

# Investigation of copigment couple between Aronia anthocyanins and ascorbic acid in temperature range

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**Abstract.** In this study, the interaction between Aronia as pigment and ascorbic acid copigment is presented. The process is observed at different concentrations and at different temperatures. The equilibrium constant of interactions between them and some thermodynamic parameters were obtained. The obtained results showed that with the increase of temperature, destruction in the pigment:copigment couple was observed. Furthermore, the kinetic parameters in the system were obtained, and the reversibility of the copigmentation process was observed.

## 1 Introduction

The copigmentation process is of great importance for the food industry and therefore, has been widely investigated in numerous studies. In [1], the copigmentation process at Mulberry anthocyanins was investigated. It was determined that the intermolecular co-pigmentation improves their stability via experiment with interaction between anthocyanins and seven copigments, including a couple of phenolic acids and vanillin. Their thermal reaction kinetics were presented, and it was observed that the strongest co-pigment reaction is obtained with Ferulic acid.

In another study [2], red grape anthocyanins were studied as pigments at 25°C (room temperature) and 35°C (storage temperature). The copigment was ascorbic acid, and rosemary polyphenols were used as an anthocyanin stabilizing agent. The obtained results showed that copigmentation with a lower content of ascorbic acid was appropriate.

The pigment:copigment couple with the influence of temperature and copigment concentration between strawberry and chlorogenic acid was investigated in [3]. The equilibrium constant, Gibbs energy, enthalpy, and entropy were determined. The kinetic parameters - activation energy and z-factor - were calculated. It was observed that the copigmentation process could be reversed.

Özdemir et al. [4] presented interactions between colorants and pigments. Different colorants as anthocyanins, carotenoids, betalains, and chlorophylls, were used. During the storage period, the stability of the colorants was low.

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In [5], the copigmentation interactions between purple potato anthocyanins and metal ions were investigated using solutions with different concentrations. The hyperchromic and bathochromic effect changes presented copigmentation interactions between them. In these anthocyanins, thermal stability was achieved with the addition of sugars in the solutions.

The aim of [6] was to calculate the thermodynamic and kinetic parameters in the pigment:copigment system. Using the temperature dependence, different calculations were presented, such as the kinetic parameters, rate constant and half-life time. The copigmentation of anthocyanin leads to an increase in the hypochromic and bathochromic effects.

In another study [7], ferulic, caffeic, chlorogenic, and rosmarinic acids were used for copigmentation interaction with blackcurrant pomace anthocyanins. Solutions with a constant molar ratio of 5:1 between acids and anthocyanins were prepared and investigated at storage temperature (20°C) and at pH interval. During the storage period, Chlorogenic acid presented high levels of anthocyanins and ferulic acid had the longest half-life. In rosmarinic acid, low stability during storage was observed.

In [8], cyanidin-3-O-glucoside of three phenolic compounds and its thermal stability were investigated. The influence of the copigment – ferulic acid, dopamine, or catechin, the pigment-copigment molar ratio 1:1 to 1:100, the pH value 3-7, and the temperatures between 20 and 50°C were investigated. The study also calculated the thermodynamic parameters of the system.

This study aims to calculate the thermodynamic and kinetic parameters in the pigment:copigment system.

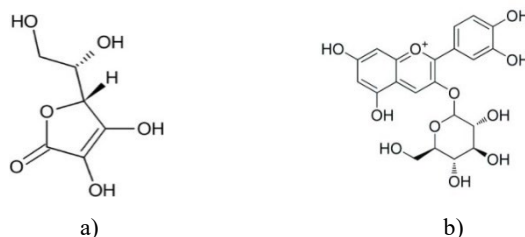
## 2 Materials and methods

### 2.1 Model solutions

McIlvaine buffer was used to preparation of stock solutions of aronia and sinapic acid. The model solutions were prepared and investigated for 30 min at 25°C.

### 2.2 Chemicals

The Aronia anthocyanins were obtained according to [9]. The ascorbic acid used was supplied by Fluka. All other reagents were pure analytical. Fig. 1 presents the structures of copigment and pigment used in this study.



**Fig. 1.** Structures of: a) ascorbic acid as copigment; b) cyanidin 3-galactoside as pigment.

### 2.3 Spectrophotometric measurements

Absorption spectra were used between 380 and 780 nm wavelength [9, 10]. Measurements were implemented with a Helios Omega spectrophotometer using 1 cm cuvettes. Before

measurements, all samples were thermostated by heating from 20°C to 60°C and then cooling from 60 °C to 20°C with a step of 10°C [11].

## 2.4 Modelling parameters

The thermodynamic parameters were presented by equation (1):

$$\Delta G = - RT \ln K \quad (1)$$

where:  $R$  – gas constant,  $J K^{-1} mol^{-1}$ ;  $T$  – temperature,  $K$ ;  $K$  – equilibrium constant.

The enthalpy was calculated by equation (2):

$$d(\ln K)/d(1/T) = - \Delta H/R \quad (2)$$

where:  $\Delta H$  – enthalpy,  $kJ mol^{-1}$

The entropy was obtained using equation (3):

$$\Delta S = (\Delta H - \Delta G)/T \quad (3)$$

where:  $\Delta S$  – entropy,  $kJ K^{-1} mol^{-1}$

The temperature dependence parameters  $Q_{10}$  and  $z$  factors were calculated according to equations (4) and (5) [12]:

$$Q_{10} = \exp\left(\frac{10E_a}{RT^2}\right) \quad (4)$$

$$z = 2.3 \frac{RT^2}{E_a} \quad (5)$$

where:  $E_a$  is the activation energy,  $kJ mol^{-1}$ .

## 3 Results and discussion

The pigment:copigment system was investigated, and results were obtained in the investigated temperature range. First, during heating between 20 and 60°C with a step of 10°C and then during cooling back to 20°C [13]. The equilibrium constant was determined and the obtained values were obtained at different temperatures. The results during the heating of the system are presented in Table 1. It is observed that the constant decrease values with the increase of temperature [14]. The highest value is presented at 30°C, and the system is the most stable. With the increase in temperature, the constant decreases, and at 60°C, it is approximately 35.

**Table 1.** Thermodynamic parameters during the heating cycle.

$t, ^\circ C$	$K, M^{-1}$	$\Delta G, kJ mol^{-1}$	$\Delta H, kJ mol^{-1}$	$\Delta S, kJ K^{-1} mol^{-1}$
20	954.99±0.1	-17.04±0.1	-24.72±0.1	-0.02±0.0
30	2130.39±0.2	-18.93±0.1	-25.89±0.1	-0.02±0.0
40	440.37±0.1	-15.39±0.3	-24.58±0.2	-0.03±0.0
50	499.63±0.1	-15.08±0.1	-23.02±0.4	-0.03±0.0
60	34.66±0.1	-8.78±0.1	-21.15±0.3	-0.04±0.0

$t$  - temperature;  $K$  - equilibrium constant;  $\Delta G$  - Gibbs free energy;  $\Delta H$  - enthalpy;  $\Delta S$  - entropy

Similar results are obtained when the system is cooling from 60 to 20°C (Table 2). The results present smaller values compared with those during heating.

**Table 2.** Thermodynamic parameters during the cooling cycle.

t, °C	K, M <sup>-1</sup>	ΔG, kJ mol <sup>-1</sup>	ΔH, kJ mol <sup>-1</sup>	ΔS, kJ K <sup>-1</sup> mol <sup>-1</sup>
50	408.21±0.1	-14.98±0.2	-22.87±0.2	-0.01±0.0
40	370.28±0.2	-15.11±0.2	-23.89±0.2	-0.02±0.0
30	2080.55±0.4	-18.44±0.1	-25.02±0.3	-0.01±0.0
20	878.14±0.2	-16.77±0.2	-24.21±0.1	-0.02±0.0

The Gibbs free energies are between 18.93 and 8.78 during heating. A better result is observed at 30°C: both during heating and cooling. At 60°C, the Gibbs energy is equal to 8.78 kJ mol<sup>-1</sup>. These results are connected with the destruction of the complex system at the same temperature. After cooling, the energy restores the high value. Two other parameters - enthalpy and entropy - were obtained after calculations. The negative values were observed during heating and cooling. The process is determined as spontaneous.

According to the presented results of calculations and experiments, the copigmentation process is presented as reversible and appropriate at temperatures around 20 to 30°C.

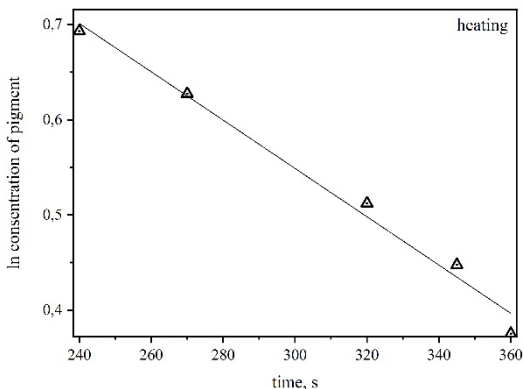
Table 3 presents results for the kinetic parameters using Arrhenius equation. The rate constants were calculated during heating and cooling. The constant increases with the increase of temperature and is high at 60°C. After cooling, it decreases again. At high temperatures, the complex is destroyed.

**Table 3.** Kinetic parameters on the copigmentation interaction between aronia and ascorbic acid.

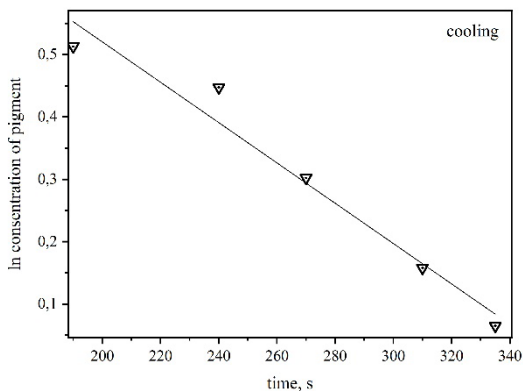
System	t, °C	k, s <sup>-1</sup>	E <sub>a</sub> , kJ mol <sup>-1</sup>	Q <sub>10</sub>	z, °C
aronia: ascorbic acid	20	0.068±0.0	29.55±0.16	1.71±0.1	40.176±0.21
	30	0.059±0.0			
	40	0.086±0.0			
	50	0.112±0.0			
	60	0.181±0.0	28.71±0.17	1.67±0.1	43.524±0.24
	50	0.103±0.0			
	40	0.079±0.0			
	30	0.054±0.0			
20	0.063±0.0				

t - temperature; k - rate constant; E<sub>a</sub> - activation energy; Q<sub>10</sub> - factor; z - factor

Depending on the order, there are three types of reactions – zero-order, first-order, and second-order. Copigmentation reactions normally present the first-order reactions connected with the change of concentration of the components. In Figs. 2 and 3, these orders are presented at heating and cooling. The correlation coefficients are at heating  $R^2 = 0.982$ , and cooling  $R^2 = 0.951$ .



**Fig. 2.** Determination of the order of the reaction at heating.



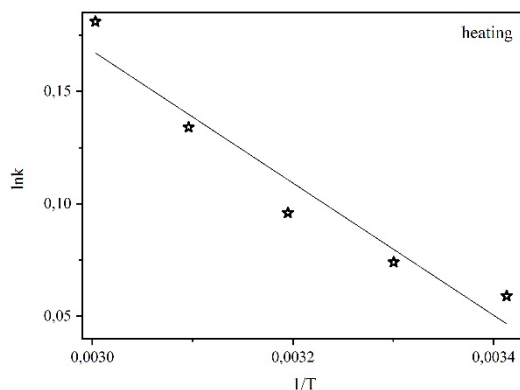
**Fig. 3.** Determination of the order of the reaction at cooling.

The kinetic parameters activation energy  $E_a$ ,  $Q_{10}$ , and  $z$ -factors were calculated too. Figs. 4 and 5 present two temperature ranges for interactions between  $\ln k$  and  $1/T$ . The activation energy is  $29.55 \text{ kJ mol}^{-1}$  at heating and  $28.71 \text{ kJ mol}^{-1}$  at cooling according to [15].

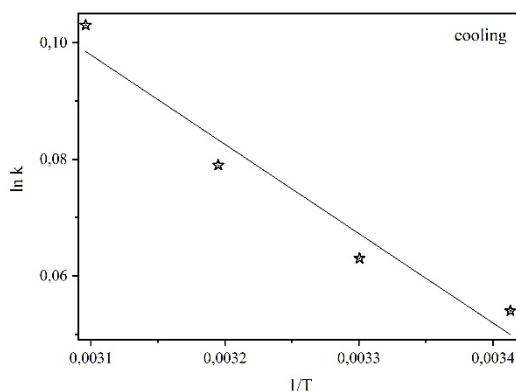
Representing the temperature dependence of a reaction as a  $Q_{10}$  factor relates the rate of a reaction. When  $Q_{10}$  is introduced, the reaction is faster if the temperature is increased by 10K.

These parameters depend on the temperature, even if the activation energy is constant. A high value of  $Q_{10}$  is associated with a greater activation energy. In general, higher  $Q_{10}$  values suggest that less temperature change is required to induce some change in oxidation rate. The results show that  $Q_{10}$  is 1.71 at heating and 1.67. at cooling. The  $z$ -factor is studied in aronia anthocyanins as pigment and ascorbic acid as copigment. Investigations were done at heating and at cooling. All results are presented in Table 3.

Thermodynamic and kinetic parameters are restoring their values when heating to 60°C and cooling to 20°C. This is confirmation again for the reversibility of the copigmentation process.



**Fig. 4.** Dependence between  $\ln k$  and  $1/T$  at heating.



**Fig. 5.** Dependence between  $\ln k$  and  $1/T$  at cooling.

## 4 Conclusion

Pigment:copigment couple aronia anthocyanin:ascorbic acid was investigated by thermodynamic and kinetic parameters. In the implemented experiment, two temperature ranges were used to investigate the copigmentation interaction. It was observed that at 30°C, both during heating and cooling, the thermodynamic and kinetic parameters showed the best results. This is a confirmation of the reversibility of the copigmentation reaction.

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