EFFECTS OF pH ON HUE ANGLE AND VISIBLE ABSORPTION MAXIMA OF CYANIDIN

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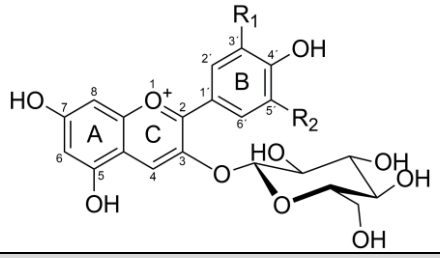
Abstract
As a major sub-group of flavonoids, anthocyanins are water soluble plant pigments responsible for the blue, purple and red color of many plant tissues. They occur primarily as glycosides of their respective aglycone anthocyanidin. Cyanidin is the most abundant anthocyanidin in fruits and vegetables (50%). In recent years, the interest in the properties and stability of anthocyanin extracts has increased. However, there remain little data in the literature relating to the properties and stability of pure anthocyanins, and especially of the anthocyanidins. The aim of the study was to compare under various pH conditions visible absorbance maxima ($\lambda_{max\text{-}vis}$) with the corresponding hue angle (h_{ab}) values of cyanidin as a color reference. Thus cyanidin aqueous solution was subjected to spectroscopic and colorimetric study to examine influence of pH value on h_{ab} and $\lambda_{max\text{-}vis}$. The cyanidin had reddish nuances at the lowest pH values. By stepwise pH increase the colour of cyanidin is gradually changed toward magenta and lilac tones, and then with further pH increase to more reddish nuances. In alkaline region cyanidin showed yellow green tones, which is gradually changed toward yellow nuances with further pH increases. For the $\lambda_{max\text{-}vis}$ the following tendency was observed: as pH increased, the $\lambda_{max\text{-}vis}$ values of cyanidin showed the bathochromic shift. The cyanidin solution exhibited variations of h_{ab}, although their $\lambda_{max\text{-}vis}$ remained stable. By contrast, solutions having shifted spectra share the same basic tonality. Measurement of the h_{ab} and the $\lambda_{max\text{-}vis}$ showed that these values were highly pH dependent. Thus we can conclude that the $\lambda_{max\text{-}vis}$ values of these cyanidin solutions at the various pHs correlate poorly with their corresponding h_{ab} and caution should be applied when using $\lambda_{max\text{-}vis}$ values for interpretation of colours.

Keywords: cyanidin, hue angle, visible absorbance maximum, spectrophotometry, colorimetry.

Introduction

Anthocyanins are the most important groupof pigments, after chlorophyll that is visible to the human eye. Chemically, anthocyanins (fromthe Greek *anthos*, a flower, and *kyanos*, dark blue) are flavonoids (flavan like). The anthocyanidins are the basic structures of the anthocyanins. The anthocyanidins (or aglycons) consist of an aromaticring [A] bonded to an heterocyclic ring [C] that contains oxygen, which is also bonded by a carbon-carbon bond to a third aromaticring [B]. When the anthocyanidins arefound in their glycoside form (bonded to a sugar moiety) they are known as anthocyanins(Figure 1) (Castañeda-Ovando et al. 2009; Delgado-Vargas et al., 2000). There is a huge variety of anthocyanins spread in nature. Up to now there are reports of more than 500 different anthocyanins and 23 anthocyanidins of which only six are the most common in vascular plants, pelargonidin, peonidin, cyanidin, malvidin, petunidin and delphinidin (Figure 1) (Castañeda-Ovando et al. 2009). The differences between individual anthocyanins are the number of hydroxyl groups in the molecule; the degree of methylation of these hydroxyl groups; the nature, number, and location of sugars attached to the molecule; and the number and the nature of aliphatic or aromatic acids attached to the sugars in the molecule (Galvano et al. 2004). The glycoside derivatives of the three non-methylated anthocyanidins (cyanidin, delphinidin and pelargonidin) are the most common in

nature, being found in 80% of pigmented leaves, 69% in fruits and 50% in flowers. The distribution of the six more common anthocyanidins in fruits and vegetables is: cyanidin 50%, delphinidin 12%, pelargonidin12%, peonidin 12%, petunidin 7%and malvidin 7% (Castañeda-Ovando et al. 2009). Cyanidins are considered the widest spread anthocyaninin the plant kingdom. They are largely distributed in the human diet through crops, beans, fruits, vegetables and red wines, suggesting that we daily ingest significant amounts of these compounds from plant-based diets (Galvano et al. 2004). Anthocyanins are of great nutritional interest because of the marked daily intake (180 to 215 mg/day in the United States), which is much higher than the intake (23 mg/day) estimated for other flavonoids, including quercetin, kaempferol, myricetin, apigenin, and luteolin. They have been reported to have positive effects in the treatment of various diseasesand are prescribed as medicines in many countries (Galvano et al. 2004). In plant tissues the anthocyanins produce blue, purple, red and intermediate hues, and appear "black" in some commodities. Their hue and structure are dependent on pH value and the presence of copigments. It has been recognised for many years that anthocyanins make a significant contribution to the colour, and hence acceptability, of many fruits, some vegetables and associated products, including beverages and preserves. Subsequently it was recognised that anthocyanin-rich extracts might have potential as food additives (Clifford, 2000). Anthocyanins have been reported to be strong antioxidants, inhibit the growth of cancerous cells, inhibit inflammation, be vasoprotectors, and have anti-obesity effects. Many of the health benefits associated with berry fruit may be due to the high concentrations of anthocyanins that they contain (McGhie and Walton 2007). The addition of natural anthocyanin extracts, to give colour toprocessed foodstuffs, could be regarded in this contextas maintaining current levels, if not actually redressing the balance, which may be desirable in view of their beneficial effects (Bridle and Timberlake 1997). Most food nowadays is processed in some way before reaching the consumer, and manufacturers have a need to replace colour lost during processing or to colour products which would otherwise be colourless and unappealing. With increasing public concern about the safety of synthetic colorants, natural pigment extracts are assuming greater prominence (Bridle and Timberlake 1997). Carotenoids and anthocyanins are amongst the most utilised vegetable colorants in the food industry. Anthocyanins are water-soluble and they are extracted from grapes, berries, red cabbage, apples, radishes, tulips, roses and orchids, amongst others (Castañeda-Ovando et al. 2009). Anthocyanins (E163) have been approved for use infoods based on very limited toxicological data (Clifford, 2000). The most common way to indicate anthocyanin colour is based on presentation of visible absorbance maxima ($\lambda_{max\text{-}vis}$) from visible absorption spectra (Fossen et al. 1998). In order to examine possibility of application spectrophotometric as well as colorimetric measurement in food industry and eventual difference between the obtained data, we compared the spectrophotometric and colorimetric parameters obtained under the same conditions.



Anthocyanin	R_1	R_2
Pelargonidin 3-glucopyranoside (Pg3Glc)	H	H
Cyanidin 3-glucopyranoside (Cy3Glc)	OH	H
Peonidin 3-glucopyranoside (Pn3Glc)	OCH_3	H
Delphinidin 3-glucopyranoside (Dp3Glc)	OH	OH
Petunidin 3-glucopyranoside (Pt3Glc)	OCH_3	OH
Malvidin 3-glucopyranoside (Mv3Glc)	OCH_3	OCH_3

Figure 1. Structure of the six most common anthocyanidin 3-monoglucosides (Cabrita et al. 2000)

Material and methods

Chemicals and reagents

The chloride salt 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) was 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 MΩ cm) (Millipore, Bedford, MA, USA).

Spectrophotometric and colorimetric measurements

The chloride salt of cyanidin was dissolved in Milli-Q water to 2×10^{-4} mol dm^{-3}. This solution was equilibrated in the dark at 25 °C, following the procedure of Brouillard et al. (Brouillardet al. 1982; Brouillard et al. 1978). Successive pH jumps of around 0.5 pH units (from pH 0.5 to 13.1) were achieved by a modified procedure to that described previously (Brouillard et al. 1982; Brouillard et al. 1978; Heredia et al. 1998; Hurtado et al. 2009). Briefly, after each addition of an aliquot (a few µL) of HCl or NaOH, the solutions were equilibrated for 5 min on a magnetic stirrer, and the pH was measured using a Seven Easy pH meter (Mettler Toledo, Schwerzenbach, Switzerlend) equipped with an InLab micro electrode (Mettler Toledo, Schwerzenbach, Switzerlend). The visible absorption spectra (380-900 nm) of the cyaniding solution was recorded at each pH at 25.0 ± 0.1 °C, using a Cary 100 Bio UV-visible 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. The hue angle (h_{ab}) for 2×10^{-4} mol dm^{-3}cyanidin solution was determined at each pH using a Konica Minolta CR-400 Chroma meter (Sensing, Inc., Osaka, Japan).

Results and discussion

Color expression of anthocyanins is dependent on the pH value (Brouillard 1982; Fossen et al. 1998; Heredia et al. 1998; Hurtado et al. 2009). Anthocyanins in rather strong acid solutions occur only as flavylium forms (Brouillard et al. 1982; Brouillard 1982; Torskangerpoll and Andersen 2005). However, when pH increases, each anthocyanin occurs as a mixture of various equilibrium forms (Brouillard et al. 1982; Brouillard 1982).The hue angle (h_{ab}), is the qualitative attribute of colour (Hurtado et al. 2009). The hue angle and the visible absorbance maximum in the aqueous solutions of cyanidin (2×10^{-4} mol dm^{-3}) were studied across the whole pH range (from pH 0.5 to 13). The cyanidin showed a reddish hue (31.41°) at the lowest pH value (pH 0.5) (Figure 2). By stepwise pH increase until pH 7.5, the colour of cyanidin is gradually changed toward magenta and purple tones (until h_{ab}=359.34°), and then with further pH increase until pH 8.8 the colour of cyanidin is changed to more reddish nuances (until h_{ab}=16.92°). At pH 9.6cyanidin showed greenish tones (h_{ab}=152.55°), which is with further pH increases gradually changed toward yellow nuances (81.36° at pH 12.5). Figure 3 shows visible absorbance maxima ($\lambda_{max\text{-}vis}$) of 2×10^{-4} mol dm^{-3} aqueous solution of cyanidin at the various pH values. The following tendency was was observed: as pH increased, the $\lambda_{max\text{-}vis}$ values of cyanidin showed the bathochromic shift. The $\lambda_{max\text{-}vis}$ of cyanidinaqueous solution shifted from 517 nm (at pH 0.5) to 599 nm (at pH 12.0) (Figure 3). In pH region 3.5 to 4.6 these shift were dramatic (from 525 to 550nm). Further increase in pH resulted in very gradually increase of $\lambda_{max\text{-}vis}$, until pH 8.8-9.6, were another bathochromic shift is observed (from 571 to 594nm). It has been postulated that as long as the $\lambda_{max\text{-}vis}$ is not shifting, the hue values is not changing. Our colorimetric analysis of cyanidin solutions (Figure 2) in comparison to the $\lambda_{max\text{-}vis}$ (Figure 3) resulted in different conclusions, the cyanidin solutions displaying huge variations of chromatic tonalities, although their spectral $\lambda_{max\text{-}vis}$ remained stable.

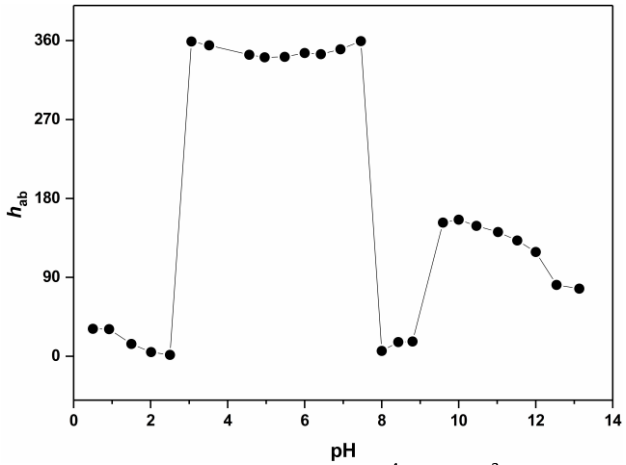


Figure 2. The hue angle (h_{ab},°) as a function of pH for the 2×10^{-4} mol dm^{-3}cyanidin aqueous solution.

For example, perceptually, the most remarkable variation for cyanidin solutions was the extent of the hue gamut covered by the cyanidin solution at pH 0.50-1.50 (sharing the same visible $\lambda_{max\text{-}vis}$, 517 nm): from orange-red hue (h_{ab}=31.41°) to a red basic hue (h_{ab}=14.05°) (Table 1). Solutions in the pH range 2.0-3.1 sharing the same $\lambda_{max\text{-}vis}$, (522-523 nm) while hues changes from red hue (h_{ab}=4.76°) to a magenta hue (h_{ab}=358.99°). With further pH increase at pH 7.5-8.0 solution again sharing the same visible $\lambda_{max\text{-}vis}$, (565 nm) while his hues ranged from magenta (h_{ab}=359.34°) to a red hue (h_{ab}=6.20°). In pH range 10.0-12.0 solutions sharing the same visible $\lambda_{max\text{-}vis}$, (599 nm) and his hues vary from green hue (h_{ab}=155.88°) to a yellow green hue (h_{ab}=118.89°). By contrast, solutions having shifted spectra share the same basic tonality. The cyanidin solutions with $\lambda_{max\text{-}vis}$ at 523 nm and 565 nm (pH 3.1 and 7.5 respectively) have the same basic tonality (h_{ab} 358.99-359.34°), and with $\lambda_{max\text{-}vis}$ at 550 nm, 559 nm and 562 nm (pH 4.6; 6.0 and 6.4 respectively) have the similar hues (h_{ab} 343.89-345.89°).

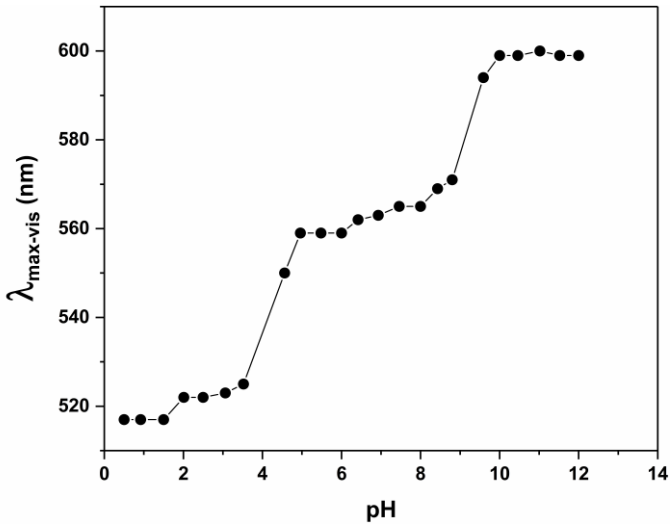


Figure 2. The visible absorbance maximum ($\lambda_{max\text{-}vis}$) values as a function of pH for the 2×10^{-4} mol dm^{-3}cyanidin aqueous solution.

Table 1. The influence of different pH values on visible absorbance maximum ($\lambda_{max\text{-}vis}$) and hue angle (h_{ab}) of $2 \cdot 10^{-4}$ mol·dm^{-3}cyanidin aqueous solution.

pH	$\lambda_{max\text{-}vis}$(nm)	h_{ab} (°)
cyanidin		
0.50	517	31.41
0.92	517	30.92
1.50	517	14.05
2.01	522	4.76
2.50	522	1.65
3.06	523	358.99
3.52	525	354.62
4.56	550	343.89
4.96	559	341.01
5.48	559	341.51
6.00	559	345.89
6.42	562	344.59
6.93	563	350.05
7.46	565	359.34
8.00	565	6.20
8.43	569	16.22
8.80	571	16.92
9.59	594	152.55
10.00	599	155.88
10.46	599	148.73
11.02	600	141.73
11.52	599	132.08
12.00	599	118.89
12.54		81.36
13.13		77.19

Conclusions

Measurement of the hue angle and the visible absorbance maximum showed that their values were highly dependent on the pH. The hue angle measurements of cyanidin aqueous solution showed that some measurable spectral variations did not correspond to a colour variation perceptible by the human visual system. Regarding the attribute of hue, the previous works unanimously considered a unique reference for anthocyanin solutions at different pH, their visible $\lambda_{max\text{-}vis}$. Consequently, when the $\lambda_{max\text{-}vis}$ remains stable, the "reference hue" was probably considered so. The colorimetric analysis of hue angle cyaniding aqueous solutions resulted in different conclusions. The solutions show huge variations in their chromatic tonalities, although their spectral $\lambda_{max\text{-}vis}$ effectively remained stable; then, *vice versa*, these solutions with shifted spectra share the same basic tonality, so $\lambda_{max\text{-}vis}$ of the cyanidin solutions at the various pHs correlated poorly with the corresponding h_{ab} and caution should be applied when using $\lambda_{max\text{-}vis}$ values for interpretation of colors.

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