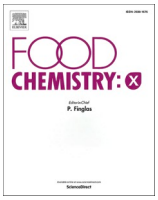
Food Chemistry: X 26 (2025) 102312

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Stability enhancement of betalain pigment extracted from *Celosia cristata* L. flower through copigmentation and degradation kinetics during storage

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ARTICLE INFO

Keywords:
Betalain
Copigmentation
Degradation kinetics
Gum Arabic
Whey protein
Celosia cristata

ABSTRACT

Celosia cristata Linn., an underutilized flower, contains betalains. The stability of betalain pigments in complex food systems is a significant challenge. In this study, we investigated the potential of copigmentation using gum arabic (0.33 % to 1 %), pectin (0.33 % to 1 %), whey protein (0.33 % to 1 %), ascorbic acid (0.05 %), and calcium carbonate (0.01 %) on betalain content, color stability, and microbial counts in betalains pigments extracted from *Celosia Cristata* L. flowers during a 90-day of storage period. A total of seven copigmentation treatments (T1 to T7) and a control (T0) without copigmentation were applied to the betalain pigments. The degradation kinetics of betalain pigments at different temperatures were also investigated. The findings revealed that among all copigmentation treatments, T7 (0.33 % gum Arabic, 0.33 % pectin, 0.33 % whey protein, 0.05 % ascorbic acid, and 0.01 % Ca^{2+}) exhibited the highest stability in terms of betalain content and color degradation.

1. Introduction

Color is a key determinant of quality for both food manufacturers and consumers. Color additives have been used in the food industry for decades to enhance the visual appeal of products. However, synthetic food colorants fhave been linked to adverse health effects, such as hyperactivity in children (McCann et al., 2007). Concerns regarding the safety of synthetic food dyes have led to a growing preference for natural colorants in commercial food production. Natural food colorants, especially those derived from plants, have gained popularity. From 2024 to 2033, natural food coloring global market is projected to grow at a compound annual growth rate (CAGR) of 8.14 % from its 2023 valuation of USD 2 billion.

This trend is further supported by the potential antioxidant properties of some natural food pigments and their potential health benefits, such as anti-inflammatory, anti-cancer, and immunomodulatory effects (Di Salvo et al., 2023; Lu et al., 2021).

Betalains are plants' secondary metabolites, water-soluble, hydrophilic in nature, and nitrogen-containing pigments. They include red-violet betacyanins and yellow betaxanthins (Calva-Estrada et al., 2022; Fathordoobady et al., 2016; Sokolova et al., 2024; Strack et al., 2003). Betalains are found in various plant parts, including fruits, leaves, seeds, stems, flowers, and roots, particularly in the *Chenopodiaceae, Cactaceae*, and Amaranthaceae families. Betalains exhibit everal bioactive properties, such as antimicrobial, anti-cancer, antioxidant, anti-inflammatory, and antilipidemic effects (Calva-Estrada et al., 2022; Ninfali et al., 2017; Pangestu et al., 2020).

Celosia cristata Linn., commonly known as Mawal, belongs to Amaranthaceae family and is cultivated globally due to its attractive and vibrant colored inflorescences (Sayeed et al., 2020). The flower of *C. cristata* contain betalains (Cai et al., 1998).

Betalains are used in different food systems, including corn oil (Attia et al. (2013), yogurt and cream (Coria-Cayupán & Nazareno, 2013), pork meat (da Silva et al. (2019), and smart packaging films (Kanatt, 2020). Azeredo (2009) reported that betalains are relatively within a pH range of 3 to 7. However, their application in different food systems is

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https://doi.org/10.1016/j.fochx.2025.102312
Received 21 January 2025; Received in revised form 19 February 2025; Accepted 21 February 2025
Available online 28 February 2025

limited due to their instability, which is inflenced by several factors, including light, water activity, oxygen, and metals (Calva-Estrada et al., 2022). Therefore, maintaining the stability of betalain pigments in complex food systems remain a significant challenge. The addition of copigments can enhance pigment stability during storage (Chatham et al., 2020). Modifying structural factors such as copigmentation, biosynthesis, and acylation is a potential solution to enhance pigment stability by forming more stable structures (Gençdağ et al., 2022). Copigments are generally colorless or slightly yellowishmolecules that exist naturally in plant cells alongside pigments that are considered an effective means to stabilize pigments against adverse environmental conditions (Zhao et al., 2020). The most commonly used copigments include organic acids, amino acids, phenolics, and alkaloids (Sharara, 2017).

The extraction of betalains from *Celosia cristata* flowers remains relatively underexplored, presenting a novel approach to utilize this flower beyond its traditional purpose. *Celosia cristata* flowers are locally grown in the Kashmir region, ensuring consistent availability for extraction without supply constraints. Investigating their betalain content can provide an alternative and potentially sustainable source of these bioactive pigments, contributing to their nutraceutical applications.

In this study, the potential of copigmentation using gum Arabic, pectin, whey protein, ascorbic acid, and calcium carbonate to improve betalain content, antioxidant activity, color stability, microbial counts, and degradation kinetics of betalain pigments extracted from *Celosia cristata* flowers during storage was examined. This research aims to enhance the stability of betalains, thereby expanding their application in functional foods and natural colorant formulations while addressing the existing gap in research on betalain extraction from *Celosia cristata* as a novel source.

2. Materials and methods

2.1. Raw materials and extraction of betalains

Fresh *Celosia cristata* flowers, free from blemishes and diseases, were procured from the local market of Shalimar in September during the harvesting period. After cleaning, the flower petals were separated manually to remove the stalk, seeds, and other unwanted parts. For extraction, 30 g *Celosia cristata* flowers were mixed with 300 mL of distilled water containing 0.1 % NaCl (*w/v*) solution. Betalains were extracted from this mixture using an ohmic heating-assisted extraction process for 3.5 min at a voltage of 131.0 V and a temperature of 49 °C. After extraction, the betalain-rich extract was stored at refrigeration temperature (4 °C) until further use.

2.2. Preparation of copigment solutions

The methodology of Chung et al. (2015) was followed to prepare copigments. Three organic compounds, i.e., gum Arabic, pectin and whey protein, were used for copigmentation. A total of eight treatments, including the control, were prepared as shown in Table 1. The pH of all treatments was adjusted to 3 using a citric acid buffer.

2.3. Storage study

A total of 30 mL of the extract, with and without copigments (pH 3), was placed in amber-colored glass vials and stored at ambient temperature (20–25 °C) in the dark for 90 days. During this period, antioxidant activity (%IA), betalain content (mg/L), total color difference (ΔE), hue angle, degradation kinetics, and total plate count were analysed every 15 days (0, 15, 30, 45, 60, 75, and 90 days). All experiments were conducted in triplicate.

Table 1
Different co-pigments and their concentrations per 100 mL of extract used for the copigmentation process.

Treatment	Copigment used for Co-pigmentation
To	Extract only
T1	Extract +1 % Gum Arabic +0.05 % ascorbic acid +0.01 % Ca^{2+}
T2	Extract +1 % Whey protein +0.05 % ascorbic acid +0.01 % Ca^{2+}
T3	Extract +1 % pectin +0.05 % ascorbic acid +0.01 % Ca^{2+}
T4	Extract +0.50 % Gum Arabic +0.50 % whey protein +0.05 % ascorbic acid +0.01 % Ca^{2+}
T5	Extract +0.50 % Gum Arabic +0.50 % pectin,0.05 % ascorbic acid +0.01 % Ca^{2+}
T6	Extract +0.50 % Whey protein +0.50 % pectin,0.05 % ascorbic acid +0.01 % Ca2+
T7	Extract +0.33 % Gum Arabic +0.33 % pectin+0.33 % whey protein +0.05 % ascorbic acid +0.01 % Ca^{2+}

2.4. Analysis during storage study

2.4.1. Betalain content (mg/L)

For betalain analysis, the methodology applied by Sokolova et al., 2022 was followed. Betalain content was determined using a spectrophotometer (Shimadzu, 1900) with a phosphate buffer (pH 6.5). The following equation (Eq.1), given by Cai et al., 1998 was used to calculate betalain content (mg/L).

$$\text{Betacyanin content} \Big/ \text{Betaxanthins content} \left(\frac{mg}{L}\right) = \frac{A \times MW \times DF \times 1000}{100 \times e \times l} \quad (1)$$

where, A = absorbance, i.e., 542 nm for betacyanins and 480 nm for betaxanthins; DF = dilution factor; l = path length (1 cm) of cuvette; e = molar extinction coefficients i.e., 60,000 L/mol cm for betacyanin and 48,000 L/mol cm for betaxanthins; and MW = molecular weight i.e., 550 g/mol and 308 g/mol for betacyanin and betaxanthins, respectively. The total betalain content was calculated by the following equation (Eq.2).

$$\text{Total Betalain content} = \text{Betacyanin content} + \text{Betaxanthins content} \quad (2)$$

2.4.2. Color analysis

The L*, a*, and b* color values were measured using a Hunter colorimeter (Model CR-2000, Minolta, Osaka, Japan) equipped with an 8 mm measuring head and "C" illumination. For calibration, the meter was set against a white plate, as recommended by the manufacturer. Color conversions were made in the L*, a*, b* color space. L* indicates the amount of whiteness or blackness in the sample; values closer to zero indicate a darker sample,while values closer to 100 indicate a lighter sample. If a* is less than zero, the color is green, and if a* is greater than zero, the color is red-purple. A positive value of b* represents yellow, while a negative b* value represents blue. The total color difference (ΔE) and hue angle were calculated using the following equations (Eq. 3 & Eq. 4), as adopted by Pathare et al. (2013).

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (3)$$

$$Hue\ angle = tan^{-}\left(\frac{b}{a}\right) \quad (4)$$

2.4.3. Kinetic calculations

Kinetic characteristics (reaction order, reaction rate constants (k), and half-life period ($t_{1/2}$) were used to assess the color properties of the material and the stability of betalains during storage. Activation energy (Ea), which measures the effect of temperature on betalain degradation, was also studied. The degradation kinetics of the extract was calculated

using standard equations for zero-order and first-order Arrhenius reaction models (Eq. 5 to Eq. 8).

$$A = A_0 - Kt \tag{5}$$

$$ln\left(\frac{A}{A_0}\right) = -Kt \tag{6}$$

where, k is the first-order rate constant (month^{-1}), t is the storage period (month), and A_0 and A are the reactant levels at time zero and time t, respectively. The Arrhenius equation was used to predict the reaction's temperature dependence.

$$t_{1/2} = \frac{ln2}{k} \tag{7}$$

$$ln\ k = lnA - \frac{E_a}{R \times T} \tag{8}$$

where k is the kinetic rate constant (time^{-1}).

2.4.4. Microbial analysis

Guidelines of the American Public Health Association (APHA. Frances and Keith, 2001) were followed for total plate counts (TPC) and yeast & mould counts. Sterilized potato dextrose agar plates were prepared and kept in a BOD incubator at 37 °C to ensure the sterility of the medium. Only clear plates, free from contamination, were used. One mL of each sample was mixed with nine mL of sterilized water, followed by serial dilutions from 10^{-2} to 10^{-5}. A total of 100 μL of the sample was spread over the surface of a potato dextrose agar plate incubated in a BOD incubator for 48 h at 37 °C. A similar procedure was followed using nutrient agar plates for TPC analysis. TPC and yeast and mould counts of the extract, with and without copigments, were analyzed every 15 days over a 90-day storage period. Results were expressed as cfu/mL.

2.4.5. Statistical analysis

Two-factor ANOVA using OPSTAT software was applied to determine the significant differences in betalain content, ΔE, hue angle, TPC, and yeast and& mould counts during the storage period.

3. Results and discussion

3.1. Effect of copigmentation and storage period on betalains content

From Table S1, it was observed that during the storage period, the mean betalain content decreased significantly ($p \leq 0.05$) from 140.31 to 61.61 mg/L. As shown in Fig. 1, treatments containing different combinations of gum Arabic, pectin, and whey protein improved the color stability of betalains. The highest degradation was observed in the control (T0) treatment, whereas the lowest degradation occurred in T7 throughout the storage period. This could be attributed to several factors, including the oxygen-scavenging ability of ascorbic acid, which helps stabilize natural pigments by reducing oxidative degradation (Gérard et al., 2019); the protective layer formation by gum Arabic (Kamel et al., 2024); the antioxidant and radical scavenging activities of whey protein, which effectively neutralize free radicals (Ren et al.2021); and pectin's ability to form gels and interact with pigments, thereby maintaining color integrity during processing and storage (Buecker et al., 2024).

Similar to the current findings, several studies (Castro-Enríquez et al., 2020; Chung et al., 2015; Kamel et al., 2024) have documented the role of gum Arabic as a natural stabilizer for color pigments in food products, contributing to improved shelf life and color retention. Kamel et al. (2024) demonstrated that pretreating dried red beetroot slices with gum Arabic reduced the degradation of betalains and antioxidants, maintaining higher sensory scores during storage. Likewise, several studies (Buecker et al., 2024; Cserjési et al., 2011; Poiana et al., 2013)

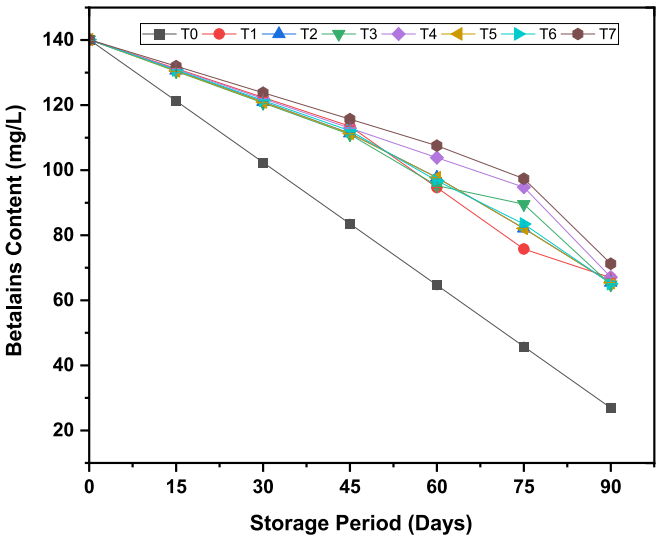

Fig. 1. Effect of copigmentation and storage period on betalains content during storage.

have established that pectin serves as an effective stabilizer for natural pigments, particularly anthocyanins, by forming protective complexes, enhancing gel formation, and providing antioxidant benefits. Buecker et al. (2024) showed that increasing the amount of pectin in pectin–phycocyanin complexes prevents precipitation, thereby improving the colloidal stability of the pigment. Similarly, Poiana et al. (2013) found that pectin improved pigment stability in blackberry jam, likely due to the formation of additional hydrogen bonds between pectin and anthocyanins. In the context of color stabilization, whey protein interacts with natural pigments, such as anthocyanins, enhancing their stability. Research indicates that whey protein can improve the color, stability, and antioxidant capacity of anthocyanin complexes (Ren, Jiménez-Flores, & Giusti, 2021). Furthermore, preheated whey protein isolate has been shown to effectively improve the color stability of rose anthocyanin extracts in beverage systems, suggesting its potential for preserving pigment integrity during storage Wang et al., 2023). Similarly ascorbic acid, while beneficial for oxygen scavenging, has been observed to accelerate the degradation of anthocyanins, leading to color loss in anthocyanin-rich beverages (Gérard et al., 2019). A combination of all three polymers, gum Arabic, pectin, and whey protein (T7), play a pivotal role in preserving the visual and functional qualities of natural pigments in food systems.

3.2. Effect of copigmentation and storage period on ΔE and hue angle during storage

During the 90-day storage period, the mean values of ΔE of extract increased significantly ($p \leq 0.05$) from 0.44 to 9.61 (Table S2). Similarly, the mean values of hue angle of the extract increased significantly ($p \leq 0.05$) from 0.24 to 0.81 (Table S3). Fig. 2(a) and Fig. 2(b) illustrate that treatments with various combinations of gum Arabic, pectin, and whey protein enhanced the color stability of betalains. The highest degradation occurred in the control treatment (T0), while the lowest degradation was observed in T7 throughout the storage period. This could be attributed to the presence of gum Arabic, which can slow down pigment degradation by reducing the mobility of reactive molecules such as oxygen radicals Cortez et al. (2017). Additionally, the oxygen-scavenging ability of ascorbic acid contributes to stabilizing natural pigments by mitigating oxidative degradation. (Gérard et al., 2019), Pectin can form non-covalent complexes with natural pigments, such as anthocyanins, through hydrogen bonding and hydrophobic interactions, shielding them from environmental factors like oxygen and light, thereby preserving color integrity (Shi et al., 2024). Furthermore, whey

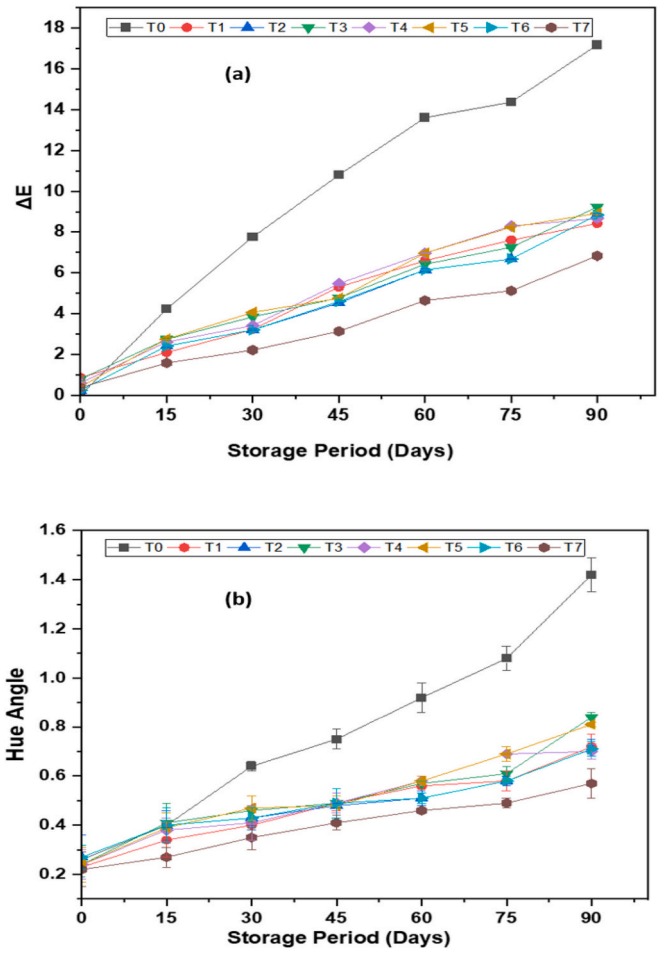

Fig. 2. Effect of copigmentation and storage period on (a) ΔE, and (b) hue angle during storage.

protein has been explored as a stabilizing agent for natural pigments due to its ability to form protective matrices around pigments, enhancing their stability (Miyagusuku-Cruzado et al., 2021). Numerous studies have demonstrated the stabilizing effects of various biopolymers on natural pigments. For example, Basavaraja et al. (2022) explored the copigmentation effects of black carrot anthocyanins and beetroot betalains, reporting a bathochromic shift from 538 nm to 564 nm, indicative of enhanced color stability. Similarly, Shi et al. (2024) emphasized the versatility of pectin in stabilizing natural pigments through various mechanisms, including the formation of protective complexes, enhancement of gel structure, and improvement of colloidal stability. These properties contribute to the development of food products with enhanced color retention and extended shelf life. Under stress conditions such as light exposure and high temperatures, copigmented betalains exhibited improved resistance to degradation. Likewise, Janjarasskul et al. (2011) reported that whey protein enhances anthocyanin stability, with higher concentrations contributing to improved chromatic properties and reduced degradation over time. Karangutkar & Ananthanarayan (2021) investigated the stability of betacyanin pigments in a model beverage system enriched with catechin, EDTA, and β-cyclodextrin. Their findings highlighted the ability of these additives to maintain the visual color properties of the beverage during storage. Measurements of hue angle (H*) and overall color change (ΔE*) confirmed enhanced color retention over time with these treatments. Miyagusuku-Cruzado et al. (2021) highlighted that whey protein improves light absorption and the tinctorial strength of model solutions containing anthocyanins. Another significant contribution comes from Qian et al. (2017), who evaluated the impact of gallic, ferulic, and caffeic acids on anthocyanin stability and color intensification in a pH 3.2 buffer. Using an accelerated stability test at 95 °C for 15 h, they observed that gallic acid offered superior protection against anthocyanin degradation, whereas ferulic and caffeic acids, though promoting some degradation, still demonstrated relatively high color retention compared to the control. Cortez et al. (2017) demonstrated that in beverage systems, gum Arabic works synergistically with ascorbic acid to prevent oxidative degradation and maintain the color stability of anthocyanins and other pigments. Collectively, these studies emphasize the critical role of biopolymers and copigmentation agents in enhancing the stability and visual quality of natural pigments, thereby supporting their application in food systems requiring extended shelf life and improved color retention.

3.3. Color degradation kinetics

The kinetics of the first-order reaction were observed in the degradation of betalains. *Celosia cristata* follows first-order kinetics for color deterioration, meaning that the rate constant (k) increases with temperature. The temperature dependency of degradation can be modelled using the Arrhenius equation. Both temperature and storage duration enhance the reaction rate constants for the degradation of betaxanthin and betacyanin. The half-life time ($t_{1/2}$), reaction order, and reaction rate constants (k) were used to assess color changes and betalain stability during storage (Table S4). Activation energy (Ea) was determined to study the effect of temperature on betalain degradation (Table S4). This study examined the impact of gum Arabic, pectin, and whey protein on the color degradation kinetics of betalain solutions at 4 °C, 20 °C and 40 °C, as shown in Fig. 3(a), (b), and (c). The results indicated that betalain degradation was minimal under these treatments, whereas the extract without copigments (T0) exhibited a significant increase in color degradation kinetics during storage (Fig. 3(a), (b), and (c)). These findings clearly demonstrate that the presence of copigments is essential for enhancing betalain stability.

Similar to the current findings, several studies (Caldas-Cueva et al., 2016; Kayın et al., 2019; Tobolková et al., 2024) have documented the first-order kinetics of betalain degradation in various food matrices under different processing and storage conditions. The first-order kinetics of betaxanthin and betacyanin degradation in red beet juice concentrates were described by Kayın et al. (2019). Similarly, Tobolková et al. (2024) observed that the degradation of betacyanins and betaxanthins in apple-beetroot juice stored at 2 °C, 7 °C, and 20 °C followed a first-order process. The degradations rate constants (k values) reported in their study were lower than those observed in the present study, ranging from 7.3×10^{-3}/day to 47.1×10^{-3}/day for betacyanin and 6.4×10^{-3}/day to 28.5×10^{-3}/day for betaxanthin. Likewise, Caldas-Cueva et al. (2016) reported that first-order reaction kinetics governed the degradation of betacyanins in an extract from ayrampo seeds (*Opuntia soehrensii*) and red beet extract, stored at 4 °C and 25 °C, at pH 4.5. They found that betacyanin stability was higher in ayrampo seed extract than in red beet extract. Futhermore, the fat content of yogurts containing red beet and ayrampo seed extractshad no significant effect on color retention, with the ayrampo seed extract demonstrating superior color stability over a 4-week storage period at 4 °C compared to the red beet extract. In contrast to the present findings, Moreno (2007) reported zero-order reaction kinetics for betalain degradation in tuna (*Opuntia elatior* Miller) and beetroot (*Beta vulgaris* L.) within four different citrus-based beverage formulations. The study found that the rate constant (k values) ranged from 12.4 to 18.1 g/100 mL × day, and the activation energy (Ea) was lower than that observed in the present study. The half-life value of betalains increased significantly under continuous light exposure after copigmentation. Specifically, $t_{1/2}$ values increased to 186.96, 226.56, and 152.88 h, demonstrating enhanced betalain stability. Conversely, when salt was added at 10%, 15%, and 18%, the $t_{1/2}$ values were 81.12, 75.36, and 83.52 hours, respectively. These findings suggest that copigmentation improves betalain thermostability, as indicated by lower rate constants (k values) and activation

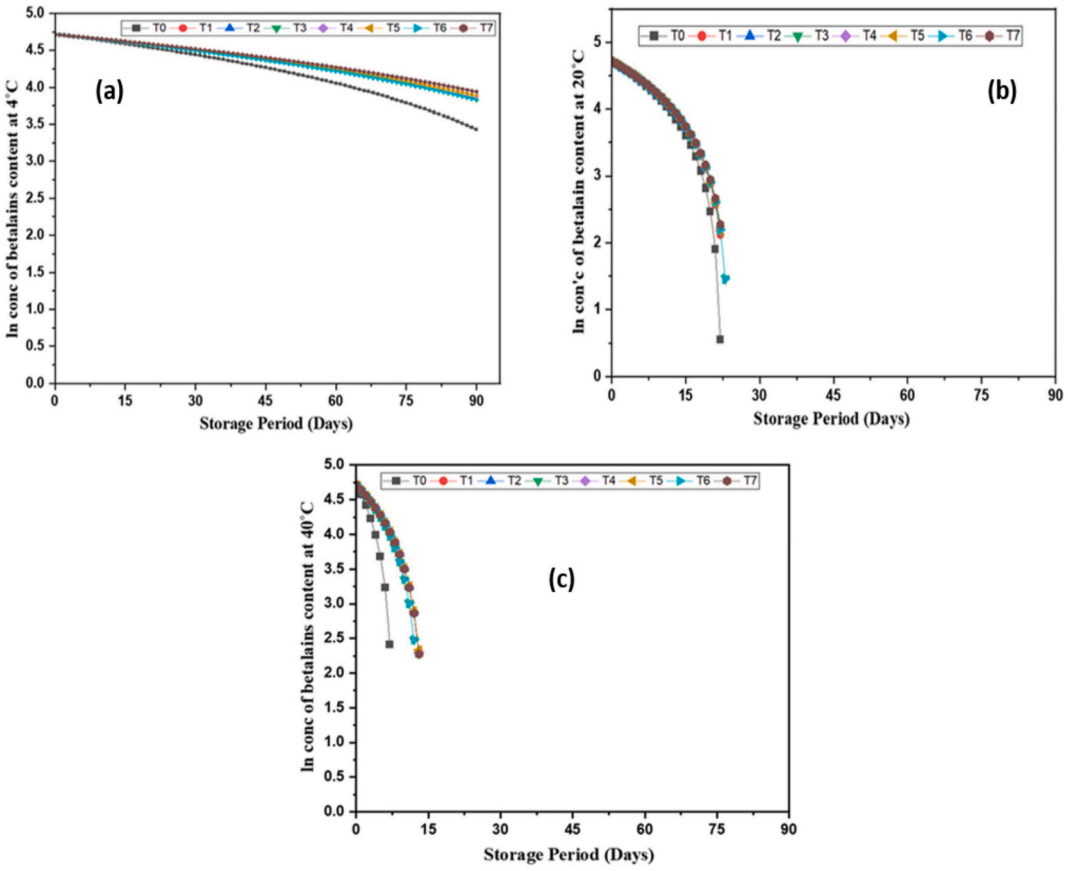

Fig. 3. First-order kinetics plots of ln concentration of betalains content versus storage period (days) (a) at 4 °C, (b) at 20 °C, and (c) at 40 °C.

energy (Ea).

3.4. *Effect of copigmentation and storage period on TPC and yeast & Moulds counts*

Table 2 and Table 3 present the TPC and Yeast and Mould counts, respectively, in different treatments. The results indicate that no microbial growth was observed in treatments T1 to T7 (containing gum Arabic, pectin, whey protein, calcium carbonate and ascorbic acid) during the first 30 days of storage. However, in the control treatment (T0), microbial growth was observed after 15 days of storage. After 30 days, microbial growth was detected in T1 to T7 as well, but the growth remained lower than in T0 throughout the entire 90-day storage period. This could be due to the formation of protective films, which act as barriers to microorganisms, potentially reducing their access to nutrients and oxygen. Additionally, the antioxidant and antimicrobial properties of the components used in the treatments may have contributed to inhibiting microbial growth (Al-Assaf, Amar, & Phillips, 2008; Saubenova et al., 2024; Liguori et al., 2021; Boudouaia et al., 2023; Baien et al., 2020). The presence of calcium ions may also have had a stabilizing effect on the copigment structure, influencing water activity and pH, thereby creating an environment hostile to microbial growth (McClements, 2004). Overall, our findings support the conclusion of Celli and Brooks (2017), who reported that lower storage temperatures are beneficial for microbial management and pigment preservation.

4. Conclusion

The results of this study suggest that the use of gum Arabic, whey protein, pectin, ascorbic acid, and calcium carbonate as copigments enhances the storage stability of betalains from *Celosia Cristata* L. flowers. The copigments used in this study also exhibited antimicrobial potential. Among all copigment treatments, T7 (0.33 % gum Arabic, 0.33 % pectin, 0.33 % whey protein, 0.05 % ascorbic acid, and 0.01 % Ca^{2+}) demonstrated the highest storage stability of betalain pigments in terms of betalain content and color retention during the storage period. The degradation kinetics of betalain pigments at 4 °C, 20 °C, and 40 °C revealed that storage at 4 °C is optimal maintaining betalain stability.

Table 2
TPC During Storage (Cfu/mL) × 10^5.

TPC	T0	T1	T2	T3	T4	T5	T6	T7	Mean
0 Day	ND	ND	ND	ND	ND	ND	ND	ND	_
15 Day	5.81 ± 0.07	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	5.81 ± 0.07
30 Day	8.43 ± 0.04	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	8.43 ± 0.04
45 Day	14.77 ± 0.05	1.66 ± 0.03	1.62 ± 0.04	1.65 ± 0.02	1.66 ± 0.01	1.64 ± 0.01	1.63 ± 0.02	1.61 ± 0.04	3.28 ± 0.02
60 Day	20.59 ± 0.02	2.88 ± 0.02	2.86 ± 0.03	2.84 ± 0.01	2.82 ± 0.02	2.83 ± 0.02	2.85 ± 0.04	2.81 ± 0.02	5.06 ± 0.02
75 Day	41.78 ± 0.03	7.55 ± 0.01	7.52 ± 0.02	7.53 ± 0.04	7.54 ± 0.04	7.52 ± 0.04	7.55 ± 0.05	7.51 ± 0.03	11.81 ± 0.03
90 Day	50.88 ± 0.01	14.0 ± 0.04	13.94 ± 0.01	13.96 ± 0.03	13.93 ± 0.03	13.97 ± 0.05	13.95 ± 0.01	13.90 ± 0.06	18.56 ± 0.02
Mean	23.71 ± 0.02	6.52 ± 0.01	6.48 ± 0.02	6.495 ± 0.02	6.48 ± 0.02	6.49 ± 0.02	6.49 ± 0.02	6.45 ± 0.02	

ND = Not determined, TFTC = Too few to count. C·D ($p \leq 0.05$); Storage days = 1.529; Treatment = 2.379; Storage days × treatment =3.63.

Table 3
Yeast and Mould During Storage (Cfu /mL) × 10^5.

Yeast & Mould	T0	T1	T2	T3	T4	T5	T6	T7	Mean
0 Day	ND	ND	ND	ND	ND	ND	ND	ND	-
15 Day	6.81 ± 0.02	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	6.81 ± 0.02
30 Day	10.13 ± 0.01	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	10.13 ± 0.01
45 Day	16.27 ± 0.01	1.76 ± 0.02	1.75 ± 0.04	1.73 ± 0.05	1.72 ± 0.04	1.74 ± 0.03	1.75 ± 0.04	1.71 ± 0.01	3.55 ± 0.03
60 Day	22.26 ± 0.02	3.54 ± 0.01	3.52 ± 0.01	3.54 ± 0.03	3.53 ± 0.01	3.52 ± 0.04	3.54 ± 0.03	3.51 ± 0.02	5.87 ± 0.02
75 Day	45.11 ± 0.04	8.66 ± 0.04	8.63 ± 0.03	8.64 ± 0.01	8.66 ± 0.02	8.63 ± 0.01	8.62 ± 0.02	8.61 ± 0.03	13.19 ± 0.02
90 Day	56.12 ± 0.03	15 ± 0.02	14.96 ± 0.02	14.94 ± 0.02	14.95 ± 0.03	14.93 ± 0.02	14.92 ± 0.01	14.87 ± 0.04	20.08 ± 0.02
Mean	26.11 ± 0.02	7.24 ± 0.01	7.21 ± 0.02	7.21 ± 0.02	7.21 ± 0.02	7.20 ± 0.01	7.20 ± 0.02	7.17 ± 0.01	

ND = Not determined, TFTC = Too few to count. C·D ($p \leq 0.05$); Storage days = 2.231; Treatment = 3.15; Storage days × treatment =7.027.

Overall, betalain pigments from the natural source *Celosia cristata*, in combination with gum Arabic, pectin, whey protein, ascorbic acid, and calcium carbonate, show potential for improved stability and commercial applications in various food systems.

CRediT authorship contribution statement

Shabnum Showkat: Writing – original draft, Methodology, Formal analysis. **Aasima Rafiq:** Writing – review & editing, Supervision, Conceptualization. **Rishi Richa:** Writing – review & editing, Validation. **Qayoom Sidique:** Writing – review & editing. **Afzal Hussain:** Writing – review & editing, Validation. **Umesh Chandra Lohani:** Writing – review & editing. **Oroofa Bhat:** Writing – review & editing. **Sanjay Kumar:** Writing – review & editing, Supervision.

Declaration of competing interest

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.

Acknowledgements

The authors are grateful to the Division of Food Science and Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Shalimar, Srinagar and the Department of Food Science & Technology, Graphic Era (Deemed to be University), Dehradun, Uttarakhand, India, for providing the necessary infrastructure for this research paper. The authors acknowledge the generous support from the researchers supporting project number (RSPD2025R980) King Saud University, Riyadh, Saudi Arabia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102312.

Data availability

Data will be made available on request.

References

Al-Assaf, S., Amar, V., & Phillips, G. O. (2008). Characterisation of gum ghatti and comparison with gum arabic. *Gums and stabilisers for the food. industry, 14*(14), 280–290.

APHA. Frances, & Keith, I. (2001). *Compendium of methods for the microbiological examination of foods.* Washington, DC.

Attia, G. Y., Moussa, M. M., & Sheashea, E. E. D. R. (2013). Characterization of red pigments extracted from red beet (*Beta vulgaris*, L.) and its potential uses as antioxidant and natural food colorants. *Egyptian. Journal of Agricultural Research, 91*(3), 1095–1110. https://doi.org/10.21608/ejar.2013.167086

Azeredo, H. M. (2009). Betalains: Properties, sources, applications, and stability–a review. *International Journal of Food Science & Technology, 44*(12), 2365–2376. https://doi.org/10.1111/j.1365-2621.2007.01668.x

Baien, S. H., Seele, J., Henneck, T., Freibrodt, C., Szura, G., Moubasher, H., & de Buhr, N. (2020). Antimicrobial and immunomodulatory effect of gum arabic on human and bovine granulocytes against *Staphylococcus aureus* and *Escherichia coli*. *Frontiers in Immunology, 10*, 3119. https://doi.org/10.3389/fimmu.2019.03119

Basavaraja, T., Joshi, A., Sethi, S., Kaur, C., Tomar, B. S., Varghese, E., & Dahuja, A. (2022). Stability enhancement of beetroot betalains through copigmentation. *Journal of the Science of Food and Agriculture, 102*(12), 5561–5567. https://doi.org/10.1002/jsfa.11939

Boudouaia, N., Bendaoudi, A. A., Benykhlef, S., Bengharez, Z., & Cantarero, A. (2023). Antibacterial action, antioxidant activity and anticoagulant effect of pectin extracted from peels of Algerian *Citrus Sinensis*. *Chemistry Proceedings, 14*(1), 69. https://doi.org/10.3390/ecsoc-27-16069

Buecker, S., Gibis, M., Bartmann, L., Bussler, S., & Weiss, J. (2024). Improving the colloidal stability of pectin–phycocyanin complexes by increasing the mixing ratio. *Journal of Food Science, 89*(2), 1086–1097. https://doi.org/10.1111/1750-3841.16917

Cai, Y., Sun, M., & Corke, H. (1998). Colorant properties and stability of Amaranthus betacyanin pigments. *Journal of Agricultural and Food Chemistry, 46*(11), 4491–4495. https://doi.org/10.1021/jf980457g

Caldas-Cueva, J. P., Morales, P., Ludeña, F., Betalleluz-Pallardel, I., Chirinos, R., Noratto, G., & Campos, D. (2016). Stability of Betacyanin pigments and antioxidants in Ayrampo (*Opuntia soehrensii* Britton and Rose) seed extracts and as a yogurt natural colorant. *Journal of Food Processing and Preservation, 40*(3), 541–549. https://doi.org/10.1111/jfpp.12633

Calva-Estrada, S. J., Jiménez-Fernández, M., & Lugo-Cervantes, E. (2022). Betalains and their applications in food: The current state of processing, stability and future opportunities in the industry. *Food Chemistry: Molecular Sciences, 4*, Article 100089. https://doi.org/10.1016/j.fochms.2022.100089

Castro-Enríquez, D. D., Montaño-Leyva, B., Del Toro-Sánchez, C. L., Juaréz-Onofre, J. E., Carvajal-Millan, E., Burruel-Ibarra, S. E., & Rodríguez-Félix, F. (2020). Stabilization of betalains by encapsulation—A review. *Journal of Food Science and Technology, 57*, 1587–1600.

Celli, G. B., & Brooks, M. S. L. (2017). Impact of extraction and processing conditions on betalains and comparison of properties with anthocyanins—A current review. *Food Research International, 100*, 501–509. https://doi.org/10.1016/j.foodres.2016.08.034

Chatham, L. A., Howard, J. E., & Juvik, J. A. (2020). A natural colorant system from corn: Flavone-anthocyanin copigmentation for altered hues and improved shelf life. *Food Chemistry, 310*, Article 125734. https://doi.org/10.1016/j.foodchem.2019.125734

Chung, C., Rojanasasithara, T., Mutilangi, W., & McClements, D. J. (2015). Enhanced stability of anthocyanin-based color in model beverage systems through whey protein isolate complexation. *Food Research International, 76*, 761–768. https://doi.org/10.1016/j.foodres.2015.07.003

Coria-Cayupán, Y., & Nazareno, M. A. (2013, October). Cactus betalains can be used as antioxidant colorants protecting food constituents from oxidative damage. In VIII international congress on Cactus pear and cochineal *1067* (pp. 319-325). Doi:10.17660/ActaHortic.2015.1067.44.

Cortez, R., Luna-Vital, D. A., Margulis, D., & Gonzalez de Mejia, E. (2017). Natural pigments: Stabilization methods of anthocyanins for food applications. *Comprehensive Reviews in Food Science and Food Safety, 16*(1), 180–198. https://doi.org/10.1111/1541-4337.12244

Cserjési, P., Bélafi-Bakó, K., Csanádi, Z., Beszédes, S., & Hodúr, C. (2011). Simultaneous recovery of pectin and colorants from solid agro-wastes formed in processing of colorful berries. *Progress in Agricultural Engineering Sciences, 7*(1), 65–80. https://doi.org/10.1556/progress.7.2011.5

Di Salvo, E., Lo Vecchio, G., De Pasquale, R., De Maria, L., Tardugno, R., Vadalà, R., & Cicero, N. (2023). Natural pigments production and their application in food, health and other industries. *Nutrients, 15*(8), 1923. https://doi.org/10.3390/nu15081923

Fathordoobady, F., Mirhosseini, H., Selamat, J., & Abd Manap, M. Y. (2016). Effect of solvent type and ratio on betacyanins and antioxidant activity of extracts from *Hylocereus polyrhizus* flesh and peel by supercritical fluid extraction and solvent extraction. *Food Chemistry, 202*, 70–80. https://doi.org/10.1016/j.foodchem.2016.01.121

Gençdağ, E., Özdemir, E. E., Demirci, K., Görgüç, A., & Yılmaz, F. M. (2022). Copigmentation and stabilization of anthocyanins using organic molecules and encapsulation techniques. *Current Plant Biology, 29*, Article 100238. https://doi.org/10.1016/j.cpb.2022.100238

Gérard, V., Ay, E., Graff, B., Morlet-Savary, F., Galopin, C., Mutilangi, W., & Lalevée, J. (2019). Ascorbic acid derivatives as potential substitutes for ascorbic acid to reduce

color degradation of drinks containing ascorbic acid and anthocyanins from natural extracts. *Journal of Agricultural and Food Chemistry, 67*(43), 12061–12071. https://doi.org/10.1021/acs.jafc.9b05049

Janjarasskul, T., Min, S. C., & Krochta, J. M. (2011). Storage stability of ascorbic acid incorporated in edible whey protein films. *Journal of Agricultural and Food Chemistry, 59*(23), 12428–12432. https://doi.org/10.1021/jf201629r

Kamel, S. M., Alqurashi, R. M., Alshebly, A. M., Saleh, F. A., Alnemr, T. M., Ali, H. F., & Alqahtani, N. K. (2024). Effect of drying method and coating with gum Arabic on physicochemical properties, Betalain content, LDL oxidation, and storage stability of dried red beetroot slices. *Journal of Food Processing and Preservation, 2024*(1), Article 8471292. https://doi.org/10.1155/2024/8471292

Kanatt, S. R. (2020). Development of active/intelligent food packaging film containing Amaranthus leaf extract for shelf life extension of chicken/fish during chilled storage. *Food Packaging and Shelf Life, 24*, Article 100506. https://doi.org/10.1016/j.fpsl.2020.100506

Karangutkar, A. V., & Ananthanarayan, L. (2021). Evaluating the effect of additives on stability of betacyanin pigments from Basella rubra in a model beverage system during storage. *Journal of Food Science and Technology, 58*(4), 1262–1273. https://doi.org/10.1007/s13197-020-04635-8

Kayın, N., Atalay, D., Türken Akçay, T., & Erge, H. S. (2019). Color stability and change in bioactive compounds of red beet juice concentrate stored at different temperatures. *Journal of Food Science and Technology, 56*(11), 5097–5106. https://doi.org/10.1007/s13197-019-03982-5

Liguori, G., Gaglio, R., Settanni, L., Inglese, P., D'Anna, F., & Miceli, A. (2021). Effect of opuntia ficus-indica mucilage edible coating in combination with ascorbic acid, on strawberry fruit quality during cold storage. *Journal of Food Quality, 2021*(1), Article 9976052. https://doi.org/10.1155/2021/9976052

Lu, W., Shi, Y., Wang, R., Su, D., Tang, M., Liu, Y., & Li, Z. (2021). Antioxidant activity and healthy benefits of natural pigments in fruits: A review. *International Journal of Molecular Sciences, 22*(9), 4945. https://doi.org/10.3390/ijms22094945

McCann, D., Barrett, A., Cooper, A., Crumpler, D., Dalen, L., Grimshaw, K., … Prince, E. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: A randomised, double-blinded, placebo-controlled trial. *The Lancet, 370*(9598), 1560–1567.

McClements, D. J. (2004). *Food emulsions: Principles, practices, and techniques*. CRC press.

Miyagusuku-Cruzado, G., Jiménez-Flores, R., & Giusti, M. M. (2021). Whey protein addition and its increased light absorption and tinctorial strength of model solutions colored with anthocyanins. *Journal of Dairy Science, 104*(6), 6449–6462. https://doi.org/10.3168/jds.2020-19690

Moreno, R. (2007). Optimising learning from animations by minimising cognitive load: Cognitive and affective consequences of signalling and segmentation methods. *Applied Cognitive Psychology, 21*(6), 765–781. https://doi.org/10.1002/acp.1348

Ninfali, P., Antonini, E., Frati, A., & Scarpa, E. S. (2017). C-glycosyl flavonoids from *Beta vulgaris* cicla and betalains from *Beta vulgaris* rubra: Antioxidant, anticancer and antiinflammatory activities—A review. *Phytotherapy Research, 31*(6), 871–884. https://doi.org/10.1002/ptr.5819

Pangestu, N. P., Miyagusuku-Cruzado, G., & Giusti, M. M. (2020). Copigmentation with chlorogenic and ferulic acid affected color and anthocyanin stability in model beverages colored with Sambucus peruviana, *Sambucus nigra*, and *Daucus carota* during storage. *Foods, 9*(10), 1476. https://doi.org/10.3390/foods9101476

Pathare, P. B., Opara, U. L., & Al-Said, F. A. J. (2013). Colour measurement and analysis in fresh and processed foods: A review. *Food and Bioprocess Technology, 6*, 36–60. https://doi.org/10.1007/s11947-012-0867-9

Poiana, M. A., Munteanu, M. F., Bordean, D. M., Gligor, R., & Alexa, E. (2013). Assessing the effects of different pectins addition on color quality and antioxidant properties of blackberry jam. *Chemistry Central Journal, 7*, 1–13.

Qian, B. J., Liu, J. H., Zhao, S. J., Cai, J. X., & Jing, P. (2017). The effects of gallic/ferulic/caffeic acids on colour intensification and anthocyanin stability. *Food Chemistry, 228*, 526–532. https://doi.org/10.1016/j.foodchem.2017.01.120

Ren, S., Jiménez-Flores, R., & Giusti, M. M. (2021). The interactions between anthocyanin and whey protein: A review. *Comprehensive Reviews in Food Science and Food Safety, 20*(6), 5992–6011. https://doi.org/10.1111/1541-4337.12854

Saubenova, M., Oleinikova, Y., Rapoport, A., Maksimovich, S., Yermekbay, Z., & Khamedova, E. (2024). Bioactive peptides derived from whey proteins for health and functional beverages. *Fermentation, 10*(7), 359. https://doi.org/10.3390/fermentation10070359

Sayeed, R., Thakur, M., & Gani, A. (2020). *Celosia cristata* Linn. Flowers as a new source of nutraceuticals-a study on nutritional composition, chemical characterization and in-vitro antioxidant capacity. *Heliyon, 6*(12). https://doi.org/10.1016/j.heliyon.2020.e05792

Sharara, M. S. (2017). Copigmentation effect of some phenolic acids on stabilization of roselle (*Hibiscus sabdariffa*) anthocyanin extract. *American Journal of Food Science and Technology, 5*(2), 45–52. https://doi.org/10.12691/ajfst-5-2-3

Shi, C., Guo, C., Wang, S., Li, W., Zhang, X., Lu, S., & Tan, C. (2024). The mechanism of pectin in improving anthocyanin stability and the application progress of their complexes: A review. *Food Chemistry: X, 101955*. https://doi.org/10.1016/j.fochx.2024.101955

da Silva, D. V. T., dos Santos Baião, D., de Oliveira Silva, F., Alves, G., Perrone, D., Del Aguila, E. M., & Paschoalin, V. M. F. (2019). Betanin, a natural food additive: Stability, bioavailability, antioxidant and preservative ability assessments. *Molecules, 24*(3). https://doi.org/10.3390/molecules24030458

Sokolova, D. V., Shvachko, N. A., Mikhailova, A. S., & Popov, V. S. (2022). Betalain content and morphological characteristics of table beet accessions: Their interplay with abiotic factors. *Agronomy, 12*(5), 1033. https://doi.org/10.3390/agronomy12051033

Sokolova, D. V., Shvachko, N. A., Mikhailova, A. S., Popov, V. S., Solovyeva, A. E., & Khlestkina, E. K. (2024). Characterization of Betalain content and antioxidant activity variation dynamics in table beets (*Beta vulgaris* L.) with differently colored roots. *Agronomy, 14*(5), 999. https://doi.org/10.3390/agronomy14050999

Strack, D., Vogt, T., & Schliemann, W. (2003). Recent advances in betalain research. *Phytochemistry, 62*(3), 247–269. https://doi.org/10.1016/S0031-9422(02)00564-2

Tobolková, B., Belajová, E., Polovka, M., & Durec, J. (2024). Effects of pasteurisation, processing atmosphere and long-term storage on orange juice quality. *Journal of Food Measurement and Characterization, 18*(1), 783–796. https://doi.org/10.1007/s11694-023-02235-6

Wang, Y., Yang, C., Zhang, J., & Zhang, L. (2023). Interaction of preheated whey protein isolate with rose anthocyanin extracts in beverage model system: Influence on color stability, astringency and mechanism. *Food Chemistry, 412*, Article 135507. https://doi.org/10.1016/j.foodchem.2023.135507

Zhao, L., Pan, F., Mehmood, A., Zhang, Y., Hao, S., Rehman, A. U., & Wang, Y. (2020). Protective effect and mechanism of action of xanthan gum on the color stability of black rice anthocyanins in model beverage systems. *International Journal of Biological Macromolecules, 164*, 3800–3807. https://doi.org/10.1016/j.ijbiomac.2020.09.027