# Effects of pH on the stability of cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside in aqueous solution

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# Abstract

The colour variation, colour intensity and stability at various pH values (2.0, 4.0, 7.0 and 9.0) of cyanidin 3-*O*- $\beta$ -glucopyranoside (Cy3Glc) and its aglycone cyanidin were investigated during a period of 8 hours storage at 25 °C. Our data showed that pH of aqueous solution had impact on spectroscopic profile of cyanidin and Cy3Glc. Beginning with the most acidic solutions, increasing the pH induce bathochromic shifts of absorbance maximum in the visible range for all examined pH values (with the exception pH 4.0 for cyanidin), while the presence of the 3-glucosidic substitution induce hypsochromic shift. Compared to cyanidin, Cy3Glc has higher colour intensity and higher stability in the whole pH range, except at pH 7.0. The 3-glucosidic substitution influences on the colour intensity of Cy3Glc in the alkaline region. After 8-hour incubation of Cy3Glc and cyanidin at pH 2.0 and 25 °C, 99% of Cy3Glc and only 27% of cyanidin remained unchanged.

*Keywords*: anthocyanins, anthocyanidins, cyanidin, cyanidin 3-glucopyranoside, colour variation, colour intensity, stability, brown index, UV–Vis absorption spectra.

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The anthocyanins are the large water-soluble group of natural pigments responsible for the attractive colours - ranging from strawberry red to blue – of most fruits, flowers, leaves, and some vegetables. More than 225 individual compounds are known. Chemically, they are glycosides of 2-phenylbenzopyrylium or flavylium salts. Anthocyanin sugars comprise monosides (glucose, galactose, rhamnose and arabinose), biosides, and triosides (linear and branched-chain). Additionally, the sugars can be acylated, notably with phenolic acids such as para-coumaric and caffeic acids which impart stability on the molecule by intra-molecular interactions [1].

Anthocyanins are commercially used in acid solutions such as soft drinks (usually within the pH range 2.5–3.8) where they are red (due to the flavyilium cation). At higher pH values (6 and upwards) they turn blue, due to formation of quinonoidal bases [1]. Since anthocyanins form the red and blue colours of most fruits and vegetables they provide the attractive colours of many fruit juices, wines, jams and preserves [2,3].

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Commercial applications of anthocyanins include sugar confectionery, jams, and bakery toppings as well as soft drinks [1]. There is worldwide interest in additional use of anthocyanins as a consequence of perceived consumer preferences as well as legislative action, which has continued the delisting of approved artificial dyes [2]. Today, there is considerable interest in the development of food colourants from natural sources to replace synthetic food colourants [4,5]. The reason behind this is to develop safe, economical, and efficient food colourants to replace the banned coal tar and azo dyes [4,6]. Many of the products so coloured are exported to countries where regulations do not permit the use of artificial colours or where there is consumer resistance to artificial additives [1]. Here, coloured anthocyanins have some advantages: they are safe, coloured especially in the red region, and relatively soluble, which simplifies their incorporation into aqueous food systems [4,7].

However, there are some limitations to the use of anthocyanins as food colourants, which include their chemical instability, their need for purification, and their tinctorial power, which is nearly 100-fold lower than that of the coal tar dyes. In food products, a number of reactions can occur, although the major problem associated with the use of anthocyanins as food colourants is their temperature, oxygen, light and enzymatic instability [4,7–12]. A particular problem is the pH influence on their behavior [3,7]. Based on observation of a few relatively simple anthocyanins in vitro, the following scheme is generally accepted [7,13,14]: at a  $pH \leq 3$ , the orange, red or purple flavylium cation predominates. As the pH increases, kinetic and thermodynamic competition occurs between the hydratation reaction on position 2 of the flavylium cation and the proton transfer reactions related to the acidic hydroxyl groups of the aglycone. While the first reaction gives a colourless hemiacetal form, which can undergo ring opening to a yellow chalcone, the latter reactions give rise to more violet quinonoidal bases. Further deprotonation of the quinonoidal bases can take place at pH values between 6 and 7 with the formation of purplish, resonance-stabilised quinonoid anions. It is generally accepted that anthocyanins exhibit their most intense colour when they are in their flavylium cation form [7]. At the pH values typical for fresh and processed fruits and vegetables, each anthocyanin will thus most probably be represented by a mixture of equilibrium forms [8].

The anthocyanins has great importance due to their demonstrated pharmacological activities [15,16]. Numerous studies have reported the beneficial health effects of consuming dietary fruits and vegetables containing anthocyanins [17-19]. They have attracted much attention in relation to their physiological activities, and their role has become an important issue in the relationship between health and human diet. In particular, the potential positive effects associated with consumption of fruit-derived foods are attributed to the presence of such natural compounds [20]. The anthocyanins have several biological activities, including antioxidant, antihepatocarcinogenic, anti-inflammatory, anti-tumor, neuroprotective, antihemolytic, anti-diabetic, hypolipidemic and cancer chemopreventive [21-35]. Epidemiological studies have suggested that anthocyanins have cardioprotective functions in human [36], and other studies have suggested that anthocyanins inhibit tumor-cell growth in vitro and suppress tumor growth in vivo [37].

An intensive research has been done to identify the content of potential anthocyanin sources [9], and the content of the principal commercial available colourant



Cyanidin 3-O- $\beta$ -glucopyranoside (Cy3glc) is a typical representative for the simple type of anthocyanins found in elderberry, blueberry, cowberry, whortleberry, blackcurrant, roselle, black chokeberry, etc. [3]. In this paper the colour and stability of Cy3Glc and his aglycone moiety of cyanidin (Figure 1), in aqueous solutions, were examined at four pH values between 2.0 and 9.0. The colour and stability changes were measured during incubation at different pH values in the period of 8 h at 25 °C. Thus, it has been possible to compare under various pH conditions and times impacts of 3-glucosidic substitution, on colour and stability. The results vary tremendously, and this emphasizes the importance of structure on anthocyanin and anthocyanidin properties.

# **EXPERIMENTAL**

#### **Chemicals and reagents**

The chloride salts of cyanidin (2-(3,4-dihydroxyphenyl)chromenylium-3,5,7-triol chloride, CAS number:



cyanidin





cyanidin 3-glucoside

528-58-5, C<sub>15</sub>H<sub>11</sub>O<sub>6</sub>Cl, molecular weight 322.7 g/mol) and cyanidin 3-*O*-β-glucopyranoside ((2*S*,3*R*,4*S*,5*S*,6*R*)--2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol chloride, CAS number: 7084-24-4, C<sub>21</sub>H<sub>21</sub>O<sub>11</sub>Cl, molecular weight 484.8 g/mol) were from Polyphenols Laboratories AS (Sandnes, Norway). Acetic acid, ammonium acetate, citric acid monohydrate, formic acid, sodium citrate and methanol were obtained from Merck (Darmstadt, Germany). Ammonium formate and ammonium hydroxide were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Aqueous solutions were prepared from Milli-Q water (resistivity >18 MΩ cm) (Millipore, Bedford, MA, USA).

#### Measurements of colour and stability

To determine the colour and stability the spectrophotometric analysis of cyanidin and Cy3Glc kept at different pH values was performed in specified time intervals in the period of 8 h. Buffer solutions of four different pH values with  $2.5 \times 10^{-2}$  mol dm<sup>-3</sup> concentration were prepared for dilution of cyanidin and Cy3Glc. The following buffers were used: ammonium formate/formic acid (pH 2.0), sodium acetate/acetic acid (pH 4.0), sodium citrate/citric acid (pH 7.0) and ammonium acetate/ammonium hydroxide (pH 9.0). The pH values of the various samples did not change during storage. The colour and stability of cyanidin and Cy3Glc were determined at 25.0±0.1 °C. The chloride salts of the cyanidin and Cy3Glc were dissolved in each buffer to a final concentration of  $5 \times 10^{-5}$  mol dm<sup>-3</sup>. The visible absorption spectra (380-800 nm) of the cyanidin and Cy3Glc solutions were recorded at specified pH values at 25.0±0.1 °C. Pure buffers were used as blank. Spectrophotometric measurements were made immediately after dissolution and then after specified time intervals in the period of 8 h. The absorbencies in the visible range of the freshly made samples were between 0.01 and 0.96 absorbance units, and none sample were diluted before measurements. The spectral behavior of anthocyanins is dependent on solvent [2], and substances present in the solution may influence the colour and stability. The impacts of atmospheric oxygen and other factors, such as the composition of the buffer solutions, were not examined in this study.

# Instruments

The accurate pH values for each buffer solution were measured by Mettler Toledo S20 Seven Easy pH meter (Mettler Toledo, Schwerzenbach, Switzerland) equipped with Mettler Toledo InLab electrode (Mettler Toledo, Schwerzenbach, Switzerland).

The spectrophotometric measurements were performed using a Cary 100 Bio UV–Visible spectrophotometer (Varian, Mulgrave, Victoria, Australia) in a thermostated 10-mm-path-length quartz cell.

## **RESULTS AND DISCUSSION**

According to Cabrita et al. [2] and Fossen et al. [3], the colour variations of cyanidin and Cy3Glc were expressed as the changes in the positions of the absorbance maximum in the visible range ( $\lambda_{max-vis}$ ), colour intensities were measured as absorbance values at visible absorbance maximum  $\lambda_{max-vis}$  immediately after dissolution  $(t_0)$  and after a certain time interval and expressed as molar absorptivities (a in  $dm^3 mol^{-1} cm^{-1}$ ), and the stability was expressed as the percentage of the absorbance remained after a certain time interval, measured at initial  $\lambda_{max-vis}$ . Brown index (BI) was expressed as the absorbance ratio at 430 nm by that at 520 nm according to the Malien-Aubert et al. [38,39]. The pH values of the dissolution have a large influence on the spectroscopic profiles of the cyanidin and Cy3Glc obtained after dissolution in the selected buffers prior to the analysis. The self-association of cyanidin and Cy3Glc occur at high concentrations (> $10^{-3}$  mol dm<sup>-3</sup>) [40]. In our investigations, cyanidin and Cy3Glc were dissolved to final concentration of  $5 \times 10^{-5}$  mol dm<sup>-3</sup>. At this low concentrations cyanidin and Cy3Glc exist as monomers in the studied solutions [41].

# Colour variation of cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside

The most common way to indicate anthocyanin colours is based on presentation of visible  $\lambda_{vis-max}$  values from UV/Vis absorption spectra. By plotting the  $\lambda_{vis-max}$  values (Table 1) obtained for the cyanidin and Cy3Glc immediately after dissolution in aqueous solutions at different pH values, a similar buffered pattern was achieved (Figure 2). The following tendency was established for Cy3Glc: beginning with the most acidic solution, increase in pH produced bathochromic shifts (Figure 2). This pattern correlates well with earlier reports for Cy3Glc [2,3,8]. At the pH 4.0, cyanidin showed no spectral band in the visible spectrum. At the other pH values, special higher pH values, maxima of absorbance in the visible range for cyanidin resulted in

Table 1. Visible absorbance maxima ( $\lambda_{vis-max}$  nm) and molar absorptivities of spectral bands in the visible range (a, dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup>) for the cyanidin and Cy3Glc ( $5 \times 10^{-5}$  mol dm<sup>-3</sup>) immediately after dissolution in buffered aqueous solutions at 25 °C

рН	Суа	anidin	Cy3Glc					
	$\lambda_{ m vis-max}$	а	$\lambda_{ m vis-m}$	nax	а			
2.0	517	5034.42	508		19297.16			
4.0	0	0	513		2429.10			
7.0	570	1467.17	549	441	7732.31	7904.24		
9.0	591	1503.02	569		12349.24			

bathochromic shift (Figure 2). The visible absorbance maxima for cyanidin were in all instances (except pH 4.0) higher than the corresponding  $\lambda_{\rm vis-max}$  values for Cy3Glc (Table 1). This reveals the impact of the 3-glycosylation in Cy3Glc on the position of the visible absorption maximum: the presence of the 3-glucosidic substitution causes hypsochromic shift.



Figure 2. Visible absorbance maxima ( $\lambda_{vis-max} / nm$ ) at different pH values, for the cyanidin and Cy3Glc ( $5 \times 10^{-5}$  mol dm<sup>-3</sup>) immediately after dissolution in buffered aqueous solutions at 25 °C.

# Colour intensity of cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside

The pH variation affects the colour intensities of the cyanidin and Cy3Glc (Table 1). Comparing the molar absorptivities of spectral bands in the visible range for cyanidin and Cy3Glc (Figures 3 and 4) it can be seen that 3-glucosidic substitution strongly increases the molar absorptivity of the aglycone moiety. The molar absorptivities were highest at pH 2.0 for the both pigments and strongly decreased toward pH 4.0, where local minimum for Cy3Glc are achieved, while cyanidin shows no spectral band in the visible spectrum. According to the previously published data simple anthocyanins, like Cy3Glc, are unstable and are quickly decolourized by hydration at the 2-position of the anthocyanidin skeleton in the pH region 5-7 [3,13], which is in good agreement with our results here. Further pH increase cause increase in molar absorptivities for the both pigments. The similarity between the curves indicates that the both pigments have the same type and distribution of equilibrium forms (Figures 5 and 6): the colourful favylium form dominates at pH 2.0, and the occurrence of colourless hemiacetal forms increases toward pH 4.0 [2,3,7,13,41,42]. At pH values above 7.0, cyanidin and Cy3Glc shows a hyperchromic effect until pH 9.0, when anthocyanins are expected to occur mainly in their guinonoidal and guinonoidal anion forms [2,7,13,41,42]. It was noticed that Cy3Glc showed relatively high *a* values in alkaline solutions. The ratio between the absorbance at the local maximum in the alkaline region at pH 9.0 and at pH 2.0 for cyanidin was 0.32, while the ratio for Cy3Glc was 0.65 indicating that 3-glucosidic substitution is favorable for colour intensity in the alkaline region. Obtained results for molar absorptivities of Cy3Glc (Table 1) is in good accordance with previously published data [2,3].



Figure 3. Molar absorptivities (a /  $dm^3 mo\Gamma^1 cm^{-1}$ ) of spectral bands in the visible range for the cyanidin and Cy3Glc (5×10<sup>-5</sup> mol  $dm^{-3}$ ) immediately after dissolution in buffered aqueous solutions at 25 °C.

Molar absorptivities of Cy3Glc solution during storage at pH 2.0 and 25 °C were high and constant, according to the flavylium cationic structure [7,13,41,42] (Figure 4A). Surprisingly, molar absorptivities of cyanidin solution during storage at pH 2.0 and 25 °C decrease, although it is in the form of flavylium cation [7,13] (Figure 4A). This indicated the shift of the coloured flavylium cation into the other structures. Molar absorptivities of Cy3Glc solution at pH 4.0 and 25 °C were significantly lower compared to the pH 2.0, but were constant during storage (Figure 4B). At pH 4.0 cyanidin does not show the spectral band in the visible spectrum (Figure 4B). At pH 7.0 Cy3Glc showed two bands with visible absorbance maximum at 441 and 549 nm (Figure 4C). The Cy3Glc showed great decrease in molar absorptivities with time at 25 °C. At the same conditions cyanidin shows low and almost constant molar absorptivity values (Figure 4C). At pH 9.0 the initial, relatively high value of Cy3Glc molar absorptivity decreases during storage at 25 °C. The molar absorptivity of cyanidin at pH 9.0 was low and slightly decreases during storage. This reveals the impact of the 3-glycosylation in Cy3Glc on the molar absorptivities: the presence of the 3-glucosidic substitution strongly increased molar absorptivities at all examined pH values and improved stability at pH 2.0 and 4.0.



Figure 4. Molar absorptivities (a) of spectral bands in the visible range (measured at  $\lambda_{vis-max}$ ) for the cyanidin ( $\bullet$ ) and Cy3Glc ( $\circ, \blacktriangle$ ) (5×10<sup>-5</sup> mol dm<sup>-3</sup>) solutions after different time of storage at 25 °C in buffered aqueous solutions at different pH values.

# Cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside stability on storage at 25 °C

The stabilities of cyanidin and Cy3Glc highly depend on pH and structure. The cyanidin and Cy3Glc were incubated for 8 h in buffered solutions at 4 different pH values at 25 °C, and their visible absorption spectra were registered at determined time intervals. According to Cabrita et al. [2] stability was described on the basis of absorbance changes measured at the cyanidin and Cy3Glc  $\lambda_{\text{vis-max}}$  for each pH value. During incubation at 25 °C and at pH 2.0, Cy3Glc showed significantly higher visible absorption values in comparison with those of cyaniding (Table 2, Figures 5A and 6A). According to the Brouillard [7], in water, for ordinary anthocyanins, the only stable coloured species is the flavylium cation, which is generally obtained for pH values lower than 3. The Cy3Glc, in accordance with this, showed stability above 98% after 8 h at 25 °C and at pH 2.0 (Figures 6A and 7A). At pH 4.0 after 8 h Cy3Glc showed stability above 90%, although the corresponding colour intensities are modest (Table 2, Figures 6B and 7B). During incubation Cy3Glc at pH 7.0 and 25 °C a strong decrease in visible absorbance took place (Table 2, Figures 6C and 7C). After 8 h of incubation it was found that stability decreased rapidly as pH increased toward pH 7.0, with stability values around 30% (Figure 7C). The stability of Cy3Glc slightly increased as pH increased into the alkaline region, and at pH 9.0 displayed around 50% stability after 8 h at this pH value (Figures 6D and 7D). Spectrophotometric analysis at pH 2.0 during 8 h revealed strong decrease of the absorption band in visible range for cyanidin solution. At this pH cyanidin exhibited low stability, despite being findings in the form of flavylium cation [7,13]. Cyanidin kept only 27% of their initial absorbance after 8 h incubation at 25 °C (Figure 7A). Figure 7A and Table 2 clearly show that the Cy3Glc is much more stable than his aglycone cyanidin. According to the Brouillard [7], since anthocyanidins have been shown to be unstable in water and much less soluble than anthocyanins, glycosilation is assumed to confer solubility and stability to the pigment. At pH 4.0 cyanidin shows no spec-



Figure 5. Visible spectra of cyanidin during 8-hour incubation at different pH values: A) 2.0; B) 4.0; C) 7.0; D) 9.0 during 8 h of storage at 25  $^{\circ}$ C. The concentration of cyanidin was 5×10<sup>-5</sup> mol dm<sup>-3</sup>, and the temperature was 25  $^{\circ}$ C.

tral band in the visible spectrum (Figure 5B). However, the stability of cyanidin improved as pH increased toward pH 7.0 (Figures 5C and 7C). In fact, cyanidin showed some degree of stability only at this pH value, although the corresponding colour intensities are low. Cyanidin displayed around 50% stability after 8 h at this pH value. On the other hand, cyanidin was very unstable at alkaline values, and kept only around 17% of their initial absorbance at pH 9.0 (Figures 5D and 7D). Only at pH 7.0 the stability of cyanidin became higher than that of Cy3Glc, while at all the other pH values Cy3Glc showed higher stability (Figure 7). From a structural point of view, it seems that the presence of the 3-glucosidic substitution strongly increase stability of aglycone moiety, possibly by protecting the flavylium nucleus from nucleophilic attack of water molecule at C-2 that, which leads to the colourless forms, hemiacetals and chalcones (Table 2, Figures 5–7).

## Brown index of cyanidin and Cy3Glc on storage at 25 °C

The initial brown index (*BI*, absorbance ratio, at 430 nm divided by that at 520 nm), for cyanidin and Cy3Glc

at lowest pH were similar and amounted around 0.3 (Figure 8). At pH 2.0 cyanidin and Cy3Glc exist predominately as red-orange flavylium cations ( $\lambda_{vis-max}$  for cyanidin was at 517 nm and  $\lambda_{\text{vis-max}}$  for Cy3Glc was at 508 nm, Table 1) [7,41,42]. The cyanidin and Cy3Glc displayed very low BI values at pH 2.0 during 8 h of storage at 25 °C. The BI for Cy3Glc remained the same during experiment (Figure 8A). The cyanidin showed very gradually increase of BI, which was accompanied with decrease in absorbance at  $\lambda_{vis-max}$  (Figure 5A and 8A). At pH 4.0 Cy3Glc was stabile (Figure 7B) with initial BI below 0.48 (Figure 8B). At this pH value BI for Cy3Glc remained almost constant during 8 hours of storage at 25 °C. At the pH 4.0 cyanidin showed no spectral bands in the visible spectrum (Figure 5B), and displayed BI values >1. The cyanidin solution at pH 4.0 thus was colourless with yellowish shades. At pH 7.0 Cy3Glc showed two spectral bands in the visible region with absorbance maximum at 441 and 549 nm (Figure 6C). Accordingly to the high absorbance values at 441 nm the BI (absorbance ratio, that at 430 nm divided by that



Figure 6. Visible spectra of Cy3Glc during 8-hour incubation at different pH values: A) 2.0; B) 4.0; C) 7.0; D) 9.0. The concentration of Cy3Glc was  $5 \times 10^{-5}$  mol dm<sup>-3</sup>, and the temperature was 25 °C.

Table 2. Absorbance of cyanidin and Cy3Glc (5×10<sup>-5</sup> mol dm<sup>-3</sup>) solutions, (measured at  $\lambda_{vis-max}$ ) at pH 2–9 during 8 h of storage at 25  $^{\circ}$ C, in the dark using air atmosphere; the upper and lower value in each interval correspond to cyanidin and Cy3Glc, respectively

	Time, h											
рн	0	0.25	0.5	1	1.5	2	3	4	5	6	7	8
2.00	0.25	0.22	0.20	0.19	0.17	0.16	0.13	0.12	0.09	0.08	0.08	0.07
	0.96	0.96	0.96	0.96	0.96	0.95	0.96	0.95	0.95	0.95	0.95	0.95
4.00	0	0	0	0	0	0	0	0	0	0	0	0
	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
7.00	0.07	0.07	0.06	0.06	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04
441 nm	0.40	0.35	0.32	0.30	0.27	0.25	0.21	0.18	0.16	0.14	0.13	0.13
449 nm	0.39	0.34	0.31	0.29	0.26	0.24	0.20	0.17	0.15	0.13	0.12	0.11
9.00	0.08	0.05	0.04	0.03	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01
	0.62	0.60	0.58	0.57	0.55	0.52	0.48	0.43	0.39	0.35	0.32	0.29

at 520 nm) for Cy3Glc was 1.09, thus giving yellowish shades [38]. The gradually increase in BI values with time (Figure 8C) was accompanied with decrease in visible absorbance at these two absorbance maxima and change in the absorbance ratio at these two wave-

lengths (Figure 6C). The initial *BI* value for cyanidin at pH 7.0 was 0.89 and gradually increases with the time (Figure 8C). The increase in *BI* values with time was accompanied with very gradual loss of colour in the visible range (decrease in absorbance, Figure 5C). How-



Figure 7. Percentages of remaining absorbance (measured at  $\lambda_{vis-max}$ ) after different time of incubation at 25 °C in buffered aqueous solutions at different pH values: A) 2.0; B) 4.0; C) 7.0; D) 9.0, for the cyanidin ( $\bullet$ ) and Cy3Glc ( $\circ$ ,  $\blacktriangle$ ) (5×10<sup>-5</sup> mol dm<sup>-3</sup>) solutions.

ever, cyanidin displayed BI values <1 during experiment (Figure 8C). The initial BI for Cy3Glc at pH 9.0 was 0.55 and gradually increase during 8 h (Figure 8D) accompanied by decrease in visible absorbance (Figure 6D). However, BI values were low and remained <1 all the time. At pH 9.0 cyanidin displayed very low absorbance values (Figure 5D) and initial BI values 0.98. The BI values after 15 min became >1, and remained higher than 1 during experiment. The gradual increase in BI values with time (Figure 8D) was accompanied with gradual decrease in visible absorption bands and changes in their position. The BI appears to be sensitive indicator of the stability of cyanidin and Cy3Glc at different pH values (Figure 5D). By comparing Figures 5-8 it can be seen that at pH 2.0 and 4.0 Cy3Glc is stabile and it has very low and constant BI values, while at pH 7.0 and 9.0 was unstable and BI values gradually increased all the time. The cyanidin was unstable at all examined pH values, which was accompanied by gradual increase in BI values during storage. After a

while, the Cy3glc turned on yellow at pH 7.0, while some effect was seen for cyanidin at pH 4.0 and 9.0.

# CONCLUSION

The cyanidin and Cy3Glc display great differences in colour variation, colour intensity, stability and BI. At an obtained pH values, such differences mainly result from the structure (presence of the sugar moieties at aglycone). Increasing the pH induces bathochromic shifts of absorbance maxima in the visible range for the both. The visible absorbance maxima for cyanidin were in all instances (with the exception pH value 4.0) higher than the corresponding visible absorbance maxima for Cy3Glc, indicating that 3-glucosidic substitution of aglycone caused hypsochromic shift. Comparing the molar absorptivities for cyanidin and Cy3Glc there is an evident high impact of the 3-glucosidic substitution on the molar absorptivity of the aglycone moiety: the 3-glucosidic substitution strongly increases molar absorptivity of the aglycone moiety, and it is favorable for colour



Figure 8. The brown index (BI) immediately after dissolution and after different time of incubation at 25  $^{\circ}$ C in buffered aqueous solutions at different pH values: A) 2.0; B) 4.0; C) 7.0; D) 9.0, for the cyanidin (•) and Cy3Glc ( $^{\circ}$ ) (5×10<sup>-5</sup> mol dm<sup>-3</sup>) solutions.

intensity in the alkaline region. During storage the presence of the 3-glucosidic substitution strongly increased molar absorptivities at all examined pH values and improved stability at pH 2.0 and 4.0. Surprisingly, spectrometric analysis revealed low stability for aglycone at pH 2.0 regardless of whether it was found in the form of flavylium cation. Only at pH 7.0 the stability of cyanidin became higher than that of Cy3Glc, while at all the other pH values Cy3Glc showed higher stability. From a structural point of view, it seems that the presence of the 3-glucosidic substitution strongly increases the stability of aglycone moiety, possibly by protecting the flavylium nucleus from nucleophilic attack of water molecule at C-2 that leads to the colourless forms. The BI appears to be sensitive indicator of the stability cyanidin and Cy3Glc at different pH values. At pH 2.0 and 4.0 Cy3Glc is stabile and it has very low and constant BI values, while at pH 7.0 and 9.0 was unstable and BI values gradually increased all the time. The cyanidin was unstable at all examined pH values which was been

accompanied by gradual increase in *BI* values during incubation. During some time period, the Cy3glc turned yellow at pH 7.0, while cyanidin at pH 4.0 and 9.0.

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# IZVOD

# UTICAJ pH VREDNOSTI SREDINE NA STABILNOST CIJANIDINA I CIJANIDIN 3-O- $\beta$ -GLUKOPIRANOZIDA U VODENOM RASTVORU

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#### (Naučni rad)

U ovom radu ispitivani su varijacija i intenzitet boje i stabilnost cijanidina i cijanidin 3-O- $\beta$ -glukopiranozida (Cy3Glc) pri različitim pH vrednostima (2,0; 4,0; 7,0 i 9,0) tokom inkubacije na temperaturi od 25 °C, u periodu od 8 sati. Dobijeni rezultati pokazuju da pH vrednost vodenog rastvora ima uticaj na spektroskopski profil cijanidina i Cy3Glc. Cijanidin i Cy3Glc su pokazali velike razlike u varijaciji i intenzitetu boje, stabilnosti i BI. Pri određenoj pH vrednosti, te razlike uglavnom rezultiraju iz strukture (prisustva šećera na aglikonu). Porast pH vrednosti izaziva batohromno pomeranje apsorpcionih maksimuma u vidljivoj oblasti spektra za sve ispitivane pH vrednosti (osim pH 4,0 za cijanidin), dok je prisustvo 3-glukozidne supstitucije dovodi do hipsohromnog pomeranja. Poređenjem molarnih apsorptivnosti cijanidina i Cy3Glc, uočen je veliki uticaj 3-glukozidne supstitucije: 3-glukozidna supstitucija jako povećava apsorptivnost aglikonskog dela i utiče na povećanje intenziteta boje u alkalnom regionu. Tokom inkubacije, prisustvo 3-glukozidne supstitucije jako je uticalo na povećanje molarne apsorptivnosti na svim ispitivanim pH vrednostima kao i na povećanje stabilnosti na pH 2,0 i 4,0. Spektroskopska analiza je pokazala nisku stabilnost aglikona na pH 2,0, bez obzira na činjenicu da se nalazi u obliku flavilijum katjona. Samo na pH 7,0 stabilnost cijanidina bila je veća od stabilnosti Cy3Glc, dok je na svim ostalim pH vrednostima Cy3Glc pokazivao veću stabilnost. Sa strukturne tačke gledišta, može se predpostaviti da 3-glukozidna supstitucija jako povećava stabilnost aglikonskog dela prema nukleofilnom napadu molekula vode na C-2 položaj aglikona, koji dovodi do formiranja bezbojnih oblika. Na osnovu dobijenih rezultata smatramo da je BI osetljivi pokazatelj stabilnosti cijanidina i Cy3Glc na različitim pH vrednostima. Na pH 2,0 i 4,0 Cy3Glc je bio stabilan i imao je veoma niske i konstantne BI vrednosti. Na pH 7,0 i 9,0 Cy3Glc je bio nestabilan, dok su BI vrednosti postepeno rasle tokom eksperimenta. Cijanidin je bio nestabilan pri svim ispitivanim pH vrednostima, što je bilo praćeno postepenim porastom BI vrednosti tokom stajanja. Tokom eksperimenta, Cy3Glc je dobio žućkastu nijansu na pH 7,0, dok je cijanidin dobio žućkastu nijansu na pH vrednostima 4,0 i 9,0.

Ključne reči: Antocijanini • Antocijanidini • Cijanidin • Cijanidin 3-glukopiranozid • Varijacija boje • Intenzitet boje • Stabilnost • Braon indeks • UV–Vis apsorpcioni spektar