Degradation of Anthocyanin Content in Sour Cherry Juice During Heat Treatment

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Summary

Sour cherry juices made from two sour cherry cultivars (Érdi bőtermő and Kántorjánosi 3) were investigated to determine their total anthocyanin content and half-life of anthocyanins during heat treatment at different temperatures (70, 80 and 90 °C) for 4 h. Before the heat treatment, Érdi bőtermő juice had higher anthocyanin concentration (812 mg/L) than Kántorjánosi 3 juice (513 mg/L). The greatest heat sensitivity of anthocyanins was measured at 90 °C, while the treatments at 80 and 70 °C caused lower thermal degradation. The loss of anthocyanins in Érdi bőtermő juice after treatment was 38, 29 and 18 %, respectively, while in Kántorjánosi 3 juice losses of 46, 29 and 19 % were observed, respectively. At 90 °C sour cherry Érdi bőtermő juice had higher half-life ($t_{1/2}$) of anthocyanins, while the Kántorjánosi 3 juice had higher $t_{1/2}$ values at 70 °C. Cyanidin-3-glucosyl-rutinoside was present in higher concentrations in both cultivars (Érdi bőtermő: 348 and Kántorjánosi 3: 200 mg/L) than cyanidin-3-rutinoside (177 and 121 mg/L) before treatment. However, during the experiment, cyanidin-3-rutinoside was proved to be more resistant to heat. Comparing the two varieties, both investigated pigment compounds were more stable in Kántorjánosi 3 than in Érdi bőtermő. Degradation rate of anthocyanins was cultivar-dependent characteristic, which should be taken into account in the food production.

Key words: Prunus cerasus, half-life of anthocyanins, heat degradation, colour changes, HPLC, juice

Introduction

Nowadays, healthy diet is becoming more and more important with key elements like fruits and vegetables. These have a high content of biologically active compounds that contribute to good health and prevention of several diseases. Sour cherry (Prunus cerasus L.) is the second most important fruit in Hungary (after apple) with the yield of 53 thousand tonnes per year (1). In the fruit industry, sour cherry is processed to puree, concentrate, juice and canned fruit. Purees are used in the bakery, confectionary or dairy industry, while juices and concentrates...
serve as the base of fruit nectars and fruit beverages. Concentrates are highly esteemed by the industry due to their intensive taste and colour (2).

The total anthocyanin content of some European sour cherry cultivars ranges from 569 to 858 mg/L, and anthocyanins mostly (approx. 80 %) consist of cyanidin compounds (2,3). In sour cherry fruit the main anthocyanin component is cyanidin-3-glucosyl-rutinoside, which gives 60–70 % of total anthocyanin content, while the second major pigment is cyanidin-3-rutinoside at 25–33 % (4,5). The following components have been found in sour cherry as well: cyanidin-3-sophoroside, cyanidin-3-glucoside (4,6), peonidin-3-glucoside (7) and peonidin-3-rutinoside (8).

However, the anthocyanin concentration in sour cherry products greatly differs depending on the method of processing. Thermal processing includes a heat treatment with maximum temperatures from 50 to 150 °C, depending on the pH of the product and the desired shelf life (9).

Anthocyanin pigments rapidly degrade during thermal processing because they are heat sensitive (10,11), which can have a dramatic impact on colour quality and may also affect nutritional properties (9). Knowledge of anthocyanin degradation kinetics, including reaction order, rate constant (k) and half-life (t1/2), is very important to predict food quality loss during thermal processing. Therefore, kinetic studies are much needed in order to minimize the undesired degradation, and to optimize the quality of specific foods (9,12). Under isothermal conditions, degradation of sour cherry anthocyanins in both juice and concentrates is characterized by first order kinetics (13).

There are several studies dealing with the effect of heat treatment on pigment concentration in fruits. Elderberry anthocyanins showed very high sensitivity to thermal treatment. After 3 h of heating at 95 °C, only 50 % of elderberry pigments remained (14). Carrying out thermal treatment at high temperature (95 °C) in combination with pasteurisation during the production of blueberry purees resulted in 43 % loss of total monomeric anthocyanins, compared to original levels found in fresh fruit (15). Other authors demonstrated that cyanidin-3-glucoside and pelargonidin-3-glucoside in blackberry and strawberry purees were significantly affected by thermal treatment at 70 °C for 2 min (16). In contrast, Rhim (17) and Kirca et al. (18) reported that anthocyanins from black carrots were reasonably stable during heating at 70, 80 and 90 °C. Based on these studies, the degree of thermal degradation of anthocyanins depends on several conditions such as magnitude and duration of heating, and characteristics of anthocyanins and raw materials.

In this study, changes of total anthocyanin content and two major anthocyanins, cyanidin-3-glucosyl-rutinoside and cyanidin-3-rutinoside are studied in two cherry juices during heat treatment at different temperatures and periods. Further aim is to determine the kinetic parameters of anthocyanin degradation in sour cherry juices in order to help to improve the predictions of quality loss.

Materials and Methods

Plant material

Érdi bőtermő and Kántorjánosi 3 (two Hungarian sour cherry cultivars) were investigated. The fruit samples were collected from the experimental field of the Research Institute for Fruit Growing and Ornamentals in Erd-Elvira major in Érd, Hungary. The fruits were picked at their optimum maturity stage in June 2011.

Chemicals and reagents

The HPLC grade solvents acetoniitrile (ACN) and methanol (MeOH) and glacial acetic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and the analytical standards of cyanidin-3-glucosyl-rutinoside (Cy-3-GR) and cyanidin-3-rutinoside (Cy-3-R) from Polyphenols Laboratories AS (Sandness, Norway). Ethanol and hydrochloric acid were obtained from Reanal (Budapest, Hungary). Fructozym P enzyme, Klar-Sol Super and ErbiGel were purchased from Kertrade Kft. (Duna-ravasâny, Hungary).

Juice preparation

Sour cherry juices from Érdi bőtermő and Kántorjánosi 3 cultivars were prepared according to industrial protocols under laboratory conditions. Sour cherries were heated to 80–85 °C in order to inactivate enzymes and to make the juice extraction easier. Then the heated samples were cooled to 40–45 °C and Fructozym P enzyme was added (0.1 mL/kg, treatment time: 60 min). The enzymatic treatment was followed by pressing of the fruits, and Fructozym P was added to the pressed juice again (0.05 mL/L, treatment time: 20–25 min). After that, pectin test was performed by adding ethanol to sour cherry juice at a ratio of 2:3. The desired clear solution (negative result) indicated that pectin molecules were broken down during enzymatic treatment. Silica 0.5 mL/L (Klar-Sol Super) and gelatin 0.1 g/L (ErbiGel) were added to the pressed juice and left to stand for approx. 30 min. The clear upper layer was then filtered under vacuum (10 mbar) through diatomaceous earth.

Heat treatment

Glass tubes were filled with clarified and filtered juices made from two sour cherry cultivars and these tubes were immersed in a water bath and heated to 70, 80 and 90 °C. The sampling was made 30, 60, 90, 120, 180 and 240 min later. After sampling, tubes were immediately cooled in ice water to stop further thermal degradation. Finally, the samples were tested with various methods to determine the effect of heat treatment on anthocyanins and on colour stability of sour cherries. The measurements were done in triplicate in each case.

Total anthocyanin content and browning ratio

Total anthocyanin content and browning ratio were determined according to the method of Füleki and Francis (19). Hydrochloric acid and ethanol were used for anthocyanin extraction. Absorbance was measured at 530 nm with U-2900A spectrophotometer (Hitachi High-Tech,
The total anthocyanin content was expressed in mg of cyanidin-3-glucoside (Cy-3-G) equivalents per L of sour cherry juice. Total anthocyanin content (in mg/L) was calculated as follows:

\[ \gamma_{\text{anthocyanin}} = A_{\text{max}} \times 15 \text{ dilution} \]

The browning can be used as a quality indicator of the samples, and it was determined based on the absorbances at minimum (410 nm) and maximum (530 nm) wavelengths (19), using the following equation:

\[ \text{Browning} = \frac{A_{\text{max}}}{A_{\text{min}}} \]

**HPLC analysis of two major anthocyanins**

The standards (0.5 mg/mL) were dissolved in methanol containing 0.1 % (by volume) hydrochloric acid and 50+ dilution was adopted as the working standard for HPLC. The Waters high-performance liquid chromatograph (Waters Co., Milford, MA, USA) was equipped with a dual absorbance detector (2487 Dual λ), a binary HPLC pump (1525), an in-line degasser, a column thermostat (set at 40 °C) and an autosampler (717 plus), and was controlled using Empower™ v. 2 software (Waters Co.). A Kinetex 2.6u C18 100A 150 mm×4.6 mm column (Phenomenex Torrance, CA, USA) was installed, and the mobile phase was 2.5 % glacial acetic acid containing water, MeOH and ACN (35:5:10). The isocratic flow rate was set to 1 mL/min, the running time was 15 min, while pressure in the column was (372±1) bar. Each injected volume was 20 μL. The samples were analyzed at 520 nm. Before injections, all samples were filtered through 0.45-μm Millex-HV PVDF syringe-driven filter unit (Millipore Co., Billerica, MA, USA). All measurements were carried out in triplicates.

**Calculation of half-life of anthocyanins**

With some exceptions (20,21), first-order kinetics (13,22–24) has been found suitable for determination of anthocyanin decomposition under both aerobic and anaerobic conditions. The first-order reaction rate constant (\(k\)) and half-life (\(t_{1/2}\)), i.e. the time needed for 50 % of anthocyanins to degrade, were calculated with the following equations (25):

\[ \ln(y/y_0) = -kt \]
\[ t_{1/2} = \ln 0.5/k \]

where \(y_0\) is the initial anthocyanin concentration and \(y_t\) is the anthocyanin concentration after time \(t\) (min) of heating at a given temperature (depending on the given experiment).

**Statistical analysis**

Statistical analysis was carried out using SPSS v. 20 software (IBM Corp., Armonk, NY, USA). A two-factor (treatment temperature and cultivar) ANOVA was done to determine their effect on total anthocyanin content and browning. In order to normalize the data of total anthocyanin content, logarithmic transformation was executed. Since the assumption of sphericity was violated both for total anthocyanin (\(\eta^2=0.61\)) and browning (\(\eta^2=0.66\)), we applied the Greenhouse-Geisser’s correction to test the within-subject effects. We also considered that the effect of size measure (denoted by partial \(\eta^2\)) is the variance explained by a given independent factor of the variance remaining after excluding the variance explained by other independent factors as well as the observed power of the test. Normality of residuals was proved using the Shapiro-Wilk’s test (p>0.05). Homogeneity of variances was tested by Levene’s test (p>0.05) and, depending on the significance level, it was followed by Tukey’s or Games-Howell’s post hoc test for the treatment temperature. Pairwise comparisons of the total anthocyanins and browning results measured after different time intervals were made by Bonferroni’s test.

**Results and Discussion**

**Changes in total anthocyanin content during heat treatment**

Total anthocyanin content of Érdi bőtermő and Kántorjánosi 3 juices was 811 and 513 mg/L, respectively (Table 1). In other studies where the pigment concentration of sour cherry juice was similarly investigated, the monomeric anthocyanin content of juice was measured within the range of 350–633 mg/L (4,5).

Table 1 shows that the treatment at 90 °C had the greatest impact on the anthocyanin content of Érdi bőtermő juices, where pigment concentration decreased from 812 to 501 mg/L (38 %). Degradation was lower at 80 °C, and the treatment for 4 h at this temperature resulted in 29 % loss of total anthocyanin content (573 mg/L) compared to the original levels found in fresh juice.

In the treatment at 70 °C, the degree of anthocyanin degradation was 18 %, resulting in 667 mg/L of anthocyanins. This shows that sour cherry pigments were the least susceptible to degradation at the lowest treatment temperature. The greatest reduction was observed after 120 min of heating which is, therefore, considered to be a critical point of heat treatment. Pigment concentration decreased more than 10 % at all treatment temperatures in samples of Érdi bőtermő.

The average total anthocyanin content in Kántorjánosi 3 juices was 513 mg/L before the treatments (Table 1), which is 37 % less than the initial value in Érdi bőtermő juices. Degradation rates in Érdi bőtermő juice at different temperatures were similar to those in Kántorjánosi 3 juice. The total pigment concentration decreased from 513 to 278 mg/L (46 % loss) at 90 °C. In a similar study, lower degradation rates of sour cherry anthocyanins were observed at 80 and 70 °C, and pigment concentration decreased by 29 and 19 % after 240 min of heat treatment. Similar degradation rates were observed in blackberry and black carrot anthocyanins at 70 and 80 °C (24,25).
was higher than 0.99 in each case. Interaction between temperature and the elapsed time or cultivar was significant ($F_{\text{temperture} \times \text{cultivar}}(2;12)=5.99$ with $p<0.05$ and $\eta^2=0.6$, and $F_{\text{temperture} \times \text{time}}(14;24)=3.26$ with $p<0.001$ and $\eta^2=0.43$). The values are expressed as mean±standard deviation (S.D.), based on $N=3$ replicates. Cy-3-G=cyanidin-3-glucoside. Different upper case letters indicate significant differences among treatments at different temperatures during the same time of treatment (read from left to right), whereas different lower case letters indicate significant differences among different treatment times at a given temperature (read from up downwards).

Concentration of two major anthocyanins in sour cherry during heat treatment

High-performance liquid chromatography analysis of sour cherry juices revealed the presence of two major anthocyanin peaks, i.e. Cy-3-GR and Cy-3-R with retention times at 4.22 and 4.86 min, respectively (Fig. 1). Other anthocyanins were present in a lower amount. The results demonstrate that Cy-3-GR is the major anthocyanin in both sour cherry cultivars, which is in accordance with the data of other authors (2,26,27). Before the heat treatment, the concentrations of Cy-3-GR and Cy-3-R in Érdi bőtermő juice were 348 and 177 mg/L, while in Kántorjánosi 3 juice they were 348 and 177 mg/L, respectively. 90 °C. Lower degradation was observed at 80 and 70 °C, which means that the concentration of this major pigment decreased to 198 and 263 mg/L, respectively, after 240 min of heating. Concentration of Cy-3-R was reduced to 58.4 mg/L at the highest treatment temperature, while 94.4 and 131 mg/L, respectively, were detected at 80 and 70 °C.

Similar trend of decrease was observed in Kántorjánosi 3 juice. The loss of Cy-3-GR was 68 %; its value decreased from 200 to 64.0 mg/L at 90 °C, to 116 mg/L at 80 °C, while 140 mg/L were measured at the lowest temperature after 240 min (Fig. 4). Concentration of Cy-3-R was also sensitive to heat treatment, as 74 % lower value (31.4 mg/L) was measured after the heating treatment at 90 °C, while the degradation at 80 and 70 °C was lower, and 67.8 and 85.6 mg/L, respectively, of Cy-3-R remained.

![Fig. 1. Typical HPLC chromatogram of sour cherry juices measured at A=520 nm](image-url)

![Fig. 2. Concentration of cyanidin-3-glucosyl-rutinoside (Cy-3-GR) and cyanidin-3-rutinoside (Cy-3-R) in Érdi bőtermő juice during heat treatment](image-url)
Comparing the two investigated sour cherry juices, the two major anthocyanin components were more stable in Kántorjánosi 3 juice although its total anthocyanin concentration was lower than in Érdi bötermő juice. Cy-3-GR is more resistant to the heat treatment than Cy-3-R, which was also reported by Verbyest et al. (22) in raspberries. The amount of Cy-3-GR is almost twice higher than of Cy-3-R in both of the two sour cherry cultivars throughout the whole experiment.

Stability of anthocyanins during heat treatment

As expected, the degradation of anthocyanins depended on temperature, being greater at higher temperatures (Table 2). This trend was similar to the results of other investigations of different sources of anthocyanins such as black carrot (25) and blood orange (29). Comparing the two sour cherry cultivars, Érdi bötermő juices had higher \( t_{1/2} \) values than Kántorjánosi 3 juices at 90 °C in most cases, which means that anthocyanins from Érdi bötermő juice were more sensitive to the treatment at the highest temperature. However, total pigment content in Kántorjánosi 3 decreased faster at 70 °C than in Érdi bötermő juices (Table 2). Based on the kinetic results, it can be concluded that heat stability of anthocyanins in sour cherry juices depended on the cultivar. Sour cherry anthocyanins were less susceptible to higher temperatures than blackberry anthocyanins. The different susceptibilities of fruit juice anthocyanins to heat might be due to their different anthocyanidin composition (30).

Table 3 shows different degrees of browning of the juices of two sour cherry cultivars after 240 min of heating, except for the Érdi bötermő juice treated at 70 °C.

![Fig. 3. Concentration of cyanidin-3-glucosyl-rutinoside (Cy-3-GR) and cyanidin-3-rutinoside (Cy-3-R) in Kántorjánosi 3 juice during heat treatment](image)

**Table 2. Kinetic parameters for the degradation of monomer anthocyanins in sour cherry juices**

<table>
<thead>
<tr>
<th>Temperature/°C</th>
<th>Érdi bötermő</th>
<th>Kántorjánosi 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>t/min ( K \cdot 10^{-3}/\text{min}^{-1} )</td>
<td>( t_{1/2}/\text{h} )</td>
<td>( K \cdot 10^{-3}/\text{min}^{-1} )</td>
</tr>
<tr>
<td>90</td>
<td>3.0 4.3 2.7</td>
<td>3.3 3.5</td>
</tr>
<tr>
<td>80</td>
<td>3.2 3.0 3.8</td>
<td>3.4 3.4</td>
</tr>
<tr>
<td>70</td>
<td>180 2.4 4.9</td>
<td>2.8 4.2</td>
</tr>
<tr>
<td></td>
<td>90 2.1 5.6 0.6 20.7</td>
<td>60 2.2 5.2 3.1 3.7</td>
</tr>
<tr>
<td></td>
<td>90 2.2 5.2 3.1 3.7</td>
<td>90 1.7 6.8 2.0 5.7</td>
</tr>
<tr>
<td></td>
<td>120 1.5 7.6 2.0 5.9</td>
<td>180 1.8 6.3 1.4 8.2</td>
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<tr>
<td></td>
<td>120 1.5 7.6 2.0 5.9</td>
<td>120 1.0 11.6 0.5 21.5</td>
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<tr>
<td></td>
<td>180 1.2 9.4 0.7 16.3</td>
<td>240 1.4 7.9 1.4 8.2</td>
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<tr>
<td></td>
<td>180 1.2 9.4 0.7 16.3</td>
<td>240 0.8 14.1 0.9 13.2</td>
</tr>
</tbody>
</table>
|               | 240 0.8 14.1 0.9 13.2  | \( k \)=first-order reaction rate constant, \( t_{1/2} \)=half-life of anthocyanins

The values are expressed as meanstandard deviation (S.D.), based on N=3 replicates. Different upper case letters indicate significant differences among treatments at different temperatures during the same time of treatment (read from left to right), whereas different lower case letters indicate significant differences among different treatment times at a given temperature (read from up downwards).
where it remained the same. However, these results suggest minimal change in the quality of the sour cherry juices. Both between-subject effects (treatment temperature and sour cherry cultivar) as well as the elapsed time (i.e., within-subject effect) had significant effects on the browning values ($F_{\text{temperature}}(2;12) = 145.54$ with $p < 0.001$; $F_{\text{cultivar}}(1;12) = 104.16$ with $p < 0.001$ and $F_{\text{temperature-cultivar}}(3.95;47.42) = 24.515$ with $p < 0.001$, respectively). Partial $\eta^2$ values were higher for between-subject effects (0.96;0.90) and lower for elapsed time effect (0.67), while the statistical power of the test was higher than 0.99 in each case. Interaction between temperature and the elapsed time was significant ($F_{\text{temperature}}(7;4.72) = 5.37$ with $p < 0.001$ and $\eta^2 = 0.47$; statistical power is higher than 0.99); however, it was insignificant between elapsed time or temperature and cultivar ($F_{\text{time}}(1;12) = 0.67$, while the partial $\eta^2$ was higher than 0.99). Interaction between elapsed time and temperature and cultivar ($F_{\text{time-cultivar}}(3.95;47.42) = 2.32$ with $p = 0.07$; $F_{\text{temperature-cultivar}}(2;12) = 2.16$ with $p = 0.16$), and for three-level type interaction ($F_{\text{time-temperature-cultivar}}(7;90;47.42;1.14$ with $p = 0.36$).

Conclusions

Sour cherry fruit is predominantly used for processing, therefore different properties of raw fruit are influenced by several technological processing steps during production. Results of this study revealed that the stability of anthocyanins in sour cherry juice depends on the level of heating. Between the two investigated juices of Hungarian sour cherry cultivars, Erdi bőtermő had higher amounts of total and cyanidin-based anthocyanins than Kántorjánosi 3 juice during the whole experiment; however, the concentration of anthocyanins in both juices was significantly affected by the thermal treatments. Comparing the two major anthocyanin components, cyanidin-3-glucosyl-rutinoside is more resistant to the heat treatment than cyanidin-3-glucosyl-rutinoside. investigating the stability of anthocyanins, we found that they degraded more quickly with the increasing temperature; however, degradation of pigments depends on the cultivar. Anthocyanin concentrations decreased, while the browning of the juices considerably increased after 4 h of treatment at 90 °C. Analysis of the combined data can be utilized for the selection of cultivars suitable for processing to health-beneficial sour cherry products. In conclusion, short-time heating at lower temperatures is recommended in order to maintain higher anthocyanin concentrations and achieve better quality parameters for sour cherry fruit juices.

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