Effects of Metal Ions on the Stabilization of Anthocyanin Pigments Powder from Butterfly Pea Flowers

Piyachat Kranpukdee [1], *Voranuch* Somsongkul[1, 2*], and *Sarinya* Shawaphun[1†]

[1]Department of Industrial Chemistry, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Wongsawang, Bang Sue, 10800 Bangkok, Thailand
[2]Integrated Chemistry Research Center for Sustainable Technology, King Mongkut's University of Technology North Bangkok, Wongsawang, Bang Sue, 10800 Bangkok, Thailand

Abstract. Butterfly pea (*Clitoria ternatea* L.) flowers have been recognized as a natural source of anthocyanins, particularly delphinidin, which is used as a natural colorant but is highly susceptible to degradation influenced by environmental factors such as pH and temperature. In this study, the co-pigmentation of butterfly pea anthocyanins with metal ions (Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, and Ca^{2+}) was evaluated under pH conditions of 4, 7, and 10, using a non-metal control for comparison. Total anthocyanin content (TAC), percentage of yield, and color measurement (L*, a*, b*, ΔE) were analyzed before and after accelerated heat and cooling cycle stability testing. The highest TAC (35.8 mg/L) and percentage of yield (98.95%) were obtained with Al^{3+} at pH 4. Minimal ΔE values (0.11–0.17) were observed in Al^{3+}treated samples across all pH levels, indicating excellent color stability, whereas significant color degradation (ΔE 4.64–8.29) was observed in the control group. These results indicate that anthocyanin stability can be significantly enhanced by Al^{3+}, followed by Fe^{2+} and Zn^{2+}, suggesting the potential of metal-assisted co-pigmentation in improving the stability and functionality of natural colorants for applications in food, cosmetics, and related industries.

1 Introduction

Butterfly pea (*Clitoria ternatea* L.) flowers are known to contain high concentrations of anthocyanins, which are utilized as natural pigments, particularly delphinidin. The hydroxyl (-OH) groups located at the 3', 4', and 5' positions on the B-ring of delphinidin have been shown to promote co-pigmentation, thereby enhancing pigment stability [1, 2]. However, anthocyanins are susceptible to degradation, which is influenced by several environmental factors, including pH, light, temperature, oxygen, and enzymatic activity; these factors collectively reduce their stability and shelf life [3, 4]. Due to the ionic nature of anthocyanin molecules, their coloration is strongly affected by pH. Four main chemical forms of anthocyanins are known to exist, depending on the pH of the solution. Under strongly acidic conditions (pH = 1), the flavylium cation form is predominantly observed; this form is characterized by a positive charge, high water solubility, and red-to-purple coloration. As the pH increases to 2–4, quinonoidal blue species are formed. At pH 5–6, carbinol pseudo base or chalcone, at pH > 7, pyrocatechol forms are observed; both are colorless and may exhibit charge balance depending on the solution environment. At pH levels above 7, anionic forms become dominant, and anthocyanin degradation is promoted due to specific substituent groups (Figure 1.) [5]. Therefore, the development of anthocyanin pigment powder in a suitable form is considered essential for enhancing stability and extending shelf life. The stability of anthocyanins can be improved through the formation of co-pigmentation complexes with metal ions, a process in which interactions occur between the ligands of anthocyanin molecules and electron-accepting metal ions, which influence both the color characteristics and stability of the pigment. In this study, five metal ions—aluminum ion (Al^{3+}), iron ion (Fe^{2+}), zinc ion (Zn^{2+}), magnesium ion (Mg^{2+}), and calcium ion (Ca^{2+})—were selected as representatives of different metal categories based on variations in charge, ionic radius, and complex-forming abilities. Al^{3+} is classified as a post-transition metal from Group 13, while Fe^{2+} and Zn^{2+} are transition metals belonging to Groups 8 and 12, respectively. Mg^{2+} and Ca^{2+} are alkaline earth metals from Group 2 of the periodic table. These metal ions are capable of interacting with functional groups present in the anthocyanin structure, potentially altering the electronic structure of the molecules and affecting both color intensity and hue. This study aims to enhance the stability of natural pigments extracted from butterfly pea flowers by means of co-pigmentation with metal ions, without the need for

* Corresponding author: voranuch.s@sci.kmutnb.ac.th
† Corresponding author: sarinya.s@sci.kmutnb.ac.th

synthetic additives. Furthermore, the potential for application in food, cosmetics, and various bioproduct

Industries are demonstrated [6-8].

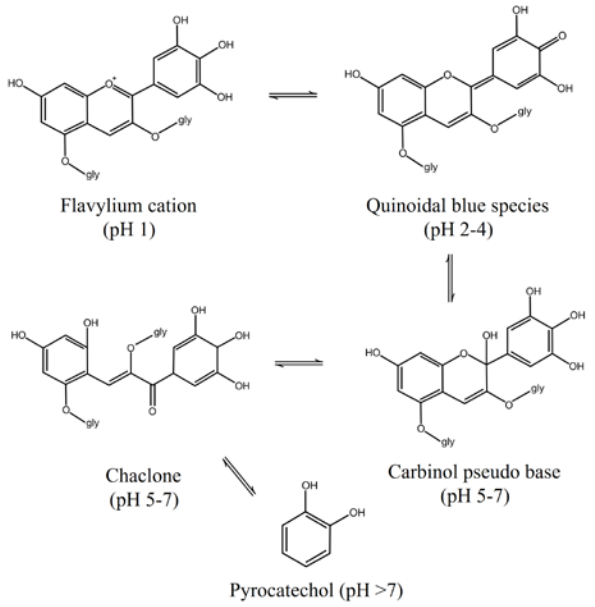

Fig. 1. Representative structural transformations of delphinidin under various pH conditions, including flavylium cation (pH = 1), quinonoidal base (pH 2–4), carbinol pseudo base or chalcone (pH 5–7), and pyrocatechol form (pH > 7).

2 Materials and Methods

2.1 Materials

Butterfly pea flower powder was obtained from Long Beach Co., Ltd. (Thailand) and subsequently stored in airtight containers under dry and dark conditions until use. Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), and anhydrous zinc chloride ($ZnCl_2$) were purchased from Kemaus (Australia), while iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) was procured from QRe™. All chemical reagents used in this study were of analytical grade.

2.2 Extraction of Anthocyanins from Butterfly Pea Flowers

The extraction process was performed by weighing 1.000 g of butterfly pea flower powder, to which distilled water was added at a ratio of 1:10. The total volume was then adjusted to 10.00 mL using a volumetric flask, and the solution was thoroughly mixed. The initial pH of the extract was measured and subsequently adjusted to 1.0 using 10 wt% hydrochloric acid (HCl). Extraction was conducted at 40 °C for 60 minutes.

2.3 Preparation of Pigment Powder Using Co-pigmentation with Metal Ions

Metal salts, including $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $AlCl_3 \cdot 6H_2O$, $ZnCl_2$, and $FeCl_2 \cdot 4H_2O$, were each weighed at 0.01 mol based on their respective molecular weights and subsequently added to the butterfly pea flower extract. And co-pigmentation with metal ions was subsequently performed at 40°C for 60 minutes. The pH of each mixture was measured and adjusted to 4, 7, and 10 using 10 wt% ammonium hydroxide (NH_4OH) solution. The mixtures were then allowed to stand at room temperature for 30 minutes to promote co-pigmentation and precipitation between anthocyanin and metal ions. The resulting precipitates were separated by centrifugation at 6,000 rpm for 15 minutes. The pigment powder was dried at 50°C for 24 hours, after which the dried powders were analyzed for percentage of yield and color values using a colorimeter based on the CIE L*ab** system.

2.4 Determination of total anthocyanin content

The total anthocyanin content was determined by diluting the butterfly pea flower extract samples co-pigmented with Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, and the control sample without metal ions at a ratio of 1:10 using two buffer systems: 0.025 M potassium chloride (KCl) at pH 1.0 and 0.4 M sodium acetate at pH 4.5. Absorbance measurements were performed using a UV–Visible spectrophotometer (SPECORD 210 PLUS, Analytic Jena, Germany) at wavelengths of 520 nm and 700 nm. The instrument was operated with a 1.0 nm slit width and medium scan speed, equipped with a silicon photodiode detector. Baseline correction was automatically applied using a solvent blank. The total anthocyanin content was determined using Equation (1):

$$Total\ Anthocyanins\ Content = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1} \quad (1)$$

where $A = (A_{520nm} - A_{700nm})_{pH1} - (A_{520nm} - A_{700nm})_{pH4.5}$; MW = molecular weight of cyanidin-3-glucoside ($g \cdot mol^{-1}$); DF = dilution factor; L = path length of the cuvette (cm); ε = molar absorptivity of cyanidin-3-glucoside ($L \cdot mol^{-1} \cdot cm^{-1}$).

2.5 Determination of the percentage of yield

The percentage yield was calculated based on the dried weight of the pigment powder obtained from the co-pigmentation of butterfly pea flower extract with Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, and the control sample, relative to the initial weight of the butterfly pea flower powder used for extraction. The percentage yield was determined using Equation 2:

$$Percentage\ of\ Yield\ (\%) = \frac{Weight_d\ (g)}{Weight_i\ (g)} \times 100 \quad (2)$$

Where W_d = Dried weight of the pigment powder obtained from the co-pigmentation between the butterfly

pea flower extract and metal ions (g), and W_i = Initial weight of butterfly pea flower powder used for extraction (g).

2.6 Heat and Cooling Cycle Stability Test

The stability of the pigment powders obtained from the co-pigmentation of butterfly pea flower extract with Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, and the control sample was evaluated using an accelerated heat and cooling cycle stability test. The samples were initially stored at 4°C in a refrigerator for 48 hours, followed by incubation at 45°C in a hot air oven for an additional 48 hours. This procedure was defined as one thermal cycle and was repeated for a total of eight cycles [10].

2.7 Color measurement

Color measurements of the pigment powders obtained from the co-pigmentation of butterfly pea flower extract with Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, and the control sample were conducted using a benchtop colorimeter (ColorFlex EZ 45-0 (LAV), HunterLab, USA). The instrument was operated under the CIE L*a*b* color system with the L*, a*, and b* scales, employing a D65 light source, a 10° observer angle, reflectance mode, and a 25 mm aperture size. Calibration was performed before each measurement using a physical white tile standard. Samples were placed in a glass cell at the measurement port, and L*, a*, and b* values were recorded in triplicate. The average values were subsequently used for analysis. The L* value indicates lightness, ranging from 0 (black) to 100 (white). The a* value corresponds to the red-green axis, with positive values indicating redness and negative values indicating greenness. The b* value corresponds to the yellow-blue axis, where positive values indicate yellowness and negative values indicate blueness [11]. The total color difference (ΔE) was determined using Equation 3.

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \quad (3)$$

Where L1, a1, and b1 were recorded as the color measurements before the heat and cooling cycle stability test, and L2, a2, and b2 were recorded as the color measurements after the heat and cooling cycle stability test

ΔE is widely recognized in color science as a quantitative indicator of chromatic alteration and was interpreted according to standard perceptibility thresholds: $\Delta E < 1$ – imperceptible to the human eye; $1 \leq \Delta E < 2$ – detectable by trained observers; $2 \leq \Delta E < 3.5$ – perceptible by untrained observers; $3.5 \leq \Delta E < 5$ – perceptible; and $\Delta E > 5$ – distinctly different in appearance.

3 Results and Discussion

3.1 Determination of total anthocyanin content

The total anthocyanin content (TAC) of butterfly pea flower extract following co-pigmentation with various metal ions—Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, and Ca^{2+}—as well as a control sample, was evaluated at pH 4, 7, and 10. At pH 4 (Figure 2). The TAC values (mg/L) were observed in the following descending order: Al4 = 216.39, Fe4 = 212.24, Zn4 = 211.60, Mg4 = 210.14, Ca4= 208.88, and Control4 = 200.98.

The highest enhancement in TAC by Al^{3+} is attributed to its high positive charge (+3) and small ionic radius (~53 pm), resulting in a high charge density. Al^{3+} acts as a strong Lewis acid, forming stable co-pigmentation with electron-donating functional groups on anthocyanin molecules, such as hydroxyl and carbonyl groups. Furthermore, the empty orbitals of Al^{3+} facilitate co-pigmentation interactions that stabilize the anthocyanin in butterfly pea extract, thus effectively inhibiting anthocyanin degradation.

Fe^{2+}, a first-row transition metal, ranked second in stabilization efficiency. Although it carries a lower positive charge than Al^{3+}, its incomplete d-orbital configuration ($3d^6$) allows ligand-to-metal charge transfer (LMCT) and π–d interactions with anthocyanins, enhancing stability via co-pigmentation and resonance mechanisms. Its moderate ionic radius (~78 pm) also supports effective bonding with functional groups on anthocyanins.

Zn^{2+} displayed similar but slightly lower TAC enhancement compared to Fe^{2+}. As Zn^{2+} possesses a filled d-orbital shell ($3d^{10}$), it cannot participate in π-back bonding, though it can still undergo co-pigmentation with electron-donating ligands to a certain extent. Its lack of redox activity and resonance capabilities accounts for its lower efficiency.

Mg^{2+} and Ca^{2+}, classified as Group 2A metals, contributed less to TAC enhancement due to the absence of vacant d-orbitals, restricting interactions to electrostatic outer-sphere coordination. Although Mg^{2+} has a smaller ionic radius (~72 pm) than Ca^{2+} (~100 pm), resulting in a higher charge density and slightly better stabilization capacity, their co-pigmentation is inherently weaker compared to transition metals [12].

The control sample, which lacked metal ions, exhibited the lowest TAC, highlighting the critical role of metal ions and anthocyanin interactions in pigment stabilization under mildly acidic to basic conditions.

Experimental results further indicated that TAC significantly declined with increasing pH. At pH 4 (Figure 3), anthocyanins predominantly existed as quinonoidal blue species, which are relatively stable. Upon increasing the pH to 7, a structural transition to the carbinol pseudo base form—less stable—was observed, leading to reduced TAC values: Al7 = 212.04, Fe7 = 207.18, Zn7 = 200.93, Mg7 = 198.42, Ca7 = 195.74, and Control^7 = 175.01 mg/L. At pH 10 (Figure 4), anthocyanins were largely present in the chalcone form, a degraded structure, resulting in a pronounced decrease in TAC: Al10 = 207.81, Fe10 = 193.78, Zn10 = 180.46, Mg10 = 170.09, Ca10 = 166.11, and Control10 = 143.95 mg/L. Nevertheless, the presence of metal ions consistently contributed to higher TAC values compared to the control

across all pH conditions, confirming their role in mitigating anthocyanin degradation. This transformation abolishes the C6–C3–C6 flavonoid skeleton and the associated conjugated system, leading to a chemically unstable species that is prone to oxidation and degradation. Nonetheless, metal ions—particularly Al^{3+} and Fe^{2+}—were still able to partially retard structural degradation, likely via electrostatic interactions or limited coordination, thus maintaining yields significantly above that of the control.

At pH 4, anthocyanins are predominantly found in the form of quinonoidal base species (Figure 5a), which are stabilized via resonance structures involving an extended conjugated double bond system across the aromatic rings. Through this structural arrangement, efficient electron delocalization over the π-system is facilitated, thereby reducing molecular susceptibility to nucleophilic attack and enhancing chemical stability. As a consequence, anthocyanins in this form can retain their coloration, and relatively high total anthocyanin content (TAC) is maintained. These quinonoidal species are less affected by pH-induced hydration or ring-opening reactions, rendering them comparatively more stable under mildly acidic conditions. At pH 7, structural transformation is triggered, disrupting the conjugated π-system and resulting in the formation of a carbinol pseudo base (Figure 5b), which is a colorless and chemically less stable intermediate. Simultaneously, tautomerization or molecular rearrangement may occur, yielding an open-chain chalcone structure (Figure 5c), which lacks aromatic stabilization and is prone to further degradation. Both the carbinol pseudobase and chalcone forms are considered thermodynamically unstable and more reactive than the quinonoidal base, thereby contributing to a significant decline in TAC. Under alkaline conditions (e.g., pH 10), extensive degradation of the anthocyanin structure is observed. The strongly basic environment promotes bond cleavage, particularly within the flavylium core, resulting in the irreversible breakdown of the chromophore. This degradation leads to the formation of smaller phenolic compounds, primarily pyrocatechol-type derivatives (Figure 5d), which no longer retain the original conjugated system essential for pigment functionality. Such irreversible transformations not only abolish the chromatic properties of anthocyanins but also substantially compromise their functional stability in food, cosmetic, and pharmaceutical applications [5, 13].

In summary, the results demonstrated a significant decrease in total anthocyanin content with increasing pH, attributable to the pH-dependent structural instability of anthocyanins in alkaline conditions. However, the co-pigmentation with metal ions was found to play a crucial role in enhancing the molecular stability of anthocyanins. The stabilization efficiency of metal ions followed the order: Al^{3+} > Fe^{2+} > Zn^{2+} > Mg^{2+} > Ca^{2+} > Control, effectively delaying pigment degradation under basic conditions [14-18].

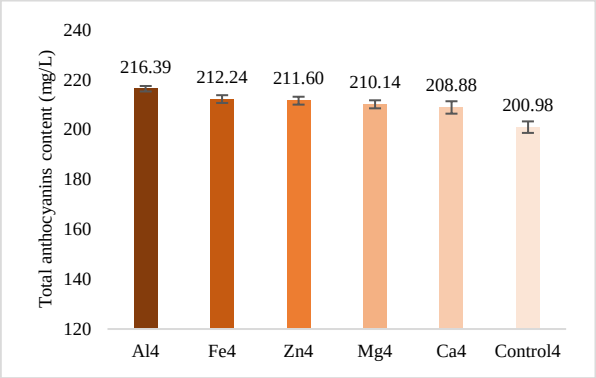

Fig. 2. Total anthocyanin content of butterfly pea flower extract after co-pigmentation with different metal ions at pH 4: Al^{3+} (Al4), Fe^{2+} (Fe4), Zn^{2+} (Zn4), Mg^{2+} (Mg4), Ca^{2+} (Ca4), and non-metal ions (Control4).

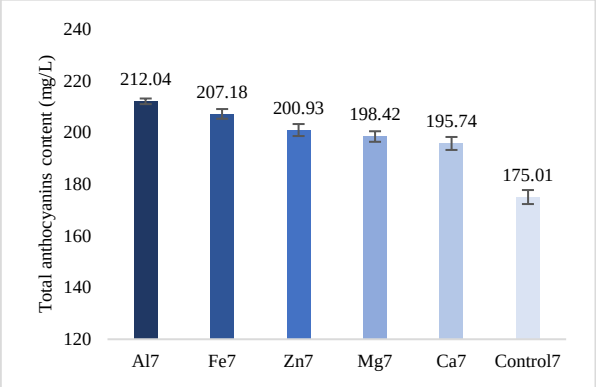

Fig. 3. Total anthocyanin content of butterfly pea flower extract after co-pigmentation with different metal ions at pH 7: Al^{3+} (Al7), Fe^{2+} (Fe7), Zn^{2+} (Zn7), Mg^{2+} (Mg7), Ca^{2+} (Ca7), and non-metal ions (Control7).

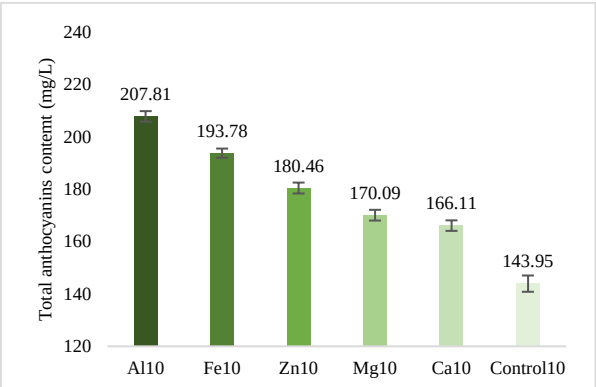

Fig. 4. Total anthocyanin content of butterfly pea flower extract after co-pigmentation with different metal ions at pH 10: Al^{3+} (Al10), Fe^{2+} (Fe10), Zn^{2+} (Zn10), Mg^{2+} (Mg10), Ca^{2+} (Ca10), and non-metal ions (Control10).

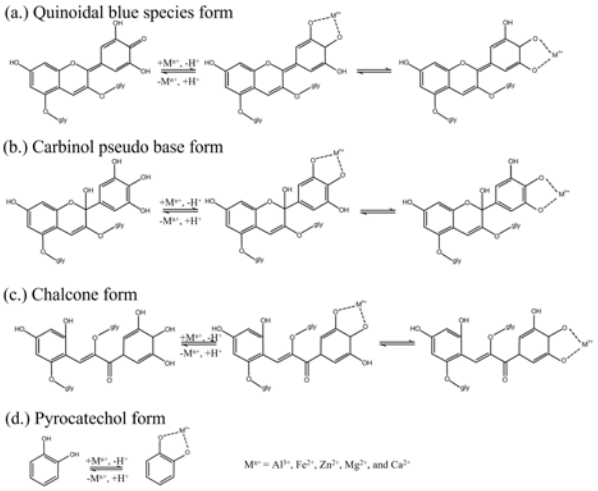

Fig. 5. Co-pigmentation between delphinidin and metal ions (M^{n+}) under different pH conditions: (a) quinonoidal blue species at pH 4, (b) carbinol pseudo base at pH 7, (c) chalcone form at pH 7, and (d) pyrocatechol form at pH 10.

3.2 Determination of the percentage of yield

The percentage yield of butterfly pea flower extract following co-pigmentation with various metal ions—Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, and Ca^{2+}—as well as a control sample, under pH conditions of 4, 7, and 10, was found to be consistent with the total anthocyanin content (TAC) previously described.

At pH 4 (Figure 6), the yields were as follows: Al4 = 98.95%, Fe4 = 88.80%, Zn4 = 75.32%, Mg4 = 67.72%, Ca4 = 52.50%, and Control4 = 35.91%. This pH level corresponds to the predominance of the quinonoidal blue species, whose structure is characterized by a conjugated system formed between the A and B rings through the flavylium backbone. The oxygen atom at the C4 position exists in the enol form, enabling interaction with hydroxyl groups on the B ring, which promotes extensive delocalization of π-electrons. This electronic delocalization contributes to the pronounced blue coloration and enhanced stability under mildly acidic conditions. The quinonoidal blue species possess a high capability for co-pigmentation with metal ions via hydroxyl and/or carbonyl groups located at the 3', 4', and 5' positions on the B-ring of delphinidin [15-17].

Al^{3+}, due to its trivalent charge and small ionic radius, exhibited the highest co-pigmentation ability, resulting in the maximum pigment yield. Fe^{2+}, as a transition metal with accessible d-electrons, facilitated stable co-pigmentation to a slightly lesser extent. Zn^{2+}, having an ionic radius similar to that of Mg^{2+} but a slightly greater coordination potential, led to a moderate pigment yield. Mg^{2+}, although able to interact through hydroxyl groups, provided lower yields due to its lower coordination energy. Ca^{2+}, with its larger ionic radius and the weakest coordination ability among the tested ions, resulted in a relatively low yield. The control group, devoid of metal ions, produced the lowest pigment yield due to the absence of stabilizing interactions [12].

At pH 7 (Figure 7), the following yields were obtained: Al7 = 89.93%, Fe7 = 74.04%, Zn7 = 60.03%, Mg7 = 52.50%, Ca7 = 40.20%, and Control7 = 25.35%. Under this condition, anthocyanins are converted into the carbinol pseudo base form or chalcone. This conversion disrupts the conjugated π-system, resulting in diminished coloration (almost colorless) and reduced chemical stability. Although this form is unfavorable for efficient co-pigmentation, residual hydroxyl groups may still participate in weak interactions with metal ions, allowing the pigment yield to remain significantly higher than the control.

At pH 10 (Figure 8), the yields decreased further: Al10 = 72.29%, Fe10 = 62.26%, Zn10 = 45.19%, Mg10 = 37.22%, Ca10 = 19.76%, and Control10 = 10.79%. Under strongly alkaline conditions, anthocyanins are predominantly present in the pyrocatechol form, an open-ring structure resulting from cleavage of the C ring.

In conclusion, structural transformations of anthocyanins induced by pH shifts have been demonstrated to exert a direct influence on their co-pigmentation efficiency with metal ions. These pH-dependent conformational changes alter the chromophore stability and, consequently, affect pigment preservation and functionality. Based on the relative structural stabilities of the major anthocyanin forms observed under different pH conditions, the following order has been established: quinonoidal blue species (pH 4) > carbinol pseudo base or chalcone (pH 7) > pyrocatechol derivatives (pH 10). This trend correlates well with the measured percentage yields of total anthocyanin content (TAC), where greater molecular stability has been associated with higher pigment retention. Despite the inherent susceptibility of anthocyanins to degradation under neutral and alkaline conditions, co-pigmentation with metal ions has been found to play a critical role in enhancing the stability of anthocyanin structures, particularly those extracted from butterfly pea flowers. Through co-pigmentation, they have been stabilized to varying extents depending on the type of metal ion involved. The metal ions investigated in this study can be ranked according to their stabilizing efficacy as follows: $Al^{3+} > Fe^{2+} > Zn^{2+} > Mg^{2+} > Ca^{2+} >$ Control. This ranking highlights the potential application of specific metal ions in mitigating pigment degradation across a range of environmental conditions, offering a practical strategy for improving the stability of anthocyanin-based colorants in food, pharmaceutical, and cosmetic formulations [14–18].

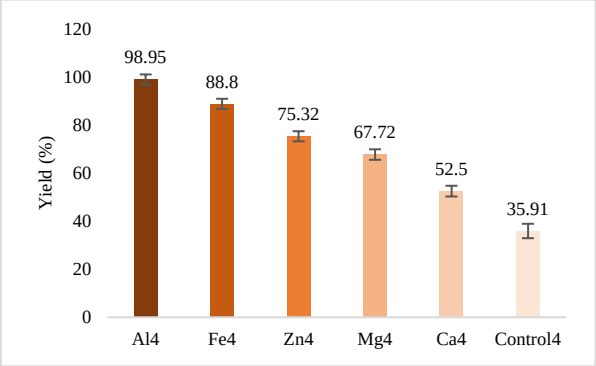

Fig. 6. The percentage yield of anthocyanin pigment powder from butterfly pea flowers after co-pigmentation with various

metal ions at pH 4: Al^{3+} (Al4), Fe^{2+} (Fe4), Zn^{2+} (Zn4), Mg^{2+} (Mg4), Ca^{2+} (Ca4), and non-metal ions (Control7).

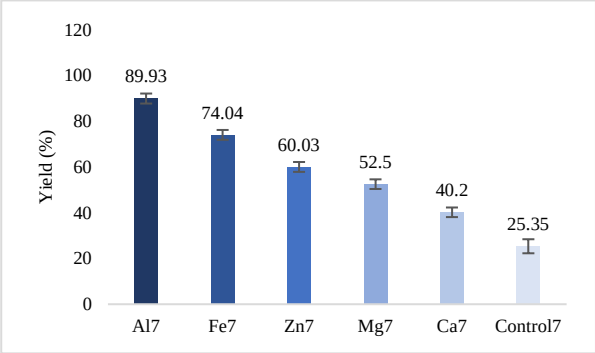

Fig. 7. The percentage yield of anthocyanin pigment powder from butterfly pea flowers after co-pigmentation with various metal ions at pH 7: Al^{3+} (Al7), Fe^{2+} (Fe7), Zn^{2+} (Zn7), Mg^{2+} (Mg7), Ca^{2+} (Ca7), and non-metal ions (Control7).

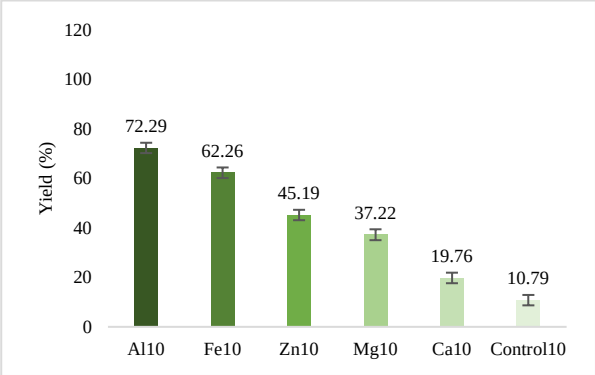

Fig. 8. The percentage yield of anthocyanin pigment powder from butterfly pea flowers after co-pigmentation with various metal ions at pH 10: Al^{3+} (Al10), Fe^{2+} (Fe10), Zn^{2+} (Zn10), Mg^{2+} (Mg10), Ca^{2+} (Ca10), and non-metal ions (Control10).

3.3 Color measurement before and after the Heat and Cooling Cycle Stability Test

The pigment powders obtained from the co-pigmentation of butterfly pea flower extract with Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+} , and control samples under pH conditions of 4, 7, and 10 are presented in Table 1. Color parameters L*, a*, b* were analyzed both before and after stability testing under heat and cooling cycle conditions, which were designed to simulate thermal stress commonly encountered during product processing or storage when color is a critical attribute. These parameters provide essential information regarding hue and saturation. ΔE was calculated to evaluate the extent of color change, serving as an indicator of pigment powder stability [11].

The color measurement of anthocyanin pigment powders obtained through co-pigmentation with various metal ions and control samples was evaluated before Stability tests under accelerated heat and cooling cycles, revealing a wide range of hue variations under different pH conditions. At pH 4, the control sample (Control4) exhibited values of L* = 34.68, a* = 3.77, and b* = –5.28, indicating a deep reddish-purple hue characteristic of anthocyanins in the quinonoidal blue species form. In comparison, Ca4 (L* = 30.80, a* = 8.52, b* = –4.35) and Mg4 (L* = 32.48, a* = 8.19, b* = –4.60) displayed similarly deep reddish-purple tones. Fe4 (L* = 30.83, a* = 5.15, b* = 11.52) exhibited a reddish-yellow hue, while Zn4 (L* = 36.85, a* = 21.90, b* = 8.03) presented a vivid red coloration. Al4 (L* = 42.65, a* = 15.01, b* = 6.46) showed a distinctly purplish-red hue. At pH 7, the control sample (Control7) demonstrated a muted green tone (L* = 39.03, a* = –1.25, b* = –4.72), reflecting the persistence of quinoidal base and hemiketal forms. Ca7 (L* = 31.85, a* = –0.15, b* = –1.12) and Mg7 (L* = 34.83, a* = –0.45, b* = –1.27) appeared grayish-green in tone. Zn7 and Fe7 exhibited identical L* values of 36.81, with a* = 5.64 and b* = 1.26, resulting in light reddish-yellow hues. Al7 (L* = 46.78, a* = –3.92, b* = 11.76) was characterized by a bright bluish-green coloration. At pH 10, the control sample (Control10) presented a light green shade (L* = 42.77, a* = –2.72, b* = 1.02), attributed to the formation of the chalcone form. Ca10 (L* = 34.31, a* = –1.17, b* = 1.84) showed a yellowish-green tone, while Mg10 (L* = 48.84, a* = –5.72, b* = 15.23) exhibited a bright light green hue. Zn10 (L* = 40.80, a* = –2.72, b* = 1.15) appeared pale green. Fe10 (L* = 34.25, a* = 3.21, b* = 18.85) showed a reddish-yellow hue, and Al10 (L* = 54.83, a* = –4.76, b* = 17.03) demonstrated a light yellowish-green tone with a grayish tint.

After undergoing heat–cooling cycle stability testing, the control samples at all pH levels exhibited significant changes in color parameters. For Control4, the L* value increased to 39.16, while a* decreased to 2.54, and b* was recorded at –5.34, resulting in a ΔE of 4.64. Control7 presented L* = 43.77, a* = –3.86, and b* = –4.05, with a corresponding ΔE of 5.46. In the case of Control10, an L* value of 50.89, a* = –1.03, and b* = 1.15 was observed, with the ΔE reaching as high as 8.29—the highest among all tested conditions. These findings indicated substantial fading and structural degradation of the pigment molecules, particularly under alkaline conditions, where anthocyanin forms are inherently less stable and more thermally labile. In contrast, all samples co-pigmented with metal ions exhibited notable chromatic stability. At pH 4, minimal changes in ΔE were observed for Ca4 (ΔE = 0.15), Mg4 (ΔE = 0.15), Fe4 (ΔE = 0.12), Zn4 (ΔE = 0.12), and Al4 (ΔE = 0.11), with Al4 showing virtually unchanged L*, a*, and b* values, reflecting the superior color retention imparted by Al^{3+}. At pH 7, similarly low ΔE values were recorded for all metal-co-pigmented samples: Ca7 (ΔE = 0.23), Mg7 (ΔE = 0.21), Zn7 (ΔE = 0.17), Fe7 (ΔE = 0.16), and Al7 (ΔE = 0.12). In particular, Al7 showed an increase in the b* value to 11.87, suggesting an effective preservation of the bluish tone through the formation of a highly stable pigment complex. At pH 10, color stability was maintained across all metal ion treatments, with Ca10 (ΔE = 0.29), Mg10 (ΔE = 0.25), Zn10 (ΔE = 0.21), Fe10 (ΔE = 0.19), and Al10 (ΔE = 0.17) displaying only minor changes in color parameters. Remarkably, Al10 exhibited L* = 54.89, a* = –4.77, and b* = 17.19 after thermal cycling, underscoring the capability of Al^{3+} to form stable complexes with anthocyanin functional groups, effectively preventing pigment degradation under alkaline conditions.

In summary, the stability of pigment powders obtained from co-pigmentation of butterfly pea flower extracts with Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, $Ca^{2+,}$ and control samples under pH conditions of 4, 7, and 10 was investigated. It was found that Al^{3+} exhibited the highest efficacy in maintaining color stability due to its +3 charge and small ionic radius, which facilitated strong complex formation with the hydroxyl groups of anthocyanins. Fe^{2+} and Zn^{2+} also contributed effectively to color preservation under acidic and neutral conditions, whereas Mg^{2+} and Ca^{2+} demonstrated lower stability, particularly under alkaline conditions. Stability tests under accelerated heat and cooling cycles indicated that anthocyanins in the quinonoidal blue species form at pH 4 exhibited the greatest stability, while the pyrocatechol form observed at pH 10 showed the highest degree of color degradation in the control samples lacking metal ions. This degradation was attributed to the absence of stabilizing complexation by metal ions. Overall, metal ions—especially Al^{3+}—significantly enhanced the stability of anthocyanin pigments, rendering them suitable for applications in the pigment industry.

Table 1. Color Measurements of anthocyanin pigment powder from butterfly pea flowers after co-pigmentation with various metal ions at pH 4, 7, and 10

		pH 4	**L***	**a***	**b***	**pH 7**	**L***	**a***	**b***	**pH 10**	**L***	**a***	**b***
Control	Before		34.68	3.77	-5.28		39.03	-1.25	-4.72		42.77	-2.72	1.02
	After		39.16	2.54	-5.34		43.77	-3.86	-4.05		50.89	-1.03	1.15
ΔE		4.64				5.46				8.29			
Ca	Before		30.80	8.52	-4.35		31.85	-0.15	-1.12		34.31	-1.17	1.84
	After		30.90	8.64	-4.35		31.96	-0.32	-1.01		34.60	-1.20	1.85
ΔE		0.15				0.23				0.29			
Mg	Before		32.48	8.19	-4.60		34.83	-0.45	-1.27		48.84	-5.72	15.23
	After		32.63	8.19	-4.60		35.00	-0.44	-1.14		48.97	-5.64	15.42
ΔE		0.15				0.21				0.25			
Zn	Before		36.85	21.90	8.03		36.81	5.64	1.26		40.80	-2.72	1.15
	After		36.93	21.82	8.03		36.94	5.52	1.26		40.87	-2.89	1.24
ΔE		0.12				0.17				0.21			
Fe	Before		30.83	5.15	11.52		36.81	5.64	1.26		34.25	3.21	18.85
	After		30.84	5.20	11.63		36.94	5.52	1.26		34.29	3.25	19.03
ΔE		0.12				0.16				0.19			
Al	Before		42.65	15.01	6.46		46.78	-3.92	11.76		54.83	-4.76	17.03
	After		42.64	14.93	6.54		46.80	-3.97	11.87		54.89	-4.77	17.19
ΔE		0.11				0.12				0.17			

4 Conclusion

The effects of co-pigmentation between anthocyanin extracts from butterfly pea flower and various metal ions (Al^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}) under pH conditions of 4, 7, and 10 were investigated and compared with control samples lacking metal ions. The highest total anthocyanin content (TAC) was observed in samples co-pigmented with Al^{3+} at pH 4, followed by Fe^{2+} and Zn^{2+}, whereas markedly lower TAC values were detected in the presence of Mg^{2+} and Ca^{2+}. Control samples consistently showed the lowest TAC across all pH levels, suggesting the essential role of metal ions in delaying anthocyanin degradation. Pigment powder yield was found to correlate with TAC results, with the highest yields obtained in Al^{3+} and Fe^{2+} treated samples. Zn^{2+} yielded intermediate results, while Mg^{2+}, Ca^{2+}, and control samples showed reduced yields. Following thermal stability testing, color parameters L*, a*, b*, and ΔE were assessed. The smallest ΔE values, indicating enhanced color retention, were associated with Al^{3+} and Fe^{2+}. Conversely, the highest ΔE values were recorded for control samples, particularly at pH 10. These results confirm that Al^{3+} provided the most effective stabilization of anthocyanins.

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