

## Research Article

## Encapsulation and copigmentation effects on sour cherry anthocyanin color stability in beverages

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**ABSTRACT-** Sour cherry is a valuable source of anthocyanins. Their utilization in food systems is constrained by the low stability of these pigments. This research builds upon our earlier study that examined how carrier agents (maltodextrin and maltodextrin combined with Persian gum) and tannic acid, as a copigment, influence the characteristics of anthocyanins in sour cherry in spray-dried powders. All formulations exhibited encapsulation efficiencies exceeding 90.37% and high solubility (> 99.94%). The anthocyanin retention in copigmented samples encapsulated with maltodextrin or maltodextrin + Persian gum was 53.23% and 38.13%, respectively, both of which were higher than those observed in the non-copigmented counterparts. This study aimed to assess the color stability of sour cherry anthocyanin powders under three different storage conditions and to examine their color stability in model beverages. All powders showed  $\Delta E$  values of 1.5–5 with similar color retention across different storage conditions. However, powders produced with maltodextrin + Persian gum carriers (without tannic acid) showed greater color loss after 28 days, consistent with the reduced anthocyanin stability. After 49 days, the color stability of beverages containing anthocyanin powders was comparable to that of control beverages formulated with fresh anthocyanin extract (with/without tannic acid). This effect likely arises from copigmentation complex disruption during pasteurization, the standardized acidic pH that levels anthocyanin stability, and matrix interactions that hinder copigmentation. In pasteurized acidic beverages, copigmentation offers limited color stability, making processing and matrix optimization crucial for anthocyanin protection. For commercialization, drying with carriers like maltodextrin is preferable to fresh extracts.

### INTRODUCTION

Anthocyanins are naturally occurring pigments that contribute to the purple, red, and blue hues in various fruits and plants (Cai et al., 2022). Anthocyanins belong to the flavonoid family and exhibit antioxidant activities that can reduce obesity, cardiovascular disease, inflammation, diabetes, and cancer (Liu et al., 2023). The low stability of anthocyanins is the main disadvantage of using them. Factors that affect their stability and color are pH, temperature, oxygen, light, ascorbic acid, sugars, enzymes, degradation products, and metallic ions (Enaru et al., 2021; Herrera-Balandrano et al., 2021). Copigmentation and encapsulation represent two primary methods to improve the stability of anthocyanins (Gençdağ et al., 2022).

Sour cherries are distinguished by their elevated levels of phenolic compounds, with a particular emphasis on anthocyanins. Cyanidin-3-glucosyl-rutinoside, cyanidin-3-

suforoside, cyanidin-3-rutinoside, and cyanidin-3-glucoside have been identified as anthocyanin compounds in sour cherries (Cairone et al., 2023).

Copigmentation is a method by which anthocyanins can form complexes or associate with copigments, like phenolic compounds, metal ions, and biopolymers. Thus, copigmentation can intensify color and antioxidant properties (Li et al., 2024; Trouillas et al., 2016). However, some studies revealed that copigmented complexes can be precipitated or separated during the food sterilization process and after interaction with the food matrix. For example, copigmented complexes decreased significantly after heating at 80 °C or via ultraviolet (UV) radiation (Tan et al., 2021; You et al., 2018). This reduction may degrade and discolor the anthocyanins. Thus, external conditions and types of copigments must be carefully optimized and selected (Sari, 2016). Phenolic acids have been identified as effective agents for the copigmentation of anthocyanins,

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enhancing both the color intensity and stability of these pigments (Molaeafard et al., 2021; Singh et al., 2025; Wang et al., 2024). In mildly acidic solutions that contain anthocyanins, phenolic acids can enhance the coloration of anthocyanins. This enhancement can occur through two mechanisms: a bathochromic shift, which involves an increase in the wavelength of maximum absorbance, and a hyperchromic effect, which results in an increase in the maximum absorbance. These phenomena may contribute to the improved color stability of anthocyanins (Lv et al., 2022). Our previous study demonstrated that incorporating tannic acid (TA) as a copigment into a sour cherry anthocyanin solution (pH 3.5) enhanced the bathochromic shift, hyperchromic effect, and color intensity compared to the non-copigmented anthocyanin solution (Moshfegh et al., 2024).

Microencapsulation by spray drying is a very useful method to increase the stability of anthocyanins, allowing their incorporation into sensitive systems (Kaderides et al., 2024). Maltodextrin (MD), Arabic gum, and emulsifying starches are the most common carrier agents (Darijani et al., 2025). MD makes bioactive compounds more stable and has many good properties, such as high solubility and low viscosity (Gómez-Gaete et al., 2024). Persian gum (PG) is a natural exudate derived from the wild almond tree and is considered a viable alternative to Arabic gum (Jafari et al., 2012; Jafari et al., 2013). Our previous study indicated that the microencapsulation of sour cherry anthocyanin extract with MD and PG via spray drying yielded powders with low moisture content, high solubility, and good quality, making them suitable for storage (Moshfegh et al., 2024). Studies have demonstrated that the combined use of carrier agents for spray drying and suitable copigments can cause color intensification and greater stability of the anthocyanins (Salati et al., 2025; Sarabandi et al., 2019; Tan et al., 2019; Xue et al., 2019).

Numerous studies have employed copigmented or encapsulated anthocyanins in model beverages as colorants. For instance, Wang et al. (2024) utilized epigallocatechin gallate, ferulic acid, and gallic acid as copigments to enhance the color stability of anthocyanins in both model and real blueberry fermented beverages. Similarly, Lin et al. (2023) examined the effects of five polyphenols as copigments on the stability of purple cabbage anthocyanins in model beverages. Burin et al. (2011) encapsulated Cabernet Sauvignon anthocyanins with MD, cyclodextrin, and Arabic gum, and evaluated their stability in an isotonic soft drink system. However, research on the combined application of copigmentation and encapsulation of anthocyanins in model beverages remains limited (Salati et al., 2025). This research builds upon our prior investigation, which examined the influence of carrier agents (MD and a combination of MD with PG) and TA as a copigment on various properties of anthocyanins derived from sour cherries. All treatments in the previous study exhibited high encapsulation efficiency, high solubility, and low water activity. Powders containing copigmented MD retained higher anthocyanin levels under various storage conditions and displayed a deeper red hue compared to

those produced using a copigmented blend of MD and PG (Moshfegh et al., 2024). The purpose of this study was to evaluate the color stability of sour cherry anthocyanin powders in 3 storage conditions as well as their application as natural food colorants in model beverages.

## MATERIALS AND METHODS

### Materials

Sour cherries, PG, and sugar were purchased from a local market. MD (DE = 11) and TA were supplied by Mixadd (Iran) and Molekula (England), respectively. All other chemicals were of analytical grade and supplied by chemical suppliers.

### Anthocyanin extraction

Sour cherries (*Prunus cerasus* L.) were bought from a local market in Shiraz (Iran) in June 2022. The sour cherries were pitted and then stored in a storage container at  $-80^{\circ}\text{C}$  until extraction. Anthocyanin extraction was done according to our previous research (Moshfegh et al., 2024).

### Formation of anthocyanin-copigment complex

The optimal concentration of TA as copigment was selected based on our previous study (Moshfegh et al., 2024). Anthocyanin extract was mixed with distilled water (1:10, v/v). TA was prepared at a concentration of 0.015 M in a buffer solution, consisting of 0.06 M phosphoric acid and 0.02 M sodium acetate, adjusted to a pH of 3.5. Subsequently, this TA solution was mixed with the diluted anthocyanin extract at a volume ratio of 1:1, and was incubated for one hour in a dark environment. Consequently, the final molar ratio of anthocyanin to TA was established at 1:0.25.

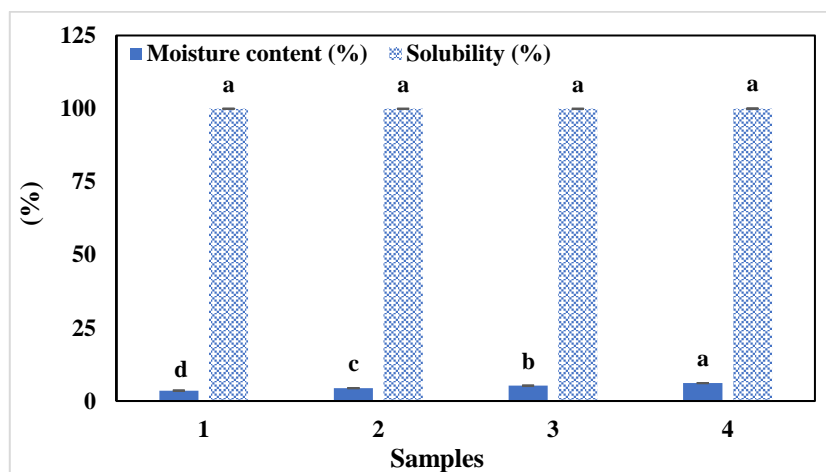
### Spray drying

MD, along with an MD-PG complex in a mass ratio of 90:10 (w/w), was utilized as carrier agents. These carrier agents added to the anthocyanin-copigment solution until the °Brix rose from 3 to 20. Controls were prepared as well (without TA). Four distinct samples were formulated: sample 1 (S1) consisted of anthocyanin combined with MD, sample 2 (S2) had copigmented anthocyanin in conjunction with MD, sample 3 (S3) comprised anthocyanin mixed with a complex of MD and PG in a ratio of 90:10 (w/w), sample 4 (S4) included copigmented anthocyanin paired with a complex of MD and PG in a 90:10 (w/w) ratio. Samples were subjected to spray drying via an industrial spray dryer (Maham Sanat, Neyshabur, Iran) under optimal conditions (Moshfegh et al., 2024). The spray-dried powders were kept at  $-18^{\circ}\text{C}$  in opaque containers until further experiments were carried out. Some characteristics of anthocyanin powders were investigated in our previous research (Moshfegh et al., 2024), and are summarized in Table 1 and Fig. 1.

**Table 1.** Characterization of sour cherry anthocyanin powders (Moshfegh et al., 2024)

Sample	Bulk density	Water activity ( $a_w$ )	Anthocyanin retention (%)	Encapsulation efficiency (%)
S1	$0.35 \pm 0.00^a$	$0.31 \pm 0.00^d$	$40.87 \pm 0.70^b$	$91.40 \pm 0.43^a$
S2	$0.36 \pm 0.02^a$	$0.33 \pm 0.00^c$	$53.23 \pm 2.74^a$	$90.37 \pm 1.10^a$
S3	$0.29 \pm 0.00^a$	$0.38 \pm 0.00^b$	$25.08 \pm 4.14^c$	$91.92 \pm 0.82^a$
S4	$0.35 \pm 0.02^a$	$0.40 \pm 0.00^a$	$38.13 \pm 0.46^b$	$90.30 \pm 0.82^a$

Data are presented as mean values  $\pm$  standard deviation. Distinct letters within the same column show a statistically significant difference among the samples ( $P < 0.05$ ).



**Fig. 1.** Moisture contents and solubility (%) of anthocyanin powders. Distinct letters above the bars signify significant differences ( $P < 0.05$ ). Samples: 1: S1; 2: S2; 3: S3; and 4: S4 (Moshfegh et al., 2024).

#### Color stability of anthocyanin powders

Spray-dried samples were subjected to storage under three distinct conditions, both in the presence and absence of light. The light exposure condition was achieved by placing the powders in a transparent Falcon tube (50 mL) and exposing them to 3000 lux of fluorescent light at ambient temperature. Conversely, the absence of light conditions involved placing the samples in a Falcon tube (50 mL) that was covered with aluminum foil, with samples maintained at an ambient temperature of 21 °C or in a refrigerator at 4 °C. All samples were stored for a duration of up to 28 days to assess their color stability (Raharjo et al., 2019).

The color of the samples was assessed using a digital colorimetric technique, according to Afshari-Jouybari and Farahnaky (2011) method. In summary, the samples were placed within a wooden enclosure that contained a light bulb as a source of illumination. A mobile camera was put vertically on top of the sample and pictures were taken immediately after spray drying and on day 28 of storage. Subsequently, the images were uploaded to a personal computer for analysis using Adobe Photoshop software. The color parameters included  $L^*$ ,  $a^*$ , and  $b^*$  were analyzed and the total color difference ( $\Delta E$ ) of samples was calculated by the following equation:

$$\Delta E = (\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2 \quad \text{Eq. (1)}$$

where  $\Delta a^*$ ,  $\Delta b^*$ , and  $\Delta L^*$  are a, b, and L differences between day 28 and day 0.

#### Color stability of beverage samples

The stability of powders was studied in a model beverage. The model beverage was prepared according to Bassijeh et al. (2020) study, with some modifications. Anthocyanin powder was incrementally incorporated into the model

beverage until the desired bright red color was achieved, resulting in a final concentration of 3.6 g of powder per 100 g of drink. Spray-dried samples (3.6 g/100 g) as colorant agent, citric acid (1 g/100 g), and sugar (10 g/100 g) were added to the distilled water with a final weight of 100 g. Control samples were prepared with extracted anthocyanin, with or without TA. The anthocyanin extract had a Brix value of 28. In the solution prepared prior to drying, 5 mL of anthocyanin extract was incorporated into 100 mL, corresponding to 1.4 g of anthocyanin. The Brix value of this solution (with wall material) was 20, indicating that it contained 7% anthocyanin. Consequently, 3.6 g of the resulting powder provided 0.25 g of anthocyanin. Based on the Brix value of 28 for the anthocyanin extract, approximately 0.9 g of the extract was required per 100 g of the control beverage. In the copigmented formulation, the pre-drying solution contained 6% TA, equivalent to 0.2 g in 3.6 g of powder; therefore, 0.2 g of TA was incorporated per 100 g of the control model beverage. Beverage samples were poured into amber glasses and pasteurized in a water bath for 1 minute. Therefore, six beverage samples were prepared: (1) S1, (2) S2, (3) extracted anthocyanin, (4) extracted anthocyanin + TA, (5) S3, and (6) S4. The beverage samples were stored in amber glass vials and were kept for 49 days at 21 °C (in the absence of light). The absorbance of the beverage samples was measured at a wavelength of 514 ( $\lambda_{max}$  anthocyanin) by a UV-Vis spectrophotometer, as color stability, on days 0, 7, 14, 20, 28, 35, 42, and 49 of storage.

#### Statistical analysis

Data was presented as mean  $\pm$  standard deviation from duplicate experiments. Statistical significance was

determined by one-way ANOVA ( $P < 0.05$ ), followed by Duncan's multiple range test using SPSS version 26.0.

## RESULTS AND DISCUSSION

### *Color stability of anthocyanin powders*

The results illustrate the  $\Delta E$  of spray-dried powders stored at 4 °C without light and in the presence or absence of light at room temperature (Table 2). The color difference is notable when  $\Delta E$  values are in the range of 1.5 to 5 and visually evident for values above 5 (Obón et al., 2009). According to the results, the  $\Delta E$  of all powders in different storage conditions was between 1.5 and 5, and the  $\Delta E$  of each sample was not significantly different in various storage conditions ( $P \geq 0.05$ ). Thus, it can be concluded that the color of powders can be preserved equally in different storage conditions. Furthermore, the findings suggested that the  $\Delta E$  values for S1, S2, and S4 under various storage conditions are significantly lower than the  $\Delta E$  value for S3 ( $P < 0.05$ ). As a result, S3 showed higher changes of color after 28 days. Consequently, the utilization of an MD and PG combination as a carrier agent, in the absence of TA, demonstrates reduced efficiency regarding color stability. This result was consistent with the anthocyanin stability observed in our previous study, where a more pronounced decline in the anthocyanin content of S3 was recorded after 28 days of storage under various conditions (Moshfegh et al., 2024). This may be attributed to the weaker molecular interactions of PG with anthocyanins, differences in encapsulation mechanisms, and its potentially less effective protective barrier during spray drying.

This point was supported by Zorić et al. (2017) who reported that only minor color changes were observed ( $\Delta E = 1.08-2.40$ ) in the sour cherry anthocyanin powder with MD as the carrier agent throughout the storage time of 1 year at 4 and 20 °C. Deng et al. (2023) examined the color difference ( $\Delta E$ ) of purple corn anthocyanins encapsulated with MD and their combination with whey protein isolate or Arabic gum. They reported no significant differences in  $\Delta E$  among the samples after 30 days of storage under various conditions. Nonetheless, an increasing trend in  $\Delta E$  was observed over time, which can be attributed to the anthocyanin degradation.

### *Color stability of beverage samples*

The color stability of the anthocyanin powders (encapsulated with the carrier agents of MD, MD + PG, and with/without TA) and extracted anthocyanin with or without TA, as controls (without carrier agents), added to the model beverages was studied at 21 °C for 49 days. Fig. 2 indicates that the absorbance of all beverages at 514 nm relatively remained stable for 49 days. The difference between day 49 and 0 shows the amount of color change, and no significant difference was observed between samples in terms of color change ( $P \geq 0.05$ ) (Fig. 3). As a result, the color stability of anthocyanin powders and that of the control group in model beverages appeared similar. This indicated that the beverages containing encapsulated and copigmented anthocyanin performed similarly to the beverages containing fresh anthocyanin extract in terms of color retention. Pasteurization (1-minute heat treatment) significantly influenced anthocyanin-copigment complexes

and initial color parameters. Previous studies have shown that thermal processing adversely affects phytochemical compounds and may disrupt copigmentation reactions (Chitgar et al., 2018). The thermal conditions applied during pasteurization can disrupt non-covalent interactions, such as hydrogen bonding and  $\pi-\pi$  stacking, that stabilize anthocyanin-copigment complexes (Wang et al., 2024). Studies have shown that thermal treatment induces conformational changes in both anthocyanins and copigments, thereby reducing their capacity to form stable complexes (Qin et al., 2018). Sari et al. (2012) reported that, at both 80 and 98 °C, free anthocyanins in the model beverage exhibited greater stability than their copigmented counterparts. Also, under acidic pH conditions, anthocyanins predominantly occur in a stable flavylium cation form, ensuring consistent color expression, irrespective of copigment presence (Babaloo & Jamei, 2018). In beverage systems with standardized acidic pH, intrinsic stability provided by the low pH environment may downregulate the proportionate role of copigmentation. Furthermore, the beverage matrix composition, typically including sugar, citric acid, and water, can substantially influence the effectiveness of copigmentation. Previous research has demonstrated that sugar content affects anthocyanin stability, with higher concentrations potentially breaking copigment-anthocyanin interactions (Molaeafard et al., 2024). In complex beverage matrices, the presence of multiple components may also lead to competitive binding scenarios, where various molecules compete for interaction with anthocyanins. Such competition can attenuate the protective effect of added copigments, resulting in more uniform stability profiles across different formulations (Zang et al., 2022).

The results indicate that in pasteurized, acidic beverage systems, copigmentation may not provide substantial long-term benefits for color stability. Instead, greater emphasis should be placed on optimizing processing parameters and matrix composition to enhance overall anthocyanin protection. Since fresh anthocyanin extracts are challenging to store and transport, drying techniques, commonly employing carriers such as MD, are preferable for commercialization and incorporation into food products.

The effectiveness of copigmentation and encapsulation of anthocyanins in beverages remains inconsistent across studies. For example, Burin et al. (2011) studied the stability of anthocyanin powders in isotonic soft drinks for 40 days and reported that the powders encapsulated with MD showed a lower percentage of color retention than samples encapsulated with a mixture of MD and Arabic gum, concluded that this combination was more efficient in terms of anthocyanin stability. Saberian and Pasban Noghabi (2023) investigated the effect of thermal treatment (90 °C for 100.8 min) on anthocyanin retention in beverages formulated with encapsulated saffron petal anthocyanins. They reported that microcapsules prepared with whey protein concentrate and MD exhibited the greatest retention of total anthocyanins, while no significant differences were observed among the other carriers tested (MD, Arabic gum, Arabic gum + PG, whey protein concentrate, MD + PG, Arabic gum + MD, Arabic gum + whey protein concentrate). Similarly, Salati et al. (2025) found that the absorbance of model beverages containing encapsulated anthocyanins with MD or MD + Arabic gum, with or

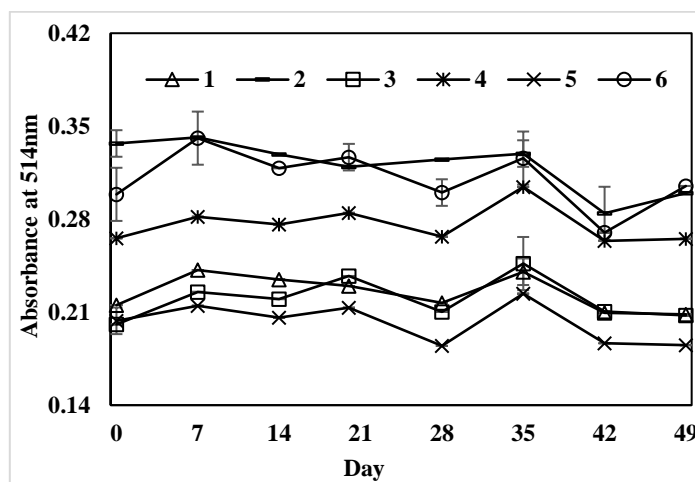
without TA, remained nearly unchanged over 49 days of storage. Xiong et al. (2025) reported that rose juice supplemented with gallic acid and  $\beta$ -cyclodextrin exhibited higher color stability compared to the control, indicating their role as effective color stabilizers during storage. In contrast, Antonio-Gómez et al. (2023) noted no significant differences in color stability between chagalapoli fruit anthocyanins encapsulated with MD and their free forms

after 42 days of storage at 25 °C. Saberian and Pasban (2022) further reported that phenolic copigments (gallic acid, ferulic acid, quercetin, and rutin) had no significant effect on the anthocyanin stability in modeled beverages. Consistently, Pangestu et al. (2020) demonstrated that both chroma and anthocyanin content declined significantly over time in modeled beverages, even in the presence of copigments such as chlorogenic and ferulic acids.

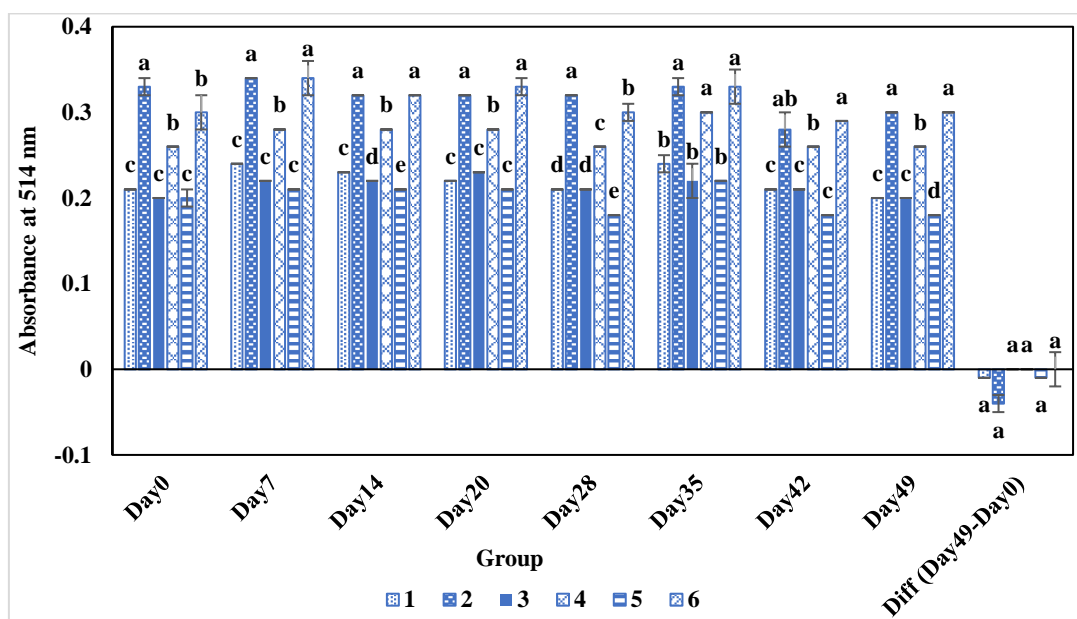
**Table 2.** Total color difference ( $\Delta E$ ) of spray-dried powders after 28 days in 3 storage conditions

Sample	$\Delta E$		
	Room temperature under light	Room temperature in darkness	Refrigerated in darkness
S1	3.24 ± 1.32 <sup>abA</sup>	1.86 ± 1.10 <sup>bA</sup>	2.45 ± 1.25 <sup>bA</sup>
S2	2.20 ± 1.06 <sup>bA</sup>	2.94 ± 1.00 <sup>abA</sup>	2.12 ± 0.77 <sup>bA</sup>
S3	4.45 ± 1.00 <sup>aA</sup>	4.24 ± 2.09 <sup>aA</sup>	4.00 ± 0.57 <sup>aA</sup>
S4	2.59 ± 1.38 <sup>bA</sup>	2.35 ± 0.60 <sup>bA</sup>	3.38 ± 1.51 <sup>abA</sup>

Data are presented as mean ± standard deviation. In the same column, distinct lowercase letters show a statistically significant difference among the samples ( $P < 0.05$ ). Conversely, different uppercase letters within the same row signify a statistically significant difference in the storage conditions ( $P < 0.05$ ).



**Fig. 2.** Color stability of model beverages in 49 days. Samples: (1) S1, (2) S2, (3) extracted anthocyanin, (4) extracted anthocyanin + TA, (5) S3, and (6) S4.



**Fig. 3.** Color stability of model beverages in 49 days. Distinct letters in each group signify a significant difference ( $P < 0.05$ ). Samples: (1) S1, (2) S2, (3) extracted anthocyanin, (4) extracted anthocyanin + TA, (5) S3, and (6) S4.

## CONCLUSION

The stability of anthocyanin powders demonstrated that the coloration of the samples was consistent across various storage conditions, including ambient temperature in the absence of light, ambient temperature in the presence of light, and refrigeration in the absence of light. Also, color stability indicated that anthocyanin powders incorporating MD, both with and without the addition of TA, and anthocyanin powder formulated with a mixture of MD and PG alongside TA caused less color change in different storage conditions, compared to the anthocyanin powder that included a mixture of MD and PG without TA. All six beverage samples exhibited comparable color retention at 514 nm over the 49-day storage period. This outcome can be attributed to the disruption of copigmentation complexes during pasteurization, the standard acidic pH (adjusted with citric acid) that tends to equalize anthocyanin stability across formulations, and the interactions of various matrix components that interfere with copigmentation effectiveness. Collectively, these factors minimized differences between copigmented and non-copigmented samples. Although copigmented sour cherry anthocyanin powders inherently demonstrated superior color and anthocyanin stability, all treatments performed similarly in the beverage matrix. Also, for the commercialization of natural colors such as anthocyanins, it would be more suitable to apply drying and confer use as powders for storage and transportation than using fresh anthocyanins. Thus, MD could be an appropriate carrier agent for this purpose.

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## CRedit AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Niloofar Moshfegh; Formal analysis: Niloofar Moshfegh; Investigation: Niloofar Moshfegh; Methodology: Niloofar Moshfegh; Writing – review & editing: Niloofar Moshfegh; Visualization: Niloofar Moshfegh; Conceptualization: Azam Abbasi; supervision: Azam Abbasi; Methodology: Azam Abbasi; Review & editing: Azam Abbasi.

## DECLARATION OF COMPETING INTEREST

The authors report no potential conflicts of interest.

## ETHICAL STATEMENT

The study protocol received approval from the ethics committee of Shiraz University of Medical Sciences and has been registered with the Iranian Registry of Clinical Trials (IRCT) under the identification number IR.SUMS.SCHEANUT.REC.1402.029.

## DATA AVAILABILITY

The dataset utilized in this research is delineated within the article.

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