

# Investigation of fluorescence properties of cyanidin and cyanidin 3-*O*- $\beta$ -glucopyranoside

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## Abstract

The absorption and fluorescence emission spectra of cyanidin and cyanidin 3-*O*- $\beta$ -glucopyranoside (Cy3Glc) at pH 5.5 in aqueous solution have been studied. The most effective fluorescence excitations of cyanidin were at the UV absorption maxima at 220 and 230 nm and at higher wavelengths at 270 and 280 nm. Cyanidin exhibits fluorescence emission maxima at 310 nm and in the visible range at 615 nm. The most effective fluorescence excitation for the Cy3Glc was at 220 and at 230 nm, and at higher wavelengths at 300 and at 310 nm. The Cy3Glc has fluorescence emission spectra with the maximum at 380 nm and does not show fluorescence emission in the visible range. If compare fluorescence emission spectra of cyanidin and Cy3Glc, it can be seen that fluorescence emission intensity for cyanidin is significantly higher than that for Cy3Glc. These results revealed the impact of 3-glucosidic substitution at C-3 of aglycone (to form Cy3Glc) on the significantly decrease in fluorescence emission intensity, and disappearance of the fluorescence emission in visible wavelength range.

**Keywords:** anthocyanins, anthocyanidins, cyanidin, cyanidin 3-glucopyranoside, fluorescence emission spectra, UV-Vis absorption spectra.

Available online at the Journal website: <http://www.ache.org.rs/HI/>

Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium (flavylium) salts, where the 3-hydroxyl group is replaced by glucose or another sugar. Cyanidin 3-*O*- $\beta$ -glucopyranoside (Cy3Glc) is a simple anthocyanin that is found in different berries, such as elderberries, blueberries, cowberries, whortleberries, blackcurrants, roselle and black chokeberries [1,2].

Anthocyanins have several biological activities, including antioxidant, antihepatocarcinogenic, anti-inflammatory, anti-tumor, neuroprotective, antihemolytic, anti-diabetic, hypolipidemic and cancer chemopreventive [3–17]. Epidemiological studies have suggested that anthocyanins have cardioprotective functions in human [18], and other studies have suggested that anthocyanins inhibit tumor-cell growth *in vitro* and suppress tumor growth *in vivo* [19].

These are natural, water soluble, non-toxic pigments that can show a variety of colours, from orange to blue [20–22]. Today, there is considerable interest in the development of food colorants from natural

SCIENTIFIC PAPER

UDC 547.973:543.42/.423

*Hem. Ind.* 69 (2) 155–163 (2015)

doi: 10.2298/HEMIND140203030R

sources to replace synthetic food colorants [23,24]. The reason behind this is to develop safe, economical and efficient food colorants to replace the banned coal tar and azo dyes [23,25]. Here, the 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 [1,23].

However, there are some limitations to the use of anthocyanins as food colorants, 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, and pH can affect their colours, although the major problem associated with the use of anthocyanins as food colorants is their temperature, oxygen, light and enzymatic instability [1,21,23,26–29].

Similar to the other anthocyanidins and anthocyanins, cyanidin and Cy3Glc exist in various structural forms in aqueous environments, which depend on the pH (Figure 1). Spectral investigations have revealed the coexistence of the flavylium cation (Figure 1, AH<sup>+</sup>), quinonoid base (Figure 1, A), two hemiacetal forms (Figure 1, B), and two chalcone forms (Figure 1, C). The equilibrium between these structures is highly pH dep-

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Paper received: 3 February, 2014

Paper accepted: 9 April, 2014

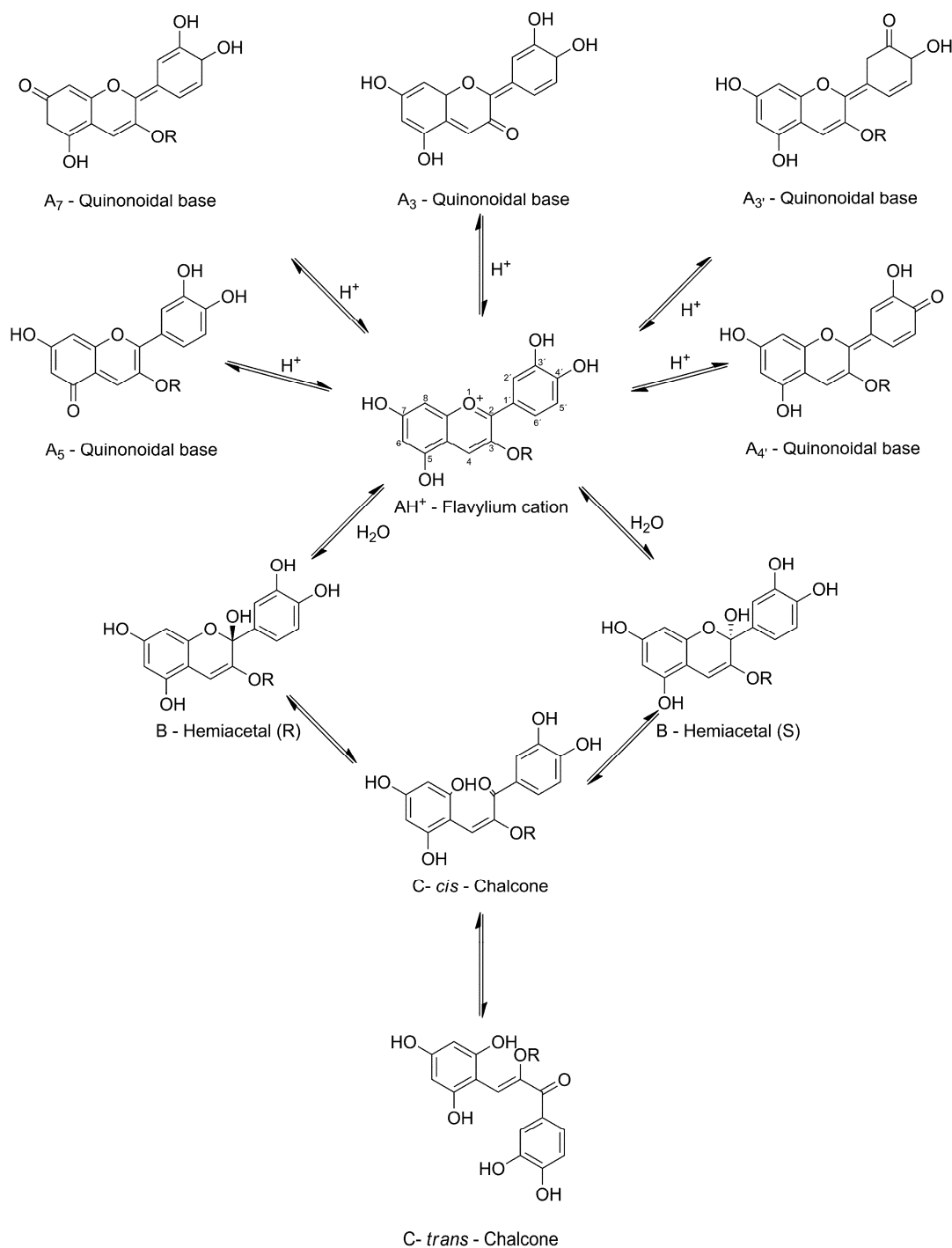


Figure 1. Transformations of cyanidin and Cy3Glc in aqueous solutions at different pH values. At C-3 for cyanidin  $R = H$ , for Cy3Glc  $R = Glc$  [23,30–32,36].

endent, and except for the *cis-trans* chalcone isomerisation, all of these transformations are fully reversible, with reaction half-times of several minutes or less. At  $pH < 2$ , the anthocyanins exist predominately as orange, red or purple flavylium cations, which are characterised by an extended  $\pi$ -electron system, and consequently show electronic absorption in the visible region [1,23,30–35].

Upon elevation of pH 4–6 kinetic and thermodynamic competition occurs between the hydration reaction on position 2 of the flavylium cation ( $AH^+$ ) and the proton transfer reactions related to the acidic hydroxyl groups of the aglycone. First reaction, hydration *via* the nucleophilic attack, leading to colorless hemiacetal structure (B, hydration equilibrium); the latter can subsequently undergo ring-opening, forming

pale yellow *cis*-chalcone ( $C_{cis}$ ) with restituted large  $\pi$ -electron system (see Figure 1), with possibility of further (usually slow) isomerisation to the *trans*-chalcone ( $C_{trans}$ ). The second reaction is the fast deprotonation of flavylum cation ( $AH^+$ ) leading to tautomeric more violet quinonoid bases (A) (prototropic tautomeric equilibrium). Further deprotonation of the quinonoid bases (A) can take place at pH between 6 and 7 with the formation of purplish, resonance-stabilised quinonoid anions ( $A^-$ ) [1,2,23,30].

The transformations of cyanidin and Cy3Glc which undergo in aqueous media are illustrated in Figure 1 [23,30–32,36].

Some anthocyanins show measurable fluorescence, but information on this topic in the literature is scarce. The study of Drabent and coworkers [37] with red cabbage extracts, containing mainly cyanidin derivatives, has shown that the colorless compounds present in these extracts fluoresce, and that this emission is pH dependent. The fluorescence emission of anthocyanins has usually been investigated using excitation in the visible region and near UV, that is, at excitation wavelength,  $\lambda_{exc} > 270$  nm [31,37–39]. Low fluorescent quantum yield, especially of colored forms of anthocyanins, is one of the reasons why it is rarely applied in analytics [31].

Previously published monographs describe fluorescence of various forms of anthocyanins in aqueous environment at various pH. In particular, it was found that chalcones (C) have fluorescence emission in the spectral range 420–450 nm ( $\lambda_{exc}$  320–340 nm), while hemiacetal form (B) at 370 nm. The flavylum cation ( $AH^+$ ) shows weak fluorescence emission in the spectral range 570–620 nm ( $\lambda_{exc} = 520$  nm). The quinonoid base (A) as anion ( $A^-$ ) has fluorescence emission spectra in the range 600–665 nm [31,37–39].

The fluorescence techniques are high sensitive and nondestructive. It can add useful information on content of anthocyanins in food and drinks. This techniques are useful tool for nondestructive monitoring of anthocyanin compounds [40,41]. In the presented study we investigated the absorption and fluorescence properties of the non-sugar, aglycone, moiety of cyanidin, and the impact of its 3-glucosidic substitution (to form Cy3Glc) on the spectral parameters. An attempt has been made to correlate the absorption and emission spectra. The fluorescence of cyanidin and Cy3Glc were excited in the UV range (210–350 nm, at every 10 nm). The low concentration of dyes indicates that anthocyanin exists as monomers in these solutions.

## EXPERIMENTAL

### Chemicals and reagents

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

528-58-5,  $C_{15}H_{11}O_6Cl$ , molecular weight 322.7 g/mol) and Cy3Glc ((2S,3R,4S,5S,6R)-2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol chloride, CAS Number: 7084-24-4,  $C_{21}H_{21}O_{11}Cl$ , molecular weight is 484.8 g/mol) were obtained from Polyphenols Laboratories AS (Sandnes, Norway). Hydrochloric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Aqueous solutions were prepared from Milli-Q water (resistivity  $> 18$  M $\Omega$  cm, Millipore, Bedford, MA, USA).

### Fluorescence emission measurements of cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside

The chloride salts of the cyanidin and Cy3Glc were dissolved in Milli-Q water to  $2 \times 10^{-5}$  mol dm $^{-3}$ . The fluorescence emission spectra of cyanidin and Cy3Glc were measured at pH 5.5. This pH is chosen according to the procedure of Drabent *et al.* [31]. The required pH was achieved by addition of hydrochloric acid or sodium hydroxide, and measured using a Seven Easy pH meter (Mettler Toledo, Schwerzenbach, Switzerland) equipped with an InLab micro electrode (Mettler Toledo, Schwerzenbach, Switzerland). These solutions were equilibrated in the dark at 25 °C, following the procedure of Brouillard *et al.* [30,42]. The fluorescence emission spectra of the cyanidin and Cy3Glc solutions were recorded at  $25.0 \pm 0.1$  °C, using a Cary Eclipse fluorescence spectrophotometer (Varian, Mulgrave, Victoria, Australia) in a thermostated 10-mm-path-length fluorescence cell, with slit widths with a normal band-pass of 5 nm for both excitation and emission. The excitation wavelengths used for cyanidin and Cy3Glc were in the UV range (210–350 nm, at every 10 nm). Each spectrum was multiplied by the dilution factor.

### UV-Vis spectrometry of cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside

The chloride salts of the cyanidin and Cy3Glc were dissolved in Milli-Q water to  $2 \times 10^{-4}$  mol dm $^{-3}$ . The UV-Vis spectra of cyanidin and Cy3Glc were measured at pH 0.4 and 5.5, according to the procedure of Drabent *et al.* and Figueiredo *et al.* [31,38]. At pH  $< 2$ , the anthocyanins exist as flavylum cations, which show absorption in the visible region [1,23,30–35], at pH 5.5 anthocyanins exist as colorless hemiacetal structure [1,21,23,30,31,43,44]. The required pH was achieved by addition of hydrochloric acid or sodium hydroxide, and measured using a Seven Easy pH meter (Mettler Toledo, Schwerzenbach, Switzerland) equipped with an InLab micro electrode (Mettler Toledo, Schwerzenbach, Switzerland). These solutions were equilibrated in the dark at 25 °C, following the procedure of Brouillard *et al.* [30,42]. The absorption spectra (190–900 nm) of the cyanidin and Cy3Glc solutions were recorded at specified pH at  $25.0 \pm 0.1$  °C, using a Cary 100 Bio UV-Vis

spectrophotometer (Varian, Mulgrave, Victoria, Australia) in a thermostated 10-mm-path-length quartz cell, with Milli-Q water as the reference. Each spectrum had the solvent spectrum subtracted and was multiplied by the dilution factor.

## RESULTS AND DISCUSSION

The absorption and fluorescence emission spectra of cyanidin and Cy3Glc at pH 5.5 in aqueous solution have been examined. The impact of the 3-glucosidic substitution at the aglycone moiety of cyanidin was examined in terms of the fluorescence emission properties. This allowed the impact of the 3-glucosidic substitution at the aglycone moiety of cyanidin to be examined in terms of the spectral properties.

The self-association of cyanidin and Cy3Glc occurs at high concentrations ( $>10^{-3}$  mol dm $^{-3}$ ) [39]. In our

investigations, cyanidin and Cy3Glc were dissolved to final concentrations of  $2 \times 10^{-5}$  for fluorescence and  $2 \times 10^{-4}$  mol dm $^{-3}$  for absorption measurements. At low concentrations cyanidin and Cy3Glc exist as monomers in the studied solutions [31].

### Absorption and fluorescence properties of cyanidin

The UV–Vis absorption spectra of cyanidin in aqueous solution at pH 0.4 and 5.5 are presented in Figure 2. Fluorescence emission spectra of cyanidin in aqueous solution at pH 5.5 for different excitation wavelength are shown at Figure 3.

At pH 0.4, the absorption band in the visible spectra for cyanidin was narrow, with a visible absorption maximum ( $\lambda_{\text{max-vis}}$ ) at 516 nm (Figure 2), which corresponds to the flavylium cation (Figure 1, AH $^+$ ) [1,23]. At this pH value, the flavylium cation is essentially the sole species present in solution [1]. The band corresponds to the

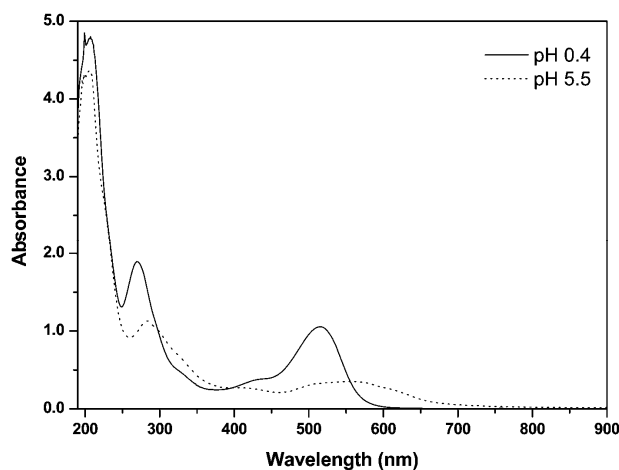


Figure 2. UV–Vis spectra of cyanidin in aqueous solution at pH 0.4 (---) and 5.5 (.....). The concentration of cyanidin was  $2 \times 10^{-4}$  mol dm $^{-3}$ , and the temperature was 25.0 °C.

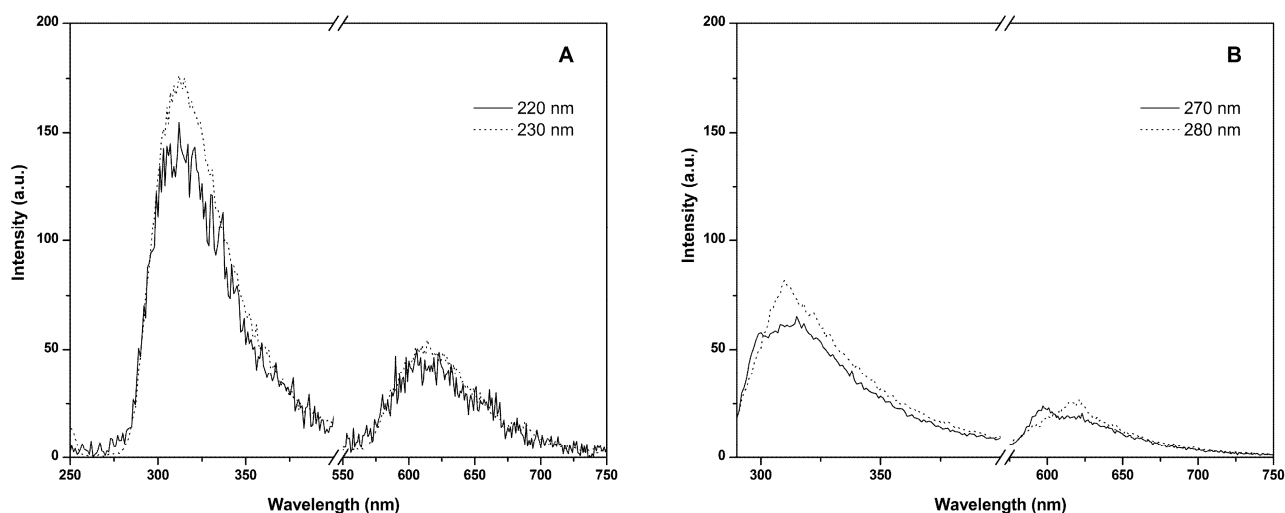


Figure 3. Fluorescence emission spectra of cyanidin in aqueous solution at pH 5.5. A) Excitation wavelength  $\lambda_{\text{exc}} = 220$  (---) and 230 (.....) nm; B) excitation wavelength  $\lambda_{\text{exc}} = 270$  (---) and 280 (.....) nm. The concentration of cyanidin was  $2 \times 10^{-5}$  mol dm $^{-3}$  and the temperature was 25.0 °C.

flavylium cation disappear at pH 5.5, whereby it completely shifts towards the colorless hemiacetal form and the coloured quinonoid bases. At the pH 5.5, the broad absorption band indicates that cyanidin is likely to be present in a few similar equilibrium structures in this pH range. There was a change in the spectral band, or a bathochromic shift, in the  $\lambda_{\max\text{-vis}}$  spectra of cyanidin, from 516 nm (at pH 0.4) to 560 nm (at pH 5.5). In the literature, this shift has been ascribed to the equilibrium between the flavylium cation and the quinonoid bases [1]. In the fast proton-transfer diffusion-controlled reactions, the protons are transferred from the oxygen atoms of the C-3, C-5, C-7, C-3' and C-4' hydroxyl groups of the flavylium cation to a water molecule. Deprotonation occurs to some extent at all of the hydroxyl groups of the flavylium cation [1,30] (Figure 1). Both the flavylium cation and the quinonoid bases strongly absorb visible light [1,30]. The colourless hemiacetal form arises due to nucleophilic addition of water to the positively charged pyrylium ring at C-2 (Figure 1). Equilibration of this reaction occurs much slower than the proton transfer reaction, as it takes a few minutes for completion at room temperature.

Comparison of absorption spectra of cyanidin at pH 5.5 with the absorption spectra of the flavylium cation (at pH 0.4) shows that in water at pH 5.5, mainly cyanidin colourless form exists (Figure 2). Their absorption spectra in UV region differ from that of the flavylium cation especially at 270–280 nm (the range used to excite the fluorescence). At the pH of 0.4 cyanidin shows band in UV range with a  $\lambda_{\max\text{-uv}}$  at 270 nm, while at pH 5.5 the  $\lambda_{\max\text{-uv}}$  is at 284 nm. This absorption bands previously assigned to the hemiacetal forms indicate the expected formation of hemiacetal form on the expense of flavylium cation [1,38]. Another absorption band of very high intensity at low wavelengths exists in the UV range. The maxima of this absorption band do

not change with pH and appear at around 210 nm and that is characteristic for polyphenolic compounds [45]. This wavelength is later used for fluorescence excitation. These results agree with the literature data reported for anthocyanins [33,38,46].

The excitation wavelengths for recording fluorescence emission spectra of cyanidin in aqueous solution at pH 5.5 were in the UV range (210–350 nm, at every 10 nm). According to the UV–Vis absorption spectra (Figure 2), the most effective excitation of fluorescence is measured at the first UV absorption maxima of cyanidin at 220 and at 230 nm (Figure 3A). At these wavelengths the molar absorptivities of the compound are high ( $\epsilon = 14731.35$  and  $11581.60 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ , respectively). The second less intensive UV absorption maximum of cyanidin were at around 280 nm, and accordingly, the second most effective fluorescence excitations were at 270 and 280 nm (Figure 3B), the characteristic absorption band of the flavonoids with no  $\pi$ -electron coupling between the two rings system (*e.g.*, hemiacetal forms) [36,38]. Obtained results are in accordance with obtained spectroscopic data ( $\epsilon = 4886.70 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 270 nm and  $5565.1 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 280 nm). It was found that cyanidin has fluorescence emission in the wavelength from 280 to 700 nm with two bands, the first one with the maximum at  $\lambda_{\max}^{\text{fl}} \approx 310 \text{ nm}$  and the second one in visible range with lower fluorescence emission intensity with maximum at  $\lambda_{\max}^{\text{fl}} \approx 615 \text{ nm}$  (Figure 3A and B). The fluorescence emission spectral band of cyanidin in the visible range is assigned to the quinonoid bases [38].

#### Absorption and fluorescence emission properties of cyanidin 3-O- $\beta$ -glucopyranoside

The UV–Vis absorption spectra of Cy3Glc in aqueous solution at pH 0.4 and 5.5 are presented in Figure 4. Fluorescence emission spectra of Cy3Glc in aqueous

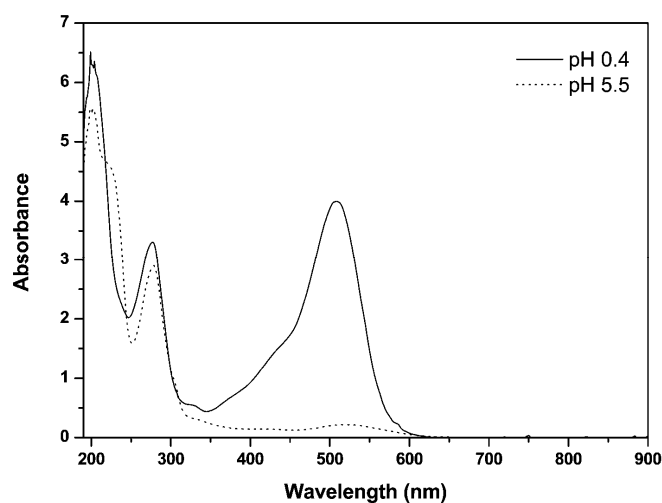


Figure 4. UV-vis spectra of Cy3Glc in aqueous solution at pH 0.4 (---) and 5.5 (.....). The concentration of cyanidin was  $2 \times 10^{-4} \text{ mol dm}^{-3}$ , and the temperature was  $25.0 \text{ }^\circ\text{C}$ .

solution at pH 5.5 for different excitation wavelength are shown at Figure 5.

Similar to cyanidin, at the lowest pH values, the absorption band in the visible spectrum for Cy3Glc was narrow with  $\lambda_{\text{max-vis}}$  at 509 nm (Figure 4), and it corresponds to the flavylum cation (Figure 1,  $\text{AH}^+$ ) [1,24,30]. Previously, the absorbance maximum of Cy3Glc determined in aqueous solution in the pH range from 0.8 to 3.7 was reported as  $511 \pm 1$  nm [2,21,24,47], which is in good agreement with our result here.

At pH 5.5, there was an extremely large decrease in the absorbance of Cy3Glc in the visible range in comparison with pH 0.4 (Figure 4). The band characteristic of the flavylum cation disappears at pH 5.5. This reflects the conversion of the coloured flavylum cation into the colourless hemiacetal form [1,23,30]. The main process in this pH range is the nucleophilic addition of water to the coloured flavylum cation. Comparison of the spectra at pH 5.5 with the absorption spectra of the flavylum cation at pH 0.4 (Figure 4) indicates that Cy3Glc exists mainly in a colourless hemiacetal and chalcone forms, formed at this pH which is in good agreement with result of Drabent *et al.* [31]. The values of molar absorptivities in visible range at 520 nm for cyanidin and Cy3Glc at pH 0.4 ( $\epsilon = 5216.00$  and  $18544.65 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ , respectively) indicates the high impact of the 3-glucosidic substitution on the absorptivity of the aglycone moiety. In the pH range from 0.4 to 5.5, a bathochromic shift in the UV–Vis spectra of Cy3Glc was observed, where the visible absorption maximum is shifted from 509 to 530 nm. This indicates that the deprotonation/protonation equilibrium that occurs corresponds to the deprotonation of the small remaining amounts of the flavylum cation, into the quinonoid bases.

Beside the visible absorption maxima, Cy3Glc shows two bands in UV range with the almost constant position of absorption maxima regardless of the pH value. The first band with lower intensity has absorption maxima at 277 nm at pH 0.4 and 278 nm at pH 5.5. The maximum of the second absorption band of very high intensity and low wavelengths did not change with pH and appeared at 204 nm, characteristic for polyphenolic compounds [45]. This wavelength is later used for fluorescence excitation. These results are in good agreement with the previously published data [33,38,46].

The excitation wavelengths for recording fluorescence emission spectra of Cy3Glc in aqueous solution at pH 5.5 were in the UV range (210–350 nm, at every 10 nm). According to the UV-vis absorption spectra (Figure 4), the most effective excitation of fluorescence is measured at the first UV absorption maxima of Cy3Glc at 220 and 230 nm (Figure 5A). At this wavelengths the molar absorptivities of the compound are high ( $\epsilon = 23333.00$  and  $21718.45 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ , respectively). The second less intensive UV absorption maxima of cyanidin was at around 280 nm, and accordingly, the second most effective excitation of fluorescence were at 300 and 310 nm (Figure 5B). Obtained results are in accordance with obtained spectroscopic data ( $\epsilon = 5791.20 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 300 nm and  $3404.50 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 310 nm). It was found that cyanidin has fluorescence emission spectra in the wavelength from 320 to 550 nm. The fluorescence emission spectra obtained here are in accordance with results of Drabent *et al.* [31]. It was found that Cy3Glc has a fluorescence emission intensity maximum at  $\lambda_{\text{max}}^{\text{fl}} \approx 380$  nm (Figure 5). The absence of the fluorescence emission band in visible range can be due to the very low concentrations of quinonoid bases, as can be seen from the absorption spectra.

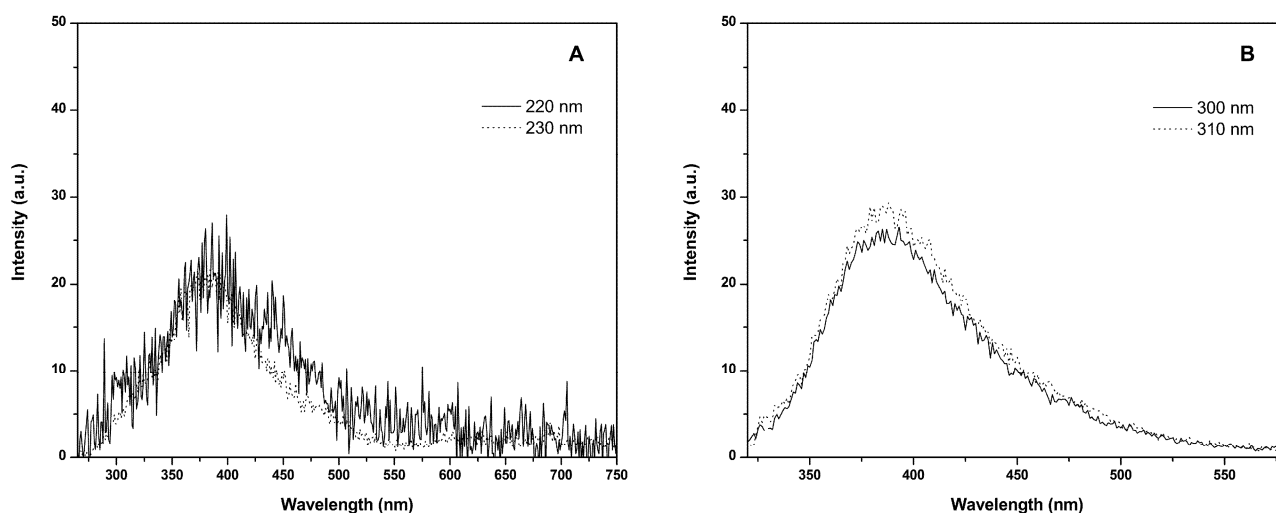


Figure 5. Fluorescence emission spectra of Cy3Glc in aqueous solution at pH 5.5. Excitation wavelength,  $\lambda_{\text{exc}}$ : A) 220 (—) and 230 (·····) nm; B) 300 (—) and 310 (·····) nm. The concentration of cyanidin was  $2 \times 10^{-5} \text{ mol dm}^{-3}$ , and the temperature was  $25.0 \text{ }^\circ\text{C}$ .

If compared fluorescence emission spectra ( $\lambda_{\text{exc}}$  was 230 nm) of cyanidin and Cy3Glc, it is seen that the fluorescence emission intensity for cyanidin is significant higher than for Cy3Glc (around 170 a.u. for cyanidin and 20 a.u. for Cy3Glc) (Figures 3 and 5). Similar results were obtained by applying  $\lambda_{\text{exc}} = 270$  nm. The obtained fluorescence emission intensity of cyanidin is significant higher than for Cy3Glc (around 70 a.u. for cyanidin and around 20 a.u. for Cy3Glc). Our results revealed the impact of its 3-glucosidic substitution at C-3 of aglycone (to form Cy3Glc) on the significantly decrease in fluorescence emission intensity, and disappearance of the fluorescence emission band in visible wavelength range. The cyanidin glycosides are regarded as non-fluorescent or exhibit very faint fluorescence, at least in neutral and weakly acidic aqueous media [32,34,36,39,46,48]. We found that both studied compounds have fluorescence emission spectra. Best to our knowledge, this work presented here is the first fluorescence characterization of cyanidin.

## CONCLUSION

The results of our work presented in this paper show that cyanidin in aqueous solution at pH 5.5 exhibits fluorescence emission spectra with the maximum of the fluorescence intensity at  $\lambda_{\text{max}}^{\text{fl}} \approx 310$  nm and surprisingly the second low intensity maxima at  $\lambda_{\text{max}}^{\text{fl}} \approx 615$  nm in the visible wavelength range. The most effective fluorescence excitation wavelengths of cyanidin were at the UV absorption maxima, the first at 220 and 230 nm and the second at the 270 and 280 nm. The fluorescence emission spectra can be obtained for Cy3Glc in aqueous solution with the maximum in the fluorescence emission at  $\lambda_{\text{max}}^{\text{fl}} \approx 380$  nm by using the excitation wavelengths 220 and 230 nm and at higher wavelengths at 300 and 310 nm. If compare fluorescence emission spectra of cyanidin and Cy3Glc, the fluorescence emission intensity for cyanidin is significantly higher than that for Cy3Glc. The Cy3Glc does not show fluorescence emission band in the visible range. These results revealed the impact of its 3-glucosidic substitution at C-3 of aglycone (to form Cy3Glc) on the significantly decrease of fluorescence emission intensity in UV range, and disappearance of the fluorescence emission band in visible range. Thus, the fluorescence spectroscopy, as a high sensitive technique, can be useful for non-destructive monitoring of anthocyanin compounds, especially in food and drinks.

## Acknowledgements

The authors would like to express their gratitude for financial support to the Slovenian Research Agency through the P4-0121 Research Programme and the Bilateral Project between the Republic of Slovenia and the

Republic of Serbia BI-RS/12-13-015. V.R. was partly financed by a CEEPUS SI-8402/2010 Bilateral Scholarship.

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## IZVOD

### PROUČAVANJE FLUORESCENTNIH SVOJSTAVA CIJANIDINA I CIJANIDIN 3-O- $\beta$ -GLUKOPIRANOZIDA

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U ovom radu ispitivana su apsorpciona i fluorescentna svojstva aglikona bez šećera, cijanidina i uticaj njegove 3-glukozidne supstitucije (do oblika Cy3Glc) na spektralne parametre. Učinjen je pokušaj da se utvrdi korelacija između apsorpcionih i fluorescentnih emisionih spektara. Ekscitacija molekula cijanidina i Cy3Glc vršena je talasnim dužinama iz UV oblasti spektra (210–350 nm, na svakih 10 nm). Poređenje apsorpcionog spektra cijanidina pri pH 5,5 sa apsorpcionim spektrom flavilijum katjona (pH 0,4) pokazuje da u vodenom rastvoru, pri pH 5,5, uglavnom postoji bezbojna forma cijanidina. Emisioni spektri cijanidina proučavani su pri ekscitaciji molekula talasnim dužinama iz UV oblasti spektra, koje su odgovarale talasnim dužinama apsorpcionih maksimuma. U skladu sa UV-Vis apsorpcionim spektrima, najefikasnija ekscitacija molekula cijanidina izmerena je na 220 i 230 nm, gde je molarna apsorptivnost jedinjenja najveća. Druga efikasna ekscitacija molekula cijanidina bila je na 270 i 280 nm. Utvrđeno je da cijanidin ima fluorescentni emisioni spektar sa dve trake, prvu u UV oblasti sa maksimumom na 310 nm i drugu u vidljivoj oblasti sa maksimumom na 615 nm. Poređenje apsorpcionog spektra Cy3Glc pri pH 5,5 sa apsorpcionim spektrom flavilijum katjona (pri pH 0,4) ukazuje na to da Cy3Glc uglavnom postoji u bezbojnom obliku pri ovoj pH vrednosti. U skladu sa UV-Vis apsorpcionim spektrima, najefikasnija ekscitacija molekula Cy3Glc izmerena je na 220 i 230 nm, gde je molarna apsorptivnost jedinjenja najveća. Druga efikasna ekscitacija molekula Cy3Glc bila je pri 300 i 310 nm. Utvrđeno je da Cy3Glc ima fluorescentni emisioni spektar sa maksimumom na 380 nm, ali ne pokazuje fluorescentnu emisiju u vidljivoj oblasti. Ako se uporede fluorescentni emisioni spektri cijanidina i Cy3Glc može se videti da je intenzitet fluorescentne emisije cijanidina značajno veći od intenziteta fluorescentne emisije Cy3Glc. Ovi rezultati ukazuju na uticaj 3-glukozidne supstitucije na C-3 atomu aglikona (do oblika Cy3Glc) na značajano smanjenje intenziteta fluorescentne emisije i gašenje fluorescencije u oblasti talasnih dužina vidljivog dela spektra.

**Ključne reči:** Antocijanini • Antocijanidini • Cijanidin • Cijanidin 3-glukopiranozid • Fluorescentni emisioni spektar • UV-Vis apsorpcioni spektar