

Kinetic Analysis of Anthocyanin Degradation and Polymeric Colour Formation in Grape Juice during Heating

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Abstract

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The degradation of total monomeric anthocyanins and changes in the proportion of polymeric colour (% PC) as well as antioxidant capacity of grape juice were studied during heating at 70–90°C. Anthocyanin degradation fitted to a first order reaction model, while the formation of % PC followed zero order reaction kinetics. High correlations ($r = 0.989–0.997$) were found between anthocyanin degradation and % PC formation during heating. The activation energies for the degradation of anthocyanins and formation of % PC were 64.89 and 50.42 kJ/mol, respectively. Trolox equivalents antioxidant capacity (TEAC) values of grape juice slightly changed during heating.

Keywords: red grape, monomeric anthocyanins, thermal processing, reaction kinetics; antioxidant activity

There is a great interest in the consumption of both grapes and grape juices which are important dietary sources of phenolic compounds including phenolic acids, resveratrol, and flavonoids (flavanols, flavonols, anthocyanins, and procyanidins) (MAKRIS *et al.* 2006). They are also good sources of antioxidants due to their high content of phenolics. Antioxidant activity of grapes has been reported to correlate positively with their phenolic composition, including anthocyanins, flavonols, flavan-3-ols, and hydroxybenzoates (BURIN *et al.* 2010; BAIANO & TERRACONE 2011).

Colour is one of the critical quality factors of grape juices which affects the consumer sensory acceptance. The attractive red colour of grape juice is mainly due to the presence of anthocyanins. The composition and total content of the anthocyanins in grapes are mainly affected by the cultivar, maturity, year of production, and environmental factors (MAZZA & FRANCIS 1995). The most abundant anthocyanins in grapes are malvidin, delphinidin, peonidin, petunidin, cyaniding, and pelargonidin (TIWARI *et al.* 2010). The stability of these anthocyanins is influenced by many factors including temperature, pH, oxygen, enzymes, the presence of copigments and metallic ions, ascorbic acid, sulfur dioxide, sugars and their degradation products (MAZZA & MINIATİ 1993), and also the methods of

grape juice processing (POZO-INSFRAN *et al.* 2007; TIWARI *et al.* 2010; PALA & TOKLUCU 2013).

The colour of the grape juice is seriously affected by the heat treatment which is the most widely used method of preserving and extending the shelf life of grape juices (PALA & TOKLUCU 2013). During heating, anthocyanins degrade due to their high reactivity. For example, POZO-INSFRAN *et al.* (2006) reported that 24% of anthocyanins were lost during thermal pasteurisation of Muscadine grape juice using a HTST unit (75°C, 15 s). Also, polymerisation of anthocyanins occurs during thermal processing of anthocyanin-containing juices (BROWNMILLER *et al.* 2008; HAGER *et al.* 2008; TURFAN *et al.* 2011). The degradation and polymerisation of anthocyanins affect the biological activity of anthocyanin containing foods. SADILOVA *et al.* (2007) reported that antioxidant capacities of elderberry, strawberry, and black carrot anthocyanins decreased during heating. Also, a 25.9% loss in antioxidant capacity of grape juice was reported after thermal pasteurisation (POZO-INSFRAN *et al.* 2006). On the other hand, there are also studies reporting no changes in antioxidant capacity after thermal processing of anthocyanin-containing products (BROWNMILLER *et al.* 2008; HAGER *et al.* 2008).

The measurement of total anthocyanin content along with polymeric colour is very useful to evaluate the colour quality of anthocyanin-containing juices during heating. In order to predict the colour loss and minimise the undesired changes, the knowledge of the degradation kinetics including the reaction order, reaction rate, and activation energy is very important (PATRAS *et al.* 2010). The thermal degradation kinetics of anthocyanins has been studied in anthocyanin-containing juices such as from blackberry (WANG & XU 2007), blueberry (KECHINSKI *et al.* 2010), and grape (HILLMANN *et al.* 2011). However, to the best of our knowledge, no study exists on the formation kinetics of polymeric colour in anthocyanin-containing juices during heating. Thus, the objectives of this study were to determine the degradation kinetics of anthocyanins and formation kinetics of % PC in grape juice during heating at 70–90°C. Since grape juice is a good source of antioxidants, the change in antioxidant capacity during heating was also investigated.

MATERIAL AND METHODS

Juice preparation. Grape juice was prepared from a red grape cultivar of Karasakız (*V. vinifera*) harvested from Bozcaada, Çanakkale, Turkey. After the harvest, the grapes were immediately brought to the laboratory, washed with tap water and removed from their stems. Juice was extracted with a juicer (Tefal, France) and filtered using a fourfold cheesecloth. Then it was depectinised with 2 ml of pectolytic enzyme per litre at 50°C for 1.5 h and filtered. The filtered juice was clarified with 10 ml of 5% bentonite, 12 ml of 5% gelatine, and 5 ml of 15% kizelsol at 50°C for 30 min and then filtered using a filter paper.

Heat treatment. The thermal stability of anthocyanins was studied at 70, 80, and 90°C. The juice samples (25 ml portions) were transferred into pyrex tubes. The tubes were well capped to avoid evaporation and placed in a thermostatic water bath (GFL, Burgwedel, Germany) preheated to a given temperature. One tube was used for controlling the temperature of the juice. For this purpose, a thermometer was placed into the tube and when the temperature of the juice reached the desired value a capped tube (zero time sample) was removed from the water bath and rapidly cooled by plunging into an ice water bath. At regular time intervals (90 min at 70°C and 60 min at 80 and 90°C), the samples were removed from the water bath and rapidly cooled. The

contents of the cooled tubes were analysed for anthocyanin content, % PC, and antioxidant capacity.

Total monomeric anthocyanin content. Total monomeric anthocyanin contents were determined using the pH differential method suggested by GIUSTI and WROLSTAD (2001). The absorbance of diluted juice samples in buffers at pH 1.0 and 4.5 were measured at 520 nm (λ_{max}) and 700 nm (haze correction) using an UV-Vis spectrophotometer (Shimadzu 1100; Shimadzu, Corp., Kyoto, Japan). Total monomeric anthocyanins were calculated as malvidine 3-glucoside using the following equation:

$$\text{Total monomeric anthocyanins (mg/l)} = (A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times l)$$

where:

A – absorbance difference

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

MW – molecular weight of malvidine 3-glucoside (493.5)

ϵ – extinction coefficient (28 000) for malvidine 3-glucoside

DF – dilution factor

L – path length of the cuvette

Percent polymeric colour (% PC). Percent polymeric colour was determined according to the bisulphite bleaching method outlined by GIUSTI and WROLSTAD (2001). The absorbance of bisulphite treated and untreated samples were measured at 420 and 520 nm for brown pigments and monomeric anthocyanins, respectively. Turbidity was corrected by measuring the absorbance at 700 nm. Colour density was determined by summing the absorbance of the untreated sample with bisulphite at 420 and 520 nm, while polymeric colour was determined as the sum of the absorbance of bisulphite treated sample at 420 and 520 nm. The percentage of polymeric colour (PC) was calculated using the following equation:

$$\% \text{ PC} = (\text{polymeric colour/colour density}) \times 100$$

Trolox equivalent antioxidant capacity. Trolox equivalent antioxidant capacity (TEAC) was determined according to the decolourisation assay which is based on the ability of a compound to scavenge the stable ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical (RE *et al.* 1999). The details of the analysis procedure were outlined in our previous study (PALA & TOKLUCU 2013).

Other analyses. Soluble solids and pH were measured at 20°C using an Abbe 5 Refractometer (Bellingham-Stanley Ltd., Kent, UK) and a pH meter (Sartorius PB-11; Sartorius AG, Goettingen, Germany),

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Table 1. Analytical data of grape juice

Soluble solid (°Brix)	18.75 ± 0.001
pH	3.34 ± 0.001
Titrateable acidity ^a (g/100 ml)	0.57 ± 0.001
Total phenols ^b	1047.50 ± 21.21
Total monomeric anthocyanin ^c (mg/l)	60.70 ± 0.184
Antioxidant capacity ^d (TEAC)	12.41 ± 0.244

^aas tartaric acid; ^bas mg of gallic acid equivalents per l of grape juice; ^cas malvidin 3-glucoside; ^das μmol of trolox equivalents per ml of grape juice

respectively. Titrateable acidity was determined by means of the potentiometric method (IFU 1968). Folin-Ciocalteu method was used for the determination of total phenolic contents (SPANOS & WROLSTAD 1990).

Statistical analyses. All assays were performed in duplicate. The arithmetic means, standard deviations and determination and correlation coefficients were calculated using Excel (Microsoft Inc., Washington, USA).

RESULTS AND DISCUSSION

Physical and chemical characteristics of the clarified grape juice are presented in Table 1. These values agree well with the values reported for grape juice obtained from the same cultivar (PALA & TOKLUCU 2013).

Degradation kinetics of total monomeric anthocyanins during heating. When the ln concentrations of monomeric anthocyanins were plotted as a function of the heating time, linear curves were obtained as seen in Figure 1. The determination coefficient values were higher than 0.98 indicating a good data fit to the first-order kinetic model. Our results are in agreement

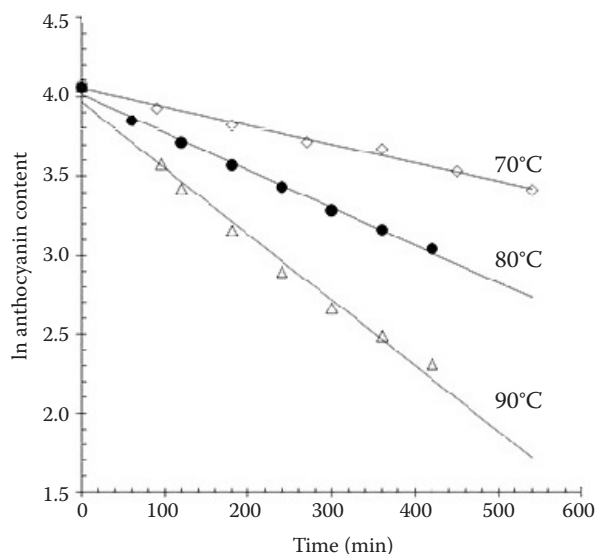


Figure 1. Anthocyanins degradation in grape juice during heating

with those from the previous studies reporting the first-order reaction model for the thermal degradation of monomeric anthocyanins (WANG & XU 2007; KECHINSKI *et al.* 2010; HILLMANN *et al.* 2011).

The first-order reaction rate constants (k_1) and half-lives ($t_{1/2}$), i.e. the time needed for 50% degradation of anthocyanins, were calculated by the Eqs (1) and (2):

$$\ln(C_t/C_0) = -k_1 \times t \quad (1)$$

$$t_{1/2} = -\ln 0.5 \times k_1^{-1} \quad (2)$$

where: C_0 – initial anthocyanin content; C_t – anthocyanin content after t minute heating at a given temperature

As expected, the degradation rate of monomeric anthocyanins increased with increasing heating temperature (Table 2). The $t_{1/2}$ values were determined as 10.03, 5.02, and 2.79 h at 70, 80, and 90°C, respectively. However, HILLMANN *et al.* (2011) found higher $t_{1/2}$

Table 2. Kinetic parameters for the degradation of anthocyanins and formation of polymeric colour in grape juice during heating

	Reaction order	Temperature (°C)	Reaction rate		Activation energy (kJ/mol)
			$-k_1 \times 10^3$ (min ⁻¹)	$k_0 \times 10^2$ (% PC/min)	
Anthocyanin degradation	1	70	1.20 (0.988)		64.89 (0.998)
		80	2.40 (0.996)		
		90	4.20 (0.998)		
Percent polymeric colour formation	0	70		3.86 (0.945)	50.42 (0.999)
		80		6.53 (0.977)	
		90		10.22 (0.962)	

Numbers in parentheses are the determination coefficients

values (21.19–5.20 h) for the anthocyanins of grape juice obtained from Bordo grapes at 70–90°C. Thus, the anthocyanins of Karasakız grape seem to be more susceptible to high temperatures compared to those of Bordo grape. This may be explained by the nature of the anthocyanins occurring in the grape varieties. Bordo grape is one of the most common varieties of *Vitis labrusca* and the most abundant anthocyanins in this variety are 3,5-diglucosides derivatives. On the other hand, Karasakız grape belongs to the *Vitis vinifera* variety, and 3-monoglucosides which have a lower stability than 3,5-diglucosides are the most commonly identified anthocyanins in this variety (LAGO-VANZELA *et al.* 2011). However, similar $t_{1/2}$ values were reported for thermal degradation of the anthocyanins in blueberry juice (8.6 and 5.1 h) at 70 and 80°C (KECHINSKI *et al.* 2010) and blackberry juice (8.8–2.9 h) at 70–90°C (WANG & XU 2007).

The dependence of the anthocyanins degradation on temperature was determined by calculating the activation energy (E_a) values from the Eq. (3):

$$k = k_0 e^{-E_a/RT} \quad (3)$$

where: k_0 – frequency factor; R – universal gas constant (8.314 J/mol·K); T – absolute temperature

The activation energy for the degradation of anthocyanins was determined as 64.89 kJ/mol at 70–90°C. Our E_a value is lower than those reported for Bordo grape anthocyanins (72.74 kJ/mol) at 70–90°C (HILLMANN *et al.* 2011) and blueberry anthocyanins (80.4 kJ/mol) at 40–80°C (KECHINSKI *et al.* 2010). Moreover, WANG and XU (2007) reported a lower E_a value (58.95 kJ/mol) for the degradation of blackberry anthocyanins at 60–90°C. Since a high activation energy value indicates a higher sensitivity of the reaction rate to temperature, the degradation of anthocyanins in Karasakız grape juice seems to be more susceptible to temperature elevations than that of blackberry juice anthocyanins, but less susceptible than the degradation of Bordo grape and blueberry juices anthocyanins.

Formation kinetics of polymeric colour during heating. % PC values during heating were plotted as a function of time and linear curves were obtained (Figure 2) indicating that the formation of % PC follows zero-order reaction kinetics. The zero-order reaction rate constants (k_0) were calculated by the Eq. (4):

$$C_t - C_0 = +k_0 \times t \quad (4)$$

where: C_0 – initial % PC; C_t – % PC after t minute heating at a given temperature

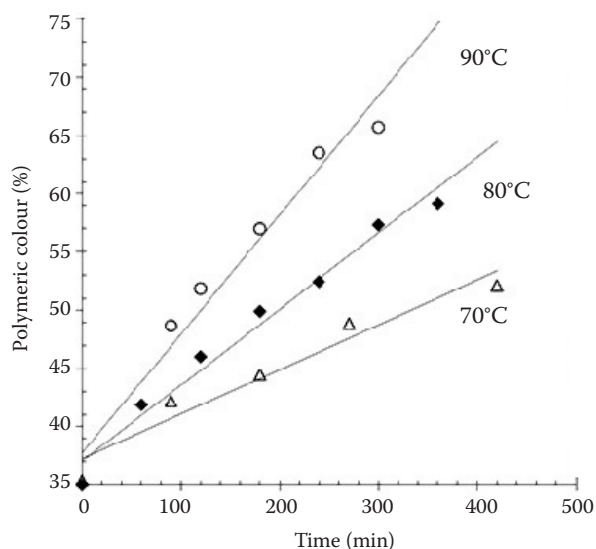


Figure 2. Formation of % PC in grape juice during heating

The rate constants (k_0) are shown in Table 2. As expected, the formation rate of % PC values increased with increasing heating temperature. The temperature dependence of the formation of % PC was also determined and E_a value for the formation of % PC in grape juice was found to be 50.42 kJ/mol at 70–90°C. No study has been found in the literature on the formation kinetics of % PC in anthocyanin containing juices during heating. However, the kinetics of % PC formation during storage of anthocyanin containing juices was reported by several researchers. TURFAN *et al.* (2012) reported that the formation of % PC followed first-order kinetic model in pomegranate juice concentrate during storage at –23, 5, 12, and 20°C. On the other hand, % PC formation was fitted to both zero- and first-order kinetics in black carrot juice concentrates during storage at –23, 5, and 20°C (TÜRKYILMAZ & ÖZKAN 2012).

The increase in % PC during thermal treatment has been reported in anthocyanin-containing juices and products. For example, TURFAN *et al.* (2011) reported a significant increase in % PC in pomegranate juice after pasteurisation (95°C, 10 min). It was also reported that % PC values increased while the concentration of monomeric anthocyanins decreased in thermally processed blackberry (HAGER *et al.* 2008) and blueberry (BROWNMILLER *et al.* 2008) products such as juice, puree, and canned fruits.

High negative correlations were found between the degradation of anthocyanins and formation of % PC in grape juice during heating, with the correlation coefficients of 0.989, 0.997, and 0.993 at 70, 80, and 90°C, respectively. Good correlations were also found between

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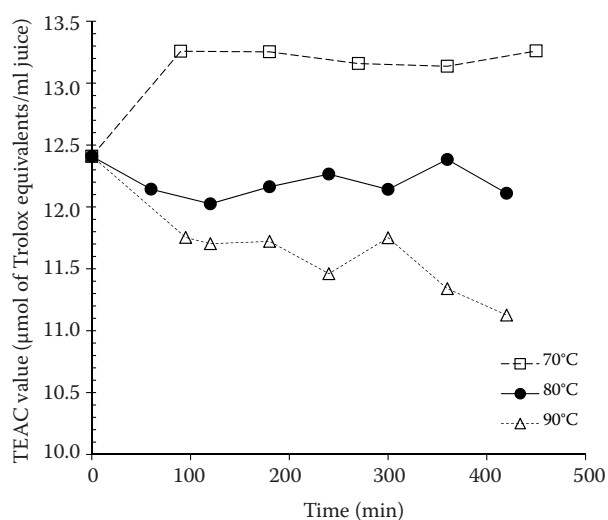


Figure 3. Effect of heat treatment on antioxidant capacity of grape juice (TEAC – Trolox equivalent antioxidant capacity)

these parameters during storage of pomegranate juice concentrate (TURFAN *et al.* 2012) and black carrot juice concentrates (TÜRKYILMAZ & ÖZKAN 2012).

Changes in antioxidant activity during heating.

As seen in Figure 3, the antioxidant activity of grape juice changed slightly during heating. However, when the TEAC values were plotted versus the heating time, no fitting kinetic model was found for the change in antioxidant activity. A 6.9% increase occurred in TEAC values at 70°C, while 2.4 and 10.3% decrease was observed at the end of a 7 h heating at 80 and 90°C, respectively. The higher decrease in the anthocyanin concentration at 90°C may have resulted in losses of antioxidant activity. In fact, there was a positive linear correlation between the decrease in TEAC values and total monomeric anthocyanin content during heating at 90°C ($r = 0.903$). However, there was not a clear relationship at 70 and 80°C. Similarly, a linear relationship was found between anthocyanin concentration and TEAC values of elderberry, strawberry and black carrot anthocyanins heated at 95°C for 4 h (SADILOVA *et al.* 2007).

In the literature, there are a few studies reporting how antioxidant capacity changed during heating of anthocyanin containing systems. HARTMANN *et al.* (2008) evaluated the effects of processing on the antioxidant capacity of strawberry juice using different methods and reported that there were no significant differences in the antioxidant capacity according to the TEAC and FRAP methods, while a considerable rise in the antioxidant capacity was found by the DPPH method after heat treatment (85°C, 15 min). Moreover, it was found that the pasteurisation of

blackberry juice (HAGER *et al.* 2008) and blueberry juice (BROWNMILLER *et al.* 2008) did not affect the antioxidant capacity (ORAC and PCL methods). However, POZO-INSFRAN *et al.* (2006) reported that 25.9% of antioxidant capacity (ORAC method) was lost during thermal pasteurisation of Muscadine grape juice using a HTST unit (75°C, 15 s).

CONCLUSION

In this study were evaluated the kinetics of both anthocyanins degradation and the formation of % PC in grape juice, as well as the changes in the antioxidant capacity of juice during heating. Grape juice anthocyanins were found to be susceptible to high temperatures. However, the antioxidant activity of juice changed slightly during heating. Anthocyanin degradation fitted to a first-order reaction model, while the formation of % PC following a zero-order reaction model. Since the heat treatment had a significant effect on monomeric anthocyanins and polymeric colour, it should be carefully optimised to decrease the anthocyanin losses and polymeric colour formation in the commercial processing of grapes into juice. Stabilisation of anthocyanins may be provided with copigmentation or in another way. Therefore, further studies are needed on the stabilisation of anthocyanins in grape juice.

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