased to 596.67 mg/L under room light and 584.12 mg/L under direct sunlight. In contrast, the MAC under dark storage increased slightly to 664.98 mg/L and remained statistically comparable to the initial value, as indicated by the common superscript. Relative to the initial value, the measured MAC therefore decreased by approximately 9.2 % under room light and 11.1 % under direct sunlight, whereas

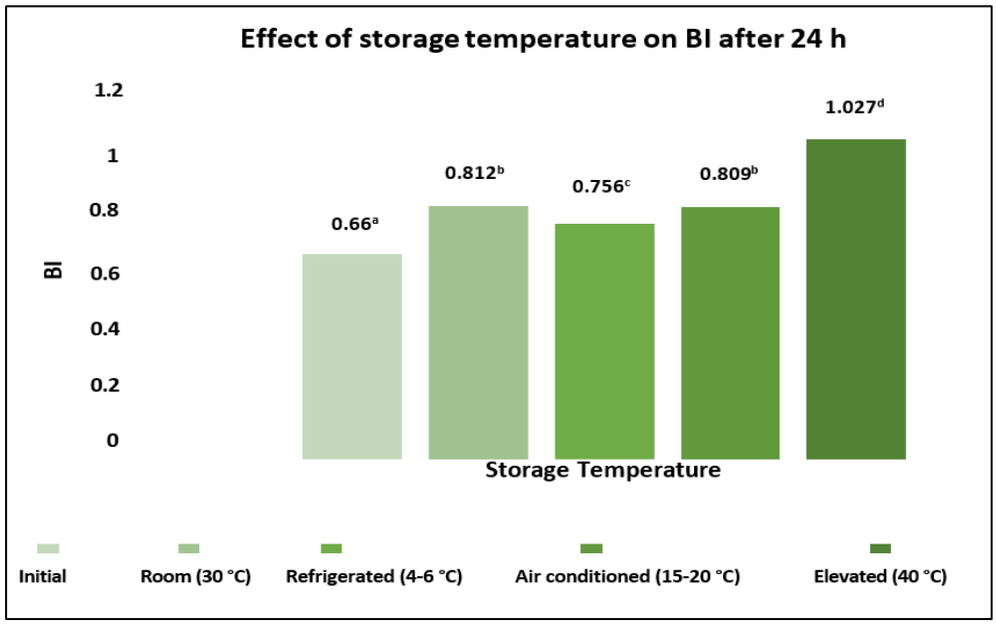
dark storage maintained the measured monomeric anthocyanin fraction. The greater loss of MAC under light exposure is consistent with the established photosensitivity of anthocyanins, for which light can promote pigment degradation and associated color changes [2, 17, 28, 29].

In contrast to MAC, BI increased from 0.50 in the initial extract to 0.850 under room light, 0.917 under direct sunlight, and 0.866 under dark storage (Fig. 5b). Although direct sunlight produced highest numerical BI, the overlapping statistical groupings indicate that the BI values among the three light treatments were not completely separated statistically. Therefore, BI provided weaker discrimination among the light treatments than under the present experimental conditions. The increase in BI is consistent with the development of brown-colored products during pigment degradation and associated secondary reactions; however, the present measurements do not permit identification of the specific chemical compounds responsible for the observed browning [16].

To further assess temperature-dependent stability, the extracts were subsequently stored under dark, temperature-controlled conditions at different temperatures. The effects of storage temperature on MAC and BI are presented in Fig. 6.



a



b

Figure 6: Change in monomeric anthocyanin content (MAC) (a) and browning index (BI) (b) of the *Ixora coccinea* pigment extract under different storage temperature during 24 h of storage.

Storage temperature had a pronounced effect on both MAC and BI (Fig.6). The initial MAC was 746.97 mg/L, while the values after 24 h were 677.46 mg/L at room temperature,726.35 mg/L under refrigeration, 679.79 mg/L under air conditioned conditions, and 620.91 mg/L at the elevated temperature condition. Refrigerated storage therefore resulted in the highest MAC among the tested storage temperatures and showed less loss of the measured monomeric anthocyanin fraction than room, air-conditioned, or elevated-temperature storage. Nevertheless, the refrigerated sample remained below the initial MAC, indicating that refrigeration reduced, but did not completely prevent, pigment loss during the 24-h storage period. The BI increased from 0.66 initially to 0.812, 0.756, 0.809, and 1.027 under room, refrigerated, air-conditioned, and elevated-temperature storage, respectively. The highest BI at the elevated temperature indicates greater development of browning under thermal stress. These temperature-dependent changes are consistent with the established sensitivity of anthocyanins to thermal degradation and with reports showing improved anthocyanin retention under lower-temperature storage [2, 16, 17, 30].

A separate pH-dependency experiment was not conducted in the present study because the use of commercial carbonated water as the extraction solvent was an integral part of the proposed extraction system; therefore, its inherent acidic pH was intentionally retained rather than independently adjusted [31]. This approach allowed the stability of the *Ixora coccinea* pigment to be evaluated under the actual physicochemical conditions of the selected commercial solvent. Anthocyanin stability is strongly influenced by pH, with acidic conditions generally favoring the colored flavylium form, whereas increasing pH can promote structural transformations and color loss [2].In addition, Patil and Datar [4] have previously investigated the stability and pH-dependent behavior of anthocyanins from *Ixora coccinea*; therefore, repeating a comparable pH.-dependency experiment was considered redundant within the scope of the present study. Accordingly, the present work focused on evaluating pigment stability under the selected carbonated-water extraction system, particularly in relation to light and temperature conditions, rather than re-evaluating the previously reported influence on pH [4].

Overall, the OFAT results indicate that light exposure primarily affected the retention of the measured monomeric anthocyanin fraction, whereas storage temperature influenced both MAC and browning responses more distinctly. Dark storage maintained MAC at a level statistically comparable to the initial extract, while refrigerated storage provided the most favorable balance between MAC retention and lower BI among the tested temperatures. These findings are relevant to anthocyanin-rich polyphenolic food-color systems; however, because total phenolic content and individual non-anthocyanin polyphenols were mot quantified in the present study, the results should not be interpreted as evidence of overall polyphenol retention [32].

3.6 Response surface optimization of anthocyanin stability under selected storage conditions

To further characterize the combined effects of storage temperature and flower loading on pigment stability, a central composite design (CCD) under response surface methodology (RSM) was applied using Design-Expert software. Fresh *Ixora coccinea* flowers were extracted with commercial carbonated water under the selected extraction conditions, and the resulting extracts were stored under dark conditions for 24 h. Storage temperature and flower amount were selected as the independent variables, while monomeric anthocyanin content (MAC) and browning index (BI) were used as the response variables. The experimental ranges and corresponding coded levels of the independent variables are presented in Table 3.

Table 3: Actual and coded levels of storage temperature and flower amount used as independent variables in the central composite design for RSM

Factor	Name with Unit	Low Actual	High Actual	Low coded	High coded	Mean	Std. dev
A	Temperature (° C)	4	35	-1.414	1.414	19.500	8.598
B	Amount of Flower (g)	4	7	-1.414	1.414	5.500	0.832

The observed and model-predicted values of MAC and BI obtained from the 13 experimental runs are presented in Table 4

Table 4: Experimental design runs and observed and model-predicted values of monomeric anthocyanin content (MAC) and browning index (BI) under different combinations of storage temperature and flower amount

Run	Flower amount (g)	Temperature (° C)	MAC (mg/L)		BI	
			Observed Response	Predicted Response	Observed Response	Predicted Response
1	6.56	30.46	537.26	522.44	0.73	0.82
2	4.00	19.50	324.1	347.29	1.19	1.10
3	5.50	19.50	485.5	474.78	0.81	0.9
4	6.56	8.54	614.62	607.41	0.68	0.68
5	4.44	30.46	369	342.15	1.08	1.11
6	5.50	19.50	476.03	474.78	0.89	0.9
7	7.00	19.50	600.25	602.27	0.78	0.69
8	5.50	19.50	458.72	474.78	0.9	0.9
9	5.50	19.50	442.2	474.78	0.99	0.9
10	4.44	8.54	452.24	427.12	0.92	0.97
11	5.50	35.00	401.24	414.69	1.05	1.0
12	5.50	19.50	482.95	474.78	0.83	0.9
13	5.50	4.00	528.02	534.86	0.8	0.8

The RSM analysis indicated that the fitted model for MAC was statistically significant ($p < 0.0001$), with both storage temperature and flower amount contributing significantly to the observed variation in MAC (Table 5). Storage temperature showed a significant effect ($F= 37.06$, $p= 0.0001$), while flower amount exerted a stronger effect within the investigated design space ($F=166.86$, $p < 0.0001$). The coefficient of determination ($R^2= 0.9533$) indicated that approximately 95.3 % of the variation in MAC was explained by the fitted model. The adjusted R^2 (0.9439) and predicted R^2 (0.9155) were also relatively close, supporting the predictive consistency of the model within the experimental domain. In addition, the lack-of-fit test was not significant ($p=0.4263$), indicating that the deviation between the fitted model and the experimental data was not significantly greater than the pure experimental error. The adequate precision value of 27.98 was substantially above the commonly used minimum criterion of 4, indicating an adequate signal for exploring the design space.

The ANOVA results further showed that both storage temperature and flower amount significantly influenced MAC and BI within the investigated deign space (Table 5).

Table 5: Analysis of variance (ANOVA) of the fitted RSM models for monomeric anthocyanin content (MAC) and browning index (BI)

	Source	Sum of Squares	DF	F value	Prob > F	
MAC	Model	79452.68	2	101.96	<0.0001	Significant
	A-Temperature	14440.99	1	37.06	0.0001	
	B-Amount of flower	65011.69	1	166.86	<0.0001	
	Residual	3896.18	10			
	Lack of Fit	2556.02	6	1.27	0.4263	Not Significant
	Pure Error	1340.16	4			
	Cor Total	83348.86	12			

Std. Dev.	19.74	R-Squared	0.9533
Mean	474.78	Adj R-Squared	0.9439
C.V. %	4.16	Pred R-Squared	0.9155
PRESS	7046.97	Adeq Precision	27.9753

	Source	Sum of Squares	DF	F value	Prob > F	
BI	Model	0.2108	2	20.52	0.0003	Significant
	A-Temperature	0.0397	1	7.73	0.0194	
	B-Amount of flower	0.1711	1	33.32	0.0002	
	Residual	0.0513	10			
	Lack of Fit	0.0314	6	1.05	0.5039	Not Significant
	Pure Error	0.0199	4			
	Cor Total	0.2621	12			

Std. Dev.	0.0717	R-Squared	0.8041
Mean	0.8962	Adj R-Squared	0.7649
C.V. %	8.00	Pred R-Squared	0.6491
PRESS	0.092	Adeq Precision	12.5889

Within the investigated design space, increasing flower amount was associated with an increase in the predicted MAC response, whereas increasing storage temperature was associated with a decrease in MAC. The stronger F-value for flower amount (166.86) compared with temperature (37.06) indicates that flower amount contributed more strongly to the variation in MAC within the fitted model. This effect should be interpreted within the experimental range of 4-7 g of flower material and 4-35 °C, rather than extrapolated beyond the design space.

The temperature-dependent decrease in MAC is consistent with the known thermal sensitivity of anthocyanins. Anthocyanin degradation during storage can involve structural transformations and loss of the colored monomeric forms, with higher temperatures generally accelerating these processes. Similar temperature-dependent decreases in anthocyanin retention have been reported in anthocyanin-rich fruit systems and other plant-derived extracts [2, 17, 19, 30]. The present RSM results therefore reinforce the OFAT observations, in which refrigerated storage showed greater retention of the measured MAC than the higher-temperature conditions.

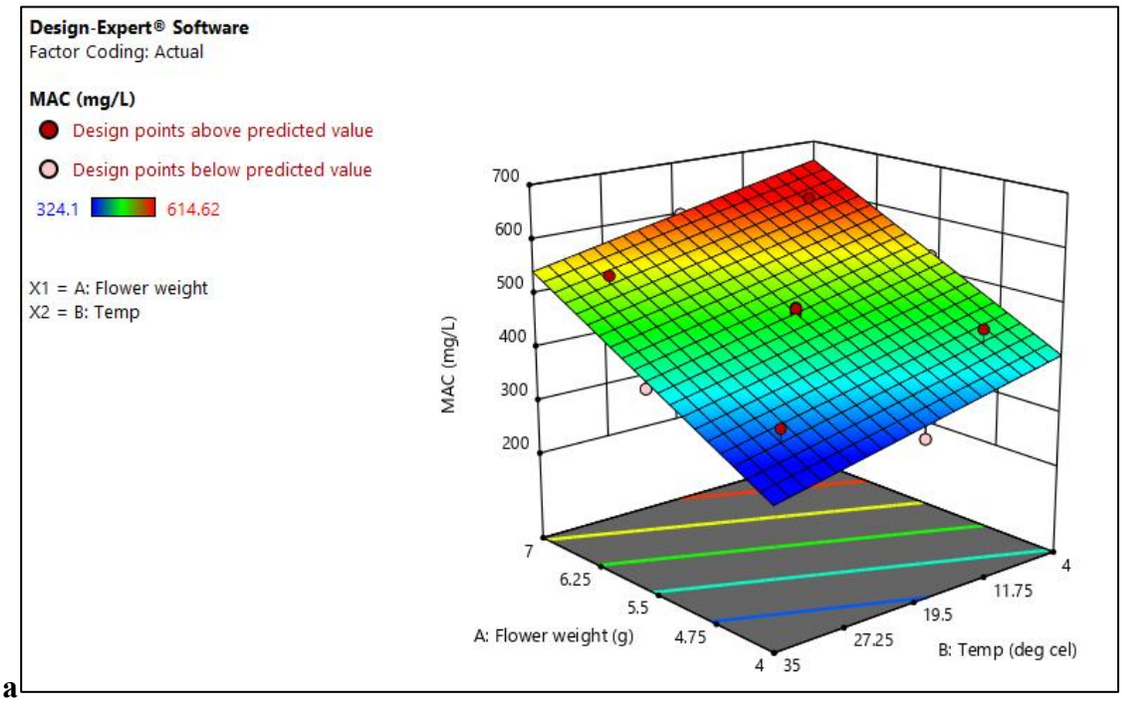
For BI, both storage temperature and flower amount were also significant model terms, with flower amount showing the stronger statistical effect ($F= 33.32$, $p= 0.0002$) compared with temperature ($F= 7.73$, $p= 0.0194$). The BI model showed and R^2 of 0.8041, with adjusted and predicted R^2 values of 0.7649 and 0.6491, respectively. Thus, the model described the browning response reasonably well, although its predictive performance was lower than that obtained

for MAC. Within the studied design space, lower storage temperatures were associated with lower BI values, indicating reduced development of the optical browning response under cooler storage conditions. This trend is consistent with previous reports showing that storage temperature influences anthocyanin degradation and associated color changes in anthocyanin-containing food systems [16, 17, 30].

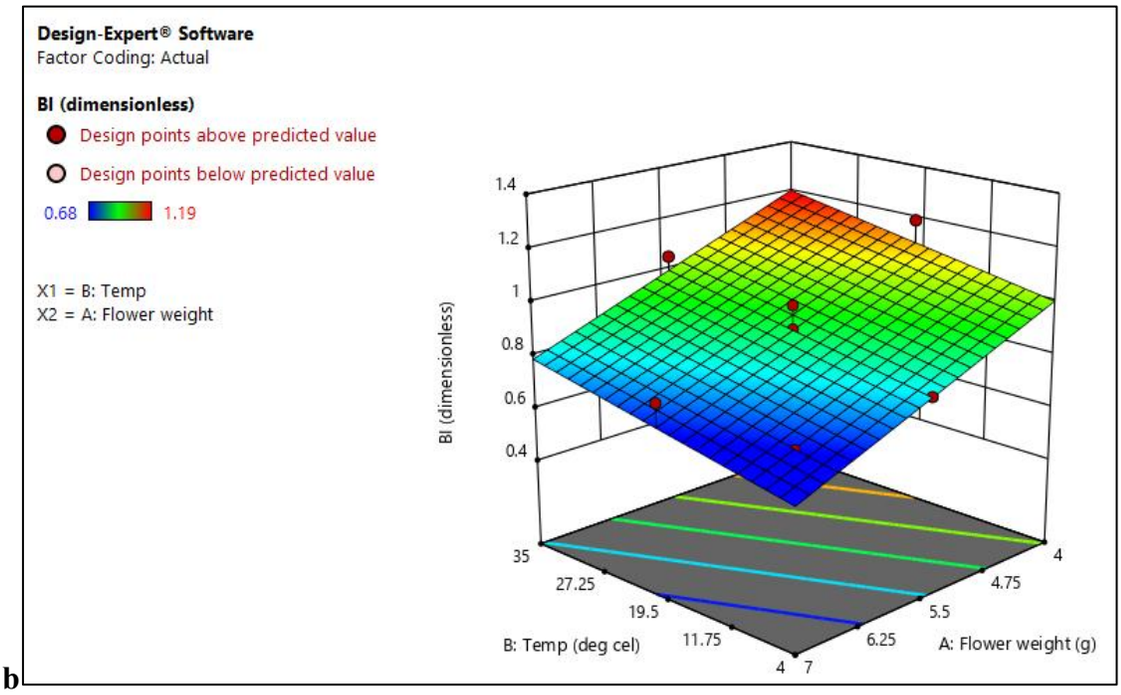
The response surface further indicated that flower amount positively influenced /mac within the investigated range, whereas its effect on BI was comparatively smaller in magnitude than its effect on MAC. This difference is also reflected in the model statistics, with flower amount showing a stronger contribution to MAC than to BI. The response-surface analysis therefore indicates that flower loading is an important process variable for controlling the measured anthocyanin concentration, while storage temperature is particularly relevant to maintaining anthocyanin retention and limiting browning during storage.

Based on the fitted response models and the specified optimization criteria, the predicted operating condition was a storage temperature of 7.3 °C and a flower amount of 6.903 g. At this combination, the model predicted a MAC of 641.276 mg/L and a BI of 0.624. The overall desirability value was 1.0, indicating that the selected combination simultaneously satisfied the response targets defined for the optimization. These values represent model-derived conditions within the investigated design space and were subsequently subjected to experimental validation.

Taken together, the response surface analysis slows that the measured monomeric anthocyanin fraction was more strongly associated with the selected process variables than the browning response. The higher MAC predicted at lower storage temperatures is consistent with the established susceptibility of anthocyanins to temperature-dependent degradation, whereas the concurrent decrease in BI indicates reduced development of browning-related optical changes Under cooler storage conditions.



a



b

Figure 7: Three dimensional response-surface plots showing the effects of storage temperature and flower amount on (a) monomeric anthocyanin content (MAC) and (b) browning index (BI). The plotted points represent experimental design runs, while the response surfaces represent model-predicted responses within the investigated design space.

These findings are relevant to the preservation of anthocyanin-rich food-color systems during storage, particularly where maintaining pigment concentration and limiting color deterioration are simultaneous processing objectives [2, 17, 30].

The predicted optimum was subsequently validated experimentally using the selected flower amount and storage temperature. The experimentally measured MAC was 686.2 mg/L compared with the model-predicted value of 641.276 mg/L, corresponding to an absolute residual error of 5.65 %. For BI, the experimental value was 0.671 compared with the predicted value of 0.624, giving an absolute residual error of 7.0% (Table 6).

Table 6: Experimental validation of the RSM-predicted optimum for monomeric anthocyanin content (MAC) and browning index (BI)

Flower Weight (g)	**Temperature (° C)**	**Predicted value**	**Experimental Value**	**Absolute residual error (%)**
6.903	7.3			
MAC (mg/L)		641.276	686.2	5.65
BI		0.624	0.671	7

The relatively low residual errors indicate reasonable agreement between the model predictions and the experimental observations under the selected validation conditions. The validation therefore supports the applicability of the fitted models within the investigated design space.

Overall, the RSM results demonstrate that storage temperature and flower amount were important determinants of the measured anthocyanin and color responses of the carbonated-water extract. Within the investigated design space, lower

storage temperature favored retention of MAC and was associated with lower BI, while increasing flower amount increased the measured MAC response. The mode-derived condition of 7.3 °C and 6.903 g of flower material provided predicted MAC and Bi values of 641.276 mg/L and 0.624, respectively, and showed reasonable agreement with the subsequent experimental validation. Because the present study quantified monomeric anthocyanins and browning responses rather than total phenolic content or individual non-anthocyanin polyphenols, these findings specifically support the stability of the measured anthocyanin fraction and associated color characteristics rather than overall polyphenol retention.

3.7 Input-output assessment and process efficiency of Ixora coccinea pigment extraction

The Input-output characteristics of the selected carbonated-water extraction process were assessed under the experimentally selected extraction conditions. Fresh *Ixora coccinea* flowers (6 g) were extracted with 60 mL of food-grade carbonated water at 80 °C for 50 min, corresponding to a flower-to-solvent ratio of 1:10. This condition was selected from the preliminary flower-to-solvent ratio experiments based on the measured color response and visual quality of the extract.

3.7.1 Input-output assessment based on measured MAC

The extract prepared from fresh flowers showed a monomeric anthocyanin content (MAC) of 657.26 mg/L under the selected analytical conditions. Following extraction and the subsequent experimentally validated storage condition, the pigment-rich extract showed a measured MAC of 686.2 mg/L, compared with the RSM-predicted value of 641.28mg/L. The difference between the predicted and experimentally measured values was associated with an absolute residual error of 5.65%, as reported in the model validation. These values indicate the concentration of the measured monomeric anthocyanin fraction in the extract and should not be interpreted as a direct measurement of total anthocyanin recovery from the original flower material. A summary of the principal input materials, extract output and quantitatively identified process residues is presented in Table 7:

Table 7: Input-output summary of the selected carbonated-water extraction process for *Ixora coccinea* pigment

Process step	**Input materials**	**Output products**	**Residual/observed changes**
Preparation	6 g fresh flower	Flower-carbonated water mixture 60 ml	Non-extractable solids
Extraction	Prepared mixture	Pigment rich extract (686.2 mg/L MAC)	Floral residue, browning-related color changes
Filtration	Pigment solution	Clear pigment extract	Filter cake (floral residue)

Estimated monomeric anthocyanin equivalents in 60 mL of extract at the measured MAC: 657.26 mg/L x 0.06 L= 39.44 mg, equivalent to 6.57 mg MAC equivalents per g of fresh flower.

Estimated monomeric anthocyanin equivalents in 60 mL of extract at the experimentally measured MAC: 686.2 mg/L x 0.06 L= 41.17 mg, equivalent to 6.86 mg MAC equivalents per g of fresh flower.

The RSM-derived flower amount of 6.903 g represents the model-predicted optimum within the investigated design space for the selected MAC and BI response criteria; it should not be interpreted as a directly measured optimum for total pigment recovery.

The observed browning response (browning index 0.671 under the experimentally validated condition) indicates color-related changes in the extract; however, the present study did not quantitatively partition anthocyanin losses among browning, polymerization, oxidation, or retention in the floral residue [16].

Overall, the input-output assessment indicates that the selected carbonated-water process generated an anthocyanin-containing aqueous extract with a measured MAC of 686.2 mg/L under the experimentally validated

condition. The corresponding extract-based estimate was 41.17 mg MAC equivalents in 60 mL of extract, or 6.86 mg MAC equivalents per g of fresh flower. These values provide a practical estimate of the measured monomeric anthocyanin fraction associated with the selected extraction system; however, a complete pigment mass balance would require quantitative determination of anthocyanins remaining in the floral residue and characterization of degradation or transformation products.

More broadly, the recovery of naturally derived bioactive compounds from underutilized biological materials is increasingly aligned with sustainable food and nutrition strategies, in which valorization of biomass can support both resource efficiency and the development of value-added bioactive ingredients [33].

3.7.2 Energy consumption and sustainability considerations

The laboratory-scale extraction required a 1000-W plate heater operated for 50 min, corresponding to an electrical energy input of approximately 0.833 kWh per batch. This value represents the direct heating energy used during the experimental extraction and does not include energy associated with flower production and transport, carbonated-water manufacture and packaging, filtration, equipment manufacture, or downstream processing. Therefore, it should be interpreted as a laboratory process-energy measurement rather than a complete environmental impact assessment.

The use of a food-compatible aqueous extraction medium nevertheless offers a practical sustainability advantage by avoiding the need for organic-solvent recovery and reducing the potential burden associated with solvent residues in a food-color application. The broader principles of solvent selection and sustainable extraction emphasize reducing hazardous solvent use and considering process requirements across the extraction chain [10, 34]. At larger scale, further reductions in resource use and environmental burden could potentially be achieved through water reuse, carbon-dioxide recovery, heat recovery, and process integration. These measures, however, require assessment at pilot or industrial scale before quantitative environmental benefits can be established. Thus, the present process demonstrates the practical feasibility if using a food-compatible carbonated-water medium at laboratory scale, while its industrial sustainability will depend on scale-up, heat recovery, solvent and water management and a complete process-level environmental assessment.

3.7.3 Preliminary environmental impact Assessment

A preliminary laboratory-scale environmental impact estimate was performed to examine the contribution of extraction heating energy to the process. Based on the experimentally measured extract-based MAC estimate of 6.86 mg MAC equivalents per g of fresh flower and a heating-energy input of 0.833 kWh per batch, the corresponding laboratory-scale energy intensity was estimated at approximately 20,233 kWh per kg of measured MAC equivalents. Using the stated Indian grid emission factor of 0.76 kg-equivalent per kWh [35], this corresponds to approximately 16 kg CO_2-equivalent per kg of measured MAC equivalents under the specific laboratory heating conditions. These values represent a process-energy estimate rather than a complete life-cycle inventory or life-cycle assessment and should therefore not be extrapolated directly to industrial-scale production.

Carbonated water may offer practical advantages over organic solvent systems in food-color processing because it is directly food-compatible and does not require removal of residual organic solvent after extraction. However, a quantitative environmental comparison with ethanol or other organic solvents requires equivalent system boundaries and functional units covering solvent production, transport, extraction, recovery, and waste management [10]. Such a comparison was beyond the scope of the present laboratory-scale study.

A complete industrial-scale life-cycle assessment incorporating flower sourcing, transportation, carbonated-water production and packaging, extraction, heating, filtration, water and CO_2 management, and downstream processing would be required to determine the overall environmental performance of the process [36].

The selected process produced a clarified, crude aqueous carbonated water followed by simple physical filtration, without chromatographic or resin-based purification. The resulting extract contained a measurable monomeric anthocyanin fraction and therefore represents a preliminary food-color system rather than a purified anthocyanin preparation. The absence of a solvent-removal step is a practical advantage for food-oriented applications; however, the crude extract may contain co-extracted sugars, organic acids, phenolic compounds, and other matrix components that can influence color stability and storage behavior. Further concentration or purification would therefore be required where a standardized or high-purity anthocyanin colorant is intended [4].

Under the selected experimental conditions, the extract-based estimate corresponded to 6.86 mg MAC equivalents per g of fresh flower. Patil and Datar reported anthocyanin values for *Ixora coccinea* in a comparable range of approximately 6.6-7.0 mg/ g of fresh flower [4].The similarity provides contextual support for the magnitude of the measured anthocyanin response obtained in the present study; however, differences in extraction conditions, solvent system, analytical procedures, and reporting basis should be considered when making direct quantitative comparisons.

Although organic and acidified solvent systems may provide high pigment recovery under specific extraction conditions, their suitability for direct food applications must also consider solvent safety, residual-solvent requirements, downstream removal, and process complexity. The use of food-compatible carbonated water therefore represents a different process-design strategy in which extraction performance is considered together with food compatibility and downstream processing requirements [10].

Among the tested storage conditions, dark storage provided the highest retention of the measured MAC in the light experiment, while refrigerated storage provided the highest MAC among the tested temperature conditions. These observations are consistent with the established sensitivity of anthocyanins to light and temperature during storage [2, 17, 28, 29, 30].Taken together, the findings indicate that food-grade carbonated water can provide an extract with a measured MAC response comparable in magnitude to previously reported *Ixora coccinea* anthocyanin values, while offering a food-compatible aqueous extraction route. This type of resource-efficient recovery of naturally occurring bioactive compounds is consistent with broader food-system strategies aimed at converting biological materials into value-added ingredients while reducing resource wastage [33].

Overall, the selected carbonated-water process provided a food-compatible aqueous extract containing a measurable monomeric anthocyanin fraction, with an extract-based estimate of 6.86 mg MAC equivalents per g of fresh flower under the experimentally validated condition. The process requires only heating and filtration at the laboratory scale and avoids a dedicated organic-solvent removal step. These characteristics support the potential of carbonated water as a practical extraction medium for anthocyanin-containing food-color systems. However, the present results should be regarded as a laboratory-scale process assessment rather than a complete pigment recovery or life-cycle analysis. Quantitative determination of anthocyanins remaining in the floral residue, identification of degradation products, pilot-scale energy assessment, and a full life-cycle assessment would be required to establish recovery efficiency and environmental performance at industrial scale.

4 Conclusion

The present study demonstrated the feasibility of using food-grade carbonated water as an aqueous extraction medium for obtaining an anthocyanin-containing pigment extract from *Ixora coccinea* flowers. Among the extraction systems evaluated, carbonated water provided a favorable color response and was therefore selected for subsequent optimization. A flower-to-solvent ratio of 1:10 (6 g flower/ 60 mL carbonated water) was selected based on the balance between pigment-related color response and visual quality of the extract. Post-harvest

assessment further showed that fresh and one-day-old flowers produced statistically comparable measured monomeric anthocyanin content (MAC), supporting the use of freshly harvested or short-term post-harvest material for subsequent processing.

The storage experiments demonstrated that anthocyanin-associated responses were influenced by both light and temperature. Dark storage maintained the measured MAC at a level statistically comparable to the initial extract, whereas exposure to room light and direct sunlight resulted in measurable decreases in MAC. Temperature also produced a clear response, with refrigerated storage showing the highest MAC retention among the tested temperature conditions, while elevated temperature resulted in greater MAC loss and the highest browning index. These findings reinforce the sensitivity of anthocyanin-containing extracts to environmental conditions during storage. Response surface methodology farther established that storage temperature and flower amount significantly affected both MAC and BI within the investigated experimental domain. Flower amount exerted the stronger effect on MAC, whereas lower storage temperature favored anthocyanin retention and was associated with lower browning. The model-derived condition of 7.3 °C and 6.903 g flower material predicted a MAC of 641.276 mg/ L and a browning index of 0.624. Experimental validation yielded 686.2 mg/ L MAC and a browning index of 0.671, corresponding to absolute residual errors of 5.65% and 7.0%, respectively, supporting the applicability of the fitted models within the investigated design space.

From a food-processing perspective, the carbonated-water system provides a simple aqueous extraction route involving heating and filtration without a dedicated organic-solvent removal step. The experimentally validated extract corresponded to an extract-based estimate of 6.86 mg MAC equivalents per g of fresh flower. This value should be interpreted as a measure derived from the concentration of the monomeric anthocyanin fraction in the extract rather than as a complete anthocyanin recovery yield, because anthocyanins remaining in the floral residue and individual degradation products were not quantitatively determined.

Overall, the findings provide preliminary evidence that *Ixora coccinea* can serve as a source of anthocyanin-rich, naturally colored extracts and that carbonated water can function as a food-compatible extraction medium. The study contributes specifically to the understanding of anthocyanin extraction and stability in a flavonoid-rich plant matrix, with practical relevance to the development of naturally derived food-color systems. However, because total phenolic content and individual non-anthocyanin flavonoids were not quantified, the present findings should not be interpreted as evidence of overall polyphenol or flavonoid retention. Future work should therefore focus on comprehensive anthocyanin and flavonoid profiling using chromatographic techniques, identification of degradation products, assessment of antioxidant and other relevant bioactivities, and evaluation of the extract in representative food matrices. Pilot-scale process assessment and a complete life-cycle evaluation would also be required to establish the industrial feasibility and environmental performance of the extraction system.

Declarations

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

Ethics, consent to participate, and consent to publish declaration: The collection of *Ixora coccinea* flowers from local gardens in Haldia, West Bengal, India, was conducted in accordance with applicable local and national guidelines. The plant material was collected from cultivated plants, and no protected or endangered species were involved. No specific collection permit was required for this study. The study is not a clinical trial; therefore,

clinical trial registration details are not applicable.

Data availability: The data generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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