Model wine solutions: Colour and composition changes during ageing

by

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S u m m a r y : The interaction between malvidin 3-glucoside, the main anthocyanin in red wine made from *Vitis vinifera* grapes, and (+)-catechin and the effect on this interaction of ferric ions and acetaldehyde was examined. In the models not containing acetaldehyde losses of malvidin 3-glucoside were observed, but there were only negligible losses of catechin; no new compounds were observed. In the presence of acetaldehyde the formation of new compounds was determined by high performance liquid chromatography; this formation coincided with rapid losses in the concentrations of malvidin 3-glucoside and catechin. A molecular ion at m/z 809 was determined by FAB MS, corresponding to a dimer consisting of malvidin 3-glucoside linked to catechin by an acetaldehyde bridge, according to a mechanism previously suggested by TIMBER-LAKE and BRIDLE (1976).

Concurrent with the losses in anthocyanins, qualitative and quantitative changes in visible colour were also observed, consisting of changes in the wavelength of maximum absorbance (λ_{max}) and in maximum absorbance intensity (A_{max}). Models containing malvidin 3-glucoside plus catechin or catechin plus ferric ions showed a marked decrease in their λ_{max} from 525 nm to 440 nm; there was little net effect of the ferric ions on these changes. The model containing acetaldehyde showed a large increase in A_{max}, while the λ_{max} showed a bathochromic shift from 524 nm to 557 nm; colour decreased after achieving a maximum and the λ_{max} decreased slightly.

Changes in colour monitored by measuring hue angle, chroma and L* value are also reported.

K e y   w o r d s : anthocyanins, wines, acetaldehyde, ageing, colour measurements, HPLC

Introduction

The colour of a red wine is one of its most important and obvious quality indicators. During maturation, red wine colour changes from bright red to a reddish-brown tint, due to the anthocyanins extracted from the grape skins during fermentation forming polymeric pigments, by condensation with other flavonoid compounds (SOMERS 1971; RIBÉREAU-GAYON 1982; RIBÉREAU-GAYON *et al.* 1983). Studies by SOMERS (1971) suggest that direct condensation of anthocyanins also occurs, at carbon 4, probably with procyanidins. Acetaldehyde has long been known to affect the polymerisation reaction; JOSLYN and COMAR (1941) discussed some evidence that wine colour changes involved a combination of acetaldehyde with pigments rather than direct oxidation of the pigment. The involvement of acetaldehyde in polymerisation reactions was further demonstrated when TIMBERLAKE and BRIDLE (1976) carried out a detailed study of polymerisation reactions in model wine solutions; they proposed the formation of highly coloured intermediates containing anthocyanin and catechin linked by CH_3CH bridges. ROGGERO *et al.* (1987) confirmed these findings, and reported the formation of two highly coloured compounds, the ratio of which depended on whether catechin or epicatechin was used as the phenolic compound.

BARANOWSKI and NAGEL (1983) studied in model wine systems the kinetics of malvidin 3-glucoside condensation in the presence of catechin and acetaldehyde, and

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found that when a molar excess of acetaldehyde is present condensation proceeds rapidly and produces two to three times as much polymeric material as when acetaldehyde is present in amounts equimolar to malvidin 3-glucoside and catechin.

However, the properties of these highly coloured compounds are not clearly documented. In order to study further the colour changes and reactions during maturation of wines, we investigated these reactions in model solutions. We studied interactions between malvidin 3-glucoside, the main anthocyanin in red wine made from *V. vinifera* grapes, and (+)-catechin; the role of acetaldehyde and ferric ions was also investigated. The latter is known to play a role in the oxidation and browning of phenolic compounds (MARKAKIS 1982). High performance liquid chromatography (HPLC) was used to measure the concentration of anthocyanins and observe the formation of new compounds. Colour changes were monitored using spectrophotometry and tristimulus measurements.

Materials and methods

M a t e r i a l s : Anthocyanins were extracted from Touriga Nacional grape skins with methanol containing 3 % (v/v) formic acid and purified by preparative HPLC. A reversed-phase column (5 µm Spherisorb Hexyl, 240 mm × 16 mm) was used. The pigments were eluted with 0.6 % aqueous perchloric acid and methanol, using the following gradient: from 30 % to 55 % methanol in 44 min, to 100 % methanol in 1 min, hold at 100 % methanol for 10 min before returning to 30 % methanol over 3 min. The flow rate was 4 ml/min and the injection volume was 1 ml. The fraction containing malvidin 3-glucoside (93 % pure) with small amounts of two other anthocyanins was collected, concentrated (rotary evaporator and Sep-Pak C_{18} cartridge) and dried under vacuum.

P r e p a r a t i o n   o f   s o l u t i o n s : Aqueous potassium hydrogen tartrate (Merck) (0.02 M; pH = 3.7) containing ethanol (10 % v/v) was used as a model wine base. Other components were dissolved in this buffer to give the following final concentrations: malvidin 3-glucoside 0.25 mM (0.12 mg/ml), (+)-catechin (Sigma) 1.03 mM (0.3 mg/ml), acetaldehyde (Merck) 0.5 % (v/v) and ferric nitrate (Analab) to give 5 mg/l Fe^{3+}.

Four model wine solutions were prepared: (i) malvidin 3-glucoside alone (control), (ii) malvidin 3-glucoside and catechin, (iii) malvidin 3-glucoside, catechin and acetaldehyde, (iv) malvidin 3-glucoside, catechin and ferric ions. These solutions were kept in four sets of stoppered vials (3 ml sample in a 7 ml vial), and allowed to react in the dark at room temperature. Samples from separate vials were analysed periodically in duplicate by HPLC and spectrophotometry.

H P L C : A Hewlett-Packard 1090M model with an auto injector (25 µl injection volume) and a diode array detector recording at 280 and 520 nm was used to measure the anthocyanin concentrations. A reversed-phase ODS Hypersil column (100 mm × 2.1 mm; particle size 5 µm) at 40 °C was used, with a flow rate of 0.3 ml/min. Using 0.6 % aqueous perchloric acid and methanol as eluants the following linear gradient was used: in 30 min from 20 % to 50 % methanol, in 0.5 min to 98 % methanol, hold for 2 min at 98 % methanol to wash the column and then return to the initial conditions (20 % methanol) to re-equilibrate for 10 min.

Malvidin 3-glucoside (93 % pure) was used as an external standard to quantify anthocyanins in the model solutions. The absorbance (A) of this standard (16 mg/l in M HCl) was measured at 520 nm in a 10 mm glass cell and the concentration (c) of

anthocyanins expressed as malvidin 3-glucoside chloride (molecular weight 529) was calculated using a molar absorptivity value of 28000 (NIKETIC-ALEKSIC and HRAZDINA 1972); thus c (mg/l) = 18.9 × A.

The concentrations of (+)-catechin were monitored at 280 nm by the same gradient elution programme and quantified using an external standard of (+)-catechin in water (23.4 mg/l).

Colour measurements: These were made using a Philips PU8740 spectrophotometer and glass cells of 2 mm path length. At the same time L*a*b* values were calculated using illuminant D65 and a 10° observer according to the CIELAB 76 convention (McLAREN 1980). The L* value is a measure of lightness, from completely opaque (0) to completely transparent (100), a* is a measure of redness and b* of yellowness (-b* blueness). Hue angle (H) is calculated from H = arctanb*/a*(°) and chroma (C) as C=[(a*)²+(b*)²]⁰·⁵. Changes in C reflect a bias towards the dominant colour component (a* or b*). The hue angle has previously been reported to correlate well with the sensory assessment of brownness in wines, while the L* value correlated well with the intensity assessment (BAKKER and ARNOLD 1993). All measurements were done in duplicate; the mean value is reported in the data.

F A B M S: The anthocyanin sample was dissolved in a few µl M HCl, and added to the clean copper tip of the FAB insertion probe and dried in the vacuum lock. Glycerol (1 µl) was mixed with the dried sample on the probe, which was then inserted in the FAB source. Spectra (+ve ion) were obtained using a MS 9/50 mass spectrometer (Kratos Analytical Ltd.), operated at a resolution of 1000, with the gun producing a beam of xenon atoms of approximately 8 kV average translatable energy.

Results and discussion

On storage, all solutions exhibited a logarithmic decrease in total anthocyanin concentration, as shown in Fig. 1. The linear loss of anthocyanins in all four model mixtures indicates a first order reaction with respect to this loss, confirming the find-
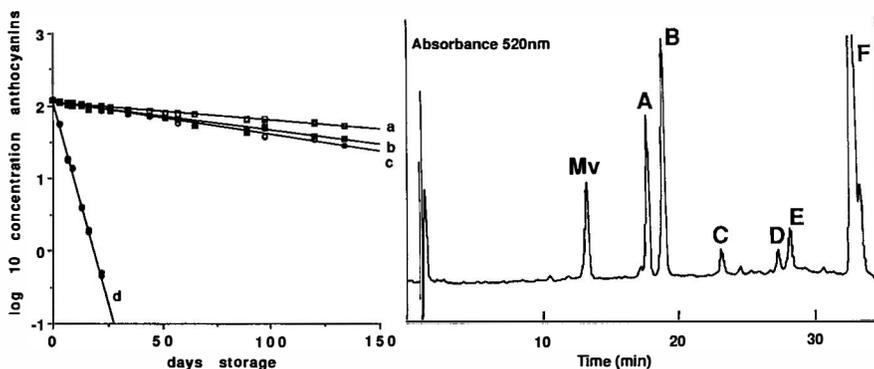

Fig. 1 (left): Logarithmic changes in malvidin 3-glucoside concentration in model wine systems. a: control, b: anthocyanins+catechin, c: anthocyanins+catechin+ferric ions, d: anthocyanins+catechin+acetaldehyde. The following equations were calculated using stepwise linear regression; the standard error is shown in brackets and the Multiple R is given at the end of the equation:
a: Y = −0.0025X(5.8E−5) + 2.0467(0.0036); 0.991 — b: Y = −0.0039X(1.04E−4) + 2.3090(0.0064); 0.989
c: Y = −0.0046X(9.3E−5) + 2.0531(0.0057); 0.993 — d: Y = −0.1106X(0.0015) + 2.0753(0.0189); 0.999.

Fig. 2 (right): Chromatogram of model wine containing anthoycanins + catechin + acetaldehyde, recorded after 9 d, showing newly formed compounds A, B, C, D, E and F.

ings of BARANOWSKI and NAGEL (1983). The control sample showed the slowest rate of anthocyanin loss, while samples containing catechin and catechin plus ferric ions lost anthocyanins at a faster rate. However, the loss was greatly accelerated when acetaldehyde was added to the anthocyanin and catechin, even though the decrease in anthocyanins was accompanied by the formation of six new coloured compounds.

The reaction rates k (day^{-1}) are shown in Tab. 1 and compared with the rates reported by BARANOWSKI and NAGEL (1983). They used equimolar concentrations of malvidin 3-glucoside, d-catechin and a fivefold molar excess of acetaldehyde. The four fold molar excess of catechin used in our models gave a greater k value with respect to the disappearance of anthocyanins for the malvidin 3-glucoside and (+)-catechin mixture. An increase in concentration from 110 mg/l acetaldehyde to 5000 mg/l in model wine led to a 13 fold increase in reaction rate with respect to anthocyanins for this mixture.

Table 1

Reaction rates with respect to the disappearance of total anthocyanins in model wine systems

Mixture	k (day^{-1})	literature value[*] k (day^{-1})
Anthocyanin	5.66×10^{-3}	6.31×10^{-3}
Anthocyanins and catechin	8.88×10^{-3}	6.74×10^{-3}
Anthocyanins, catechin and ferric ions	10.50×10^{-3}	
Anthocyanins, catechin and acetaldehyde	254.76×10^{-3}	19.00×10^{-3}

[*] Data calculated from Baranowski and Nagel (1983), calculated with respect to malvidin 3-glucoside.

Tab. 2 shows that the rapid decrease in the anthocyanin concentration is accompanied by the formation of five new peaks. After only 3 d, the anthocyanin concentration was more than halved, and almost 40 % of the remaining concentration was due to the new compounds. The transient nature of the new peaks, lasting no more than 22 d, indicates that they are intermediates in the polymerisation reaction. Peaks A and B showed the highest concentration when analysed after 3 d storage, even though

Table 2

Concentrations of anthocyanins (mg/l) determined during storage of model wine containing malvidin 3-glucoside, catechin and acetaldehyde. Peaks A, B, C, D and E show the concentrations of new peaks formed during storage

Storage time (days)	Anthocyanin concentration (mg/l)	Peonidin 3-glucoside (mg/l)	Malvidin 3-glucoside (mg/l)	Concentration new peaks				
				A	B	C (mg/l)	D	E
0	125.3	7.4	117.4		0.6			
3	54.6	1.5	31.5	7.4	11.7	0.8	0.7	1.1
7	18.9	0.0	4.5	3.8	6.7	1.0	0.7	1.7
9	14.0		2.5	3.4	5.3	0.8	0.6	1.3
13	4.0		0.4	0.8	1.3	0.4	0.4	0.7
16	2.6		0.2	0.5	0.9	0.3	0.3	0.6

their concentrations continued to decrease; together they accounted for more than 50 % of the anthocyanins in the mixture. Peaks C, D and E were present in much lower concentrations, but formed an increasingly large percentage of the total.

A typical chromatogram showing the new compounds and their retention times is shown in Fig. 2 (see p. 113). Peaks A and B (retention times were 17.5 and 18.7 min, respectively), were both more violet than malvidin 3-glucoside and showed their λ_{max} at 544 nm (Fig. 3). Peak A has a more pronounced shoulder at 460 nm than peak B. The concentrations of peaks C, D and E were too low to record a spectrum. After 7 d, compound B was the main pigment (35.3 % of the total concentration), until after 9 d a new bluer compound (F), eluting at 32.8 min (λ_{max}= 550nm) appeared (Fig. 3). After 26 d this was the only compound detected at 520 nm. Since this compound eluted during the wash-off procedure of the column, it is suspected that F consists of a mixture of fairly immobile polymerised material, and hence has not been listed in Tab. 2.

The changes in the catechin concentrations are shown in Fig. 4. The models containing malvidin 3-glucoside and catechin without acetaldehyde show very little change in catechin concentrations. This indicates that no dimers were formed by direct condensation. In contrast, BISHOP and NAGEL (1984) reported the formation of a colourless bicyclic condensation product from the condensation of malvidin 3,5-diglucoside and (+)-catechin, after 7 d storage in 10 mM HCl at 38 °C. However, our model containing malvidin 3-glucoside, catechin and acetaldehyde showed a rapid decrease in the catechin concentration. These results indicate that in the absence of acetaldehyde the observed losses in anthocyanin concentrations, as discussed for Fig. 1, may be due to the instability of the pigment itself, rather than any condensation reaction with catechin. However, the rapid loss of both the anthocyanin and the catechin concentration in the presence of acetaldehyde confirms that a condensation reaction is occurring between malvidin 3-glucoside and catechin, mediated by acetaldehyde.

Further proof of this reaction giving a dimer was obtained by the determination of its mass weight. A sample containing a mixture of peaks A and B was subjected to positive ion FAB MS and a molecular ion was determined at m/z 809. This corresponds exactly to a dimer consisting of malvidin 3-glucoside linked to catechin by an acetaldehyde bridge, according to a mechanism previously suggested by TIMBERLAKE
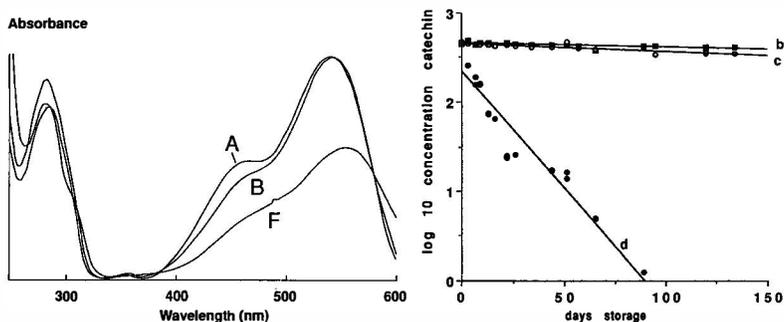

Fig. 3 (left): Absorbance spectra of peaks A and B (A_{max} 544 nm) and peak F (visible A_{max} 550 nm).

Fig. 4 (right): Logarithmic changes in (+)-catechin concentration in model wine systems. b: anthocyanins+catechin, c: anthocyanins+catechin+ferric ions, d: anthocyanins+catechin+acetaldehyde. The following equations were calculated using stepwise linear regression; the standard error is shown in brackets and the Multiple R is given at the end of the equation.
b: Y = –0.0004X(6.9E–5) + 2.658(0.0042); 0.736 — c: Y = –0.0009X(9.2E–5) + 2.654(0.0054); 0.878 — d: Y = –0.0262X(0.0017) + 2.351(0.0614); 0.957.

and BRIDLE (1976). They proposed a number of substitution possibilities at both the catechin and the malvidin 3-glucoside molecule. Hence one or both of these newly formed compounds may correspond to this molecular ion, since A and B are probably isomers. Further studies are necessary to determine the substitution sites.

Not all the polymerised compounds remained in solution. A light violet precipitate was observed in samples containing acetaldehyde from the third day of storage and this increased considerably after the 9th day. These results are in agreement with previous work (ROGGERO *et al.* 1987), although, in our model system, the reaction proceeded more quickly. By the 16th day all malvidin 3-glucoside had disappeared, whereas ROGGERO *et al.* (1987) still had just over 40 % of the original concentration remaining. This difference in reaction rate is probably due to the greater molar excess of acetaldehyde in our model wine.

During these reactions, quantitative and qualitative changes in visible colour were also observed. In the control model only small changes in absorbance values were measured. The other three models showed changes in both λ_{max} and A_{max}. Fig. 5 illustrates changes as follows: models containing additionally catechin or catechin plus ferric ions showed a marked decrease in their λ_{max} from 525 nm to 440 nm, occurring after 57 and 34 d, respectively. Both models showed a slow decrease in absorbance at 525 nm, concurrent with an increase at 440 nm, the new λ_{max}. There was little net effect from the ferric ions on these changes. The model containing acetaldehyde exhibited a 68 % increase in A_{max} during the first 7 d of storage, reaching a plateau at day 7 lasting till day 16, thereafter decreasing to the initial value. The λ_{max} showed a bathochromic shift from 524 nm to 557 nm; on further loss of colour after 37 d λ_{max} started to decrease, reaching 545 nm after 120 d at the end of the experiment.
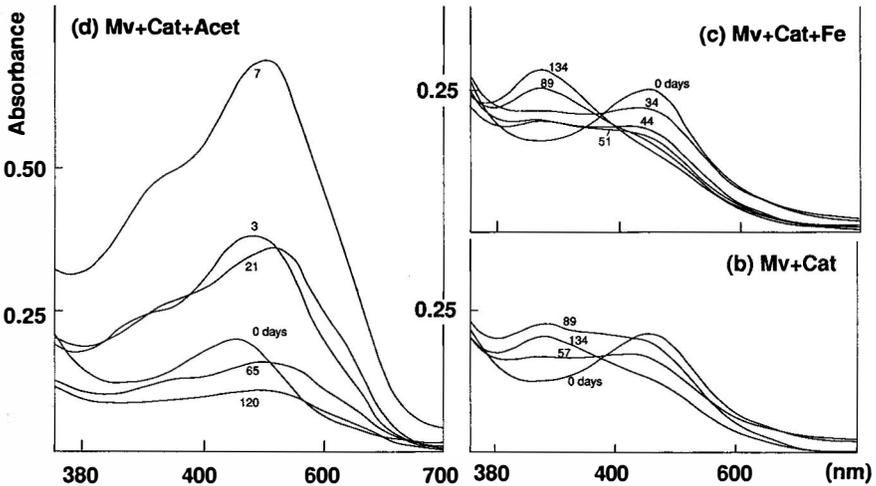

Fig. 5: Absorbance spectra changes with time for models b: anthocyanins+catechin, c: anthocyanins+catechin+ferric ions, d: anthocyanins+catechin+acetaldehyde.

There are considerable changes in the hue angle concurrent with the observed changes in A_{max} and λ_{max} as shown in Fig. 6. Control samples showed a small increase in hue angle of 20° over 134 d, while models containing catechin and catechin with ferric ions show a much greater increase in hue angle, indicating an increase in brownness. The ferric ions appear to accelerate the browning process. In contrast, the hue angle decreased rapidly in the model containing acetaldehyde, indicating an increasingly violet hue.

The changes in L^* values during storage for the sample containing anthocyanins, catechin and acetaldehyde is shown in Fig. 7. The sample became rapidly darker with L^* reaching its minimum value (60.6) at day 7; after day 16 it became lighter, with the L^* value increasing and reaching the initial value again. In contrast the other samples showed little changes in L^* (not shown); the averages were as follows: control 89.6 (range 88.9 to 91.4), anthocyanins and catechin 89.3 (range 88.1 to 90.6) and anthocyanins, catechin and ferric ions 88.7 (range 86.0 to 90.7).
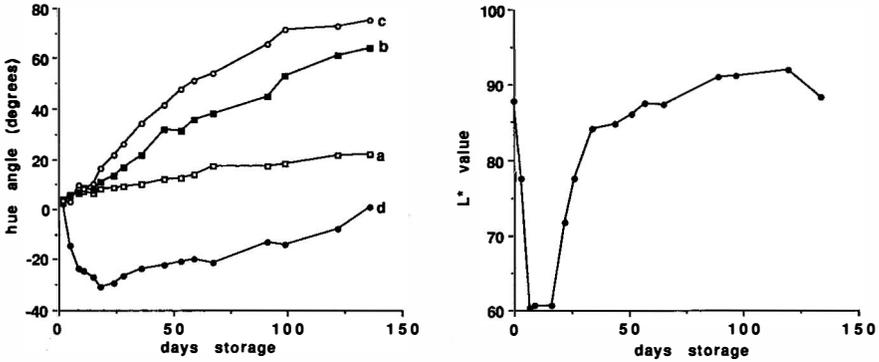

Fig. 6 (left): Changes in hue angle (degrees) in models a: control, b: anthocyanins+catechin, c: anthocyanins+catechin+ferric ions, d: anthocyanins+catechin+acetaldehyde.

Fig. 7 (right): Changes in L^* values in model containing anthocyanins+catechin+acetaldehyde.

Fig. 8 shows the changes in chroma in all the model solutions with time. Chroma decreased in control samples, whereas in the samples containing (+)-catechin a decrease occurred during the first 65 d only, followed by a slight increase until the end of the experiment. Samples containing both (+)-catechin and ferric ions showed no change during the first 60 d of storage, thereafter a gradual increase continued until the end of the experiment. In samples containing acetaldehyde, a rapid increase in chroma was observed during the first 7 d of storage, indicating a major contribution of the b^* value to the colour observed. This was followed by a rapid decrease until after 25 d there was just a steady decline in chroma.
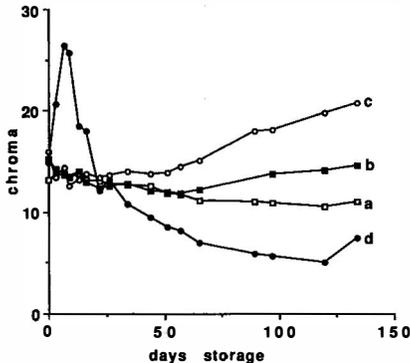

Fig. 8: Changes in chroma in models a: control, b: anthocyanins+catechin, c: anthocyanins+catechin+ferric ions, d: anthocyanins+catechin+acetaldehyde.

Acknowledgements

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