

## Effect of SO<sub>2</sub> on the extraction of individual anthocyanins and colored matter of three Portuguese grape varieties during winemaking

by

C. DALLAS and O. LAUREANO

Universidade Técnica de Lisboa, Instituto Superior de Agronomia, Laboratório Ferreira Lapa, 1399 Lisboa, Portugal

**S u m m a r y :** Periquita, Tinta Roriz and Tinta Barroca grapes were fermented under standard conditions with 3 levels of SO<sub>2</sub> concentration. The anthocyanin monomers were separated by HPCL and identified by DAS on the grape skin extracts.

Periquita was the wine with the highest color density and the higher content in total anthocyanins. Malvidin 3-glucoside, peonidin 3-glucoside and petunidin 3-glucoside were found in largest amounts in Periquita wines, while delphinidin 3-glucoside and malvidin 3-glucoside-*p*-coumarate were present in higher concentrations in Tinta Barroca wines. Malvidin 3-glucoside acetate and peonidin 3-glucoside coumarate were found in the highest concentration in Tinta Roriz wines.

The influence of SO<sub>2</sub> on the extraction of total anthocyanins and the major part of monomeric anthocyanins was very evident, but the rate of the extraction depends also on the nature of the anthocyanins and the characteristics of each variety. The high concentration of SO<sub>2</sub> slowed down the formation of polymeric pigments.

**K e y w o r d s :** anthocyanins, color, berry skin, HPCL-DAS, SO<sub>2</sub>, vinification, red wine, Periquita, Tinta Roriz, Tinta Barroca.

### Introduction

The composition and concentration of anthocyanins in grapes depend on the variety and the degree of fruit maturation. FLANZY *et al.* (1972) and BOURZEIX *et al.* (1983) found that the concentration of anthocyanins can be ten times higher in some varieties than others. The composition of anthocyanins can be different between clones of the same variety (FONG *et al.* 1971; ROGGERO *et al.* 1986).

The initial color of a red wine is mainly due to anthocyanins extracted from the solid parts of grapes. The transfer into the wine of anthocyanins and other phenolic compounds depends on the mode of extraction employed. During extraction some factors increase anthocyanin transfer, others decrease it. Several studies of different factors influencing color extraction (maceration time, alcohol, temperature, SO<sub>2</sub>, processing method) have been reported (POUX and AUBERT 1968; AUBERT and POUX 1969; RIBEREAU-GAYON *et al.* 1970; GUETOV *et al.* 1972; BOURZEIX 1976; TIMBERLAKE and BRIDLE 1976; SOMERS and EVANS 1979).

The importance of the addition of SO<sub>2</sub> at the various stages of wine-making is well known. It is the preferred agent to control undesirable microorganisms, to restrain browning and to serve as an antioxidant. The general opinion is that SO<sub>2</sub> aids the extraction of pigments during fermentation. SUDRAUD (1963) reported an increase of 5–6 % of phenolic compounds with the use of SO<sub>2</sub>. RIBEREAU-GAYON *et al.* (1970) observed an increase of anthocyanin and tannin after addition of SO<sub>2</sub> in a model solution. They found that SO<sub>2</sub> could have a dissolving effect, retaining more free SO<sub>2</sub>. BERG and AKIYOSHI (1962) confirm the greater extraction of color in the presence of SO<sub>2</sub>, and greater retention of color in wines fermented with SO<sub>2</sub>.

The use of high performance liquid chromatography (HPCL) has had a great impact on analyses of the anthocyanins, and allowed their direct measurement in red wines, since they appear as discrete peaks without any interference from polymeric pigments (in young wines, at least). WULF and NAGEL (1978), BERTRAND and GAUTHIER (1982) and ROGGERO *et al.* (1992) have followed the evolution of the individual anthocyanins during maceration and fermentation using HPLC. The development of diode array spectroscopy (DAS) combined with HPCL permitted rapid and simultaneous separation and identification of each of the different anthocyanins according to their spectral properties (HEBRERO *et al.* 1988).

Considering the importance of the addition of SO<sub>2</sub> during fermentation, and the fact that previous studies only reported the total anthocyanins, we have evaluated the effect of SO<sub>2</sub> on the extraction of monomeric anthocyanins and the colored complexes.

### Materials and methods

1. **G r a p e s :** Three portugese grape varieties, Periquita (region of Ribatejo, north of Lisbon), Tinta Roriz and Tinta Barroca (region of Douro Valley) were harvested in 1991 and 1992 at technical maturity (22.7, 24.0 and 18.4 °Brix; pH = 3.7, 3.8 and 3.9 and titratable acidity 3.5, 4.9 and 5.0 g/l as tartaric acid, resp.).

Ten frozen berries of each cultivar were peeled and the skins ground for 1 min in a blender with 40 ml of methanol containing 0.1 % HCl. The resulting slurry was centrifuged for 10 min (4000 g), concentrated at low temperature (30 °C) and filtered through a 0.45 µm filter before injection into the chromatograph.

2. **Vinification**: Approximately 300 kg of each variety were used for the vinification. The red wines were made under standard conditions of fermentation in order to allow comparisons between wines from the three different cultivars.

The grapes of each variety were destemmed, crushed and divided into three homogenous batches. Every batch was supplied with 0, 75 and 150 mg/kg of SO<sub>2</sub> (as potassium metabisulfite) and fermented at controlled temperature (22 °C). The fermenting mash was punched down twice daily during the active part of fermentation. At specific gravity 1.005 the fermenting mash of each batch was pressed in a hydraulic vertical press. Both press and free run wines were then combined, and after malolactic fermentation, red wines were racked, filtered and SO<sub>2</sub> adjusted prior to bottling.

During fermentation contents of individual anthocyanins and colored matter were monitored daily.

3. **Equipment**: The equipment used for analytical HPCL was a Perkin-Elmer system, equipped with a 410-LC pump, a solvent programmer model 420, a UV-Vis detector model LC-95 and a channel integrator LC1-100 were connected to integrate peaks. A Rheodyne injector model 7125-A fitted with a 20 µl loop was used to inject 20 µl samples. A reversed phase Superpher 100, C<sub>18</sub> (Merck, Darmstadt, Germany) column (5 µm packing, 250 mm x 4.6 mm i.d.) protected with a guard column of the same material was used.

The evolution of the anthocyanin pigments were analysed using conditions similar to those of ROGGERO *et al.* (1988).

The solvent A was 40 % formic acid, solvent B was CH<sub>3</sub>CN and solvent C was H<sub>2</sub>O bidistilled. The initial conditions were 25 % of A, 6 % of B and 69 % of C for 15 min followed by a linear gradient to 25 % of A, 25.5 % of B and 49.5 % of C during 70 min. We finished with 20 min of 25 % of A, 25.5 % of B and 49.5 % of C. The flow rate was 0.7 ml/min and the detector wave-length 520 nm. The solvents and samples were filtered (0.45 µm) before analysis.

The diode-array spectroscopy (DAS) system employed was a MERCK-HITACHI L-6200-A pump connected to a MERCK DAD model L-3000. Data treatment utilized the DAD-Manager software. The same column and the same condition of elution was used as described previously.

4. **Standardisation of HPLC**: The concentration of monomeric anthocyanins in wines was quantified by using malvidin 3-glucoside chloride (obtained from Extrasynthese, Lyon, France) as an external standard. Peak area were converted to mg/l of malvidin 3-glucoside using a standard curve prepared by different concentrations of malvidin 3-glucoside chloride in methanol 0.1 % HCl (25, 50, 75, 100, 200 and 500 mg/l). 20 µl of each sample were injected in triplicate in HPLC.

The analysis of variance of the linear regression (R<sup>2</sup> = 99.87 %) between the area and the concentration of the samples are highly significant (P < 0.001).

5. **Color measurement**: The following measurements related to colored matter:

- Color density (CD) defined as the sum of absorbances at 620, 520 and 420 nm (GLORIES 1984).

- Color hue defined as the ratio of the absorbances at 420 and 520 nm.

- Total pigment color WCA = E<sub>520</sub> (HCl) was measured in 1 M HCL at 520 nm (SOMERS and EVANS 1977).

- The percentage of total polymeric material can be estimated from [(WCA-ACA)/WCA] x 100, where WCA is the total pigment color and ACA is the total anthocyanins color calculated from the measured HPCL content (BAKKER *et al.* 1986).

- The total anthocyanin concentration was calculated by the method of RIBEREAU-GAYON and STONESTREET (1965).

- Total phenolics were estimated by Folin - Ciocalteu reagent (RIBEREAU-GAYON *et al.* 1972).

The samples of must/wine were centrifuged for 10 min at 4000 g and filtered (0.45 µm) prior to analysis. The color absorbancy measurements were made with a Pye Unicam SP8-100 spectrophotometer, and all readings were converted to absorbancy E (10 mm).

6. **Statistical analysis**: Statistical calculations were performed using Statgraphics Statistical Computer Package. To determine the specific difference an one-way analysis of variance was done. Duncan's multiple range test was used to separate the means of the main effects.

## Results and discussion

1. **Identification of anthocyanins**: Fig. 1 displays the chromatograms at 520, 310 and 280 nm of Tinta Roriz grape skin extracts. The identification of anthocyanins and especially the *p*-coumarates and acetates were carried out by the simultaneous use of HPLC and DAS, according to their spectral properties and elution orders.

Tab. 1 shows the retention times, the maximum absorption wave-lengths reported in the literature (HEBRERO *et al.* 1988) and the experimental values of the different chromatographic peaks of T. Roriz grape skin extracts. Some differences observed for the 3 acetic acid acylated anthocyanins (delphinidin, cyanidin, petunidin) are due to the small absorption of these anthocyanins on UV. A shoulder in UV spectra was present and as a consequence a lower precision for the determination of the maximum wave-length.

It is well known that the elution order of anthocyanins in reversed-phase chromatography is related to the polarity of these compounds.

However, a difference in the elution of delphinidin 3-glucoside-*p*-coumarate was present in our chromatograms compared to other authors (WULF and NAGEL 1978; PIERGIOVANNI and VOLONTERIO 1980; ROGGERO *et al.* 1984; ROGGERO *et al.* 1986). Delphinidin 3-glucoside-*p*-coumarate (peak 10) appears between the peonidin 3-glucoside-acetate

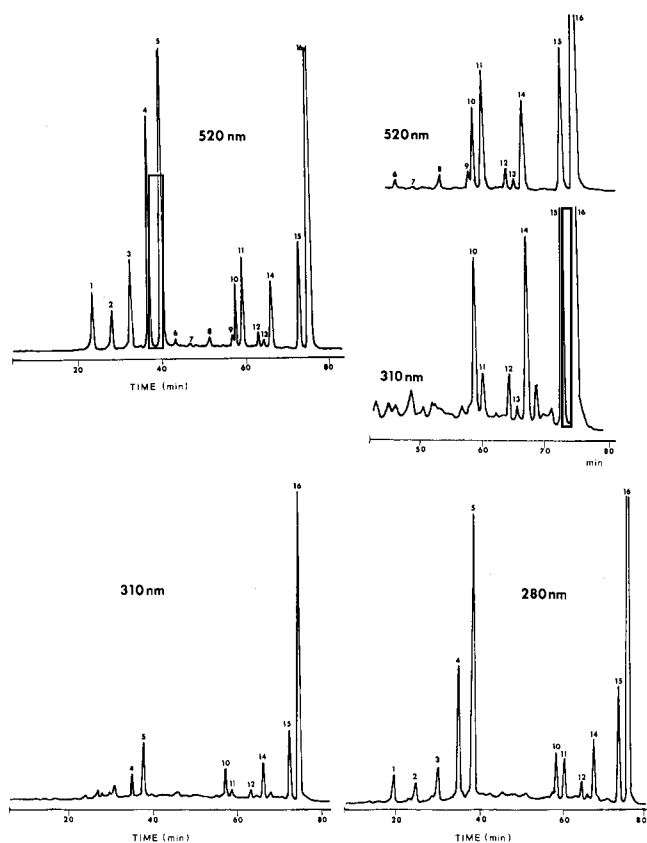


Fig. 1: HPCL chromatograms of Tinta Roriz grape skin extracts recorded at 520, 310 and 280 nm. At the right top side the end part of chromatogram 520 and 310 nm. Peak identification is shown in Tab. 1.

(peak 9) and malvidin 3-glucoside-acetate (peak 11), and not as expected after the malvidin 3-glucoside-acetate. The different selectivity of the column (Superpher) from the other commercial columns could be the explanation of this particularity. ROGGERO *et al.* (1988), using the same type of column for his experiments, observed the same elution or-

der for delphinidin 3-glucoside-*p*-coumarate.

Peaks 1 to 5 are known to correspond, respectively, to the 3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin.

Peaks 6, 7, 8, 9 and 11 on Fig. 1 correspond to the acetic acid acylated derivatives of the 3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin. As pointed out above, the presence of an anthocyanin coumarate (delphinidin 3-glucoside-*p*-coumarate, peak 10) between the last two anthocyanin acetates (peonidin 3-glucoside-acetate, peak 9 and malvidin 3-glucoside acetate, peak 11) has been proved by the spectral properties of these compounds (Fig. 2). The occurrence of an absorption maximum belonging to the monoglucoside can be seen together with a maximum at 310 nm, typical of *p*-coumaric acid in the case of delphinidin 3-glucoside-*p*-coumarate (peak 10) and not for peonidin 3-glucoside-acetate (peak 9) and malvidin 3-glucoside-acetate (peak 11).

Moreover the relationship between the peak area of the chromatograms recorded at 310 and 520 nm (Fig. 1, right top side) is much greater in the case of the delphinidin 3-glucoside-*p*-coumarate (peak 10) than in the case of peonidin and malvidin 3-glucoside-acetate.

Peaks 10, 12, 14, 15 and 16 correspond, respectively to the *p*-coumarates of the 3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin. The UV absorption spectra of all these compounds in Fig. 2 show an additional maximum at 310 nm (typical of *p*-coumaric acid) together with a maximum characterising the monoglucoside. The identity of these pigments can also be confirmed by comparing the chromatograms recorded at 520, 310, and 280 nm (Fig. 1).

Fig. 3 shows the chromatograms recorded at 520 nm of Periquita and T. Barroca grape skin extracts. These chromatograms were quite similar to T. Roriz chromatogram. Only the peak 14a observed on the chromatogram of Periquita variety (Fig. 3) could be malvidin 3-glucoside-cafeate.

Table 1

Retention time, reported (HEBRERO *et al.* 1988) and experimental maximum absorption wavelengths (nm) and identification of the chromatographic peaks of Tinta Roriz grape skin extracts.

Peak n°	Retention time (mn)	$\lambda_{\max}$ reported	$\lambda_{\max}$ experimental	Identification
1	25.69	523-277	525-277	Delphinidin-3-monoglucoside
2	30.29	517-279	517-280	Cyanidin-3-monoglucoside
3	34.93	526-277	527-278	Petunidin-3-monoglucoside
4	39.45	517-277	518-279	Peonidin-3-monoglucoside
5	42.60	526-277	528-278	Malvidin-3-monoglucoside
6	45.97	526-277	529-284	Delphinidin-3-monoglucoside-acetate
7	49.28	520-279	524-285	Cyanidin-3-monoglucoside-acetate
8	54.10	530-278	530-283	Petunidin-3-monoglucoside-acetate
9	59.58	520-279	523-277	Peonidin-3-monoglucoside-acetate
10	60.39	529-310-279	530-310-282	Delphinidin-3-monoglucoside- <i>p</i> -coumarate
11	62.04	530-278	530-279	Malvidin-3-monoglucoside-acetate
12	66.09	521-310-279	523-310-283	Cyanidin-3-monoglucoside- <i>p</i> -coumarate
13	67.45			Unknown
14	69.12	530-310-279	533-310-282	Petunidin-3-monoglucoside- <i>p</i> -coumarate
14 a	69.72			Malvidin-3-monoglucoside-cafeate ?
15	76.05	522-310-281	522-310-284	Peonidin-3-monoglucoside- <i>p</i> -coumarate
16	78.38	531-310-281	533-310-283	Malvidin-3-monoglucoside- <i>p</i> -coumarate

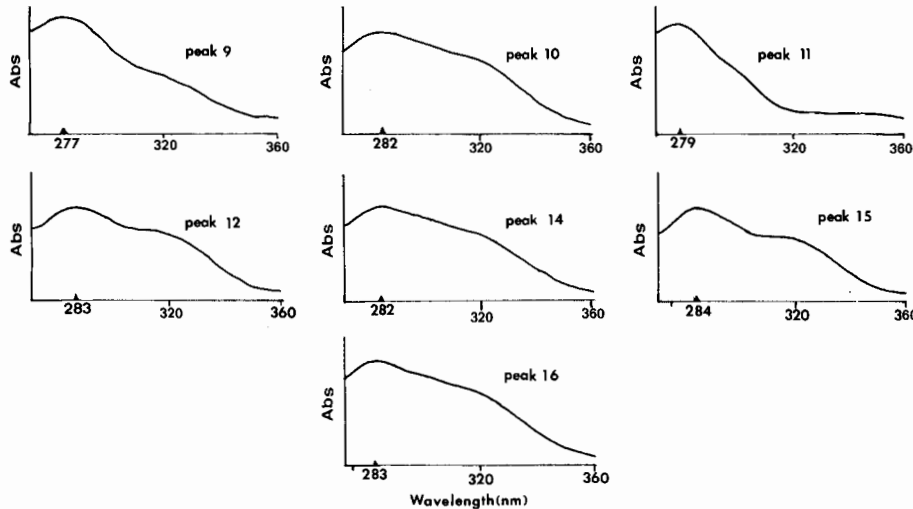


Fig. 2: UV absorption spectra of different anthocyanins of Tinta Roriz grape skin extract as recorded by diode array spectrophotometric detection. Peak 9 = peonidin-3-monoglucoside-acetate; 10 = delphinidin-3-monoglucoside-*p*-coumarate; 11 = malvidin-3-monoglucoside-acetate; 12 = cyanidin-3-monoglucoside-*p*-coumarate; 14 = petunidin-3-monoglucoside-*p*-coumarate; 15 = peonidin-3-monoglucoside-*p*-coumarate; 16 = malvidin-3-monoglucoside-*p*-coumarate.

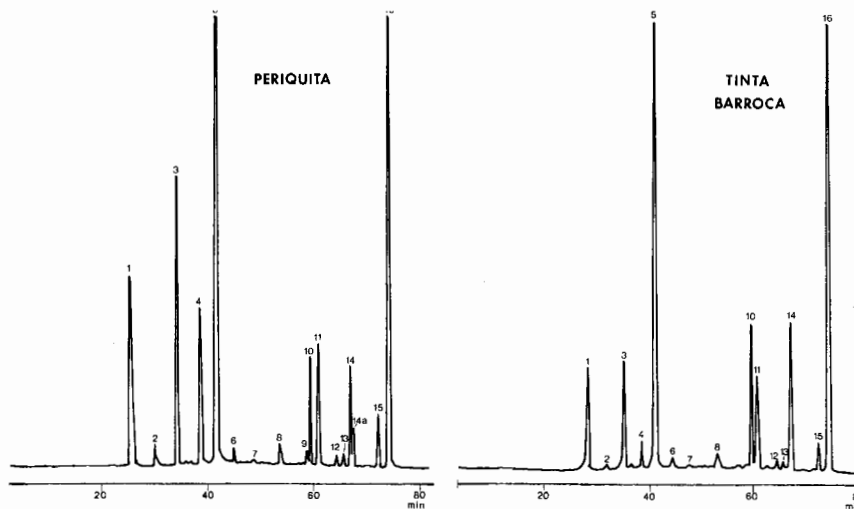


Fig. 3.: HPLC separation of anthocyanins of Periquita and Tinta Barroca grape skin extracts. 1) Dp-Gl; 2) Cy-Gl; 3) Pt-Gl; 4) Pn-Gl; 5) Mv-Gl; 6) Dp-Gl-Ac; 7) Gy-Gl-Ac; 8) Pt-Gl-Ac; 9) Pn-Gl-Ac; 10) Dp-Gl-Coum; 11) Mv-Gl-Ac; 12) Cy-Gl-Coum; 13) Unknown; 14) Pt-Gl-Coum; 14a) Mv-Gl-Caf; 15) Pn-Gl-Coum; 16) Mv-Gl-Coum.

Dp = delphinidin, Cy = cyanidin, Pt = petunidin, Pn = peonidin, Mv = malvidin, Gl = monoglucoside, Ac = acetate, Coum = *p*-coumarate; Caf = caffeate.

It may equally be assumed that peak 13 could be peonidin 3-glucoside-caffeate. However, as these compounds were not found in sufficient amount to obtain reliable spectra, it was not possible to identify them.

## 2. Anthocyanin content of the wines:

The wines were analysed at the end of fermentation and we present the mean of the duplicate analysis of each anthocyanin. Tab. 2 shows the monomeric anthocyanins identified and found in significant amounts in the Periquita, Tinta Roriz and Tinta Barroca wines.

There are significant differences between the wines, reflecting the different composition of the grapes used. Periquita wine is characterised by a high content in non-acylated anthocyanins (89 % of known anthocyanins) and

by a low content in acylated anthocyanins (7 %). In particular, the concentration of malvidin 3-glucoside (190.9, 214.3 and 278.4 mg/l respectively on the wines prepared with 0, 75 and 150 mg/l of SO<sub>2</sub>) and peonidin 3-glucoside (13.5, 15.2 and 22.1 mg/l, respectively) were higher compared to the other wines. Only petunidin 3-glucoside was found in similar concentration in Periquita and T. Barroca wines. The wines prepared from the varieties T. Roriz and T. Barroca were characterised by a higher content of acylated anthocyanins (35 %) compared to Periquita wines. However T. Barroca is the wine with the higher concentration of malvidin 3-glucoside-*p*-coumarate and T. Roriz wine presents the higher concentration of malvidin 3-glucoside acetate and peonidin 3-glucoside-*p*-coumarate. Concerning the non-acylated anthocyanins T. Barroca presents the

Table 2

Influence of SO<sub>2</sub> on the extraction of individual anthocyanins (mg/l).

SO <sub>2</sub> content (mg/kg)	Variety	Delp-3 gluc	Petun-3 gluc	Peon-3 gluc	Malv-3 gluc	Malv-3 acetate	Delp-3 coumarate	Petun-3 coumarate	Peon-3 coumarate	Malv-3 coumarate	Total known
0	Periquita	5.9 a*	13.3 a	13.5 a	190.9 a	7.3 a	1.1 a	—	2.2 a	16.3 a	251.3 a
75		8.3 b	16.3 b	15.2 a	214.3 b	8.6 b	1.9 ab	—	3.0 a	23.9 b	293.0 b
150		10.6 c	22.0 c	22.1 b	278.4 c	11.0 c	2.5 b	—	4.5 b	30.7 b	383.0 c
0	Tinta Roriz	0.2 a	3.7 a	2.9 a	97.8 a	19.7 a	3.7 b	1.9 a	5.3 a	36.3 a	171.3 a
75		1.0 b	5.1 a	4.1 b	118.3 b	27.6 b	2.8 a	3.7 b	6.0 b	37.1 a	206.0 b
150		1.9 c	7.0 b	5.7 c	153.4 c	33.8 c	5.0 c	3.9 b	8.0 c	45.8 b	264.4 c
0	Tinta Barroca	8.1 a	14.6 a	3.7 b	124.0 a	18.9 a	5.6 a	4.9 a	2.2 a	37.8 a	220.0 a
75		9.2 a	16.9 b	4.0 b	134.6 b	21.2 b	7.5 b	6.5 b	2.7 b	45.9 b	248.5 b
150		13.2 b	20.5 c	2.8 a	132.5 b	23.0 c	8.1 c	7.3 c	4.6 c	48.5 c	260.5 c

\* Means separated by Duncan's multiple range test at the 5 % level; not significantly different when followed by the same letter.

higher level of delphinidin 3-glucoside compared to the other wines.

The concentration of SO<sub>2</sub> added during fermentation is responsible for the significant difference of the extraction of total anthocyanins (Tab. 2). The wines made with a high content of SO<sub>2</sub> (150 mg/l) show a higher concentration of total anthocyanins than the wine made with 75 mg/l and the control wine (0 mg/l). The monomeric anthocyanins indicate also significant differences in concentration. However some exceptions are present; the concentration of peonidin 3-glucoside and peonidin 3-glucoside-*p*-coumarate (15.2 and 3.0 mg/l, respectively) in the Periquita wine (75 mg/l of SO<sub>2</sub>) is not significantly different from the wines made without SO<sub>2</sub> (13.5 and 2.2 mg/l). In T. Roriz wines (control and 75 mg/l of SO<sub>2</sub>), the concentration of petunidin 3-glucoside (3.7 and

5.1 mg/l) and malvidin 3-glucoside-*p*-coumarate (36.3 and 37.1 mg/l) were quite similar. There are no significant differences in extraction of delphinidin 3-glucoside (8.1 and 9.2 mg/l) and peonidin 3-glucoside (3.7 and 4.0 mg/l) in T. Barroca wine. The addition of 75 mg/l of SO<sub>2</sub> did not produce a significant increase in the concentration of these pigments compared to the control wine.

The above results indicate that the effect of SO<sub>2</sub> on the extraction of monomeric anthocyanins is not the same for all anthocyanins, but depends on the nature of each anthocyanin (in the case of Periquita wines, an increase of 46, 50 and 88 % was observed respectively for malvidin 3-glucoside, malvidin 3-glucoside-acetate and malvidin 3-glucoside-*p*-coumarate concentration) and on the variety used (Fig. 4). For the same anthocyanins mentioned increases of 57, 71 and 26 %, respectively, were observed in T. Roriz wines, but these percentages were different for T. Barroca wines (7, 22 and 28 %, respectively).

3. Color measurements (Tab. 3): The analysis of the different wines showed that Periquita was the most highly colored wine of the cultivars (Fig. 5). However the total pigment (WCA) was lower in the Periquita and Tinta Barroca wines than in Tinta Roriz. The total phenol content was higher in the wines if Periquita and T. Roriz.

There are considerable differences in these measurements as the result of the SO<sub>2</sub> addition during processing. The color density values, the total pigment (WCA) and total anthocyanin contents were higher in the wines made with 75 and 150 mg/kg of SO<sub>2</sub> than the control wine. As a consequence of the greater anthocyanin concentration, the color hue values were lower for the wines made with 150 mg/l of SO<sub>2</sub>.

The formation of polymeric pigments begins at grape crushing and reaches considerable proportion during fermentation (LAUREANO and VIEIRA 1983; BAKKER *et al.* 1986). The high concentration of SO<sub>2</sub> (150, 75 mg/kg) in Periquita and T. Roriz wines decreased the polymeric pigment as indicated in the percentage of pigment color value, but significant differences in T. Barroca wines were not observed (Fig. 5). The high SO<sub>2</sub> concentration which slow down the

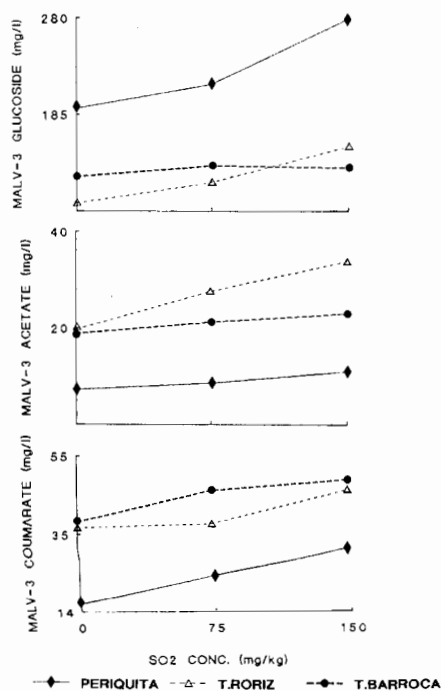


Fig. 4: Effect of SO<sub>2</sub> on the extraction of malvidin 3-glucoside, malvidin 3-glucoside-acetate and malvidin-3-monoglucoside-*p*-coumarate on the Periquita, Tinta Roriz and Tinta Barroca wines.

Table 3

Effects of SO<sub>2</sub> on the colored matter of Periquita, Tinta Roriz and Tinta Barroca red wines.

SO <sub>2</sub> content (mg/kg)	Variety	Colour density	Colour hue	Total pigment (WCA)**	% Polymeric pigment***	Total phenols (index)	Total anthocyanins (mg/l)
0	Periquita	8.7 a*	0.52 c	19.6 a	28.2 b	29.0 a	496.0 a
75		9.7 b	0.49 b	22.1 b	26.5 b	32.8 b	551.8 b
150		10.6 c	0.47 a	26.5 c	17.3 a	35.5 c	630.7 c
0	T. Roriz	8.7 a	0.63 b	23.1 a	52.2 c	30.6 a	463.2 a
75		8.8 b	0.62 b	25.2 b	44.6 b	32.9 ab	489.0 b
150		8.7 a	0.59 a	26.8 c	31.8 a	34.5 b	532.9 c
0	T. Barroca	6.6 a	0.64 c	19.8 a	33.9 a	20.5 a	440.4 a
75		6.7 b	0.60 b	21.6 b	32.3 a	22.2 a	494.8 b
150		7.2 c	0.54 a	22.4 b	31.8 a	22.4 a	516.7 c

\* Means separated by Duncan's multiple range test at the 5 % level; not significantly different when followed by the same letter.

\*\* WCA = E<sup>10</sup> (HCl 1M)

\*\*\* % Polymeric pigment = [(WCA-ACA)/WCA] x 100

direct condensation of anthocyanins with other flavanoid compounds or bind the acetaldehyde could be the explanation of this decrease. BAKKER (1986) observed the lowest percentage of polymeric pigment in a Porto wine made with 100 mg/l of SO<sub>2</sub> compared to the control wine. The percentage of polymeric pigment was negatively correlated ( $r = -0.74$ ;  $P < 0.05$ ) with the amount of total anthocyanins.

When SO<sub>2</sub> is added during fermentation the color of the wine is affected. The influence of the SO<sub>2</sub> is different for each of the fractions of the color complex and is also dependent on grape variety.

### Acknowledgements

The authors thank Dr. J. M. RICARDO-DA-SILVA for his helpful advice, G. RODRIGUES and M. J. BARATA for the technical assistance and EC for funding this work under FLAIR project (No. 89053).

### References

- AUBERT, S.; POUX, C.; 1969: Extraction des composés phénoliques du raisin. II. Taux de passage dans les vins. *Ann. Technol. Agric.* **18**, 111-127.
- BAKKER, J.; 1986: HPLC of anthocyanins in port wines: Determination of ageing rates. *Vitis* **25**, 203-214.
- -; PRESTON, N. W.; TIMBERLAKE, C. F.; 1986: The determination of anthocyanins in ageing red wines; comparison of HPLC and spectral methods. *Amer. J. Enol. Viticult.* **37**, 121-126.
- BERG, H. W.; AKIYOSHI, M.; 1962: Color behavior during fermentation and ageing of wines. *Amer. J. Enol. Viticult.* **13**, 126-132.
- BERTRAND, A.; GAUTHIER, M.; SALAGOITY-AUGUST, M.-H.; 1982: Etude de l'évolution des anthocyanes en fonction de la durée de macération. *Connaiss. Vigne Vin* **16**, 145-148.
- BOURZEIX, M.; 1976: Les composés phénoliques des raisins et du vin. Leurs effets sur la qualité. *Rev. Franç. Oenol.* **63**, 53-69.
- -; HEREDIA, N.; KOVAC, V.; 1983: Richesse de différents cépages en composés phénoliques totaux et en anthocyanes. *Prog. Agric. Vitic.* **17**, 421-428.
- FLANZY, M.; BOURZEIX, M.; HEREDIA, N.; DUBERNET, M.; 1972: La teneur et la répartition des divers composés phénoliques dans le raisin et la rafle de douze cépages. *C. R. Acad. Agricult. France* **58**, 452-460.
- FONG, R. A.; KEPNER, R. E.; WEBB, A. D.; 1971: Acetic-acid-acylated anthocyanin pigments in the grape skins of a number of varieties of *Vitis vinifera*. *Amer. J. Enol. Viticult.* **22**, 150-155.
- GLORIES, Y.; 1984: La couleur des vins rouges, 2<sup>ème</sup> partie. Mesure originale et interprétation. *Connaiss. Vigne Vin* **18**, 195-217.
- GUETOV, G.; NEDELTCHEV, N.; TZAKOV, O.; 1972: Evolution des anthocyanes au cours de la vinification et de la conservation des vins. *Bull. O.I.V.* **43**, 338-345.

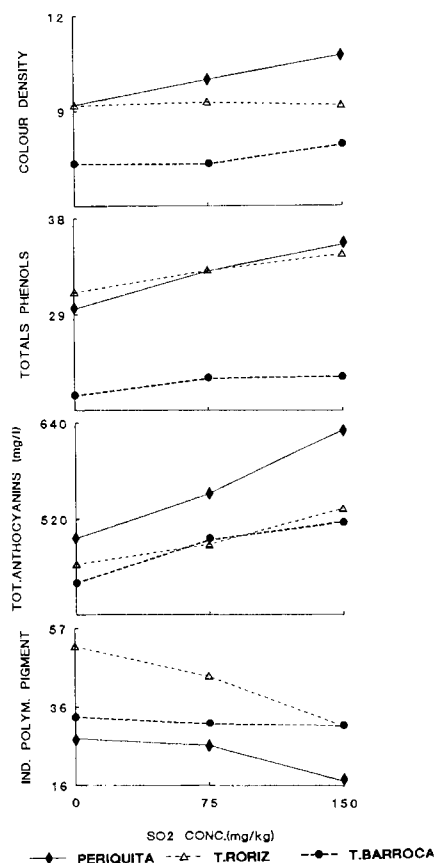


Fig. 5: Effects of SO<sub>2</sub> on the color density, total phenols, total anthocyanins and percentage of polymeric pigments of Periquita, Tinta Roriz and Tinta Barroca wines.

- HEBRERO, E.; SANTOS-BUELGA, C.; RIVAS-GONZALO, J. C.; 1988: High performance liquid chromatography-diode array spectroscopy identification of anthocyanins of *Vitis vinifera* variety Tempranillo. *Amer. J. Enol. Viticult.* **39**, 227-233.
- LAUREANO, O.; VIEIRA, M.; 1983: O desengace na composição fenólica do vinho tinto. *Enologia* **2**, 16-25.
- NAGEL, C. W.; WULF, L. W.; 1979: Changes in the anthocyanins, flavanoids and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet Sauvignon. *Amer. J. Enol. Viticult.* **30**, 111-116.
- PIERGIOVANNI, L.; VOLONTERIO, G.; 1980: Tecnica cromatografica nello studio della frazione antocianica delle uve. *Riv. Viticolt. Enol.* **33**, 289-308.
- POUX, C.; AUBERT, S.; 1968: Corrélation entre la constitution en composés phénoliques des vins, la température de vinification et les caractéristiques des vins, la température de vinification et les caractéristiques générales de l'année. *Ann. Technol. Agric.* **17**, 299-313.
- RIBÉREAU-GAYON, J.; PEYNAUD, E.; SUDRAUD, P.; RIBÉREAU-GAYON, P.; 1972: Sciences et techniques du vin. Vol. 1. Analyse et contrôle des vins. Dumond, Paris.
- ; STRONESTREET, E.; 1965: Le dosage des anthocyanes dans le vin rouge. *Bull. Soc. Chim. France* **9**, 2649-2655.
- ; SUDRAUD, P.; MILHÉ, J. C.; CAMBAS, R.; 1970: Recherches technologiques sur les composés phénoliques des vins rouges. II. Les facteurs de dissolution des composés phénoliques. *Connaiss. Vigne Vin* **4**, 133-144.
- ROGGERO, J. P.; ARCHIER, P.; COEN, S.; 1992: Étude par HPLC des compositions phénoliques et anthocyaniques d'un moût de raisin en fermentation. *Sci. Aliments* **12**, 37-46.
- ; COEN, S.; RAGONNET, B.; 1986: High performance liquid chromatography survey on changes in pigment content in ripening grapes of Syrah. An approach to anthocyanin metabolism. *Amer. J. Enol. Viticult.* **37**, 77-83.
- ; LARICE, J. L.; ROCHEVILLE-DIVORNE, C.; ARCHIER, P.; COEN, S.; 1988: Composition anthocyanique de cépages. I - Essai de classification par analyse en composantes principales et par analyse factorielle discriminante. *Rev. Franç. Oenol.* **112**, 41-48.
- ; RAGONNET, B.; COEN, S.; 1984: Analyse fine des anthocyanes des vins et des pellicules de raisin par la technique HPLC. Étude de quelques cépages méridionaux. *Vignes Vins* **327**, 38-42.
- SOMERS, T. C.; EVANS, M. E.; 1977: Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO<sub>2</sub>, 'chemical age'. *J. Sci. Food Agricult.* **28**, 279-287.
- ; --; 1979: Grape pigment phenomena: Interpretation of major colour losses during vinification. *J. Sci. Food Agricult.* **30**, 623-633.
- SUDRAUD, P.; 1964: Étude expérimentale de la vinification en rouge. Thèse Ingénieur-Docteur Bordeaux, France.
- TIMBERLAKE, C. F.; BRIDLE, P.; 1976: The effect of processing and other factors on the color characteristics of some red wines. *Vitis* **15**, 37-42.
- WULF, L. W.; NAGEL, C. W.; 1978: High pressure liquid chromatographic separation of anthocyanins of *Vitis vinifera*. *Amer. J. Enol. Viticult.* **29**, 42-49.

Received July 6, 1993