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Kinetic parameters of the copigmentation effect of caffeic acid and strawberry anthocyanins

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The effect of temperature on the stability of the copigmentation complex of strawberry anthocyanin extract as pigment and caffeic acid as copigment was investigated. The system was studied with a high concentration of caffeic acid 1:20 to 1:100 molar ratio. Different temperatures and copigment concentrations were used for the investigated pigment:copigment interaction and kinetic parameters such as activation energy (Ea), z – factor and degradation rates (k) were calculated. According to the calculated results, at high temperatures (50ºC) destruction of the complex was observed. Decreasing the temperature in the range of 20–30ºC did not lead to restoration of the complex, indicating irreversibility of the copigmentation process.

Key words: Copigmentation, Caffeic acid, Anthocyanins, Kinetic parameters

INTRODUCTION

Anthocyanins belong to the class of flavonoids and represent one on the most important and most widely spread groups of plant pigments [1]. They are known for their ability to form copigments with other phenolic compounds, thus affecting the colour appearance of plant material and anthocyanin-rich foods. There are many studies on the copigmentation process and the stability of copigment complexes in different foods. For example, spectrophotometric measurements ($\Delta\lambda$, ΔA) revealed that there is an interaction between a crude anthocyanin extract from Cabernet Sauvignon grape extracts and caffeic acid suggesting copigmentation in both model and yoghurt systems [2]. The addition of caffeic acid (1:1 w/v) significantly increased the stability of anthocyanins in both systems. Petrova *et al*. studied the interactions between strawberry anthocyanins as pigment and caffeic acid as copigment finding no reversibility of the copigmentation process [3]. Chang *et al*., investigated the copigmentation effects of exogenous caffeic acid and ferulic acid on anthocyanins stability in blackberry juice using visible absorption spectra and HPLC-DAD-MS [4]. The results showed that both caffeic acid and ferulic acid significantly increase the absorption intensity and wavelength maximum (λmax) of anthocyanins in blackberry juice. The copigmentation is an effective way to enhance the colour of anthocyanins [5]. For example, Sharara [6] investigated the possibility to increase the stability of anthocyanins in roselle extract during storage by the addition of some phenolic acids (ferulic, cinnamic and coumaric) as a natural alternative instead of harmful synthetic ones. Copigmentation effects of citric acid, DL-malic acid, tartaric acid, caffeic acid and ferulic acid on purple sweet potato anthocyanins were studied. Results showed that these five organic acids increased the absorption value of purple sweet potato anthocyanins, but did not change the anthocyanin composition. The half-life of anthocyanin degradation (15.3 h) was increased by citric acid, DL-malic acid and tartaric acid to 19.1, 19.0 and 16.9 h at 90ºC, but was reduced by caffeic acid and ferulic acid to 1.8 and 1.6 h, respectively, indicating an unfavorable effect on the thermal stability of purple sweet potato anthocyanins. Sari observed investigated the copigmentation effect of *Berberis crataegina* anthocyanins with phenolic acids (i.e., tannic, gallic, ferulic and caffeic acids) at 90ºC in various pigment:copigment molar ratios (1:10; 1:50; 1:100) [7]. Copigmentation increased the stability of anthocyanins, but the increase in pigment:copigment ratios resulted differently on each phenolic acid compound, i.e., as the molar ratio increased in gallic acid copigmented samples, the anthocyanin degradation decreased, but conversely, it increased in all the other acids used. Additionally, the compatibility of the degradation of copigmented anthocyanins to first order and Weibull distribution models was studied. New colorimetric variables have been defined in the

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uniform CIELAB colour space to assess the quantitative and qualitative colour changes induced by copigmentation and their incidence on visual perception [8]. The copigmentation process was assayed in a model solution between malvidin 3-glucoside and three phenolic compounds (catechin, epicatechin and caffeic asid) as a function of pH and the pigment:copigment molar ratio. Increasing copigment concentration induced perceptible colour changes at molar ratios higher than 1:2, consisting in a bluish and darkening effect of anthocyanin solutions. Among the different CIELAB attributes, hue difference was the best-correlated parameter with the increase of copigment concentration, providing the relevance of this physicochemical phenomenon on the qualitative changes of anthocyanin colour.

Patras *et al*. [9] investigated the effect of storage time and temperature on the degradation of bioactive compounds such as ascorbic acid, anthocyanins, total phenols, colour and total antioxidant capacity of strawberry jam. The results indicated that lightness (*L*) value decreased significantly over 28 days of storage at 4 and 15°C, with lower values measured at higher temperatures. Anthocyanins, ascorbic acid and colour degradation followed first-order kinetics where the rate constant increased with an increase in the temperature. Wang *et al.* [10] studied the thermal and storage stabilities of anthocyanins in blackberry juice and concentrate over the temperature ranges of 60–90°C and 5–37°C. Results indicate that the thermal degradation of anthocyanins followed first-order reaction kinetics. The temperature-dependent degradation was adequately modelled by the Arrhenius equation. The activation energy value for the degradation of blackberry anthocyanins during heating was 58.95 kJ/mol for the 8.90 Brix blackberry juice. During storage, antocyanins in the 65.0 Brix blackberry juice concentrate degraded more rapidly than that in 8.90 Brix blackberry juice, with activation energies of 65.06 kJ mol^{-1} and 75.5 kJ mol^{-1}, respectively.

The aim of this work was to calculate the kinetic parameters of the copigmentation effect between caffeic acid and strawberry anthocyanins, and to determine the stability of the system as a function of temperature and copigment concentration.

EXPERIMENTAL

Chemicals

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

Strawberry anthocyanins extraction and isolation

Strawberry anthocyanins were extracted and purified *via* the following procedure: Frozen strawberries were thawed and manually squeezed in a beaker. The homogenized purée was extracted overnight at 4°C using methanol acidified with hydrochloric acid (1%, v/v) at a solvent/solid ratio of 2.5:1 (v/w). The extraction mixture was filtered and the organic solvent was evaporated under vacuum (30°C). To remove sugars, salts, and amino acids from the crude extracts, samples were purified using a column (465 × 30 mm i.d.) filled with adsorption resin Amberlite XAD 16N (Sigma Aldrich Co., St. Louis, MO, USA). Prior to sample application, the resin was conditioned and equilibrated by rinsing with 500 ml of methanol and 1000 ml of water, and acidified with trifluoroacetic acid (TFA, pH 2). Subsequently, 250 ml of the aqueous strawberry extract were applied and the column was rinsed with 1000 ml of acidified water (pH 2). For elution of the pigments at least 500 ml of a mixture of methanol and acidified water (TFA, pH 2) (95:5, v/v) was applied until the column was colorless. The organic solvent of the eluate was evaporated under vacuum (30°C). To separate anthocyanins from colorless polyphenols, further purification was performed by extracting the aqueous phase three times with the same volume of ethyl acetate. After evaporation and concentration under vacuum (30 °C), the residue was lyophilized for 72 h. Total monomeric anthocyanins were assessed by the pH-differential method.

Sample preparation

Stock solutions of strawberry extract with concentration 1×10^{-4} M (on the basis of total anthocyanins expressed as pelargonidin 3-glucoside equivalents) and caffeic acid in different concentrations (2.10^{-3}, 4.10^{-3}, 6.10^{-3}, 8.10^{-3}, 1.10^{-2}), were prepared in McIlvaine buffer (0.1 M, pH 3.4). Model solutions of strawberry anthocyanins and caffeic acid were obtained by mixing equal volumes (5 ml) of the corresponding stock solutions and were left for equilibration for 30 min at room temperature. Before spectrophotometric measurements, model solutions of samples (model solutions) were thermostated (VEB MLW Prüfgerätewerk Medingen, Sitz Freital, Germany)

at 20, 30, 40 or 50°C at heating and after that at cooling to 40, 30 and 20ºC, respectively.

Spectrophotometric measurements

Absorption from 380 to 780 nm was measured using 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.

Modelling of kinetic parameters

For calculation of kinetic parameters two models were chosen.

– The first is a conventional chemical kinetic model, Arrhenius model [11, 12]. There is a linear relationship between ln K and 1/T:

$$k = k_0 e^{-\frac{E_a}{RT}} \quad (1)$$

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 Ball's model [11. 13], which defines a decimal reduction time which is related to temperature *via* a factor z.

$$D = \ln 10 k \quad (2)$$

$$D = D_0 10^{-\frac{T}{z}} \quad (3)$$

where: D is 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. The z value could be estimated from Ea using the relationship:

$$z = \ln(10) \frac{RT^2}{E_a} \quad (4)$$

Statistical analysis

The results reported in the present study are the mean values of at least two determinations and the coefficients of variation were found to be below 2.5 % in all cases. Linear regression analysis was performed using the statistical package of Microsoft Excel®.

RESULTS AND DISCUSSION

The kinetic investigation of the copigmentation system in our study was done using the following kinetic parameters: degradation rate (k), activation energy (Ea) and z-factor [11]. According to the results and especially the high value of the correlation coefficient (R), the interaction between pigment and copigment could be described as first order reaction (Table 1). These results are in compliance with the results of Shikov *et al*. [14] and Gonnet [15] and the correlation coefficient (R) > 0.9 in all cases confirms that the degradation of visual colour follows a first order reaction at all temperatures [18]. Anthocyanin degradation is connected with the half-life ($t_{1/2}$) and degradation rates (k), but Ea is connected with the z-factor.

Table 1. Kinetic parameters for thermal investigation of pigment:copigment interactions between strawberry anthocyanin and caffeic acid following the Arrhenius and Ball models.

System	t, ºC	k, s^{-1}	R^2	$t_{1/2}$, s	Ea, kJ mol^{-1}	R^2	z, ºC
Anthocyanins :caffeic acid 1:20/1:100	20	0.00463	0.941	149.709	20.533	0.927	88.561
	30	0.00521	0.917	133.042			
	40	0.00695	0.931	99.733			
	50	0.00834	0.921	83.111			
	50/40	0.00927	0.924	74.773	15.801	0.841	111.319
	40/30	0.00758	0.912	91.444			
	30/20	0.00731	0.873	94.822			

The constant k was obtained for the corresponding system when changing the concentration for a specified time. Then the rate constants for the different temperatures were calculated. The increase in anthocyanin half-life degradation time ($t_{1/2}$) means decreasing the rate of their degradation [16], whereas low values of the constant are related to low degradation rate of the reaction and high stability of the system. During heating, the lowest rate constant was at 20°C, which proves system stability at this temperature. The high values of the degradation rate during cooling is an evidence for the destruction of the complex and the irreversibility of the copigmentation process.

The anthocyanin degradation rate constants obtained for each model solution were plotted as a function of heating temperature and linear dependence was observed (Figure 1). The obtained results are in agreement with previous studies including strawberry anthocyanins [14, 15, 17] Using linear regression, the degradation rate was analyzed using Eq. (1) to determine the overall

order and rate constant (k) for the degradation reaction.

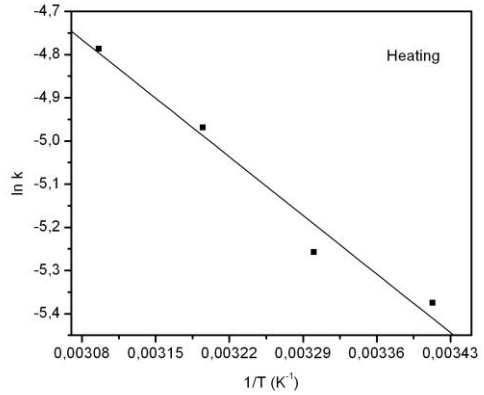

Fig.1. Arrhenius plots for degradation of anthocyanins in strawberry fruits extracts at heating.

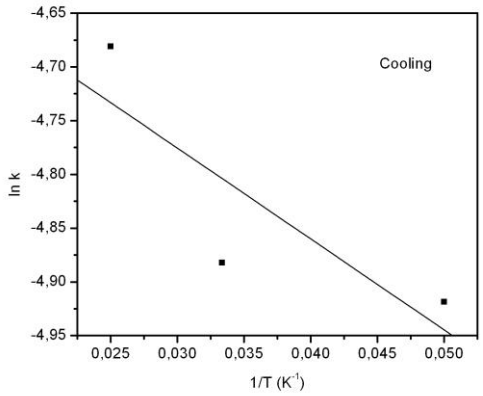

Fig. 2. Arrhenius plots for degradation of anthocyanins in strawberry fruits extracts at cooling.

Figure 2 shows a similar dependence of degradation rates (k) *versus* 1/T at cooling. There is some deviation of the points from the straight line, which is connected with the destruction of the copigment complex without subsequent restoration after cooling.

Based on the experimental results, the activation energy (Ea) and z-factor were calculated. It was observed that this system is characterized with low Ea and high z–factor. These results are connected with the low stability of the system and the irreversibility of the copigmentation process during cooling. The activation energies (Ea) ranged between 20 kJ mol^{-1} at heating to 15 kJ mol^{-1} at cooling and z-factor ranged from 88 to 111°C. According to these results, it could be concluded that higher activation energy implies lower rate of degradation and higher stability of the system. Similar Ea were reported for colour degradation of green vegetable purées. Activation energies of 28.55, 41.15 and 34.01 kJ mol^{-1} for spinach purée, mustard leaves and a mixed purée, respectively, were reported by Ahmed *et al*. [18].

Besides the kinetic parameters, we determined the colour parameters of the system at all temperatures (Table 2). Some authors connect these parameters with the kinetics of the process [12, 13]. These parameters are: *L* for lightness, *a* for redness and *b* for yellowness. According to the colorimetric investigation, the decrease of *L* values was related to the copigmentation process. We observed that lightness (L) decreased at 20ºC and pigment:copigment ratio of 1:100, which is an evidence for a copigmentation effect. Increasing the temperature to 50ºC led to elevation of L values, but the subsequent cooling did not decrease L values. This is a confirmation for the irreversible destruction of the complex at higher temperatures, which is not restored at lower temperature.

Table 2. CIELab colour parameters of strawberry anthocyanin:caffeic acid complex at heating and cooling.

Molar ratio of pigment:copigment	λmax.	Amax.	L	a	b
t = 20ºC					
1:0	501	0.575	84.9	28.4	21.5
1:20	504	0.647	81.9	33.2	20.9
1:40	504	0.697	80.2	36.7	20.9
1:60	506	0.756	78.2	39.9	21.1
1:80	509	0.773	74.0	40.1	20.1
1:100	510	0.779	73.2	41.9	20.1
t = 30ºC					
1:0	501	0.526	85.8	26.6	19.6
1:20	503	0.587	83.6	30.6	19.9
1:40	505	0.640	81.5	33.9	19.6
1:60	506	0.704	79.5	37.5	20.4
1:80	507	0.706	79.1	37.8	19.0
1:100	508	0.722	78.0	37.0	20.6
t = 40ºC					
1:0	501	0.535	84.1	25.5	19.4
1:20	503	0.562	83.9	29.3	19.1

1:40	504	0.607	81.9	31.6	18.9
1:60	506	0.684	79.2	34.2	20.4
1:80	504	0.680	81.5	34.0	18.3
1:100	504	0.626	81.0	34.5	18.2
t = 50ºC					
1:0	500	0.530	84.6	25.7	19.8
1:20	502	0.571	83.7	29.6	19.4
1:40	504	0.626	81.1	32.0	19.0
1:60	506	0.961	61.7	30.1	17.4
1:80	507	0.637	80.3	34.2	18.4
1:100	507	0.703	77.8	35.0	21.0
t = 50/40ºC					
1:0	500	0.790	81.9	33.8	29.9
1:20	501	0.891	78.2	39.0	30.1
1:40	503	0.913	77.9	41.2	29.7
1:60	506	0.924	77.5	42.0	30.1
1:80	507	0.918	77.1	41.3	30.2
1:100	504	0.940	76.8	41.4	29.7
t = 40/30ºC					
1:0	500	0.793	81.7	33.9	30.0
1:20	502	0.884	78.8	39.3	30.2
1:40	504	0.913	77.4	40.4	29.6
1:60	506	0.918	77.5	41.0	29.8
1:80	506	0.953	76.4	41.8	29.8
1:100	508	0.954	76.1	41.3	28.9
t = 30/20ºC					
1:0	500	0.806	81.5	34.1	30.4
1:20	504	0.897	78.5	40.2	30.1
1:40	505	0.923	77.4	40.8	29.6
1:60	505	0.926	77.4	41.3	29.6
1:80	507	0.956	76.3	41.1	29.7
1:100	507	0.960	76.2	41.7	29.9

CONCLUSIONS

The thermal stability of strawberry anthocyanin:caffeic acid (1:20 to 1:100) complex was proved first by heating the system to 50ºC and then cooling to 20ºC. At high temperatures (around 40-50ºC) destruction was observed and there was not restoration of the complex at 20ºC. This was confirmed by the calculated kinetic parameters and the colorimetric investigation of the system. On the basis of the calculated kinetic parameters it could be concluded that the copigmentation process is possible only at a
temperature of 30ºC or lower.

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КИНЕТИЧНИ ПАРАМЕТРИ НА КОПИГМЕНТАЦИОННИТЕ РЕАКЦИИ МЕЖДУ КАФЕЕНА КИСЕЛИНА И АНТОЦИАНИ ОТ ЯГОДИ

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(Резюме)

Изследван е ефектът на температурата върху стабилността на комплекса между антоциани от ягоди като пигмент и кафеена киселина като копигмент. Системата е изследвана при високи концентрации на кафеената киселина с моларно съотношение от 1:20 до 1:100. Използвани са различни температури и концентрации на копигмента за изследване на системата пигмент:копигмент, изчислени са кинетични параметри като активираща енергия (Ea), z - фактор и скоростна константа (k). Според изчислените резултати, при високи температури (50 °C) се наблюдава разрушаване на комплекса. Понижаването на температурата в диапазона 20-30 °C не доведе до възстановяване на комплекса, което доказва необратимост на процеса на копигментация.