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Chromatic characterization of anthocyanins from red grapes—I. pH effect

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Five anthocyanins (the 3-monoglucoside of delphinidin, cyanidin, petunidin, peonidin and malvidin), isolated from red grape skins, were subjected to a spectroscopic study to characterize their chromatic properties in a model solution imitating wine in the pH range 1.5–7.0. Tristimulus colorimetry (CIEXYZ, CIELUV and CIELAB colour spaces) proved to be a useful tool for the chromatic characterization of these anthocyanins. Significant colour differences were found among them, related to the number and type of substituents in their B rings. Two-substituted compounds were located in the area of orange hue, while three-substituted compounds were in the area of red–purple. As the methoxylation degree increased, a shift was observed toward purple. Also, a relationship was found between saturation and chroma and the number of hydroxy groups in the B ring. In all the anthocyanins studied, the increase in pH provoked a curve displacement in chromaticity, approaching that of the illuminant. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Anthocyanins are natural pigments, responsible for a wide range of colours in vegetables, fruits, and derived products, such as jams, juices or red wines. Because of consumers' concerns for synthetic colorants, pigments obtained from natural sources, and particularly anthocyanins, have gained increasing interest in the food industry. The skins of grapes obtained as a by-product of the winemaking process are currently the main source for the extraction of anthocyanins for their use as food colorants (Francis, 1989). Grape anthocyanins are based on five aglycones, which differ by their pattern of substitution in the B ring. They occur exclusively as 3-glucosides in *Vitis vinifera* varieties, while 3,5-diglucosides also occur in American species (*V. riparia, V. rupestris*, etc.) and hybrid varieties.

There are some limitations to the use of anthocyanins as food colorants. The influence of pH on their colour is well known, as well as the bleaching effect of the sulphite addition. A number of physical conditions also affect anthocyanin stability, such as temperature, light, oxygen, metals, etc. In aqueous solutions, common anthocyanins exist as a mixture of several structures in chemical equilibrium: flavylium cation (red), quinonoidal anhydrobase (blue), carbinol pseudobase (colourless), and chalcone (colourless or light yellow). At pHs below 2, anthocyanins exist basically in cationic form; as the pH increases, a rapid deprotonation occurs to afford quinonoidal forms. In aqueous media, the hydration of the flavylium cation gives the hemiacetal form in equilibrium with a chalcone. At room temperature, and in slightly acidic media, the equilibrium between the carbinol and chalcone forms is very slow and takes hours to be reached. The increase of temperature displaces the equilibria towards the chalcone forms. The hydration equilibrium is established faster for the anthocyanin 3,5-diglucosides than for the corresponding 3-glucosides. The relative amounts of all these structural forms vary with both pH and the structure of the anthocyanin. For the grape anthocyanins, the major product formed at pH values above 3 is the hemiacetal form and that is the reason why slightly aqueous solutions of anthocyanins are usually colourless (Iacobucci and Sweeny, 1983; Cheminat and Brouillard, 1986).

Anthocyanin equilibria are also influenced by pigment concentration and the presence of other compounds. In their natural media, anthocyanins are frequently associated to colourless molecules (mainly, polyphenols and other flavonoids), which exert a

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determining influence on the colour. The process of copigmentation has been extensively studied in model solutions (Mazza and Brouillard, 1990; Brouillard *et al.*, 1991; Dangles and Brouillard, 1992; Baranac *et al.*, 1996) and plant extracts (Giusti and Wrolstad, 1996; Figueiredo *et al.*, 1996) and its influence on colour expression in flowers (Goto and Kondo, 1991) is well known. In solutions containing mixtures of different anthocyanins, such as fruit juices and red wine, the contribution of each and every pigment to the colour should also be considered.

The application of colorimetric systems, including uniform colour spaces (CIELUV and CIELAB) and non-uniform colour spaces (CIEXYZ), is of great value in the quantification and characterization of anthocyanin chromatic properties. Tristimulus colorimetry has been widely applied to food and food products with different purposes, such as the assessment of colour changes during food processing (Maga and Kim, 1990; Dodds *et al.*, 1991) or wine alteration (Cheynier *et al.*, 1990), as well as a maturity index (Delwiche and Baumgardner, 1985; Delwiche, 1989). Also, chromaticity value has been related to pigment concentration (Little, 1977; Berset and Caniaux, 1983; Singha *et al.*, 1991; Ihl *et al.*, 1994) and to physicochemical parameters (Heredia and Guzmán, 1995).

The aim of the present work was to establish the chromatic characteristics of the main anthocyanins found in red grapes, and their changes with regard to pH modification. The application of tristimulus colorimetry offers an objective measurement of colour, due to the fact that it is based on the consideration of the whole visible spectrum, and allows the real chromatic profile of each compound to be obtained. This work is part of a wider study on the different factors that influence the colour of anthocyanins and their mixtures. Quantitative colour measurements have been carried out by spectrophotometry in solutions of anthocyanins previously isolated from red grape skins.

MATERIALS AND METHODS

Anthocyanin extraction

Anthocyanins were extracted by repeated maceration of red grape skins (*V. vinifera*) in methanol containing 5% of 1 N HCl at 4°C. Methanol phases were successively pooled, concentrated under vacuum at 30°C, redissolved in water and washed with *n*-hexane to remove liposoluble substances. The aqueous extract was concentrated and placed on a column (50 × 2.5 cm) filled up to 30 cm with a mixture of Polyclar AT (20%), Silicagel 60 for column chromatography (70%) and Silicagel 60 G for thin-layer chromatography (TLC; 10%), prepared as described by Bourzeix *et al.* (1980). Sugars and other polar substances were washed with water and the anthocyanins eluted with acidic methanol (0.1% HCl). The eluate was concentrated under vacuum and redissolved in a minimum volume of water.

Anthocyanin isolation

The water extract was fractionated by semipressure liquid chromatography using Büchi equipment equipped with a model 681 pump, a model 687 gradient former and a UV-VIS model 683 detector. A 46 × 2.6 cm column was used, packed at the laboratory with Lichoprep RP-18 of 24–40 μm (Merck, Darmstadt, Germany). A gradient was established between 10% acetic acid (A) and methanol (B): isocratic 0%B over 40 min; 0%B to 5%B over 40 min; isocratic 5%B over 80 min, and 5%B to 10%B over 60 min, at a flow rate of 6 ml min^{-1}. Anthocyanin detection was made at 520 nm and the peaks were collected in a Frac-100 (Pharmacia, Uppsala, Sweden) fraction collector. Impure anthocyanins collected were purified by HPLC in a Waters Model 600 controller pump using a Nova-pak C18 6 μm (25 × 100 mm) column. The solvents were 5% acetic acid (A) and gradient grade methanol (B), establishing the following gradient: isocratic 5%B over 22 min; 5%B to 10%B over 3 min; isocratic 10%B over 5 min, and 10%B to 15%B over 15 min, at a flow rate of 15 ml min^{-1}. Detection was carried out at 280 nm with a Waters model 486 Turnable absorbance detector. The compounds isolated were transferred to an aqueous solution and lyophilized. The purity of the lyophilized anthocyanins was determined by HPLC/DAS (Diode Array Spectroscopy). Anthocyanins obtained were the 3-monoglucoside derivatives of delphinidin (Dp3g), cyanidin (Cy3g), petunidin (Pt3g), peonidin (Pn3g) and malvidin (Mv3g).

HPLC/DAS analysis

This was carried out according to Rivas-Gonzalo *et al.* (1995) in a Hewlett–Packard series 1050 pump with a Lichrospher 100 RP-18 5 μm column (25 × 0.4 cm I.D.) thermostatted at 30°C; 5% formic acid (A) and gradient grade acetonitrile (B) were used as solvents, establishing a linear gradient between 10%B and 35%B over 50 min at a flow rate of 1.5 ml min^{-1}. Detection was carried out at 250, 280, 330, 440 and 520 nm with an HP-1040M series II diode array detector coupled to a HP79994A data treatment station.

Molar absorptivity measurement

Solutions containing 10^{-4} M of each anthocyanin were prepared in 0.5% tartaric acid (w/v) with 10% ethanol, adjusted at a pH value of 0.5 with HCl, in order to completely displace the equilibria towards the flavylium form. Molar absorptivities of the flavylium cations were calculated from the absorbance measured at the maximum wavelength of each anthocyanin, in a Perkin-Elmer Lambda 3B spectrophotometer using a 10 mm pathlength quartz cell.

Spectral measurements

Solutions of 10 ml were prepared containing 10^{-4} M of each anthocyanin in 0.5% tartaric acid (w/v) with 10% ethanol, adjusted at an initial pH value of 1.5 with HCl. Successive pH jumps of 0.2 units (from pH 1.5 to pH 8.0) were achieved by adding small volumes of NaOH 1 M or 10 M (< 25 μl); at the final pH value the dilution of the solutions was lower than 5%. Measurements of pH were carried out in a Crison Micro pH 2000 pH meter. Absorption spectra (380–770 nm) of the solutions were recorded at every pH value using a Perkin–Elmer Lambda 3B spectrophotometer in a 10 mm pathlength quartz cell, with water as a reference.

Tristimulus colorimetry

From the visible spectra obtained, tristimulus values were obtained by the weighted-ordinate method ($\Delta\lambda$ = 1 nm), with the CIE 1964 standard observer (10° visual field) and the CIE standard illuminant D_{65}, as references. Chromatic analyses were carried out following CIE recommendations (CIE, 1986, 1995): CIE 1964 (x,y) system (CIEXYZ), CIE 1976 ($L^*u^*v^*$) space (CIELUV), and CIE 1976 ($L^*a^*b^*$) space (CIELAB). Calculations were made using PCROM®, an original PC software (Álvarez and Heredia, 1994).

RESULTS AND DISCUSSION

The purities of the isolated anthocyanins, calculated from the chromatographic areas of their peaks in the HPLC chromatograms registered at 280 nm, were 90% for Dp3g and Pt3g, 93% for Pn3g and 94% for Mv3g. The impurities detected were due to other grape skin components, and occasionally anthocyanins with similar reverse-phase–high performance liquid chromatography (RP–HPLC) behaviour. No products of degradation were detected from any of these anthocyanins in the HPLC chromatograms, such as anthocyanidins, or products of their partial hydrolysis, such as syringic acid (from malvidin) or vanillic acid (from peonidin). The purity of Cy3g (81%) was lower, due to its higher instability and lower concentration in the grape skin, making its isolation more difficult. The main impurities detected were Pt3g (5%) and another peak showing absorbance only in the UV region that could be assigned to a product of the degradation of Cy3g.

Significant differences can be observed among the spectra of the anthocyanins obtained at pH 1.5, where the flavylium cation is dominant. The maximum absorption is slightly shifted towards lower wavelengths in the anthocyanins with two substituents in the B ring (Cy3g and Pn3g, λ_{max} around 512 nm), as compared with those showing three (Dp3g, Pt3g, Mv3g, λ_{max} between 518 and 520 nm). It can be observed that, at this pH, the absorbance at maximum wavelength increases with the number of hydroxyl groups in the B ring; thus, Dp3g (three –OH) shows the highest value, followed by Pt3g and Cy3g (two –OH) and Mv3g and Pn3g (one –OH). On the other hand, the absorbance decreases when hydroxyl groups are substituted by methoxyl groups, as shown by comparison of the series with three (Dp3g > Pt3g > Mv3g) and two (Cy3g > Pn3g) B ring substituted anthocyanins.

At pH 1.5, the absorbance of the solutions (10^{-4} M), at the maximum wavelength of each anthocyanin, ranged between 1.356 and 2.074 units (10 mm quartz cell). These differences are partially explained by the different molar absorptivities of their flavylium cations, which are the dominant structural forms at pH 1.5. The molar absorptivities have been calculated taking into account the purity of each anthocyanin established from their HPLC chromatograms, the values being: 23 700 for Dp3g, 18 800 for Cy3g, 18 900 for Pt3g, 14 100 for Pn3g and 20 200 Mv3g. However, a different distribution of species in equilibrium must also exist for each anthocyanin at this pH. This would explain the lower absorbance of the Mv3g solution when compared with those of Pt3g and Cy3g, in spite of the greater molar absorptivity of its flavylium form. It must be assumed that, at pH 1.5, a higher percentage of Mv3g exists as colourless forms, which could be related to the methoxylation of the B ring, as it is also observed in pH 3.5 solutions (see below).

An important hypochromic effect can be observed in all the anthocyanin spectra at pH 3.5, considered as a reference value for wine pH, where the maximum absorbance ranges between 0.141 and 0.315 units. It is evident that the hydration reaction, leading to colourless structures, predominates over the proton transference equilibrium in these solutions. Nevertheless, the slight bathochromic shift (2 to 4 nm) observed in the λ_{max} of the anthocyanin spectra when compared with their λ_{max} at pH 1.5, indicates that certain amounts of the coloured quinonoidal bases also occur in the solutions at pH 3.5. The absorbance at pH 3.5 seems to be influenced by the methoxylation in the B ring. Cy3g (two hydroxyl and no methoxyl groups) shows the highest absorbance, followed by Dp3g > Pt3g > Pn3g > Mv3g (two methoxyl, one hydroxyl). According to Iacobucci and Sweeny (1983), lower percentages of coloured quinonoidal bases are expected to be formed as the number of methoxyl groups increases in the molecule, which leads to a higher proportion of colourless anthocyanin pseudobases. This could also explain the slightly greater bathochromic displacement (4 nm) of the λ_{max} observed at pH 3.5 for Dp3g, (the one with the greater number of hydroxyl groups in the B ring) than for the other anthocyanins (2 nm).

The chromatic characteristics of the pigments studied are shown in Tables 1–3. Dominant wavelengths, calculated as by Heredia and Guzmán (1992), for the red–orange forms (Cy3g, Pn3g) are located in the λ_d = 600–620 nm interval, with excitation purity values, $p_e \sim 50\%$. The bluer forms (Mv3g and Pt3g) are distributed into

the non-spectral purple triangle, with complementary wavelengths values, λ_c, around 490 nm. Dp3g is located in an intermediate zone (λ_d = 637.9 nm), outside the non-spectral purple area. The luminance factor (Y) is related to the concentration of the solution (50 ppm), and, therefore, it should be considered in terms of relative values; it ranges from 31.8% for the darker solution (Pt3g), to 45.4% for the lighter one (Pn3g).

The greater uniformity of the colour spaces CIELUV and, especially, CIELAB, offers good chromatic characterizations of the five compounds studied. The main differences among them are found in terms of hue, with a variation coefficient of 37% for h_{ab} and 64% for h_{uv}, while other more quantitative variables (L^*, C^*_{ab}, C^*_{uv}, s^*_{uv}) have variation coefficients lower than 20%. These results confirm that the differences observed among the chromatic characteristics of anthocyanins are qualitative, although they can also be quantified.

In the (a^*,b^*) diagram, Pn3g and Cy3g (with two substituents in the B ring) are located in the area of the orange hues, while Dp3g, Pt3g and Mv3g (three substituents in the B ring) are located in the red–purple area. The h_{ab} value is also influenced by methoxylation: the higher the number of methoxyl groups (Cy3g < Pn3g, Dp3g < Pt3g < Mv3g), the higher the displacement towards purple hues. The quantitative value of chromaticity (chroma C^*_{ab}) is related to the number of hydroxyl groups in the B ring, showing Dp3g

Table 1. CIE 1964 (x, y) colour system (CIEXYZ)

Anthocyanin	pH	Tristimulus values			Chromaticity coordinates		Dominant wavelength	Excitation purity
		X	Y	Z	x	y	λ_d	p_e
Dp3g	1.5	53.33	32.62	18.20	0.51	0.31	637.9	50.80
	3.5	78.59	72.48	80.26	0.34	0.31	494.0[a]	2.32
Cy3g	1.5	61.42	41.37	20.47	0.50	0.34	609.6	53.25
	3.5	79.63	72.45	73.62	0.35	0.32	488.4[a]	8.17
Pt3g	1.5	51.12	31.79	21.77	0.49	0.30	487.1[a]	41.44
	3.5	80.87	76.69	83.87	0.34	0.32	493.3[a]	2.20
Pn3g	1.5	64.61	45.43	28.45	0.47	0.33	616.4	42.16
	3.5	83.85	81.03	84.44	0.34	0.33	488.5[a]	4.64
Mv3g	1.5	58.97	37.77	31.71	0.46	0.29	488.4[a]	30.50
	3.5	85.33	83.61	91.75	0.33	0.32	495.0[a]	0.91

[a]Values corresponding to complementary wavelength λ_c (colour points located in the non-spectral purples triangle).

Table 2. CIE 1976 ($L^*u^*v^*$) colour space (CIELUV)

Anthocyanin	pH	Chromatic coordinates			Chroma	Hue	Saturation
		L^*	u^*	v^*	C^*_{uv}	h_{uv}	s^*_{uv}
Dp3g	1.5	63.85	132.25	18.28	133.51	7.87	2.09
	3.5	88.20	29.39	−6.63	30.12	−12.74	0.34
Cy3g	1.5	70.44	121.43	28.68	124.77	13.30	1.77
	3.5	88.18	36.42	0.55	36.43	0.89	0.41
Pt3g	1.5	63.17	120.55	10.42	121.00	4.96	1.92
	3.5	90.18	23.80	−4.79	24.28	−11.38	0.27
Pn3g	1.5	73.17	107.48	21.15	109.54	11.14	1.50
	3.5	92.14	21.78	0.17	21.78	0.46	0.24
Mv3g	1.5	67.85	114.16	1.91	114.18	0.98	1.68
	3.5	93.28	16.38	−4.28	16.93	−14.65	0.18

Table 3. CIE 1976 ($L^*a^*b^*$) colour space (CIELAB)

Anthocyanin	pH	Chromatic coordinates			Chroma	Hue
		L^*	a^*	b^*	C^*_{ab}	h_{ab}
Dp3g	1.5	63.85	68.55	26.97	73.66	21.48
	3.5	88.20	20.55	−1.89	20.63	−5.28
Cy3g	1.5	70.44	60.06	33.90	68.97	29.46
	3.5	88.18	22.69	3.23	22.92	8.14
Pt3g	1.5	63.17	65.71	18.97	68.40	16.13
	3.5	90.18	16.53	−1.16	16.57	−4.03
Pn3g	1.5	73.17	55.63	25.27	61.09	24.45
	3.5	92.14	13.81	1.81	13.93	7.46
Mv3g	1.5	67.85	65.36	11.37	66.34	9.89
	3.5	93.28	11.70	−1.41	11.78	−6.87

to be the purest colour and Pn3g the least saturated one (closest point to the illuminant). As can be observed in Table 4, colour differences higher than one unit are obtained among the samples (ΔE^*_{ab} from 8 to 35 units), indicating that they can be visually discriminated, even by an untrained eye (Melgosa *et al.*, 1997).

Anthocyanins are situated in the first quadrant of the (u^*,v^*) diagram. Saturation (s^*_{uv}), a parameter only defined in CIELUV space, is used to demonstrate the relevance of the number of hydroxyl groups in the B ring, and the number of substituents, by this order, in the final colour. According to it, the purest colour is shown by Dp3g, followed by Pt3g > Cy3g > Mv3g > Pn3g.

As pH increases, the colour of the different anthocyanins moves to the non-spectral purple area (Fig. 1) and it approaches to the illuminant taken as a reference (D_{65}), which means a progressive loss of colour. Similar behaviour is observed in the (x, y) and (u^*, v^*) diagrams (Fig. 2); the slight curvatures for the five anthocyanins are adjusted to a three-grade polynomic equation (Table 5). However, in the (a^*, b^*) diagram, four-grade polynomic equations are needed to adjust the curves (Fig. 3), due to the influence of lightness (L^*) on chromaticity points in CIELAB space.

Figures 4–7 show the evolution of several chromatic parameters with pH. Brightness expressions (luminance

Table 4. Colour and hue differences

	pH	Colour differences		Hue differences	
		ΔE^*_{uv}	ΔE^*_{ab}	ΔH^*_{uv}	ΔH^*_{ab}
Dp3g–Cy3g	1.5	16.300	12.788	17.367	11.921
	3.5	10.049	5.549	11.865	6.003
Dp3g–Pt3g	1.5	14.100	8.516	18.846	9.987
	3.5	6.209	4.540	8.291	5.760
Dp3g–Pn3g	1.5	26.600	16.021	34.588	18.106
	3.5	10.900	8.640	13.180	10.198
Dp3g–Mv3g	1.5	24.700	16.418	31.127	17.525
	3.5	14.163	10.216	18.675	12.525
Cy3g–Pt3g	1.5	19.600	17.541	18.666	15.973
	3.5	13.848	7.824	18.314	9.876
Cy3g–Pn3g	1.5	16.000	10.077	21.983	12.498
	3.5	15.171	9.826	20.715	12.716
Cy3g–Mv3g	1.5	27.800	23.289	29.692	23.294
	3.5	21.235	12.974	28.376	16.322
Pt3g–Pn3g	1.5	19.600	15.534	20.428	13.955
	3.5	5.703	4.479	5.910	4.815
Pt3g–Mv3g	1.5	11.600	8.932	12.640	7.882
	3.5	8.058	5.745	10.457	6.807
Pn3g–Mv3g	1.5	21.000	17.782	20.889	17.761
	3.5	7.090	4.015	8.514	4.409

Table 5. Polynomic regressions between chromaticity coordinates

	Equation	n	r^*	p
CIEXYZ				
Dp3g	$y = -7.771x^3 + 12.162x^2 - 6.077x + 1.279$	26	0.999	< 0.001
Cy3g	$y = -7.485x^3 + 11.155x^2 - 5.286x + 1.126$	19	0.977	< 0.001
Pt3g	$y = -11.486x^3 + 16.916x^2 - 8.116x + 1.570$	27	0.970	< 0.001
Pn3g	$y = -9.185x^3 + 13.000x^2 - 5.938x + 1.201$	28	0.940	< 0.001
Mv3g	$y = -10.771x^3 + 15.806x^2 - 7.592x + 1.490$	21	0.999	< 0.001
CIELUV				
Dp3g	$v^* = 9.938 \times 10^{-6}(u^*)^3 + 0.002(u^*)^2 - 0.260(u^*) - 0.659$	26	1.000	< 0.001
Cy3g	$v^* = -8.774 \times 10^{-7}(u^*)^3 + 0.003(u^*)^2 - 0.190(u^*) + 2.619$	19	0.998	< 0.001
Pt3g	$v^* = 5.019 \times 10^{-6}(u^*)^3 + 0.003(u^*)^2 - 0.391(u^*) + 2.327$	27	0.944	< 0.001
Pn3g	$v^* = -4.912 \times 10^{-6}(u^*)^3 + 0.004(u^*)^2 - 0.192(u^*) + 2.431$	28	0.999	< 0.001
Mv3g	$v^* = 8.030 \times 10^{-6}(u^*)^3 + 0.002(u^*)^2 - 0.310(u^*) + 0.340$	21	0.997	< 0.001
CIELAB				
Dp3g	$b^* = 7.193 \times 10^{-6}(a^*)^4 - 0.001(a^*)^3 + 0.027(a^*)^2 - 0.395(a^*) - 0.316$	26	0.998	< 0.001
Cy3g	$b^* = 1.100 \times 10^{-5}(a^*)^4 - 0.001(a^*)^3 + 0.042(a^*)^2 - 0.539(a^*) - 3.243$	19	1.000	< 0.001
Pt3g	$b^* = 1.000 \times 10^{-5}(a^*)^4 - 0.001(a^*)^3 + 0.046(a^*)^2 - 0.741(a^*) - 2.638$	27	0.994	< 0.001
Pn3g	$b^* = 9.561 \times 10^{-6}(a^*)^4 - 0.001(a^*)^3 + 0.037(a^*)^2 - 0.436(a^*) - 2.511$	28	0.998	< 0.001
Mv3g	$b^* = 3.376 \times 10^{-6}(a^*)^4 - 3.250 \times 10^{-4}(a^*)^3 + 0.014(a^*)^2 - 0.306(a^*) + 0.613$	21	0.999	< 0.001

Y, and lightness L^*) increase progressively, reaching a maximum between pH 4 and 5 (Fig. 4), due to the formation of colourless forms. On the other hand, at pH higher than 5, brightness slightly decreases, indicating that other coloured forms are being formed.

Chroma (C^*_{uv} and C^*_{ab}), defined in the uniform colour spaces, undergoes a linear decrease as pH increases (Fig. 5). It reaches values close to 0 at pH 5, which means that the colour approaches the grey line (lowest chromaticity area), or, similarly, that there is a maximum contribution of the white light to the global colour impression. In the same way, saturation (s^*_{uv}), defined in CIELUV, and excitation purity (p_e), in CIEXYZ, decreases to minimum values (area close to the illuminant D_{65}; Fig. 6).

Figure 7 shows the evolution of the hue angle (h_{uv}, h_{ab}), with pH as a numerical expression for qualitative measurement of chromaticity. In most cases, a slight decrease to blue hues exists between pH 4 and 5,

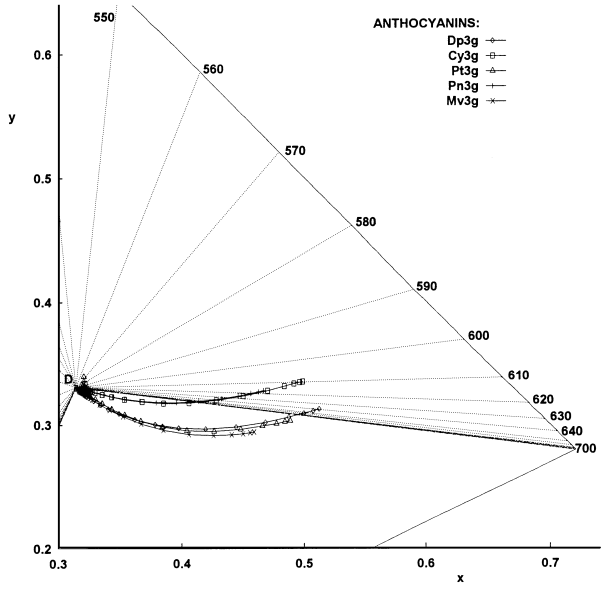

Fig. 1. Colour changes of the anthocyanins studied with pH on CIE 1964 (x, y) chromaticity diagram.

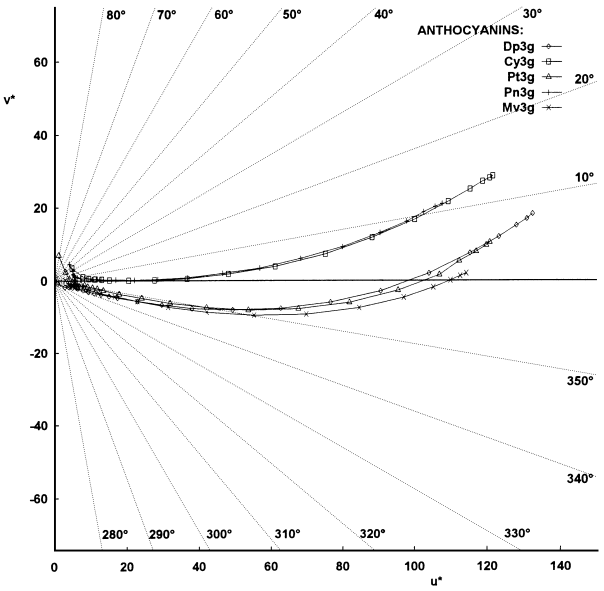

Fig. 2. Colour changes of the anthocyanins studied with pH on CIE 1976 (u^*, v^*) chromaticity diagram.

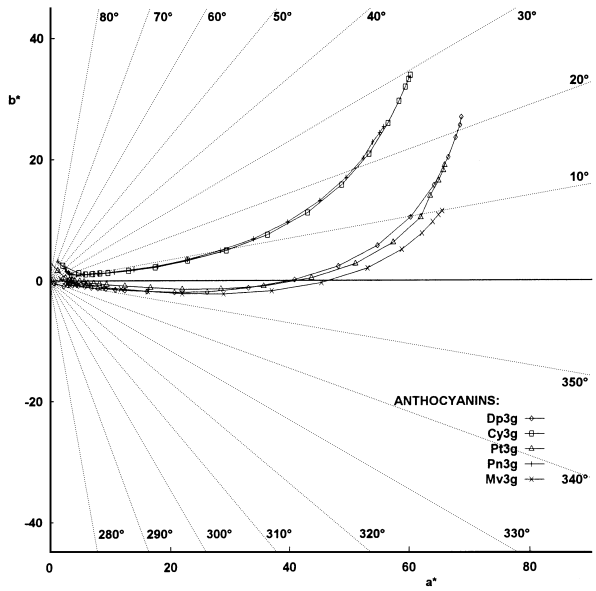

Fig. 3. Colour changes of the anthocyanins studied with pH on CIE 1976 (a^*, b^*) chromaticity diagram.

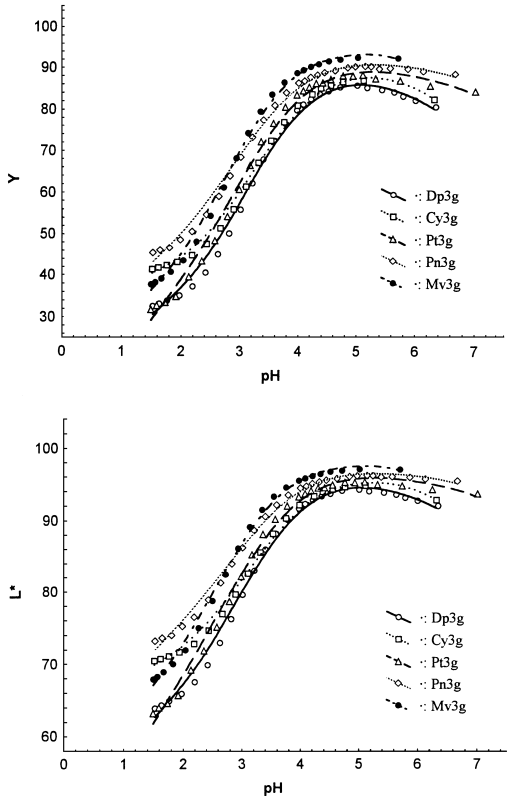

Fig. 4. Changes of brightness with pH (luminance Y, lightness L^*).

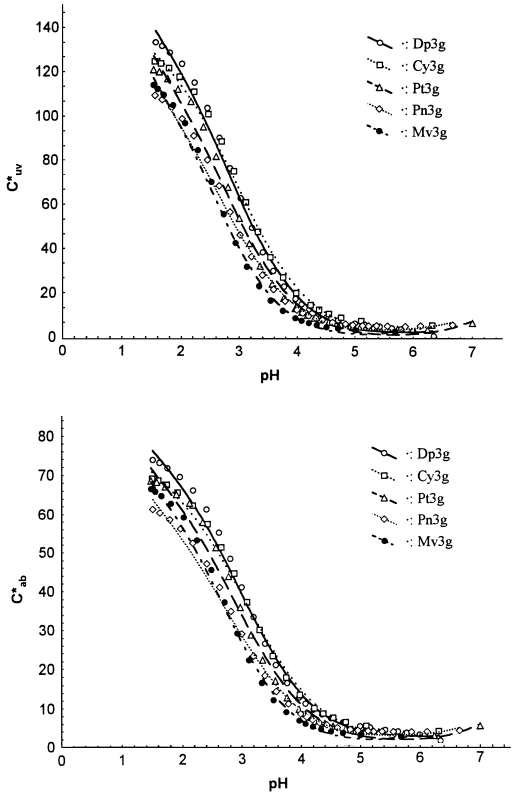

Fig. 5. Changes of chroma with pH (C^*_{uv}, C^*_{ab}).

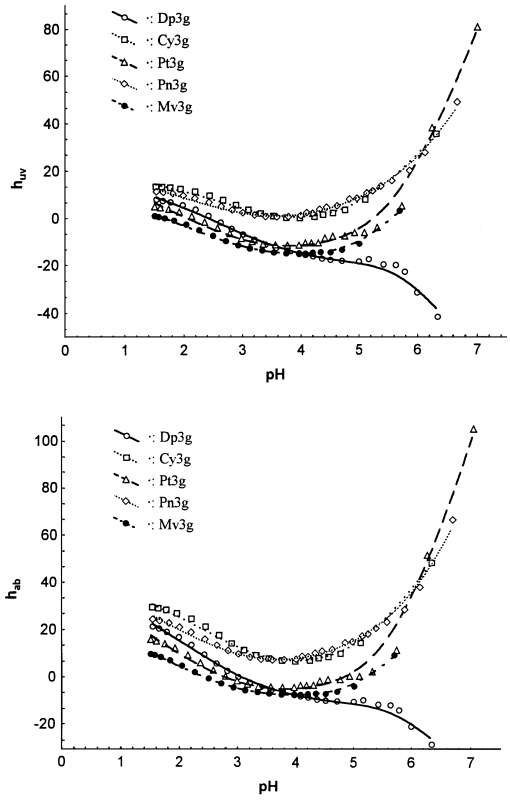

Fig. 7. Changes of hue with pH (h_{uv}, h_{ab}).

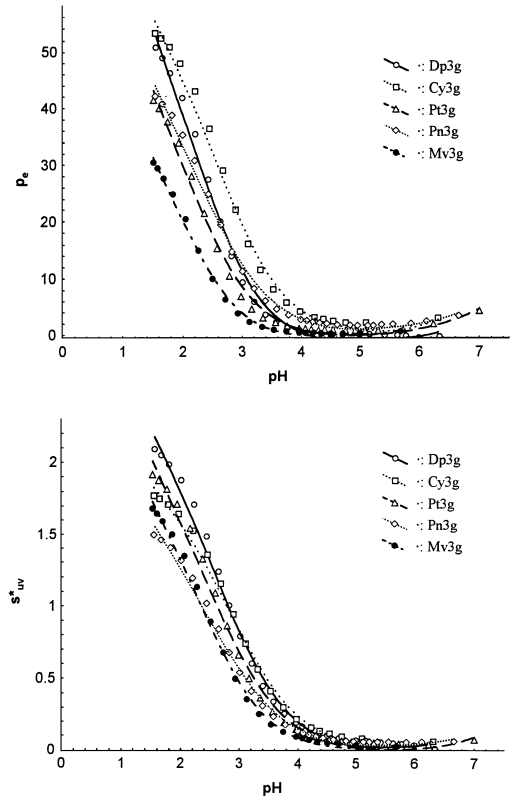

Fig. 6. Changes of purity with pH (excitation purity p_e, saturation s^*_{uv}).

followed by a sharp increase to green–yellow zones, at pH 5, when chroma values decrease. Different behaviour is shown by Dp3g, decreasing to bluer forms when pH values get close to 7.

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