THE COLOUR AND STABILITY OF CYANIDIN AND CYANIDIN 3-O- β -GLUCOPYRANOSIDE

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The relationships between the color variation, colour intensity, thermal stability and brown index (BI) of the aglycone moiety of cyanidin and its 3-glucosidic substitution in cyanidin 3-O- β -glucopyranoside (Cy3Glc) were studied in the aqueous solution at pH 7.0 and under the conditions of the elevated temperature (55.0 ± 0.1 °C) which simulate the rapid food ageing. Both studied compounds showed a hypsochromic shift during the storage. The cyanidin showed a significantly higher colour intensity and greater thermal stability compared to Cy3Glc. The gradual loss of the colour of the Cy3Glc solution was accompanied by the increase in BI. The BI for cyanidin was 0.63 and <1 while for Cy3Glc it was 1.09 and >1 all the time thus giving yellowish shades to the Cy3Glc aqueous solution. These facts are important from the point of view of using these compounds as food colorants, indicating a higher tinctorial power and higher stability of cyanidin compared to the Cy3Glc.

Keywords: cyanidin, cyanidin 3-O- β -glucopyranoside, colour variation, colour intensity, thermal stability, brown index

Introduction

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- β -glucopyranoside (Cy3Glc) is a simple anthocyanin that is found in different berries, such as elderberries, blueberries, cranberries, whortleberries, blackcurrants, strawberries, raspberries, blackberries, and black chokeberries [1–3].

Anthocyanins have several biological activities, including antioxidant, antihepatocarcinogenic, anti-inflammatory, anti-tumor, neuroprotective, antihemolytic, anti-diabetic, hypolipidemic and cancer chemopreventive [4–18]. Epidemiological studies have suggested that anthocyanins have cardioprotective functions in humans [19], and other studies have suggested that anthocyanins inhibit the tumor-cell growth *in vitro* and suppress the tumor growth *in vivo* [20].

In recent decades, the development of the increased interest in the chemistry of anthocyanins, a family of glycosidic pigments responsible for most of orange, red and blue colors of flowers and fruits, was noticed. These are natural, water soluble, non-toxic pigments that can show a variety of colours, from orange to blue [21–23]. The anthocyanins have a long history as a part of the human diet [1]. The motivation to extend the use of these water-soluble pigments as food colorants is also increasing, along

with their potential positive health effects. Today, there is a considerable interest in the development of food colorants from natural sources to replace synthetic food colorants [24,25]. The reason behind this is to develop safe, economical, and efficient food colorants to replace the banned coal tar and azo dyes [24,26]. 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 [1,24].

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 [1,3,22,24,27–29].

In recent years, the interest in the properties and stability of anthocyanin extracts has increased. However, there are little data in literature relating to the properties and stability of pure anthocyanins, and especially of anthocyanidins. The major reason for this is that most of the anthocyanins are difficult to purify and they have a limited commercial accessibility, especially in large

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quantities. It is important to know how structural transformations, such as glucosidation, influence the properties of these compounds. These facts are important from the point of view of the possibility to use these compounds as natural food colorants.

The aim of this paper was to describe the colour variation, colour intensity, thermal stability and brown index of the aglycone moiety of cyanidin, and the impact of its 3-glucosidic substitution (to form Cy3Glc) (Figure 1) on these parameters during storage at pH 7.0 and under the conditions of the elevated temperature (55.0 ± 0.1 °C) which simulated the rapid food ageing.

Figure 1. Structures of cyanidin and cyanidin 3-glucoside.

Experimental

Chemicals and reagents

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 484.8 g/mol) were from Polyphenols Laboratories AS (Sandnes, Norway). The 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was from Sigma Aldrich (Steinheim, Germany). Aqueous solutions were prepared from Milli-Q water (resistivity >18 M Ω cm) (Millipore, Bedford, MA, USA).

Colour and stability measurement of cyanidin and Cy3Glc at pH 7.0 and 55.0 °C

Thermal stabilities of cyanidin and Cy3Glc were determined in 20 mM HEPES, pH 7.0, at 55.0 ± 0.1 °C. The chloride salts of cyanidin and Cy3Glc were dissolved in 20 mM HEPES, pH 7.0 to the final concentration of 0.25 mM. The absorption spectra (190-900 nm) of these cyanidin and Cy3Glc solutions were recorded at 55.0 ± 0.1 °C using a Cary 100 Bio UV-vis spectrophotometer (Varian, Mulgrave, Victoria, Australia) in a thermostated 10-mm path length quartz cell, using a digital thermocouple that allowed direct temperature measurements of the samples, and with 20 mM HEPES, pH 7.0 as the reference. The spectra were recorded at specific time intervals. Each spectrum was corrected for the blank solution. Brown index (BI) is expressed as the absorbance ratio at 430 nm by that at 520 nm.

Results and discussion

According to Cabrita et al. and Fossen et al. [30,31], the colour variation of cyanidin and Cy3Glc was expressed as the changes in the positions of the absorbance maximum in the visible range ($\lambda_{\max-\text{vis}}$), color intensities were measured as absorbance values at visible absorbance maximum $\lambda_{\max-\text{vis}}$ at time zero (t_0) and after a certain time interval and molar absorptivity (ϵ , in $M^{-1} \text{ cm}^{-1}$), and the colour stability was expressed as the percentage of the absorbance remaining after a certain time interval, measured at $\lambda_{\max-\text{vis}}$. Brown index (BI) is expressed as the absorbance ratio at 430 nm by that at 520 nm.

Colour variation of cyanidin and Cy3Glc at pH 7.0 and 55.0 ± 0.1 °C

By plotting the visible absorbance maximum, $\lambda_{\max-\text{vis}}$, the values obtained for cyanidin and Cy3Glc at the time zero (t_0) and after a certain time interval in the aqueous solution at pH 7.0 and 55.0 ± 0.1 °C, a similar pattern was achieved (Figure 2). Cyanidin and Cy3Glc showed a gradual hypsochromic shift up to 180 min, and then a dramatic hypsochromic shift up to 300 min. The initial $\lambda_{\max-\text{vis}}$ value at the t_0 for cyanidin was 9 nm higher than that for Cy3Glc. The curve for the change in the position of the absorbance maximum with time for cyanidin was almost parallel to the time-axis during the first 180 min, while the curve for Cy3Glc gradually decreased in this time interval. After 300 min, a hypsochromic shift in the visible absorption band (Figure 2) of near 60 nm can be seen for both compounds. This revealed the impact of 3-glycosylation in Cy3Glc on the position of the visible absorbance maximum: the presence of the 3-glucosidic substitution affected the visible absorbance maximum shifts towards lower wavelengths (hypsochromic shift) at pH 7.0 and 55.0 ± 0.1 °C.

Figure 2. Visible absorbance maximum ($\lambda_{\max-\text{vis}}$) of 0.25 mM cyanidin (●) and Cy3Glc (○) as a function of time, in 20 mM HEPES, pH 7.0, and 55.0 ± 0.1 °C.

Colour intensity of cyanidin and Cy3Glc at pH 7.0 and $55.0 \pm 0.1^\circ\text{C}$

The storage of cyanidin and Cy3Glc at pH 7.0, and at $55.0 \pm 0.1^\circ\text{C}$ affected their colour intensities. The molar absorptivities for cyanidin were significantly higher than for Cy3Glc all the time (Figure 3). The curves which show the change in molar absorptivities with time for both compounds show a hypochromic effect. The decrease in the molar absorptivities for cyanidin was gradual all the time, while Cy3Glc showed a significant decrease in the molar absorptivities in the first 20 min, and then very low and almost constant values of molar absorptivities. The molar absorptivities for cyanidin after the dissolution (0 min) were $8166.88\text{ M}^{-1}\text{ cm}^{-1}$ and after 300 min they were $4801.92\text{ M}^{-1}\text{ cm}^{-1}$, which are higher values than molar absorptivities for Cy3Glc immediately after the dissolution (0 min), $4083.32\text{ M}^{-1}\text{ cm}^{-1}$. These data clearly indicated higher tinctorial power of cyanidin compared to Cy3Glc. This fact is important from the point of view of the use of these compounds as food colorants. The pre-sence of the 3-glucosidic residue significantly decreased the colour intensity of Cy3Glc at pH 7.0 and the temperature of $55.0 \pm 0.1^\circ\text{C}$.

Figure 3. Molar absorptivities of 0.25 mM cyanidin (●) and Cy3Glc (○) as a function of time in 20 mM HEPES, pH 7.0, and $55.0 \pm 0.1^\circ\text{C}$.

Cyanidin and Cy3Glc stability at pH 7.0 and at $55.0 \pm 0.1^\circ\text{C}$

Both examined compounds showed the stability above 50 %, after 300 min at pH 7.0, and $55.0 \pm 0.1^\circ\text{C}$. Cy3Glc showed lower temperature stability values than cyanidin. It was found that the stability of Cy3Glc decreased rapidly in the first minutes after the dissolution. After 10 minutes 78.83 % of Cy3Glc and 96.47 % of cyanidin were intact. Figure 4 clearly shows greater thermal resistance of cyanidin compared to Cy3Glc at pH 7.0. From the structural point of view, it seems that the presence of 3-glucosidic substitution decreases the stability of Cy3Glc under the condition of elevated temperatures in a neutral aqueous solution.

Figure 4. Percentages of the remaining absorbance (measured at $\lambda_{\text{max-vis}}$) of 0.25mM cyanidin (●) and Cy3Glc (○) as a function of time in 20 mM HEPES, pH 7.0, and $55.0 \pm 0.1^\circ\text{C}$.

Brown index of cyanidin and Cy3Glc at pH 7.0 and $55.0 \pm 0.1^\circ\text{C}$

The initial BI for cyanidin was 0.63 and remained <1 all the time (Figure 5). The initial BI for Cy3Glc was 1.09 and it remained >1 all the time thus giving yellowish shades. The gradual loss of the colour (a decrease in molar absorptivities, (Figure 3) was accompanied by the increase in BI. Under higher thermal conditions which simulated a rapid food ageing, the BI increase was concomitant with a hypsochromic shift in the visible absorption band (Figure 2) of nearly 60 nm.

Figure 5. Brown index of 0.25 mM cyanidin (●) and Cy3Glc (○) as a function of time, in 20 mM HEPES, pH 7.0, and $55.0 \pm 0.1^\circ\text{C}$.

Conclusion

The colour, the intensity of the colour, the stability and brown index of the aglycone moiety of cyanidin and Cy3Glc under the conditions of the elevated temperature which simulated a rapid food ageing, were different. The visible absorbance maximum for cyanidin was higher than that

for Cy3Glc, indicating the influence of the 3-glucosidic substitution on a hypsochromic shift of the visible absorbance maximum. Both studied compounds showed a hypsochromic shift during the storage. All the time, the molar absorptivities for cyanidin were significantly higher than those for Cy3Glc. These data clearly indicated a higher tinctorial power of cyanidin compared to Cy3Glc. The presence of 3-glucosidic residue significantly decreased the colour intensity of Cy3Glc compared to cyanidin under given conditions. Cyanidin showed greater thermal stability compared to Cy3Glc. It seems that the presence of 3-glucosidic substitution decreased the stability of Cy3Glc under the condition of elevated temperatures in a neutral aqueous solution. BI for cyanidin was 0.63 and <1, while BI for Cy3Glc was 1.09 and >1 all the time thus giving yellowish shades. Cyanidin showed a significantly higher colour intensity and greater thermal resistance compared to Cy3Glc. These facts are important from the point of view of using these compounds as food colorants, indicating a higher tinctorial power and higher stability of cyanidin compared to Cy3Glc.

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Izvod

BOJA I STABILNOST CIJANIDINA I CIJANIDIN 3-O- β -GLUKOPIRANOZIDA

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U ovom radu ispitivana je veza između varijacije boje, intenziteta boje, termičke stabilnosti i braon indeksa (BI) aglikonskog dela cijanidina i njegove 3-glukozidne supstitucije do cijanidin 3-O- β -glukopiranozida (Cy3Glc) u vodenom rastvoru pri pH 7.0 i u uslovima povišene temperature (55.0 ± 0.1 °C), koji simulira ubrzano starenje hrane. Oba proučavana jedinjenja pokazuju hipsohromni pomak u vidljivom delu spektra tokom stajanja. Cijanidin pokazuje znatno veći intenzitet boje i veću termičku stabilnost u odnosu na Cy3Glc. Postepeni gubitak boje rastvora Cy3Glc praćen je povećanjem BI. Za cijanidin BI bio je 0.63 i <1, dok je za Cy3Glc BI bio 1.09 i >1 sve vreme, dajući žućkastu nijansu vodenom rastvoru Cy3Glc. Ove činjenice su važne sa tačke gledišta moguće upotrebe ovih jedinjenja kao prehrambenih boja, ukazujući na veću moć bojenja i veću stabilnost cijanidina u odnosu na Cy3Glc.

Cljučne reči: cijanidin, cijanidin 3-O- β -glukopiranozid, varijacija boje, intenzitet boje, termička stabilnost, braon indeks