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Colours of pure anthocyanidin 3-glucosides in aqueous solutions during storage

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ABSTRACT.

A study on anthocyanin stability and colour variation (intensity, λ_{max}, ε) during storage at 10° and 23°C over the pH range 1-12 was conducted for the 3-glucosides of the six common anthocyanidins.

Although it has been believed that anthocyanins are stable only at low pH values, this study revealed a more complex behaviour. Under relative strong acidic conditions (pH<3) the stability and intensity for the red colours of the anthocyanin 3-glucosides are similar and very high. Stability and intensity then decrease in the slightly acidic region, but in the neutral/alkaline region these anthocyanins experience an increase in both stability and colour intensity. It is mostly in the alkaline region that differences between the individual anthocyanidin 3-glucosides are enhanced. Pg3glc, Pn3glc and Mv3glc exhibit a remarkable stability and develop intense bluish colours around local maxima at pH 8-9. Cy3glc also displays an improved stability in the alkaline region but above pH 8 its colour turns red instead of blue. Dp3glc and Pt3glc are purple/blue between pH 6-8 but turn red at higher pH values. Their stability and colour intensity are low in the alkaline region, but they show some colour stability in the pH 5-7 region, although the corresponding colour intensities are modest.

These results show that some anthocyanins may provide stable and intense bluish colour for extended application in slightly alkaline food products. For instance, there are some Mexican food products (varieties of tacos) containing anthocyanins under alkaline conditions.

INTRODUCTION.

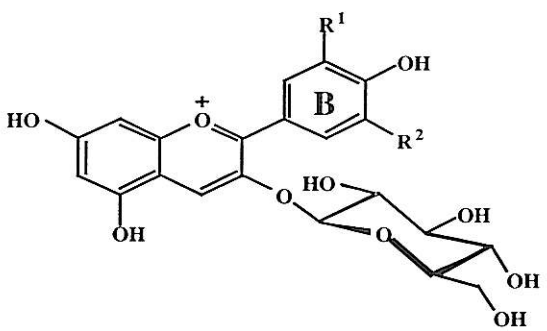
The anthocyanins form the red and blue colours of most fruits and vegetables, and provide therefore attractive colours of many fruit juices, wines, jams and preserves. There is additional interest in the use of anthocyanins as food colorants

as a consequence of consumer preferences, beneficial health effects and legislative action. The anthocyanins are affected by a number of chemical and physical factors (Jackman *et al.*, 1987; Francis, 1989) including pH, temperature, oxidative environment and light.

The aim of this work is to study the changes in colour intensity and stability of six pure anthocyanidin 3-glucosides in aqueous solutions upon pH variation (range 1-12) at 10° and 23°C. In a recent paper similar parameters were used to compare cyanidin 3β -glucoside and the more complex anthocyanin, petunidin 3-[6-(4-*p*-coumaroyl-α-rhamnosyl)-β-glucoside]-5-β-glucoside, petanin (Fossen *et al.*, 1998).

EXPERIMENTAL.

Pigments were extracted with acidified methanol from various sources: strawberry, *Fragaria ananassa* (Pg3glc); rice, *Oryza* sp. (Cy3glc and Pn3glc); *Abies koreana* (Dp3glc, Pt3glc); *Vaccinium* sp. (Mv3glc). Crude extracts were partitioned against ethyl acetate, and purified anthocyanins were obtained after successive use of chromatographical techniques (Amberlite XAD-7, Droplet counter-current chromatography, Sephadex LH-20, preparative HPLC). The individual pigments were transferred to vials, dried, and dissolved in 17 different buffers solutions (pH 1.0-11.6)



	R^1	R^2
Pg3glc:	H	H
Cy3glc:	OH	H
Pn3glc:	OCH_3	H
Dp3glc:	OH	OH
Pt3glc:	OCH_3	OH
Mv3glc:	OCH_3	OCH_3

giving solutions with final concentration of 1.0×10^{-4} M. Two sets of samples were prepared in this way to be stored at different temperatures (10° and 23°C).

UV-Vis measurements were made after 1h, 1, 2, 5, 8, 15 and 60 days using the buffer solutions as the reference. Absorption values at λ_{max} for each of the pH

values were registered and their respective colour intensities were expressed as molar absorptivities. Colour stability was expressed as % retained colour.

RESULTS AND DISCUSSION.

Colour variation

The colour intensities of the six anthocyanidin 3-glucosides change considerably (Fig. 1). The absorptivities of all pigments were highest at pH 1 and decreased towards pH 5-6: the red flavylium ion dominates at pH 1, whereas at pH 5 the colourless carbinol pseudo-base forms predominate. A further pH increase revealed individual colour changes, and an unexpected hyperchromic effect with local maxima between pH 8-10, when at least the anthocyanins 3-glucosides with only one hydroxyl group on the B-ring (Mv3glc, Pn3glc and Pg3glc) are supposed to occur in their bluish quinonoidal and quinonoidal anion forms. Whereas the colour intensity of all anthocyanins changes in a quite similar way in the acid region, under alkaline conditions larger differences arise: the absorptivities of Pn3glc and Mv3glc are higher than those of the other monoglucosides, indicating that methoxylation in addition to the lack of *ortho*-dihydroxylation on the aglycone B-ring is favourable for colour intensity in the alkaline region.

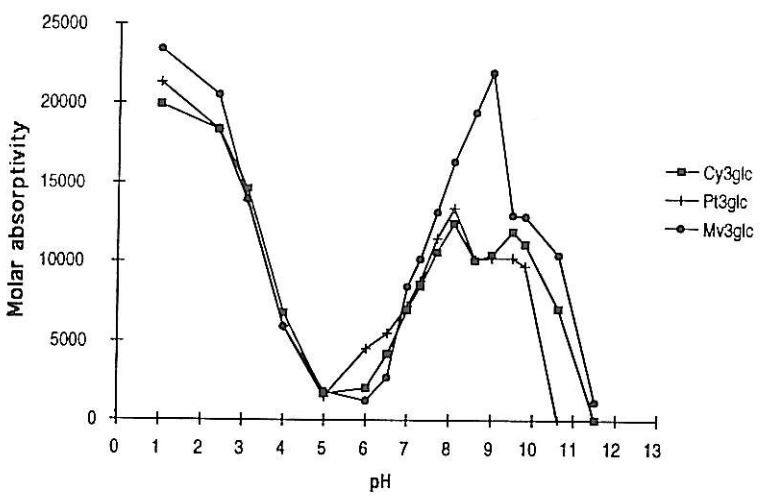


Figure 1. Absorptivity of cyanidin 3-glucoside, petunidin 3-glucoside and malvidin 3-glucoside (1.03×10^{-4} M) recorded 1 hour after dissolution at 23°C.

Colour stability

Stability of the individual pure anthocyanins varies with pH and temperature. In general, anthocyanin 3-glucosides showed high and similar stability to more complex anthocyanins like petanin at 10°C under acidic conditions (pH 1-3) (Fossen *et al.*, 1998). More than 70% of the pigment colour was intact after 60 days of storage. Colour stability then decreased towards neutrality but some anthocyanins would further experience a stability increase, which culminated at local maxima around pH 8-9. For instance, the 3-glucosides of malvidin, peonidin and pelargonidin (Fig 2, left) still displayed 20–60% colour after 8 days at these pH values, where they also show their most bluish colours. The presence of methoxyl groups and the absence of *ortho*-dihydroxylation on the B-ring seems to favour this blue colour stability in the alkaline region.

Dp3glc and Pt3glc exhibited a notably different behaviour (Fig. 2, right): They didn't suffer the dramatic stability decrease typical for the other anthocyanidin 3-monoglucosides in the pH 5-7 range, but on the other hand their stability in the alkaline region is reduced. At pH 6-7 these anthocyanins formed purple precipitates, which resulted in decolourisation of the solutions. The precipitates were dissolved/suspended upon shaking to give purple solutions. The presence of ortho-dihydroxyl groups seems to favour stability in this pH region.

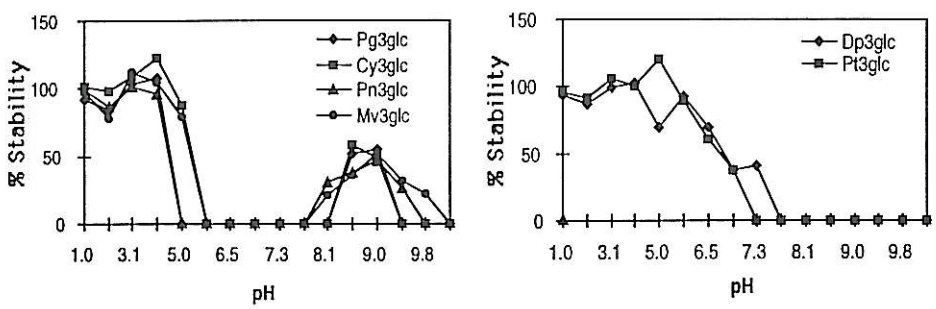


Figure 2. The stability of the six common anthocyanidin 3-glucosides after 8 days storage at 10°C at various pH values.

At 23°C a similar pattern was observed, however, the corresponding colour stabilities were somewhat lower than at 10°C, especially in the alkaline region. Around pH 1-2 all anthocyanins were relatively stable, and conserved 40% or more

of their initial colour after 60 days storage. At pH 8–9, only Pn3glc and Mv3glc kept some bluish colour after 5 days of storage (12-22%).

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