

# Characterisation of Colour Components and Polymeric Pigments of Commercial Red Wines by Using Selected UV-Vis Spectrophotometric Methods

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**The composition of the colour components of commercial red wines from Italy was analysed in terms of total colour, co-pigmentation, SO<sub>2</sub>-resistant pigments, small and large polymeric pigments, and tannins. A total of 128 wines, including Sangiovese, Cabernet Sauvignon, Nero d'Avola, Merlot, Marzemino, Negroamaro, Aglianico, Cannonau and Rossese di dolceacqua from five vintages (1999 to 2003) were analysed. The composition of the wines showed significant differences in terms of total colour and co-pigmentation. Total colour at 520 nm was highest in Marzemino (7.50±1.5 AU, absorbance units), and then in Aglianico (7.10±2.8 AU) and Cabernet Sauvignon (5.01±2.50 AU). The level of large and small polymeric pigments in Sangiovese wines showed a range from 0.05 to 0.40 and 0.10 to 0.27 AU 520 nm respectively. The variation in the level of polymeric pigments is affected by a number of factors, including vintage, grape composition, fermentation and storage conditions.**

## INTRODUCTION

The colour components of wine are important parameters that contribute to the sensory characteristics (*e.g.* colour and astringency) and the antioxidant properties of wine (Kanner *et al.*, 1994; Hagerman *et al.*, 1998; Waterhouse, 2002; López-Vélez *et al.*, 2003). The colour of red wine is the result of an interesting and challenging, complex mixture of several components, including free monomeric anthocyanins (Mazza, 1995), the enhancement of their colour due to co-pigmentation with other non-coloured phenolics (Boulton, 2001), and polymeric pigments (Somers, 1971). The colour of a red wine continues to change throughout its life and can be affected by a number of winemaking practices and environmental conditions. During fermentation, and especially during aging, the monomeric anthocyanins are thought to be gradually incorporated into polymeric pigments and this confers colour stability to a wine (Singleton and Esau, 1969; Somers and Evans, 1974; Ribéreau-Gayon, 1982). The mechanisms, the products and the kinetics of polymer formation in wine are only partially known (Fulcrand *et al.*, 2004; Kennedy and Hayasaka, 2004).

Objective measurement of the components of wine colour is an essential part of the modern concept of winemaking. In this view, the availability of new methods for the measurement of polymeric pigments provides important tools for a better understanding of the chemistry of red wine colour. The spectrophotometric assays developed thus far are based on the assumption that the polymeric pigments are much less sensitive than the anthocyanins to SO<sub>2</sub> and to changes in pH. The classic spectrophotometric assay of Ribéreau-Gayon and Stonestreet (1965) measures the polymeric pigments of wine after the bisulphite bleaching of anthocyanin

monomers carried out on an acidified sample (pH <1). Somers and Evans (1974; 1977) have developed a set of spectrophotometric measures to determine the amount of total anthocyanins and polymeric pigments in red wines. This procedure is based upon two assumptions: (i) bisulphite bleaches only the monomeric anthocyanins; and (ii) the colour of the polymeric pigments increases by a factor of 5/3 on acidification. Bakker *et al.* (1986) have shown that oligomeric pigments are also bleached to some extent by bisulphite.

Recently, Harbertson *et al.* (2003) proposed a new method for the analysis of the polymeric pigments in wine based on a combination of protein precipitation and the bleaching effect of bisulphite. This method allows for the quantification of two classes of non-bleachable polymeric pigments: small polymeric pigments (SPP) that are not precipitable with protein, and large polymeric pigments (LPP), which are precipitable with protein. The SPP fraction probably contains several anthocyanin-derived compounds in various proportions that have been formed through different mechanisms, such as cross-linking reaction, direct reaction and cyclo-addition. The LPP fraction would be expected to contain anthocyanins that have reacted directly with polymeric flavan-3-ols or polymeric pigments that have been formed by acetaldehyde cross-linking. The levels of SPP and LPP have been monitored in grape berries, and information on their content in commercial wines is limited to Zinfandel, Pinot noir, Cabernet Sauvignon and Syrah (Adams *et al.*, 2004).

The aim of this study was to characterise selected colour components, including co-pigmentation, small and large polymeric pigments and tannins, of a variety of commercial red wines from Italy, especially from Sangiovese grapes.

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## MATERIALS AND METHODS

### Wine samples

A total of 128 samples (n) of commercial bottled red wines from nine regions in Italy, including Sangiovese (n = 70 samples), Cabernet Sauvignon (n = 13), Nero d'Avola (n = 10), Merlot (n = 9), Marzemino (n = 7), Negroamaro (n = 6), Aglianico (n = 5), Cannonau (n = 4), and Rossese di dolceacqua (n = 4), were analysed (see Table 1). The map in Figure 1 shows the regions of origin of the red wines analysed in this study. The wines were analysed at between three and 48 months of age (vintage years: 1999 to 2003).

### Spectrophotometric method of Boulton

For each wine (128 samples), the following parameters were measured by spectrophotometric assay according to the literature: total colour, co-pigmentation and SO<sub>2</sub>-resistant pigments (Boulton *et al.*, 1999). The samples were filtered through a 0.45 µm polytetrafluoroethylene membrane (PTFE) syringe filter and the wine pH was adjusted to 3.60, before spectrophotometric analysis was done using a Shimadzu 1240 model (Shimadzu, Milan, Italy). Twenty microlitres of 10% (v/v) acetaldehyde was added to 2 mL of wine and, after 45 min at room temperature, the *total colour* of the wine was measured at 520 nm. To another 2 mL of wine, 160 µL of 5% (w/v) SO<sub>2</sub> was added, and then the value of the SO<sub>2</sub>-resistant pigments was measured at 520 nm. For *co-pigmentation*, the absorbance of wine at 520 nm was read after a sample dilution of 1:19 in a 12% ethanol, pH 3.60 potassium bitartrate buffer, corrected for the dilution. There are pH effects on the free monomeric anthocyanin ionisation, the colour of the co-pigmented form, and the coloured polymers. The selected pH value (3.60) provides the only rational basis on which to compare colour components, which are independent of pH effects across all wines.

### Spectrophotometric method of Adams

A set of 40 Sangiovese wines from the Emilia-Romagna region was further analysed by spectrophotometric methods as described in the literature for the following parameters: small and large polymeric pigments (Harbertson *et al.*, 2003), and tannin (Harbertson *et al.*, 2002).

Simultaneous analysis of the *polymeric pigments* and *tannin* in wines required two 1.5 mL microfuge tubes for each sample. The first tube was made up by dispensing 1 mL of the acetic acid/



FIGURE 1

Map of Italy showing the origin of the red wines analysed in this study. Regions: (A) Campania; (B) Emilia-Romagna; (C) Liguria; (D) Puglia; (E) Sardegna; (F) Sicilia; (G) Trentino; (H) Umbria; (I) Veneto.

NaCl buffer (containing 200 mM acetic acid and 170 mM NaCl adjusted to pH 4.9 with NaOH) into the tube and then adding 500 µL of the diluted wine (with a buffer of 12% aqueous ethanol (v/v), containing 5 g/L potassium bitartrate adjusted to pH 3.3 with HCl). One mL of the mixture was transferred to a cuvette and the absorbance at 520 nm was determined (*reading A*). Then 80 µL of 0.36 M potassium metabisulphite was added and mixed, and the absorbance at 520 nm was redetermined after a 10 min incubation (*reading B*). For the second tube, 1 mL of the acetic acid/NaCl buffer containing bovine serum albumin at 1 mg/mL (BSA Fraction V powder, Sigma, Milan, Italy) was put into a 1.5 mL microfuge tube, and 500 µL of the diluted wine was added. The mixture was allowed to incubate at room temperature for 15 min with slow agitation. After incubation, the sample was cen-

TABLE 1

Origin, type and number of red wines from the different regions of Italy.

Code	Region	Wine (number of samples)	Total
A	Campania:	Aglianico (5)	5
B	Emilia-Romagna:	Merlot (2), Sangiovese (68), Cabernet Sauvignon (11)	81
C	Liguria:	Rossese di dolceacqua (4)	4
D	Puglia:	Sangiovese (2), Negroamaro (6)	8
E	Sardegna:	Cannonau (4)	4
F	Sicilia:	Merlot (2), Nero d'Avola (10)	12
G	Trentino:	Merlot (1), Marzemino (7)	8
H	Umbria:	Merlot (1)	1
I	Veneto:	Merlot (3), Cabernet Sauvignon (2)	5
Total			128

trifuged for 5 min at 13 500 g to pellet the tannin-protein precipitate. One millilitre of the supernatant was put into a cuvette, 80 µL of 0.36 M potassium metabisulphite was added and, after a 10 min incubation, the absorbance was determined at 520 nm (*reading C*). The absorbance due to MP, SPP and LPP is given as (A-B), C and (B-C) respectively.

The absorbance due to polymeric pigments in the protein-tannin precipitate was determined by redissolving the tannin-protein precipitate (from the second tube above) and measuring the absorbance at 510 nm. The tannin-protein pellet from tube two was first washed with 250 µL of the acetic acid/NaCl buffer, and the sample was recentrifuged for 1 min at 13 500 g. The wash solution was discarded, after which 875 µL of a buffer containing 5% triethanolamine (TEA) (v/v) and 5% sodium dodecyl sulphate (SDS; lauryl sulphate, sodium salt, Sigma, Milan, Italy) (w/v) was added. After incubation for 10 min at room temperature, the tube was vortexed and the absorbance at 510 nm was determined. This absorbance represents the amount of polymeric pigments that precipitated with the protein. For tannin analysis, 125 µL of the ferric chloride hexahydrate (Sigma, Milan, Italy), was added and the absorbance at 510 nm was determined after a 10 min incubation. The absorbance due to tannins in the sample is calculated as the final absorbance minus the background (absorbance value due to the reagents without the sample) and expressed in catechin equivalents by comparison with a standard curve pre-

pared with standard solution of (+)-catechin (Extrasynthese, Genay, France), using eight concentration levels ranging from 50 to 500 mg/L ( $R^2 = 0.999$ ). All data are expressed in absorbance units (AU) with 1 cm length path and corrected by dilutions, unless otherwise specified.

### Statistical analysis

The statistical analysis of the data, including linear regression analysis, analysis of variance (ANOVA) and post-hoc comparison by Tukey HSD test for unequal sample size at 5% probability level ( $p \leq 0.05$ ), was performed using Statistica 5.1 (StatSoft, Tulsa, OK).

### RESULTS AND DISCUSSION

This investigation was initiated as a survey of Sangiovese wines because of the economic importance of this grape cultivar in the Emilia-Romagna region. Taking into account the scientific and practical interest in the analysis of the colour components of red wine and the availability of additional wines, it was decided to include wines from two international varieties in this study, viz. Cabernet Sauvignon and Merlot, as well as wines from local emerging grape varieties, such as Cannonau, Marzemino and Nero d'Avola.

The first step in the study focused on the analyses of total colour, co-pigmentation, and SO<sub>2</sub>-resistant pigments in the wines using Boulton's method. The composition of the wines showed a

TABLE 2

Composition and statistical analysis of 128 red wines from Italy. All parameters are expressed as AU at 520 nm.

Wine	N. sample	Total color	Copigmentation	SO <sub>2</sub> resistant pigments
		Mean $\pm$ SD*	Mean $\pm$ SD*	Mean $\pm$ SD**
Sangiovese	70	4.17 $\pm$ 1.31a	0.35 $\pm$ 0.38a	2.36 $\pm$ 1.00
Cabernet Sauvignon	3	5.01 $\pm$ 2.50ab	0.41 $\pm$ 0.42a	3.25 $\pm$ 2.16
Cannonau	4	3.71 $\pm$ 0.87a	0.71 $\pm$ 0.64ab	1.65 $\pm$ 0.76
Merlot	9	4.70 $\pm$ 1.63a	0.60 $\pm$ 0.37ab	2.09 $\pm$ 0.99
Aglianico	5	7.10 $\pm$ 2.90b	0.39 $\pm$ 0.30ab	3.03 $\pm$ 0.86
Negroamaro	6	4.51 $\pm$ 1.19a	0.80 $\pm$ 0.53ab	2.29 $\pm$ 0.78
Marzemino	7	7.50 $\pm$ 1.50b	1.18 $\pm$ 0.63b	1.91 $\pm$ 0.40
Nero d'Avola	10	3.68 $\pm$ 1.18a	0.32 $\pm$ 0.30a	1.85 $\pm$ 0.57
Rossese	4	2.48 $\pm$ 0.34a	0.62 $\pm$ 0.66ab	0.86 $\pm$ 0.32

\*Group comparisons by means of Tukey HSD test for unequal sample size. Means within each column with changed letter are significantly different using Tukey HSD test ( $P \leq 0.05$ ).

\*\*Non-significant difference at  $p$ -level  $\leq 0.05$ .

TABLE 3

Composition of 40 Sangiovese wines from Emilia-Romagna region, Italy.

Year	No samples	LPP	SPP	LPP+SPP	LPP/SPP	Tannins
		AU 520 nm	AU 520 nm	AU 520 nm	–	g/l
		mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD
2003	1	0.33	0.71	1.04	0.46	0.23
2002	14	1.09 $\pm$ 0.37	0.99 $\pm$ 0.33	2.08 $\pm$ 0.61	1.17 $\pm$ 0.43	0.49 $\pm$ 0.19
2001	14	1.21 $\pm$ 0.35	1.15 $\pm$ 0.32	2.35 $\pm$ 0.35	1.13 $\pm$ 0.38	0.67 $\pm$ 0.29
2000	8	1.64 $\pm$ 0.41	1.18 $\pm$ 0.38	2.82 $\pm$ 0.57	1.56 $\pm$ 0.69	0.88 $\pm$ 0.44
1999	3	1.35 $\pm$ 0.23	1.11 $\pm$ 0.24	2.46 $\pm$ 0.35	1.25 $\pm$ 0.37	0.82 $\pm$ 0.04

significant difference in terms of total colour and co-pigmentation, while there was no significant difference between the wines regarding SO<sub>2</sub>-resistant pigments (see Table 2). Marzemino and Aglianico showed the greatest total colour at 520 nm, followed by Cabernet Sauvignon, and then the others wines. The level of co-pigmentation was highest in Marzemino wines, and lowest in Sangiovese. The highest content of SO<sub>2</sub>-resistant pigments was found in Cabernet Sauvignon ( $3.25 \pm 2.16$  AU 520 nm), whereas Sangiovese wines showed an intermediate level of SO<sub>2</sub>-resistant pigments ( $2.36 \pm 1.00$  AU 520 nm). In terms of colour, Marzemino and Aglianico wines seem to be suitable for aging due to the high level of their total colour and SO<sub>2</sub>-resistant pigments.

The general levels of SO<sub>2</sub>-resistant pigments found in the analysed wines are comparable with the values reported in other papers concerning studies of polymeric pigments in different wines from Canada (Mazza *et al.*, 1999), Italy (Piergiovanni *et al.*, 1986; Boselli *et al.*, 2004), Spain (Gomez-Plaza, *et al.*, 1999), the USA (Nagel and Wulf, 1979), and Australia (Somers and Evans, 1974).

It is well documented in the literature that the grape cultivar affects the amount of total colour and the co-pigmentation of wine to a large extent (Boulton *et al.*, 1999; Mazza *et al.*, 1999; Boselli *et al.*, 2004; Versari *et al.*, 2004). The data presented here support this view. The co-pigmentation of anthocyanins in wines is a competitive equilibrium involving several anthocyanins and many cofactors. Wines made from grapes high in cofactors and/or with a prevalence of acylated forms of the non-malvidin pigments in their anthocyanin pattern may have a higher level of co-pigmentation (Boulton, 2001). This is partly the reason for poorer co-pigmentation in the Sangiovese wines, which lack the acylated pigments (Baldi and Romani, 1992), whereas Merlot and Cabernet Sauvignon wines contain high levels of acylated anthocyanins (Mattivi *et al.*, 1995), followed by Aglianico (Calò *et al.*, 1994) and Cannonau (Castia *et al.*, 1992). Furthermore, co-pig-

mentation is typical of young wines, which can explain up to 50% of the total colour (Boulton, 2001).

To obtain more insight into the composition of Sangiovese wines in terms of colour components, the SO<sub>2</sub>-resistant pigments were analysed using a more selective method proposed by Harbertson *et al.* (2003), which allows the measurement of two groups of polymeric pigments: SPP and LPP. This part of the investigation was conducted on a set of 40 Sangiovese wines that were between three and 48 months old (vintage years: 1999 to 2003) at the time of these analyses. The SO<sub>2</sub>-resistant pigments accounted for the average percentage of 62%, 72%, 65% and 54% of the total colour of four-year-old (1999), three-year-old (2000), two-year-old (2001) and one-year-old (2002) Sangiovese wines respectively. Table 3 shows the level of LPP, SPP and tannins in Sangiovese wines. The comparison between vintages showed an increasing level of LPP, SPP and tannins in Sangiovese wines during the first three years, *i.e.* 2003 to 2000. The variation in the level of polymeric pigments is affected by a number of factors, including vintage, grape composition, fermentation and storage conditions. In particular, the decrease in polymeric pigments may be due to precipitation and/or the lighter hue of the larger polymers. There is general consensus on the increase in LPP content in red wines with age (De Beer *et al.*, 2004).

The levels of LPP (range 0.3 to 2.3 AU) and SPP (range 0.6 to 2.0 AU) found in Sangiovese wines are generally higher than the values reported for wines made from local red grape varieties in the Marche region, Italy (Boselli *et al.*, 2004), and for red wines from the USA (De Beer *et al.*, 2004). The distribution of the LPP/SPP ratio in Sangiovese wines covered a range of 0.15 to 2.78 (see Figure 2), and was in agreement with the values reported on Pinot Noir (range 0.14 to 2.20), Cabernet Sauvignon (0.18 to 3.93), and Syrah (0.28 to 1.71) (Harbertson *et al.*, 2003).

The highest correlation value was found between the determination of SO<sub>2</sub>-resistant pigments by Boulton's assay at pH 3.6 and the sum of LPP and SPP as determined by Adams' assay using

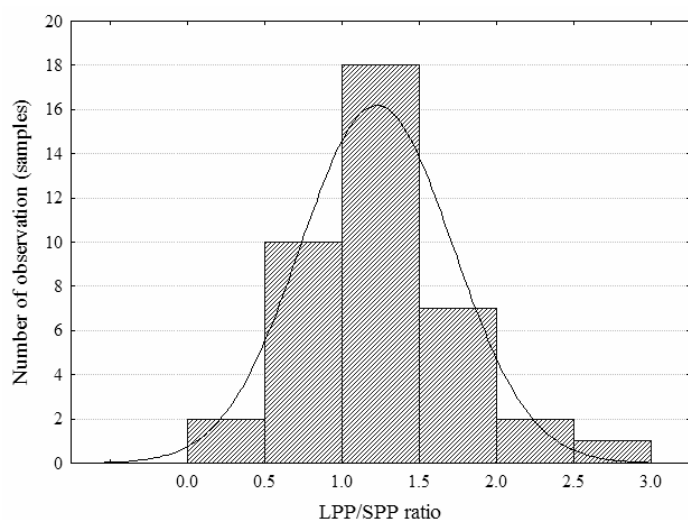


FIGURE 2

Frequency distribution of LPP/SPP ratio found in 40 Sangiovese wines from the Emilia-Romagna region (Italy).

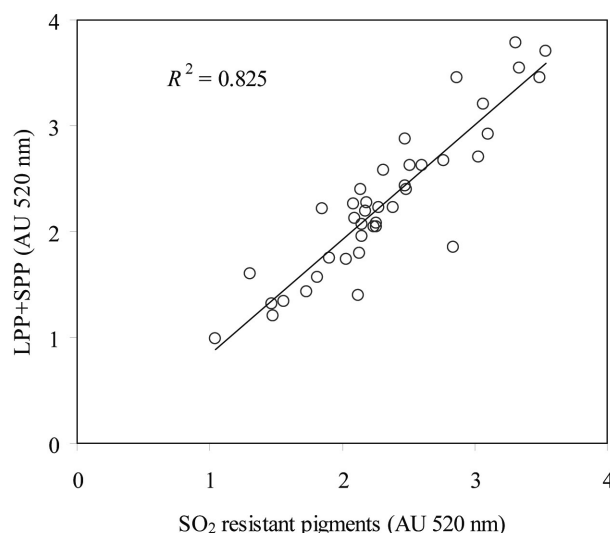


FIGURE 3

Correlation between the amount of SO<sub>2</sub>-resistant pigments determined by Boulton's assay and LPP+SPP determined by Adams's assay found in 40 Sangiovese wines from the Emilia-Romagna region (Italy).



combined protein precipitation and bisulphate bleaching at pH 4.9 ( $r = 0.91$ ;  $R^2 = 0.825$ ) (see Figure 3). This finding confirms that Adams's assay fractionates polymeric pigments into two classes, those that precipitate with protein and those that do not (Harbertson *et al.*, 2003).

## CONCLUSIONS

In conclusion, the recent availability of selective UV-Vis methods for the measurement of parameters that have not been taken into account in traditional wine colour measurements represents a valuable opportunity for researchers and winemakers. The choice of a proper analytical method is critical, thought in many cases the results that are obtained differ on the basis of the analytical procedure.

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