Vitis **33**, 31–35 (1994)

Model wine solutions: Effect of sulphur dioxide on colour and composition during ageing

by

ANNA PICINELLI, J. BAKKER and P. BRIDLE

Institute of Food Research, Reading Laboratory, Reading, Berkshire, United Kingdom

S u m m a r y : The effect of sulphur dioxide and acetaldehyde on the interaction between malvidin 3-glucoside, the main anthocyanin in red wine made from *Vitis vinifera* grapes, and (+)-catechin was examined. In all model wines the molar losses of malvidin 3-glucoside were significantly greater than the losses of catechin. The concentration of malvidin 3-glucoside decreased fastest in the presence of acetaldehyde while the most rapid loss of catechin occurred in the model containing malvidin 3-glucoside and catechin only. In the presence of sulphur dioxide, these losses still occurred, but much more slowly, indicating that condensation reactions may take place even in the presence of sulphur dioxide. Polymerisation was most prominent in the model containing malvidin 3-glucoside, catechin and acetaldehyde. Concurrent with the losses in anthocyanin and catechin, qualitative and quantitative changes in visible colour were also observed. Changes in colour monitored by measuring hue angle and chroma are also reported.

K e y w o r d s : model wines, anthocyanins, acetaldehyde, sulphur dioxide, catechin, HPLC, colour measurements, ageing.

Introduction

One of its most important quality indicators of a red wine is its colour. 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 mechanisms summarised and discussed previously (BAKKER *et al.* 1993).

Sulphur dioxide is widely used in wine making as an antioxidant and an inhibitor of undesirable microbial growth; if added prior to fermentation, it can increase the extraction of colour from grape skins when making red wines, and stabilise the colour when added at bottling (BURROUGHS 1974). However, this is achieved at the expense of reversible bleaching of the monomeric anthocyanin colour of red wine due to the formation of anthocyanin-4-bisulphite, a stable colourless compound (JURD 1964; TIMBERLAKE and BRIDLE 1967). Furthermore, sulphur dioxide binds strongly to acetaldehyde (BURROUGHS and SPARKS 1973), hence the presence of sulphur dioxide would be expected to influence the colour changes during maturation. The effect of sulphur dioxide on wine polymerisation reactions has not been studied hitherto.

The aim of this work is to extend the study of polymerisation in model red wine solutions and examine the effect of sulphur dioxide on these reactions. We studied interactions between malvidin 3-glucoside, the main anthocyanin in red wine made from *Vitis vinifera* grapes, and (+)-catechin; the role of acetaldehyde and sulphur dioxide was also investigated. High performance liquid chromatography (HPLC) was used to measure the anthocyanin and catechin concentrations. Colour changes were monitored using spectrophotometry and tristimulus measurements.

Materials and methods

S a m p l e p r e p a r a t i o n : Anthocyanins were extracted from the skins of various Portuguese grape varieties, with methanol and formic acid (97:3, v/v), and malvidin 3-glucoside was separated and purified by preparative HPLC (BAKKER *et al.* 1993). Reagents were analytical grade: (+)-catechin (Sigma), acetaldehyde (Aldrich) and sodium metabisulphite (Merck). Solvents were HPLC grade.

M o d e l s o l u t i o n s : Reactants were dissolved in a sterilised model wine medium of potassium bitartrate (Merck) buffer (0.02 M, pH = 3.7), containing ethanol (10 % v/v). The reactants were used in amounts calculated to give the following final concentrations in reaction vials (3 ml sample in a 7 ml vial): malvidin 3-glucoside 0.25 mM (0.12 mg/ml), (+)-catechin 0.4 mM (0.12 mg/ml), acetaldehyde 0.9 mM (0.04 mg/ml) and sulphur dioxide 0.9 mM (0.05 mg/ml). Five different experimental mixtures were prepared, filtered (0.45 µm sterile membrane filters) and dispensed into 5 sets of vials as follows:

(A) malvidin 3-glucoside (control),
(AC) malvidin 3-glucoside and (+)-catechin,
(ACA) malvidin 3-glucoside and (+)-catechin and acetaldehyde,
(ACS) malvidin 3-glucoside and (+)-catechin and sulphur dioxide,
(ACAS) malvidin 3-glucoside and (+)-catechin and acetaldehyde and sulphur dioxide.

All samples were allowed to react in the dark at ambient temperature, and were analysed in duplicate from separate vials over a period of 95 d.

C o l o u r m e a s u r e m e n t s : A Philips PU8740 spectrophotometer and 2 mm path length glass cells were used for CIELAB 76 colour measurements (BAKKER *et al.*

Correspondence to: Dr. JOKIE BAKKER, Institute of Food Research, Reading Laboratory, Earley Gate, Whiteknights Road, Reading, Berkshire RG6 2EF, United Kingdom.

1993). Colour measurements of samples containing malvidin 3-glucoside and (+)-catechin and sulphur dioxide were made immediately after the addition of 500 mg/l acetaldehyde, to ensure that the base colour was measured, rather than the colour of the anthocyanins bleached by sulphur dioxide.

Total pigments: Total pigments and total phenols were determined in triplicate by spectrophotometry using 10 mm path length UV cells. Samples were diluted as required with 0.1 M HCl and absorbances were measured at 520 and 280 nm respectively. The total pigment absorbance measurement was expressed as concentration (c) of malvidin 3-glucoside (molar mass 529) using the molar absorptivity value of 28,000 at 520 nm (NIKETIC-ALEKSIC and HRAZDINA 1972). Hence, c(mg/l) = 18.89 absorbance.

Anthocyanin and (+)-catechin analysis: A Hewlett-Packard 1090 M HPLC with an auto injector (25 µl) and a diode array detector recording at 280 and 520 nm was used to measure the anthocyanin and (+)-catechin concentrations using malvidin 3-glucoside chloride and (+)-catechin as external standards (BAKKER *et al.* 1993).

Sulphur dioxide analysis: Free and total sulphur dioxide were determined using a Dionex 4500 ion chromatograph (Dionex Corporation, Sunnyvale, CA, USA). Immediately before analysis samples were diluted as required in buffer: for total SO_2 a pH 9 buffer was used (20 mM Na_2HPO_4) and for free SO_2 a pH 2 buffer was used (10 mM H_2SO_4). Samples (50 µl) were analysed using a Dionex Ionpac ICE-AS1 column, with 10 mM H_2SO_4 as eluent at 1 ml/min flow. Detection was by pulsed amperometry, using a Pt electrode at +0.7 V. Quantification was done using an external standard prepared from sodium metabisulphite in pH 9 buffer.

Acetaldehyde analysis: Free acetaldehyde was determined by headspace gas chromatography using a Perkin Elmer 8700 model, equipped with a HS-101 headspace sampler. Separation was accomplished on a capillary CP-WAX 57CB (50 m x 0.32 mm i.d.) column (Chrompak). The oven was held at 45 °C for 1 min, followed by a 30 °C/min increase to 250 °C, and held 5 min at this temperature. Other parameters were: flame ionization detector 300 °C, injector temperature 300 °C, carrier gas (N_2) flow rate 1.4 ml/min. The determination was calibrated using an external acetaldehyde standard. Total acetaldehyde was determined by an enzymatic method (Boehringer Mannheim GmbH, food analysis enzymatic kit).

Statistical analysis: Linear regression analysis of variance was done using SPSS.

Results and discussion

Colour changes: During ageing the hue angle of all models increased, indicating that they had all developed some browning (Fig. 1). The browning reactions started slowly in all models; after about 20 d of storage there were only small differences between the samples, with the exception of ACS; however, there were large differences in the extent of browning by the end of the experiment.

Model AC browned most, while the least browning was observed in this sample when sulphur dioxide was also present (ACS). The formation of yellow xanthylium pigments, noted by JURD and SOMERS (1970) and later in the context of long-term wine reactions by TIMBERLAKE and BRIDLE (1976), is the probable explanation of the browning reactions occurring in mixture AC. It is interesting to note therefore, that this reaction is apparently retarded in the presence of sulphur dioxide (ACS).

Model ACA showed only a very small increase in browning during the first 50 d, after which time this model had browned less than control A. This very slow browning in the presence of acetaldehyde can be explained by the superimposed blueing effect of acetaldehyde - mediated reactions with anthocyanin and catechin. Previously, BAKKER *et al.* (1993) observed in a model containing 0.5 % v/v acetaldehyde, a decrease in hue angle of 25 ° indicating blueing during the first 25 d of storage, after which a slow increase in hue angle was observed indicating the onset of browning; at the end of the experiment (140 d) the authors reported that the hue angle was the same as that of the starting mixture. Under the current experimental conditions, model ACA did not show a rapid increase in browning until after 50 d storage. At this time the concentration of acetaldehyde had decreased to about 10 mg/l (Fig. 2), and it is probable that this concentration became a limiting factor, since there was still 50 mg/l free malvidin 3-glucoside (Fig. 3) remaining in this model.

There was only a small difference in browning pattern between samples ACA and ACAS, even though equimolar concentrations of sulphur dioxide and acetaldehyde were added in the latter model. Sulphur dioxide has a high af-

Fig. 1: Changes in hue angle (degrees) for all models. A(Δ): malvidin 3-glucoside (control); AC (●): malvidin 3-glucoside and (+)-catechin; ACA (○): malvidin 3-glucoside and (+)-catechin and acetaldehyde; ACS (□): malvidin 3-glucoside and (+)-catechin and sulphur dioxide; ACAS (■): malvidin 3-glucoside and (+)-catechin and acetaldehyde and sulphur dioxide.

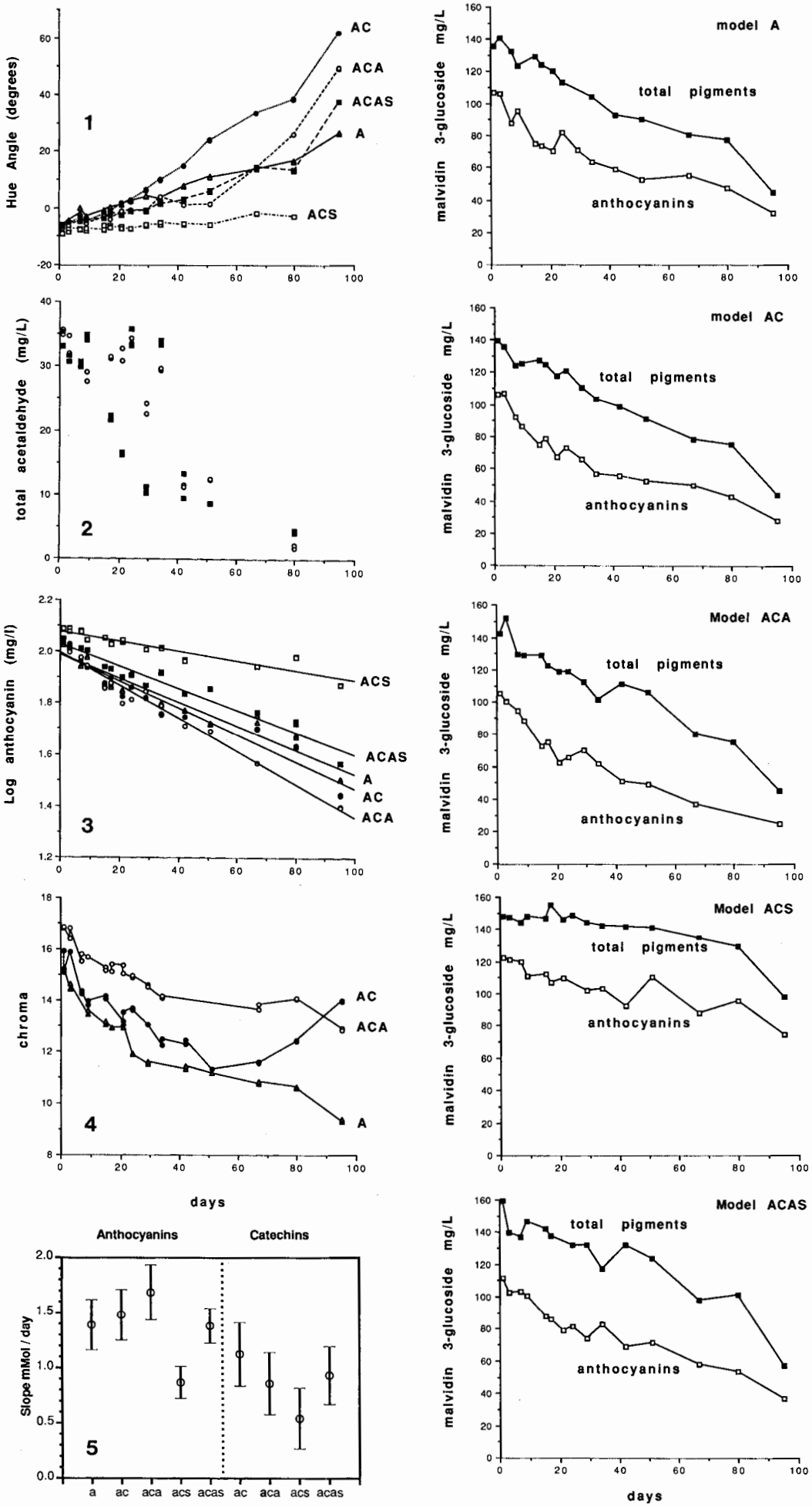
Fig. 2: Changes in total acetaldehyde concentration in models ACA and ACAS. ACA, ACAS: see Fig. 1.

Fig. 3: Changes in malvidin 3-glucoside concentration for all samples. 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 = -0.0047X (2.76E-4) + 1.9902 (1.19E-2); 0.995; AC: Y = -0.0052X (2.64 E-4) + 1.9883 (1.14E-2); 0.966; ACA: Y = -0.0064X (2.20E-4) + 1.9963 (8.62E-3); 0.985; ACS: Y = -0.0019X (1.48E-4) + 2.0764 (6.46.E-3); 0.933; ACAS: Y = -0.0043X (2.05E-4) + 2.0273 (8.85E-3); 0.969. A, AC, ACA, ACS, ACAS: see Fig. 1.

Fig. 4: Changes in chroma for models A, AC and ACA. A, AC, ACA: see Fig. 1.

Fig. 5: Slopes calculated for decrease in anthocyanin and catechin concentrations expressed in mMol/d, with 95 % confidence regions. A, AC, ACA, ACS, ACAS: see Fig. 1.

Fig. 6 (right column): Decrease in total pigment measurement and anthocyanin concentration determined by HPLC, both expressed in mg/l. A, AC, ACA, ACS, ACAS: see Fig. 1.


Hue Angle (degrees)
AC
ACA
ACAS
A
ACS
1
malvidin 3-glucoside mg/L
model A
total pigments
anthocyanins
total acetaldehyde (mg/L)
2
model AC
Log anthocyanin (mg/l)
ACS
ACAS
A
AC
ACA
3
Model ACA
chroma
AC
ACA
A
4
Model ACS
days
Anthocyanins
Catechins
Slope mMol / day
5
a ac aca acs acas ac aca acs acas
Model ACAS
days

finity for acetaldehyde at this pH (BURROUGHS and SPARKS 1973), and hence should bind the available acetaldehyde, leaving very little free to react. Thus model ACAS would have been expected to have a browning pattern similar to model AC, if exactly equimolar concentrations of acetaldehyde and sulphur dioxide had been added. However, the average free acetaldehyde in ACAS was 7.9 mg/l, whereas in the sample not containing sulphur dioxide (ACA) all the acetaldehyde is available for reactions with the flavonoid components.

The chroma (Fig. 4) of control A showed a steady decrease, while model AC showed a more gradual decrease during the first 50 d of storage, followed by an increase in chroma, in agreement with earlier findings (BAKKER *et al.* 1993). In contrast to these earlier observations made in the presence of much higher concentrations of acetaldehyde, when an increase in chroma was observed during the first 7 d of storage, indicating an increase in the amount of colour expressed by the mixture, we now note a slow decrease in chroma during the entire storage period of ACA. These observations accord well with the fact that no new peaks were observed by HPLC, just a gradual decline in the concentration of anthocyanins and catechin.

Acetaldehyde and sulphur dioxide changes: The total acetaldehyde concentration in models ACA and ACAS decreased during the first 80 d of the experiment, from 35 mg/l to less than 5 mg/l (Fig. 2). The variation between the measuring points is due to inter-vial variation. The effect of sulphur dioxide on the loss of acetaldehyde cannot be determined from these data. The free acetaldehyde concentration in the ACAS model did not change during the course of the experiment, remaining at an average of 7.9 mg/l. The sulphur dioxide concentration decreased slowly in both ACS and ACAS, and no obvious effect of acetaldehyde on the rate of loss was observed.

Anthocyanin and catechin losses: On storage, all solutions exhibited a logarithmic decrease in total anthocyanin concentrations (Fig. 3). The linear loss of anthocyanins in all four model mixtures indicates a first order reaction with respect to this loss, confirming the findings of BARANOWSKI and NAGEL (1983). In models AC and ACA the rate of loss was greater than in control A, while sulphur dioxide had a slowing effect on this process in both cases, although in ACAS the rate of loss was only marginally less than in control A.

The reaction rates k (day^{-1}) are shown in the Table and compared with rates reported previously (BAKKER *et al.* 1993; BARANOWSKI and NAGEL 1983). Using a 3.6-fold molar excess of acetaldehyde, the observed increase in reaction rate for the model ACA was less than when the molar excess was 5-fold. Surprisingly, even in the presence of a 3.6-fold molar addition of sulphur dioxide (ACAS), the anthocyanin concentration still decreased, although at a much slower rate. The slowing effect of sulphur dioxide on the rate of anthocyanin loss is shown even better comparing AC and ACS reaction rates. The apparent equilibrium constant for acetaldehyde with sulphur dioxide at pH 4 is 1.4×10^{-6} (BURROUGHS and SPARKS 1973), while for malvidin 3-glucoside with sulphur dioxide it is 6×10^{-5} (BURROUGHS, pers. comm.). Thus in the presence of both acetaldehyde and malvidin 3-glucoside, sulphur dioxide would mostly be bound to acetaldehyde, as can also be determined from the lack of bleaching. Although the anthocyanin was to some extent protected against loss by sulphur dioxide, its degradation still occurred.

The losses of catechin in all four models were also logarithmic with time. In order to compare the losses of anthocyanins with those of catechin (not shown), the regression lines were calculated on the concentration expressed in mMol (Fig. 5). The rate of loss of catechin was always significantly slower ($p < 0.05$) than for anthocyanins in corresponding models. This is attributable to the anthocyanin and catechin condensation which is superimposed on the degradation of anthocyanins alone, as indicated by the loss in model A. The extent to which both reactions occur cannot be ascertained, but the differences in colour changes for the models indicate that different reactions occur. Adding acetaldehyde increased the slope of anthocyanin loss, but decreased the slope of catechin loss. The implication appears to be that the condensation involving acetaldehyde, anthocyanin and catechin involves

Table

Reaction rates with respect to the disappearance of total anthocyanins in model wine systems, calculated with respect to malividin 3-glucoside

Mixture	k (day^{-1})	k (day^{-1})†	literature value* k (day^{-1})
Anthocyanin	10.77×10^{-3}	5.66×10^{-3}	6.31×10^{-3}
Anthocyanins and catechin	12.07×10^{-3}	8.88×10^{-3}	6.74×10^{-3}
Anthocyanins, catechin and acetaldehyde	14.73×10^{-3}	254.76×10^{-3}	19.00×10^{-3}
Anthocyanins, catechin and sulphur dioxide	4.32×10^{-3}		
Anthocyanins, catechin, acetaldehyde and sulphur dioxide	9.81×10^{-3}		

† Bakker, Bridle and Picinelli (1993), using a 400-fold molar excess.
* Data calculated from Baranowski and Nagel (1983), using a 5-fold molar excess.

relatively more anthocyanin molecules than the condensation occurring in the absence of acetaldehyde.

Comparison of total pigment measurement with anthocyanin concentration: The total pigment absorbance measurement expresses the sum of the concentration of monomeric anthocyanins and the colour exhibited by polymerised material. Since the molar absorptivity value of newly formed polymerised material is not known, the concentration from this absorbance measurement is expressed as malvidin 3-glucoside, assuming the same molar absorptivity value for both. The difference between the total pigment data and the anthocyanins determined by HPLC both expressed in concentration terms (Fig. 6), gives an indication of the contribution of the coloured polymers to the overall colour. Over the course of the experiment, the largest difference was observed in the model containing anthocyanins, catechin and acetaldehyde, e.g. after about 50 d, there was double the colour concentration as expressed by the total pigment measurement, i.e. 50 % of the colour was due to polymers. In contrast the difference for the model containing anthocyanins, catechin and sulphur dioxide remained much smaller.

Conclusion: In the absence of acetaldehyde sulphur dioxide reduces the rate of anthocyanin loss and slightly reduces polymerisation. When acetaldehyde is also present these effects of sulphur dioxide are no longer observed.

Acknowledgements

The authors wish to thank IAN WAKELING and DUNCAN HEDDERLEY for statistical analysis, the EC for funding this project under FLAIR (project no. 89053) and NATO's Scientific Committee, Spain, for financial support for Dr. ANNA PICINELLI.

References

BAKKER, J.; PICINELLI, A.; BRIDLE, P.; 1993: Model wine solutions: Colour and composition changes during ageing. Vitis **32,** 111-118.

BARANOWSKI, E. S.; NAGEL, C. W.; 1983: Kinetics of malvidin 3-glucoside condensation in model wine systems. J. Food Sci. **48**, 419-421, 429.

BURROUGHS, L. F.; 1974: Browning control in fruit juices and wines. Chem. Ind. (Sep.) 718-720.

- -; SPARKS, A. H.; 1973: Sulphite binding power of wines and ciders. I. Equilibrium constants for the dissociation of carbonyl bisulphite compounds. J. Sci. Food Agricult. **24**, 187-198.

JURD, L.; 1964: Reactions involved in sulphite bleaching of anthocyanins. J. Food Sci. **29**, 16-19.

- -; SOMERS, T. C.; 1970: The formation of xanthylium salts from procyanidins. Phytochemistry **9**, 419-427.

NIKETIC-ALEKSIC, G. K.; HRAZDINA, G.; 1972: Quantitative analysis of the anthocyanin content in grape juices and wine. Lebensm.-Wiss. Technol. **5**, 163-165.

TIMBERLAKE, C. F.; BRIDLE, P.; 1967: Flavylium salts, anthocyanidins and anthocyanins. II. Reactions with sulphur dioxide. J. Sci. Food Agricult. **18**, 479-485.

- -; - -; 1976: Interactions between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines. Amer. J. Enol. Viticult. **27**, 97-105.

Received May 12, 1993