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Food Chemistry 89 (2005) 427–440
Food Chemistry
www.elsevier.com/locate/foodchem

Colour stability of anthocyanins in aqueous solutions at various pH values

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Received 2 July 2003; received in revised form 4 March 2004; accepted 4 March 2004

Abstract

This study focuses on the impact of anthocyanin structures such as 5-glucosidic substitution and aromatic acylation on anthocyanin, colour and stability at various pH values. Two concentrations (0.05 and 0.15 mM) of cyanidin 3-glucoside (**1**), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**), and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) in aqueous solutions were studied at fourteen pH levels between 1.1 and 10.5 during a 98 day period at 10 °C. The three pigments represent the structural variation of many anthocyanins isolated from fruits and vegetables. Colours were expressed by the CIELAB coordinates h_{ab}, (hue angle), C^* (chroma = saturation), and L^* (lightness), as well as by visible absorption maxima (λ_{max}) and molar absorption coefficients (ε). Limitations of using only spectral parameters, such as λ_{max} and ε, to express colour variations, were revealed. Pigment **2** was more unstable than **1** at most pH values, showing lower C^* and higher L^* values after storage for only hours in neutral and weakly acid solutions. Pigment **3** showed higher colour stability than the nonacylated forms at all pH values but pH 1.1, where all pigments retained their colours in the storage period, and in the most alkaline solutions, where all pigments experienced dramatic colour changes. Of potential importance for some food products, in solutions with pH 4.1 and 5.1, **3** maintained nearly the same h_{ab}, C^*, and L^* values during the whole measuring period (98 days), in contrast to pigments **1** and **2**. The hue angle shift towards bluish tones in freshly made samples of anthocyanins with 5-glucosidic substitution, were amplified with aromatic acylation (**3**) throughout the entire pH range except pH 10.5. The variations in the results emphasizes the importance of structure for anthocyanin properties in fresh and processed fruits and vegetables.

Keywords: Anthocyanins; Colour; Stability; CIELAB; NMR

1. Introduction

Anthocyanins provide colours ranging from salmon-pink through red, and violet to nearly black in a variety of plant sources. They play a critical role in the colour quality of many fresh and processed fruits. The interests in and motives for extended use of these colorants are influenced by their potential beneficial health effects (Clifford, 2000). It is well known that anthocyanin properties, including colour expression, are highly influenced by anthocyanin structure and pH, etc. (Cabrita, Fossen, & Andersen, 2000; Francis, 1989; Jackman, Yada, Tung, & Speers, 1987). However, precise information about colours of individual anthocyanins during storage is very limited.

The parameters employed for describing colour variation of anthocyanin solutions have mainly been shifts of the visible λ_{max} as a measure for hue variations, and absorptivity changes for variations of colour intensity. However, Gonnet (1998) has shown that an adequate description of colour variations of anthocyanins, caused for instance by pH, requires: (i) that spectral variations considered should be those affecting the entire spectral curve, not only its visible λ_{max}, (ii) that three colour attributes (hue, saturation and lightness) should be used to describe colour, and (iii) that these should refer to light source and observer conditions. Bakker, Bridle, and Timberlake (1986), Heredia and Guzmanchozas (1993), Pérez-Magariño and San-José (2002), and Almela, Javaloy, Fernández-López, and López-Roca

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doi:10.1016/j.foodchem.2004.03.002

(1995) have shown that the CIEL*a*b* system is useful for describing colours in wine. The CIELAB scale has also been used to describe colours of juices, extracts and flowers (Bakker, Bridle, & Bellworthy, 1994; Biolley & Jay, 1993; Hashimoto, Tanaka, Maeda, Fukuda, Shimizu, & Sakata, 2002; Hosoki, Hamada, Kando, Moriwaki, & Inaba, 1991; Lee, 2002; Nørbæk, Christensen, & Brandt, 1998; Robbins & Moore, 1990; Rodriguez-Saona, Giusti, & Wrolstad, 1999). There are a few reports dealing with CIELAB colours of pure anthocyanins (Bakker & Timberlake, 1997; Giusti, Rodriguez-Saona, & Wrolstad, 1999; Gonnet, 1998; Gonnet, 1999; Gonnet, 2001; Heredia, Francia-Aricha, Rivas-Gonzalo, Vicario, & Santos-Buelga, 1998; Stintzing, Stintzing, Carle, Frei, & Wrolstad, 2002); however, none addresses colour variation during storage for months over the entire pH range.

More than 600 different anthocyanins, with a variety of building blocks, have been reported (Andersen, 2001). Based on observations of some relatively simple anthocyanins in vitro, the following scheme, related to pH changes, is generally accepted (Brouillard, 1988): at a pH of approximately 3 or lower, the orange, red or purple flavylium cation predominates. As the pH is raised, kinetic and thermodynamic competition occurs between the hydration reaction on position 2 of the flavylium cation and the proton transfer reactions related to its acidic hydroxyl groups. While the first reaction gives colourless carbinol pseudo-bases, which can undergo ring opening to yellow retro-chalcones, the latter reactions give rise to more violet quinonoidal bases. Further deprotonation of the quinonoidal bases can take place at pH between 6 and 7 with the formation of more bluish resonance-stabilised quinonoid anions. At the pH values typical for fresh and processed fruits and vegetables, each anthocyanin will thus most probably be represented by a mixture of equilibrium forms.

It is well known that aromatic acyl groups may influence colour and improve stability, and several mechanisms, including intra- and intermolecular copigmentation as well as self-association, have been suggested for explanation of these effects (Brouillard, 1988; Malien-Aubert, Dangles, & Amiot, 2001; Nerdal & Andersen, 1992; Yoshida, Toyama, Kameda, & Kondo, 2000). Molecular optimizations have shown that aromatic acyl groups, in highly substituted anthocyanins participating in intramolecular copigmentation, protect the aglycone against hydration in the 2- and 4-positions (Figueiredo, George, Tatsuzawa, Toki, Saito, & Brouillard, 1999).

In this paper the colour variation of three anthocyanins (or their degradation products), at two different concentrations in aqueous solutions, were examined at fourteen pH-values between 1.1 and 10.5. The colours were measured, both as CIELAB colour coordinates and UV–Vis absorption spectra, during storage at 10 °C for 98 days. The anthocyanins include cyanidin 3-glucoside (**1**), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) and cyanidin 3-(2″-(6‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**), which are representative of the structural variation of most anthocyanins found in fruits and vegetables (Mazza & Miniati, 1993). Thus, it has been possible to compare under various pH conditions, especially the impact of 5-glucosidic substitution and aromatic acylation, for colour, solubility and stability. The results vary tremendously, and this emphasizes the importance of structure on anthocyanin properties in fresh and processed fruits and vegetables.

2. Materials and methods

2.1. Sources

Pigment **3** was obtained from red cabbage (*Brassica oleracea*) bought in the local food market. A pure anthocyanin mixture isolated from red cabbage was subjected to alkaline hydrolyses giving **2**. Pigment **1** was isolated from black rice (*Oriza sativa*), also bought in the local food market. The purity of pigments **1**–**3** was determined to be higher than 95%, based on HPLC online detection in the aromatic region (270–370 nm). The purity of the individual pigments was also checked by the NMR data.

2.2. Pigment isolation

Red cabbage was extracted with methanol containing 1.0% TFA, while rice was extracted with methanol containing 0.1% HCl. Each extract was concentrated under reduced pressure, and thereafter purified by partition against ethyl acetate.

Each of the partly purified samples, including the sample which was subjected to alkaline hydrolysis (see 2.3), was applied on Amberlite XAD-7 columns. The adsorbed samples were washed with water, before the anthocyanins were eluted from the column using acidified methanol. The pigments **1** and **2** were individually isolated by Sephadex LH-20 column (100 cm × 50 mm and 100 cm × 26 mm, respectively) chromatography. The pigments were isolated with a stepwise gradient from 20% to 70% methanol in water containing 0.1% HCl. Pigments **1** and **2** were eluted at approximately 40% and 20% acidified methanol, respectively. Pigment **3** was isolated by using high speed countercurrent cromatography. The HSCCC-instrument (Model CCC-1000, Pharma-Tech Research Corp., Baltimore, Maryland, USA) was equipped with 3 preparative coils, connected in series (tubing i.d. 2.6 mm; total volume 850 ml). A two-phase solvent-system, consisting of *t*-butyl methyl ether: *n*-butanol:acetonitrile:water (2:2:1:5 v/v/v/v) acidified with 0.01% TFA, was used in descending

Table 1
^{1}H and ^{13}C spectral data for **1, 2** and **3** dissolved in CD_3OD:CF_3COOD (19:1) at 25 °C

Aglycone	**1**, ^{1}H δ (ppm)	**1**, ^{13}C δ (ppm)[a]	**2**, ^{1}H δ (ppm)	**2**, ^{13}C δ (ppm)[a]	**3**, ^{1}H δ (ppm)	**3**, ^{13}C δ (ppm)[a]
2		164.20[b]		165.05		163.85
3		145.53		146.26		145.29
4	9.04 s	136.60	9.16 s	135.87	8.85 s	134.16
5		159.09		157.08		156.2
6	6.75 s	103.31	7.14 s	105.74	7.01 s	105.42
7		170.31		169.46		169.08
8	6.98 s	95.15	7.17 s br	97.44	6.71 s	97.06
9		157.48		156.80		156.2
10		113.22		113.21		112.67
1′		121.08		121.11		120.85
2′	8.11 s br	118.25	8.13 d; 1.8	118.71	7.85 s br	117.93
3′		147.27		147.58		147.44
4′		155.67		156.51		156.52
5′	7.09 d; 8.2	117.37	7.15 d; 8.9	117.77	7.11 d; 8.7	117.28
6′	8.32 d br; 7.9	128.29	8.35 dd; 8.7, 1.8	129.10	8.30 d br; 8.4	129.54
3-glc						
1″	5.39 d; 7.4	103.58	5.58 d; 7.4	102.40	5.74 d; 6.1	100.25
2″	3.77 t; 8.0	74.71	4.17 t; 8.2	81.63	4.24 t; 7.2	79.65
3″	3.67	78.01	3.88 t; 8.8	78.08	3.93 m	76.49
4″	3.54 t; 8.7	71.01	3.60 m	70.97	3.81 m	71.08
5″	3.65	78.68	3.72 m	78.68	3.97 m	75.14
6A″	4.01 d; 11.8	62.29	4.04 d br; 12.1	62.4[c]	4.64 d br; 11.4	63.37
6B″	3.81 dd; 12.2, 6.0		3.84 dd; 12.1, 5.4	62.4[c]	4.46 dd; 11.8, 4.5	63.37
2″-glc						
1‴			4.89 d; 7.6	104.55	5.33[d]	99.91
2‴			3.29 m	75.79	4.97 t; 8.7	74.91
3‴			3.39 m	77.70	3.74 m	75.92
4‴			3.32 t; 9.3	71.26	3.54[e] m	70.91[e]
5‴			3.05 m	77.96	3.46[e] m	78.05[e]
6‴			3.57 m	62.4[c]	3.97 m	62.48
5-glc						
1⁗			5.26 d; 7.8	102.61	5.25 d; 7.5	102.39
2⁗			3.78 t; 8.8	74.50	3.83 m	74.52
3⁗			3.66 t; 8.8	77.68	3.65[f] m	77.68[f]
4⁗			3.55 m	71.12	3.53 m	70.99
5⁗			3.68 m	78.62	3.66[f] m	78.58[f]
6A⁗			4.04 d br; 12.1	62.4[c]	4.03 m	62.27
6B⁗			3.84 dd; 12.1, 5.4	62.4[c]	3.78 m	62.27
6″-sin						
C=O						168.42
α					6.22 d; 15.9	115.00
β					7.34 d; 15.9	146.94
1						125.80
2					6.60 s	106.51
3						148.92
4						139.44
5						148.92
6					6.60 s	106.51
OMe					3.80 s	56.56
2‴-sin						
C=O						167.91
α					6.29 d; 15.9	115.41
β					7.42 d; 15.8	147.08
1						126.00
2					6.66 s	106.38
3						149.09
4						139.40
5						149.09
6					6.66 s	106.38
OMe					3.85 s	56.55

Table 1 (*continued*)

See Fig. 1 for structures.

s:singlet; d:doublet; t:triplet; m:multiplet; br:broad. For some signals, the coupling constant(s) (in Hz) are presented after the multiplicity.

[a] ^{13}C chemical shifts for **1** and **2** were achieved from CAPT spectra. Heteronuclear NMR techniques (HSQC and HMBC) were used for shift assignments of **3**.

[b] Weak signal.

[c] The ^{13}C shifts of the three glucosyl C6 were almost identical.

[d] Hidden under the solvent peak. The ^{1}H chemical shift was found from the HSQC spectrum.

[e] Signals with the same superscript may be reversed.

[f] Signals with the same superscript may be reversed.

mode (the lightest phase as stationary) (Degenhardt, Knapp, & Winterhalter, 2000; Torskangerpoll, Chou, & Andersen, 2001). The revolutionary speed of the centrifuge was set to 1000 rpm, and the solvents were pumped into the column at a flow rate of 5 ml min^{-1}. Pigments **1**–**3** after isolation, were stored under dried condition in sealed vials at −23 °C before further use.

The HPLC-results were obtained with an HP-1050 system (Hewlett-Packard) using an ODS Hypersil column (25 cm × 0.4 cm, 5 μm). The elution profile consisted of a linear gradient, from 10% B to 100% B, during the first 17 min, then an isocratic elution using 100% B for 6 min, and finally a linear gradient back to 10% B for the last minute, where A is H_2O:HCOOH (18:1, v/v) and B is MeOH:H_2O:HCOOH (10:8:1, v/v/v). The flow rate was 0.75 ml min^{-1}, and aliquots of 6 to 12 μl were used. The diode array detector (DAD) was set to monitor anthocyanins at 520 ± 20 nm. The t_R-values for **1**–**3** were 14.2, 10.2 and 19.8 min, respectively. Pigment **1** was co-chromatographed with cyanidin 3-glucoside from black currant.

2.3. Alkaline hydrolysis

A mixture of acylated anthocyanins (114.2 mg) was dissolved in 20 ml methanol and 10 ml 2.0 M NaOH. The initial green solution was left for 2 h at room temperature. Then this yellow-brownish solution changed to red after addition of 15 ml 2.0 M HCl. The sample was dried under nitrogen for 2 h.

2.4. Pigment identification

The NMR experiments, one-dimensional ^{1}H, ^{13}C (CAPT), ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC, were performed on a Bruker DRX-600 instrument at 600.13 and 150.92 MHz for ^{1}H and ^{13}C, respectively. A TBI probehead was used for all experiments, except for CAPT, where a BBO probehead was used. As secondary references, the deuteriomethyl ^{13}C and the residual ^{1}H signal of the solvent (CF_3COOD–CD_3OD; 1:19 v/v) were used (δ 49.0 and δ 3.4 from TMS for ^{13}C and ^{1}H, respectively). Assignments of chemical shifts and coupling constants of **1**–**3** are presented in Table 1. A strong crosspeak between C3 (145.53 ppm) and the only anomeric proton (5.39 ppm) in the HMBC spectrum of **1**, showed that the sugar unit was connected to the aglycone in position 3. The NMR data on **1** were in accordance with cyanidin 3-glucopyranoside (see Fig. 1).

On the basis of chemical shifts and coupling-patterns in the ^{1}H NMR spectrum of **2**, the AMX system at 8.13, 7.15 and 8.35 ppm were assigned to H-2′, H-5′ and H-6′, respectively, the 2H AX system at 7.14 and 7.17 ppm to H-6 and H-8, and the singlet at 9.16 ppm to H-4 of the aglycone, cyanidin. This spectrum also showed three anomeric protons, together with a crowded sugar region typical for three glucose units (Table 1). Crosspeaks in the HMBC spectrum of **2** at 5.26/157.08 ppm (H1′′′′/C5) and 5.58/146.26 ppm (H1′′/C3) showed two of the glucose units to be attached at the aglycone 5 and 3 positions. The third glucose moiety was located at the sugar 2′′-position by the two HMBC crosspeaks at 4.17/104.55 ppm (H2′′/C1′′′) and 4.89/81.63 ppm (H1′′′/C2′′) and the downfield shift of H2′′ (4.17 ppm). Pigment **2** was thus identified as cyanidin 3-(2′′-glucopyranosylglucopyranoside)-5-glucopyranoside.

Compared to **2**, **3** contains, in addition, two aromatic acyl groups, which were identified as two sinapic acid moieties. Both acyl groups showed a typical cinnamoyl acid pattern with a J_{HH} of 15.9 Hz between the two alkene protons in accordance with the *E*-configuration (Table 1). Crosspeaks in the HMBC spectrum of **3** at

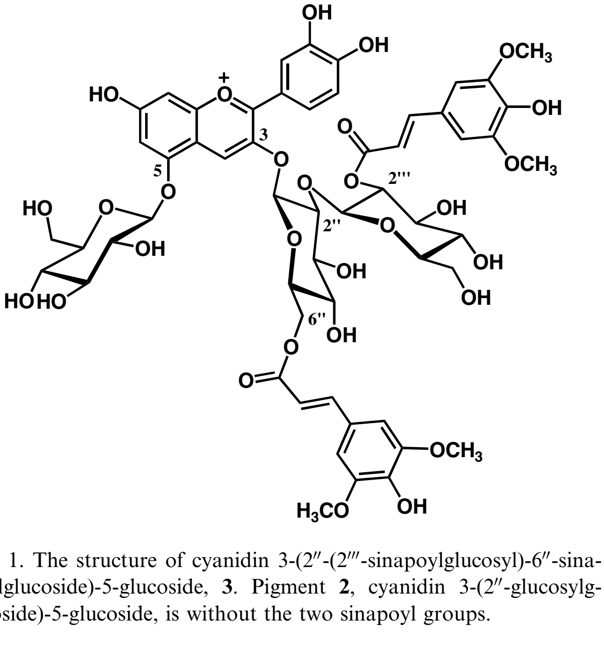

Fig. 1. The structure of cyanidin 3-(2′′-(2′′′-sinapoylglucosyl)-6′′-sinapoylglucoside)-5-glucoside, **3**. Pigment **2**, cyanidin 3-(2′′-glucosylglucoside)-5-glucoside, is without the two sinapoyl groups.

Table 2
Solvent proportions (v/v) used in the buffer solutions

pH	A	B	C	D	E	F	G	H
1.1	271.7	728.3						
3.0			691.6	308.4				
4.1			998.0	2.0				
5.1			688.7		311.3			
6.0					100.7	899.3		
6.6					247.0	753.0		
6.8					309.4	690.6		
6.9					367.9	632.1		
7.2					409.7	590.3		
7.3					438.8	561.2		
8.0				290.8			709.2	
8.9				84.2			915.8	
9.9					267.9		732.1	
10.5					75.8			924.2

A: 0.2 M KCl, B: 0.2 M HCl, C: 0.1 M $KHC_8O_4H_4$, D: 0.1 M HCl, E: 0.1 M NaOH, F: 0.1 M KH_2PO_4, G: 0.025 M borax, H: 0.05 M Na_2HPO_4.

4.64/168.42 (H6A″/C=O), 4.46/168.42 (H6B″/C=O), and 4.97/167.91 (H2‴/C=O) confirmed the linkage positions of the acyl groups in accordance with cyanidin 3-(2″-(6‴-sinapoylglucopyranosyl)-6″-sinapoylglucopyranoside)-5-glucopyranoside. The structure of **3** was also confirmed by the molecular ion at m/z 1185 in the electrospray MS spectrum. To our knowledge, this is the first report on ^{13}C NMR data for this anthocyanin.

2.5. Buffer solutions and anthocyanin solubility

Preparation of anthocyanin solutions: 10.2, 4.5 and 27.3 mg of **1**, **2** and **3**, respectively, were dissolved in acidified methanol. Each pigment solution was then divided into 14 equal portions, dried under nitrogen, and dissolved in appropriate volumes of buffers to achieve 0.05 mM solutions. The 0.15 mM anthocyanin solutions

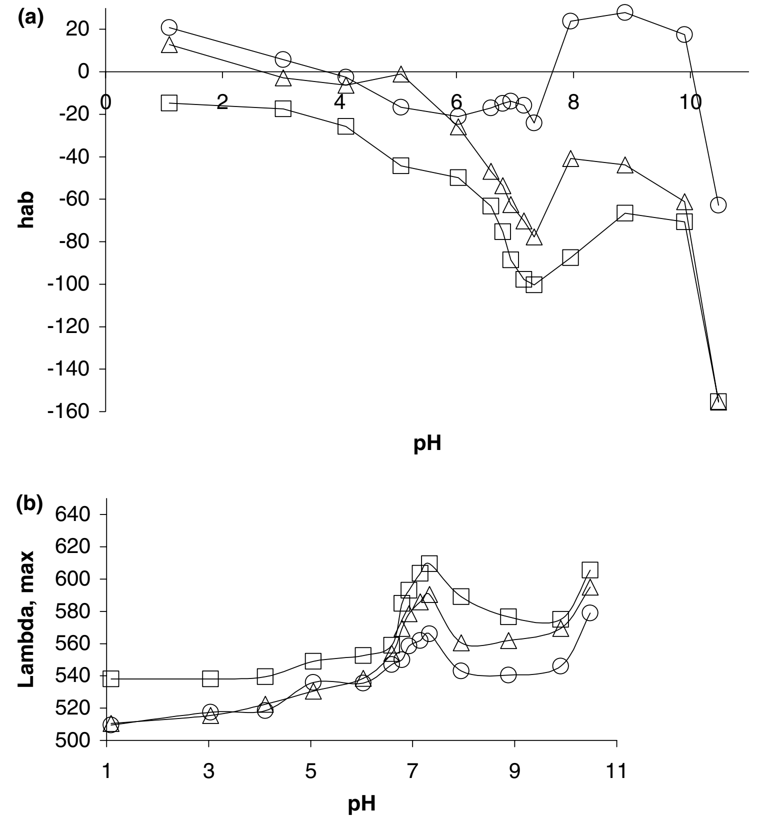

Fig. 2. (a) h_{ab}, values of freshly made 0.05 mM solutions of cyanidin 3-glucoside (**1**) (○), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) (△) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) (□) at pH-values from 1.1 to 10.5. (b) λ_{max} values of freshly made 0.05 mM solutions of cyanidin 3-glucoside (**1**) (○), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) (△) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) (□) at pH-values from 1.1 to 10.5.

were similarly prepared. All solutions were sealed, and kept at 10 °C during storage. The solvents used for preparations of the buffer-solutions were 0.2 M KCl (A), 0.2 M HCl (B), 0.1 M $KHC_8O_4H_4$ (C), 0.1 M HCl (D), 0.1 M NaOH (E), 0.1 M KH_2PO_4 (F), 0.025 M borax (G), 0.05 M Na_2HPO_4 (H), and Table 2 shows the solvent proportions. The accurate pH-values were measured with a Hanna HI 9224 pH-meter equipped with a Hanna HI 1330B pH electrode. The pH values of the various samples did not change during storage.

Pigment **1**–**3** showed different solubilities in the various buffer solutions. While all solutions of **2** dissolved easily in the buffer solutions, the following samples of **1** were not completely dissolved initially: At pH 5.1 the 0.05 mM solution was completely dissolved 2 days after preparation, while the corresponding samples, with pH 6.0, 6.6, 6.8 and 6.9, required 1 day, and the sample with pH 7.2 needed 1 h for the process. A similar pattern was observed for 0.15 mM solutions of **1**, with the exemption of pH 1.1 (1 h), pH 5.1 (7 days) and pH 7.2 (1 day). For **3** compared to **1**, the pH interval with incomplete dissolution also included 0.05 mM solutions at pH 4.1 and 7.2 (1 day) and pH 5.1 (2 days), 0.05 mM solution at pH 7.3 and 0.15 mM solutions at pH 1.1, 3.0 and 8.0 (1 h), and 0.15 mM solutions at pH 6.6 and 6.8 (2 days).

2.6. Colour measurements

UV/Vis absorption spectra were recorded between 240 and 700 nm for both 0.05 and 0.15 mM solutions of **1**–**3** at fourteen different pH-values (see Table 2) on a Varian Cary3 UV–Vis Spectrophotometer. As references, the respective buffer solutions were used. The UV/Vis spectra were recorded for freshly made samples ("0"), after 1 h, 1 day, and then after 2, 5, 7, 14, 21, 28, 35, 49, 70 and 98 days. The absorbances of the freshly made samples were between 0.04 and 3.22 absorbance units, and no samples were diluted before measurements. The samples were kept in a refrigerator (10 °C) between the measurements. The colours of the same samples were also measured using an Ultra Scan XE Hunter colorimeter (Hunter Associates Laboratories Inc., Reston, VA, USA), giving the various CIELab parameters presented in Tables 4–9. The impacts of atmospheric oxygen and other factors, such as the composition of the buffer solutions, were not addressed in this study.

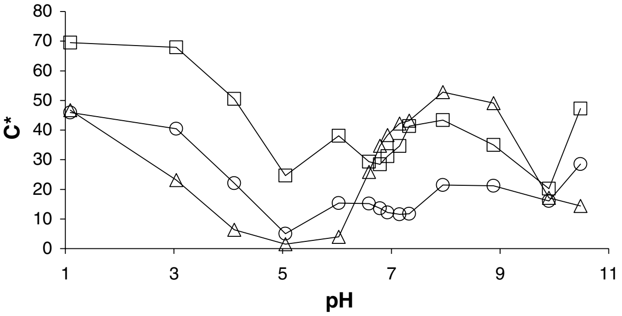

Fig. 3. C^* values of freshly made 0.05 mM solutions of cyanidin 3-glucoside (**1**) (○), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) (△) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) (□) at pH-values from 1.1 to 10.5.

Table 3
Absolute molar absorptivities, ε (l/(mol cm)) for 0.05 mM cyanidin 3-glucoside (**1**), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) at pH-values from 1.1 to 10.5

pH	1	2	3
1.1	16520	19260	23460
3.0	12240	6060	22380
4.1	5920	1620	16360
5.1	3540	760	9680
6.0	8120	7740	12380
6.6	8340	12120	12960
6.8	8360	13340	13260
6.9	8620	15180	16260
7.2	8600	17640	18140
7.3	9180	19340	20960
8.0	8340	16220	20820
8.9	8020	16740	20000
9.9	8240	14420	15080
10.5	14380	20420	15340

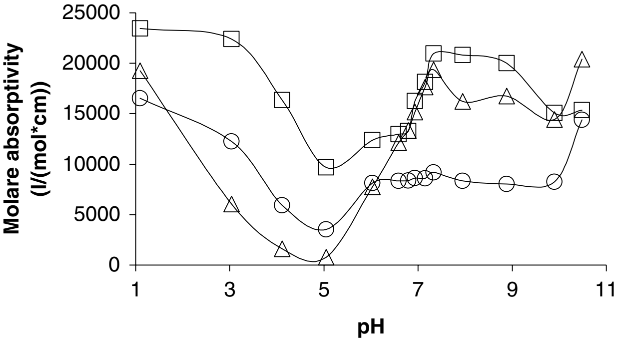

Fig. 4. Absolute molar absorptivities as a function of pH for 0.05 mM cyanidin 3-glucoside (**1**) (○), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) (△) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) (□).

The most common way to indicate anthocyanin colours is based on presentation of visible λ_{max}-values from UV/Vis absorbtion spectra. Since anthocyanins in rather strong acid solutions occur only as flavylium forms, the λ_{max}-values may be reasonably representative of colour at these pH values. However, when pH increases, each anthocyanin occurs as a mixture of various equilibrium forms (Brouillard, 1988) in proportions which are unknown for practically all anthocyanins. At these pH-values the corresponding single λ_{max}-values may, to a lesser degree, be representative of the solution colour. Thus, a system such as CIELab (CIE, 1986), which takes into account the absorption/transmission at all wavelengths, is preferable. The L^*, a^* and b^* values can be outlined from the X, Y and Z tristumulus values using the following equations: $L^* = 116(Y/Y_n)^{1/3} - 16$; $a^* = 500[(X/X_n)^{1/3} - (Y/Y_n)^{1/3}]$; $b^* = 200[(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}]$, where $X_n = 94.825$, $Y_n = 100.00$ and $Z_n = 107.399$ using the D_{65} illuminant and 10° observer condition, and X/X_n, Y/Y_n, $Z/Z_n > 0.008856$. When X/X_n, Y/Y_n or Z/Z_n are less than 0.00856, other equations should be used (Hunter & Harold, 1987). From the L^*, a^* and b^* cartesian coordinates, the polar coordinates may be calculated from:

$$C^* = \sqrt{a^{*2} + b^{*2}} \text{ and } h_{\mathrm{ab}} = \arctan\left(\frac{b^*}{a^*}\right).$$

For converting a^* and b^* values into h_{ab}, the signs of a^* and b^* must also be taken in account (McGuire, 1992). Red colours are represented by h_{ab}, values around 0°, while blue colours are described by values close to 270° (or −90°). Thus, a lilac colour gives a value of 315° (or −45°). Yellow is illustrated with hue angles near 90° and orange colours around 45°, while green colours are found close to 180° (or −180°).

Table 4

h_{ab}, values of 0.05 mM cyanidin 3-glucoside (**1**), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) at pH-values from 1.1 to 10.5 measured initially (0), after 1 hour (1 h), 1 day (1 d), 2, 5, 7, 14, 21, 28, 35, 49, 70 and 98 days at 10 °C

	pH 1.1	pH 3.0	pH 4.1	pH 5.1	pH 6.0	pH 6.6	pH 6.8	pH 6.9	pH 7.2	pH 7.3	pH 8.0	pH 8.9	pH 9.9	pH 10.5
(**1**)														
0	20.8	5.7	−2.5	−16.8[a]	−21.2[a]	−17.1[a]	−14.8[a]	−13.8[a]	−15.8[a]	−24.2	23.9	27.9	17.6	−62.8
1 h	20.9	5.7	−2.4	−10.8[a]	−24.9[a]	−25.4[a]	−21.7[a]	−18.0[a]	−17.8	−24.7	24.3	28.4	19.4	−63.3
1 d	20.9	5.7	−2.1	−6.4[a]	−2.2	−13.7	−21.0	−25.3	−25.7	−26.6	27.5	31.4	29.9	75.0
2 d	20.9	5.6	−2.2	−6.8			−8.4	−11.3	−16.6	−19.0	28.3	32.9	39.4	80.1
5 d	20.8	5.3	−2.1	−5.2			20.8	25.9	26.2	28.6	33.9	39.4	68.3	83.7
7 d	20.8	5.2	−2.2	−4.4					47.7	48.9	37.5	43.9	78.9	85.2
14 d	20.6	5.0	−2.2	1.4						69.6	50.2	63.4	88.7	89.4
21 d	20.6	5.1	−0.9								59.4	78.8	91.0	91.8
28 d	20.3	4.8	3.4								62.2	83.0	91.1	91.9
35 d	20.2	4.8	15.1								65.0	85.6	92.1	92.6
49 d	20.2	4.7	52.9								68.5	87.5	93.9	93.5
70 d	20.3	4.5									71.6	89.6	95.3	94.7
98 d	19.7	4.4									72.4		96.4	94.3
(**2**)														
0	12.8	−2.8	−6.3[b]	−1.1[b]	−26.0[b]	−46.8	−53.7	−62.6	−70.2	−77.8	−40.9	−43.8	−61.2	−155.5[c]
1 h	12.9	−2.8	−5.9		22.1	−28.4	−54.9	−67.3	−75.0	−81.7	−42.0	−44.8	111.3	107.9
1 d	13.1	−3.1	−5.6				80.2	103.6	121.9	140.6	28.6	104.8	106.1	104.8
2 d	13.4	−2.8	−4.5									99.6	101.3	102.9
5 d	12.7	−4.2	−8.9									91.0	95.3	99.6
7 d	13.0	−3.9	−7.6									81.4	94.3	97.5
14 d	12.8	−3.8	−7.5									80.6	94.7	97.1
21 d	12.9	−3.6	−6.9									81.2	95.1	96.9
28 d	13.0	−3.5	−6.1									81.6	95.4	96.8
35 d	13.0	−3.1	−5.6									82.3	96.2	96.7
49 d	13.4	−2.8	−5.6									83.1	97.2	96.3
70 d	13.2	−1.3	−2.8									85.5	99.3	97.2
98 d	13.2	−0.2	−1.6									86.1	99.1	97.2
(**3**)														
0	−14.8	−17.4	−25.8[a]	−44.3[a]	−49.9[a]	−63.3[a]	−75.4[a]	−88.5[a]	−97.7[a]	−100.4[a]	−87.5	−66.5	−70.6	−155.4
1 h	−15.1	−17.5	−25.3[a]	−43.9[a]	−50.0[a]	−63.5[a]	−74.4[a]	−83.9[a]	−91.8[a]	−97.7	−91.8	−72.7	−95.2	175.5
1 d	−15.1	−17.6	−25.3	−44.9[a]	−50.6	−65.2	−76.7	−87.7	−96.9	−104.2	−98.8	−87.2	113.5	109.2
2 d	−14.6	−17.4	−25.2	−45.5	−50.6	−65.8	−78.2	−90.3	−100.5	−108.6	−101.4	−107.5	110.7	105.7
5 d	−14.3	−17.6	−25.2	−45.8	−51.1	−67.0	−80.7	−94.5	−106.3	−116.1	−111.4	156.6	107.4	104.5
7 d	−14.1	−18.2	−25.2	−45.9	−51.1	−67.3	−81.3	−95.8	−108.1	−118.4	−114.4	145.6	106.8	104.4
14 d	−13.4	−18.2	−25.2	−45.8	−51.5	−70.0	−86.2	−103.0	−115.7	−125.8	−122.1	119.2	105.3	103.4
21 d	−13.1	−18.3	−25.3	−46.2	−52.4	−74.1	−92.4	−109.8	−121.4	−129.7	−126.9	111.2	104.8	102.6
28 d	−12.4	−18.2	−25.2	−46.1	−52.3	−76.5	−95.5	−112.5	−123.7	−131.9	−129.0	108.5	104.7	102.4
35 d	−11.4	−18.1	−25.1	−45.9	−52.3	−78.2	−97.5	−114.1	−125.1	−133.3	−131.2	106.4	103.8	101.4
49 d	−10.5	−18.0	−25.2	−46.2	−53.3	−82.3	−101.6	−117.8	−128.5	−136.8	−138.8	104.6	103.0	100.5
70 d	−8.7	−17.7	−25.5	−46.6	−54.0	−85.5	−105.3	−122.0	−133.1	−141.5	−152.1	103.3	102.5	100.4
98 d	−7.5	−17.9	−25.3	−47.5	−56.1	−89.0	−109.2	−126.8	−138.2	−147.1	−164.0	102.5	102.4	100.1

When the L^*-value became higher than 95, and the C^*-value lower than 5, no more measurements were recorded.

[a] Some undissolved pigment was still present.

[b] The colour turned lighter during the preparation time.

[c] The colour turned more green (less blue) during the preparation time.

3. Results and discussion

3.1. Colours of anthocyanins in freshly made samples at pH 1.1 to 10.5

The hue angles (h_{ab}), of cyanidin 3-glucoside (**1**), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**), and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) are given in Tables 4 and 5. Although the colour differences between the three pigments vary with pH, the hues changed with pH along the same lines for each of the pigments (Fig. 2(a)). The three pigments had reddish nuances at the lowest pH values. By stepwise pH increase until 7.3, the colours gradually changed toward more bluish tones. At this pH in freshly made 0.05 mM solutions, **1** showed red-lilac nuance ($h_{ab} = -24°$), while **2** and **3** showed blue tones ($h_{ab} = -78°$ and $-101°$, respectively). At higher pH-values the hue angles increased again. For each pigment they were nearly similar in the pH interval 8.0 to 9.9. At pH 10.5, the hue angles

Table 5
h_{ab}, values of 0.15 mM cyanidin 3-glucoside (**1**), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) at pH-values from 1.1 to 10.5 measured initially (0), after 1 hour (1 h), 1 day (1 d), 2, 5, 7, 14, 21, 28, 35, 49, 70 and 98 days at 10 °C

	pH 1.1	pH 3.0	pH 4.1	pH 5.1	pH 6.0	pH 6.6	pH 6.8	pH 6.9	pH 7.2	pH 7.3	pH 8.0	pH 8.9	pH 9.9	pH 10.5
(**1**)														
0	42.4	25.6	5.0[a]	−50.0[a]	−47.1[a]	−50.7[a]	−46.8[a]	−50.0[a]	−47.0[a]	−39.7[a]	24.1	26.9	23.4	−53.6
1 h	42.2	25.8	5.1	−28.0[a]	−51.8[a]	−64.1[a]	−61.9[a]	−61.2[a]	−55.8[a]	−44.1[a]	24.1	27.3	24.5	−56.5
1 d	42.2	25.5	5.0	−21.6[a]	−27.3	−53.3	−58.2	−63.9	−64.6	−56.6	25.3	29.5	31.8	68.3
2 d	41.9	25.1	4.4	−22.5[a]	−24.8	−44.7	−48.2	−54.3	−55.6	−50.2	25.5	31.1	40.8	76.4
5 d	41.8	24.0	4.3	−21.6[a]	−19.5	−34.5	−33.4	−33.7	−33.1	−23.6	28.7	38.4	68.6	79.3
7 d	41.8	23.6	4.3	−20.0	−14.9	−28.9	−24.8	−22.1	−17.7	−2.9	31.5	43.9	76.3	80.3
14 d	41.7	22.5	3.5	−5.1	−8.7	−10.0	10.3	25.7	39.3	50.0	43.6	66.2	82.2	83.2
21 d	41.8	22.1	5.5	37.9	1.1	12.0	38.2	53.2	59.0	64.2	50.6	76.9	85.2	85.3
28 d	41.8	21.5	12.3	57.0	14.3	31.4	53.3	61.1	64.1	67.8	53.1	79.6	86.9	86.5
35 d	41.6	21.1	24.3	59.8	32.0	46.4	59.8	64.8	66.8	69.6	55.4	81.1	88.4	87.5
49 d	41.5	20.1	56.9	60.4	33.5	57.3	65.9	69.1	69.8	71.5	59.1	83.3	90.7	89.3
70 d	41.2	19.6	74.0	62.1	49.5	65.9	70.1	71.6	71.7	73.1	62.7	85.8	92.7	91.0
98 d	41.1	17.6	78.1	64.0	52.9	69.5	71.8	72.7	72.5	73.7	64.7	87.7	94.2	92.1
(**2**)														
0	38.0	4.4	−8.0[b]	−13.6[b]	−28.9[b]	−42.3	−46.3	−51.5	−56.1	−59.8	−34.2	−34.8	−46.9	−171.4[c]
1 h	38.1	4.5	−7.9	−10.5	−6.5	−40.0	−57.7	−63.6	−67.0	−68.4	−36.3	−36.1	106.8	103.3
1 d	38.4	4.3	−7.5	−7.8	9.9	29.6	49.6	94.2	146.0	170.1	−12.5	100.6	101.8	99.7
2 d	38.7	3.7	−7.3	−5.9			61.0	90.9	125.3	140.2	52.6	91.6	96.9	98.9
5 d	38.8	2.4	−8.5	−9.0			43.0	81.7	125.3	137.8	60.2	80.6	89.9	97.5
7 d	39.0	2.0	−7.8	−5.4			60.6	84.5	109.4	124.1	58.0	70.4	83.6	95.1
14 d	39.2	1.9	−7.8	−3.3			55.6	77.5	101.7	116.5	55.0	69.3	84.2	93.6
21 d	39.3	2.0	−7.4	−0.1			61.4	76.3	92.0	103.4	52.2	70.3	85.6	94.0
28 d	39.4	2.3	−6.8	2.6			65.7	76.1	86.3	96.7	52.2	71.2	86.6	94.3
35 d	39.4	2.4	−6.5	4.6			66.9	75.6	83.9	91.3	52.7	72.1	87.4	94.3
49 d	39.7	2.7	−6.1	7.7			70.9	76.3	80.6	85.5	53.9	73.6	88.7	94.1
70 d	39.4	3.6	−4.8	14.3			75.8	77.9	78.9	81.2	56.5	75.4	90.7	93.5
98 d	39.4	4.8	−3.9	21.3			77.7	78.2	78.3	78.9	58.5	76.3	91.7	92.5
(**3**)														
0	−9.7[a]	−5.2[a]	−22.2[a]	−41.2[a]	−43.0[a]	−58.2[a]	−67.4[a]	−72.1[a]	−87.7[a]	−97.4[a]	−77.4[a]	−79.4	−73.9	−153.0
1 h	−1.1	−4.2	−19.5[a]	−39.2[a]	−42.9[a]	−55.1[a]	−61.4[a]	−65.4[a]	−78.0[a]	−82.0[a]	−79.6	−88.6	−105.2	−167.4
1 d	−0.1	−4.1	−18.9	−38.2	−43.8	−55.1[a]	−60.4[a]	−65.8	−70.7	−75.4	−86.3	−104.0	116.2	102.7
2 d	0.3	−4.1	−18.9	−38.3	−43.9	−55.8	−61.6	−67.4	−72.8	−77.8	−91.0	−116.4	106.1	96.7
5 d	0.0	−4.7	−19.3	−38.7	−44.4	−56.9	−63.1	−69.8	−76.0	−81.4	−100.2	−152.8	98.4	95.7
7 d	0.2	−4.9	−19.3	−38.8	−44.6	−57.1	−63.6	−70.5	−76.9	−82.7	−101.8	−169.9	97.5	95.8
14 d	1.2	−4.9	−19.4	−39.0	−45.5	−59.8	−67.6	−75.6	−82.8	−88.9	−113.5	123.5	96.2	96.0
21 d	1.2	−5.5	−19.5	−39.0	−46.4	−62.7	−71.7	−80.5	−88.3	−94.5	−122.5	106.9	96.6	96.0
28 d	2.4	−5.4	−19.4	−39.1	−47.2	−65.0	−75.0	−84.4	−92.4	−98.7	−126.6	103.0	97.0	96.0
35 d	3.9	−5.2	−19.5	−39.4	−48.2	−67.2	−78.2	−88.4	−97.0	−103.3	−130.2	100.9	96.9	95.6
49 d	5.1	−5.5	−19.5	−39.6	−50.0	−71.3	−84.0	−95.6	−104.8	−111.2	−140.1	98.9	96.9	95.3
70 d	7.7	−4.9	−19.8	−40.2	−52.5	−75.7	−90.2	−103.9	−114.2	−121.7	−157.6	97.2	96.9	94.9
98 d	8.6	−5.2	−19.9	−40.7	−55.0	−79.8	−95.6	−109.9	−121.2	−130.3	−172.3	96.3	97.4	94.7

When the L^*-value became higher than 95, and the C^*-value lower than 5, no more measurements were recorded.

[a] Some undissolved pigment was still present.

[b] The colour turned lighter during the preparation time.

[c] The colour turned more green (less blue) during the preparation time.

were lowered dramatically. At this pH, anthocyanins achieved immediately after dissolution, bluish or greenish tones, which disappeared very rapidly (Tables 4 and 5).

Fig. 2(a) shows that the three cyanidin derivatives had different hues at nearly all pH-values from 1.1 to 10.5. It is noticeable that **1** had the highest and **3** the lowest h_{ab}, values throughout nearly the entire pH-region. The aromatic acyl groups of **3** clearly influenced the aglycone chromophore by inter-/intramolecular association, creating more purple or bluish tones. As shown by the hue angles (Fig. 2(a)), glucosidic substitution at the aglycone 5 position (pigments **2** and **3**) compared to non-substitution of this position (**1**), produced a relative large decrease of the h_{ab}, values in alkaline solutions. For instance at pH 8.0, in 0.05 mM solutions, **1** showed a colour between red and orange ($h_{ab} = 24°$) and **2** a lilac colour ($h_{ab} = -41°$), while **3** had a blue colour ($h_{ab} = -88°$).

Fig. 2(b) shows visible λ_{max}-values of freshly made 0.05 mM solutions of **1**–**3** at the various pH-values.

Table 6

C^* values of 0.05 mM cyanidin 3-glucoside (**1**), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) at pH-values from 1.1 to 10.5 measured initially (0), after 1 hour (1 h), 1 day (1 d), 2, 5, 7, 14, 21, 28, 35, 49, 70 and 98 days at 10 °C

	pH 1.1	pH 3.0	pH 4.1	pH 5.1	pH 6.0	pH 6.6	pH 6.8	pH 6.9	pH 7.2	pH 7.3	pH 8.0	pH 8.9	pH 9.9	pH 10.5
(**1**)														
0	45.9	40.5	22.1	5.0[a]	15.4[a]	15.2[a]	13.6[a]	12.2[a]	11.6[a]	11.8	21.5	21.2	16.1	28.5
1 h	45.8	40.5	22.1	4.3[a]	4.3[a]	9.9[a]	11.0[a]	10.9[a]	11.0	11.4	21.7	21.4	16.1	23.8
1 d	45.7	39.9	21.2	4.1[a]	2.4	2.9	3.5	4.2	5.2	6.4	19.3	19.8	12.8	9.3
2 d	45.3	39.9	21.9	4.0			2.8	3.0	3.6	4.4	17.7	18.3	10.4	10.0
5 d	45.2	37.8	21.3	4.1			2.2	2.2	2.4	2.7	14.5	14.1	7.0	9.2
7 d	45.2	37.4	21.0	4.0					2.6	2.9	13.1	12.0	6.6	8.8
14 d	45.1	36.8	18.8	3.7						3.8	9.5	7.2	6.2	7.5
21 d	45.0	36.6	15.2	3.4							7.8	5.5	5.8	6.7
28 d	44.9	35.9	10.4	3.7							7.1	4.8	5.4	6.3
35 d	44.7	35.2	6.4								6.7	4.6	5.2	5.8
49 d	44.7	33.4	3.8								6.2	4.4	4.9	5.4
70 d	44.4	31.6									5.8	3.9	4.5	4.9
98 d	44.3	28.6									5.0		3.6	4.6
(**2**)														
0	46.7	23.1	6.3[b]	1.5[b]	4.0[b]	25.9	34.7	38.3	42.2	43.2	52.8	49.1	17.2	14.4[c]
1 h	46.8	23.0	6.3		0.8	1.2	4.6	13.2	23.3	31.1	40.3	26.1	11.4	20.6
1 d	47.0	19.0	6.1				0.7	0.8	1.0	1.4	1.3	6.9	11.4	16.4
2 d	47.2	18.2	5.9									6.9	9.1	14.0
5 d	47.4	17.1	6.2									6.3	7.5	12.2
7 d	47.4	16.9	5.9									5.8	7.1	11.4
14 d	47.5	17.0	5.8									5.5	6.8	10.4
21 d	47.6	16.6	5.8									5.4	6.5	9.8
28 d	47.7	16.8	5.7									5.2	6.2	9.1
35 d	47.7	16.7	5.7									5.2	6.0	8.6
49 d	47.9	16.0	5.7									5.0	5.8	7.9
70 d	46.6	14.7	5.4									4.7	5.4	6.5
98 d	47.7	14.2	5.6									4.7	5.2	5.7
(**3**)														
0	69.5	67.9	50.5[a]	24.7[a]	38.1[a]	29.4[a]	28.4[a]	31.2[a]	34.7[a]	41.4[a]	43.4	35.0	20.2	47.2
1 h	69.3	68.0	56.8[a]	36.7[a]	42.7[a]	41.3[a]	39.0[a]	41.5[a]	42.9[a]	44.9	43.0	33.2	8.5	37.3
1 d	69.3	68.1	59.3	41.8[a]	40.5	37.8	37.0	37.6	38.9	40.8	38.7	20.5	31.4	34.5
2 d	69.1	67.8	58.8	40.0	39.1	35.7	34.4	34.9	36.2	38.5	34.9	10.6	31.8	30.7
5 d	68.9	67.7	58.6	39.2	37.7	32.9	31.0	31.3	32.6	35.2	29.4	6.9	29.0	26.8
7 d	68.9	66.4	58.7	39.2	38.4	33.1	30.8	30.8	32.1	34.6	28.1	7.8	28.3	25.7
14 d	69.0	66.4	57.9	37.1	34.3	27.8	25.9	26.5	28.8	31.8	23.0	10.8	25.5	21.5
21 d	68.8	66.2	57.6	35.9	31.3	23.9	22.5	23.8	26.3	29.6	20.0	13.9	22.8	18.5
28 d	68.5	65.9	56.5	34.2	29.1	21.9	20.8	22.5	25.0	27.7	17.4	16.1	21.0	16.9
35 d	68.5	65.6	55.4	32.7	27.7	20.5	19.8	21.5	23.9	26.3	15.1	17.2	19.6	15.7
49 d	68.2	65.3	54.7	31.0	24.5	18.1	17.9	19.4	21.5	23.4	11.7	17.9	17.5	14.6
70 d	67.5	64.3	52.5	28.5	20.9	16.0	15.8	16.7	18.1	19.6	8.4	17.7	15.4	13.1
98 d	67.0	63.8	51.9	27.3	19.2	13.9	13.9	14.1	15.2	16.1	6.8	16.8	13.4	11.9

When the L^*-value became higher than 95, and the C^*-value lower than 5, no more measurements were recorded.

[a] Some undissolved pigment was still present.

[b] The colour turned lighter during the preparation time.

[c] The colour turned more green (less blue) during the preparation time.

The following tendency was the same for all three pigments: starting with the most acidic solutions, increments in pH produced bathochromic shifts. Just around neutrality these shifts were dramatic, with the highest visible $\lambda_{\max}$-values at pH 7.3 for all three pigments. Further increase in pH resulted in hypsochromic shifts until new bathochromic shifts were revealed above pH 9. This pattern correlates well with earlier reports on anthocyanidin 3-monoglucosides; Cabrita et al. (2000) have shown that anthocyanins containing aglycones with two or three hydroxyl groups on the B-ring (like cyanidin) achieved the same variation in the alkaline region as described above, contrary to anthocyanidin 3-monoglucosides with only one hydroxyl group on the B-ring, which showed almost constant $\lambda_{\max}$ values for each pigment in this pH region. Thus, the pattern shown in Fig. 2(b) indicated that more complex anthocyanins, such as **3** (having two aromatic acyl groups and 3,5-diglycosidic substitution) may involve the same type of aglycone equilibrium forms at

Table 7
C^* values of 0.15 mM cyanidin 3-glucoside (**1**), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) at pH-values from 1.1 to 10.5 measured initially (0), after 1 hour (1 h), 1 day (1 d), 2, 5, 7, 14, 21, 28, 35, 49, 70 and 98 days at 10 °C

	pH 1.1	pH 3.0	pH 4.1	pH 5.1	pH 6.0	pH 6.6	pH 6.8	pH 6.9	pH 7.2	pH 7.3	pH 8.0	pH 8.9	pH 9.9	pH 10.5
(**1**)														
0	81.9	70.2	49.8[a]	22.6[a]	32.5[a]	27.7[a]	22.9[a]	22.3[a]	21.9[a]	24.0[a]	47.2	46.9	40.6	49.7
1 h	81.5	70.4	50.0	13.8[a]	24.7[a]	29.1[a]	24.3[a]	23.6[a]	22.5[a]	23.6[a]	47.6	47.4	40.7	44.4
1 d	81.4	70.0	49.0	13.0[a]	11.7	17.7	17.5	19.4	20.4	20.6	44.5	45.3	32.3	17.5
2 d	83.9	72.4	51.0	13.9[a]	11.0	15.0	14.4	15.9	17.0	17.6	42.9	43.6	26.0	25.4
5 d	83.7	71.0	50.5	13.7[a]	9.9	12.1	10.8	10.5	10.4	9.7	35.4	33.8	18.2	25.4
7 d	83.6	70.4	50.3	13.3	9.0	10.8	9.5	9.0	8.5	7.9	31.9	28.8	17.4	24.6
14 d	83.6	69.1	45.4	10.5	8.5	8.3	7.3	7.2	7.8	8.9	24.0	18.4	16.5	21.7
21 d	83.7	68.7	35.5	10.4	7.7	7.1	7.5	8.6	9.9	11.3	21.2	15.4	15.2	19.5
28 d	83.3	67.6	25.4	16.2	7.4	7.3	8.6	9.8	11.2	12.7	20.2	14.3	14.1	17.8
35 d	82.9	66.9	19.0	20.5	7.3	7.7	9.2	10.4	11.9	13.5	19.5	13.8	13.3	16.7
49 d	82.8	65.5	15.4	24.0	7.6	8.7	10.2	11.5	12.9	14.5	18.6	13.1	12.2	15.3
70 d	82.1	64.2	17.6	24.8	8.8	9.8	11.1	12.3	13.7	15.3	17.5	12.2	11.1	13.5
98 d	82.2	60.8	19.1	24.7	9.1	10.3	11.3	12.6	13.8	15.3	16.7	11.3	9.9	12.0
(**2**)														
0	78.6	52.9	20.9[b]	6.1[b]	13.1[b]	64.7	75.7	74.6	73.4	71.5	81.2	77.7	32.5	35.6[c]
1 h	78.6	53.1	20.8	5.7	3.3	5.1	16.6	39.9	54.5	62.3	75.7	60.2	26.4	52.9
1 d	79.0	52.4	20.4	5.2	3.1	2.1	1.5	1.1	2.1	3.9	5.2	18.7	33.6	50.7
2 d	79.3	50.9	20.1	5.0			2.0	1.9	2.9	5.0	4.8	21.5	30.8	48.3
5 d	79.6	48.9	20.1	5.6			1.9	1.5	2.8	5.3	6.2	21.4	27.9	43.2
7 d	79.8	50.0	19.6	5.0			2.6	2.7	3.8	6.0	7.0	22.2	24.9	37.1
14 d	80.2	46.6	19.2	5.3			3.0	3.1	4.3	6.6	8.9	21.2	23.2	33.7
21 d	80.4	46.3	18.9	5.1			4.0	4.3	5.7	8.0	10.7	20.3	21.8	31.9
28 d	80.4	46.1	18.9	5.1			5.0	5.6	7.2	9.2	12.4	19.6	20.5	30.6
35 d	80.5	45.8	18.7	5.2			5.8	6.5	8.5	10.9	13.7	19.0	19.4	29.1
49 d	80.7	45.1	18.8	5.1			7.6	8.5	11.1	13.5	15.5	18.1	18.0	26.6
70 d	78.4	41.6	17.6	5.2			10.5	11.6	14.7	17.2	16.8	17.3	16.0	23.4
98 d	80.4	39.9	17.7	5.4			13.7	15.2	18.7	21.4	18.5	17.8	15.9	23.4
(**3**)														
0	79.6[a]	80.6[a]	79.0[a]	65.4[a]	75.8[a]	64.4[a]	57.8[a]	59.7[a]	48.9[a]	46.6[a]	56.1[a]	38.8	24.3	42.3
1 h	78.1	77.6	77.5[a]	71.5[a]	72.4[a]	66.9[a]	63.8[a]	63.1[a]	55.9[a]	55.6[a]	53.6	40.1	23.6	50.4
1 d	77.9	77.6	77.9	73.8	71.3	66.8[a]	64.7[a]	62.5	60.1	57.7	51.1	35.2	40.2	55.2
2 d	77.9	77.6	77.9	73.7	71.1	65.9	63.6	61.2	58.7	56.4	49.3	30.1	47.4	54.2
5 d	80.8	80.6	81.0	76.4	73.5	67.2	64.3	61.6	59.0	56.7	48.3	19.5	51.7	53.3
7 d	80.7	80.6	80.8	76.2	73.5	67.1	64.0	61.1	58.4	56.0	47.4	16.6	52.8	52.5
14 d	80.8	80.7	81.2	75.7	71.8	63.5	59.7	57.0	54.7	52.9	42.8	18.9	53.9	48.3
21 d	80.7	80.9	81.3	75.5	69.8	59.3	55.1	52.9	51.3	50.1	38.2	28.5	51.4	44.7
28 d	80.2	80.4	80.8	74.6	67.5	55.7	51.1	49.7	48.7	47.8	34.1	34.1	49.6	42.4
35 d	80.2	80.3	80.5	73.5	65.6	52.8	48.2	47.1	46.6	46.2	30.5	37.4	47.9	40.5
49 d	80.2	80.5	80.4	72.4	61.6	46.9	42.2	42.5	42.9	43.3	24.6	40.2	44.5	38.0
70 d	80.0	80.0	80.1	69.8	54.6	39.9	35.8	37.3	39.2	40.1	18.1	41.4	40.8	35.6
98 d	80.2	80.1	80.2	68.8	49.4	34.8	31.0	33.2	35.5	36.6	14.9	40.5	37.1	33.4

When the L^*-value became higher than 95, and the C^*-value lower than 5, no more measurements were recorded.

[a] Some undissolved pigment was still present.

[b] The colour turned lighter during the preparation time.

[c] The colour turned more green (less blue) during the preparation time.

the various pH values as the anthocyanidin 3-monoglucosides.

The λ_{max}-values of freshly made 0.05 mM solutions of **1**–**3** at the various pH-values correlated poorly with the corresponding h_{ab}, values, and the correlation coefficients (0.41, 0.80 and 0.90 for **1**–**3**, respectively) showed that caution should be applied when using λ_{max}-values for interpretation of colours. For instance, **1** showed the same λ_{max}-value (546–547 nm) in aqueous solutions at both pH 6.6 and 9.9; however, its corresponding h_{ab}, values were −17° and 18°, respectively.

3.2. Colour saturation (chroma) of anthocyanins in freshly made samples at pH 1.1–10.5

The C^* values (Tables 6 and 7) show the distance away from the greytone (i.e., degree of colour saturation) in a scale from 0 to 100. Fig. 3 shows how the chroma varied

Table 8

L^* values of 0.05 mM cyanidin 3-glucoside (**1**), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) at pH-values from 1.1 to 10.5 measured initially (0), after 1 hour (1 h), 1 day (1 d), 2, 5, 7, 14, 21, 28, 35, 49, 70 and 98 days at 10 °C

	pH 1.1	pH 3.0	pH 4.1	pH 5.1	pH 6.0	pH 6.6	pH 6.8	pH 6.9	pH 7.2	pH 7.3	pH 8.0	pH 8.9	pH 9.9	pH 10.5
(**1**)														
0	81.1	82.3	89.5	94.9[a]	82.6[a]	75.8[a]	75.2[a]	74.3[a]	73.7[a]	72.5	74.8	75.0	73.2	62.0
1 h	81.1	82.3	89.5	96.4[a]	95.2[a]	85.0[a]	80.8[a]	77.8[a]	76.0	74.0	75.1	75.2	73.8	65.9
1 d	81.1	82.6	90.0	96.7[a]	97.3	95.2	93.5	91.1	88.0	84.7	78.6	77.8	80.3	92.8
2 d	81.2	82.6	89.7	96.7			94.5	93.0	91.1	88.9	80.7	79.8	85.4	94.6
5 d	81.2	83.5	90.0	96.8			95.8	95.1	94.4	93.6	85.3	85.0	93.6	96.0
7 d	81.3	83.7	90.1	96.8					95.5	94.8	87.5	87.8	95.7	96.5
14 d	81.3	84.0	91.1	96.9						96.2	92.8	94.2	97.3	97.4
21 d	81.4	84.1	92.8								95.3	97.0	97.8	98.0
28 d	81.5	84.3	94.6								96.1	97.9	98.1	98.0
35 d	81.5	84.5	96.2								96.4	97.9	98.2	98.3
49 d	81.6	85.2	97.5								96.8	98.1	98.5	98.6
70 d	81.5	85.9									97.0	98.7	98.6	98.7
98 d	81.8	87.2									97.8		99.3	98.8
(**2**)														
0	81.5	90.5	96.5[b]	98.2[b]	96.6[b]	80.7	73.2	69.3	65.4	64.4	67.1	67.3	80.6	83.3[c]
1 h	81.5	90.6	96.6		98.6	97.6	94.7	87.9	79.8	73.9	74.2	79.6	96.3	96.9
1 d	81.4	92.1	96.7				98.2	98.0	97.7	97.2	96.5	97.1	97.9	97.5
2 d	81.4	92.4	96.9									97.5	97.8	97.5
5 d	81.6	93.2	96.9									97.7	98.1	97.8
7 d	81.6	93.2	96.8									97.4	98.1	97.6
14 d	81.6	93.1	96.2									97.6	98.2	97.8
21 d	81.4	93.1	97.1									97.8	98.4	97.9
28 d	81.5	93.3	97.0									98.0	98.5	98.0
35 d	81.5	93.2	97.1									98.1	98.6	98.1
49 d	81.4	93.5	97.1									98.2	98.7	98.2
70 d	78.9	90.6	94.0									95.5	96.0	95.6
98 d	81.3	93.6	96.7									98.5	98.9	98.8
(**3**)														
0	66.0	66.5	72.5[a]	82.7[a]	72.1[a]	76.0[a]	75.3[a]	72.2[a]	69.3[a]	63.2[a]	57.8	56.3	55.3	57.2
1 h	66.0	66.5	68.0[a]	74.3[a]	68.5[a]	65.5[a]	65.5[a]	62.1[a]	60.8[a]	59.4	58.8	57.3	64.1	72.5
1 d	66.2	66.6	67.9	70.4[a]	70.1	68.0	66.7	65.6	64.7	64.0	62.9	65.8	93.2	95.3
2 d	66.2	66.6	68.1	71.3	71.0	69.4	68.7	67.7	67.1	66.3	65.7	73.6	94.7	96.2
5 d	66.3	66.6	68.3	71.6	72.0	71.3	71.0	70.6	70.3	69.8	71.1	83.8	95.7	96.6
7 d	66.3	67.2	68.2	71.6	71.5	71.1	71.1	70.9	70.8	70.5	72.5	85.4	96.0	96.7
14 d	66.6	67.6	69.0	73.2	74.4	74.8	74.9	74.6	74.0	73.6	77.3	89.2	97.0	97.2
21 d	66.7	67.6	69.3	73.8	76.2	77.2	77.2	76.8	76.3	75.5	79.8	91.9	97.2	97.3
28 d	66.9	67.6	69.5	74.5	77.1	78.1	78.1	77.6	77.0	76.3	81.4	93.5	97.2	97.3
35 d	67.1	67.8	69.9	75.3	77.7	78.8	78.6	78.0	77.6	77.3	82.9	94.4	97.3	97.4
49 d	67.7	68.1	70.6	76.2	79.8	80.3	79.9	79.6	79.4	79.3	85.5	95.6	97.5	97.4
70 d	68.0	68.2	71.4	77.2	81.2	80.8	80.7	80.7	81.0	81.2	87.5	96.1	97.5	97.6
98 d	68.8	68.7	71.5	77.7	82.4	81.7	81.5	82.6	83.0	83.3	89.4	96.8	98.2	97.9

When the L^*-value became higher than 95, and the C^*-value lower than 5, no more measurements were recorded.

[a] Some undissolved pigment was still present.

[b] The colour turned lighter during the preparation time.

[c] The colour turned more green (less blue) during the preparation time.

with pH in freshly made samples of **1**–**3**. The highest values were found at the lowest pH-values, except for **2**, which had its highest C^* values in a region around pH 8.0. Freshly made samples of **2** and **3** had significantly higher C^* values than **1** in the pH region 6.6 to 8.0, revealing the impact of 5-glucosidation on colour saturation. The molar absorptivities (ε) of **1**–**3** (Table 3) followed a similar colour strength pattern (Fig. 4) to that described above. When the ε values of petunidin 3-($4'''$-coumaroylrhamnosyl)glucoside-5-glucoside, petanin, were compared with similar values of the six common anthocyanidin 3-glucosides (Cabrita et al., 2000; Fossen, Cabrita, & Andersen, 1998), it was noticed that petanin in contrast to anthocyanin 3-monoglucosides, showed relatively high ε values in slightly alkaline solutions. This hyperchromic effect in slightly alkaline solutions should thus be attributed to 3,5-diglycosidic substitution and not aromatic acylation of the anthocyanin.

Table 9

L^* values of 0.15 mM cyanidin 3-glucoside (**1**), cyanidin 3-($2''$-glucosylglucoside)-5-glucoside (**2**) and cyanidin 3-($2''$-($2'''$-sinapoylglucosyl)-$6''$-sinapoylglucoside)-5-glucoside (**3**) at pH-values from 1.1 to 10.5 measured initially (0), after 1 hour (1 h), 1 day (1 d), 2, 5, 7, 14, 21, 28, 35, 49, 70 and 98 days at 10 °C

	pH 1.1	pH 3.0	pH 4.1	pH 5.1	pH 6.0	pH 6.6	pH 6.8	pH 6.9	pH 7.2	pH 7.3	pH 8.0	pH 8.9	pH 9.9	pH 10.5
(**1**)														
0	63.7	63.0	69.6[a]	65.5[a]	39.9[a]	33.5[a]	35.3[a]	33.3[a]	32.2[a]	31.1[a]	38.7	38.7	39.3	24.3
1 h	63.4	63.1	69.9	80.7[a]	60.7[a]	41.4[a]	40.5[a]	37.1[a]	35.0[a]	33.7[a]	39.4	39.1	40.3	28.2
1 d	63.6	63.3	70.6	82.6[a]	81.7	67.6	63.7	56.5	51.3	48.2	44.7	43.8	53.9	75.4
2 d	66.3	66.1	73.6	86.4[a]	87.0	76.4	73.8	67.6	62.8	58.9	51.4	51.1	68.6	85.9
5 d	66.3	66.6	73.9	86.3[a]	87.9	79.6	78.2	75.2	73.3	71.4	61.4	64.7	86.6	89.1
7 d	66.3	66.9	74.0	86.7	88.7	80.6	79.8	77.7	76.8	75.6	66.2	71.5	90.2	90.1
14 d	66.5	67.5	76.6	87.7	88.4	82.9	83.5	83.4	83.7	82.5	77.8	87.5	92.7	92.3
21 d	66.7	67.7	81.2	88.2	88.6	84.7	86.1	86.6	86.5	85.2	82.4	92.4	94.0	93.5
28 d	66.8	68.1	85.5	86.6	88.5	85.2	87.2	87.1	87.0	85.9	84.1	93.7	94.5	94.2
35 d	66.9	68.2	88.6	86.3	90.4	87.1	88.3	87.9	87.5	86.3	85.2	94.2	95.2	94.7
49 d	67.0	68.9	91.3	85.6	88.0	87.5	89.0	88.6	88.3	87.3	87.0	94.6	95.9	95.2
70 d	66.6	69.5	92.3	84.5	87.9	88.5	89.4	89.2	88.9	88.2	88.8	95.5	96.6	96.2
98 d	67.2	71.1	92.7	86.3	87.7	89.5	90.4	90.1	89.9	89.1	90.1	96.2	97.3	97.0
(**2**)														
0	69.5	76.9	89.4[b]	94.5[b]	89.2[b]	49.9	36.9	31.3	29.1	27.6	35.6	35.1	52.3	55.5[c]
1 h	69.6	77.1	89.6	95.0	92.7	89.5	79.7	60.8	47.2	38.0	43.1	47.3	84.8	87.7
1 d	69.4	77.5	89.9	95.3	95.9	95.3	94.6	93.5	92.0	89.6	88.1	87.4	90.2	90.4
2 d	69.4	78.2	90.1	95.4			94.6	93.6	92.4	90.7	91.1	90.7	92.4	93.3
5 d	69.6	79.2	90.3	95.4			94.2	93.4	92.2	90.7	91.3	89.9	92.2	93.5
7 d	69.6	80.0	89.8	95.2			94.7	94.1	92.8	91.4	91.8	89.0	91.9	93.1
14 d	69.4	80.0	90.3	95.4			94.2	93.3	92.2	90.5	91.3	90.1	92.8	93.1
21 d	69.4	80.4	90.7	95.2			94.3	93.4	92.3	90.9	90.6	91.2	93.6	93.5
28 d	69.2	79.9	90.7	95.1			94.2	93.4	92.2	90.3	90.2	92.0	94.2	93.9
35 d	69.4	80.5	90.7	94.9			93.7	92.8	91.4	90.0	90.1	92.4	94.7	94.1
49 d	69.4	80.6	90.8	95.0			93.4	92.4	90.9	89.5	90.0	93.1	95.2	94.3
70 d	67.2	78.6	87.8	91.8			89.9	88.8	87.1	85.8	87.2	90.7	93.0	91.6
98 d	69.4	82.1	90.6	94.6			91.9	90.5	88.6	87.3	89.8	93.5	96.1	94.3
(**3**)														
0	56.7[a]	48.1[a]	50.3[a]	52.5[a]	35.4[a]	42.3[a]	46.6[a]	41.8[a]	53.6[a]	57.3[a]	28.2[a]	22.7	19.6	27.6
1 h	46.0	45.1	40.8[a]	40.7[a]	33.7[a]	33.3[a]	33.8[a]	30.9[a]	39.5[a]	38.4[a]	27.8	24.2	23.5	35.8
1 d	46.1	45.1	39.9	36.4	35.4	31.6[a]	30.4[a]	29.4	29.6	29.8	30.6	29.1	73.8	82.5
2 d	46.2	45.1	39.9	36.5	35.6	32.3	31.4	30.7	30.8	30.9	32.5	35.2	80.2	85.3
5 d	48.5	47.3	42.1	39.0	38.2	35.1	34.4	34.0	34.3	34.5	38.8	53.3	86.3	90.4
7 d	48.5	47.3	42.3	39.2	38.1	34.9	34.3	34.0	34.4	34.8	39.7	57.4	87.0	90.8
14 d	48.9	47.5	42.6	40.0	39.7	37.5	37.3	36.9	37.2	37.5	46.2	69.9	89.9	91.7
21 d	49.0	47.4	42.7	40.0	41.1	40.0	40.3	39.9	40.1	40.3	52.0	77.6	91.1	91.9
28 d	49.2	47.5	42.9	40.6	42.0	41.6	41.9	41.7	41.6	41.6	55.2	81.5	91.4	92.1
35 d	49.8	47.6	43.3	41.7	42.7	42.7	43.7	43.0	42.9	42.9	58.0	83.9	91.8	92.3
49 d	50.2	47.8	43.6	42.4	44.8	46.2	47.3	46.5	45.9	45.8	63.4	87.0	92.4	92.8
70 d	50.8	47.9	44.2	44.2	48.6	50.6	52.0	49.8	49.1	49.0	69.2	88.4	92.4	92.7
98 d	51.6	48.1	44.5	44.7	50.8	53.2	53.3	52.3	51.8	52.2	72.7	89.5	93.6	93.4

When the L^*-value became higher than 95, and the C^*-value lower than 5, no more measurements were recorded.

[a] Some undissolved pigment was still present.

[b] The colour turned lighter during the preparation time.

[c] The colour turned more green (less blue) during the preparation time.

Freshly made samples of the acylated pigment (**3**) also had much higher colour saturation than the corresponding solutions of nonacylated pigments (**1** and **2**) in the pH range 3.0–6.6. For instance, at pH 6.0, the anthocyanins **1** and **2** showed very weak colours, while a 0.15 mM solution of **3** had a C^*-value of 76 (Table 7), an effect of significant importance in the search for colour additives for weakly acid solutions.

3.3. Colour variation of anthocyanins in the pH range 1.1–10.5 during storage

According to the CIELAB parameters of **1**–**3** during storage (Tables 4–9), their colour stabilities depend highly on pH and anthocyanin structure. As revealed in Fig. 5 and Table 4, the hues changed little at low pH values (pH 1.1 and 3.0) for all pigments. At pH 4.1 the most simple anthocyanin (**1**), maintained nearly the same reddish shade for 20 days; however, thereafter, the colour changed toward orange until all of the pigment was gone after ca. 2 months. At this pH, the acylated pigment (**3**) maintained its initial hue during the whole period (98 days). Pigment **2** showed an intermediate course by having a relative stable hue; however, its chroma was relative low (Tables 6 and 7). Pigment **2** was significantly more unstable than **1** at most pH values, showing very low C^* and very high L^* values after storage for only some hours in neutral and weakly acid solutions (Tables 6–9).

By comparing Tables 6 and 7 (and Tables 8 and 9) it was observed that the most concentrated samples (0.15 mM) had the highest C^*-values (and lowest L^*-values) as expected. The hue angles were clearly lowest for the less concentrated solutions (0.05 mM) (bathochromic effect) in the most acidic buffers for all three pigments (Tables 4 and 5). In the three most alkaline solutions, all pigments turned into yellow colours during storage regardless of concentration. In the pH range 5.1 to 8.0, the impact of concentration on anthocyanin colour was more complex. However, in this range, the colour stabilities of, in particular, the nonacylated pigments (**1** and **2**) were clearly greater in 0.15 mM solutions.

Pigment **3** showed greater colour stability, expressed by h_{ab}, C^* and L^* parameters, than the nonacylated forms (**1** and **2**) at all pH values but pH 1.1, where all pigments retained their colours in the storage period, and in the most alkaline solutions, where all pigments experienced dramatic colour changes. Interestingly, the hue of 0.05 mM solution of **3** at pH 6.9 decreased just around 40° after more than three months of storage, going from a pure blue tone (h_{ab}, around −90°) to a slightly different shade (−127°). At this pH, both **1** and **2** started to change colours just after 1 h. In solutions with pH 4.1 and 5.1, **3** maintained nearly the same h_{ab}, C^*, and L^* values during the whole measuring period (98 days), in contrast to its non-acylated analogues, **1** and **2** (Tables 4–9).

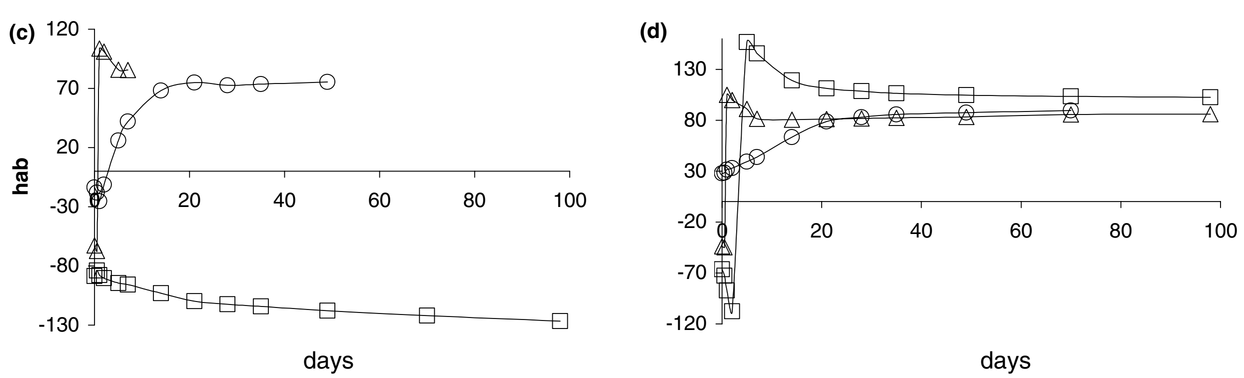

Fig. 5. h_{ab} values variations with time (days) for 0.05 mM cyanidin 3-glucoside (**1**) (○), cyanidin 3-(2″-glucosylglucoside)-5-glucoside (**2**) (△) and cyanidin 3-(2″-(2‴-sinapoylglucosyl)-6″-sinapoylglucoside)-5-glucoside (**3**) (□) at pH 1.1 (a), pH 4.1 (b), pH 6.9 (c) and pH 8.9 (d).

Acknowledgements

The authors thank Polyphenols Laboratories AS for instrumental support. K.T. acknowledges The Norwegian Research Council for a fellowship.

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