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Copigmentation effect of phenolic compounds on red currant juice anthocyanins during storage

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Summary

Copigmentation has been suggested as a main colour stabilising mechanism in plants protecting the coloured flavylium cation from the nucleophilic attack by the water molecule. In this study influence of phenolic compounds addition (catechol, 4-methyl catechol, (+)-catechin and gallic acid) on stability of red currant juice anthocyanins (copigment:pigment molar ratio 50:1 and 100:1) during 30 days of storage at 4 °C was investigated. Stability of anthocyanins was evaluated through determination of anthocyanins, total colour difference (ΔE*), kinetic parameters and anthocyanin retention. The initial anthocyanin content of red currant juice was 44.34 mg/100 g. During storage degradation of anthocyanins occurred. After storage anthocyanin content of red currant juice was 38.87 mg/100 mL. However, in samples with addition of phenolic compounds degradation was less pronounced due to formation of pigment-copigment complex (i.e. copigmentation). Anthocyanin content in samples with addition of phenolic compounds ranged from 39.2 to 43.83 mg/100 mL, depending on phenolic compound, its concentration and storage time. The lowest degradation was observed when gallic acid was added. Monitoring only λ_{max} of absorption spectrum of juices, one can get incomplete picture of colour stability of red currant juice. It was important to monitor total colour change (ΔE*) with CIELAB colour system since all parameters are taken into account. The lowest ΔE*, after 30 days of storage, had samples with addition of catechol and (+)-catechin (0.83 and 0.86, respectively), while the highest values had samples with addition of gallic acid (1.26).

Keywords: anthocyanins, phenolic compounds, copigmentation, juice storage

Introduction

The great variety of red, blue and purple tones of the flowers and fruits in nature come from anthocyanins, glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts, important plant pigments of the flavonoid class. It has been recognized that anthocyanin-rich plant extracts might have potential as natural food colorants. So far, anthocyanins have not been broadly used in foods and beverages due to their low stability. In fact, the colour stability of anthocyanins depends on a combination of various factors, like the structure and concentration of the anthocyanin, pH, temperature, and presence of complexing agents (phenols, metals) (Mazza and Brouillard, 1990; Bąkowska et al., 2003). Important stabilisation processes result in the interaction of anthocyanins between themselves (self-association) or with other chemicals of the medium, such as metal cations (metal complexation) and copigments (copigmentation) (Asen et al., 1972; Dangles, 1997; Berké and de Freitas, 2005). Hydration is countered by these phenomena; water molecules are removed from the surface of the chromophore, thus stabilising the colour form. Copigmentation of anthocyanins is extremely important, as it is responsible for the increase in absorbance intensity (hyperchromism) and for a positive shift in the visible wavelength (bathochromism). Besides, the anthocyanin and copigment types and their relative concentration, copigmentation is shown to be dependent upon ionic strength, pH, solvent, the presence of metal salts or macrocycles and temperature (Mazza and Brouillard, 1990). Those factors have been studied in the case of fruit-derived products (Mazza and Brouillard, 1987) and wine (Brouillard and Dangles, 1994; Dariaz-Martín et al., 2002). 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, amino acids, organic acids, nucleotides, polysaccharides, metals, and anthocyanins themselves (Mazza and Brouillard, 1990, Bąkowska et al., 2003).

The possibility of using copigmentation phenomenon to improve anthocyanin color stability for food applications has been widely studied (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; Bąkowska et al., 2003; Rein and

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Heinonen, 2004; Mazzaracchio et al., 2004; Mollov et al., 2007; Awika, 2008; Oszmiański et al., 2009). The most commonly studied copigments include ferulic acid, rutin, quercetin, caffeic acid, chlorogenic acid, tannin acid (gallotannins) and rosmarinic acid. In this study influence of different phenolic compounds (catechol, 4-methyl catechol, (+)-catechin and gallic acid) on stability of red currant juice anthocyanins (copigment:pigment molar ratio 50:1 and 100:1) during 30 days of storage at 4 °C was investigated.

Materials and methods

Material

Red currant fruit (*Ribres rubrum*) 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 and gallic acid) in two different concentrations; phenolic compound:anthocyanin 50:1 and 100:1 molar ratio. Samples were stored for 30 days at 4 °C.

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 MW cyanidin-3-glucoside molecular weight (449.2), DF was dilution factor 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 was initial anthocyanin content and c_t anthocyanin content after heating time at the given temperature.

Colour measurement

Colour changes of red currant juices were monitored by colorimeter (Minolta CR-300). The chromatic values L*, a*, b* were used to calculate the total colour difference (ΔE*) of the samples. The higher L*, a*, b* values meant higher lightness, red colour, and yellow colour, respectively. Total colour difference (ΔE*) was calculated according the following formula:

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (5)$$

For each sample five measurements were conducted.

Statistical analysis

Anthocyanin content was analyzed by the analysis of variance (ANOVA) and Fisher's least significant difference (LSD) with significance defined at $P < 0.05$. All statistical analyses were carried out using the software program STATISTICA 8 (StatSoft, Inc, USA). The results were expressed as means ± standard deviation.

Results and discussion

Anthocyanin content

Results of study of influence of phenolic compounds (catechol, 4-methyl catechol, (+)-catechin and gallic acid) on anthocyanin content of red currant juice during storage are presented in Table 1. Next to influence of phenolic compounds, amount of phenolic compounds on anthocyanin stability was

also investigated. Anthocyanin content in red currant juice was 44.34 mg/100mL. After 15 days of storage, samples with addition of catechol (RCJ+C) and 4-methyl catechol (RCJ+4-MC) had lower anthocyanin content than control sample (sample without phenolic compounds addition) while samples with addition of (+)-catechin (RCJ+CT) and gallic acid (RCJ+G) had higher anthocyanin content. Anthocyanin content of red currant juice decreased from 44.34 mg/100 mL to 41.08 mg/100 mL, while this decrease was from 40.33 to 40.95 mg/100 mL when catechol and 4-methyl catechol were added and from 41.83 to 43.83 mg/100 mL when (+)-catechin and gallic acid were added. After 30 days of storage, different tendency was observed. All samples with addition of phenolic compounds improved anthocyanin stability in higher or lesser extent. Red currant juice (control sample) had a decrease of up to 38.07 mg/100 mL, while samples with addition of phenolic compounds had anthocyanin content from 39.20 to 41.58 mg/100 mL, thus in all cases higher amount than control sample. Addition of different amount of phenolic compounds had influence on anthocyanin content with some exceptions. After 15 days of storage there were no significant difference between samples with addition of catechol in different amounts, while after 30 days of storage there were no significant difference between samples with addition of catechol, 4-methyl catechol and (+)-catechin.

Table 1. Anthocyanin content (mg/100 mL) of red currant juice without and with addition of phenolic compounds during 30 days of storage at 4 °C

Samples	15 days	30 days
RCJ	41.08 ± 0.25^{a}	38.07 ± 0.35^{a}
RCJ + C 50:1	40.58 ± 0.14^{b}	39.83 ± 0.21^{b}
RCJ + C 100:1	40.45 ± 0.09^{b}	$40.20 \pm 0.14^{b,d}$
RCJ + 4-MC 50:1	40.33 ± 0.21^{b}	$39.58 \pm 0.17^{b,c}$
RCJ + 4-MC 100:1	40.95 ± 0.11^{a}	39.20 ± 0.53^{c}
RCJ + CT 50:1	41.83 ± 0.09^{c}	$40.33 \pm 0.08^{b,d,e}$
RCJ + CT 100:1	42.83 ± 0.11^{d}	40.80 ± 0.14^{e}
RCJ + GA 50:1	42.46 ± 0.18^{e}	40.83 ± 0.13^{e}
RCJ + GA 100:1	43.83 ± 0.10^{f}	41.58 ± 0.18^{f}

RCJ – red currant juice; C – catechol; 4-MC – 4-methyl catechol; CT – (+)-catechin; GA – gallic acid

Values in the same column with different superscripts (a-f) are significantly different ($P<0.05$) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD).

Rein and Heinonen (2004) showed that during 103 days of storage, sinapic acid induced the strongest colour in strawberry juice, ferulic and sinapic acid improved raspberry juice colour equally, and rosmarinic acid enhanced the colour of lingonberry and cranberry juices the most. Results of Bąkowska et al. (2003) showed that addition of quercetin, rutin, tannic acid and flavons had stabilising effect on cyanidin-3-glucoside during 3 months of storage.

Results of our experiment after 15 days of storage of samples with addition of catechol and 4-methyl catechol were not in accordance with results of Bąkowska et al. (2003) and Rein and Heinonen (2004), while after 30 days of storage the same effect of phenolic compounds were also observed in our case as in studies of previously mentioned authors, suggesting that copigmentation effect for some phenolic compounds was evident after longer storage period. Anthocyanin–copigment complexation does not always lead to visible copigmentation (enhanced coloration or improved colour stability) (Salas et al., 2004; Awika, 2008), what probably happened in our samples with catechol and 4-methyl catechol after 15 days of storage. Influence of phenolic compounds on anthocyanin stability can be explained by formation of anthocyanin/copigment complex (copigmentation). During copigmentation positive shift of wavelength occurs, in our case the shift was 3-4 nm, depending on added phenolic compounds (Kopjar et al., 2009). Berké and de Freitas (2005) observed the bathochromic effect only for monomers ((-)-epicatechin, (-)-epicatechin gallate and (+)-catechin), but the maximum shift was for (-)-epicatechin, from 527 to 532 nm.

Copigmentation phenomenon is not always predictable, and it is not well understood how different factors enhance or reduce this phenomenon. The structure of the anthocyanin aglycone seems to significantly affect rate and degree of copigmentation (Mazzaracchio et al., 2004), probably by influencing the degree of intramolecular copigmentation, as well as the hydration efficiency of the pyranic ring. Other important factors that influence the degree of copigmentation include pH, ionic strength of solution, temperature and pigment to copigment molar ratio (Davies and Mazza, 1993).

Kinetic parameters

Calculation of kinetic parameters and anthocyanin retention was also conducted (Table 2). All samples with addition of phenolic compounds had lower reaction rate constant (0.0021 to 0.0041 $days^{-1}$), higher half-lives (193.8 to 323.5 days) and anthocyanin retention (89.8 to 93.7 %) in comparison to control sample (0.0051 $days^{-1}$, 136.3 days and 85.8 %, respectively for k, $t_{1/2}$ and AR).

The lowest k values, the highest values of $t_{1/2}$ and AR were in samples with addition of gallic acid i.e. samples which had the highest anthocyanin content.

Table 2. Kinetic parameters and anthocyanin retention (AR) of red currant juice without and with addition of phenolic compounds during 30 days of storage at 4 °C

Samples	k ($days^{-1}$)	$t_{1/2}$ (days)	AR (%)
RCJ	0.0051	136.3	85.8
RCJ + C 50:1	0.0036	193.8	89.8
RCJ + C 100:1	0.0033	212.1	90.6
RCJ + 4-MC 50:1	0.0038	183.1	89.2
RCJ + 4-MC 100:1	0.0041	168.7	88.4
RCJ + CT 50:1	0.0032	219.3	90.9
RCJ + CT 100:1	0.0028	249.9	92.0
RCJ + GA 50:1	0.0027	252.9	92.0
RCJ + GA 100:1	0.0021	323.5	93.7

RCJ – red currant juice; C – catechol; 4-MC – 4-methyl catechol; CT – (+)-catechin; GA – gallic acid

Colour determination

Monitoring only λ_{max} of absorption spectrum of juices, one can get incomplete picture of colour stability of red currant juice. The CIELAB colour system enables an approach to the changes of juice colour where all parameters are taken into account (Table 3).

Table 3. Colour difference (ΔE*) of red currant juice without and with addition of phenolic compounds during 30 days of storage at 4 °C

Samples	15 days	30 days
RCJ	0.774	0.991
RCJ + C 50:1	0.621	0.825
RCJ + C 100:1	0.799	0.829
RCJ + 4-MC 50:1	0.951	1.238
RCJ + 4-MC 100:1	0.972	1.083
RCJ + CT 50:1	0.358	0.871
RCJ + CT 100:1	0.438	0.856
RCJ + GA 50:1	0.431	1.262
RCJ + GA 100:1	0.426	1.028

After 15 days of storage the lowest colour change expressed as total colour difference (ΔE*) had samples with addition of (+)-catechin and gallic acid (0.358 - 0.438), and the change was even lower than in control sample (0.774). The highest colour difference had samples with addition of 4-methyl catechol (0.951 - 0.972). After 30 days of storage the colour difference increased and tendency of this parameter changed.

Colour difference of control sample was 0.991, and the lowest colour difference was in samples with addition of catechol and (+)-catechin (0.825 - 0.871). The highest colour difference was in samples with addition of 4-methyl catechol and gallic acid (1.028 - 1.262). Interestingly the highest change in colour difference between samples after 15 days of storage and 30 days of storage was in samples with addition of (+) catechin and gallic acid i.e. samples which had the lowest colour difference after 15 days of storage. Rein and Heinonen (2004) investigated influence of addition of different phenolic acid on colour enhancement of berry juices. They showed that lingonberry and cranberry juices with rosmarinic acid, had the lowest ΔE* during 103 days of storage, suggesting stabilisation of colour. In the case of raspberry juice the similar effect was observed when ferulic acid was added, while in strawberry juice there was no significant influence of phenolic acids observed.

Conclusions

Addition of phenolic compounds to red currant juice was proven to be valuable tool for improvement of anthocyanin stability during storage. The highest anthocyanin content had samples with addition of gallic acid. Those samples also had the highest values of half lives and anthocyanin retention and the lowest values of reaction rate constant. The lowest impact on anthocyanin stability had addition of 4-methyl catechol. Taking into account overall factors influencing the colour of juice, results showed that the highest colour difference had samples with

addition of gallic acid and 4-methyl catechol while the lowest colour difference was in samples with addition of catechol and (+)-catechin.

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