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Prevention of thermal degradation of red currant juice anthocyanins by phenolic compounds addition

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Summary

The aim of this study was investigation of prevention of thermal degradation of the red currant juice anthocyanins by addition of different phenolic compounds (catechol, 4-methyl catechol, catechin, chlorogenic acid and gallic acid). Phenolic compounds were added in 50:1 and 100:1 copigment:pigment molar ratio. Red currant juice samples were heated at 30, 50, 70 and 90 °C for 1 hour. Thermal degradation of anthocyanins was investigated through determination of anthocyanin content and calculation of the reaction rate constant, half-life of degradation, activation energy and anthocyanin retention. Anthocyanin content ranged from 34.32 mg/100 mL to 47.97 mg/100 mL in a red currant juice, depending on temperature. When phenolic compounds were added anthocyanin content ranged from 34.94 mg/100 mL to 50.41 mg/100 mL, depending on both temperature and added phenolic compounds. In all cases when phenolic compound were added, anthocyanin content, half-lives of degradation increased and the reaction rate constants decreased. The thermal degradation of anthocyanins was lower when phenolic compounds were added due to copigmentation effect. With increase of temperature copigmentation effect was less pronounced. The most contributing phenolic compounds on anthocyanin stability at 30 °C was gallic acid, at 50 °C and 70 °C catechin, and at 90 °C 4-methyl catechol. Concentration of phenolic compounds also had influence on investigated parameters.

Key words: anthocyanins, thermal degradation, addition of phenolic compounds, red currant juice

Introduction

Anthocyanins, the biggest group of water-soluble natural pigments, are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts. They are responsible for attractive colours of flowers, fruits (especially berries) and vegetables, as well as their products (Mazza and Brouillard, 1990). It has been recognized that anthocyanin-rich plant extracts might have potential as natural food colorants. 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 and Miniati, 1993; Wang et al., 1997; Cliford, 2000; Espin et al., 2000). The major problem of anthocyanins use as natural food colorants is their instability, either in simple or in complex food formulations. Anthocyanins are stable under acidic conditions, but under normal processing and storage conditions they transform to colourless compounds and subsequently to insoluble brown pigments. Number of factors influences the stability of anthocyanins, like temperature, pH, light, oxygen, enzymes, presence of ascorbic acid, sugars, sulphite salts, metal ions and copigments (Jackman et al., 1987; Francis, 1989; Bąkowska et al., 2003; Gradinaru et al., 2003; Tsai and Huang, 2004). 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 (Wilska-Jeszka and Korzuchowska, 1996; Baranac et al., 1996; Baranac et al., 1997a; Baranac et al., 1997b; Baranac et al., 1997c; Dimitrić-Marković et al., 2000; Boulton, 2001; Hubbermann et al., 2002; Bąkowska et al., 2003; Rein and Heinonen, 2004; Mazzaracchio et al., 2004; Hubbermann et al., 2006; Kopjar et al., 2007; Mollov et al., 2007; Awika, 2008; Kopjar et al., 2008; Oszmiański et al., 2009; Kopjar et al., 2009). Copigmentation has been suggested as a main colour stabilising mechanism in plants protecting the coloured flavylium cation from the nucleophilic attack of the water molecule (Mazza and Brouillard, 1987; Baranac et al. 1997a). The attack by water converts the flavylium ion to colourless pseudobase resulting in colour loss. Formation of complex between the pigment and copigment causes a hyperchromic effect (ΔA) and a bathochromic shift ($\Delta\lambda_{max}$). While hypochromic effect means an increase in colour intensity, the bathochromic shift presents shift of the maximum absorbance wavelength (Chen and Hrazdina, 1981; Mazza and Miniati, 1993). A copigment may be one of flavonoids, alkaloids,

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amino acids, organic acids, nucleotides, polysaccarides, metals and anthocyanins themselves (Mazza and Brouillard, 1990).

Thermal degradation of anthocyanins was extensively studied (Garzon and Wrolstad, 2002; Kırca and Cemoroğlu, 2003; Gradinaru et al., 2003; Tseng et al., 2006; Kırca et al., 2007; Harbourne et al., 2008) but there are only few studies on thermal degradation of anthocyanins when phenolic compounds were added (Bąkowska et al., 2003; Gradinaru et al., 2003).

In this study, stability effect of catechol, 4-methyl catechol, catechin, chlorogenic acid and gallic acid on red currant juice anthocyanins during heating at 30, 50, 70 and 90 °C was investigated.

Materials and methods

Material

Red currant fruit was bought at local market and kept at -20 °C before sample preparation. Phenolic compounds were obtained from Sigma, Germany and potassium chloride and sodium acetate from Kemika, Croatia.

Sample preparation

Red currant juice was prepared by pressing through cheese cloth and filtered through rough filter paper. Samples of juice were prepared without and with addition of selected phenolic compounds (catechol, 4-methyl catechol, catechin, chlorogenic acid and gallic acid) in two different concentrations; phenolic compound:anthocyanin 50:1 and 100:1 molar ratio. Samples were left for one and a half hour to stabilise.

Degradation studies

The thermal stability of anthocyanins in a red currant juice was studied at 30, 50, 70 and 90 °C. Aliquots of red currant juice (20 mL) were put into glass tubes which were well capped for avoiding evaporation of samples. Tubes were put in water bath which was preheated at desired temperature. Samples were heated for 1 hour at certain temperature, removed from water bath and rapidly cooled in ice-cooled water to the room temperature. After cooling determination of monomeric anthocyanins were conducted.

Measurement of monomeric anthocyanins

Determination of monomeric anthocyanins was conducted by pH-differential method (Giusti and Wrolstad, 2001). Total monomeric anthocyanins were expressed as cyanidin-3-glucoside. Sample absorbance was read against a blank cell containing distilled water. The absorbance (A) of the sample was then calculated according the following formula:

$$A = (A_{\lambda vis} - A_{700})_{pH\ 1.0} - (A_{\lambda vis} - A_{700})_{pH\ 4.5} \quad (1)$$

where $A_{\lambda vis}$ was wavelength at which maximal absorbance of samples was achieved.

The monomeric anthocyanin pigment content in the original sample was calculated according the following formula:

$$\text{Anthocyanin content (mg/L)} = (A \times MW \times DF \times 1000) / (\varepsilon \times l) \quad (2)$$

where DF was dilution factor, MW cyanidin-3-glucoside molecular weight (449.2) and ε molar absorptivity (26,900).

Measurements were done in duplicates.

Calculation of kinetic parameters of anthocyanin degradation

The first-order reaction rate constants (k), half-lives ($t_{1/2}$) i.e. the time which is necessary for degradation of 50 % of anthocyanins, were calculated using following equations:

$$\ln (c_t/c_0) = -k \times t \quad (3)$$

$$t_{1/2} = -\ln (0.5)/k \quad (4)$$

where c_0 is initial anthocyanin content and c_t anthocyanin content after heating time at the given temperature.

According to Arrhenius equation, there is linear relationship between lnk and 1/T:

$$k = k_0 \exp(-E_a/RT) \quad (5)$$

where E_a is the activation energy, R gas constant and T temperature.

Results and discussion

Anthocyanin content

One of the main factors effecting stability of anthocyanins is the temperature. Thermal degradation of red currant juice anthocyanins at different temperatures, 30, 50, 70 and 90 °C for 1 hour was investigated. As it was proven in many studies on thermal degradation of anthocyanins, earlier (Garzon and Wrolstad, 2002; Kırca and Cemoroğlu, 2003; Gradinaru et al., 2003; Tseng et al., 2006; Kırca et al., 2007, Harbourne et al., 2008), in our case degradation of red currant juice anthocyanins also occurred with increase of the temperature. Simpson (1985) suggested that two mechanisms are responsible for thermal degradation of anthocyanins: (1) hydrolysis of the 3-glycoside linkage to form the more labile aglycon; and (2) hydrolytic opening of the pyrilium ring to form a substituted chalcone, which then degrades to a brown insoluble compound of a polyphenolic nature. In our study prevention of thermal degradation by addition of phenolic compounds in 2 different concentrations to a red currant juice was investigated. Anthocyanin content in a red currant juice without and with addition of phenolic compounds (copigment: pigment molar ratio 50:1 and 100:1) during heating at different temperatures for 1 hour is presented in Table 1. Fresh prepared juice before any treatment contained 50.85 mg/100 mL of anthocyanins. After heating at different temperatures, anthocyanin content in red currant juice decreased in comparison to fresh prepared juice before any treatment. The lowest anthocyanin content had red currant juice (34.32 to 47.97 mg/100 mL), regardless of the heating temperature. All juice samples with addition of phenolic compound had higher anthocyanin content in comparison to red currant juice which is prove that phenolic compounds prevented thermal degradation of anthocyanins. From the results (Table 1) it is obvious that concentration of phenolic compounds played important part in prevention of degradation of anthocyanins. At temperatures below 90 °C, in all samples when phenolic compounds were added in higher concentration, the higher anthocyanin content was obtained, with exception of catechol at 30 °C. Depending on temperature, all phenolic compounds caused higher prevention of anthocyanins from thermal degradation which can be noted through anthocyanin content. At 30 °C the highest anthocyanin content had samples with addition of gallic acid and catechin, 50.41 and 50.35, respectively. Addition of catechol and catechin caused the highest anthocyanin (content) at 50 °C, 47.59 and 47.47, respectively. Samples with addition of catechin and 4-methyl catechol had the highest anthocyanin content at 70 °C, 43.96 and 43.33, respectively. Not all samples with higher concentration of phenolic compound, exposed to the highest heating temperature (90 °C), had higher anthocyanin content, thus anthocyanin content of samples with addition of 50:1 and 100:1 of 4-methyl catechol was 37.95 and 37.32, for catechin 37.70 and 37.20 and with addition of chlorogenic acid 37.45 and 37.45, respectively.

Table 1. Anthocyanin content (mg/100 mL) of red currant juice during heating at different temperatures

Samples	30 °C	50 °C	70 °C	90 °C
Control	47.97±0.49	45.59±0.42	39.33±0.29	34.32±0.34
C 50:1	49.97±0.50	46.46±0.15	41.45±0.36	34.94±0.24
C 100:1	49.72±0.35	47.47±0.26	42.08±0.34	36.45±0.22
4-MC 50:1	48.72±0.28	46.34±0.35	41.83±0.53	37.95±0.31
4-MC 100:1	50.09±0.51	47.22±0.39	43.33±0.15	37.32±0.39
CT 50:1	49.85±0.43	46.97±0.49	42.46±0.36	37.70±0.19
CT 100:1	50.35±0.23	47.59±0.42	43.96±0.37	37.20±0.21
CA 50:1	49.60±0.25	46.59±0.25	40.95±0.42	37.45±0.31
CA 100:1	49.60±0.36	47.09±0.35	42.83±0.37	37.45±0.22
GA 50:1	49.60±0.46	46.21±0.41	40.33±0.38	36.70±0.18
GA 100:1	50.41±0.24	47.22±0.35	41.58±0.25	37.32±0.17

(C – catechol; 4-MC – 4 methyl catechol; CT – catechin; CA – chlorogenic acid; GA – gallic acid)

Calculation of anthocyanin retention (Table 2) followed anthocyanin content. Again, with increase of temperature (30, 50, 70 and 90 °C), degradation of anthocyanins was higher, and that was shown through decrease of anthocyanin retention. Addition of phenolic compounds, namely catechol, 4-methyl catechol, catechin, chlorogenic acid and gallic acid caused prevention of thermal degradation of red currant juice anthocyanins. Depending on phenolic compound thermal degradation was prevented in higher or lesser extent.

Table 2. Anthocyanin retention (%) of red currant juice during heating at different temperatures

Samples	30 °C	50 °C	70 °C	90 °C
Control	94.34	89.66	77.34	67.49
C 50:1	98.27	91.37	81.51	68.71
C 100:1	97.78	93.35	82.75	71.68
4-MC 50:1	95.81	91.13	82.26	74.63
4-MC 100:1	98.50	92.86	85.21	73.39
CT 50:1	98.03	92.37	83.50	74.14
CT 100:1	99.01	93.59	86.45	73.16
CA 50:1	97.54	91.62	80.53	73.65
CA 100:1	97.54	92.61	84.23	73.65
GA 50:1	97.54	90.86	79.31	72.17
GA 100:1	99.13	92.86	81.77	73.39

(C – catechol; 4-MC – 4 methyl catechol; CT – catechin; CA – chlorogenic acid; GA – gallic acid)

Kinetic parameters

Prevention of anthocyanins from thermal degradation was also proven by calculation of the reaction rate constants and half-lives. Assuming that degradation of anthocyanins fits first-order reaction model, as it was proven in many studies earlier (Garzon and Wrolstad, 2002; Kırca and Cemoroğlu, 2003; Tseng et al., 2006; Kırca et al., 2007), it was possible to calculate the reaction rate constants and half-life of anthocyanin degradation. The reaction rate constants (k) are presented in Table 3. Red currant juice had k value 0.0583 h^{-1} at 30 °C, while with addition of all phenolic compounds that value decreased (0.0087 to 0.0428) meaning that degradation reaction of anthocyanins slowed down after addition of phenolic compounds. The same tendency was observed at higher temperatures. With increase of temperature k values increased proving that degradation rate accelerates with increase of temperature.

Results of half-life calculation are presented in Table 3. Anthocyanins of red currant juice had the lowest half-life, only 11.89 h, while with addition of phenolic compounds those values increased, from 16.20 to 79.96, at 30 °C. Heating of juice samples caused decrease of half-lives, for all samples but the lower values were obtained for red currant juice.

Kırca et al. (2007) studied anthocyanin stability of black carrots during heating at 70, 80 and 90 °C at different pH and with different solid content. They found out that half-lives for their samples were between 2.3 h and 25.1 h depending on temperature, pH and solid content. For thermal degradation at 60, 70 and 80 °C, of sour cherry anthocyanins, Cemoroğlu et al. (2004), obtained $t_{1/2}$ from 2.8 to 24 h depending on solid content. Kırca and Cemoroğlu (2003) determined $t_{1/2}$ from 0.4 to 3.4 h depending on solid content after thermal (70, 80 and 90 °C) degradation of blood orange anthocyanins. $t_{1/2}$ values of red current juice were similar, however, after addition of phenolic compounds higher values were obtained.

Table 3. Kinetic parameters for thermal degradation of anthocyanins of red currant juice

Samples	Temperature	k (h^{-1})	$t_{1/2}$ (h)	E_a (kJ/mol)
Control	30 °C	0.0583	11.89	33.08 (0.9870)*
	50 °C	0.1092	6.35	
	70 °C	0.2569	2.70	
	90 °C	0.3932	1.76	
C 50:1	30 °C	0.0175	39.71	35.30 (0.9970)
	50 °C	0.0903	7.68	
	70 °C	0.2044	3.39	
	90 °C	0.3753	1.85	
C 100:1	30 °C	0.0225	30.84	39.04 (0.9856)
	50 °C	0.0688	10.08	
	70 °C	0.1893	3.66	
	90 °C	0.3329	2.08	
4-MC 50:1	30 °C	0.0428	16.20	38.88 (0.9835)
	50 °C	0.0929	7.46	
	70 °C	0.1953	3.55	
	90 °C	0.2926	2.37	
4-MC 100:1	30 °C	0.0150	46.03	45.75 (0.9607)
	50 °C	0.0741	9.36	
	70 °C	0.1600	4.33	
	90 °C	0.3093	2.24	
CT 50:1	30 °C	0.0199	34.89	36.99 (0.9955)
	50 °C	0.0794	8.73	
	70 °C	0.1803	3.84	
	90 °C	0.2992	2.32	
CT 100:1	30 °C	0.0099	70.15	47.37 (0.9677)
	50 °C	0.0663	10.46	
	70 °C	0.1456	4.76	
	90 °C	0.3126	2.22	
CA 50:1	30 °C	0.0249	27.85	45.75 (0.9607)
	50 °C	0.0875	7.92	
	70 °C	0.2165	3.20	
	90 °C	0.3058	2.27	
CA 100:1	30 °C	0.0249	27.85	46.37 (0.9854)
	50 °C	0.0768	9.02	
	70 °C	0.1716	4.04	
	90 °C	0.3059	2.27	
GA 50:1	30 °C	0.0249	27.85	43.36 (0.9842)
	50 °C	0.0959	7.24	
	70 °C	0.2318	2.99	
	90 °C	0.3261	2.13	
GA 100:1	30 °C	0.0087	79.76	43.79 (0.9578)
	50 °C	0.0741	9.36	
	70 °C	0.2012	3.44	
	90 °C	0.3094	2.24	

* Number in parentheses presents correlation coefficient of relation between lnk and 1/T

(C – catechol; 4-MC – 4 methyl catechol; CT – catechin; CA – chlorogenic acid; GA – gallic acid)

Since the study was conducted at different temperatures it was possible to determine relation between lnk and 1/T. Correlation coefficients were high, between 0.9578 and 0.9970, so the temperature dependence of reaction rate constants followed the Arrhenius relationship, typical of many deteriorative processes in food products, thus calculation of E_a was possible (Table 3). The lowest E_a had samples of red current juice, 33.08 kJ/mol, while samples with addition of phenolic compounds had higher E_a. The lowest values of E_a, when phenolic compounds were added, had samples with catechol 50:1 and catechin 50:1, 35.3 kJ/mol and 36.99 kJ/mol, respectively, while addition of gallic acid 50:1 and chlorogenic acid 50:1 caused the highest values, 43.36 kJ/mol and 45.75 kJ/mol, respectively. When the phenolic compounds were added at higher concentration, different tendency was observed. The lowest value of E_a had, again, sample with addition of catechol, 39.04 kJ/mol. Other samples had similar values. It is interesting that higher concentration of chlorogenic acid did not cause increase of E_a. Conducting investigation of thermal degradation of anthocyanins of black carrots Kırca et al. (2007) obtained results for E_a from 42 kJ/mol to 95 kJ/mol depending on pH and solid content, the values higher than in our case. Gradinaru et al. (2003) investigated influence of chlorogenic acid on *Hibiscus sabdariffa L.* anthocyanins and reported values for E_a from 55 to 63 kJ/mol with varying temperature from 55 to 98 °C.

Copigmentation effect

Positive effect of phenolic compounds addition to a red currant juice against thermal degradation could be explained by copigmentation effect. Several factors influence copigmentation, among which copigment type is the most important one (Mazza and Brouillard, 1990; Dimitrović-Marković et al., 2000; Bąkowska et al., 2003). Results of determination of λ_{max} are presented in Table 4. Red currant juice without addition of phenolic compounds had λ_{max} at 513 nm, while red currant juice with addition of phenolic compounds had higher λ_{max}. The difference in increase of λ_{max} ($\Delta\lambda_{max}$), i.e. bathochromic shift for different phenolic compounds was different. $\Delta\lambda_{max}$ were 3 nm for catechol and 4-methyl catechol, 4 nm for gallic acid and 5 nm for catechin and chlorogenic acid. Mollov et al. (2007) found out that addition of polyphenolic copigments extracted from distilled rose petals to strawberry anthocyanins caused $\Delta\lambda_{max}$ of 6 nm. Bąkowska et al. (2003) reported that shift of λ_{max} of cyanidin were 21.4, 14.9, 5.5, 3.8 and 2.5 nm after addition of flavones of *Scutellaria baicalensis* Georgi, quercetin-5´-sulphonic acid, sodium salt of morin-5´-sulphonic acid, tannic acid and chlorogenic acid, respectively. Also they reported that absorbance i.e. hyperchromic effect increased with addition of copigments in higher or lesser extent. Their results showed that the lowest hyperchromic effect was observed for the complex of cyanidin and phenolic acids. In our case absorbance also increased with addition of phenolic compounds which had been shown through increase of anthocyanin content. Mollov et al. (2007) demonstrated that the addition of polyphenolic copigments extracted from distilled rose petals reduces the thermal degradation of strawberry anthocyanins, thus improving colour stability of the processed strawberries. In strawberry beverages, an increase in colour intensity has been observed after addition of phenolic acids acting as copigments (Boulton, 2001; Rein and Heinonen, 2004).

While at 30 °C the difference between determined parameters for red currant juice and red currant juice with addition of phenolic compounds is high, with increase of temperature that difference becomes smaller, meaning that addition of phenolic compounds had higher positive effect on anthocyanins at lower temperatures. The interaction between the copigment and the pigment is exothermic and the temperature increase cause dissociation of the pigment-copigment complex resulting in colourless compounds, thus colour loss occurs (Mazza and Brouillard, 1990). Brouillard and Dangles (1994) also reported that with increase of temperature pigment-copigment complex becomes less stable, and solvation effect on the flavylium ions play a dominant role i.e. flavylium ions become more liable first to non-covalent hydration and than to covalent hydration, leading to colourless species of carbinol pseudobase and chalcone. With increase of temperature, competition between hydration and copigmentation occurs and turns against copigmentation.

Table 4. Bathochromic shift of red currant juice after addition of phenolic compounds

Samples	λ_{max} (nm)	$\Delta\lambda_{max}$ (nm)
Control	513	
Catechol	516	3
4-methyl catechol	516	3
Catechin	518	5
Chlorogenic acid	518	5
Gallic acid	517	4

Conclusions

Addition of phenolic compounds could be valuable tool for prevention of anthocyanin degradation due to copigmentation effect. Additionally, phenolic compounds possess the ability to reduce oxidative damage associated with many diseases, including cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, asthma, hepatitis, liver injury, arthritis, immune deficiency diseases and ageing (Pietta et al., 1998; Lee et al., 2000; Middleton et al., 2000). Both of those properties could improve nutritional value of food products and also could be used in food product formulation, especially functional foods.

Increase of anthocyanin content, increase the half-life, anthocyanin retention and decrease of reaction rate constants in a red currant juice samples when the phenolic compounds were added, indicates the stabilisation of anthocyanins through formation of pigment-copigment complex. Depending on temperature different phenolic compound contribute mostly to anthocyanin stability. At 30 °C the highest impact on anthocyanin stability had addition of gallic acid and catechin, at 50 °C catechin and catechol, at 70 °C catechin and 4-methyl catechol, and at 90 °C 4-methyl catechol and catechin.

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