

The effect of pH on the formation of coloured compounds in model solutions containing anthocyanins, catechin and acetaldehyde

by

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S u m m a r y : The qualitative and quantitative effect of pH on the formation of new coloured compounds from the interaction between malvidin 3-glucoside, (+) catechin and acetaldehyde was studied. Five different solutions were prepared at pH 2, 3, 3.7, 4 and 5, and the concentration of the reactants and newly formed compounds was monitored by HPLC. In all mixtures there was a decrease in the concentration of malvidin 3-glucoside and catechin, coupled with the formation of two principal new compounds thought to be dimers (labelled A and B), linked at different positions, and several minor compounds. The rate of loss of the reactants and the longevity and concentrations of A and B varied according to pH, with the greatest amounts and most rapid formation of these compounds occurring at pH 2. The catechin concentration still decreased when there was no malvidin 3-glucoside remaining. This observation indicated that further polymerisation reactions were occurring, involving catechin molecules only, or catechin and coloured polymers.

When cyanidin 3-rutinoside was used instead of malvidin 3-glucoside, similar reactions were observed, although new coloured compounds were formed more slowly.

K e y w o r d s : model wines, anthocyanins, catechin, acetaldehyde, pH, HPLC.

Introduction

The chemistry underlying the colour changes observed as red wines mature has been the subject of numerous papers over the years and has been reviewed recently by MACHEIX *et al.* (1990). The role of anthocyanins and flavan 3-ols in these reactions has been studied (TIMBERLAKE and BRIDLE 1976) and the additional presence of acetaldehyde is known to produce rapid changes in colour hue and intensity, *via* an acid-catalysed BAEYER reaction (SINGLETON *et al.* 1964, TIMBERLAKE and BRIDLE 1976, ROGGERO *et al.* 1987). This effect is particularly apparent during the early stages of port wine maturation (BAKKER and TIMBERLAKE 1986), since these wines generally contain higher concentrations of acetaldehyde than table wines. The increase in colour observed during this latter process is due to the formation of new purple-coloured pigments, which were studied in model by BAKKER *et al.* (1993). These authors reported two new compounds showing absorbance maxima at 544 nm, which were tentatively identified as dimers from the polymerisation of malvidin 3-glucoside and catechin linked by acetaldehyde.

The aim of the present work was to establish the quantitative and qualitative effects of pH on this reaction to find the conditions of maximum formation of the new pigments, thus enabling their ready preparation for future full characterisation. The major anthocyanin occurring in red wines, malvidin 3-glucoside, was used in our studies, although the availability of cyanidin 3-rutinoside also made it possible to study this reaction using a non-wine anthocyanin.

Materials and methods

P r e p a r a t i o n o f s o l u t i o n s : Potassium hydrogen tartrate buffer (0.02 M, pH 3.7), containing 10 % ethanol (v/v) was used as a model wine base. The reactants were dissolved in buffer to give the following final concentrations: 0.12 mg/ml of malvidin 3-glucoside (0.24 mM), 0.69 mg/ml of catechin (2.4 mM) and 0.5 % of acetaldehyde (v/v).

The pH 3.7 buffer mixtures were adjusted to give four other pH values, by addition of Na₂CO₃ (pH 4 and 5), tartaric acid (pH 3) and HCl (pH 2). Reaction mixtures (20 ml) were kept in screw-top vials and stored in the dark at room temperature. The pH value was measured at each analysis.

H P L C a n a l y s i s : The samples were filtered and analysed (in duplicate) by HPLC (Hewlett-Packard 1090 M Series II) equipped with a diode array detector, an auto-injector (25 µl) and a ODS-Hypersil reversed-phase column (100 x 2.1 mm, particle size 5 µm), at 40 °C. Elution was performed with acidified water (0.6 % perchloric acid) and methanol, flow rate 0.3 ml/min, using a linear gradient starting with 20 % methanol increasing to levels of 52 % at 32 min, 98 % at 32.5 min and 98 % at 34 min returning to 20 % at 35 min. Chromatograms were recorded at 280 and 520 nm.

Malvidin 3-glucoside and catechin were used as standards as previously reported (BAKKER *et al.* 1993).

Results and discussion

A general decrease in the concentrations of malvidin 3-glucoside and catechin occurred at all pH values, concurrent with the formation of two principal new coloured compounds, labelled A and B. Changes were monitored by HPLC and it was necessary to make frequent measurements, particularly at the lower pH values due to the speed of reaction. Fig. 1 is a typical HPLC chromatogram showing imalvidin 3-glucoside and the two new dimers.

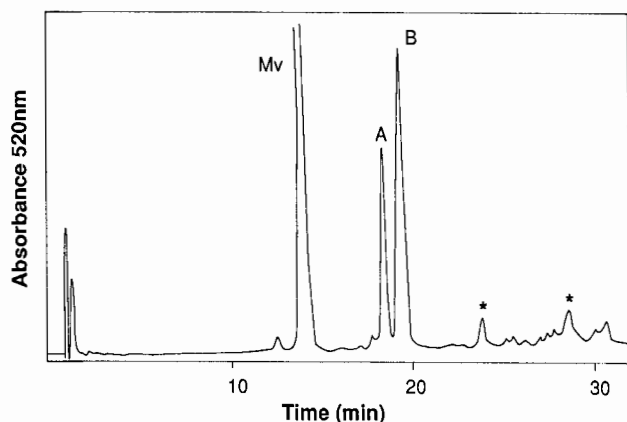


Fig. 1: HPLC chromatogram showing malvidin 3-glucoside (Mv), compounds A and B and later eluting polymeric pigments (*).

At pH 2 (Fig. 2) the formation of compounds A and B began after 1 h, reaching a maximum after 10 h (12.8 and 20.9 mg/l, respectively). A and B then began to decrease and were not detectable after 8 d (B) and 10 d (A) (Fig. 3, pH 2.0). The malvidin 3-glucoside concentration was too small to measure after 2 d. Samples at pH 3 (Fig. 3, pH 3.0) showed a rapid decrease of malvidin 3-glucoside during the first 2 d, by which time the levels of compounds A and B had reached a maximum (10.8 and 15.8 mg/l, respectively). After the 3rd day no malvidin 3-glucoside was measurable and A and B had both disappeared after 5 d.

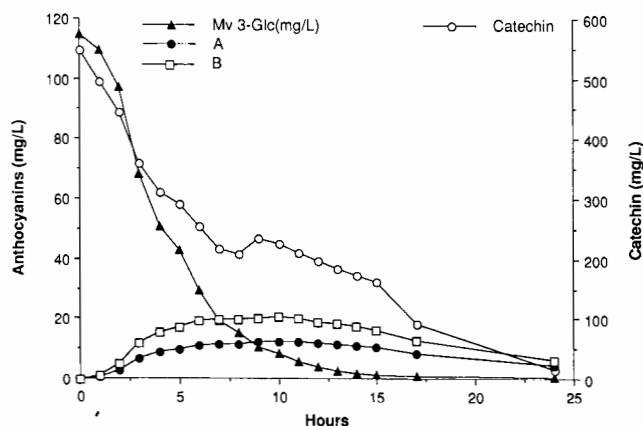


Fig. 2: Changes in the concentrations of malvidin 3-glucoside, compounds A and B and catechin, at pH 2 during the first 24 h.

At pH 3.7 and pH 4.0 (Fig. 3) malvidin 3-glucoside decreased more slowly and was not detectable after 5 d (pH 3.7) and 10 d (pH 4.0). Compounds A and B increased to a maximum (7.5 and 12.8 mg/l respectively, at pH 3.7

and 6.4 mg/l and 10.7 mg/l respectively, at pH 4.0) by the 2nd day, then decreasing and disappearing by day ten.

Different behaviour was shown by the pH 5.0 solution (Fig. 3), where malvidin 3-glucoside decreased much more slowly and disappeared after 12 d. However, A and B reached their maximum levels on the 5th day (1.5 and 2.4 mg/l, respectively) and disappeared after 8 d (A) and 10 d (B).

The results show that in general, the formation of new compounds A and B occurs most rapidly at the lowest pH values accompanied by the most rapid losses of malvidin 3-glucoside. Differences in the speed of reaction according to pH may be related to the differences in the theoretical proportion of flavylum cation (AH⁺) present at each pH (TIMBERLAKE and BRIDLE 1980). Thus the higher the concentration of the flavylum cation the faster the reaction; i. e. lowest pH (pH 2 = 80 % AH⁺) has the fastest reaction, while highest pH (pH 5 = 0 % AH⁺) has the slowest reaction. It also appears that the amount of A and B formed at various pH values is related to the concentration of this cation. Thus pH has a double effect, influencing both the speed of formation and the amounts of compounds A and B. The greatest amounts of compounds A and B are formed after 10 h at pH 2; their stability however, was less dependent of pH. The short lives of A and B especially at pH 2 and pH 3, may be ascribed to the speed of the continuing polymerisation reaction in the presence of the remaining catechin (JURD 1969), (without or with the involvement of acetaldehyde) - even though there was no malvidin 3-glucoside available to react at this stage. The catechin concentrations (Fig. 4) showed a gradual decrease at all pH values disappearing most rapidly at pH 2 (not detectable after 2 d) and slowest at pH 5 (76 % loss by day 12).

Although compounds A and B were formed at all pH values, B was invariably present at the higher concentration. It has been proposed that A and B are isomers (BAKKER *et al.* 1993, TIMBERLAKE and BRIDLE 1976), with linkage occurring at positions 6 or 8 of the flavonoid ring of the anthocyanin. The option for their formation *via* position 8

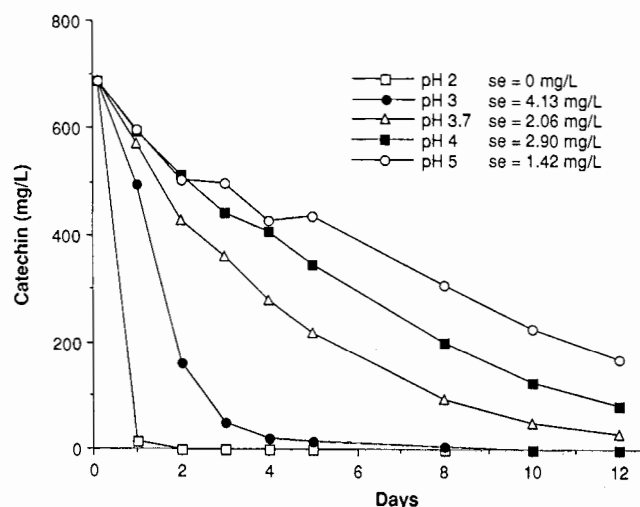


Fig. 4: Changes in the concentration of catechin with time, according to pH, with pooled within sample residual standard errors (se) for each pH.

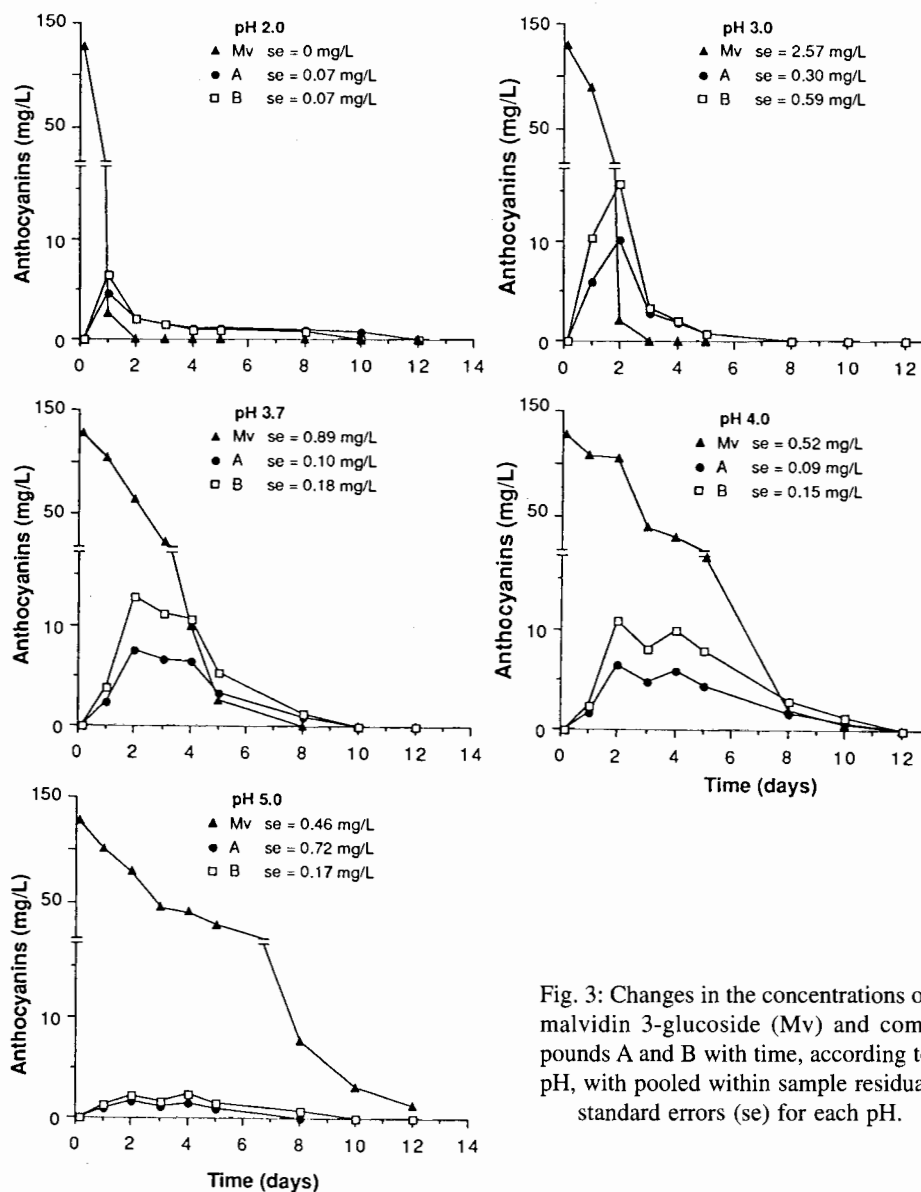


Fig. 3: Changes in the concentrations of malvidin 3-glucoside (Mv) and compounds A and B with time, according to pH, with pooled within sample residual standard errors (se) for each pH.

is more favourable than *via* position 6, by analogy with simple flavylum salt behaviour (TIMBERLAKE and BRIDLE 1976) and also because of the higher net negative ground state charge of position 8 (BENDZ *et al.* 1967). Hence, it is possible that B is a dimer linked *via* position 8 of malvidin 3-glucoside and A is linked *via* position 6.

In addition to A and B, other coloured peaks appeared on the first day at pH 2 and pH 3 and on the 2nd day at the higher pH values. Since these peaks were present at very low concentrations (Fig. 1), their spectra could not be measured. It is probable that they represent larger polymers formed by the addition of extra molecules of catechin in the presence of acetaldehyde, but without the involvement of malvidin 3-glucoside. The ratios of the rates of loss of catechin and malvidin 3-glucoside are shown below as averages according to pH:

pH	catechin/malvidin 3-glucoside
2.0	5.93
3.0	6.72
3.7	5.95
4.0	7.46
5.0	7.38

The rate of disappearance of catechin was always greater than the rate for malvidin 3-glucoside, indicating that in addition to the above reaction, catechin may also be undergoing some self-polymerisation (JURD 1969), evidenced by some new peaks appearing in the 280 nm HPLC chromatogram (not shown) eluting after catechin itself.

At the end of the experiment the solutions were still coloured at all pH values, although there were no discrete compounds detectable by HPLC. This effect may be explained by the fact that the dimers A and B polymerise further to higher molecular weight coloured compounds both in the presence of acetaldehyde (HASLAM and LILLEY 1988) or without the intervention of acetaldehyde (SOMERS 1971). These polymers may eventually become so large as to be insoluble, indeed, we observed some precipitation in samples at all pH values.

Similar behaviour was observed when malvidin 3-glucoside was replaced by cyanidin-3-rutinoside over the same range of pH (data not shown). Generally, the reaction was always slower and the maximum levels of A and B were formed at pH 2 and pH 3 at day 3 and at day 18 for the other pH values. Compound B was again always present

in a higher proportion, but after 45 d a quantifiable amount of cyanidin 3-rutinoside remained, as well as compounds A and B. In contrast with malvidin 3-glucoside, at pH 5 the degradation of cyanidin 3-rutinoside was faster than at pH 4 or 3.7. These observed differences when another anthocyanin moiety is used, are attributable to differences in the ring substitution patterns. However, A and B were better separated from each other by HPLC using malvidin 3-glucoside, hence this is the anthocyanin of choice for scaling-up their preparation for confirmation of their structures.

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