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# The effect of processing and other factors on the colour characteristics of some red wines

by

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## Der Einfluß der Weinbereitung und anderer Faktoren auf die Farbmerkmale von Rotweinen

Zusammenfassung. — In Long Ashton wurden aus demselben Lesegut von Cascade-Trauben (Seibel 13.053) Rotweine nach drei verschiedenen Verfahren — (a) Vergärung auf der Maische, (b) Maischeerhitzung, (c) Kohlensäuremazeration — hergestellt und in bestimmten zeitlichen Abständen analysiert. Der Wein nach (b) war viel intensiver gefärbt als derjenige nach (a); er enthielt jedoch weniger Anthocyan- und mehr polymere Farbstoffe. Der Wein nach (c) war am schwächsten gefärbt, obgleich er einen ähnlichen Anthocyangehalt aufwies wie nach (b) hergestellter Wein; außerdem war er stärker braun getönt. Die Unterschiede in der Rotfärbung wurden auf Schwankungen im physiko-chemischen Zustand der Anthocyane zurückgeführt und zahlenmäßig durch den Ionisationsgrad ausgedrückt. Zwischen der optischen Dichte der Rotweine und dem Ionisationsgrad ihrer Anthocyane bestand eine auffällig genaue lineare Beziehung.

Bei den Weinen aus Long Ashton und einigen jungen Beaujolais-Weinen aus dem Handel wurden die Faktoren untersucht, welche die Ionisierung der Anthocyane beeinflussen. Einwirkung von Luft verstärkte die Färbung, steigerte die Polymerisation der Anthocyane und erhöhte bei einigen kommerziellen Weinen, wohl durch Oxidation unbekannter farbloser Vorstufen, den tatsächlichen Anthocyangehalt. Ein Zusatz von Acetaldehyd war noch wirkungsvoller: Ein beträchtlicher Zuwachs ionisierter Anthocyane war begleitet von Anthocyanverlusten durch verstärkte Umwandlung in polymere Farbstoffe. Bei den Weinen aus Long Ashton überwog in den nach (b) hergestellten das Acetaldehyd gegenüber dem Bisulfit, wodurch wahrscheinlich die Färbung vertieft wurde.

Die unterschiedliche Ausprägung der Rotweinfärbung ist in erster Linie durch das angewandte Extraktionsverfahren bedingt, wobei Anthocyane und Phenole in verschiedener Weise miteinander reagieren. Im Verlauf der Gärung und Lagerung wird der Farbcharakter durch weitere Faktoren abgewandelt.

## Introduction

The colour of red wines and the changes that occur during the wine making and maturing processes have been reviewed recently (Singleton and Esau 1969, Somers 1972, Ribéreau-Gayon 1974) but have assumed greater prominence following the association of colour with overall quality of red wines (Somers and Evans 1974, Somers 1975). It seems that the colour of a red wine depends not only on its anthocyanin content but largely on the physico-chemical state of these pigments and their environment. The concept of degree of ionisation of anthocyanins, defined as the percentage ratio of anthocyanin colour at wine pH to that at pH less than 1.0, provides a numerical basis for a fuller understanding of changes in wine colour and their significance. We have used this concept and the analytical techniques described by Somers and Evans (1974) to study the differences between wines made at Long

Ashton from the same batch of grapes by three processing methods and the subsequent effects of storage and other treatments both on these and some commercial wines.

## Materials and Methods

### Preparation of wines

Small quantities of wines were made from one batch of grapes (Cascade — Seibel 13.053) divided into three portions as follows:

Wine A — Fermented on the skins: The grapes were cooled to 7 °C, de-stemmed and crushed with added $SO_2$ (50 ppm) appropriate to its pH. The pulp was held in a plastic bag, heavily inoculated with yeast after 12 h and punched down at intervals for 2 days. Care was taken to ensure that $CO_2$ was present from the start. The juice was pressed out, sugar added to sp. gr. 1090 and fermented to dryness at 10—15 °C under air locks. The wine was then treated as for a dry white wine.

Wine B — Thermovinification: Grapes were de-stemmed, crushed and weighed at ambient temperature and $SO_2$ (30 ppm) was added to the pulp. The pulp was rapidly heated to 60 °C and 0.4% w/w paper pulp added and slowly stirred in for 30 min. After cooling to 45 °C, the pulp was treated with a pectin-degrading enzyme and held for a further 30 min. The juice was pressed out, chilled to 15 °C, centrifuged, filtered, the sp.gr. increased to 1090, $SO_2$ (50 ppm) added and a yeast inoculum mixed in after 6 h. The juice was fermented to dryness and sulphited (50 ppm).

Wine C — Carbonic maceration: Weighed bunches of grapes were placed in a stainless steel vessel which was then filled with water (40 °C), containing $SO_2$ (200 ppm) at pH 3.5 (acidity adjusted with malic acid). When the temperature had cooled to 32—35 °C, the liquid was displaced completely with $CO_2$ and a small volume of depectinising enzyme added. The grapes were maintained at this temperature under $CO_2$ for 7 days, de-stemmed, pressed and the juice treated as for a white wine.

All the wines were finally pasteurised in bottles.

### Analytical procedure

The analyses were as described by Somers and Evans (1974). Wine colour can be regarded simply as the sum of two components, the colour of the monomeric anthocyanins and that due to the polymeric pigments. Their relative amounts can be estimated by using sodium metabisulphite which bleaches the former but not the latter. The visible spectrum of the wine was recorded without dilution and again 1 min after adding sodium metabisulphite solution (0.8 ml of 20% to 10 ml of wine). The optical density values (E) were re-calculated to those which would have been obtained in a 10 mm cell, as were all subsequent measurements. The wine colour density was then $E_{520nm} + E_{420nm}$, and the colour due to the polymeric pigments was the residual $E_{520nm}$ in the presence of sodium metabisulphite. The proportions of colour at wine pH due to monomeric anthocyanins and polymeric pigments respectively, were readily calculated. The tint (T) of the wine colour was the ratio $E_{420nm}/E_{520nm}$ (Sudraud 1958).

The total colour of the wine was measured at $E_{520nm}$ at pH $< 1$ (1.0 ml wine + 10.0 ml n HCl; measured after 4—6 h). The colour was again the sum of that due to monomeric anthocyanins and the polymeric pigments. Under these acid conditions, the contribution of the anthocyanins is a much greater proportion of the total colour, because the colour of the polymeric pigments is much less affected by pH.

The contribution of the polymeric pigments could not be obtained directly, since sodium metabisulphite is ineffective in acid, but was calculated as $5/3 \times E_{520nm}$ of the polymeric pigment at wine pH. Subtraction of this value from the total colour then gave $E_{520nm}$ due to the anthocyanins, which was converted to anthocyanin content using an $E^{1\%}_{10mm}$ value of 500 at 520 nm. The factor 5/3 for the polymeric pigments was assumed constant irrespective of the age of the wine examined. More recent work suggests that this may not be strictly true and that in reality the degree of ionisation of the older wines may be a little lower than those quoted in the "Results". However, the differences are likely to be small and in no way affect the overall conclusions.

Expressed in terms of proportion of total colour at $pH < 1$, the significance of the polymeric pigments appeared misleadingly low because they are much less coloured than the monomeric anthocyanins. It was considered more realistic to express them on a weight basis, however approximate. The $E^{1\%}_{520nm}$ values of the polymeric pigments vary from 10—44 according to solvent (Somers 1966, 1967, 1968). As a first simple approximation, we assumed a value of 50, i.e. one-tenth of the value for the monomeric anthocyanins, and calculated the polymeric pigment content accordingly.

It should be emphasised that this simple concept supposes a sharp distinction between monomeric and polymeric anthocyanins in wine. In reality, there is probably a transition through oligomers of intermediate size, some evidence of which has already been indicated (Burroughs 1975).

Free $SO_2$ in the wines was determined (using 10 ml samples) by three measurements of $E_{520nm}$ (a) on the untreated wine, (b) 30 min after adding 0.1 ml 10% v/v acetaldehyde and (c) 10 min after adding 0.1 ml 3% sodium metabisulphite solution, according to Burroughs (1975). Free and total $SO_2$ and total acetaldehyde contents were also determined by more conventional distillation procedures (Burroughs and Sparks 1964, 1973).

Total phenols were estimated by Folin-Ciocalteau reagent (Singleton and Rossi 1965) and catechin-type phenolics were estimated using vanillin (Swain and Hillis 1959).

Free and total acidities were determined by titration with alkali before and after cation exchange treatment, and expressed as tartaric acid (%).

## Results

### Long Ashton wines

The Long Ashton wines were analysed when they were approximately 4, 15 and 21 months old (Table 1). There were striking differences in colour. Wine B made by thermovinification was nearly twice as coloured as wine A, fermented on the skins, despite containing less anthocyanins and being of higher pH, which should have lessened the colour. Wine C made by carbonic maceration was only one-third as coloured as wine B although containing almost the same initial concentration of anthocyanins. The colour differences were reflected in the variations in degree of ionisation of the anthocyanins in the wines, B being much the greatest, followed by A and then C, the least. A further pronounced difference was the greatly increased polymeric pigment content of wine B compared with wines A and C.

During storage in bottle, the content of anthocyanins decreased and that of the polymeric pigments increased in all three wines, as was expected. Two wines (B and C), however, increased in colour despite the anthocyanin losses. The remaining

Table 1

Analyses of Long Ashton wines
Analysen von Weinen aus Long Asthon

| Processing method and wine pH | Line | Age of wine (mths) | Further treatment | Colour density | Anthocyanins (g/l) | Polymeric pigments (g/l) | Degree of ionisation (%) | Tint | Polymeric pigment at wine pH (%) |
|---|---|---|---|---|---|---|---|---|---|
| Wine A | 1 | 4 | — | 3.30 | 0.65 | 0.14 | 5.3 | 0.57 | 19 |
| (fermented on | 2 | 15 | — | 3.16 | 0.34 | 0.23 | 6.5 | 0.79 | 38 |
| skins; | 3 | 15 | + acetaldehyde (30 min) | 3.80 | 0.34 | 0.23 | 9.3 | 0.70 | 30 |
| pH 3.55) | 4 | 15 | expose to air for 15 days | 4.42 | 0.34 | 0.27 | 10.0 | 0.83 | 32 |
| | 5 | 15 | + catechin, expose to air for 15 days | 4.58 | 0.31 | 0.27 | 11.0 | 0.85 | 32 |
| | 6 | 21 | — | 3.11 | 0.31 | 0.24 | 6.6 | 0.83 | 41 |
| Wine B | 7 | 4 | — | 6.20 | 0.45 | 0.37 | 11.3 | 0.70 | 30 |
| (thermo- | 8 | 15 | — | 6.33 | 0.21 | 0.60 | 16.5 | 0.81 | 51 |
| vinification; | 9 | 15 | + acetaldehyde (30 min) | 6.35 | 0.21 | 0.60 | 17.0 | 0.79 | 51 |
| pH 3.80) | 10 | 15 | expose to air for 15 days | 7.28 | 0.19 | 0.64 | 20.0 | 0.91 | 51 |
| | 11 | 15 | + catechin, expose to air for 15 days | 7.63 | 0.18 | 0.66 | 21.6 | 0.93 | 50 |
| | 12 | 21 | — | 6.20 | 0.19 | 0.60 | 17.0 | 0.83 | 53 |
| Wine C | 13 | 4 | — | 1.95 | 0.42 | 0.15 | 2.6 | 0.95 | 45 |
| (carbonic | 14 | 15 | — | 2.15 | 0.27 | 0.20 | 3.3 | 1.05 | 58 |
| maceration; | 15 | 15 | + acetaldehyde (30 min) | 2.76 | 0.27 | 0.20 | 6.3 | 0.89 | 42 |
| pH 3.90) | 16 | 15 | expose to air for 15 days | 3.41 | 0.26 | 0.26 | 7.1 | 1.04 | 46 |
| | 17 | 15 | + catechin, expose to air for 15 days | 3.60 | 0.26 | 0.26 | 7.7 | 1.06 | 44 |
| | 18 | 21 | — | 2.19 | 0.26 | 0.21 | 3.5 | 1.07 | 59 |

anthocyanins therefore must have become more coloured, as shown by increased values of degree of ionisation (Table 1 — compare line 7 with 8, and line 13 with 14). The colour changes of red wines during storage were therefore governed by these two effects and, depending upon their relative magnitudes, some wines may increase in colour (B and C) while others may fall (e.g. A).

In wines, the colour of anthocyanins and their degrees of ionisation are influenced by many factors, one of the most obvious being the depressive action of sulphur dioxide. To eliminate the effect of variable $SO_2$ content on the degree of ionisation, acetaldehyde was added and the wine colours were measured after 30 min, when all the $SO_2$ had formed acetaldehyde-bisulphite. These measurements were part of the procedure also used for determining free $SO_2$ in the wines (Burroughs 1975) (Table 2). Wine B was very little affected, but wines A and C (15

Table 2

Analyses of Long Ashton wines (age 15—16 months)
Analysen von Weinen aus Long Ashton (Alter 15—16 Monate)

| | A | B | C |
|---|---|---|---|
| Total phenols (g/l) | 1.57 | 1.46 | 1.75 |
| Catechin-type phenolics (g/l) | 0.73 | 0.61 | 0.98 |
| Free acid (% as tartaric) | 0.60 | 0.70 | 0.46 |
| Total acid (% as tartaric) | 0.96 | 1.12 | 0.82 |
| Total $SO_2$ (ppm) | 43 | 20 | 69 |
| Free $SO_2$ (ppm — by aeration) | 12 | pr.nil | 22 |
| Free $SO_2$ (ppm — by acetaldehyde-$SO_2$) | 2 | pr.nil | 6 |
| Total acetaldehyde (ppm) | 19 | 26 | 23 |

months old) increased appreciably in colour and degree of anthocyanin ionisation, signifying the presence of some free $SO_2$. Nevertheless, the effect of the variable amounts of free $SO_2$ in the wines was small compared to the differences produced by processing treatments (Table 1 — compare lines 3, 9 and 15).

The wines containing acetaldehyde were kept exposed to air. After their immediate increase in colour due to combination of the free $SO_2$, the colour of wines A and C continued to increase markedly until precipitation occurred after 8 days (Fig. 1 (c)). In contrast, the colour of wine B was little affected but nevertheless precipitation occurred after the same period. Turbidities and deposits caused by acetaldehyde added to red wines have been observed previously (Joslyn and Comar 1941).

Oxygen also affects the degree of ionisation of anthocyanins since it is known that the colour of wine can increase when exposed to air. It has been postulated that this phenomenon may be due to re-oxidation of colourless anthocyanin forms, possibly flavenes, formed under reducing conditions (Ribéreau-Gayon 1971, 1973, 1974). Samples of the three wines, when 15 months old, were accordingly left exposed to air and examined at intervals. The colour of all three wines increased more than that attributable to oxidation of the free $SO_2$ present (Fig. 1 (a)). Wine C exhibited the greatest colour increase, but this was not enough to affect the original relative order of wine colour.

The total colour obtained on acidification was smaller for each wine than that found before exposure to air. Thus, there was no evidence of any production of anthocyanins and the increased colour of the wines seemed due solely to augmentation of the existing anthocyanin colour, as indicated by increases in degree of ionisation (Table 1 — compare lines 2 and 4, 8 and 10, and 14 and 16).

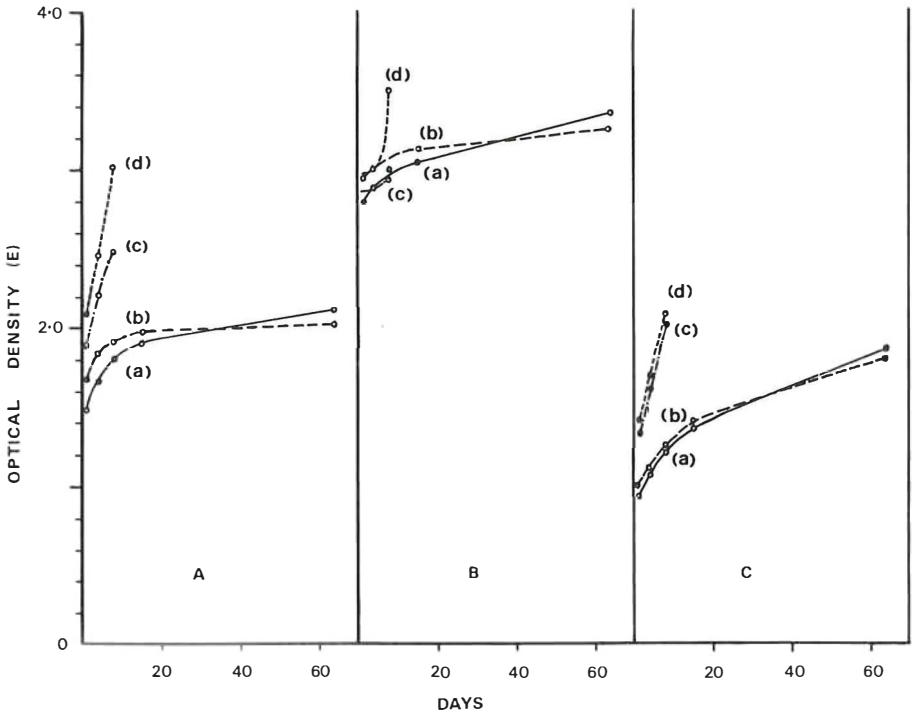


Fig. 1: Colour changes ($E_{520}$) of Long Ashton wines exposed to air: (a) wine alone = ———, (b) wine + (+)-catechin (0.1%) = — — —, (c) wine + acetaldehyde (0.2%) = — . — . — ., (d) wine + acetaldehyde (0.2%) + (+)-catechin (0.1%) = - - - - - - -.

Farbveränderungen ($E_{520}$) von Weinen aus Long Ashton nach Lufteinwirkung: (a) Wein ohne Zusatz = ———, (b) Wein + (+)-Katechin (0,1%) = — — —, (c) Wein + Acetaldehyd (0.2%) = — . — . —, (d) Wein + Acetaldehyd (0,2%) + (+)-Katechin (0.1%) = - - - - - -.

It is known also that anthocyanins can become more coloured by co-pigmentation with many flavonoid compounds (Asen *et al.* 1972), although such complexes are dissociated by adding ethanol. It was thus interesting to examine how much augmentation was possible in wines. For this purpose (+)-catechin (1 g/l), a typical wine phenolic, was dissolved in the wines. There was a small instantaneous colour increase (Fig. 1 (b)), signifying that the phenomenon occurs to some extent in wines despite their ethanol content. On keeping the samples exposed to air, the colours increased further but gradually fell below those of the wines without added catechin.

In all wines the most rapid and greatest colour increase occurred in the presence of both added catechin and acetaldehyde, until precipitation occurred (Fig. 1 (d)).

## Phenolic contents

Table 2 shows the phenolic contents of the three wines. Wine C, made by carbonic maceration, surprisingly contained most total phenolics and most catechin-type phenolics. Previously this procedure has yielded wines reduced in colour and acid (free and total), as happened here, but has also given reduced tannin contents (Singleton and Esau 1969, Beelman and McArdle 1974). At 4 months, wine C could be regarded as more mature than the others (Laszlo *et al.* 1966) because the poly-

meric pigments contributed a higher proportion of its wine colour (45%) than in the other wines (19 and 30%), and it was the most browned (Table 1).

Wine B, made by thermovinification, possesed least total and catechin-type phenolics, perhaps because some of the phenolics had already combined with the anthocyanins to give polymeric pigments which reacted less with the colorimetric reagents used to estimate them. However, until more is known about the analysis of the polymers, or the phenolic components can be estimated individually, the figures merely reflect the inadequacy of such determinations, and no certain conclusions should be drawn.

### Sulphur dioxide and acetaldehyde contents

It is known that normal chemical methods (involving acidification and $SO_2$ removal by aeration *in vacuo* or titration) exaggerate the free $SO_2$ content of anthocyanin-containing media such as red wines and fruit juices; this is because such methods include the $SO_2$ bound to the anthocyanins at wine pH (Timberlake and Bridle 1967 b). The acetaldehyde-$SO_2$ method of Burroughs (1975) overcomes these difficulties and gave much smaller values of free $SO_2$ in wines A and C (Table 2).

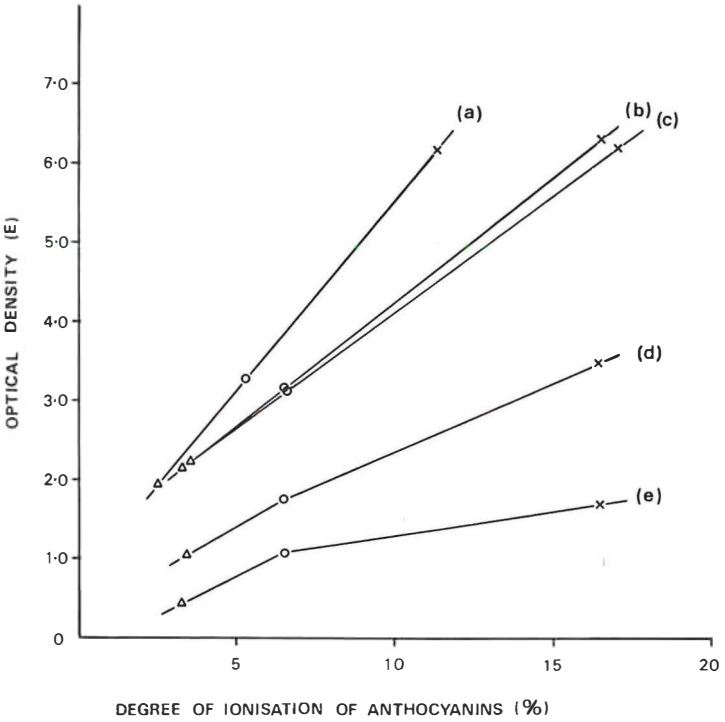


Fig. 2: Plots of optical density (E) against degree of ionisation (%): (a) wine colour density ($E_{520} + E_{420}$) at 4 months, (b) at 15 months, (c) at 21 months, (d) $E_{520}$ of wine at 15 months, (e) $E_{520}$ due to anthocyanins alone at 15 months. Wine A = O, wine B = ×, wine C = △.

Optische Dichte (E) in Beziehung zum Ionisationsgrad (%): (a) Weinfärbung ($E_{520} + E_{420}$) nach 4 Monaten, (b) nach 15 Monaten, (c) nach 21 Monaten, (d) $E_{520}$ von Wein nach 15 Monaten, (e) $E_{520}$ der Anthocyane allein nach 15 Monaten. Wein A = O, Wein B = ×, Wein C = △.

Table 3

Analyses of commercial wines (Beaujolais)
Analysen kommerzieller Weine (Beaujolais)

| Wine age (months) | Treatment | Wine D | | | | | Wine E | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Colour density | Antho-cyanins (g/l) | Polymeric pigments (g/l) | Degree of ionisation (%) | Tint | Colour density | Antho-cyanins (g/l) | Polymeric pigments (g/l) | Degree of ionisation (%) | Tint |
| 7 | — | 2.65 | 0.140 | 0.18 | 14.0 | 0.71 | 4.75 | 0.188 | 0.23 | 24.0 | 0.62 |
| 15 | — | 3.23 | 0.068 | 0.25 | 32.7 | 0.70 | 4.15 | 0.105 | 0.32 | 25.8 | 0.73 |
| 15 | exposure to air for 11 days | 3.68 | 0.068 | 0.28 | 37.4 | 0.73 | 4.92 | 0.116 | 0.38 | 27.0 | 0.82 |
| 16 | — + acetaldehyde (0.1%) | 3.23 | 0.068 | 0.26 | 34.6 | 0.67 | 3.95 | 0.106 | 0.32 | 26.0 | 0.72 |
| 16 | measure after 30 min | 3.30 | 0.068 | 0.26 | 36.1 | 0.67 | 4.06 | 0.106 | 0.32 | 27.8 | 0.70 |
| 16 | measure after 7 days | 4.21 | 0.058 | 0.32 | 47.5 | 0.73 | 5.26 | 0.072 | 0.39 | 46.2 | 0.76 |

Wine B contained no measurable free $SO_2$ but rather an excess of acetaldehyde. Thus, if all the 20 ppm $SO_2$ in wine B was bound with acetaldehyde, the major sulphite binding compound in wines, it would have required 14 ppm of acetaldehyde. However, the wine contained 26 ppm acetaldehyde, suggesting that some free acetaldehyde was available to interact with anthocyanins and phenolics.

### Correlation between colour parameters

Somers and Evans (1974) found a linear relationship between wine colour density and the degree of ionisation of the anthocyanins. Values for individual wines were scattered about a regression line, the slope of which differed according to the variety of grape. When we plotted similar data for the Long Ashton wines, the three points lay on perfect straight lines at each time of sampling (Fig. 2 (a), (b) and (c)). The slopes of the lines decreased as the wines aged. The perfect linearity was quite unexpected considering the widely differing processes used, and is at yet unexplained. Corresponding plots of $E_{520nm}$ (Fig. 2 (d)) and $E_{520nm}$ due to anthocyanins alone (Fig. 2 (e)) against degree of ionisation were not linear.

A linear relationship between wine colour density and the concentrations of ionised anthocyanins has also been reported (Somers and Evans 1974). However, this is less surprising because a linearity is inherent in the method of calculating the latter; the scatter of the values for individual wines then represents merely differences in the extent of browning and the amounts of polymeric pigments they contain. Because of these last factors the appropriate values for the Long Ashton wines did not lie on straight lines but statistical regression lines and significance values could have been calculated, if required.

### Commercial wines

Studies were also made on five young Beaujolais wines approximately 7, 15 and 16 months after making. Analytical data are given on only two of the wines (Table 3) as examples. At 7 months, the degree of ionisation of the anthocyanins varied from 14% to 31%. These values are much greater than those for the Long Ashton wines which contained anthocyanin 3,5-diglucosides known to be much less coloured than the 3-monoglucosides of *Vitis vinifera* (Timberlake and Bridle 1967 a).

Storage produced the expected changes viz. reduction in anthocyanin contents and production of polymeric pigments. Changes in the degree of ionisation were variable — three wines (including D) increased appreciably, one wine increased only slightly, and one wine fell. Two wines increased in colour; in these, increased ionisation evidently more than counterbalanced the anthocyanin losses. The remaining three wines lost colour.

Exposure of the 15-month-old wines to air for 11 days increased colour density and the degrees of ionisation. In contrast to the Long Ashton wines, the total colour obtained on acidification increased in three wines, notably in wine E. The anthocyanin content of wine E also increased but was unchanged in the other two wines (including D), despite increased formation of polymeric pigments. In these three wines therefore, the colour increase appeared to be due to production of anthocyanins (possibly by oxidation of reduced colourless forms) as well as colour augmentation of the existing anthocyanins, indicated by increased ionisation values. In the remaining two wines the anthocyanins were reduced; thus, in these wines the colour increase must be attributed largely to the augmentation effect.

That free $SO_2$ was not a factor in the above changes was confirmed by adding acetaldehyde to freshly-opened bottles (16 months old) and measuring the increases in optical density after 30 min (Table 3). The very small increases indicated very

little free $SO_2$ (0—1.3 ppm). The wines containing excess acetaldehyde were kept at room temperature. Despite losses of anthocyanins and increases in polymeric pigments, there were considerable increases in colour, and the degrees of ionisation were correspondingly large (7 days storage — Table 3). The wavelength peaks of the wines underwent a hypsochromic shift of 4—8 nm.

After 11 days further storage, precipitation commenced and the colour diminished, however, the degrees of ionisation of the pigments still soluble attained very high values (50—67%). At this stage the wines contained little anthocyanin (0.039—0.048 g/l) but largely polymerised pigments (0.44—0.52 g/l).

Table 3 shows values of tint (T), that provide an index of browning or maturity. Those for wine D fell during ageing although there was a considerable increase in polymeric pigments. Moreover, in all the wines after adding acetaldehyde, the tint values altered only slightly and did not adequately represent the considerable polymerisation which had occurred. The ratios of the colours due to monomeric and polymerised pigment forms or their approximate contents as calculated using the appropriate extinction coefficients by the Somers' technique were therefore much more meaningful in this particular case.

## Discussion

Sufficient evidence has been produced here and by Somers (1971) to indicate that acetaldehyde has important effects on colour changes in red wines. Thus, adding it to wines induced large colour increases and accelerated polymerisation of the anthocyanins, leading to their partial precipitation. Its action was characterised by much increased ionisation of the anthocyanins. In experiments in model systems, which will be reported elsewhere, using known pure anthocyanins and phenolic compounds, we have observed similar effects and have concluded that acetaldehyde has little immediate action on anthocyanins alone, but a pronounced effect when phenolic compounds are also present. Acetaldehyde appears to react initially with the phenolic moiety forming a reactive species which then interacts with the anthocyanin, possibly via Baeyer-type reactions involving $CH(CH_3)$ bridges (Singleton *et al.* 1964, Somers 1971). In wines, acetaldehyde must undoubtedly act slower than in these experiments because of its lower concentration and the conditions of limited oxygen availability. Thus, it is significant that the thermovinified wine (B) which exhibited the greatest colour and the largest degree of ionisation should also contain an excess of acetaldehyde over sulphur dioxide. The excess of acetaldehyde in this wine also explains why adding more had little effect on the wine colour, in contrast to the changes it induced in wines A and C which contained free sulphur dioxide. The effect of acetaldehyde in wine B seemed to have attained a maximum and could be stimulated only by adding further phenolic substrate e.g. (+)-catechin (Fig. 1, B (d)) which thus appeared to be a limiting factor. In wine A and particularly wine C, the opposite situation prevailed; the phenolics were not limiting, since acetaldehyde produced a large colour effect which was only slightly increased by adding (+)-catechin.

Acetaldehyde can be formed in wines (with other aldehydes) by microbial action during fermentation and a range of reactive carbonyl compounds, which may induce similar effects, could be formed by photochemical decomposition of tartaric acid catalysed by traces of iron in the wines (Pollard and Timberlake 1971). Also, acetaldehyde can be formed more slowly from ethanol by coupled oxidation with

phenolic compounds (WILDENRADT and SINGLETON 1974). The point at which it occurs in excess of sulphur dioxide in wines must be critical since when this is reached reactions are initiated, dependent upon the types of phenolic compounds present, which are not possible as long as free sulphur dioxide is present. Oxygen must play a part in this process, since by oxidising sulphur dioxide, it will hasten the formation of free aldehyde. Beyond this, oxygen appears to have a dual effect in augmenting anthocyanin colour and, in some wines, also producing anthocyanins. Oxidation of flavenes proposed by RIBÉREAU-GAYON (1971, 1973, 1974) could explain the latter observation, but although flavenes can be formed from synthetic flavylium salts (for references see TIMBERLAKE and BRIDLE 1975) they have yet to be isolated from the anthocyanins themselves. The mechanism of both effects thus remains obscure. It should be noted that the wines exposed to air were kept in darkness as far as possible to avoid any aldehyde effect caused by photochemical decomposition of tartaric acid, as mentioned earlier.

By manipulating the Long Ashton wines in the ways described, it was not possible to adjust their colours to be proportional to the amounts of anthocyanins present or to bring their anthocyanins to the same degree of ionisation. Therefore, the effects already discussed must have been superimposed upon the main cause of the wide divergences of colour, namely the extraction method employed. The figures for total phenolics in wines A and B suggest that about the same amounts of phenolic compounds and anthocyanins were extracted from the fruit. The heat treatment, possibly aided by the depectinising enzyme, is thus implicated as the main causative agent; although the finished wines were pasteurised in bottle, to prevent microbiological spoilage, this procedure was a common factor, the effects of which will be investigated. Inactivation of oxidising or other deleterious enzymes by heat during extraction may explain the increased colour and degree of ionisation, but a more probable explanation is the effect of heat on the types of phenolics extracted and on the nature of the phenolic-anthocyanin interactions. It is known that the mechanism of anthocyanin degradation can vary according to the environmental conditions and that it is particularly affected by temperature. The increased polymeric pigments in the thermovinified wine B (Table 1) is an obvious result of greater phenolic-anthocyanin interactions; but at the same time, the increased ionisation of the anthocyanins suggests that the process was not simply the acceleration of the normal ageing reactions at ambient temperatures. Wine C made by carbonic maceration contained most phenolics and adequate anthocyanins, but nevertheless exhibited lowest colour and degree of ionisation, due in part to the high pH. Although the grapes were warm for an extended period during this process, the cell contents were evidently not in such intimate contact as if the grapes had been crushed. Some alcoholic fermentation was observed, but alcohol has been reported a normal component of carbonic maceration (PEYNAUD and RIBÉREAU-GAYON 1971). The results indicate that very different but as yet unexplained mechanisms operate during extraction depending on whether the solubilising agent is alcohol (fermented on the skins), heat (thermovinification) or alcohol-carbon dioxide (carbonic maceration).

## Summary

Red wines were made from the same batch of Cascade grapes (Seibel 13.053) at Long Ashton by three different processing methods (a) extraction on the skins, (b) thermovinification and (c) carbonic maceration and were analysed at intervals. The

wine made by thermovinification was much more coloured than that fermented on its skins, but it contained less anthocyanin and more polymeric pigment. The wine made by carbonic maceration was the least coloured, despite containing anthocyanins similar in amount to those in the thermovinified wine, and it appeared more brown. The red colour differences were attributed to variations in the physicochemical state of the anthocyanins and were expressed numerically in terms of their degrees of ionisation. There was a remarkably exact linear relationship between wine colour density and the degree of ionisation of the anthocyanins in the three wines.

Factors affecting anthocyanin ionisation were studied in the Long Ashton wines and in some commercial young Beaujolais wines. Exposure to air augmented colour, increased anthocyanin polymerisation and, in some of the commercial wines, actually increased the amount of anthocyanins, presumably by oxidation of unknown colourless forms. The effects of adding acetaldehyde were even more striking. Large increases in the degree of ionisation of the anthocyanins occurred concurrently with losses of anthocyanins by further transformations into polymeric pigments. Of the Long Ashton wines, that made by thermovinification contained an excess of acetaldehyde over bisulphite, which was probably a factor augmenting its colour.

It was concluded that these fermentation and storage factors were superimposed upon the main effects which were attributed to the different mechanisms of anthocyanin-phenolic interactions operative under the various extraction procedures.

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