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ORIGINAL ARTICLE

# Influence of copigmentation and phenolic composition on wine color

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**Abstract** Chromatic characteristics and their relationships with copigmentation and phenolic composition were studied in 160 bottled red wines. Free anthocyanins, copigmented anthocyanins and polymeric pigments contributing to color were calculated according to Boulton protocol and related to main changes produced in wine visible spectra after destroying any copigmented anthocyanins effect. Color differences between copigmented and non copigmented wines were quantified and related with ageing, cultivar and phenolic profile. Phenomenon of co-pigmentation visually increases the colour at 420, 520 and 620 nm for most of wines. Copigmented wines showed a mean value of 8.26 CIELab units higher than non copigmented ($\Delta E_{ab(c-nc)}$), being this shift deeper for young wines than for aged wines. Copigmentation mostly changed hue and decreased L, a* and b* values therefore resulted into purplish and darker wine. Visual variations in color caused by copigmentation was related to particularly anthocyanins and copigments (mostly flavonols and hydroxycinnamic acids).

**Keywords** Red wine · Color · Copigmentation · CIELab · Phenolic composition

**Highlights**
- Copigmentation decreases hue, $h_{ab}$, and L* CIELab components but increases color intensity
- Visual consequences of copigmentation would be noticeable even by a non-trained person.
- Blended wines show higher copigmentation values than single-cultivar ones.
- Copigmentation visual effects are significantly correlated with the concentration of most flavonols.
- Blue color component seems to be particularly related to wine ageing.

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## Introduction

Wine color is an important quality parameter carefully observed by professional tasters and consumers, as it reports possible deficiencies from the winemaking process and it evolves while ageing (Parpinello et al. 2009).

Anthocyanins are the main compounds involved on red wine color, whose visible expression depends among other factors on pH. Red colored flavylium cation is the major form present in highly acidic media. As pH increases, anthocyanins become partly a flavylium quinone purplish base and partly a non-colored carbinol (Brouillard et al. 1978). Alternatively, this colorless carbinol can be converted into cis- and trans- chalcones, which exhibit light yellow color (Furtado et al. 1993). Anthocyanins might also react with other molecules and produce new pigments (Francia-Aricha et al. 1997; He et al. 2012).

Moreover, wine color is strongly conditioned by copigmentation, a phenomenon based on anthocyanins interactions between themselves or with other molecules, called copigments. This fact reduces the formation of the colorless hydrated base (carbinol) and enhances equilibrium towards color compounds as

described by Mazza and Brouillard (1990). Wine copigments are phenolic acids, flavonoids and amino acids (He et al. 2012).

Copigmentation not only increases wine color (hyperchromic property), but also changes its hue and attributes by bathochromic or hypsochromic shifts; therefore, wine shows different color depending on the copigments available (Brouillard and Dangles 1994). In this sense, red wine color is strongly conditioned by its phenolic content in three groups of substances: free anthocyanins, copigmented anthocyanins and polymeric pigments. Non anthocyanin phenolic compounds (mainly hydroxycinnamic acids and flavonols) can also affect color characteristics through copigmentation with anthocyanins (Darias-Martín et al. 2002).

In this work, the visual influence of the copigmentation on red wine color and its relation with the phenolic content was estimated for the first time in a large number of samples of young and shortly aged wines. Moreover, colorimetric differences were also quantified and related with ageing and cultivars used. A new methodology (Garcia-Marino et al. 2013), which determines such influence in the CIELab color space, was used to establish the influence of co-pigmentation on the color, in addition to the method developed by R.B. Boulton (1996).

## Material and methods

### Samples

A total of one hundred sixty bottled red wines from vintages 2008–2012 were selected. All samples accomplished with legal quality standards for commercial wines and were stored at 20 ± 5 °C until analysis. Cultivar distribution was 70 Listán Negro (LN), 21 Baboso (B), 14 Listán Prieto (LP), 7 Castellana (C), 6 Vijariego (V), 6 Syrah (S), 6 Negramoll (N), 6 Merlot (M), 6 Tintilla (T), 6 Ruby Cabernet (R) and 12 Blending (BL) of LN and N cultivars.

### Analytical methods

#### *Wine color characteristics*

All spectrophotometric measurements were obtained with a λ25 Perkin-Elmer spectrophotometer. A "synthetic wine" was used as blank and for any sample dilution (12 % ethanol, 5 g/l tartaric acid and 3.6 pH, all chemicals from Panreac, Spain).

Wine color intensity (ICM = $A_{420} + A_{520} + A_{620}$) and color hue ($A_{420}/A_{520}$) were determined following Glories methodology (1984). Tristimulus CIELab parameters ($h_{ab}$*, L*, C*, a* and b*) were determined following recommendations of the Commission Internationale de L'Eclariage (OIV 2014) in a 0.1 cm path length cuvette (Hanna, USA) using the 380–780 nm wine spectra. Absorbance measurements were automatically corrected to 10 mm path length.

Wine spectrum was firstly obtained at its natural pH, then with pH adjusted to 3.6 (adding HCl or NaOH 0.1 N depending on the wine initial pH), and finally from a 20 rate wine dilution, which avoids any copigments color effect. CIELab coordinates for non-copigmented wines were estimated using diluted samples wavelengths measurements (Garcia-Marino et al. 2013). This dilution leads to copigments-anthocyanin structures destruction, therefore only free anthocyanins and polymeric pigment color fractions remained and wine color without copigmentation can be measured. Absorption results were multiplied by the dilution factor (20) and non-copigmented wine CIELab coordinates calculated using $A_{450}$, $A_{520}$, $A_{570}$ and $A_{630}$ following Pérez Caballero et al. (2003) procedure once copigmentation complexes were completely dissociated.

Color differences between two color points in the CIELab space ($\Delta E_{ab}$) were calculated as the Euclidean distance between their locations in a three dimensional scale following Gonnet (1999):

$$\Delta E_{ab} = \left(\Delta L^2 + \Delta a^2 + \Delta b^2\right)^{0.5} \quad (1)$$

Therefore, color differences between copigmented/uncopigmented wines with pH adjusted can be defined as:

$$\Delta E_{ab(c-nc)} = \left[(L_c - L_{nc})^2 + (a_c - a_{nc})^2 + (b_c - b_{nc})^2\right]^{0.5} \quad (2)$$

Wines color visual descriptors were determined by the Perkin-Elmer Colvin software following the CIELab scale described by the UNE 72031/83 standard.

#### *Copigmentation*

Free anthocyanins, copigmented anthocyanins and polymeric pigments color fractions were obtained according to Boulton (1996). Wine was previously filtered (0.45 μm) and pH adjusted to 3.6.

Total wine color ($A^{acet}$) was quantified by measuring absorbance at 520 nm after elimination of any $SO_2$ bleaching effect; that is, adding 20 μl of 10 % acetaldehyde to 2 ml of wine and performing the measurement after reaction time (45 min.). Color due to polymeric pigments ($A^{SO2}$) was evaluated by measuring absorbance at 520 nm after a 160 μl addition of 5 % $SO_2$ solution to 2 ml wine. Color without copigmented anthocyanins is assumed to be $A^{20}$, obtained by measuring at the same wavelength (520 nm) the wine dilution prepared with "synthetic wine" and multiplying by the dilution factor (×20). This dilution leads to copigment complexes dissociation while free anthocyanins and polymeric pigments color contributions remain constant. All absorbance

readings were converted to 10 mm path length and color contribution fractions were calculated as follows:

$$\text{Free anthocyanins color fraction}\left(X_{\text{Free Anthocyanin}}\right) = \left(A^{20}–A^{SO2}\right)/A^{acet} \quad (3)$$

$$\text{Copigmented anthocyanins color fraction}\left(X_{\text{Copigmentation}}\right) = \left(A^{acet}–A^{20}\right)/A^{acet} \quad (4)$$

$$\text{Polymeric pigments color fraction}\left(X_{\text{Polymeric Pigment}}\right) = A^{SO2}\Big/A^{acet} \quad (5)$$

Phenolic compounds were estimated at 280 nm ($A_{280}$). Flavonols cofactor content was obtained directly with a 365 nm measurement ($A_{365}$). Hydroxycinnamic acids were measured at 320 nm ($A_{320}$) and flavonoids were quantified based on hydroxycinnamic acids and phenol content. Monomeric anthocyanins were obtained following Cayla et al. (2002) with an acidic dilution and a 520 nm measurement.

*Individual phenolic compounds*

Main wine phenolic compounds were quantified by using HPLC-DAD procedure described by Ibern-Gómez et al. (2002). Separation was performed on a Waters 2690 and detection with a Waters 996 Photodiode Array Detector (DAD). 15 μL of previously filtered samples were injected on a thermostated (30 °C) reversed-phase Nova-Pak C18 column (3.9 × 150 mm; 4 μm particle; Waters). Chromatograms were processed at 280, 320, 365 and 520 nm while peaks were identified by their retention times and spectral data. Some compounds were directly compared with external standards and the rest were identified by their relative retention times and spectral data published in similar conditions (Lamuela-Raventós and Waterhouse 1994; Vivar-Quintana et al. 2002; Baiano and Terracone 2011).

Compounds identified were phenolic acids (gallic, syringic, protocatechuic, caftaric, caffeic, coutaric and coumaric acids), flavanols (catechin and epicatechin), flavonols (myricetin, myricetin-3-O-glucoside, myricetin-3-O-glucuronide, laricitrin-3-O-glucoside, kaempferol-3-O-glucoside, isorhamnetin, isorhamnetin-3-O-glucoside, syringetin-3-O-glucoside, quercetin, quercetin-3-O-glucoside, quercetin-3-O-glucuronide and rutin), monomeric anthocyanins (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, cyanidin-3-O-(6-acetyl)-glucoside, petunidin-3-O-glucoside, petunidin-3-O-(6-acetyl)-glucoside, peonidin-3-O-glucoside, peonidin-3-O-(6-acetyl)-glucoside, peonidin-3-O-(6-p-coumaroyl) glucoside, malvidin-3-O-glucoside, malvidin-3-O-(6-acetyl)-glucoside, and malvidin-3-O-(6-p-coumaroyl) glucoside as well as the stilbenoid resveratrol.

Phenols identified at 280 nm were quantified using gallic acid as standard and expressed as mg- gallic acid equivalent (GAE)/L; hydroxycinnamic acids were identified at 320 nm and expressed as mg- caffeic acid equivalent (CAE)/L; flavonols (365 nm) were quantified as mg- quercetin equivalent (QE)/L; and anthocyanins (520 nm) were quantified with a oenin calibration and expressed as mg- oenin equivalent (OE)/L. Detection and quantification limits were calculated according to the three and ten sigma criterion. Calibration curves were constructed from chromatograms as peak area (absorbance) vs. concentration (mg-/L). All phenolic standards presented linear calibration curves within the concentration range studied ($r = 0.9942–0.9999$).

*Statistics*

Statistics were performed using SPSS 17.0 and analytical measurements were obtained in triplicate. Correlation analyses were carried out using Pearson coefficient (r). Analysis of variance, simple correlations and multiple regressions were considered statistically significant with at least $p < 0.05$.

## Results and discussion

### Parameters of color

There are different hues for red wine color, most of the used descriptors were violet red, purple red, garnet red, cherry red, ruby red, brick red, chesnut red and brown red, described in a scale from less evolved wine colors (violet red = 1) to a maximal oxidation influence in color (brown red = 8). Main red colors developed by wines were violet (1) and purple red (2). Shortly aged red wines (2008, 2009 and 2010 vintages) presented higher ($p < 0.05$) values of hue ($A_{420}/A_{520}$), b* and $h_{ab}$* (data not shown) than the young red wines produced in 2011 and 2012.

*pH influence on color*

pH influences in red wine color are well known (Torskangerpoll and Andersen 2005; Kontoudakis et al. 2011;). Boulton (1996) recommended homogenizing all samples at pH 3.6 before pursuing any color measurement in order to avoid its critical influence. In the present study, color measurement was done directly at wine natural pH and also after pH adjustment in order to observe significant change in the colorimetric characteristics.

For 10 % of samples, changes in visual color descriptors (e.g. from violet red to granet red) were already involved with pH adjustment, being this change to darker or brighter hues

depending on the wine initial pH. Consequently, changes in absorbance ($\Delta A_{420}$, $\Delta A_{520}$, $\Delta A_{620}$), color intensity ($\Delta ICM = \Delta A_{420} + \Delta A_{520} + \Delta A_{620}$), and CIELab ($\Delta L^*_{winepH\text{-}pH3.6}$, $\Delta b^*_{winepH\text{-}pH3.6}$, $\Delta a^*_{winepH\text{-}pH3.6}$) were either positive or negative depending on the initial pH of wine. Most of wines with an "acid" (initial $pH_{wine} < 3.6$) decreased their L* value once pH was adjusted, whereas wines with a "basic" (natural $pH_{wine} > 3.6$) increased their L* coordinate when pH was adjusted. Hence as pH decreased wine color became lighter (higher L*). Similarly, chromacity (C*), red/green (a*), and yellow/blue (b*) coordinates evolved to lower values when pH was increased. Therefore, red and yellow tonalities increase as wine pH becomes lower, giving more "lively" tonalities and red hot hues.

Consequently, when pH was increased for most naturally "acid" wines, the hue ($A_{420}/A_{520}$) increases and color evolves to more oxidized tonalities. Similarly, 95 % of those naturally "basic" wines showed lower hues when pH decreased therefore their natural color changed from oxidized shades to more "young" looking red wines.

$\Delta E_{ab(winepH\text{-}pH3.6.)}$ evaluates global colorimetric differences between wines at their initially natural pH and once it is standardized. According to Martínez et al. (2001) $\Delta E_{ab}$ values as low as 2.7 CIELab Units (C.U.) represent chromatic changes commonly perceived by the human eye; average $\Delta E_{ab(winepH\text{-}pH3.6.)}$ was 3.88 ± 3.53 and almost half of the samples presented chromatic differences greater than 2.7 C.U. Therefore, these color changes, exclusively due to modifications of pH, would be visually detected in 50 % of the cases.

*Copigmentation derived colorimetric changes*

Color changes due to copigmentation are detailed in Table 1. Copigmentation modified wine color ranging from 1.52 to 23.31 C.U., with a mean value of 8.26 ± 4.17 C.U. Garcia-Marino et al. (2013) reported that copigmentation color varies from 5.9 to 14.9 C.U., developing blending wines the highest $\Delta E_{ab(c\text{-}nc)}$. In this study, blending (BL) also exhibited high copigmentation color changes in comparison with most samples. Cultivars used for blending (LN and N) displayed lower colorimetric changes due to copigmentation when they were analyzed separately in comparison to the visual changes observed when the same cultivars were part of blending wines. This probably means that wine blending enhanced the visual effect of copigments and the expression of anthocyanins, increasing copigmentation visual effects in the final color. Red wines produced from N and R cultivars showed higher $\Delta E_{ab(c\text{-}nc)}$ values than the remaining ($p < 0.05$). Furthermore, copigmentation influenced the color which was detected by a non-trained person in 93 % of the samples ($\Delta E_{ab(c\text{-}nc)} > 2.7$ C.U.).

$\Delta L^*_{(c\text{-}nc)}$ was negative for all cultivars; supporting the assumption that copigmentation prevents the colour evolution of wine while aging, maintaining darker colors. Lightness decreased due to copigmentation which was maximal for Listán Prieto (LP) cultivar (−12.5 C.U.); $\Delta b^*_{(c\text{-}nc)}$ variation due to copigmentation revealed to be negative for most samples; therefore copigmentation brought changes to smaller yellow chromacities. This $\Delta b^*_{(c\text{-}nc)}$ negative trend also suggested that copigmentation decreased wine hue. In fact, hue [$\Delta(A_{420}/A_{520})_{(c\text{-}nc)}$] changed, showing lower values when copigmentation was present. This suggested that copigmentation contributed to less oxidized hues, mainly because it resulted in higher increase $A_{520}$ than $A_{420}$. Copigmentation increased absorbance at almost every wavelength ($\Delta A_{420(c\text{-}nc)}$, $\Delta A_{520(c\text{-}nc)}$, $\Delta A_{620(c\text{-}nc)}$), being maximal at 520 nm (e.g. 1.60 ± 1.36 U.A. for R). Color intensity also changed ($\Delta ICM_{(c\text{-}nc)} = \Delta A_{420(c\text{-}nc)} + \Delta A_{520(c\text{-}nc)} + \Delta A_{620(c\text{-}nc)}$), presenting highest average value (2.44 ± 2.18 U.A.) for R. R cultivar also showed the highest average copigmentation factor (Table 3).

All these qualitative changes could be interpreted in a visual way stating that copigmentation diminished wine clarity, increasing dark red colors. Copigmentation also decreased the yellow component (b*) and therefore wines evolve to lower yellow hues. Additionally, this phenomena increased color in all its components ($A_{420}$, $A_{520}$, $A_{620}$), but particularly at $A_{520}$, producing changes in hue and evolving wine color to darker red hues; just like a decrease in wine pH increases global wine color.

Colorimetric results according to geographical area (data not shown) were similar to those already explained by cultivar, wines from warm areas developed higher $\Delta ICM_{(c\text{-}nc)}$ and $\Delta E_{ab(c\text{-}nc)}$ values. This fact is consistent with a previous study (Heras-Roger et al. 2014) where wines from warm areas developed higher color intensity and copigmentation factors.

Copigmentation results ($X_{Copigmentation}$) and related change in color ($\Delta E_{ab(c\text{-}nc)}$) according to the age of wines are presented in Table 2. The influence of copigmented anthocyanins on wine color decreased with ageing, being maximal for young wines (2011–2012), although no significant differences were observed. In contrast, polymeric pigment color factor ($X_{Polymeric\ Pigment}$) significantly increased with ageing, presenting its maximum for wines produced in 2008. Vintages data indicated role of copigmentation factor ($X_{Copigmentation}$) in bringing the color changes ($\Delta E_{ab(c\text{-}nc)}$). Vintages developing relatively high copigmentation factors also presented important chromatic variations ($\Delta L^*_{(c\text{-}nc)}$, $\Delta a^*_{(c\text{-}nc)}$, $\Delta b^*_{(c\text{-}nc)}$) due to this phenomenon, although no significant differences were obtained. $\Delta A_{620(c\text{-}nc)}$ decreased with ageing, which forecast a possible inverse relationship with changes derived from copigmentation measured at this wavelength. Young wines with high copigmentation factors developed a higher blue percentage in their color.

**Table 1** Copigmentation influence on color parameters obtained for the red wines produced from different grape cultivars

| | $\Delta E_{ab(c\text{-}nc)}$ (C.U.) | $\Delta L^*_{(c\text{-}nc)}$ (C.U.) | $\Delta a^*_{(c\text{-}nc)}$ (C.U.) | $\Delta b^*_{(c\text{-}nc)}$ (C.U.) | $\Delta h_{ab}{}^*_{(c\text{-}nc)}$ (C.U.) | $\Delta A_{420(c\text{-}nc)}$ (U.A.) | $\Delta A_{520(c\text{-}nc)}$ (U.A.) | $\Delta A_{620(c\text{-}nc)}$ (U.A.) | $\Delta ICM_{(c\text{-}nc)}$ (U.A.) | $\Delta Hue_{(c\text{-}nc)}$ (U.A.) |
|---|---|---|---|---|---|---|---|---|---|---|
| LN | $8.60^{ab}$ | $-4.4^{ab}$ | $-2.1^{ab}$ | $-6.1^{abc}$ | $-4.1^{abc}$ | $0.26^{ab}$ | $0.82^{abcd}$ | $0.14^{abc}$ | $1.22^{abc}$ | $-0.11^{a}$ |
| B | $6.28^{ab}$ | $-2.7^{b}$ | $-2.8^{ab}$ | $-4.3^{abc}$ | $-2.9^{abc}$ | $0.30^{ab}$ | $0.68^{abcd}$ | $0.15^{abc}$ | $1.13^{abc}$ | $-0.09^{a}$ |
| LP | $7.49^{ab}$ | $-4.4^{ab}$ | $-0.2^{b}$ | $-4.4^{abc}$ | $-3.4^{abc}$ | $-0.06^{a}$ | $0.11^{a}$ | $0.05^{a}$ | $0.10^{a}$ | $-0.10^{a}$ |
| C | $6.71^{ab}$ | $-1.7^{b}$ | $-0.4^{b}$ | $-2.6^{c}$ | $-1.9^{c}$ | $-0.14^{a}$ | $0.49^{ab}$ | $0.06^{ab}$ | $0.41^{ab}$ | $-0.12^{a}$ |
| V | $6.67^{ab}$ | $-3.6^{ab}$ | $-1.6^{ab}$ | $-5.3^{abc}$ | $-3.0^{abc}$ | $0.16^{ab}$ | $0.42^{ab}$ | $0.09^{abc}$ | $0.67^{ab}$ | $-0.07^{a}$ |
| N | $11.41^{b}$ | $-7.6^{a}$ | $-1.8^{ab}$ | $-7.9^{ab}$ | $-6.1^{a}$ | $0.17^{ab}$ | $0.48^{ab}$ | $0.19^{abc}$ | $0.84^{abc}$ | $-0.14^{a}$ |
| S | $8.71^{ab}$ | $-4.1^{ab}$ | $-3.9^{ab}$ | $-6.5^{abc}$ | $-4.6^{abc}$ | $0.53^{b}$ | $1.50^{cd}$ | $0.22^{bc}$ | $2.25^{bc}$ | $-0.11^{a}$ |
| T | $4.95^{a}$ | $-2.0^{b}$ | $-2.1^{ab}$ | $-3.5^{bc}$ | $-2.6^{bc}$ | $0.09^{ab}$ | $0.80^{abcd}$ | $0.16^{abc}$ | $1.05^{abc}$ | $-0.08^{a}$ |
| R | $12.18^{b}$ | $-5.8^{ab}$ | $-4.9^{a}$ | $-9.2^{a}$ | $-5.6^{ab}$ | $0.59^{b}$ | $1.60^{d}$ | $0.25^{c}$ | $2.44^{bc}$ | $-0.11^{a}$ |
| M | $6.09^{ab}$ | $-2.8^{b}$ | $-2.5^{ab}$ | $-4.8$ | $-3.1^{abc}$ | $0.18^{ab}$ | $0.55^{abc}$ | $0.13^{abc}$ | $0.86^{abc}$ | $-0.10^{a}$ |
| BL | $10.17^{ab}$ | $-4.8^{ab}$ | $-3.4^{ab}$ | $-8.1^{ab}$ | $-5.3^{abc}$ | $0.36^{ab}$ | $1.29^{bcd}$ | $0.19^{abc}$ | $1.84^{abc}$ | $-0.14^{a}$ |

## Phenolic content and copigmentation

Cultivar copigmentation parameters and those phenolic compounds presenting significant differences are shown in Table 3. Copigmentation influence in color varies importantly between red wines from different grape cultivars. Most red wines presented 50 % of their color due to free anthocyanin, copigmentation levels around 14–26 %, and those cultivars showing high color percentages due to polymeric pigment presented also low copigmentation. Darias-Martín et al. (2007) obtained 22.3 % copigmentation after a year of alcoholic fermentation from exclusively Listán Negro (LN) wines, which is consistent with the 19 % found in this paper (Table 3), where five different vintages were considered.

No significant differences between red wines according to cultivar were obtained for any benzoic acid (gallic, syringic, protocatechuic acid), caffeic acid, tyrosol, epicatechin, or minor anthocyanins (cyanidin, petunidin, peonidin and delphinidin type), and therefore their contents are not shown. However, red wine samples were significantly grouped by cultivar according to malvidin derivatives content and flavonol profiles, as it was previously described by Hermosín-Gutiérrez et al. (2005). Significant differences were also observed for caftaric and coumaric acids, as well as resveratrol and catechine.

**Table 2** Copigmentation and its influence on color according to vintage

| | 2008 $n=6$ | 2009 $n=6$ | 2010 $n=7$ | 2011 $n=95$ | 2012 $n=46$ |
|---|---|---|---|---|---|
| $X_{Copigmentation\ (parts\ per\ unit)}$ | $0.05^{a}$ | $0.12^{a}$ | $0.09^{a}$ | $0.19^{a}$ | $0.18^{a}$ |
| $X_{Free\ Anthocyan\ (parts\ per\ unit)}$ | $0.32^{a}$ | $0.41^{a}$ | $0.40^{a}$ | $0.44^{a}$ | $0.46^{a}$ |
| $X_{Polymeric\ Pigment\ (parts\ per\ unit)}$ | $0.63^{b}$ | $0.47^{ab}$ | $0.51^{ab}$ | $0.37^{a}$ | $0.36^{a}$ |
| $\Delta E_{ab(c\text{-}nc)}$ (CIELab Units) | $2.77^{a}$ | $7.04^{a}$ | $6.15^{a}$ | $8.59^{a}$ | $8.02^{a}$ |
| $\Delta L^*_{(c\text{-}nc)}$ (CIELab Units) | $-1.40^{a}$ | $-3.50^{a}$ | $-2.81^{a}$ | $-4.46^{a}$ | $-3.70^{a}$ |
| $\Delta a^*_{(c\text{-}nc)}$ (CIELab Units) | $-0.39^{a}$ | $-2.03^{a}$ | $-1.40^{a}$ | $-2.24^{a}$ | $-1.95^{a}$ |
| $\Delta b^*_{(c\text{-}nc)}$ (CIELab Units) | $-2.36^{a}$ | $-5.76^{a}$ | $-5.05^{a}$ | $-6.26^{a}$ | $-4.93^{a}$ |
| $\Delta h_{ab(c\text{-}nc)}$ (CIELab Units) | $-2.38^{a}$ | $-2.99^{a}$ | $-3.72^{a}$ | $-4.09^{a}$ | $-3.60^{a}$ |
| $\Delta A_{420(c\text{-}nc)}$ (Absorbance Units) | $0.05^{a}$ | $0.07^{a}$ | $0.12^{a}$ | $0.27^{a}$ | $0.16^{a}$ |
| $\Delta A_{520(c\text{-}nc)}$ (Absorbance Units) | $0.41^{a}$ | $0.27^{a}$ | $0.45^{a}$ | $0.88^{a}$ | $0.60^{a}$ |
| $\Delta A_{620(c\text{-}nc)}$ (Absorbance Units) | $0.07^{a}$ | $0.09^{a}$ | $0.12^{a}$ | $0.14^{a}$ | $0.14^{a}$ |
| $\Delta ICM_{(c\text{-}nc)}$ (Absorbance Units) | $0.53^{a}$ | $0.43^{a}$ | $0.69^{a}$ | $1.29^{a}$ | $0.90^{a}$ |
| $\Delta Hue_{(c\text{-}nc)}$ (Absorbance Units) | $-0.11^{a}$ | $-0.10^{a}$ | $-0.09^{a}$ | $-0.11^{a}$ | $-0.10^{a}$ |

## Correlations

Color parameters and phenolic compounds have been correlated in order to find out relevant relationships. Copigmentation depends on anthocyanins and relationships between themselves or available copigments. Table 4 shows the correlations obtained between visual phenomena and most representative phenolic compounds. Obviously, it was directly related with anthocyanins and flavonols, which may act as copigments. Similarly, ratio of Anthocyanin/Flavonol was significantly correlated with visual changes produced by copigmentation ($Ratio_{Anthocyanin/Flavonol}$-$\Delta ICM_{(c\text{-}nc)}$ $r = 0.571$).

Changes in global intensity due to copigmentation ($\Delta ICM_{(c\text{-}nc)}$) were related with every anthocyanin quantified, especially with malvidin derivatives but also with other phenolic compounds which may act as copigments. Main relationships were obtained with coumaric and coutaric acids, as well as with resveratrol. No relationship was obtained with benzoic acid quantified, while almost every flavonol was significantly related to visual copigmentation changes. Figure 1 described the highly significant correlation ($r = 0.701$) obtained between rutin concentration and the copigmentation fraction in color, revealing the importance of wine copigments in this phenomenon. Similarly, Fig. 2 shows the relationship ($r = 0.346$) between wine global color intensity changes due to copigmentation

**Table 3** Copigmentation parameters and phenolic compounds in red wines

| | LN | B | LP | C | V | N | S | T | R | M | BL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_{Copigmentation\ (parts\ per\ unit)}$ | $0.19^{ab}$ | $0.15^{ab}$ | $0.14^{ab}$ | $0.24^{b}$ | $0.08^{a}$ | $0.11^{ab}$ | $0.21^{ab}$ | $0.16^{ab}$ | $0.26^{b}$ | $0.07^{a}$ | $0.22^{ab}$ |
| $X_{Free\ Anthocyan\ (parts\ per\ unit)}$ | $0.46^{ab}$ | $0.42^{ab}$ | $0.46^{ab}$ | $0.51^{b}$ | $0.34^{a}$ | $0.41^{ab}$ | $0.39^{ab}$ | $0.40^{ab}$ | $0.48^{ab}$ | $0.45^{ab}$ | $0.45^{ab}$ |
| $X_{Polymeric\ Pigment\ (parts\ per\ unit)}$ | $0.35^{ab}$ | $0.43^{abc}$ | $0.40^{abc}$ | $0.25^{a}$ | $0.58^{c}$ | $0.48^{bc}$ | $0.40^{abc}$ | $0.44^{abc}$ | $0.26^{a}$ | $0.48^{bc}$ | $0.32^{ab}$ |
| $A_{365(Flavonols)}$ (Units of Absorbance) | $14.3^{ab}$ | $17.4^{abc}$ | $17.7^{abc}$ | $11.0^{ab}$ | $20.1^{abc}$ | $18.1^{abc}$ | $22.6^{bc}$ | $29.8^{c}$ | $6.2^{a}$ | $20.3^{abc}$ | $10.2^{ab}$ |
| Hydroxycinnamic acids (mg/l caffeic acid) | $283.8^{abc}$ | $262.3^{ab}$ | $297.7^{abc}$ | $298.3^{abc}$ | $236.7^{a}$ | $262.8^{ab}$ | $303.8^{abc}$ | $370.7^{c}$ | $237.8^{a}$ | $343.3^{bc}$ | $298.6^{abc}$ |
| Total Flavonoids (Units of Absorbance) | $29.4^{a}$ | $43.0^{bc}$ | $47.6^{bc}$ | $34.6^{ab}$ | $38.6^{abc}$ | $29.0^{a}$ | $39.4^{abc}$ | $48.4^{c}$ | $26.7^{a}$ | $43.9^{bc}$ | $34.4^{ab}$ |
| Caftaric acid (mg CAE/L) | $62.4^{bc}$ | $34.9^{ab}$ | $79.9^{c}$ | $44.6^{abc}$ | $44.4^{abc}$ | $66.9^{bc}$ | $15.8^{a}$ | $30.4^{ab}$ | $18.8^{a}$ | $46.6^{abc}$ | $48.9^{abc}$ |
| Coumaric acid (mg CAE/L) | $6.6^{a}$ | $4.3^{a}$ | $2.1^{a}$ | $4.8^{a}$ | $3.9^{a}$ | $4.0^{a}$ | $15.8^{bc}$ | $20.4^{c}$ | $16.6^{bc}$ | $4.3^{a}$ | $10.2^{ab}$ |
| Resveratrol (mg CAE/L) | $7.6^{bc}$ | $3.9^{ab}$ | $4.8^{abc}$ | $5.1^{abc}$ | $3.4^{ab}$ | $4.1^{ab}$ | $7.2^{abc}$ | $3.2^{a}$ | $6.9^{abc}$ | $8.5^{c}$ | $7.3^{abc}$ |
| Catechin (mg catechin/L) | $36.1^{abc}$ | $45.0^{bcd}$ | $27.1^{ab}$ | $48.1^{cd}$ | $35.0^{abc}$ | $23.1^{a}$ | $44.0^{bcd}$ | $56.6^{d}$ | $33.7^{abc}$ | $34.0^{abc}$ | $44.6^{bcd}$ |
| Malvidin-3-O-gluc. (mg OE/L) | $51.8^{abc}$ | $41.6^{ab}$ | $30.6^{ab}$ | $99.0^{c}$ | $19.9^{ab}$ | $19.9^{ab}$ | $59.1^{abc}$ | $65.8^{bc}$ | $98.9^{c}$ | $13.2^{a}$ | $69.3^{bc}$ |
| Malvidin-6-acet-3-O-gluc.(mg OE/L) | $6.1^{ab}$ | $3.8^{a}$ | $2.7^{a}$ | $4.4^{a}$ | $2.3^{a}$ | $2.1^{a}$ | $13.1^{bc}$ | $8.9^{ab}$ | $18.8^{c}$ | $3.6^{a}$ | $13.9^{bc}$ |
| Malvidin-6-cou-3-O-gluc.(mg OE/L) | $7.9^{a}$ | $7.2^{a}$ | $7.8^{a}$ | $7.2^{a}$ | $4.8^{a}$ | $0.9^{a}$ | $7.6^{a}$ | $23.7^{b}$ | $8.9^{a}$ | $4.1^{a}$ | $10.6^{a}$ |
| Myricetin (mg QE/L) | $8.0^{bcd}$ | $4.9^{ab}$ | $5.0^{ab}$ | $10.2^{cd}$ | $2.9^{a}$ | $4.2^{ab}$ | $11.9^{cd}$ | $7.0^{abc}$ | $12.2^{d}$ | $8.5^{bcd}$ | $10.7^{cd}$ |
| Myricetin-3-O-glucoside (mg QE/L) | $11.6^{bc}$ | $6.5^{ab}$ | $15.0^{c}$ | $8.3^{abc}$ | $8.3^{abc}$ | $12.6^{bc}$ | $2.8^{a}$ | $5.7^{ab}$ | $3.5^{a}$ | $8.6^{abc}$ | $9.2^{abc}$ |
| Quercetin (mg/L) | $1.6^{ab}$ | $2.1^{abc}$ | $4.0^{bc}$ | $2.2^{abc}$ | $1.9^{abc}$ | $2.0^{abc}$ | $2.3^{abc}$ | $2.6^{abc}$ | $1.0^{a}$ | $4.5^{c}$ | $2.2^{abc}$ |
| Quercetin-3-O-glucur. (mg QE/L) | $13.9^{bcd}$ | $9.3^{abc}$ | $14.9^{bcd}$ | $15.6^{bcd}$ | $4.7^{a}$ | $9.2^{abc}$ | $20.4^{d}$ | $7.8^{ab}$ | $18.5^{d}$ | $21.3^{d}$ | $17.2^{cd}$ |
| Isorhamnetin (mg QE/L) | $2.7^{abc}$ | $2.8^{abc}$ | $2.2^{ab}$ | $2.8^{abc}$ | $2.4^{ab}$ | $1.6^{a}$ | $4.3^{c}$ | $3.2^{abc}$ | $2.2^{ab}$ | $3.5^{bc}$ | $3.6^{bc}$ |
| Isorhamnetin-3-O-gluc. (mg QE/L) | $3.5^{abc}$ | $3.6^{abc}$ | $3.9^{abc}$ | $3.8^{abc}$ | $2.9^{ab}$ | $2.1^{a}$ | $5.4^{c}$ | $4.8^{bc}$ | $3.5^{abc}$ | $4.9^{bc}$ | $4.8^{bc}$ |
| Laricitrin-3-O-glucoside (mg QE/L) | $2.7^{abcde}$ | $1.6^{ab}$ | $1.9^{abc}$ | $2.4^{abcd}$ | $1.6^{ab}$ | $2.5^{abcde}$ | $3.5^{de}$ | $3.2^{cde}$ | $4.0^{e}$) | $1.3^{a}$ | $3.1^{bcde}$ |

and myricetin, similar correlations were obtained for almost every flavonol quantified.

Additionally, copigmentation influences on color hue presented negative correlations with anthocyanins and some potential cofactors as caffeic acid, resveratrol, and most of flavonols. However, no significant relationships were obtained with coumaric, coutaric or benzoic acid. Changes in wine hue showed negative correlations with anthocyanins and cofactors because the copigmentation increases $A_{520}$ more than $A_{420}$.

**Table 4** Correlation coefficients (r) between most relevant phenolic compounds and copigmentation parameters

| | $\Delta ICM_{(c\text{-}nc)\ (U.A.)}$ | $\Delta Hue_{(c\text{-}nc)\ (U.A.)}$ | $\Delta E_{ab(c\text{-}nc)\ (C.U.)}$ | $X_{Copigmentation}$ |
|---|---|---|---|---|
| Anthocyanins | 0.641** | −0.254** | 0.350** | 0.656** |
| Malvidin derivatives | 0.501** | −0.256** | 0.268** | 0.682** |
| Malvidin-3-O-(6-acetyl)-glucoside | 0.560** | −0.248** | 0.334** | 0.421** |
| Flavonols | 0.390** | −0.181* | 0.261** | 0.402** |
| Rutin | 0.468** | −0.264** | 0.252** | 0.701** |
| Myricetin | 0.586** | −0.254** | 0.346** | 0.616** |
| Laricitrin-3-O-glucoside | 0.356** | −0.306** | 0.284** | 0.321** |
| Resveratrol | 0.417** | −0.214** | 0.227** | 0.456** |
| Caffeic acid | 0.075 | −0.294** | 0.162 | 0.200** |
| Coumaric acid | 0.431** | −0.150 | 0.284** | 0.271** |
| Coutaric acid | 0.274** | −0.110 | 0.240** | 0.257** |

Correlation is significant at 0.01 (**) and 0.05 (*) level

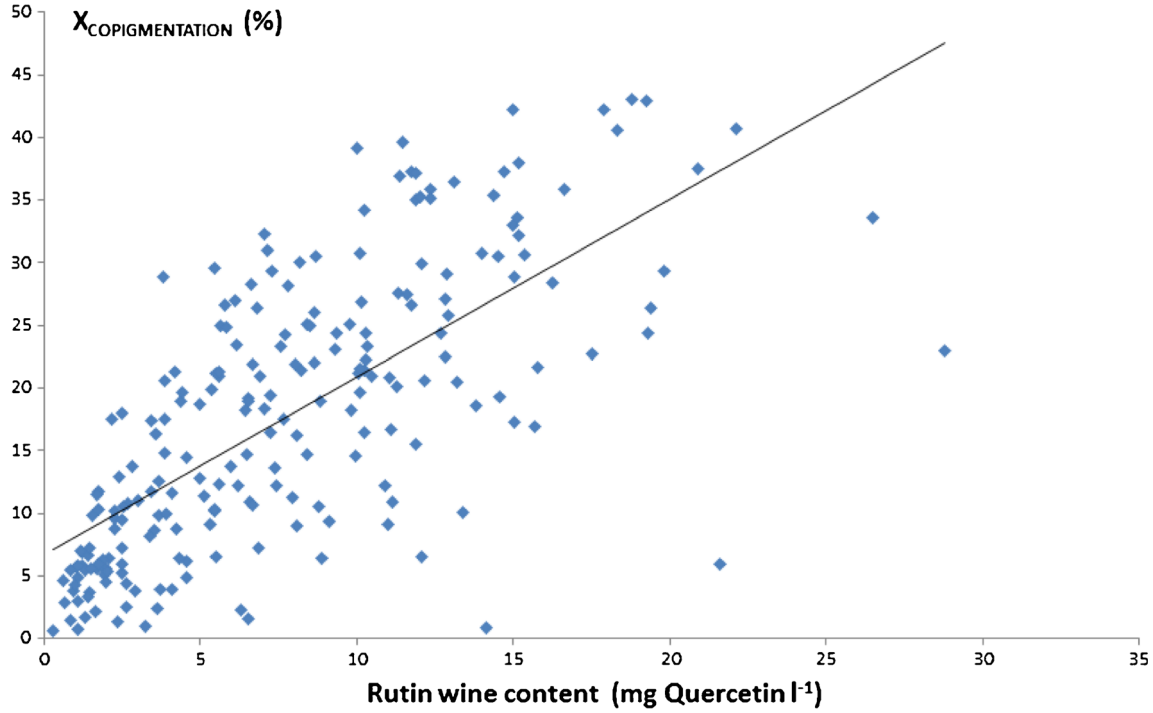


**Fig. 1** Relationship between rutin concentration and wine copigmentation color fraction

Copigmentation color differences were related to changes in absorbance, particularly at 620 nm ($\Delta E_{ab(c\text{-}nc)}$-$\Delta A_{420(c\text{-}nc)}$, $r$ = 0.598; $\Delta E_{ab(c\text{-}nc)}$-$\Delta A_{520(c\text{-}nc)}$, $r$ = 0.598; $\Delta E_{ab(c\text{-}nc)}$-$\Delta A_{620(c\text{-}nc)}$, $r$ = 0.711), showing a high significant relationship between blue color ($A_{620}$) and the copigmentation phenomenon.

$X_{Copigmentation}$ and $X_{Polymeric\ Pigment}$ were inversely related ($r$ = −0.778), which was consistent with Boulton (2001) descriptions about the copigmentation role during oxidation and ageing reactions in red wines. Copigmentation color fraction influences color shifts at all wavelengths studied ($X_{Copigmentation}$-$\Delta A_{420(c\text{-}nc)}$, $r$ = 0.550; $X_{Copigmentation}$-$\Delta A_{520(c\text{-}nc)}$, $r$ = 0.633; $X_{Copigmentation}$-$\Delta A_{620(c\text{-}nc)}$, $r$ = 0.430), being particularly related with global color intensity changes ($X_{Copigmentation}$-$\Delta ICM_{(c\text{-}nc)}$, $r$ = 0.662). These correlations

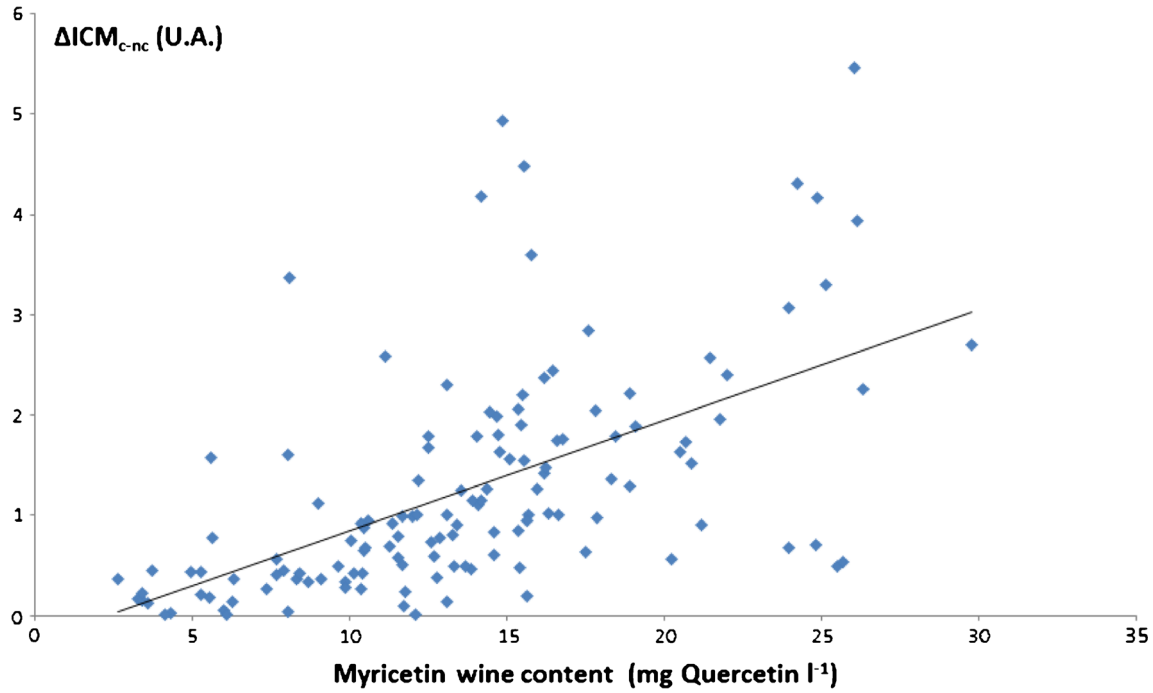


**Fig. 2** Relationship between myricetin content and color changes due to copigmentation

indicate that copigmentation involves a direct noticeable visual change in wine color, which is higher for young wines where the copigmentation fraction is maximal and polymeric pigment content is marginal.

## Conclusion

Copigmentation alters visual perception for most wines. A minor change in pH modifies anthocyanin equilibrium, which involves a wine color shift. Copigmentation involve every chromatic component, particularly decreasing L*, b* and $h_{ab}$* and increasing color ($\Delta E_{ab(c\text{-}nc)}$) and individual absorbances ($\Delta A_{420(c\text{-}nc)}$, $\Delta A_{520(c\text{-}nc)}$, $\Delta A_{620(c\text{-}nc)}$). Moreover copigmentation phenomena improve color, producing wines with lower clarity and darker red hues, which visually are less evolved.

Anthocyanins and copigments were the main compounds involved in copigmentation visual changes, being particularly relevant for $\Delta a^*_{(c\text{-}nc)}$ and $\Delta b^*_{(c\text{-}nc)}$ malvidin-3-O-(6-acetyl)-glucoside, flavonols (mostly myricetin derivatives and rutin), resveratrol, coutaric and coumaric acids. Lightness variations due to copigmentation ($\Delta L^*_{(c\text{-}nc)}$) were related with copigments but unexpectedly no relationship was found with any monomeric anthocyanin. Changes in hue ($\Delta Hue_{(c\text{-}nc)}$) presented interesting correlations with caffeic acid, a phenolic copigment not related with changes in other colorimetric variables. No relationship was found between copigmentation and benzoic acid derivatives, except for syringic acid.

In summary, copigmentation phenomena revealed to be a highly important factor for the color of red wines involving great influences on the changes associated with ageing.

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