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Effect of Heating, UV Irradiation and pH on Stability of the Anthocyanin Copigment Complex

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Abstract: The influences of temperature, UV irradiation, pH and copigment concentration on the stability of a copigmentation complex were investigated. The copigments selected for the study included: Catechin, Chlorogenic acid and Gallic acid. Five levels of copigment concentration as: 0, 120, 240, 480, 960 mg L^{-1} were examined. The copigmentation effect increased with copigment contain. UV irradiation had a stronger degradation effect on the copigmentation complex than heating at 80°C. The suitable pH for copigmentation complex was in pH 3.5. In this study, catechin predominate among copigments.

Key words: Anthocyanin, copigmentation, UV irradiation, colour stability, temperature, red cabbage

INTRODUCTION

Anthocyanins are natural pigments widely distributed in nature. The anthocyanins (glycosylated polyhydroxy derivatives of 2-phenylbenzopyrylium salts) are natural, water-soluble, non-toxic pigments responsible for some colours of fruits, vegetables, flower and other plant tissues (Mazza and Brouillard, 1990).

At pH below 2 anthocyanins exist primarily in the form of red flavylium cations. When the pH increases to 6, the flavylium cation converts into purple quinonoidal bases. These compounds are labile and, upon nucleophilic attack by water, transform to the colourless carbinol pseudobases and chalcone pseudobases.

So far, anthocyanins have not been broadly used in foods and beverages, since they are not as stable as synthetic dyes. In fact, the colour stability of anthocyanin depends on a combination of various factors, such as the structure and concentration of the anthocyanin, pH, temperature and presence of complexing agents (phenols, metals) (Mazza and Brouillard, 1990). Recent investigations (Davies and Mazza, 1993; Mazza and Brouillard, 1987, 1990; Bakowska *et al*., 2003) have suggested that the molecular copigmentation of anthocyanins with other compounds (copigments) is the main colour-stabilizing mechanism in plants. A copigment alone is usually colourless, but when added to an anthocyanin solution it greatly enhances the colour of the solution. A copigment may be one of flavonoids, alkaloids, metals and anthocyanins themselves (Mazza and Brouillard, 1990). Copigments have electronrich pi systems, which are able to associate with the comparatively electron-poor flavylium ion. This association provides protection from the nucleophilic addition of water on the flavylium ion (Mazza and Brouillard, 1987). The attack by water converts the flavylium ion into the colourless pseudobase, which consequently results in the loss of colour. The complexation of a copigment with an anthocyanin causes a hyperchromic effect (ΔA) and a bathochromic shift ($\Delta\lambda$) (Chen and Hrazdina, 1981; Mazza and Miniat, 1993).

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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), morin and rutin (Baranac *et al*., 1997, 1996), have been identified as good copigments.

Some of these copigments are expensive or synthetic substances and further studies are required to find their natural cheap equivalents.

The overall aim of this research to exam enhance and stabilize of anthocyanin with copigments and factors (such as pH, heating, UV irradiation) that effects on copigmentation phenomena. Since there have been only limited reports on the effect of UV irradiation on anthocyanin-copigment complex, our effort was to exam this factor.

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In this study three substances were examined as copigments: Catechin, Chlorogenic acid and Gallic acid. The stability of colorants was investigated at pH 2.5, 3.5 and 4.5 under UV irradiation and heating, in the presence and absence of copigment.

MATERIALS AND METHODS

The red cabbage used for this study that were cultured in Dezful city at Ahvaz province in the southwest of Iran. The samples were frozen and stored at -18°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°C (Abyari *et al*., 2006). Substances used in the experiments were Catechin (Sigma), Chlorogenic acid (Merk) and Gallic acid (Merk).

Buffer solutions of three different pH values were prepared by mixing sodium acetate (0.02 mol dm^{-3}) and phosphoric acid (0.06 mol dm^{-3}). The ionic strength of the solutions was adjusted by sodium choloride (0.02 mol dm^{-3}) (Marcovic *et al*., 2005).

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°C for 2 h.

For UV stability colorant solution with and without copigment (10 mL) in glass open cuvets, were placed under UV light (LB 301.1 BAKMED lamb, 253.7 nm, 2.1 mW cm^{-2}) for 2 h.

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 recorded in quartz cuvets 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 copigments to the red cabbage 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 three copigments at the pH 3.5 buffer solution are shown in Fig. 1-3.

Concentration of the copigments were 0, 120, 240, 480, 960 (mg L^{-1}). As shown in this figures, in all of anthocyanin-copigment complex the highest anthocyanin-copigment concentration is 960 (mg L^{-1}). Type of copigment is an important factor that effects on copigmentation. Figure 4 shows absorption spectra of red cabbage anthocyanin with and without copigments. This figure indicates the biggest bathochromic shift was induced by Catechin with red cabbage anthocyanin. ($\Delta A = 0.14$, $\Delta\lambda = 5.5$ nm). With other copigments, this effects are lower and amount to:

$\Delta A = 0.071$, $\Delta\lambda = 4.5$ nm for Chlorogenic acid;
$\Delta = 0.04$, $\Delta\lambda = 3$ nm for Gallic acid.

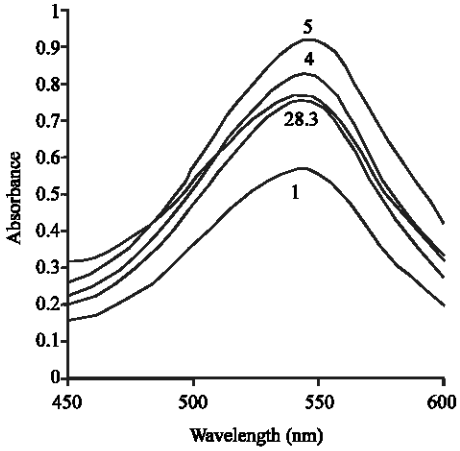

Fig. 1: The absorption of anthocyanin and Catechin with different concentration: 1 (0 mg L^{-1}); 2 (120 mg L^{-1}); 3 (240 mg L^{-1}); 4 (480 mg L^{-1}); 5 (960 mg L^{-1}) at pH 3.5

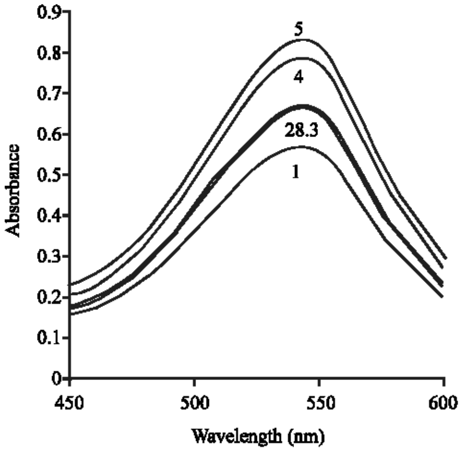

Fig. 2: The absorption of anthocyanin and Chlorogenic acid with different concentration: 1 (0 mg L^{-1}); 2 (120 mg L^{-1}); 3 (240 mg L^{-1}); 4 (480 mg L^{-1}); 5 (960 mg L^{-1}) at pH 3.5

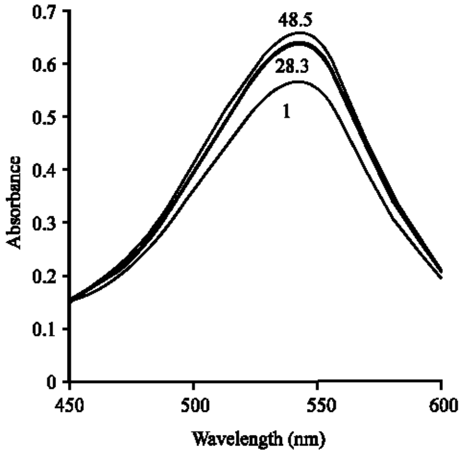

Fig. 3: The absorption of anthocyanin and gallic acid with different concentration: 1 (0 mg L^{-1}); 2 (120 mg L^{-1}); 3 (240 mg L^{-1}); 4 (480 mg L^{-1}); 5 (960 mg L^{-1}) at pH 3.5

Of the copigments, Catechin were the best copigment for red cabbage anthocyanin. Gallic acid seems to be rather poor copigments.

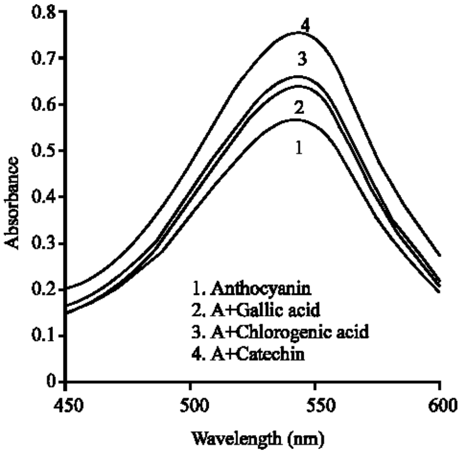

Fig. 4: The absorption spectra of anthocyanin with and without copigment (concentration 120 mg L^{-1}) at pH 3.5

Table 1: Influence of 2 h heating at 80°C on bathochromic shift and the hyperchromic effect in solutions of copigments and anthocyanin at pH 3.5[a]

	$\Delta\lambda$ (nm)		ΔA	
Copigment	Before	After 2 h 80°C	Before	After 2 h 80°C
Catechin	5.5	3	0.137	0.086
Chlorogenic acid	4.5	2	0.071	0.034
Gallic acid	3	1	0.037	0.023

[a] $\Delta\lambda$ and ΔA are change of wavelength and absorbance of visible maximum at 525 nm upon addition of copigments

The influence of heating on the copigmentation effect: 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°C for 2 h, at pH 3.5 and copigment concentration 120 (mg L^{-1}), are shown in Table 1. The highest bathochromic shift was observed for anthocyanin with Catechin complex. The lowest bathochromic shift was observed with the complex of anthocyanin and Gallic acid.

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 temperature was found to be a significant factor that determine the thermodynamic conditions of the copigmentation process. In all reported research a temperature increase produces a decrease of the copigment bond intensity 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).

Copigmentation is affected by pH. It has been demonstrated that the copigment effect occurs from pH values near 1 to neutrality (Wiliams and Hrazdina, 1979).

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 copigments (Fig. 5), can be interpreted by the mechanism proposed by Brouillard *et al.* (1991) to describe the intermolecular copigmentation effect. At pH 1-2, anthocyanin exists essentially in the flavylium form and the hyperchromic shift in the spectral maximum, observed in the presence of the copigment, is due to the interaction of the anthocyanidin flavylium cation with the copigment. The molecular extinction coefficient at the visible maximum of the complex is identical to that of the uncopigmented cation. At pH 3, there is an important colour loss for anthocyanidin alone and significant colour retention for the solution containing the anthocyanin and the copigment. Thus, the copigment effect is to reduce the production of the colourless carbinol pseudobase. At pH 4-6, the solutions containing only anthocyanidin are practically colourless, whereas the solution with both anthocyanidin and copigment are still coloured. In this pH range quinoidal bases are formed and, again, colour retention is due to decrease in the amount of the carbinol pseudobase in the solution. Figure 5 indicates that, at pH 2.5, the colour of anthocyanidin decreases shaply during the first 30 min. Then it decreases more slowly up to 120 min. At pH 3.5, sharp absorbance decrease takes place over the first 30 min whilst, at pH 4.5, absorbance decrease slowly all the time. The colour of complexes slowly decrease during heating and after 120 min all investigated copigments stabilized anthocyanin colour at all pH values. In among investigated copigments, for red cabbage anthocyanins, Catechin were predominated over other copigments.

Table 2: Influence of 2 h UV irradiation on bathochromic shift and the hyperchromic effect in solutions of copigments and anthocyanin at pH 3.5[a]

	$\Delta\lambda$ (nm)		ΔA	
Copigment	Before	After 2 h UV	Before	After 2 h UV
Catechin	5.5	3.5	0.137	0.099
Chlorogenic acid	4.5	2.5	0.071	0.051
Gallic acid	3	2	0.037	0.027

[a] $\Delta\lambda$ and ΔA are change of wavelength and absorbance of visible maximum at 525 nm upon addition of copigments

Influence of UV irradiation on the copigmentation effect: UV irradiation is one of the several factors anthocyanin degradation. This research illustrated that presence of copigments in anthocyanin solution inhibited the degradation influence of UV irradiation on anthocyanin (Table 2). As can be seen in this table, anthocyanin-Catechin complex has the biggest hyperchromic effects and anthocyanin with Gallic acid has the lowest hyperchromic effects. As shown in Table 2, UV irradiation induced anthocyanin degradation after 60 min irradiation. This degradation of anthocyanin and decrease of anthocyanin colour identificated by decrease of hyperchromic effects after 60 min.

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 6 shows influence of UV irradiation time on anthocyanin stability. As can be seen, the absorbance at 525 nm decreases with increasing irradiation time at all investigated pH values. This decrease

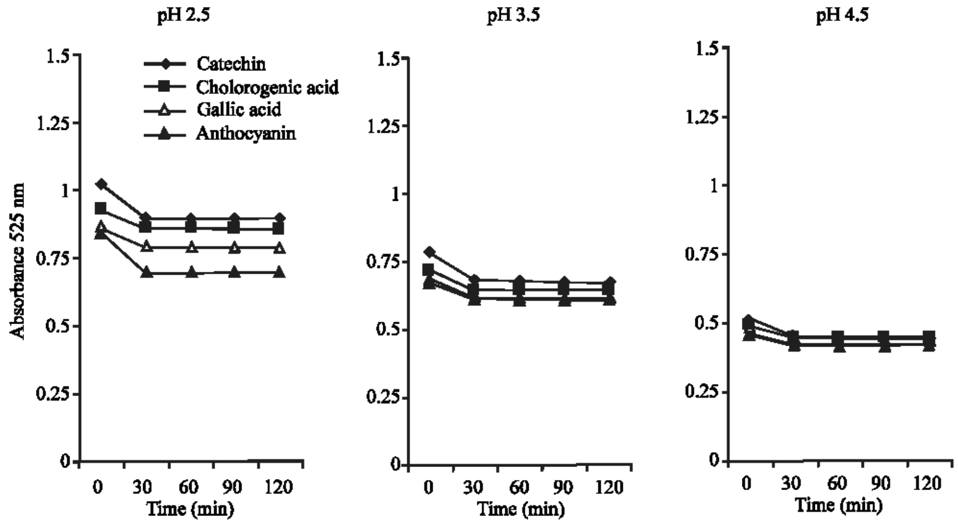

Fig. 5: Influence of time heating at 80°C and pH value (2.5-4.5) on absorbance at 525 nm of anthocyanin, with and without copigments: concentration 120 mg L^{-1}

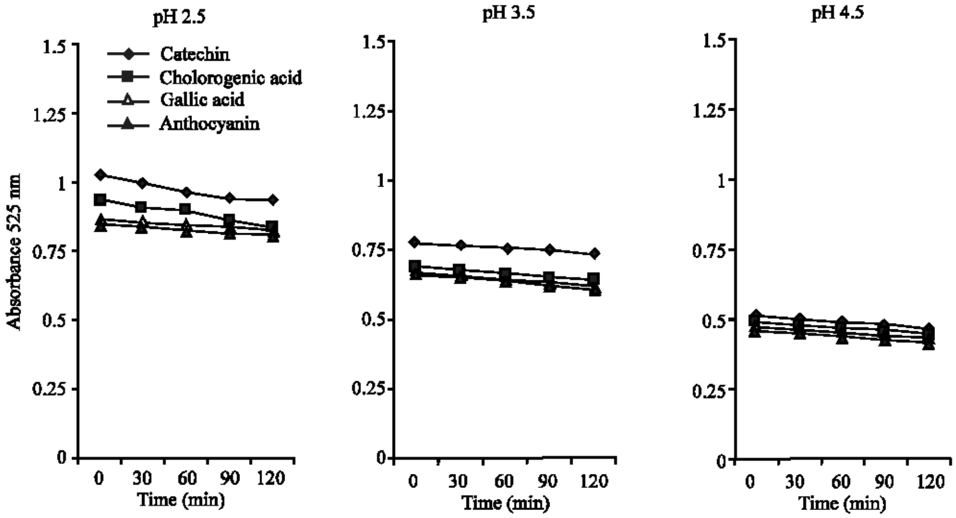

Fig. 6: Influence of time of UV irradiation and pH value (2.5-4.5) on absorbance at 525 nm of anthocyanin, with and without copigments: concentration 120 mg L^{-1}

is famous at pH 2.5. Catechin as the copigment, prevented the UV degradation and inhibited the degradation influence of UV and stabilized the anthocyanin better than other copigments. At pH 4.5, absorbance decrease is very slowly during UV irradiation and after 30 min anthocyanin was stabilized at all the time. The results of this investigated were good agreement with the results reported by Bakowska *et al.* (2003), Kucharska *et al.* (1998) and Abyari *et al.* (2006) who studied influence of UV irradiation time on anthocyanin-copigment stability.

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