

# Stability of Seedless Red grape anthocyanin under the effect of Chlorogenic acid copigment, heating and UV irradiation

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**ABSTRACT:** Copigments are colourless substances which can form a coloured cluster with anthocyanins. The colour changes in fruits, vegetables and flowers may be caused by these reactions between anthocyanins and various organic compounds present in higher plants. In this study copigmentation of anthocyanin in Seedless Red grape were investigated with chlorogenic acid copigment. The dependence of the copigmentation process on the heating, UV and copigment concentration was studied. Five levels of copigment concentrations as: 0, 120, 240, 480, 960 (mg/l) were examined. The copigmentation effect increased with copigment contain. During UV irradiation, anthocyanin-copigmentation complex showed higher stability in comparison with anthocyanins. Also UV irradiation has the biggest effect on the copigmentation complex in comparison with heating.

**Keywords:** Anthocyanin, Copigmentation, UV irradiation, heating, Seedless Red.

## INTRODUCTION

Anthocyanin color molecules are a subclass of flavonoids. Anthocyanins are unstable and easily susceptible to degradation. The stability of anthocyanins is affected by pH, temperature, presence of enzymes, UV, structure and concentration of the anthocyanins. The stability of anthocyanin color can be improved by copigmentation, where the anthocyanin molecule reacts with other natural plant components directly or through weak interactions, resulting in an enhanced and stabilized color (Darias-Martin et al., 2002; Talcott et al., 2003). Copigmentation is a solution phenomenon in which pigments and other noncolored organic components form molecular associations or complexes (Boulton, 2001). These associations cause the pigments to exhibit far greater color than would be expected from their concentration. The phenomenon has long been recognized in flowers and fruits. Malvidin-3-glucoside was the major pigment had isolated from grapes (Kallithraka et al., 2005; Willstatter and Zollinger, 1915; Willstatter and Zollinger, 1916). This association between the pigments and their copigmentation cofactors involves the anthocyanin glucosides, certain phenolic acids, flavonoids, and, in particular, derivatives of the flavonol and flavone subgroups. It accounts for almost half of the observed color of young grape juice (Neri and Boulton, 1995). As well as resulting in higher absorbance values (a hyperchromic shift ( $\Delta A$ )), certain cofactors lead to a bathochromic shift ( $\Delta\lambda$ ) in the wavelength at which the maximum absorbance is observed, typically 5 to 20 nm higher, providing a blue-purple tone in an otherwise red solution (Chen and Hrazdina, 1981; Mazza and Miniati, 1993). The hyperchromic effect means an increase in colour intensity, while the bathochromic shift consists of a shift of the wavelength of maximum absorbance. Various substances, such as chlorogenic acid (Brouillard et al., 1989; Dangles et al., 1992; Wilska-Jeszka and Korzuchowska, 1996) have been identified as good copigments. In this study were examined enhance and stabilize of anthocyanin with chlorogenic acid copigment, and factors (such as heating, UV irradiation, copigment concentrations) that effects on copigmentation phenomena. Five levels of copigment concentrations as: 0, 120, 240, 480, 960 (mg/l) were examined. The stability of colorants was investigated at pH 3.5 under UV irradiation and heating, in the presence and absence of copigment.

## MATERIALS AND METHODS

Seedles Red grape variety (*Vitis vinifera*) used for this study that were cultured in Bijar city at Kurdistan province in the west of Iran. The samples were frozen and stored at  $-18^{\circ}\text{C}$ . Methanole (250 ml) containing 0.01% Hcl used for extraction of anthocyanins and the juice samples were concentrated under reduced pressure with a rotary evaporator (Buchi Rotavapor-r) keeping the temperature of the water bath below  $35^{\circ}\text{C}$ . Substance used in the experiments was Chlorogenic acid (Merk). Buffer solutions of three different pH values were prepared by mixing sodium acetate ( $0.02 \text{ mol / dm}^{-3}$ ) and phosphoric acid ( $0.06 \text{ mol / dm}^{-3}$ ). The ionic strength of the solutions was adjusted by sodium chloride ( $0.02 \text{ mol / dm}^{-3}$ ). ( For thermal stability colorant solution with and without copigment (10 ml) in glass tubes with screw caps were placed in a water bath at  $80^{\circ}\text{C}$  for 2 hours. For UV stability colorant solution with and without copigment (10 ml) in glass open cuvetts, were placed under UV light (LB 301.1 BAKMED  $\lambda_{\text{max}}$ , 253.7 nm,  $2.1 \text{ mW/cm}^2$ ) for 2 hours. Absorption spectra of the anthocyanin solutions, with and without copigment, were recorded in the visible wavelength range from 450 to 610 nm. Spectrophotometric measurements were carried out by using a Spectrophotometer (WAP biowave S2100 Diode Array). The spectra were recored in quartz cuvetts of 1 cm optical path length. All experiments were repeated in triplicate.

## RESULTS AND DISCUSSION

### General

The colour of anthocyanins can be stabilized and enhanced by the addition of Chlorogenic acid to Seedles Red grape juice.

This addition induced change in bathochromic shifts and hyperchromic effects that manifest copigmentation of anthocyanin in the buffer solutions. According Mazza and Brouillard, (1990) studies, pH, pigment, copigment concentration, type of anthocyanin, temperature, ionic strength and solvent influence the copigmentation of anthocyanin. The result of the addition of copigments at five concentration levels showed that the outcome of copigmentation is dependent on copigment concentration, which is in agreement with previous works (Davies and Mazza, 1993; Asen et al., 1972). Increasing anthocyanin concentration, increases absorbance and the bathochromic shifts. The magnitude of the increase in absorbance is depend on the copigment-anthocyanin ratio for a given anthocyanin. Increasing the copigment-anthocyanin ratio also increases the magnitude of the bathochromic shifts and hyperchromic effects.

Because the anthocyanin concentration was constant in each solution, it seems obvious that the magnitude of the bathochromic shifts and hyperchromic effects depended on the concentration of copigment.

Absorption spectra of anthocyanin and spectra of the copigment formed with copigment at the pH 3.5 buffer solution are shown in figure (1).

Concentration of the copigments were 0, 120, 240, 480, 960 (mg/ l). As shown in this figures, in all of anthocyanin-copigment complex the highest anthocyanin-copigment concentration is 960 (mg/l). Copigment is an important factor that effects on copigmentation. Figure (2) shows absorption spectra of Seedles Red grape anthocyanin with and without copigment. This figure indicait the biggest bathochromic shift was induced by Chlorogenic acid with Seedles Red grape anthocyanin. ( $\Delta A = 0.137$ ,  $\Delta \lambda = 5.5 \text{ nm}$ ).

### The influence of heating

One of the factors, which affects copigmentation, is high temperature. Increasing time and temperature of heating resulted in changes in anthocyanin and copigment contents and the copigmentation complex, which resulted in the hyperchromic effect, and a bathochromic shift in the main absorption peaks. Changes in the bathochromic shift and in the hyperchromic effect as a function of heating at  $80^{\circ}\text{C}$  for 2 h, at pH 3.5, and copigment concentration 120 (mg / l), are shown in Table 1. The highest bathochromic shift was observed for anthocyanin with Chlorogenic acid complex. The lowest bathochromic shift was observed with anthocyanin. In the previous research, Bakowska et al, (2003) were reported similar results about effects of heating on copigmentation. Also these results are in good agreement with that found by Kucharska et al, (1998) and Davies and Mazza (1993). Marcovic et al, (2000) reported the temprature was found to be a significant factor that determine the termodinamic conditions of the copigmentation process. In all reported research a temperature increase produces a decrease of the copigment bond intencity and its hyperchromic shift. According to Mazza and Brouillard, (1990), the interaction between the pigment and the copigment causes dissociation of the copigmentation complexes, giving colourless compounds, thus resulting in a loss of colour. In this research, a decrease of the copigmentation effect with increase of temperature was also observed (Table 1).

The changes in absorbance as function of pH and heating time, observed in model experiments for anthocyanin in the absence and in the presence of the copigment (Fig. 3), can be interpreted by the mechanism proposed by Brouillard et al, (1991) to describe the intermolecular copigmentation effect. The colour of complex slowly decrease during heating, and after 120 min investigated copigment stabilized anthocyanin colour at all pH values. For Seedles Red anthocyanins, Chlorogenic acid-complex were predominated over anthocyanins.

**Influence of UV irradiation**

UV irradiation is one of the several factors anthocyanin degradation. This research illustrated that presence of copigment in anthocyanin solution inhibited the degradation influence of UV irradiation on anthocyanin (table 2). As can be seen in this table, anthocyanin-Chlorogenic acid complex has the biggest hyperchromic effects and anthocyanin has the lowest hyperchromic effects. As shown in table 2, UV irradiation induced anthocyanin degradation after 60 minute irradiation. This degradation of anthocyanin and decrease of anthocyanin colour identified by decrease of hyperchromic effects after 60 minute.

Bakowska et al, (2003) and Kucharska et al, (1998) previously investigated the influence of UV irradiation on the stability of the anthocyanin-copigment complex. They found similar results that we obtained in our research. Figure 4 shows influence of UV irradiation time on anthocyanin stability. As can be seen, the absorbance at 525 nm decreases with increasing irradiation time at pH 3.5. Chlorogenic acid as the copigment, prevented the UV degradation and inhibited the degradation influence of UV, and stabilized the anthocyanin better than other copigments. The results of this investigated were good agreement with the results reported by Bakowska et al, (2003); Kucharska et al, (1998) who, studied influence of UV irradiation time on anthocyanin-copigment stability.

Table 1. Influence of 2-h heating at 80°C on bathochromic shift and the hyperchromic effect in solutions of copigment and anthocyanin at pH 3.5<sup>a</sup>

copigment	$\Delta\lambda$ (nm)		$\Delta A$	
	Before	After 2 h 80°C	Before	After 2 h 80°C
Chlorogenic acid	5.5	3	0.137	0.086
Anthocyanin	3	1	0.037	0.023

<sup>a</sup>  $\Delta\lambda$  and  $\Delta A$  are change of wavelength and absorbance of visible maximum at 525 nm upon addition of copigment

Table 2. Influence of 2-h UV irradiation on bathochromic shift and the hyperchromic effect in solutions of copigment and anthocyanin at pH 3.5<sup>a</sup>

copigment	$\Delta\lambda$ (nm)		$\Delta A$	
	Before	After 2 h UV	Before	After 2 h UV
Chlorogenic acid	5.5	3.5	0.137	0.096
Anthocyanin	3	2	0.037	0.027

<sup>a</sup>  $\Delta\lambda$  and  $\Delta A$  are change of wavelength and absorbance of visible maximum at 525 nm upon addition of copigment.

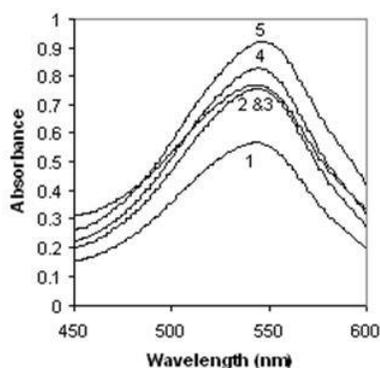


Figure 1. The absorption of anthocyanin and chlorogenic acid with different concentration: 1 (0 mg/l); 2 (120 mg/l); 3 (240 mg/l); 4 (480 mg/l);5(960 mg/l) at pH

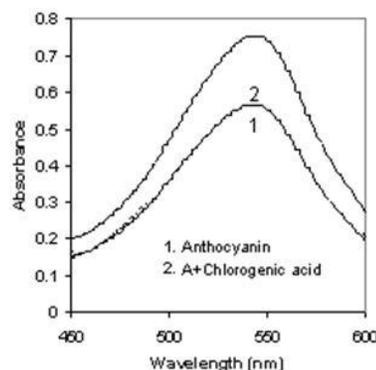


Figure 2. The absorption spectra of anthocyanin with and without copigment (concentration 120 mg/l) at pH 3.5

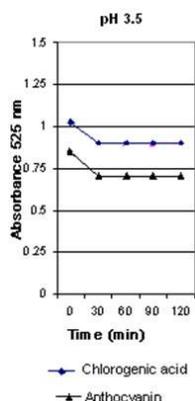


Figure 3. Influence of time heating at 80°C and pH value (3.5) on absorbance at 525 nm of anthocyanin, with and without copigments: concentration 120 mg/l

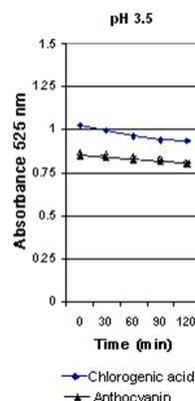


Figure 4. Influence of time of UV irradiation and pH value (3.5) on absorbance at 525 nm of anthocyanin, with and without copigments concentration 120 mg/l

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