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Isolation and structural characterization of new anthocyanin-alkyl-catechin pigments

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Abstract

A condensation reaction between malvidin 3-glucoside (mv3gl) and catechin (cat) mediated by acetaldehyde and propionaldehyde was conducted in model solutions at two pH values (at 1.5 and 3.2). Acetaldehyde led to the formation of mv3gl-8-ethyl-8-cat (m/z at 809) widely reported in the literature, whereas propionaldehyde led to the formation of mv3gl-8-propyl-8-cat (m/z at 823) and its structural characterization is elucidated herein for the first time by 1D and 2D NMR. The formation of these alkyl-linked pigments is favoured at lower pH value as the alkyl linkage requires the formation of an aldehyde carbocation for a further condensation.

Keywords: Pigment; Anthocyanin; Catechin; Kinetic; NMR; Acetaldehyde; Propionaldehyde

1. Introduction

Wine aging is a very complex process in which major organoleptic properties, such as colour and flavour are likely to change. The colour of red wine changes from an initial bright red to a more reddish-brown hue, mainly due to progressive structural changes of anthocyanins. The reaction of these pigments with tannins, directly or mediated by other molecules such as acetaldehyde, is supposed to play a crucial role in colour evolution (Dallas, Ricardo-da-Silva, & Laureano, 1996; Rivas-Gonzalo, Bravo-Haro, & Santos-Buelga, 1995; Somers, 1971; Timberlake & Bridle, 1976) with the formation of new and more stable pigments with different chromatic features (Mateus, Silva, Rivas-Gonzalo, Santos-Buelga, & De Freitas, 2002; Mateus et al., 2003; Rivas-Gonzalo et al., 1995; Timberlake & Bridle, 1976).

Acetaldehyde is the most abundant aldehyde present in Port wine and may arise from different sources, such as a fermentation intermediary product, from oxidation of ethanol (Santos-Buelga, Bravo-Haro, & Rivas-Gonzalo, 1995; Wildenradt & Singleton, 1974), or result from the addition of wine spirit to the must in order to stop the fermentation (Rogerson & De Freitas, 2002). Effectively, common wine spirits usually used in the Port wine industry have rich aldehyde content within a range of 30–250 mg/l (acetaldehyde, propionaldehyde, isovaleraldehyde, isobutyraldehyde, benzaldehyde and others).

The effect of acetaldehyde on the increase of the tannin mean degree of polymerization and its involvement in anthocyanin–tannin associations is well known (Dallas et al., 1996; Rivas-Gonzalo et al., 1995; Timberlake & Bridle, 1976). The ethyl-linked pigments resulting from acetaldehyde mediation have already been detected in wines by LC–MS (Mateus et al., 2002;

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Vivar-Quintana, Santos-Buelga, Francia-Aricha, & Rivas-Gonzalo, 1999). The unstability of these ethyl-linked adducts in model solutions was suggested to be due to the cleavage of the ethyl linkage. This feature leads to further structural reorganization (Escribano-Bailón, Álvarez-Garcia, Rivas-Gonzalo, Heredia, & Santos-Buelga, 2001), with the formation of many other compounds, such as pyranoanthocyanin pigments, which were recently isolated and structurally characterized by Mateus et al. (2002, 2003). The adducts resulting from the reaction between anthocyanins and flavanol, mediated by minor aldehydes, such as propionaldehyde should be difficult to detect in wine due to their low concentrations and their instability by analogy to the acetaldehyde-mediated anthocyanin–flavanol adducts.

Recent studies have shown that other aldehydes present in wine spirit used for Port wine fortification induce some chromatic changes in wine model solutions (Pissarra, Mateus, Rivas-Gonzalo, Santos-Buelga, & De Freitas, 2003). These changes are due to the formation of adducts between anthocyanins and flavanols, linked by alkyl groups originating from different aldehydes (propionaldehyde, isovaleraldehyde, benzaldehyde, isobutyraldehyde and others).

The aim of the present work is to structurally characterize and to study the formation of the newly formed pigments resulting from the interaction between malvidin 3-glucoside (mv3gl) and (+)-catechin (cat) in the presence of two of the most abundant aldehydes (acetaldehyde and propionaldehyde) present in commercial wine spirit used for Port wine fortification.

2. Materials and methods

2.1. Standards

(+)-Catechin was purchased from Sigma Chemical Co (St. Louis, MO, USA). Acetaldehyde and propionaldehyde were obtained from Fluka Chemika (Buchs, Switzerland) and Merck (Darmstadt, Germany), respectively.

2.2. Isolation of malvidin 3-glucoside

Anthocyanins were extracted from red grape skins (*Vitis vinifera*) with 40% aqueous ethanol. Grape skin extract was applied onto a TSK Toyopearl gel HW-40(S) (TOSOH, Japan) column (300 × 25 mm) and eluted with water acidified with HCl (2%) at a flow rate of 0.8 ml/min in order to isolate non-acylated anthocyanins. The isolation of mv3gl was performed by semi-preparative HPLC using a reversed-phase C18 column (250 × 4.6 mm i.d.), as reported elsewhere (Pissarra et al., 2003).

2.3. Anthocyanin-derived pigment synthesis

For each aldehyde (acetaldehyde and propionaldehyde), 200 ml of synthetic solution (ethanol/water 12% (v/v), pH 1.5) composed of mv3gl (2 mM)/cat/aldehyde (molar ratio of 1:4:10) was prepared. The solutions were kept at 35 °C and protected from light in order to achieve the maximum amount of adduct of the respective aldehyde. The appearance of newly formed pigments was followed by HPLC-DAD.

The kinetic studies were performed in two sets, at pH 1.5 and 3.2, following similar experimental conditions to those described above.

2.4. HPLC analysis

The samples were analyzed by HPLC-DAD (detector L-7450A; Merck) using a Merck reversed-phase C18 column (Lichrospher, 250 × 4.6 mm i.d.) at 25 °C. Solvents were (A) water/formic acid (95:5), and (B) acetonitrile. The elution gradient was performed using a L-7100 Merck pump from 10 to 65% B for 50 min at a flow rate of 1.5 ml min^{-1}.

2.5. Isolation of the anthocyanin-derived pigments

The solvent was evaporated using a rotator evaporator at 30 °C to a volume of approximately 20 ml. The solution was applied on a TSK Toyopearl gel HW-40(S) column for chromatography (300 × 25 mm) and the pigments were eluted with water/ethanol (7:3) acidified with HCl (pH <2.0) at a flow rate of 0.8 ml min^{-1}. The fraction containing the major anthocyanin-derived pigments was collected and each compound was isolated by semi-preparative HPLC using a reversed-phase C18 column (Merck, Lichrospher, 250 × 4.6 mm i.d.). The solvents were (A) water/formic acid (90:10), and (B) water/formic acid/acetonitrile (6:1:3), with the following gradient: 20% to 80% B over 30 min, 80% to 100% B over 5 min, isocratic 100% B over 10 min, from 100% to 20% B over 5 min, at a flow rate of 1.0 ml min^{-1}. The pigments were submitted to a final purification by TSK Toyopearl gel HW-40(S) column chromatography, eluted with distilled methanol acidulated with 2% HCl. The solvent was partially evaporated in a rotary evaporator at 30 °C, and the sample was freeze-dried and stored at −18 °C until used.

2.6. LC/MS analysis

A Hewlett–Packard 1100 series liquid chromatograph, equipped with an AQUA™ (Phenomenex, Torrance, CA, USA) reversed-phase column (150 × 4.6 mm, 5 μm, C18) thermostatted at 35 °C was used. Solvents were (A) aqueous 0.1% trifluoroacetic acid, and (B)

acetonitrile, establishing the gradient as reported by Pissarra et al. (2003). The capillary voltage was 3 V and the capillary temperature 190 °C. Spectra were recorded in positive ion mode between m/z 120 and 1500. The mass spectrometer was programmed to do a series of three scans: a full mass, a zoom scan of the most intense ion in the first scan, and a MS–MS of the most intense ion using a collision energy of 30 V.

2.7. NMR analysis

^{1}H NMR (500.13 MHz) and ^{13}C NMR (125.77 MHz) spectra were measured in CD_3OD/TFA (98:2) on a Bruker-DRX500 spectrometer at 298 K and with TMS as internal standard. ^{1}H chemical shifts were assigned using 1D and 2D ^{1}H NMR (gCOSY) while ^{13}C resonances were assigned using 2D NMR (gHMBC and gHSQC) techniques (Bax & Subramanian, 1986; Bax & Summers, 1986). The delay for the long range C/H coupling constant was optimized to 7 Hz.

2.8. Pigment A1: mv3gl-8-ethyl-8-cat

^{1}H NMR (500.13 MHz, CD_3OD/TFA (98:2)); ethyl linkage: δ 1.79 (d, $J = 7.6$ Hz, –CH_3); δ 5.21–5.25 (m, –CH); anthocyanin moiety: δ 3.90 (s, OMe); δ 6.66 (s, H-6A); δ 7.88 (s, H-2′6′B); δ 8.77 (s, H-4C); flavanol moiety: δ 2.45 (dd, $J = 9.5$ Hz, $J = 16.1$ Hz, H-4βF); δ 2.97 (dd, $J = 5.9$ Hz, $J = 16.1$ Hz, H-4αF); δ 3.63–3.65 (m, H-3F); δ 4.03 (d, $J = 8.8$ Hz, H-2F); δ 5.95 (s, H-2′E); 5.97 (d, $J = 8.0$ Hz, H-5′E); δ 6.11 (s, H-6D); δ 6.36 (d, $J = 8.0$ Hz, H-6′E); sugar moiety: δ 3.55 (H-4); δ 3.58 (H-3); δ 3.65 (H-5); δ 3.69 (H-2); δ 3.85 (H-6a); δ 4.03 (H-6b); δ 5.32 (H-1).

^{13}C NMR (125.77 MHz, CD_3OD/TFA (98:2)); ethyl linkage: 18.5 (–CH_3); 25.9 (–CH); anthocyanin moiety: 55.6 (OMe); 102.0 (C-6A); 109.0 (C-2′6′B); 111.7 (C-4aA); 112.7 (C-8A); 118.7 (C-1′B); 133.6 (C-4C); 143.5 (C-3C); 144.0 (C-4′B); 147.9 (C-3′5′B); 152.4 (C-8aA); 154.7 (C-5A); 160.7 (C-2C); 165.7 (C-7A); flavanol moiety: 28.7 (C-4F); 66.6 (C-3F); 81.9 (C-2F); 100.6 (C-4aA); 108.2 (C-8D); 114.5 (C-2′E); 116.0 (C-6′E); 118.4 (C-5′E); 129.3 (C-1′E); 146.7 (C-3′E); 146.7 (C-4′E); 154.0 (C-8aD); sugar moiety: 60.5 (C-6); 69.5 (C-4); 73.6 (C-2); 77.0 (C-3); 77.3 (C-5); 102.0 (C-1).

2.9. Pigment A2: mv3gl-8-ethyl-8-cat

^{1}H NMR (500.13 MHz, CD_3OD/TFA (98:2)); ethyl linkage: δ 1.76 (d, $J = 7.77$ Hz, –CH_3); δ 5.32–5.34 (m, –CH); Anthocyanin moiety: δ 3.96 (s, OMe); δ 6.63 (s, H-6A); δ 7.88 (s, H-2′6′B); δ 8.82 (s, H-4C); flavanol moiety: δ 2.42 (dd, $J = 9.5$ Hz, $J = 16.2$ Hz, H-4βF); δ 2.97 (dd, $J = 5.6$ Hz, $J = 16.2$ Hz, H-4αF); δ 3.63 (m, H-3F); δ 4.19 (d, $J = 7.8$ Hz, H-2F); δ 5.93 (d, $J = 8.0$ Hz, H-5′E); δ 5.97 (s, H-2′E); 6.11 (s, H-6D); δ 6.27 (d, $J = 8.0$ Hz, H-6′E); sugar moiety: δ 3.56 (H-4); δ 3.59 (H-3); δ 3.67 (H-5); δ 3.72 (H-2); δ 3.88 (H-6a); δ 3.99 (H-6b); δ 5.39 (d, $J = 7.7$ Hz, H-1).

^{13}C NMR (125.77 MHz, CD_3OD/TFA (98:2)); ethyl linkage: 19.9 (–CH_3); 25.3 (–CH); anthocyanin moiety: 55.5 (OMe); 102.8 (C-6A); 109.2 (C-2′6′B); 112.2 (C-4aA); 112.2 (C-8A); 118.8 (C-1′B); 133.1 (C-4C); 144.0 (C-3C); 144.0 (C-4′B); 147.8 (C-3′5′B); 152.4 (C-8aA); 155.1 (C-5A); 161.0 (C-2C); 165.7 (C-7A); flavanol moiety: 29.0 (C-4F); 67.5 (C-3F); 82.5 (C-2F); 100.7 (C-4aA); 107.0 (C-8D); 113.4 (C-2′E); 115.0 (C-6′E); 118.0 (C-5′E); 130.0 (C-1′E); 144.0 (C-3′E); 144.0 (C-4′E); 154.0 (C-8aD); sugar moiety: 60.4 (C-6); 69.2 (C-4); 73.5 (C-2); 77.0 (C-3); 77.8 (C-5); 100.1 (C-1).

3. Results and discussion

3.1. NMR analysis

The reaction between mv3gl and cat, mediated by acetaldehyde, as commonly reported in the literature, led to the formation of two major coloured pigments (Fig. 1), which correspond to two enantiomers of mv3gl-8-ethyl-8-cat (A1 and A2, at m/z 809) differing in the stereochemistry of the asymmetric carbon of the ethyl linkage. The NMR data of pigments A1 and A2 (see Section 2) are consistent with the data previously reported by other authors (Escribano-Bailón, Dangles, & Brouillard, 1996).

The propionaldehyde-mediated condensation of mv3gl and cat led to the appearance of only one chromatographic peak which was found to correspond to mv3gl-8-propyl-8-cat (P) by mass spectrometry ($[M]^+$ m/z at 823), as previously reported (Pissarra et al., 2003).

The MS analysis of this new pigment (P) showed a typical fragmentation with a loss of the catechin moiety ($[M\text{-}290]^+$ m/z at 533) and a loss of the glucose moiety ($[M\text{-}162]^+$ m/z at 661) already reported elsewhere (Pissarra et al., 2003).

However, its NMR data showed a duplication of signals for the protons H-4C, H-6A, H-2′6′B and OMe of the anthocyanin moiety, and for the protons of the –CH_2 group of the propyl linkage. The remaining protons and the carbons have similar chemical shifts (Table 1). These two sets of signals could correspond to different conformers, or to a mixture of two isomers with the same retention time in the HPLC chromatogram (Fig. 1). This latter hypothesis is more probable by analogy with the condensation reaction mediated by acetaldehyde, or other aldehydes described in literature, yielding two enantiomers (Pissarra et al., 2003). Based on the signal intensity observed in the ^{1}H NMR spectrum, the signal of one structure (P1) is approximately 3-fold higher than the other structure (P2).

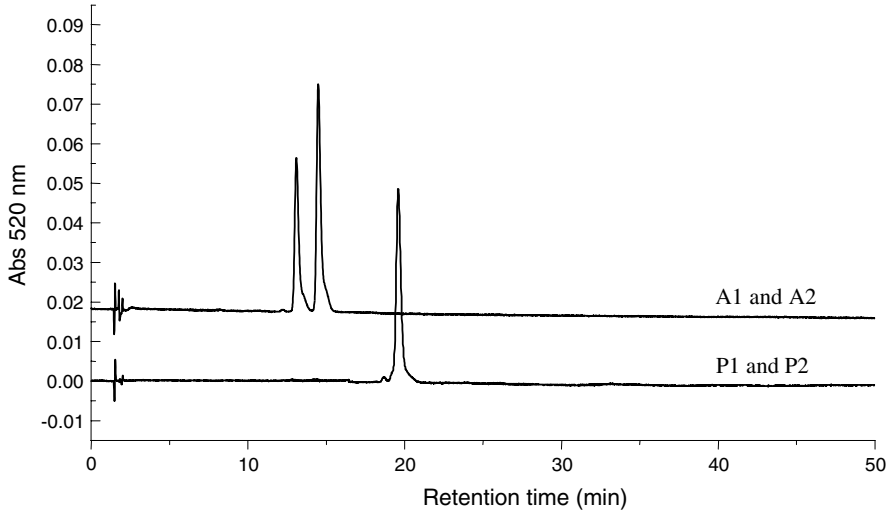

Fig. 1. Chromatograms recorded at 520 nm, obtained after purification by TSK Toyopearl gel HW-40(S) column chromatography, of the new anthocyanin-alkyl-flavanol pigments formed from the reaction between mv3gl and cat in the presence of acetaldehyde (upper part) and propionaldehyde (lower part).

The ^{1}H and ^{13}C NMR chemical shifts of each coloured compound in CD_3OD/TFA (98:2) are indicated in Table 1. The ^{1}H chemical shifts were assigned using 1D and 2D NMR techniques (COSY), and the assignment of the carbon resonances was made using 2D techniques (HSQC and HMBC). The HMBC spectra were optimized in order to visualize long distance coupling ^{1}H–^{13}C in $^{2}J_{C,H}$ and $^{3}J_{C,H}$ in order to assign most of the carbons, especially the ones of the alkyl linkage.

3.2. Anthocyanidin moiety

All the protons of the mv3gl moiety were easily assigned by comparison with those of anthocyanin-ethyl-catechin adducts A1 and A2 (see Section 2). The ^{1}H spectrum showed the presence of H-2′,6′B as a singlet at 7.96 and 7.91 ppm for structures P1 and P2, respectively, and the two methoxyl groups of ring B were also attributed to singlets at 4.01 (P1) and 3.96 ppm (P2). Proton H-4C was attributed to a singlet at 8.89 and 8.85 ppm for both structures, P1 and P2, and proton H-6A was assigned to a singlet at 6.64 (P1) and 6.66 ppm (P2). All the carbons were easily assigned through HSQC and HMBC correlations (Table 1). The assignments of carbon 4C at 134.2 ppm, carbon 6A at 104.4 ppm, carbons 2′,6′B at 110.6 ppm and the methoxyl carbons at 57.1 ppm were obtained by HSQC. The ^{1}H–^{13}C correlations, found in the HMBC spectrum, allowed the assignment of the other carbons of the structure, as shown in Fig. 2. Indeed, the long distance coupling, $^{2}J_{C,H}$ and $^{3}J_{C,H}$, allowed the assignment of all the carbons of the anthocyanidin moiety. The chemical shift found for carbon 7A (167.5 ppm) reinforces that this carbon in both structures, P1 and P2, is much more deprotected than carbon 5A and 8aA, similarly to those of pigments A1 and A2.

3.3. Flavanol moiety

The flavanol moiety was found to consist of a (+)-catechin unit, as seen from the correlation between proton H-2F of the flavanol pyran ring (4.34 ppm) and proton H-3F which was assigned as a multiplet at 3.55–3.65 ppm for both structures, P1 and P2. Indeed, the relative 2,3-stereochemistry is concluded to be *trans*, as proton H-2F resonates as a large douplet ($J = 7.63$ Hz) instead of a broad singlet or a doublet with a small constant coupling (0–3 Hz), characteristic of a (−)-epicatechin unit (Fletcher, Porter, Haslam, & Gupta, 1977). Protons H-4αF and H-4βF were easily assigned to the double doublets at 2.45 and 2.91 ppm, respectively, through the characteristic AMX spin system of the flavanol pyran ring observed in the COSY spectrum.

3.4. Sugar moiety

The anomeric carbon of the glucose moiety (around 100 ppm) and its correspondent proton (around 5 ppm) were easily assigned in all the pigments. All the other glucose protons and carbons were attributed through correlations found in the COSY and HSQC spectra, respectively.

3.5. Alkyl linkage

The alkyl linkage CH–CH_2CH_3 is assumed to be situated between ring A of the mv3gl moiety and ring D of the cat moiety, similarly to the linkage described in the literature for pigment mv3gl-8-ethyl-8-cat and also by comparison with the NMR data of a (+)-catechin molecule (as it helps to clarify the position of proton 6D). The –CH proton resonates as a singlet, integrating

Table 1
^{1}H, ^{13}C NMR data and HMBC and HSQC correlations of pigments P1 and P2, determined in CD_3OD/TFA (98:2)

Pigment P1 and P2: malvidin 3-glucoside-propyl-catechin				
Position	δ^{1} H; *J* (Hz)	δ^{13} C	HMBC	HSQC
Anthocyanidin moiety				
2C	–	162.2	H-4C; H-2′,6′B	–
3C	–	145.8	H-4C	–
4C–P_1	8.89; s			
4C–P_2	8.85; s	134.2	–	H-4C
4aA	–	111.7	H-6A	–
5A	–	156.6	H-6A; H-4C	–
6A–P_1	6.64; s			
6A–P_2	6.66; s	104.4	–	H-6A
7A	–	167.5	H-6A	–
8A	–	113.2	H-6A; CH; CH_3	–
8aA	–	153.9	H-4C	–
1′B	–	120.1	H-2′,6′B	–
2′,6′B–P_1	7.96; s			
2′,6′B–P_2	7.91; s	110.6	–	H-2′,6′B
3′,5′B	–	149.3	OMe; H-2′,6′B	–
4′B	–	145.8	H-2′,6B	–
OMe–P_1	4.01; s			
OMe–P_2	3.96; s	57.1	–	OCH_3
Flavanol moiety				
1′E	–	131.6	H-6′E	–
2′E	5.96; s	114.3	–	H-2′E
2F	4.34; d, 7.63	83.9	–	H-2F
3′E	–	145.8	H-5′E	–
3F	3.55–3.65; m	69.0	–	H-3F
4aD	–	102.4	H-4F	–
4′E	–	145.8	H-6′E; H-5′E	–
4αF	2.45; dd, *			
4βF	2.91; dd, 5.4/16.5	28.7	–	H-4F
5D	–	na	–	–
5′E	5.92; d, 7.90	119.3	–	H-5′E
6D	6.02; brs	na	–	–
6′E–P_1	6.25; d, 8.20			
6′E–P_2	6.31; d, 8.03	116.2	–	H-6′E
7D	–	na	–	–
8D	–	111.7	H-4F; CH_2	–
8aD	–	na	–	
Sugar moiety				
Gl-1	5.41, d, 7.69	103.1	–	H–Gl-1
Gl-2	3.73*	75.2	–	H–Gl-2
Gl-3	3.60*	78.0	–	H–Gl-3
Gl-4	3.58*	70.8	–	H–Gl-4
Gl-5	3.70*	78.3	–	H–Gl-5
Gl-6a	3.94*	62.1	–	H–Gl-6a
Gl-6b	4.04*	–		
Propyl bridge				
CH	5.31–5.34 s	34.5	–	CH
CH_2	2.41 m			
CH_2	2.11 m	26.7	–	CH_2
CH_3	0.95; t 7.2	14.0	–	CH_3

Key: P_1 and P_2 – isomers; * – unresolved; s – singlet; bs – broad singlet; m – multiplet; d – doublet; dd – double doublets; na – not attributed.

one proton at 5.35 ppm, whereas the two –CH_2 protons are non equivalent as they resonate as two multiplets at 2.41 and 2.11 ppm, integrating one proton each, and the –CH_3 group resonates as a triplet integrating three protons at 0.95 ppm. The presence of proton H-4C (anthocyanin moiety) proves that the alkyl group is not linked to this position. Likewise, the alkyl group is not linked to position 4 of ring F of the flavanol moiety, since protons H-4αF and H-4βF were easily assigned through the characteristic AMX spin system. Moreover,

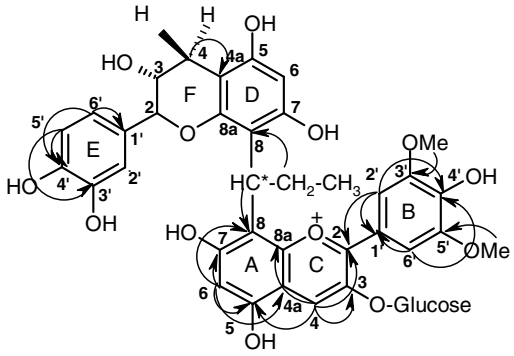

Fig. 2. Structure proposed for pigments P1 and P2 showing the long distance ^{1}H–^{13}C correlations found in the HMBC spectrum.

a ^{1}H–^{13}C correlation found in the HMBC spectrum between protons $–CH_2$ and carbons 8A (anthocyanin moiety), and between protons –CH and $–CH_3$ from the alkyl bridge and 8D (catechin moiety) proves that the alkyl group is linked through the position 8 of both anthocyanin and flavanol moieties (Fig. 2).

3.6. Kinetics

The formation of new coloured pigments resulting from the condensation reaction between mv3gl and cat, mediated by different aldehydes (acetaldehyde and propionaldehyde), is dependent on the pH of the medium. Two different pH values were tested (1.5 and 3.2).

According to the formation mechanism already proposed in the literature, the reaction involves first the protonation of the aldehyde with the formation of the respective carbocation ($R–HC^{+}OH$), followed by addition to a nucleophilic position of the flavanol, most likely at carbon C8, which can be explained by the higher negative ground state charge at this position, as previously reported (Bendz, Martensson, & Nilsson, 1967). A dehydration of the resulting protonated adduct occurs, yielding a new carbocation, which suffers a nucleophilic attack from the anthocyanin. Low acidic media could prevent the protonation of the aldehyde and consequently react with catechin to a lesser extent, thus explaining the low condensation rate observed at pH 3.2 comparatively to pH 1.5.

At pH 1.5, the anthocyanin is almost totally present in its flavylium cation form comparatively to pH 3.2 (pH close to the one found in wine) where the anthocyanin flavylium form is supposed to be partially displaced into its quinonoidal form and above all into the colourless hemiacetal form (Brouillard & Delaporte, 1977). Nevertheless, the reaction might not be strongly affected by the form in which the anthocyanin is present (Rivas-Gonzalo et al., 1995).

The reactivities of the different aldehydes led to differences in the rate of the pigment formation, easily observed from the curves represented in Fig. 3 at pH 1.5, in which we can observe that the maximum concentration of pigments A1 + A2 (mv3gl-8-ethyl-8-cat) was obtained in 24 h, whereas pigments P1 + P2 (mv3gl-8-propyl-8-cat) took about 72 h to reach maximum concentration. Acetaldehyde was found to be more reactive than propionaldehyde at both pH 1.5 and 3.2, although the reactivity differences between these aldehydes was not so significant at pH 3.2 (data not shown).

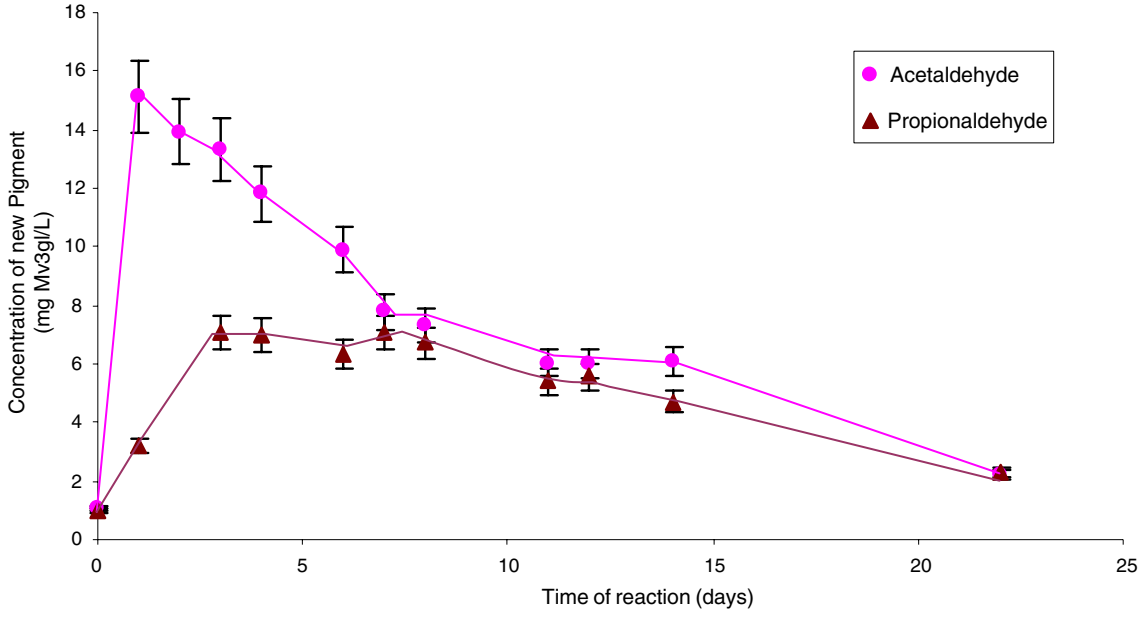

Fig. 3. Monitoring of new pigment formed in model solutions (pH 1.5) in the presence of acetaldehyde and propionaldehyde. The concentration of new pigment is expressed in mg of mv3gl per litre.

After reaching their maximum level at pH 1.5, the rate of degradation of pigments A1 + A2 was shown to be higher than for pigments P1 + P2. Thus, mv3gl-8-propyl-8-cat adducts are formed at a lower rate but they remain stable for a longer period of time than to mv3gl-8-ethyl-8-cat.

The higher reactivity shown by acetaldehyde might be explained by a higher stabilization of the intermediate carbocation (CH_3–$^+$CH–OH) by a methyl group that promotes a stronger hyperconjugation effect than the ethyl group (CH_3–CH_2–$^+$CH–OH) resulting from propionaldehyde (Chapman & Shorter, 1978). On the other hand, the propyl group is responsible for a higher steric hindrance, that might reduce the formation of the respective adduct (Pissarra et al., 2003).

4. Conclusion

The present work shows that aldehydes other than acetaldehyde may contribute to the formation of new pigments, especially in young red wines. Thus, and especially for the Port wine industry, the proper knowledge of aldehyde composition of the commercial wine spirit could be an important factor to better stabilize wine colour. Besides the aldehyde composition, its availability and the reaction pH could favour the appearance of new pigments with different chromatic features. This new pigment family (anthocyanin-alkyl-flavanol) seems to have an important role in the initial stages of red wine ageing.

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