Agronomy Research **13**(4), 892–899, 2015

Effects of degradation preventive agents on storage stability of anthocyanins in sour cherry concentrate

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Abstract. In this study the effects of sugar (sucrose, 25%), gallic acid (700 mg kg^{-1}) and ascorbic acid (700 mg kg^{-1}) were used in sour cherry concentrate in order to prevent the degradation of main anthocyanin compounds (cyanidin-3-glucosylrutinoside (Cy-3GR), cyanidin-3-rutinoside (Cy-3R) and cyanidin-3-glucoside (Cy-3G)) which are natural bioactive pigments responsible for red, blue and purple color of many fruits and vegetables. Thermal degradation of anthocyanins was evaluated by determination of anthocyanin content and calculation of the reaction rate constant, half-life of degradation, activation energy. Anthocyanin content decreased at all of the storage temperatures, as an example; there were 75, 51 and 55% reductions in Cy-3G contents of control samples (with no preventive agent) stored at 45, 24 and 4°C, respectively. The values of half-life time were above 200 days in most cases at all storage temperatures for sugar treated samples. Cy-3-GR (activation energy values 35.6-84.4 kJ mol^{-1}) was found to be the most unstable among the other anthocyanins. The most contributing agent on anthocyanin stability was sugar, whereas ascorbic acid exhibited the lowest effect in terms of preventing anthocyanin degradation.

Key words: sour cherry, anthocyanins, degradation kinetics, storage.

INTRODUCTION

Anthocyanins, the biggest group of water-soluble natural pigments, are a sub-class of flavonoids. They give attractive colours of flowers, fruits (especially berries) and vegetables, as well as their products (Mazza & Brouillard, 1990). Except as colorants, anthocyanins have multiple biological roles, e.g. antioxidant activity, anti-inflammatory action, inhibition of blood platelet aggregation and antimicrobial activity, treatment of diabetic retinopathy and prevention of cholesterol-induced atherosclerosis (Mazza & Miniati, 1993; Espin et al., 2000). Anthocyanins are stable under acidic conditions, but under normal processing and storage conditions they transform to colourless compounds and subsequently to insoluble brown pigments. Thermal treatments and storage temperature have the most important influence on anthocyanin stability (Zorić et al., 2014).

Number of factors influences the stability of anthocyanins, like temperature, pH, light, oxygen, enzymes, structure and concentration of the anthocyanins, presence of ascorbic acid, sugars, sulphite salts, metal ions and copigments (Gradinaru et al., 2003; Tsai & Huang, 2004). Garcia-Viguera et al. (1999) and Iacobucci & Sweeny (1983) proposed a free radical mechanism where cleavage of the pyrilium ring resulted as a consequence of oxidation initiated by activation of molecular oxygen induced by

ascorbic acid. Anthocyanin copigmentation reactions are common in nature and result from the association of metal ions or colorless polyphenolics (cofactors) to anthocyanins under acidic conditions. Copigment complexes also serve to enhance color and stability characteristics of anthocyanins in low acid conditions where anthocyanins are normally colorless. Several studies have suggested an increase in anthocyanin stability in the presence of cofactors (Boulton, 2001; Brenes et al., 2005).

Many studies were conducted with the aim of improving stability of anthocyanins through addition of different additives, like acids, sugars, salts, hydrocolloids and different phenolic compounds (Rein & Heinonen, 2004; Brenes et al., 2005). Fortification of fruit and berry juices with ascorbic acid is a common method to protect against oxidation and to increase the nutritional value of a food product. Ascorbic acid is thought to have several different roles in anthocyanin color stability. In fruit and berry products, by copigmentation the color of juices (Wilska-Jeszka & Korzuchowska, 1996; Dufour & Sauvaitre, 2000), purees, jams and syrups could be enhanced and stabilized, improving consumer acceptance and prolonging product shelf-life. Brenes et al. (2005) reported the total anthocyanins in grape juice ranging between 600-800 mg L^{-1}. The anthocyanin content of twenty different pomegranate varieties from Iran was reported between 5.56–30.11 mg $100g^{-1}$ (Tehranifer et al., 2010). The values of total anthocyanins in red currant juices were reported between 34.3–47.9 mg $100g^{-1}$ by Kopjar et al. (2009). Filiman et al. (2011) determined anthocyanins between 107–176 mg $100g^{-1}$ in four sour cherry varieties grown in Romania.

The chemical stability of anthocyanins in the presence of ascorbic acid, gallic acid and sucrose and storage temperature effects has not been studied upon sour cherry concentrate until now. The aim of this research was to study the kinetics of individual anthocyanins in sour cherry concentrate, in order to advance the knowledge of the thermal stability of the main anthocyanins, and to apply previously reported mathematical models enabling the prediction of the degradation of these compounds during storage.

MATERIALS METHODS

Laboratory-scale preparation of sour cherry concentrate

Sour cherries were purchased from a local market in June 2010. The samples were immediately transported to laboratory and subsequently pitted, washed and squeezed in a fruit juicer (Philips fruit juicer, HR1861). A juice of 14–16 °Brix was obtained. The obtained juice was pasteurized in an autoclave at 85°C for 30 min. The juice was filtrated through a filter paper. Before concentration it was partitioned into four batches for application of treatments: gallic and ascorbic acids were added seperately to contain final concentration in the concentrate models of 700 mg kg^{-1}. Sucrose was incorporated in order to have a final level of 25%. The initial sugar concentration of sour cherry juice is approximately 8%. For a 100 mL of juice, 2 g of sugar was added and the solution was concentrated with a volumetrically ratio of 2 : 5. The final sugar concentration was about 25%. A non-fortified batch was prepared as control. The samples were concentrated by using a vacuum evaporator at 40°C until a °Brix of 55 was obtained. Treatments were sealed in 100 mL screw-cap glass bottles. The samples were stored at three different temperatures, 4°C (in refrigerator), 24°C (room temperature) and at 45°C (in oven) for 6 months. The anthocyanin contents of samples were determined at 0, 20, 50, 90, 120 and 150^{th} days of storage.

Extraction of anthocyanins

The extraction of anthocyanins from sour cherry concentrate was performed according to a previously described procedure (Elez Garofulić et al., 2013). 4 g concentrate was mixed with 8 mL of 80% aqueous methanol solution containing 0.1% HCl (by volume), in a water bath at 60°C for 20 min. Afterwards, the extracts were filtered through Whatman No. 40 filter paper (Whatman, GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), transferred into 10 mL volumetric flasks, and made up to volume with extraction solvent. Extracts were stored at -20°C in an inert nitrogen gas atmosphere before the analysis.

HPLC analysis

The anthocyanins were simultaneously analysed by a direct injection of the extracts, previously filtered through a 0.45 mm pore size membrane filter (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Chromatographic separation was performed using HPLC analysis with Agilent 1260 Infinity quaternary LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with diode array detector (DAD), an automatic injector and ChemStation software. The separation of phenolic compounds was performed on a Nucleosil 100-5C18, 5 mm (250 mm × 4.6 mm i.d.) column (Macherey-Nagel). The solvent composition and the gradient conditions used were as described previously by Mitić et al. (2012) and Zorić et al. (2014). Mobile phase A contained 3% of formic acid in water, while solution B contained 3% of formic acid in 80 % acetonitrile. The used elution program was as follows: from 0 to 28 min 0% B, from 28 to 35 min 25% B, from 35 to 40 min 50% B, from 40 to 45 min 80% B, and finally for the last 10 min again 0% B. The flow rate was 0.8 mL min^{-1} and the injection volume was 50 µL. Detection was performed with UV/VIS–photo diode array detector by scanning from 220 to 570 nm. Identification of phenols was carried out by comparing retention times and spectral data with those of the authentic standards (anthocyanins were identified at 520 nm).

The quantifications of anthocyanins were made by the external standard method. All anthocyanin standards, cyanidin-3-glucoside (Cy-3-G) and cyanidin-3-rutinoside (Cy-3-R) were prepared as stock solutions in acidified methanol (1% of formic acid in methanol, by volume) at a concentration of 100 mg L^{-1}. Cyanidin-3-glucosylrutinoside (Cy-3-GR) identification was done in comparison with Cy-3-G. Identification was made by matching the retention time of the separated peaks and the retention time of the authentic standards. Additionally, identification was confirmed using characteristic UV/VIS spectra, polarity, and previous literature reports (Del Bo' et al., 2010; Fracassetti et al., 2013). Under the current chromatographic conditions, the limit of detection (LOD) and limit of quantification (LOQ) were determined to be 100 ng mg^{-1} (S : N (signal-to-noise ratio) > 5) and 200 ng mg^{-1} (S : N > 10), respectively.

Degradation Kinetic Studies

The thermal degradation of anthocyanins was performed according to the method reported by Kechinski et al. (2010). Degradation is a temperature-dependent process, as described by the Arrhenius equation: $k = k_0 \times e^{-Ea / RT}$, where: k_0 is the frequency factor (per min), Ea the activation energy (J mol^{-1}), R the universal gas constant (8.314 J mol^{-1} K^{-1}), and T the absolute temperature (K).

Statistical Analysis

Data were reported as mean values of at least four experiments. Statistical analysis was performed by means of Statistica software (Statsoft Inc., Tulsa, OK, USA). One-way ANOVA was performed to determine the variation among the samples stored at different temperatures. Differences between means were evaluated by the Duncan test. Differences were considered to be significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the presented research, the major anthocyanins determined in freeze-dried sour cherries were Cy-3-GR, Cy-3-R and Cy-3-G with the initial values in control group 245.6 ± 22.1, 187.4 ± 18.4 and 41.5 ± 4.0 mg kg^{-1}, respectively. The initial and final contents of anthocyanins in sour cherry concentrate after storage at different temperatures of 4, 24 and 45°C was plotted and presented in Fig. 1. Mass fractions at all temperatures followed the first order reaction kinetics with a coefficient of determination R^2, ranging from 0.84 to 0.99 (Table 1). These results are in accordance with previous reports (Zorić et al., 2014; Harbourne et al., 2008).

Anthocyanins displayed decay, directly related to the storage temperature. For all of the three compounds, the correlation indices (R^2) were > 0.85, demonstrating a direct correlation between anthocyanin concentration decrease and storage time. The increases in degradation rate of anthocyanins during processing and storage as the temperature rises were also previously reported (Maccarone et al., 1985; Fracassetti et al., 2013).

Cy-3-GR was the most unstable anthocyanin in all three storage temperatures (E_a = 35.57-84.28 kJ mol^{-1}). Cy-3-G followed it (E_a = 124.04-266.62 kJ mol^{-1}), whereas this compound was the most unstable with the addition of gallic acid, ascorbic acid and sucrose at 45°C.

The most stable anthocyanin compound Cy-3-R (Ea = 95.44-825.33 kJ mol^{-1}) showed an exception for ascorbic acid added samples at 45°C storage with the lowest half-life time ($t_{1/2}$). Ascorbic acid has been reported to show different roles in anthocyanin stability. Ascorbic acid accelerated decomposition of anthocyanins and enhances polymer pigment formation and bleaches anthocyanin pigments (Marti et al., 2002). Direct condensation between anthocyanins and ascorbic acid has been postulated as a mechanism for anthocyanin degradation (Poei-Langston & Wrolstad, 1981). Also the formation of hydrogen peroxide from ascorbic acid oxidation can influence anthocyanin stability (Talcott et al., 2003). However, the stability of acylated anthocyanins has been observed to increase in the presence of ascorbic acid (Del Pozo-Insfran et al., 2004).

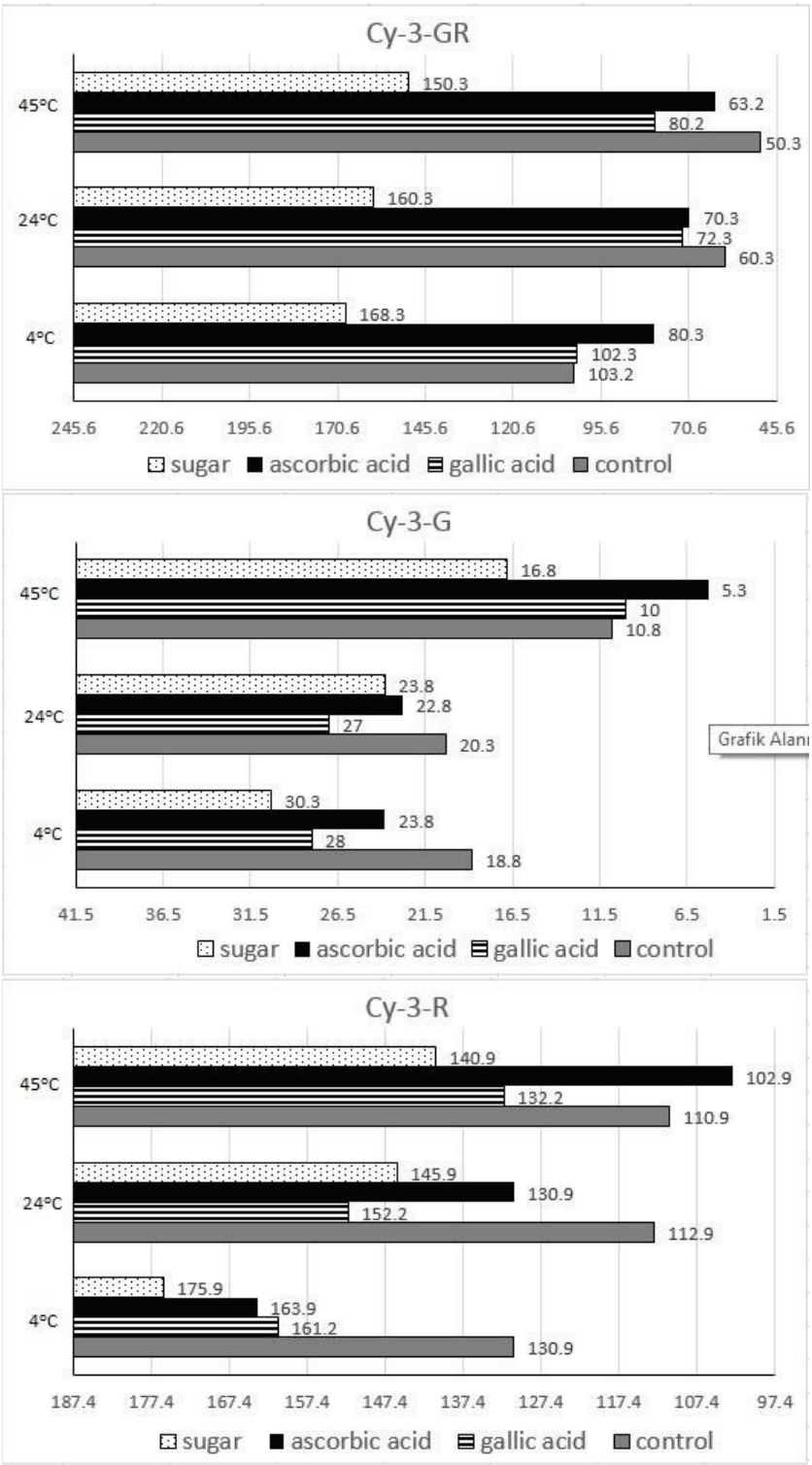

Figure 1. The initial and final contents (mg L^{-1}) of anthocyanins in sour cherry concentrate after storage at 4, 24 and 45°C for 5 months.

The $t_{1/2}$ values of anthocyanins were presented in Table 1. The half-life at 45°C of Cy-3-G and Cy-3-R was 53.3 and 6.5 days, respectively, which were lower than the values obtained for the control group samples. This indicates a negative effect of ascorbic acid on these compounds (a more dramatic reduction of half-life of Cy-3-R) at high storage temperatures. Cy-3-R was more susceptible to high temperatures than Cy-3-GR, while Cy-3-G was unstable.

Table 1. Activation energy (E_a) and half-life ($t_{1/2}$) of individual anthocyanins of the sour cherry concentrate stored at 4, 24, and 45°C.

	Compound	Treatments	$t_{1/2}$ (days)	E_a (kJ mol^{-1})	R^2
Control	Cy-3-GR[a]	4	80.0	35.57	0.92
		24	77.0		
		45	63.0		
	Cy-3-G	4	138.6	143.16	0.84
		24	173.3		
		45	73.0		
	Cy-3-R	4	346.5	95.44	0.93
		24	231.0		
		45	224.0		
Ascorbic acid (700 mg kg^{-1})	Cy-3-GR	4	99.0	44.89	0.99
		24	86.6		
		45	77.0		
	Cy-3-G	4	231.0	259.31	0.87
		24	173.3		
		45	53.3		
	Cy-3-R	4	599.0	825.33	0.84
		24	342.0		
		45	6.5		
Gallic acid (700 mg kg^{-1})	Cy-3-GR	4	138.6	84.38	0.95
		24	99.0		
		45	86.6		
	Cy-3-G	4	346.5	266.62	0.92
		24	231.0		
		45	77.0		
	Cy-3-R	4	693.0	155.72	0.98
		24	693.0		
		45	346.0		
Sugar (25%)	Cy-3-GR	4	346.0	71.79	0.89
		24	297.0		
		45	231.0		
	Cy-3-G	4	348.0	124.04	0.99
		24	230.0		
		45	173.3		
	Cy-3-R	4	758.0	538.99	0.93
		24	690.0		
		45	346.5		

([a]Cy-3GR: cyanidin-3-glucosylrutinoside, Cy-3R: cyanidin-3-rutinoside, Cy-3G: cyanidin-3-glucoside).

Among the degradation preventive agents, sucrose was the most effective, gallic and ascorbic acid followed. This effect was more evident for Cy-3-GR at 45°C. The protective effect of sucrose on degradation of anthocynins was attributed to the inhibition

of enzymatic reactions or the hindering of different condensation reactions (Wrolstad et al.,1990) and also its lowering of water activity (De Ancos et al., 1999b).

CONCLUSION

The results supported that the duration and temperature of storage have a strong influence on the anthocyanin stability. Rapid and high anthocyanin degradation indicates that it is very important to identify suitable storage conditions, for which further studies are still needed. The enhancement of sour cherry concentrate with sucrose and gallic acid can be regarded as a promising mean of improving stability of anthocyanins. The use of ascorbic acid should be avoided at elevated temperatures of storage, as ascorbic acid led to a dramatic reduction of half-life of Cy-3-R during storage at 45°C.

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