

Improving Thermal and Light Stability of Black Grape Anthocyanins Using Cobalt Complexation

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ABSTRACT: Anthocyanins (ANCs) are water-soluble pigments with antioxidant properties, offering potential as alternatives to synthetic food colorants. However, their stability is compromised by factors such as pH, temperature, and light exposure. Previous research demonstrated improved pH stability in black grape ANCs through cobalt ion (Co^{+2}) complexation. This study investigates the effects of Co^{+2} complexation on the thermal and light stability of black grape ANCs extracted from pomace. These ANCs were purified, complexed with Co^{+2} (ANC- Co^{+2}), and diluted in a pH 4.5 buffer solution. Purified ANCs were also diluted in a pH 3.5 buffer solution. Both ANCs and ANC- Co^{+2} were heated to 40°C, 60°C, and 80°C for 7 h to assess thermal stability. To evaluate light stability, ANCs and ANC- Co^{+2} were stored separately under ultraviolet (UV) light and daylight for one week. Thermal stability results revealed that complexation could significantly ($P \leq 0.05$) extend ANC half-life by more than threefold and reduce temperature sensitivity by approximately 50%. Thermal degradation of ANCs was endothermic, nonspontaneous, and more structured in the transition state, as indicated by thermodynamic parameters (activation enthalpy (ΔH) > 0, free energy of inactivation (ΔG) > 0, and activation entropy (ΔS) < 0). Light stability tests revealed that complexation significantly ($P \leq 0.05$) extended ANC half-life by over tenfold and twentyfold under UV and daylight exposure, respectively. Therefore, Co^{+2} complexation represents an effective technique to enhance the thermal and light stability of grape ANCs, making them more suitable for use as food colorants.

Keywords: anthocyanins, cobalt, grapes, *Vitis*

INTRODUCTION

Anthocyanins (ANCs) are water-soluble pigments found in various colorful plants, including in their fruit, vegetables, and flowers. Their colors arise from conjugated double bonds in their structure (Grotewold, 2006). ANCs can appear red, pink, purple, or blue, depending on the dominant anthocyanidins, the pH of their environment, and the presence of copigments, metals, or other molecules (Amr et al., 2022). As a major class of flavonoids, ANCs possess antioxidant and anticarcinogenic properties (Khoo et al., 2017). However, their stability is affected by factors such as pH levels, light exposure, temperature, oxygen concentrations, and enzyme activity, which can accelerate their degradation (Amr and Al-Tamimi, 2007).

Heat treatment is essential for extending food shelf life and enhancing safety, but it also impacts many food components, including heat-sensitive components, such as ANCs. The degree and duration of heating strongly influence ANC content in the final product. High temperatures generally degrade ANCs, generating browning by-products (Ursu et al., 2020). Light is another factor that compromises ANC. Although light is necessary for ANC synthesis in plants, it also accelerates their breakdown (Cavalcanti et al., 2011). Thermal and light-induced degradation of ANCs follow different pathways, although the end products are similar (Furtado et al., 1993).

The thermal degradation of ANCs involves a ring-opening reaction or the formation of chalcone, a pale-yellow to colorless aromatic ketone (Patras et al., 2010). This reaction is consistently endothermic, with higher tem-

Received 11 July 2024; Revised 28 August 2024; Accepted 28 August 2024; Published online 31 December 2024

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peratures favoring the chalcone form over other forms, i.e., the quinoidal base, flavylum cation, and carbinol pseudobase. Chalcone undergoes deglycosylation, producing anthocyanidin chalcone and sugars, with the former breaking down into derivatives of benzoic acid and aldehyde (Oancea, 2021). Light-induced ANC degradation occurs in several steps. First, ANCs are degraded into intermediates with hydroxyl groups at carbon number 4 of the B ring. These intermediates are hydrolyzed and opened at the C2 position of the B ring, forming chalcone, which quickly degrades into benzoic acid, aldehyde derivatives, and other by-products (Jiang et al., 2019).

Metal complexation, encapsulation, and copigmentation are techniques used to improve ANC stability. For complexation with metal cations, the anthocyanidin must have at least two free hydroxyl groups on the B ring, typically found in cyanidin, delphinidin, and petunidin. As the cation approaches the anthocyanidin molecule, it displaces hydrogen ions, converting the flavylum cation (red molecule) to a quinonoidal base (blue molecule) (Schreiber et al., 2010). The metal ion then chelates another anthocyanidin molecule, stabilizing the complex (Sigurdson, 2016). Metal cations, such as Fe^{2+} and Fe^{3+} (Tachibana et al., 2014), Al^{3+} (Sigurdson and Giusti, 2014; Sigurdson, 2016), and Zn^{2+} (Zhao et al., 2020), have been used to form complexes with ANCs, resulting in bathochromic and hyperchromic shifts in maximum wavelength absorbance.

Black grapes, *Vitis vinifera*, are rich in ANCs, which give wine and grape juice their violet-reddish color (Georgiev et al., 2014). Black grapes are a key ingredient in wine and grape juice production by numerous wineries and plants worldwide, generating considerable amounts of ANC-rich waste in the form of skin and pomace (Iannone et al., 2021). Despite the predominance of malvidin in grape ANCs, which lacks an ortho-dihydroxyl group on the B ring (Rustioni, 2015), they were effectively complexed with cobalt ions (Co^{+2}) in a prior study (Amr et al., 2022), likely due to the participation of minor anthocyanidins, such as cyanidin, delphinidin, and petunidin, during complexation, resulting in hypsochromic and hyperchromic shifts in λ_{max} . ANC and Co^{+2} complex (ANC- Co^{+2}) stability at different pH levels was investigated in four pH buffer solutions (pH 3.5, 4.5, 5.5, and 6.5) over a one week storage period, with complexation at pH 4.5 extending ANC half-life by up to eightyfold compared with noncomplexed ANCs at the same pH (Amr et al., 2022).

Notably, the thermal and light stability of grape ANC- Co^{+2} has not been reported. Therefore, this study aimed to determine the degradation kinetics and thermodynamic parameters of black grape ANCs before and after complexation with Co^{+2} at different temperatures over 7 h of heating in buffer systems. Additionally, this study

assessed the degradation kinetics of ANCs and ANC- Co^{+2} under two light settings over one week of storage in buffer systems.

MATERIALS AND METHODS

Plant material and chemicals

Fresh black grapes (*V. vinifera*) of the “Black Magic” cultivar were purchased from a local grocery in Amman, Jordan. The grapes were manually separated from their stems and seeds and frozen (-18°C) in plastic bags until extraction. Diaion HP-20, cobalt acetate tetrahydrate (reagent grade), methanol (laboratory reagent grade), citric acid monohydrate, and disodium hydrogen phosphate (Sigma-Aldrich Co.) were purchased from a local supplier in Amman.

Extraction and purification of anthocyanins (ANCs)

Frozen pomace was thawed at room temperature and dried in an oven at 50°C until a constant weight was reached. The dried pomace was ground using a coffee grinder (model Tsm6A011W, Bosch) and passed through a $600\text{-}\mu\text{m}$ mesh sieve. The obtained fine particles were mixed with acidified pure methanol (0.01% HCl), the mixture was vacuum filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator (model CH-9230, Buchi). The obtained concentrate was diluted with deionized water. To purify ANCs, the crude extract was loaded onto a column partially filled with Diaion HP-20. Deionized water was eluted through the column several times, followed by pure methanol to desorb and elute the purified ANCs. The solvent was evaporated using a rotary evaporator, and the extract was reconstituted with deionized water before centrifugation at $5,376\text{ g}$ for 10 min (model Z32-HK, Hermle). The obtained supernatant was transferred to a screw-capped flask flushed with nitrogen and stored in a freezer until further use.

Quantification of total monomeric ANCs

The pH differential method described by Giusti and Wrolstad (2001) was employed to determine the monomeric ANC content in black grape pomace (expressed as cyanidin-3-glucoside equivalent in mg/L) using the following equation:

$$\text{Total ANC content (mg/L)} = \frac{A \times \text{MW} \times \text{DF} \times 10^3}{\epsilon}$$

$$A = (A_{520\text{ nm}} - A_{700\text{ nm}})_{\text{pH } 1.0} - (A_{520\text{ nm}} - A_{700\text{ nm}})_{\text{pH } 4.5},$$

where A denotes absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF represents the

dilution factor, 10^3 is the factor used to convert g to mg, and ϵ indicates the molar extinction coefficient of cyanidin-3-glucoside (26,900 L/mol·cm).

Metal complexation of ANCs

ANCs were complexed with Co^{+2} following the method of Amr et al. (2022). Cobalt acetate tetrahydrate was added at a concentration 1,000 times the concentration of ANC (ppm) and left to stand in the dark for 50 min.

Stability tests

Heat stability: One part ANC was diluted with four parts citrate-phosphate buffer solution (0.1 M citric acid monohydrate with 0.2 M disodium hydrogen phosphate) at pH 3.5. In a parallel experiment, ANC- Co^{+2} was diluted with four parts of the same buffer solution at pH 4.5. The buffer pH levels were selected based on a previous study (Amr et al., 2022). Known aliquots of ANCs and ANC- Co^{+2} were divided into three equal portions, transferred into amber screw-capped flasks, and placed in three separate water baths at 40°C, 60°C, and 80°C, respectively, for 7 h. At the end of each 1 h interval, the flasks were removed, cooled in an ice bath for 2 min, and the absorbance was measured at the corresponding λ_{max} at hours 0, 1, 2, 3, 4, 5, 6, and 7. Pigment retention (PR) was calculated using the following equation:

$$\text{PR} = \frac{\text{Absorbance of ANCs at any time}}{\text{Absorbance of ANCs at zero time}}$$

Light stability: Complexed and noncomplexed ANCs were separately diluted with the same buffer solutions used in the heat stability tests. Known aliquots of ANCs and ANC- Co^{+2} were divided into two equal portions, transferred into screw-capped flasks, and either placed under a ultraviolet (UV) light source (a deuterium lamp in a wooden box) or left on a benchtop exposed to daylight. Both sets were maintained at room temperature for seven days. Absorbance was measured on days 0, 1, 2, 3, 4, 5, 6, and 7, and PR was calculated as in the heat stability tests.

Kinetics modeling of ANCs

The degradation kinetics of ANCs were modeled using a first-order kinetics model. The reaction rate constant (k) was determined using the following equation:

$$\ln \frac{\text{PR}_t}{\text{PR}_0} = -kt,$$

where PR_t is the PR at any given time, PR_0 is the PR at zero time, k represents the reaction rate constant, and t denotes time.

The half-life ($t_{1/2}$) was calculated as follows:

$$t_{1/2} = \frac{0.693}{k}$$

The relationship between the reaction rate constant (k) and temperature, or the effect of temperature on ANC degradation, was described by the Arrhenius equation (Labuza and Riboh, 1982):

$$k = k_A \times e^{-\frac{E_a}{RT}},$$

where k_A is the Arrhenius constant, E_a is the activation energy (J/mol), R represents the universal gas constant (8.314 J/mol·K), and T indicates the absolute temperature in Kelvin.

By transforming the equation into a natural logarithm, the following relationship was obtained:

$$\ln k = \ln A - \frac{E_a}{RT}$$

Thermodynamic analysis

Thermodynamic parameters, namely activation enthalpy (ΔH), free energy of inactivation (ΔG), and activation entropy (ΔS), were calculated using the following equations (Marangoni et al., 2020):

$$\Delta H = E_a - R \times T,$$

$$\Delta G = -R \times T \times \ln \left(\frac{k \times h}{k_B \times T} \right),$$

$$\Delta S = \frac{\Delta H - \Delta G}{T},$$

where E_a is the activation energy for thermal ANC degradation (J/mol), R is the ideal gas constant (8.314 J/mol·K), T denotes temperature (Kelvin), k represents the kinetic rate constant (s^{-1}), h is Planck's constant (6.6262×10^{-34} J·s), and k_B indicates the Boltzmann constant (1.3806×10^{-23} J/K).

Statistical analysis

All experiments were conducted in triplicate. Data were analyzed using analysis of variance in the Statistical Package for Social Sciences (SPSS version 22.0, IBM Corp.). Differences among means were evaluated using the least significant difference test and considered significant at $P \leq 0.05$. Degradation kinetic graphs, Arrhenius plots, regression line equations, and correlation coefficients (R^2) were generated using Microsoft Office Excel in Windows 11.

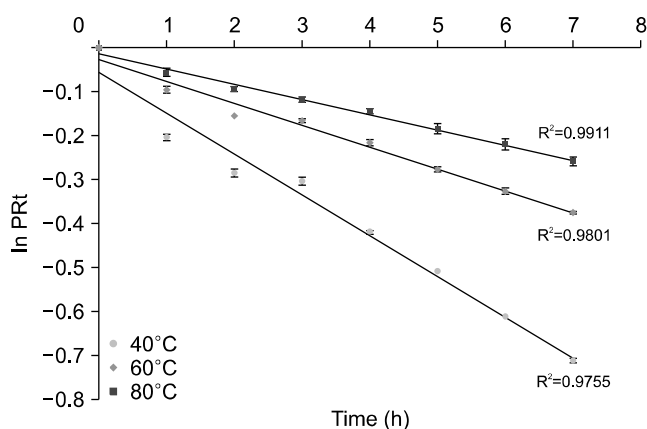


Fig. 1. ANC degradation at various temperatures. Each regression line indicates the correlation between the dependent variable (ln PRt, i.e., the natural logarithm of ANC retention after a specific heating time) and independent variable (heating time in hours) at a given temperature. R^2 is the correlation coefficient (where 1.0 indicates a perfect fit). Each point represents the mean \pm standard deviation of three measurements.

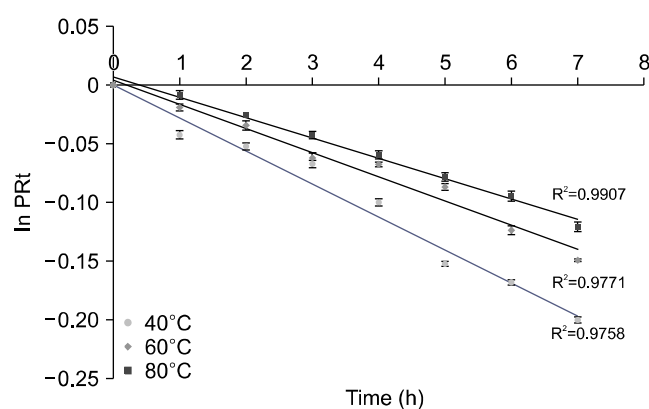


Fig. 2. ANC- Co^{+2} degradation at various temperatures. Each regression line indicates the correlation between the dependent variable (ln PRt, i.e., the natural logarithm of ANC- Co^{+2} retention after a specific heating time) and independent variable (heating time in hours) at a given temperature. R^2 is the correlation coefficient (where 1.0 indicates a perfect fit). Each point represents the mean \pm standard deviation of three measurements.

RESULTS AND DISCUSSION

Thermal degradation kinetics

Fig. 1 and 2 show that plotting the natural logarithms of PRt against time (h) yielded high correlation coefficients ($R^2 > 0.9$), indicating that the thermal degradation of ANCs (both before and after complexation) followed a first-order reaction model. This finding is consistent with data from previous studies, including ANCs from “Italia” grapes (Peron et al., 2017), blackberry juice (Wang and Xu, 2007), black rice (Hou et al., 2013), purple potato flesh (Li et al., 2013), roselle (Duangmal et al., 2008), and açai pulp (Marangoni et al., 2020).

From the slopes of the regression lines, k (the kinetic parameter from which half-life is estimated) and $t_{1/2}$ (half-life) were calculated (Table 1). Both are crucial for predicting the degradation and stability of ANCs; a lower k indicates a higher $t_{1/2}$, suggesting greater ANC stability (Hou et al., 2013). The half-life of ANCs decreased with increasing temperature, from 19.91 h at 40°C to 13.86 and 7.44 h as temperature increased to 60°C and 80°C, respectively. Similarly, for complexed ANCs, the half-life

decreased from 40.06 h at 40°C to 33.64 and 24.66 h as temperature increased to 60°C and 80°C, respectively.

At 40°C, complexation significantly ($P \leq 0.05$) increased the half-life of ANCs to at least twice its initial value, from 19.91 to 40.06 h. Complexation also significantly ($P \leq 0.05$) increased the half-life by 2.5-fold at 60°C. Notably, increasing the heating temperature to 80°C led to a threefold significant ($P \leq 0.05$) increase in half-life, from 7.44 to 24.66 h, due to complexation with Co^{+2} .

Peron et al. (2017) reported that the half-life of “Juçara” grape ANCs heated to 60°C was 114 h but declined to 18 h at 80°C. In contrast, for the “Italia” grape cultivar, ANC half-life was only 25 h at 60°C and decreased to 4 h at 80°C, despite heating at the same temperatures and pH 2.5. Ekici et al. (2014) found that 11.82% of ANCs from “Okuzgozu” black grapes degraded after 2 h, with temperatures of 80°C and 90°C resulting in 12.36% and 50.00% losses of the initial ANC concentration after 120 min, respectively, even when kept in an acidic buffer solution (pH 3) during heat treatment. This suggests that ANCs’ thermal degradation depends not only on the ANC source or the pH of the surrounding media but also on the plant cultivar.

Table 1. Kinetic parameters of ANC degradation before and after Co^{+2} complexation during heating at different temperatures (unit: per hour)

	Temperature (°C)	$k \times 10^2$	$t_{1/2}$
ANCs	40	3.48 ± 0.01^a	19.91 ± 0.17^b
ANC- Co^{+2}		1.73 ± 0.01^b	40.06 ± 0.11^a
ANCs	60	5.00 ± 0.01^a	13.86 ± 0.13^b
ANC- Co^{+2}		2.06 ± 0.01^b	33.64 ± 0.10^a
ANCs	80	9.31 ± 0.01^a	7.44 ± 0.12^b
ANC- Co^{+2}		2.81 ± 0.01^b	24.66 ± 0.16^a

Values represent mean \pm standard deviations.

Different letters (a and b) represent significant differences at $P \leq 0.05$ based on least significant difference test results. ANCs, anthocyanins; Co^{+2} , cobalt ion; k , reaction rate constant; $t_{1/2}$, half-life.

Data are limited regarding the thermal stability of purified ANCs, although a few studies have found that they are less stable than their unpurified counterparts (Oancea, 2021). For instance, Zozio et al. (2011) found that the half-life of purified ANCs extracted from black carrot was 44.7 h when heated to 50°C. Purified ANCs isolated from purple potato and thermally treated at 100°C and 150°C in a buffer system (pH 6) had half-lives of 26.456 and 2.428 min, respectively (Nayak et al., 2011).

At high temperatures, chalcone, a pale-yellow to colorless compound, is preferentially formed owing to its endothermic nature. The tautomeric equilibrium between carbinol pseudobase and chalcone shifts toward chalcone formation as temperature rises. As a result, the color imparted by monomeric ANCs fades and is lost (Alappat and Alappat, 2020). Cobalt addition markedly affects the thermal stability of ANCs as it may disrupt chalcone formation. However, the prolonged heating and the substantial temperature increase from 40°C to 80°C could weaken the interaction between ANCs and Co^{+2} , promoting chalcone formation, which eventually degrades into colorless aldehyde and phenolic acid. Another critical factor is the pH of the medium. ANC- Co^{+2} was maintained in a buffer solution at pH 4.5 during heating, where the predominant ANC form at this pH is the colorless carbinol pseudobase (Wrolstad, 2004). Consequently, excessive and prolonged heating may have partially dissociated ANC- Co^{+2} , leaving some ANCs in their free form and turning colorless at pH 4.5.

Studies on the thermal degradation of complexed ANCs are limited. However, Tachibana et al. (2014) showed that the cyanidin- Fe^{3+} complex was more thermally stable than cyanidin alone when heated to 60°C for 80 min under low acidic conditions, aligning with the current findings. Metals bind ANCs in their flavylum cation form, preventing conversion to colorless carbinol pseudobase, known for its poor heat stability (Mollaamin and Monajjemi, 2020).

Fig. 3 shows that the activation energy (kJ/mol) for ANCs before and after complexation was determined from the slope of the line obtained by plotting the reciprocal of temperature ($1/T$) against the natural logarithm of k (Table 2). The activation energy of ANCs before complexation (22.47 kJ/mol) was significantly ($P \leq 0.05$) higher (twofold) compared with after complexation (11.20 kJ/mol).

Activation energy values for ANC degradation in the present study were lower than those for ANCs isolated from “Italia” and “Juçara” grapes (93.62 and 99.77 kJ/mol, respectively; Peron et al., 2017), gooseberry juice (33.08 kJ/mol; Kopjar et al., 2009), *Morus nigra* L. (46.32 kJ/mol; Kara and Erçelebi, 2013), açai pulp (24.16 kJ/mol; Costa et al., 2018), acerola (68.04 kJ/mol; Silva et al., 2017), and blueberry juice (80.4 kJ/mol; Kechinski et al., 2010).

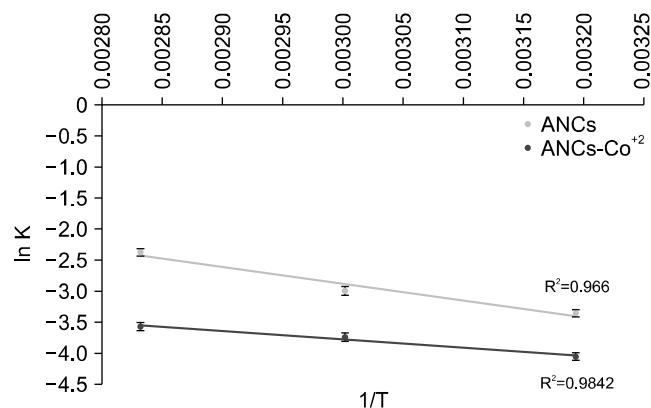


Fig. 3. Arrhenius plots for ANCs and ANC- Co^{+2} degradation. Each regression line indicates the correlation between the dependent variable ($\ln k$) and independent variable (the reciprocal of temperature). $\ln k$ is the natural logarithm of the reaction rate constant (k) as a function of the reciprocal temperature ($1/T$), used to determine the activation energy (E_a). R^2 is the correlation coefficient (where 1.0 indicates a perfect fit). Each point represents the mean \pm standard deviation of three measurements.

Table 2. Activation energy of ANCs before and after complexation with Co^{+2} (unit: kJ/mol)

	E_a
ANCs	22.47 \pm 0.21 ^a
ANC- Co^{+2}	11.20 \pm 0.16 ^b

Values represent mean \pm standard deviations. Different letters (a and b) represent significant differences at $P \leq 0.05$ based on least significant difference test results. ANCs, anthocyanins; Co^{+2} , cobalt ion; E_a , activation energy.

Higher activation energy values indicate slower thermal degradation at lower temperatures and faster degradation at higher temperatures (Kim et al., 2022). Thus, lower activation energy reflects greater stability of ANCs against temperature fluctuations (Marangoni et al., 2020). This suggests that the thermal degradation of grape ANCs (whether alone or complexed) is more stable and less dependent on temperature changes compared with other previously reported ANCs, with ANC- Co^{+2} being twofold ($P \leq 0.05$) more stable compared with ANCs alone.

Thermodynamic analysis

Table 3 presents the thermodynamic parameters (ΔH , ΔG , and ΔS) for ANCs and ANC- Co^{+2} at 40°C–80°C. All ΔH values (kJ/mol) were positive, indicating an endothermic reaction where heat energy is required for the reaction to proceed from the reactants to the transition state (Marangoni et al., 2020). Additionally, ΔH decreased with increasing temperature, indicating that the energy barrier for bond breakage between reactants decreases at higher temperatures (Oliveira and Antelo, 2020). This behavior aligns with findings from Mercali et al. (2015), Peron et al. (2017), and Marangoni et al. (2020).

Table 3. Thermodynamic parameters of ANCs degradation at different temperatures (unit: kJ/mol)

	Temperature (°C)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (kJ/mol·K)
ANCs	40	19.87±0.21 ^a	106.88±1.23 ^a	-0.28±0.001 ^a
ANCs-Co ⁺²		8.60±0.01 ^b	108.70±1.15 ^a	-0.32±0.001 ^b
ANCs	60	19.70±0.16 ^a	112.88±1.05 ^a	-0.28±0.001 ^a
ANC-Co ⁺²		8.43±0.01 ^b	115.33±1.55 ^a	-0.32±0.001 ^b
ANCs	80	19.54±0.14 ^a	118.00±1.80 ^a	-0.28±0.001 ^a
ANC-Co ⁺²		8.26±0.01 ^b	121.52±2.08 ^a	-0.32±0.001 ^b

Values represent mean±standard deviations.

Different letters (a and b) represent significant differences at $P \leq 0.05$ based on least significant difference test results. ANCs, anthocyanins; Co⁺², cobalt ion; ΔH , activation enthalpy; ΔG , free energy of inactivation; ΔS , activation entropy.

ΔG reflects the spontaneity of the reaction and represents the energy difference between the reactants and the transition state (Oliveira and Antelo, 2020). ΔG values (Table 3) were positive at all temperatures for both ANCs and ANC-Co⁺², indicating that the thermal degradation of ANCs is nonspontaneous. Similar positive ΔG values (108.0–133.5 kJ/mol) were reported by Peron et al. (2017) for grape ANCs from different cultivars (“Italia” and “Juçara”). Oliveira and Antelo (2020) also observed positive ΔG values in their thermodynamics study of “Rio Grande” cherry ANCs.

Negative ΔS values suggest that molecules are less organized at the beginning of the reaction compared with the transition state, reflecting lower reactivity (Mercali et al., 2015). Negative ΔS values were also observed by Peron et al. (2017) in grapes, Oliveira and Antelo (2020) in cherries, and Marangoni et al. (2020) in açai pulp.

Light degradation kinetics

Fig. 4 and 5 illustrate that plotting the natural logarithm of PRT against time (h) yielded high correlation coefficients ($R^2 > 0.9$), suggesting that ANC degradation (both

before and after complexation) under light exposure followed a first-order reaction model. This observation is consistent with the findings of Contreras-Lopez et al. (2014) for *Rubus fruticosus* L. ANCs and Karaoglan et al. (2019) for turnip juice ANCs. Reaction rate constants (k) and half-lives ($t_{1/2}$) of ANCs were calculated from the slopes of each regression line (Table 4).

The half-lives of ANCs (whether alone or complexed) stored under daylight conditions were at least twice as long as those stored under UV light. Compared with storage in darkness, as reported by Amr et al. (2022), the half-life under daylight conditions was 2.0-fold lower for noncomplexed ANCs and 1.5-fold lower for complexed ANCs, while under UV light, the half-life was 3.5-fold lower for noncomplexed ANCs and 5.0-fold lower for complexed ANCs.

Complexation significantly ($P \leq 0.05$) increased the half-life of ANCs under UV light exposure by at least tenfold, from 5.50 to 60.79 days. Similarly, Co⁺² complexation significantly ($P \leq 0.05$) increased the half-life of ANCs under daylight exposure by more than twentyfold (from 9.75 to 192.50 days).

Although light enhances ANC production in plants, it

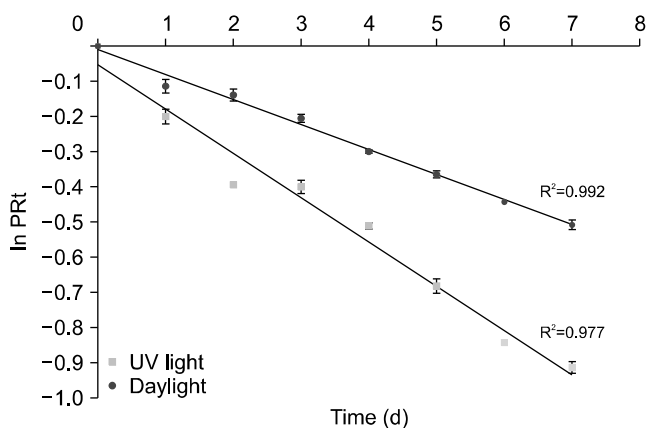


Fig. 4. ANC degradation during storage under ultraviolet and daylight conditions. Each regression line indicates the correlation between the dependent variable (ln PRT, i.e., the natural logarithm of ANC retention after a specific storage time) and independent variable (storage time in days) for each light condition. R^2 is the correlation coefficient (where 1.0 indicates a perfect fit). Each point is the mean±standard deviation of three measurements.

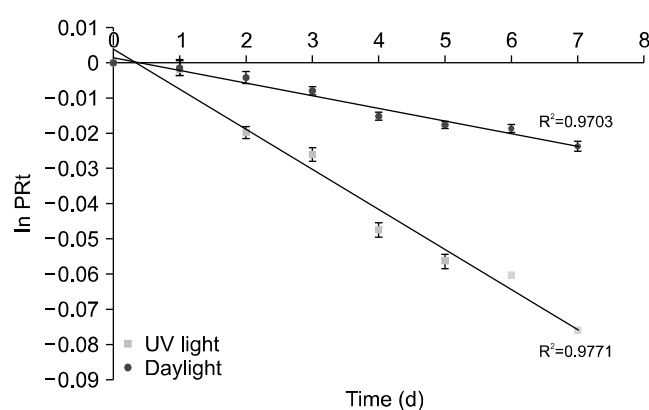


Fig. 5. ANC-Co⁺² degradation during storage under ultraviolet and daylight conditions. Each regression line indicates the correlation between the dependent variable (ln PRT, i.e., the natural logarithm of ANC-Co⁺² retention after a specific storage time) and independent variable (storage time in days) for each light condition. R^2 is the correlation coefficient (where 1.0 indicates a perfect fit). Each point represents the mean±standard deviation of three measurements.

Table 4. Kinetics parameters of ANCs during storage under different light conditions (unit: per day)

Sample	Light condition	$k \times 10^2$	$t_{1/2}$
ANCs	Daylight	7.11 ± 0.02^a	9.75 ± 0.02^b
ANC-Co ⁺²		0.36 ± 0.01^b	192.50 ± 2.30^a
ANCs	UV light	12.60 ± 0.21^a	5.50 ± 0.01^b
ANC-Co ⁺²		1.14 ± 0.01^b	60.79 ± 0.23^a

Values represent mean \pm standard deviation.

Different letters (a and b) represent significant differences at $P \leq 0.05$ based on least significant difference test results. ANCs, anthocyanins; Co⁺², cobalt ion; UV, ultraviolet; k, reaction rate constant; $t_{1/2}$, half-life.

also accelerates their degradation (Yin et al., 2021). ANCs are photodegradable when exposed to light, likely due to the formation of chalcone, a colorless ANC form that further degrades into benzoic acid and aldehyde derivatives (Jiang et al., 2019). UV light proved more damaging than daylight in the present study, as UV light was applied continuously while daylight exposure alternated with night periods. The effect of complexation on ANCs' light stability was notable; metals appeared to stabilize the chemical structure of ANCs, limiting chalcone formation under light exposure. Nevertheless, light accelerated ANC degradation relative to samples stored in darkness at the same pH.

Amr and Al-Tamimi (2007) found that light exposure during storage increased ANC degradation in *Ranunculus asiaticus* L., and Yin et al. (2021) demonstrated that UV radiation had twice the effect on "Guizhou" blueberry ANC degradation compared with daylight, aligning with the current findings. Similarly, Palamidis and Markakis (1975) reported more pronounced degradation of ANCs in grape juice stored under conditions with light compared to darkness. Aramwit et al. (2010) found that mulberry fruit ANCs degraded when exposed to light, with degradation intensifying over time. Bononi and Tateo (2007) observed that cranberry ANC extract was more sensitive to light relative to dark storage, consistent with our findings.

The present study suggests that complexed ANCs could be used for coloring various food products owing to their stability under minimal heat treatment and light exposure. However, ensuring the safety of food additives containing colorants is crucial for preventing potential health risks. A previous study assessed the impact of cobalt salt concentration in ANC-Co⁺² on cell viability using human fibroblasts; a concentration range of 0.0085 – 85 ppm cobalt acetate tetrahydrate was considered safe, with cell viability remaining above 96% (Amr et al., 2022).

The current study has certain limitations, including the use of temperatures lower than those typically applied in food heat treatments, which can reach up to 121°C. Additionally, the study assessed thermal and light degradation of ANCs and ANC-Co⁺² in a buffer system at a single pH level; degradation in more complex food ma-

trices, such as ANC-enriched foods may differ. Further investigation is required to determine the thermal stability of ANCs and their complexed forms at temperatures exceeding 100°C. It is also important to evaluate the thermal and light-induced degradation of ANC-Co⁺² in various pH buffer systems, with gradual pH increases up to 6 or 7. Moreover, assessing the storage stability of ANC-Co⁺² in real food systems, which may contain additional components affecting ANC stability, is crucial.

Food products, such as fruit juices, flavored yogurt, syrups, sorbet, and cream cheese, may benefit from the addition of ANCs in their Co⁺²-complexed form as a colorant. These products typically have a pH of around 4.5 and require minimal heat treatment, such as pasteurization, with some displayed in transparent packaging to showcase their color.

ACKNOWLEDGEMENTS

The authors would like to thank the University of Jordan for their financial support.

FUNDING

This research was funded by a grant from the deanship of Scientific Research at the University of Jordan (grant number 19/2021/43/).

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: SJ, AA. Analysis and interpretation: AA, SJ, RA, HT, IH. Data collection: SJ, HT, RA, SA. Writing the article: SJ. Critical revision of the article: SJ. Final approval of the article: all authors. Statistical analysis: SJ, HT, RA, SA. Obtained funding: AA, IH, HA. Overall responsibility: all authors.

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