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Colour effects of co-pigmentation of anthocyanins revisited—1. A colorimetric definition using the CIELAB scale

Jean-François Gonnet

Laboratoire B.M.P., Université Lyon I, 43 boulevard du 11 Novembre, F-69622 Villeurbanne,France

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This method is based on a spectral curve recorded and considered over the full range of visible light and a subsequent colorimetric specification (CIELAB system, L^*, C^*, h notation for the D65/10° illuminant/observer condition) using the three attributes of colour perception, hue, lightness and saturation for a complete definition of the colours of anthocyanin solutions. Spectral and colour measurements were performed on model solutions of pure and co-pigmented (by quercetin 3-rhamno-glucoside) cyanin (cyanidin 3,5-diglucoside) at pH from 2.5 to 5.5. Regarding the colour of anthocyanin solutions, major discrepancies were found between the results of this colorimetric analysis and those formerly reported which were only based on variations of the visible λ_{max}. As their concentration increased, pure cyanin solutions exhibited huge variations of hue (from magenta to orange) in their colour, although their λ_{max} remained stable. Contrary to usual reports concerning 'the blueing effect' of a spectral bathochromic shift, it was observed that the colour of solutions, as their visible λ_{max} moved higher, shifted either yellower or bluer, depending on the pH and concentration. As for colour variations caused by absorbance changes, this study showed that the currently employed term 'intensity' was quite ambiguous, indistinctly representing variations of two attributes of colour, saturation and lightness. Consequently, a revision of the effects of some chemical parameters on the colour of anthocyanin pigments is presented. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

With carotenoids, anthocyanins represent a major class of colouring matters in flowers, displaying a wide palette of shades in the 'cyanic' range (Haslam, 1995). These phenolic pigments are also present in foods, either as native colorants in vegetables, fruits and beverages or as additives in many other products (Markakis, 1982; Francis, 1993). Anthocyanins are in the stable and coloured form of flavylium cation in very acidic solutions only. As the pH increases, the anthocyanic nucleus is affected by important structural changes (Brouillard and Dubois, 1977) causing a dramatic loss of absorptivity (Asen *et al.*, 1975; Brouillard, 1983). In particular, in weakly acidic solutions corresponding to the pH values most frequently recorded in the vacuoles of flower petal cells (Stewart *et al.*, 1975), pure anthocyanins are predominantly accumulated as virtually colourless pseudobases. Consequently, the vivid colours displayed by flowers result from a phenomenon protecting or restoring stable and strongly absorbing forms of the anthocyanins, known as co-pigmentation (Robinson and Robinson, 1931). In short, it consists of the stacking of pigment (anthocyanin) and co-pigment (flavonoids, simpler phenolics or aliphatic acids) molecules, involving different chemical mechanisms: inter-, intra-molecular co-pigmentation, self-association,... (Goto, 1987; Mazza and Brouillard, 1987). Against those of the corresponding pure anthocyanins, the spectra of co-pigmented structures display a bathochromic shift of the visible λ_{max} coupled with a strong increase of absorptivity (hyperchromic effect), the magnitude of which depends on many factors: pH (Williams and Hrazdina, 1979), temperature (Mazza and Brouillard, 1987, 1990; Baranac *et al.*, 1996), the nature of both the anthocyanic chromophore (Williams and Hrazdina, 1979; Chen and Hrazdina, 1981; Baranac *et al.*, 1996) and the co-pigment (Asen *et al.*, 1972; Baranac *et al.*, 1996), the co-pigment to pigment molar ratio and the pigment absolute concentration (Asen *et al.*, 1972; Asen, 1976; Mazza and Brouillard, 1990).

Regarding its colour effects, Robinson and Robinson's original statement was that co-pigmentation 'may be defined as the phenomenon which makes the colour of anthocyanins *bluer, brighter* and stabler'. This consistently reported *blueing effect* was interpreted as the consequence of the bathochrome shift affecting the pigment visible absorption band, while 'increase in *colour* (or *tinctorial*) *intensity*' was the current term employed in describing the subsequent effect of hyperchromy.

In fact, this description of the colour effects of co-pigmentation is incorrect: (i) A 'basic' description of colour requires using three attributes: hue (or chromatic tonality), saturation (meaning 'purity' or vividness) and lightness (dark or light sensation). (ii) Although colour originates in a selective absorption of visible light radiations by objects (or pigments), it is not a physical phenomenon but a psychosensorial one, at the interface of three elements: a light source, an object (a pigment solution in the present context) and an observer (the eye and visual cortex in the human brain). It results that changes affecting the spectral definition of any of these elements will cause variations in the perception of colour; the functioning of this colour triplet has been examined in two previous papers (Gonnet, 1993, 1995).

Consequently, an adequate description of the colour variations of anthocyanins caused by co-pigmentation or pH requires (i) that spectral variations considered should be those affecting the entire spectral curve, not only its visible λ_{max}; (ii) that the three cited colour attributes should be employed; and (iii) that these should refer to one (or more) light source(s) and observer(s) condition(s). Although the colours of anthocyanins in foods (Price and Wrolstad, 1995; Giusti and Wrolstad, 1996) or flowers (Gonnet and Hieu, 1992) are currently assessed using this complete colorimetric notation, a comparable description of the colour effects of co-pigmentation of anthocyanins is rare. An example is Timberlake and Briddle (1984) who reported the hue, saturation and lightness variations of differently co-pigmented anthocyanin solutions using the CIELAB colour scale, but the illuminant (light source) and observer conditions employed were missing.

Based on the above considerations, a revision of the colour effects of co-pigmentation of anthocyanins appeared necessary. This paper is the first in a series addressing this topic and is focused on the methodological aspects of a new approach, using the concepts and methods currently in use for industrial or food product colour determination. The few examples considered here are from an extensive survey of the above-mentioned influential parameters on the colour of anthocyanins in aqueous media. The pigment and co-pigment used in the experiments were two widespread molecules: the anthocyanin cyanin (cyanidin 3,5-diglucoside), and the flavonol rutin (quercetin 3-rhamno-glucoside), respectively.

MATERIAL AND METHODS

Pigments

Cyanin was obtained from repeated preparative chromatography of a crude methanolic extract of rose petals; its purity was checked by high performance liquid chromatography (HPLC) and UV–visible spectrum. Rutin was commercially obtained from Extra-Synthèse (Genay, France) and recrystallized twice from methanol.

Stock solutions of pigment and co-pigment were prepared in MacIlvaine citrate-phosphate buffers (pH 2.5, 3.5, 4.5 and 5.5); each co-pigment–pigment solution was prepared by mixing equal volumes of cyanin and rutin at adequate concentrations, then vigorously stirred and left (in darkness) for 10 min at 22°C (thermostatic water bath) before measurement.

Spectrophotometric assays

Spectral curves were recorded (regular transmission, from 380 to 780 nm with a 2 nm bandwith) in 10 mm optical path quartz cuvettes using a Kontron Uvikon 943 spectrophotometer.

Colorimetric calculations

All the colour calculations were performed by a specially developed computer program run on a Zeos PC machine. From the transmittance spectral curves, the X, Y and Z tristimulus values were computerized for a couple of CIE illuminant/observer conditions: D_{65} (diffuse daylight type) and A (tungsten light), both for the 'supplementary', or 10°, CIE observer, according to the weighted ordinate method (Billmeyer and Fairman, 1987) at the recommended 5 nm step (CIE, 1986). The spectral relative energy data of the CIE illuminants and the 10° observer colour matching functions were those of the ISO/CIE 10526 and 10527 norms, respectively (ISO, 1991). From the corresponding X, Y and Z values, the L^*, a^*, b^* CIELAB co-ordinates were calculated first using the following equations (Sève, 1993):

$$L^* = f\left(\frac{Y}{Y_0}\right)$$

$$a^* = \frac{500}{116}\left[f\left(\frac{X}{X_0}\right) - f\left(\frac{Y}{Y_0}\right)\right]$$

and

$$b^* = \frac{200}{116}\left[f\left(\frac{Y}{Y_0}\right) - f\left(\frac{Z}{Z_0}\right)\right]$$

with $f(R) = 116R^{\frac{1}{3}} - 16$ when $f(R) \geq 8$, otherwise $f(R) = \left(\frac{29}{3}\right)^3 R$.

For each CIE illuminant/observer condition considered, X, Y, Z and X_0, Y_0 and Z_0 are the tristimulus values of the solution and the illuminant, respectively, while R alternatively corresponds to X/X_0, Y/Y_0 and Z/Z_0 ratios. Metric chroma, C^*, and hue angle, h_{ab} (CIELCH notation) were finally obtained by the transformation of a^* and b^* cartesian co-ordinates into polar ones according to the following equations:

$$C^* = (a^*2 + b^*2)^{0.5} \text{ and } h_{ab} = tang^{-1}(b^*/a^*)$$

On the chromatic circle in Fig. 1, hue angle values are stepped counterclockwise from h_{ab} 0°–360° (magenta–red) across a continuously fading hue circle, the other remarkable values of which are 90° (yellow), 180° (bluish-green) and 270° (blue).

Total colour difference (ΔE^*) and its components (ΔL^*, ΔC^* and ΔH^*) between a sample pair (1 and 2) were obtained using the following formulas: $\Delta E^* = (\Delta L^*2 + \Delta C^*2 + \Delta H^*2)^{0.5}$, with $\Delta L^* = L_1^* - L_2^*$ and $\Delta C^* = C_1^* - C*_2$ (CIE, 1986).

The perceived hue difference ΔH^* calculation requires that the hue angle difference ($\Delta h = h_1 - h_2$) is weighted by chroma:

$$\Delta H^* = 2\sin(\Delta h/2) \times (C_1^* \times C_2^*)^{0.5} \text{ (Sève, 1991).}$$

Although the visual perception of colour differences changes with the location of colour within the CIELAB space—and with the direction of the difference—a mean threshold value of $\Delta E^* = 1$ will be assumed as a worthwhile basis for just perceptible colour differences between two solutions.

RESULTS AND DISCUSSION

Compared with all prior studies on the co-pigmentation of anthocyanins, the first difference in this work was that the spectra of solutions covered the full range of wavelengths perceived by the human visual system. Then, the functioning of the colour vision triplet was simulated in the subsequent colorimetric calculations: the spectral data of each solution were combined with those of the other two elements of the triplet, a reference light source (namely a relative spectral distribution curve of a CIE 'illuminant') and a reference visual system (namely a set of three spectral response curves, of a CIE 'observer') in the computation of CIE 'tristimulus values' X, Y and Z (CIE, 1931). These represent the basic numerical specification of the colour of solutions, as seen by the observer under the specified light conditions. Further transformations of the tristimulus values into the CIELAB colour space (CIE, 1986) finally gave colorimetric co-ordinates which represent the psychometrical correlates of the natural attributes of human colour perception, hue, saturation and lightness, expressed in terms of hue angle (h_{ab}), metric chroma (C^*) and lightness (L^*), respectively (CIELCH notation, Fig. 1). More information about the principles of colour calculations and the arrangement of the CIELAB system have been presented previously (Gonnet, 1993, 1995).

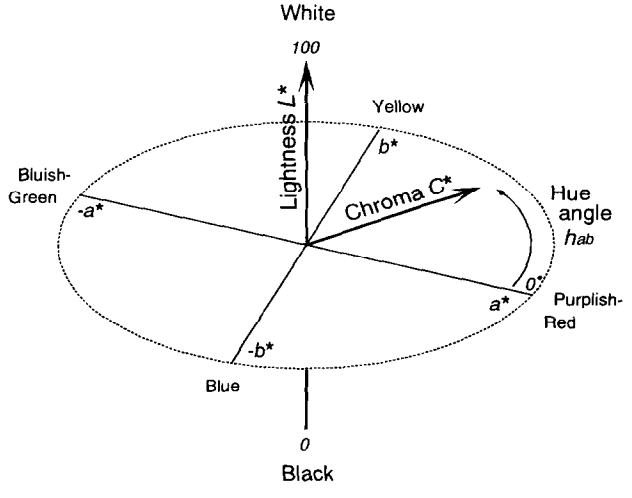

Fig. 1. CIELAB colour space..

Spectral variations in cyanin and cyanin–rutin solutions

Table 1 presents selected examples of the 224 curves recorded which are synthetical of the different spectral variations caused by the presence of rutin in cyanin solutions. These are considered here mainly to determine the accordance of the present results with those previously published.

As pH increased, the λ_{max} of pure cyanin solutions shifted from 510 nm (at pH 2.5) to 526 nm (at pH 5.5) for the 5×10^{-4} M solution. This bathochromic shift was accompanied by a dramatic hypochromic effect: the absorbance at pH 5.5 was only about 3.5% of the absorbance recorded at pH 2.5. Changes in both of these spectral effects caused by the addition of rutin were amplified as:

- pH changed from 2.5 to 5.5, with a maximal efficiency at pH 4.5.
- Co-pigment to pigment molar ratio increased. At the 1:1 ratio no or very slight changes occurred at the λ_{max}, while these became striking at a molar ratio of 8:1.
- Concentration of cyanin was higher. Against those recorded in solutions at 5×10^{-5} M, both of the spectral effects observed in corresponding solutions at 5×10^{-4} M had a stronger amplitude (more than two-fold for some, especially at higher pH values).

Consistent results were obtained throughout the multiple batches of model co-pigment–pigment solutions realized. In particular, the maximal spectral (bathochromic and hyperchromic) effects were always

Table 1. Effects of pH, anthocyanin and co-pigment concentrations on λ_{max} and absorbance in the spectra of cyanin/rutin solutions (citrate-phosphate buffer, optical path 1 cm, temperature 22°C)

Cyanin concentration		pH 2.5		pH 3.5		pH 4.5		pH 5.5	
		λ_{max}	A_{max}	λ_{max}	A_{max}	λ_{max}	A_{max}	λ_{max}	A_{max}
5×10^{-5} M		511 nm	0.480	511 nm	0.083	520 nm	0.021	523 nm	0.017
	Rutin:cyanin ratio	$\Delta\lambda$	A/A_0	$\Delta\lambda$	A/A_0	$\Delta\lambda$	A/A_0	$\Delta\lambda$	A/A_0
	1:1	0 nm	1.04	0 nm	1.04	+1 nm	1.19	0 nm	1.16
	8:1	+4 nm	1.13	+5 nm	1.47	+7 nm	1.73	+5 nm	1.45
		λ_{max}	A_{max}	λ_{max}	A_{max}	λ_{max}	A_{max}	λ_{max}	A_{max}
5×10^{-4} M		510 nm	4, 953[a]	512 nm	0.863	519 nm	0.202	526 nm	0.163
		$\Delta\lambda$	A/A_0	$\Delta\lambda$	A/A_0	$\Delta\lambda$	A/A_0	$\Delta\lambda$	A/A_0
	1:1	+5 nm[a]	1.16[a]	+3 nm	1.59	+9 nm	1.62	+9 nm	1.47
	8:1	+21 nm[a]	1.45[a]	+19 nm	2.33	+21 nm	3.74	+16 nm	3.74

$\Delta\lambda$ denotes the importance of the bathochrome shift in the presence of co-pigment. A/A_0 is the ratio of absorbances at λ_{max} in the spectra of co-pigmented (A) and pure cyanin (A_0) solutions.
[a]Calculated from spectral records in 2 mm optical path cuvette.

recorded in the solution in each series with the highest co-pigment to pigment ratio. Consequently, regarding the spectral effects of co-pigmentation of anthocyanins, all the present results completely matched those reported in previous studies and their subsequent colour expression can be adequately considered.

Colour changes of solutions with the light source

To emphasize how essential the reference to a light source is for a correct description of colours, two illuminant conditions were employed for the calculation of colour co-ordinates. These represent a reversed balance of long vs short visible wavelengths in their power spectral distribution: CIE D_{65} illuminant depicts typical diffuse daylight (colour temperature 6504K, predominance of short wavelengths), while CIE illuminant A represents an incandescent lamp (colour temperature 2864K, predominance of long wavelengths).

With reference to their colour observed under illuminant D_{65}, most of the pigment solutions exhibited striking colour changes when viewed under illuminant A (Table 2). With the exception of virtually colourless solutions (cyanin at the lowest concentrations and pH 3.5–5.5), the total difference (ΔE^*) between their colour under these two light sources exceeded by a large margin the visual perceptible threshold, reaching $\Delta E^* = 25$ units for some. With no exception, the colour of cyanin solutions—co-pigmented or not—became lighter (L^* increased) under illuminant A; the other two colour parameters were differently affected by the light changes as shown in the examples reporting the different types of variation observed.

- For solution 1, the prevailing factor originating the perceived colour difference was—increasing—chroma; simultaneously, a perceptible shift of hue occurred (ΔH^* was positive, meaning that hue moved yellower).
- The 'dense' colour of solution 2 was strongly modified under illuminant A ($\Delta E^* > 21$ units), becoming far lighter, saturated and yellower, with a comparably important variation in the three attributes of colour.
- Under D_{65} light, the colours of solutions 3 and 4 had chroma and hue in a close range, but strongly differed by their lightness ($\Delta L^* > 35$). When moved under type A light, the colour of both solutions showed a comparable major shift of hue (ΔH^* about 17 units, in the yellow direction). By

Table 2. CIELAB colour co-ordinates and colour differences of five model solutions when successively observed under daylight (D_{65}/10°) and tungsten light (A/10°)

	(D_{65}/10°)			(A/10°)			Colour differences(A-D_{65})/10°			
	L^*	C^*	h_{ab}	L^*	C^*	h_{ab}	ΔE^*	ΔL^*	ΔC^*	ΔH^*
Solution 1	68.1	26.76	5.5	71.5	32.78	10.9	7.45	3.37	6.02	2.80
Solution 2	32.1	87.56	38.9	42.8	99.56	47.4	21.25	10.63	12.00	13.94
Solution 3	32.4	65.14	2.8	41.4	65.48	17.7	19.20	8.98	0.34	16.96
Solution 4	68.2	62.54	1.4	76.0	56.10	18.4	20.17	7.80	−6.44	17.45
Solution 5	82.2	17.54	61.8	84.1	22.20	42.0	8.46	1.95	4.66	−6.79

Solution 1: Cyanin 5×10^{-4} M, co-pigment ratio: 8:1, pH 5.5. Solution 2: Cyanin 10^{-3} M, co-pigment ratio: 4:1, pH 2.5. Solution 3: Cyanin 2.5×10^{-3} M, co-pigment ratio: 1:1, pH 4.5. Solution 4: Cyanin 10^{-4} M, co-pigment ratio: 8:1, pH 2.5. Solution 5: Cyanin 2.5×10^{-4} M, co-pigment ratio: 16:1, pH 5.5.

contrast, there was no perceptible change in chroma for solution 3, while it significantly decreased in the colour of solution 4. It is also noteworthy that these two solutions displaying very close hues under both illuminants—solution 4 having either the bluest or the yellowest basic hue, depending on the illumination (h_{ab} 1.4°, vs 2.8° under D_{65} and h_{ab} 18.4° vs 17.7° under A)—had their λ_{max} far apart, 520 and 536 nm.

- For solution 5, the parameter most affected by light change was again hue (L^*, slightly decreased, C^* increased); here, the hue angle shifted clockwise (ΔH^* −6.79) under illuminant A, meaning that unlike most samples in this survey, the chromatic tonality of the solution moved to the blue pole of the chromatic circle.

These few examples show how intensely and specifically the perception of the colour attributes—and the subsequent colour variations between samples—was affected when the light conditions change, although it was spectrally originating on a constant absorption curve for each solution. Consequently, these results definitively emphasize that reporting on colour without the specification of reference light conditions cannot be achieved correctly; the same remark also applies to the observer condition.

In this study—and those to come—the colour of solutions will be considered for the CIE $D_{65}/10°$ illuminant/observer condition. This corresponds to the colour stimulus perceived by an observer viewing (10° observation field) a perfectly white background illuminated by daylight (D_{65} type) through a pigment solution of 1 cm thickness.

The revised description of anthocyanin colours: examples of pure cyanin solutions

Among the shortcomings of the previous studies, was a flawed description of the colour variations resulting from pH and/or co-pigmentation effects and also of the 'reference' colour of pure anthocyanin solutions. As reported before, the parameters employed for describing the colour of anthocyanin solutions were spectral data: mainly shifts of the visible λ_{max} as a correlate for hue variations and absorptivity changes for those of colour 'intensity'. These topics will be re-examined with the examples of solutions of pure cyanin at different pH and concentrations (Table 3), covering a comparable range to those analysed in earlier works.

First, CIELAB co-ordinates combining a very high L^* (approximately 95 or more) with very low C^* (approximately 4 or less) correspond to a virtually achromatic stimulus (i.e. white). This phenomenon was observed here for solutions at pH 4.5 and 5.5 in the 10^{-5}–10^{-4} M bracket, and for those at 10^{-5} M and 2.5×10^{-5} M at pH 3.5. Although coherent spectral—and colorimetric—variations were measured between these solutions, these do not correspond to any visually perceptible stimulus under the reference conditions of observation, and are consequently meaningless regarding their colour effect.

Regarding the attribute of hue, the previous works unanimously considered a unique reference for non-co-pigmented solutions at different concentrations, their visible λ_{max}. Consequently, since the λ_{max} generally remains stable as the concentration of solutions changes, the 'reference hue' was probably considered so. The colorimetric analysis resulted in strikingly different conclusions, the solutions in the four series studied consistently displaying huge variations of chromatic tonalities, although their spectral λ_{max} effectively remained stable (excepted for the solutions at pH 5.5) when their concentration increased.

Perceptually, the most remarkable variation was the extent of the hue gamut covered by the cyanin solutions at pH 2.5 (sharing the same visible λ_{max}, 510–511 nm): from a magenta-red hue (h_{ab} 358.4°) in the colour of the solution at 10^{-5} M it hardly shifted to an orange basic hue (h_{ab} 48.3°, solution at 10^{-3} M) and then moved backwards to red (h_{ab} 29.4°, solution at 5×10^{-3} M).

Comparable effects of concentration were observable in the batch of solutions at pH 3.5 from 5×10^{-5} M (λ_{max}, 511–512 nm) with a more restricted amplitude of nuances, from magenta-red (h_{ab} 1°) to orange-red (h_{ab} 38.2, then back to 35.8°).

Table 3. CIELAB co-ordinates ($D_{65}/10°$) and visible λ_{max} (nm) of pure cyanin solutions

Cyanin concentration (M)	pH 2.5				pH 3.5				pH 4.5				pH 5.5			
	L^*	C^*	h_{ab}	λ_{max}	L^*	C^*	h_{ab}	λ_{max}	L^*	C^*	h_{ab}	λ_{max}	L^*	C^*	h_{ab}	λ_{max}
10^{-5}	96.3	8.67	358.4	510	98.8	1.23	1.4	511	99.0	0.33	16.8	520	99.4	0.19	26.8	523
2.5×10^{-5}	92.7	17.89	1.4	511	97.9	3.25	1.3	511	98.9	0.65	14.7	520	99.1	0.45	22.9	523
5×10^{-5}	87.5	30.99	4.8	511	96.5	6.49	1.0	511	98.4	1.33	9.4	520	98.7	0.98	23.1	523
10^{-4}	80.0	47.87	11.0	511	93.9	12.54	1.5	511	97.4	2.70	5.9	520	97.6	2.11	24.1	523
2.5×10^{-4}	69.2	69.69	26.9	510	86.8	27.99	2.9	511	94.7	7.03	3.1	520	94.6	5.39	22.5	523
5×10^{-4}	60.1	90.00	41.2	510	76.8	46.95	5.9	512	89.7	14.28	2.3	519	90.3	10.41	12.1	526
10^{-3}	48.3	106.86	48.3	–	62.1	66.25	17.7	512	79.9	28.73	0.5	520	80.6	20.97	7.9	528
2.5×10^{-3}	31.7	85.26	39.7	–	42.2	87.89	38.2	–	58.8	54.10	3.4	521	57.2	45.79	9.1	528
5×10^{-3}	16.6	57.89	29.4	–	24.5	71.91	35.8	–	35.9	67.79	17.2	522	31.6	62.39	16.9	528

–, not measurable, absorbance over 4.5.

At the highest pH, variations in the chromatic tonalities of perceptibly coloured solutions at pH 4.5 (λ_{max}, 520–522 nm) covered the magenta-red (h_{ab} 3-0°) to red portion (h_{ab} 17.2°); at pH 5.5 (λ_{max}, 523–528 nm) hues started from red (h_{ab} 22.5°, 2.5×10^{-4} M), shifted bluer to red-magenta tints (h_{ab} 7.9°, 10^{-3} M), then moved yellower, to red (h_{ab} 16.9°, 5×10^{-3} M).

Another concern in the previous reports concerning the hues of solutions is the 'blueing effect' coupled with the bathochromic shift of the visible λ_{max}. In Table 3, the major trend of spectral variation as pH increased from 2.5 to 5.5 was effectively that general bathochromic shift of the λ_{max} of all solutions. Colour measurement revealed that the actual hue changes did not meet the previous statements. At all pigment concentrations, the hue angles corresponding to the bluest hues were not precisely those measured for the solutions having the highest λ_{max}. For instance, in a batch of four solutions having chroma and lightness in the same bracket—cyanin at 2.5×10^{-5} M (pH 2.5), 10^{-4} M (pH 3.5), and 5×10^{-4} M (pH 4.5 and 5.5)—the first three had their respective λ_{max} at 511, 511, 519 nm, while the hue angles measured were h_{ab} 1.4, 1.5 and 2.3°, meaning that their colour shared identical chromatic tonalities. Finally, the fourth solution having the highest λ_{max} (526 nm) displayed a distinctly yellower hue (h_{ab} 12.3°). By contrast, an important blueing effect on hue was observed for the solutions at 2.5×10^{-4} M when pH increased from 2.5 (h_{ab} 26.9°) to 3.5 (h_{ab} 2.9°), while little change was observed at their λ_{max} (510 and 511 nm). Finally, in the colour of all the solutions at pH 5.5 vs those at pH 4.5, a remarkable inverse, i.e. yellowing, effect occurred (hue angles moved counterclockwise), although the $\Delta\lambda_{max}$ were consistently shifted from 519–522 nm to 526–528 nm.

Regarding the hypochromic spectral effect resulting from lowering the pigment concentration and/or increasing the pH of anthocyanin solutions, all the papers reviewed agreed that 'colour *intensity* subsequently decreased or even vanished'. CIELAB measurement of solutions at different concentrations and pH (Table 3) revealed that colour 'intensity' changes corresponded to simultaneous and extensively variable variations of lightness and chroma. For all the solutions in Table 3, increasing the absorptivity of the solutions (by increasing the pigment concentration at each pH or lowering the pH at each concentration), always caused a lower lightness, i.e. their colour became darker.

The variations of chroma with absorbance changes were more complex. It frequently increased with pigment concentration (all the solutions in the batches at pH 4.5 and 5.5) or decreased with pH (solutions at 2.5×10^{-4} M to 10^{-3} M when pH varied from 2.5 to 5.5). For the darkest solutions at lower pH, further increased absorptivity—by pigment concentration or pH effects—caused a correlative reversed effect on chroma, which progressively decreased from a maximal value measured at a lower concentration (example of C^* decreasing from 106.86 (10^{-3} M) to 57.89 (5×10^{-3} M) for the solutions at pH 2.5).

CONCLUSION

The results of this colorimetric approach applied to a series of model solutions of cyanin and some cyanin–rutin mixtures revealed that the spectral variations resulting from the co-pigmentation phenomenon were previously incorrectly interpreted, regarding their effects on anthocyanin colours.

First, because of its triplicate intrinsic origin, there is not 'absolute' colour (of a solution here); consequently, colour analysis requires that reference light and observer conditions are first fixed. This problem is a general consideration, applying to all the fields concerned with colour, and in particular, to food products containing colouring matters (anthocyanins, for instance, co-pigmented or not), which are presented to customers in stores under widely variable light conditions.

Colour measurement of anthocyanin solutions showed that some measurable spectral variations—especially those recorded at the lowest pigment concentrations and higher pH—did not correspond to a colour variation perceptible by the human visual system.

This study also addressed the issue of the colour of the non-co-pigmented anthocyanin solutions serving as the reference for the description of colour variations caused by co-pigmentation or pH, especially regarding the attribute of hue. In this field, the visible λ_{max} of the pure pigment solutions was formerly the unique reference considered. CIELAB analysis consistently revealed that, although the λ_{max} of pure cyanin solutions at different concentrations remained stable, their colour displayed huge and complex variations of chromatic tonalities, varying from magenta to orange for some. Again concerning hue, another major discrepancy between the present results and the prior ones was the relationships of this attribute with the shifted visible λ_{max} of solutions, especially regarding the 'blueing effect' on colour. In this survey, different examples were those of solutions within a series or between series having the highest λ_{max} which were not displaying the bluest hues—by a large margin for some. Reversed effects, i.e. the yellowest chromatic tonalities measured for the solutions exhibiting the highest λ_{max} in their spectrum, were even observable in some batches of solutions.

Similarly, CIELAB measurement showed that colour 'intensity' is an ambiguous term for the description of anthocyanin colours, since it indistinctly covered simultaneous or alternate variations of two attributes of colour, lightness and chroma, which are differently influential in the appreciation of products' colours. Generally, variations of the saturation level are producing more attractive effects than those of lightness on the consumer's eye.

These consistent results emphasized that the absorption changes recorded at the visible λ_{max} of anthocyanin solutions only represent some of the multiple spectral factors originating the colour variations perceived by a human observer. Consequently, reporting on the effects of co-pigmentation or/and pH on the colour of anthocyanins, and especially with a view to using these molecules more beneficially as food additives for the production of more attractive colours based on natural products, requires that all the parameters examined in this paper are considered. Attention must be paid to the variations affecting all the portions of the spectral curves, recorded over the full extent of the human visual system sensitivity (380–780 nm). Regarding the colour effects of these spectral variations, a synthetic and realistic view can be fully achieved by means of the colorimetric analysis of solutions, especially using the CIELAB system. Basically, this method is considering all the parameters involved in the perception of colours and their variations, which are described by using numerical specifications representing the psychometrical correlates of the three natural attributes of colour perception, hue, lightness and saturation.

In this regard, a survey of the revised colour effects of the co-pigmentation of the anthocyanin cyanin by the flavonol rutin—including all the influential parameters reviewed in the introduction–and using the above-described colorimetric method will appear in a subsequent paper.

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