Effect of temperature on the copigmentation interaction between aronia anthocyanins and sinaptic acid with different methods

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Abstract. The effect of temperature and concentrations on the stability of anthocyanin extract from aronia as pigment and sinapic acid as copigment was evaluated. Different temperatures and copigment concentrations were used for the investigation pigment:copigment interaction. Equilibrium constant of copigmentation process was determined. Thermodynamic parameters Gibbs free energy (ΔG), enthalpy (ΔH), entropy (ΔS) and kinetic parameters activation energy (Ea) and z – factor were calculated. At high temperatures, around 80 °C destruction was observed and after that at 25 °C, restoration of the complex was observed again according the calculations. This was conformation for reversibility of copigmentation process.

1. Introduction
Anthocyanins represent one on the most important and most widely spread groups of plant pigments of the class of flavonoids. This group of pigments was responsible for the existence of most of the red, blue, and purple colors in flowers and fruits [1]. The last years pigment:copigment couple between different acids as copigment and different pigments were studied. Intermolecular copigmentation reactions between five anthocyanins and five phenolic acids acting as copigments were invesigated [2]. Thermodynamic and kinetic parameters of copigmentation proces were studied by Dangle et al. [3]. The temperature-dependence visible spectrum of the copigmented solutions yields the copigmentation enthalpy and entropy changes. The experiments were carried out at pH 3.5 and pH 5.5. Copigmentation appears to be clearly enthalpically driven (exothermic process with unfavourable entropy change) in the case of chlorogenic acid and (+)-catechin. Relaxation kinetic measurements on the malvin-rutin system give striking evidence of the strong slowing down that a good copigment causes to the anthocyanin fading process (hydration). From the decrease in the apparent first order rate constant of hydration on copigment addition, a general method for a quick determination of the copigmentation stability constants was proposed. Changes in the visible spectra of strawberry anthocyanins were studied depending on the addition of quercetin as a copigment [4]. The investigations were provide at different temperatures between 20 °C and 50 °C. The thermodynamic parameters of the system, ΔG, ΔH, and ΔS were calculated as a function of temperature. The couple of authors investigated kinetic of pigment:copigment interactions. According the Shakeel et al. [5], the solubilities of sinapic acid (SA) were determined in eleven different neat solvents at five different temperatures (T = 298.15 K to 318.15 K) and atmospheric pressure. Experimental solubilities expressed in mole fraction of SA were correlated well with Apelblat and van't Hoff models. The results of thermodynamic analysis indicated an endothermic and entropy-driven dissolution of SA in most of the neat solvents. Gallic acid was evaluated as copigment...
for an anthocyanin crude extract of Cabernet Sauvignon grape skins [6]. Comparison of the stability of the grape anthocyanins and their interaction with gallic acid were investigated using kinetic parameters. The highest half life values for anthocyanin and percent color retention were reached when the samples were maintained at pH 3.0, temperature of 4 ± 1 °C and in the absence of light. Extracts from purple carrot and red flesh potatoes showed higher stability according Reyes et al. [7]. Stability to thermal degradation of extracts (pH=3) followed first order kinetics. Degradation parameters such as half life (t½), degradation rates (k), D-values, z-factor and Q10-values were determined. Elderberry (Sambucus nigra L.) was considered an interesting fruit as food ingredient, due to its high content of anthocyanins that give products an attractive red colour [8]. Activation energy, degradation rates (k) and half life were obtained. Kinetic parameters calculated for elderberry juice can be used to design a thermal treatment to obtain a high retention of colour and bioactive compounds. The effect of temperature on the stability of three purified anthocyanin sources in a soft drink (pH= 3, 10 °Brix) stored at (4, 20, 30 and 50) °C for 60 days was investigated by Zozio et al. [9]. Two classical empirical approaches (Arrhenius and Ball models) were used to describe the thermal degradation kinetic of these three anthocyanins. At all temperatures, the degradation rate constant (k) for black carrot anthocyanins was less than those in açai and blackberry (0.42 × 10−2, 0.77 × 10−2 and 1.08 × 10−2) d−1, respectively, at 30 °C. Anthocyanins in black carrot degraded less rapidly than those in açai and Andean blackberry. The activation energy (Ea) for degradation of black carrot anthocyanins was 63.2 ± 4.3 kJ mol−1, and 66.3 ± 2.7 kJ mol−1 and 91.2 ± 0.4 kJ mol−1 for açai and blackberry anthocyanins, respectively, at 20–50 °C. These higher Ea of blackberry anthocyanins as compared with those of black carrot and açai imply that a small temperature increase was sufficient to degrade them more rapidly. The kinetic studies on thermal stability of anthocyanins isolated from the dry calyces of Hibiscus sabdariffa L. (roselle) in aqueous solutions (55–98 °C) was studied by Gradinaru et al. [10]. The rate constants for degradation were obtained from first order reaction kinetic plots. The scope of this work is to calculate the thermodynamic and kinetic parameters at different temperatures and copigment concentrations using experimental data.

2. Experimental

2.1. Chemicals

The copigment sinapic acid was used from Sigma – Aldrich, 98 % (Germany). The reagents used for the McIlvaine buffer pH 3.4 citric acid monohydrate and disodium hydrogen phosphate dodecahydrate, were from Merck (Darmstadt, Germany). The adsorbent Amberlite XAD 16N resin was purchased from Sigma Aldrich Co. (St. Louis, MO, USA). All other reagents and solvents used were of analytical grade.

2.2. Extraction, purification and determination of anthocyanins

Aronia anthocyanins were extracted and purified as described by Shikov et al. [11]. The total monomeric anthocyanins were assessed by the pH-differential method as described in Shikov et al. [12].

2.3. Preparation of model solutions

Stock solutions of aronia extract, on the basis of the total anthocyanins, and sinapic acid were prepared in McIlvaine buffer (0.1 M, pH 3.4). Model solutions of aronia anthocyanins (1 x 10−3 M) were obtained by mixing equal volumes (5 ml) of the corresponding stock solutions and were left for equilibration (30 min at 25 °C).

2.4. Spectrophotometric measurements

Spectral changes were followed by recording the absorption spectra of model solutions from 400 to 700 nm with Halo RB–10 spectrophotometer (Dynamica Scientific, United Kingdom), using 1 cm path length cuvettes. Before measurements, samples were incubated in a thermostatic shaker water bath (NUVE, Turkey) for 30 min at 25 °C. Before measurements the samples were thermostated (VEB MLW Prufgepate-Werk Medingen sitz Freital, Germany) at 25, 40, 60 or 80 °C, respectively, at heating and then cooling to 60, 40, 25 °C.
2.5. Modelling of thermodynamic and kinetic parameters

After spectrophotometer measurements the equilibrium constant was calculated using the following equation:

$$\ln\left(\frac{A - Ao}{Ao}\right) = \ln[K] + n \times \ln[C]$$

where A and Ao - the absorption maximum values of the anthocyanin solution with and without added copigment; C - the molar copigment concentration; K - the equilibrium constant and n - the stoichiometric ratio of the reaction [4].

Thermodynamic parameters Gibbs free energy, enthalpy and entropy were calculated using the following equations:

$$\Delta G = -RT \ln K_p$$  
(2)

where R - universal gas constant (R = 8.314 J K^{-1} mol^{-1}), T - absolute temperature (K), ΔG - Gibbs free energy (kJ mol^{-1}), K – equilibrium constant.

The enthalpy was calculated by applying the Vant-Hoff equation.

$$\frac{d \ln K}{d(1/T)} = -\frac{\Delta H}{R}$$

(3)

ΔH - enthalpy for the copigmentation reaction (kJ mol^{-1}).

Once the Gibbs free energy and the enthalpy were obtained, the entropy were determined by using classic thermodynamic equation (4):

$$\Delta S = \frac{(\Delta H - \Delta G)}{T}$$

(4)

ΔS - entropy for the copigmentation reaction (kJ K^{-1} mol^{-1}).

For calculation of kinetic parameters two models Arrhenius and Ball’s were chosen.

- The first was a conventional chemical kinetic model, Arrhenius model [9, 13]. The linear relationship between lnk and 1/T was observed:

$$k = k_0 e^{-\frac{E_a}{RT}}$$

(5)

where Ea - activation energy (kJ mol^{-1}); R - universal gas constant (R = 8.314 J K^{-1} mol^{-1}), T - absolute temperature (K).

- The second model was a Ball’s model [9, 14], defines a decimal reduction time which was related to temperature via a factor z.

$$D = \ln 10k$$

(6)

$$D = D_0 10^{\frac{T}{z}}$$

(7)

where D - decimal reduction time at temperature T (s); D_0 - value of D extrapolated at 0 °C; z - expressed in °C.

The model’s parameters were identified, using linear regression on the logarithmic curves of experimental data. Although the z value was estimated from Ea using the relationship:

$$z = \ln(10) \frac{RT^2}{E_a}$$

(8)

2.6. Statistical analysis

The results reported in the present study were the mean values of two independent determinations. The data of different samples were analyzed independently by Origin Pro 7.0 software and the standard deviations of the parameters were determined.

3. Results and discussion

In this study the model solutions were prepared with stoichiometry quantity aronia anthocyanins and variations of sinapic acid as copigment change between 1:0 to 1:50 concentration. The thermodynamic parameters of the copigmentation reaction are presented in Table 1.
Table 1. Equilibrium constants and thermodynamic parameters for the copigmentation interaction between aronia anthocyanins and sinapic acid at different temperatures

| Temperature (°C) | K     | ΔG [kJ.mol⁻¹] | ΔH [kJ.mol⁻¹] | ΔS [kJ.mol⁻¹] |
|------------------|-------|--------------|---------------|--------------|
| 25               | 10046.16±2.21 | -23.83±0.81 | -28.25±0.78 | -0.014±0.009 |
| 90               | 9063.58±1.47  | -22.71±0.18 | -27.53±0.99 | -0.015±0.009 |
| 141             | 1412.14±5.66 | -19.47±0.77 | -27.25±1.15 | -0.024±0.003 |
| 26.54±1.71      | 26.54±1.71   | -9.07±0.65  | -13.94±0.49 | -0.029±0.004 |
| 1530.98±2.91    | 1530.98±2.91 | -19.69±0.42 | -27.22±1.57 | -0.023±0.004 |
| 6049.08±4.24    | 6049.08±4.24 | -22.65±0.57 | -27.35±0.64 | -0.017±0.005 |
| 9727.47±0.94    | 9727.47±0.94 | -22.75±0.84 | -28.05±1.27 | -0.015±0.006 |

The equilibrium constant exhibited different values at different temperatures. The constant (K) displayed a strong dependence on the temperature. The gradual heating from 25 to 80 °C resulted in a significant decrease of the K values at high temperature, indicating thermal instability of the pigment:copigment complex. The subsequent cooling shifted the reaction toward the product formation, regenerating the complex. This thermal reversibility of the copigmentation reaction was of particular importance, implying that the anthocyanin-stabilizing ability of the added copigments would not be cancelled by the heat treatments.

At 25 °C the constant showed the highest value. At the same temperature the Gibbs energy exhibited the highest value too: -23.83±0.81 kJ mol⁻¹. This result was similar at heating system and shows that the system was most stable at 25 °C. At temperatures above 25 °C, the stability decreased and interactions between pigment and copigment slowed down. At all temperatures in two temperature regimes negative Gibbs energies were calculated. These results were connected with the spontaneous process of copigmentation leading to formation of the products. On the basis of Gibbs energy changes (Table 1) at all the temperatures it can be concluded that the process of copigmentation is possible only at temperatures to 25 °C. According the Petrova et al. [4] appears the similar Gibbs free energy in system, but in this work observed reversibility of copigmentation process.

![Figure 1](image_url)

Figure 1. Dependence between Gibbs free energy and enthalpy and equilibrium constant of the copigmentation interaction between aronia anthocyanins and sinapic acid in model solutions upon heating system.

The enthalpy and entropy changes of the process were also negative at all temperatures. It can be concluded that such a dependence on temperature a consequence of the exothermic copigmentation
and enthalpically driven ($\Delta H < 0$) process was observed. The negative value of the entropy, $\Delta S$, indicates that copigment formation establishes greater order in the system.

The graphical view of Gibbs free energy and enthalpy dependence on the equilibrium constant was presented in Figure 1. The stability of the system was determined by the decrease in Gibbs energy to a minimal negative value. On the figure the minimal value was observed at 25 °C. The second temperature close to the first one was 40 °C. At the other temperatures, the Gibbs energy increased to values, which was connected to the break-down of the pigment: copigment bond and destroyed of the system. Enthalpy of the process exhibit the similar results. At low temperature shown the small negative enthalpies and with increase of temperature enthalpy values increase too. This two results connected with stability of pigment:copigment system at low temperature.

The kinetic investigation of system was provide by the kinetic parameters - degradation rates ($k$), Activation energy ($E_a$) and z-factor [9]. According the experimental results and calculations, interaction between pigment and copigment described as first order reaction. This result was connected with result of Gradinaru et al. [10]. Anthocyanin degradation was connected with degradation rates constant ($k$). The values of constant $k$, activation energy and z-factor are presented in Table 2.

### Table 2. Kinetic parameters for thermal investigation of aronia anthocyanin:sinapic acid interactions following the Arrhenius and Ball models.

| System               | t, °C | k, s$^{-1}$ | $E_a$, kJ mol$^{-1}$ | z, °C   |
|----------------------|-------|-------------|---------------------|--------|
| Aronia: sinapic acid | 25    | 0.0241±0.0141 | 85.868±2.12        | 21.177±1.76 |
|                      | 40    | 0.0338±0.0282 |                   |        |
|                      | 60    | 0.0651±0.0062 |                   |        |
|                      | 80    | 0.0879±0.0071 |                   |        |
|                      | 80/60 | 0.0597±0.0191 | 59.901±3.55        | 29.965±2.32 |
|                      | 60/40 | 0.0411±0.0132 |                   |        |
|                      | 40/25 | 0.0301±0.0209 |                   |        |

The kinetic investigation of system was provide the kinetic parameters degradation rates ($k$), Activation energy ($E_a$) and z-factor using two classical kinetic models Arrhenius and Ball’s. The rate constant was the smallest at 25 °C at heating and which was proof of system stability at this temperature. After heating system to 80 °C and foloweing cooling the rate constant increases and after that decreases there values. These results were associated with the reversibility of the copigmentation process in the system. Figure 2 presented dependance betwen ln$k$ and 1/T. Linear dependance and good corelations observed at two temperature range.

The activation energies ($E_a$) ranged was calculated between 85.868±2.12 at heatin to 59.901±3.55 at cooling, and the z-factor ranged between 21.177±1.76 and 29.965±2.32 compared with values of Zozio et al. [9]. This values were associated with a low z-factor and with the degradation of anthocyanin rate, which was comparatively slower at high activation energy ($E_a$). When the temperature in the system increases, the values of z-factor increases and the activation energy $E_a$ decreases. On cooling system $E_a$ was have an extremely high value again and a correspondingly low z-factor. These results were expected and were related to the irreversibility of the copigmentation process on the system. According the Kopjar et al. [15] activation energies ($E_a$) ranged between (63 and 91) kJ mol$^{-1}$, and the z-factor ranged between (18 and 27) °C confirm results obtained in this work at heating and at cooling of the system.

Similar activation energy ($E_a$) [6] was observed in grape extract 81.34 kJ mol$^{-1}$ too. In two determinations by thermodynamic (low negative Gibbs free energy) and kinetic (higher activation energy and less rate degradation) parameters the results confitm the reversibility of the copigmentation process and higher stability of system at low temperature.
4. Conclusions
The thermal stability of aronia anthocyanin:sinapic acid was provided first by heating system to 80 °C and then cooling to 25 °C. At the same temperatures destruction was observed and after that restoration of complex was observed according to the calculations. The thermodynamic and kinetic parameters confirm this result. On the basis of calculations according to the thermodynamic and kinetic parameters obtained in all temperature range it can be concluded that the process of copigmentation is possible only to temperature 40 °C or lower.

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References
[1] Nikkhah E, Khayamy M, Heidari R, Jame R 2007 Journal of Biological Sciences 7 (8) 1412
[2] Eiro M and Heinonen M 2002 Journal of Agricultural and Food Chemistry, 50 (25) 7461
[3] Dangles O and Brouillard R 1992 Can. J. Chem. 70 2174
[4] Petrova I, Shikov V, Gandova V, Mihalev K, Dimitrov D 2017 Bulgarian Chemical Communications 49 (1) 115
[5] Shakeel F, Haq N, Raish M, Anwer MK, Al-Shdefat R 2016 Journal of Molecular Liquids 222 167
[6] Falcao, A. Falcao, E. Gris, M. Bordignon-Luiz 2008 Brazilian Journal of Food Technology 11 (1) 63
[7] Reyes L and Cisneros-Zevallos L 2007 Food Chemistry 10 (3) 885
[8] Casatia C, Baezaa R, Sanchez V, Catalanob A, L’opezb P, Zamora M 2015 Journal of Berry Research 5 (4) 29
[9] Zozio S, Pallet D, Dornier M 2011 Fruits 66 (3) 203
[10] Gradinaru G, Biliaderis C, Kallithraka S, Kefalas P, Garcia-Viguera C 2003 Food Chemistry 83 (3) 423
[11] Shikov V, Kammerer D, Mihalev K, Mollov P, Carle R 2008 Journal of Agricultural and Food Chemistry 56 (18) 8521
[12] Shikov V, Mihalev K, Yoncheva N, Karagyozov V, Mollov P 2012 Journal of EcoAgriTourism 8 (2) 231
[13] Cisse M, Vaillant F, Acosta O, Dhuique-Mayer C, Dornie M 2009 Journal of Agricultural and Food Chemistry 57 (14) 6285
[14] Kopjar M, Jaksic K, Pilizota V 2012 Journal of Food Processing and Preservation 36 (6) 542
[15] Kopjar M, Pilizota V, Subaric D, Babic J 2009 Croatian Journal of Food Science and Technology 1 (1) 24

Figure. 2. Correlation relationships between degradation rate constants as a function of temperature 1/T: a) presented system at heating; b) presented system at cooling.