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Thermodynamic And Kinetic Investigation In Copigmentation Reaction Between Strawberry Anthocyanins And Chlorogenic Acid

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Abstract
The effect of temperature on the stability of anthocyanin extract from strawberry as pigment and chlorogenic 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 ΔG, ΔH, ΔS and kinetic parameters Ea and z – factor were calculated. At high temperatures, around 50 ^{o}C destruction was observed and after that at 20 ^{o}C, restoration of the complex was observed again according the calculations. This is conformation for reversibility of copigmentation process.

Keywords: *Chlorogenic acid, Anthocyanins, Thermodynamic and kinetic parameters.*

1. Introduction

Anthocyanins, rich in many fruits, are regarded as colours and bioactive compounds with high antioxidative capacity. The colour of red fruity products is unstable and susceptible to degradation [1]. The many factors which influence of colour stability are oxygen, temperature, pH and etc. [2]. The copigmentation reaction exhibited interactions as intermolecular and intramolecular copigmentation. The interactions between strawberry anthocyanin and chlorogenic acid presented intermolecular copigmentation [3]. The last years pigment:copigment couple between chlorogenic acid as copigment and different pigments were studied. Intermolecular copigmentation reactions between five anthocyanins and five phenolic acids acting as copigments were invesigated [4]. The couple of authors investigated kinetic of pigment:copigment interactions. Gallic acid was evaluated as copigment for an anthocyanin crude extract of Cabernet Sauvignon grape skins [5]. 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. The effect of pH and temperature on the stability and visual colour of aqueous anthocyanin extracts from purple- and red-flesh potatoes was evaluated and compared to commercial extracts from grape and purple carrot [6]. Extracts from purple carrot and red flesh potatoes showed higher stability than grape and purple-flesh potato extracts. Stability to thermal degradation of extracts (pH 3) followed first-order kinetics. Degradation parameters such as $t_{1/2}$, k-, D-, z- and Q10-values were determined. Elderberry (*Sambucus nigra L.*) is considered an interesting fruit as food ingredient, due to its high content of anthocyanins that give products an attractive red colour [7]. 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 [8]. 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 is 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. [9]. The rate constants for degradation were obtained from first-order reaction kinetic plots. Copigmentation of anthocyanins with chlorogenic acid did not seem to improve their stability in solution. Influence of addition of sugars and chlorogenic acid on anthocyanin content in blackberry juice were investigated [10]. Addition of chlorogenic acid caused increase of anthocyanin content. Rose petal polyphenols were used as stabilizing agents in heated model system by Shikov et al [11].

The scope of this work is to calculate thermodynamic and kinetic parameters at different temperatures using experimental data and determined stability of system according influence of temperature and copigment concentration.

2. Materials and methods

2.1 Chemicals

Strawberry anthocyanins were extracted and purified as described by Shikov et al. [11].

The copigment chlorogenic acid used by Fluka AG, 97%. The reagents used for the McIlvaine buffer pH 3.4 citric acid monohydrate and disodium hydrogen phosphate dodecahydrate, were from Merck (Darmstadt, Germany). All other reagents and solvents used were of analytical grade.

2.2. Spectrophotometric measurements

Absorption from 380 to 780 nm measure with a Helios Omega UV-Vis spectrophotometer equipped with VISION*lite* software (all from Thermo Fisher Scientific, Madison, WI, USA) using 1 cm path length cuvettes. Before measurements the samples (model solutions) were thermostated (VEB MLW PRUFGEPATE-WERK Medingen sitz Freital, Germany) at 20, 30, 40 or 50 °C at heating and after that at cooling to 40, 30 and 20^{o} C, respectively.

2.3. Modelling of thermodynamic and kinetic parameters

After spectrophotometer measurements the equilibrium constant was calculated using the following equation: ln[(A - Ao)/Ao] = ln[K] + n × ln[C], where A and Ao are the absorption maximum values of the anthocyanin solution with and without added copigment; C is the molar copigment concentration; K is the equilibrium constant and n is the stoichiometric ratio of the reaction [12]. The dependence of ln[(A - Ao)/Ao] on the copigment concentration, ln[(A - Ao)/Ao] = *f*(ln[C]), is a straight line with a slope and intercept equal to n and ln[K].

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

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

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

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

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

ΔH is enthalpy for the co-pigmentation reaction (kJ mol^{-1}).

Once the Gibbs free energy and the enthalpy were obtained, the entropy can be determined by using classic thermodynamic equation (3):

$$\Delta S = \frac{(\Delta H - \Delta G)}{T} \quad (3)$$

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

For calculation of kinetic parameters two models were chosen.

– The first is a conventional chemical kinetic model, Arrhenius model [8, 13]. There is linear relationship between lnK and 1/T:

$$k = k_0 e^{-\frac{E_a}{RT}} \quad (4)$$

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

– The second model is a Ball's model [8, 14], defines a decimal reduction time which is related to temperature via a factor z.

$$D = \ln 10k \quad (5)$$

$$D = D_0 10^{-\frac{T}{z}} \quad (6)$$

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

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

$$z = \ln(10)\frac{RT^2}{E_a} \quad (7)$$

2.4. Data analysis

The results obtained in this work are values of two or three determinations and the coefficients of variation were less of 2.5 %. Linear regression analysis was performed using OriginPro 7.0.

3. Results and discussion

The studies in model pigment:copigment system were provided for two experiments at different temperatures. First the model systems were heated from 20 to 50 ^{o}C and after that were cooled to 20 ^{o}C.

At all temperatures equilibrium constant was determined. The equilibrium constant exhibited different values at two provide experiments. First the equilibrium constant decrease values with increase of temperature (Table 1). The smaller value is at 50 ^{o}C. After that, with decrease of temperature again the constant increase values and at 20 ^{o}C after cooling the constant is 5264.310 M^{-1} (Table 1) compared with value at the same temperature 20^{o} C at heating 6681.981 M^{-1}. At this temperature the constant showed the highest value. In the same temperature the Gibbs energy exhibited maximal value - 21.820 kJ mol^{-1}. This result show, that the system was the most stable at 20 ^{o}C. With increase of temperature more to 50 ^{o}C stability decrease and interactions between pigment:copigment couple decompose. At 50 ^{o}C the Gibbs energy is equal to - 16.986 kJ mol^{-1}. After heating to 50 ^{o}C and following cooling to 20 ^{o}C the Gibbs energy restore their value and equal to - 21.229 kJ mol^{-1}. The negative values of Gibbs free energy were observed in all cases. These results connected with spontaneous copigmentation process and reversibility of copigment interaction.

Table 1. Equilibrium constants and thermodynamic parameters for the copigmentation interaction between strawberry anthocyanins and chlorogenic acid at different temperatures

t, ^{o}C	K [M^{-1}]	ΔG [kJ mol^{-1}]	ΔH [kJ mol^{-1}]	ΔS [kJ K^{-1} mol^{-1}]
20	6681.981	-21.820	-26.819	-0.0168
30	3718.791	-20.369	-26.189	-0.0195
40	2775.720	-19.644	-25.874	-0.0209
50	949.541	-16.986	-24.720	-0.0259
50/40	1151.140	-17.463	-24.927	-0.0250
40/30	2673.802	-19.551	-25.834	-0.0211
30/20	5264.310	-21.229	-26.563	-0.0179

According to the results shown in Table 1, the pigment:copigment complex is restore when cooling the system. The reversibility of the copigmentation process was observed from another authors [15]. On the basis of Gibbs energy changes in the all temperatures it can be concluded that the process of copigmentation is possible only at temperatures 30 ^{o}C or lower.

The calculated enthalpy and entropy of the copigmentation process were negative at all temperatures too. It can be concluded that dependence on temperature is a exothermic copigmentation process, ΔH < 0. The effect of temperature in pigment:copigment system presented by Chatelier's principle and can be used to predict the effect of change in conditions on a chemical equilibrium. It can be stated as: When any system at equilibrium is subjected to change in concentration, temperature, volume or pressure, then the system readjusts itself to counteract the effect of the applied change and a new equilibrium is established. According the equilibrium constant values, exothermic process and heating of system adjusts the equilibrium in direction of formation of the reagents. When in system observed exothermic process and decrease temperature (cooling system) the equilibrium adjusts in direction of formation of the products. These results confirm, that the enthalpy increases to positive values when temperature is increased to 50 ^{o}C and system pigment:copigment decreases stability. The negative value of the entropy (ΔS) indicates that copigment formation establishes greater order in the system. Dependence between enthalpy-entropy compensation is presented in Figure 1.

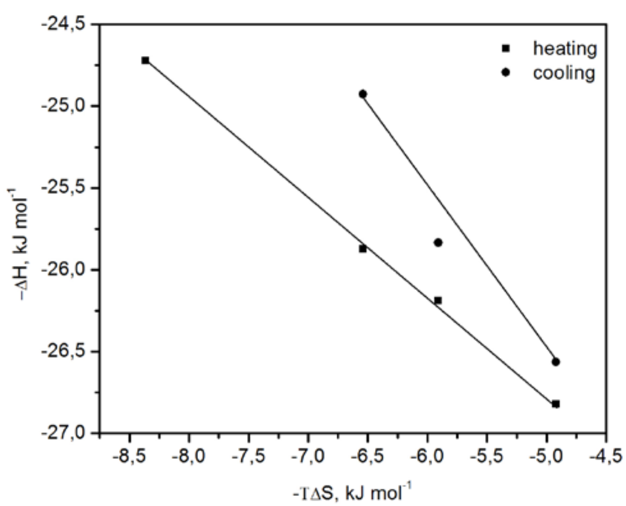


Fig. 1. Dependance of enthalpy-entropy compensation for the copigmentation interaction between strawberry anthocyanins and chlorogenic acid at heating and at cooling.

In the figure good correlation was observed between enthalpy-entropy changes at different temperatures. This figure presents two experimental results, one – connected with increase of temperature and one – decrease to initial temperature, 20 ^{o}C.

Table 2. CIELab colour parameters of strawberry anthocyanin:chlorogenic acid at heating and cooling of thermal treatment performed at different temperatures

Molar ratio pigment:copigment	Δλmax.	ΔAmax.	L	a	b
t=20° C					
1:0	500	0.546	84.8	28.1	20.0
1:2	501	0.553	84.8	28.6	20.1
1:4	501	0.557	83.8	29.6	20.2
1:6	501	0.566	83.7	29.9	20.6
1:8	500	0.572	83.8	31.0	20.5
1:10	502	0.585	83.1	31.4	20.3
t=30° C					
1:0	500	0.581	85.0	27.1	21.9
1:2	500	0.583	85.0	28.0	21.9
1:4	501	0.606	84.5	27.9	22.2
1:6	501	0.608	84.4	28.5	22.2
1:8	500	0.621	84.5	29.6	22.7
1:10	500	0.636	83.4	29.4	22.8
t=40° C					
1:0	500	0.546	85.9	26.5	19.9
1:2	500	0.555	85.8	27.2	20.4
1:4	501	0.596	86.5	29.1	21.2
1:6	501	0.608	85.4	30.0	21.9
1:8	500	0.574	85.1	28.1	20.8
1:10	500	0.588	84.5	28.3	21.0
t=50° C					
1:0	500	0.534	85.4	16.5	20.1
1:2	500	0.536	85.3	27.2	20.0
1:4	504	0.577	84.1	29.2	20.6
1:6	503	0.572	84.2	29.4	20.5
1:8	503	0.546	85.0	28.2	20.1
1:10	502	0.556	84.3	27.6	20.2
t=50/40° C					
1:0	500	1.139	87.8	16.7	12.1
1:2	500	0.522	85.8	26.5	19.4
1:4	500	0.530	85.3	27.1	19.3
1:6	500	0.823	86.5	23.5	18.0
1:8	500	0.569	84.3	28.9	20.6
1:10	500	0.576	84.1	29.4	20.4
t=40/30° C					
1:0	500	0.532	85.2	26.5	19.7
1:2	500	0.525	85.4	26.7	19.1
1:4	500	0.533	85.3	27.3	19.6
1:6	501	0.554	84.4	28.0	19.9
1:8	501	0.578	83.6	28.7	20.7
1:10	500	0.597	82.8	29.2	20.7
t=30/20° C					
1:0	500	0.541	85.2	26.8	20.2
1:2	500	0.532	85.2	26.9	19.4
1:4	501	0.541	85.1	27.4	19.9
1:6	500	0.560	84.3	28.2	20.0
1:8	500	0.585	83.5	29.0	20.8
1:10	500	0.605	82.5	29.5	20.8

The spots exhibit almost linear dependence. This is connected with the gradual increase of temperature and decrease of enthalpy change.

After determined stability of pigment:copigment couple with dates of equilibrium constant and thermodynamic parameters the spectrophotometer measurements were provide at all temperatures and colour parameters were determined after heat treatment. Some authors connect this parameters with kinetic of the process [13, 14]. In Table 2 presented the values of L, a and b. The colour parameters are: L for lightness, a for redness and b for yellowness. Colorimetric determinations for system stability presented by Gonet et al. [16] and Shikov [17].

According the colorimetric investigation of system [16, 17] decrease of L values connected with copigmentation process. In Table 2 observed that at 20 °C decrease lightness L and increase colour parameter a (associated to red colour). At the same temperature at higher copigment concentration L decrease again and this confirm previous investigation [17] that high copigment concentration stabilized system. With increase of temperature to 50 °C the L values increase and with following cooling decrease again. This is conformation for destroy system at higher temperature and restore pigment:copigment interaction at lower temperature. Big differences at L and a values at heating and cooling at 20 °C do not seen.

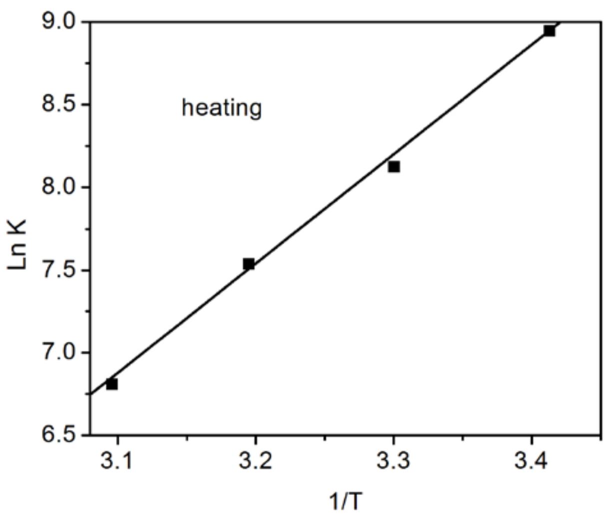
The effect of temperature for pigment:copigment interaction was investigated using Arrhenius and Ball models. Arrhenius equation expressed by the linearized plotting ln K against 1/T. The temperature dependence of K was quantified by the activation energy Ea. z - factor is another kinetic parameter, determined stability of investigated system express connection between decimal reduction $\log_{10}$ (D) as a function of temperature. The results of Ea and z presented in Table 3. Between Ea and z - factor had reciprocal dependence and the appropriate results observed in same table. Activation energy and z - factor determined for two temperature range. At heating system to 50 °C and at cooling to 20 °C.

Table 3. Kinetic parameters for thermal investigation of strawberry anthocyanin:chlorogenic acid interactions following the Arrhenius and Ball models

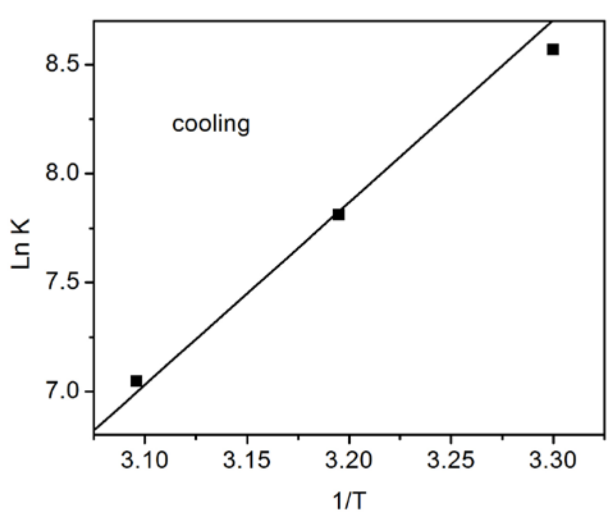
Pigment:copigment	Ea, kJ mol^{-1}	R^2	z, °C
Strawberry anthocyanin: chlorogenic acid 1:0/1:10	51.175	0.965	35.535
	57.955	0.997	30.348

Two activation energies exhibited close values and this is prove again for restore copigment complex at lower temperature. The activation energies (Ea) ranged is between (51 and 57) kJ mol^{-1}, and the z - factor ranged between (30 and 35) °C. According [8] activation energies (Ea) ranged between (63 and 91) kJ mol^{-1}, and the z - factor ranged between (18 and 27) °C. The authors conclude that higher activation energy implies the less rate degradation and higher stability of system. The results obtained in this work is closed with this observed in literature.

The graphical view between Ln K and 1/T are presented in Figure 2 a and b.



2 a)



2 b)

Fig. 2. Plot of Ln K for the copigmentation interaction between strawberry anthocyanins and chlorogenic acid as a function of reciprocal temperature.

In the figure good correlation at different temperatures were observed. The figures shows that plot between logarithm of K and 1/T is a linear. Two activation energies at heating and cooling obtained by the slope of the graphic.

Conclusion

The thermal stability of strawberry anthocyanin:rose polyphenols was provide first by heating system to 50 ^{o}C and then cooling to 20 ^{o}C. At the same temperatures destruction was observed and after that the observed complex was restored. The thermodynamic parameters exhibited negative values at all temperatures and this is proof for a stable complex. The colour and kinetic parameters confirm too destroy and restore of complex at different temperatures. On the basis of thermodynamic and kinetic parameters obtained in all temperature range it can be concluded that the process of copigmentation is possible only at temperature 30^{o} C or lower.

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