TITLE: **Colour-copigmentation study by Tristimulus colorimetry (CIELAB) in red wines obtained from Tempranillo and Graciano varieties**

ABBREVIATED RUNNING TITLE: **Colorimetry study of wines copigmentation**

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Abstract

A study of the evolution of copigmentation in wines of different varieties has been undertaken. Colorimetric measurement of Tempranillo [T] and Graciano [G] monovarietal wines, wines from vinification with both grapes [M], and wines from blending Tempranillo and Graciano wines [W] was performed by spectrophotometric determination. Significant differences (*$p<0.05$*) were found among the wines. Graciano afforded somewhat darker and more colourful wines. The colour difference value ΔE^*_{ab} was in the 1.16 -6.12 unit range, which suggests that by co-vinification or coupage [W] the wines obtained are more similar to [T], whereas with co-maceration [M] the wines show a behaviour more similar to that of [G].

The colour difference between copigmented and non-copigmented wines was 13.58 CIELAB units in the initial stages of winemaking and 9.27 in the final stages. Evaluation of this parameter, as well as confirming the importance of this process during the early stages of the vinification, affords information as to whether changes in colour due to copigmentation are visually relevant. The wines from grape blending had higher copigmentation values than the Graciano and Tempranillo wines.

The [W] wine was the most stable, and the wines from grape blending [M] showed a similar behaviour to Graciano wines. Thus, vinification with grape blending gave rise to less stable and more different wines than vinification with wine blending.

Keywords:

Copigmentation, colour, CIELAB, co-winemaking, polyphenols

1. INTRODUCTION

The colour of red wine is one of its most important quality parameters, and determines sensory evaluation to a significant extent. Generally, it is the first characteristic perceived, and therefore plays a key role in the decision-making process of the consumer, who usually tends to prefer wines with a deep colour and hue (Kunsági-Máté, Stampel, Kollár & Pour Nikfardjam, 2008).

Phenolic compounds, which are responsible for the colour of wines, are transferred from the skin and seeds of grapes and diffuse into the must and wine during the maceration stage. The bright red colour of young wines is mainly due to free anthocyanins, self-association, and the copigmentation of anthocyanins with other phenols present in these wines such as flavanols, flavonols and hydroxycinnamic acids (Haslam, 1980). In this sense, the colour of wines is first determined by the pigment content of the grapes and, second, by the pigments and copigments formed during the vinification process, because the latter exert an important influence on the greater or lesser stability of colour during ageing.

Copigmentation involves hydrophobic interactions (π−π stacking) between the planar polarisable nuclei of the coloured forms of anthocyanins (pigments) and copigment molecules (flavonoids, simpler phenolics or aliphatic acids), involving different chemical mechanisms: inter- and intra-molecular copigmentation, self-association, etc. (Goto, 1987; Maza & Brouillard, 1987). Copigmentation complexes adopt a sandwich configuration that protects the flavylium chromophore from nucleophilic attack by water, thus reducing the formation of colourless hemiketal and chalcone forms. The final result is that anthocyanin solutions show a more intense colour than would be expected according to the pH value of the medium (Goto & Kondo, 1991). In addition, anthocyanin chromophores may also associate among themselves (self-association), as well as with aromatic residues of their own molecule (intramolecular copigmentation). It appears that colour extraction and retention in wine is strongly influenced by the levels of cofactors in it. Not all varieties of grapes are rich in cofactors, and neither do they all have the same quantities of anthocyanins and polyphenols. Accordingly, co-maceration of different grape varieties could favour an increase in the content of anthocyanins (García-Marino, Santos-Buelga, Rivas-Gonzalo & Escribano-Bailón, 2009) and could contribute to an increase in the copigmentation process (Moreno-Arribas & Polo, 2008). Likewise, blends from different wines afford wines with a more balanced anthocyanin/flavanol ratio (Monagas, Bartolomé & Gómez-Cordovés, 2006).

The *vitis vinifera* L. cv. Tempranillo is a very suitable red grape variety for the elaboration wines destined for ageing. The musts obtained from Tempranillo have an intense colour, which represents a good base wine for blending. On the other hand, *V. vinifera* L. cv. Graciano is also a red grape variety traditionally used as a complement in the vinification of wines elaborated with other varieties and its musts show a vivid red colour, are very aromatic and have high acidity, being used to improve the characteristics of Tempranillo, and affording a long shelf-life, and higher colour intensity and aroma to the mixture. Studies performed previously have also unveiled differences between varieties as regards their composition. Thus, in the case of anthocyanins, the skins of grapes from the Graciano variety have a higher content of peonidin in comparison with malvidin than Tempranillo grapes. In contrast, Tempranillo grapes have higher contents of delphinidin and petunidin in comparison with malvidin than Graciano grapes (Núñez, Monagas, Gómez-Cordovés & Bartolomé, 2004). These differences between the varieties are also seen in their flavanols, since it has been reported that the absolute content of these flavan-3-ols is higher in Graciano grapes than in the Tempranillo variety (Núñez, Gómez-Cordovés, Bartolomé, Hong & Alysone Mitchell, 2006).

According to Monagas, Gómez-Cordovés, Bartolomé, Laureano and Ricardo Da Silva (2003) the seeds of grapes of the Graciano variety have higher concentrations than those of Tempranillo grapes. Likewise, the seeds the Graciano variety grapes have higher contents of monomers than those of the Tempranillo variety (Núñezet al., 2006); the concentration of the monomer epicatechin is higher or similar to that of catechin (Monagas, Gómez-Cordovés, Bartolomé, Laureano & Ricardo Da Silva, 2003).

Thus, mixtures between these varieties by co-maceration or by the blending of wines, “coupage”, could lead to a product with a phenolic material, allowing the elaboration of wines that are more stable in colour over time. Colorimetric study of the original wines, as well as their mixtures, may lead to a better knowledge of the influence of the particular grape variety on the colour of the wine.

According to Boulton (2001), the presence of copigments in the grape exerts a strong influence on the colour density of young red wine and on the greater or lesser stability of the colour during the ageing of the wine (Darías-Martín, Carrillo, Díaz & Boulton, 2001; Darías-Martín, Martín-Luis, Carrillo-López, Lamuela-Raventós, Díaz-Romero & Boulton, 2002; Schwarz, Picazo-Bacete, Winterhanlter & Hermosín-Gutiérrez, 2005).

In previous studies it has been shown that during the winemaking process, with the passage of time the colour due to the copigmentation and the presence of free anthocyanins diminishes, and the contribution of the polymer pigments increases (Schwarz et al., 2005). This decrease in the copigmentation of wine with the passage of time has also been studied by Hermosín-Gutiérrez, Sánchez-Palomo and Vicario-Espinosa (2005), who demonstrated the differences between three varieties (Cabernet

Sauvignon, Cencibel and Syrah) with regard to the phenolic composition and the different levels of copigmentation.

Boulton (1996) developed a spectrophotometric method (by measurement of the visible λ_{max}) to evaluate the magnitude of copigmentation in red wines, improving the method proposed by Somers and Evans (1977). From the colorimetric point of view, an adequate description of the colour variations of wines caused by copigmentation requires the consideration that the spectral variations observed would affect the entire spectral curve, and not only its visible λ_{max} (Figure 1a, b). In this respect, Gómez-Míguez, González-Manzano, Escribano-Bailón, Heredia and Santos-Buelga, (2006) carried out preliminary tests using tristimulus colorimetry to explain the copigmentation phenomenon. Tristimulus colorimetry, through calculation of the ΔE^*_{ab} parameter (difference in colour), among others, allows the interpretation of copigmentation at the visual level.

Research into the industrial evaluation of differences in colour has undergone significant progress in recent years. Many antecedents concerning the application of colorimetry in different fields of the industry are known, such as the reproduction of colour in manufactured products and in systems of communication, or studies addressing the degradation of colour in works of art and foodstuffs, and the changes in colour which some fruits and vegetables undergo during ripening (Mackinney & West, 1940; Hoffman & Kanapaux, 1955; Walker, 1964; Francis & Clydesdale, 1975; Ramaswamy & Richards, 1980; Chen & Ramaswamy, 2002).

Nonetheless, there is very little previous information about the application of the differences in colour to the study of copigmentation. Thus, the aim of the present work was to study copigmentation changes in wines of different varieties (Graciano cv. and Tempranillo cv.), offering a colorimetric interpretation of the above mentioned effect.

2. METHODS

2.1. Samples

The wines were elaborated by Bodegas RODA (Haro, La Rioja, Spain) and correspond to the 2005 and 2006 vintages. The [T] and [G] wines were made from *V. vinifera* cv. Tempranillo and Graciano fresh grapes, respectively. [M] wines result from a mixture of Tempranillo/Graciano (80:20) grapes, and [W] wines from a blending of [T] and [G] wines (80:20) after malolactic fermentation. The enological parameters determined in wines during the vinification process of these grape varieties are shown in Table 1.

Samples were collected periodically during the winemaking process and correspond to the following stages:

- Initial stages (alcoholic fermentation, post-fermentative maceration, start and middle stage of malolactic fermentation): step 1-step 4
- Final stages (end of malolactic fermentation; after 3, 6, 12 and 14 months in oak barrels, and after 5, 9 and 12 months in bottles): step 5-step 12.

The number of samples was 88. These samples corresponded to 12 steps for the [T], [G] and [M] wines and 8 steps for the [W] wine. All samples were taken in triplicate and analyzed separately.

2.2. Chemical Analyses

2.2.1. Wine sample handling conditions

For the analysis of anthocyanins and flavonols, 1 mL of wine sample was diluted (1:1) with 0.1N HCl. Then, the samples were filtered through 0.45 µm Millex® syringe-driven filter units and injected directly into the chromatographic system.

The analysis of flavanols and phenolics was carried out according to García-Marino et al. (2009). With a view to eliminating the red pigments, 2 mL of each wine sample were eluted through Oasis® MCX 3cc (60 mg) cartridges (Waters Corporation Milford, Massachussets, USA) previously conditioned with 2 mL of methanol and 2 mL of water. After washing with 4 mL of ultrapure water, the flavan-3-ols and the phenolic acids were eluted with 8 mL of methanol, the anthocyanins and the flavonols being retained in the cartridges. A small volume of water was added to the eluate, and this was concentrated under a vacuum at a temperature lower than 30 °C until the complete elimination of methanol was achieved. The volume of the aqueous residue was adjusted to 0.5 mL with ultrapure water (MilliQ), filtered (0.45 mm), and analysed by HPLC–DAD–MS.

2.2.2. HPLC-DAD-MS analysis

HPLC-DAD analyses were performed with a Hewlett-Packard 1100 series liquid chromatograph. The LC system was connected to the probe of the mass spectrometer via the UV cell outlet. Mass analyses were performed using a FinniganTM LCQ ion-trap detector (Thermoquest, San Jose, CA, USA) equipped with an API source, using an electrospray ionisation (ESI) interface. The HPLC-DAD-MS analysis conditions used to carry out the analyses of red pigments and flavonols were in accordance with García-Marino, Hernández-Hierro, Rivas-Gonzalo & Escribano-Bailón (2010), selecting an additional wavelength at 360 nm to achieve the analysis of flavonols. Analyses of flavan-3-ols and phenolic acids were carried out as described by García-Marino, Ibañez, Rivas-Gonzalo & García-Moreno (2006), selecting an additional wavelength at 330 nm to achieve the analysis of phenolic acids.

2.2.3. Quantification

For the quantitative analyses, calibration curves were obtained using standards of anthocyanins (delphinidin, cyanidin, petunidin, peonidin and malvidin 3-O-glucosides), flavonols (myricetin, quercetin and kaempferol), flavanols (catechin, gallocatechin, epicatechin gallate, dimer B2 and trimer epicatechin-4,8-epicatechin-4,8-catechin) and phenolic acids (3,4-dyhydroxybenzoic acid and 4-hydroxycinnamic acid). Anthocyanins were purchased from Polyphenols Labs. (Sandnes, Norway). Myricetin, kaempferol, (+)-gallocatechin and (-)-epicatechin gallate were purchased from Extrasynthèse (Genay, France). Quercetin, (+)-catechin, 3,4-dyhydroxybenzoic acid and 4-hydroxycinnamic acid were purchased from Sigma (Steinheim, Germany). Procyanidin dimer and trimer were obtained at our laboratory by Escribano-Bailón, Gutiérrez-Fernández, Rivas-Gonzalo and Santos-Buelga (1992).

The total content of the different groups of phenolic compounds studied was calculated from the sum of the individual concentrations obtained for each individual compound, expressed in mg/L of wine.

2.3. CIELAB Colour Space

The colorimetric implications of the copigmentation phenomenon as regards the total colour of wine were evaluated by tristimulus colorimetry from the entire visible spectrum (380-770 nm). In this study, the wine colour with the copigmentation effect was obtained from the absorbance spectrum of the wines. The wine colour without the copigmentation effect was reconstituted from the absorbance spectrum of the wine sample after diluting 20-fold with a wine-like solution (pH 3.6) and multiplying by the dilution factor. That dilution led to the dissociation of the complex responsible for the copigmentation. Spectrophotometric measurements of the original and diluted wines were performed. The whole visible spectrum (380-770 nm) was recorded at constant intervals ($\Delta\lambda$=2 nm) with a Hewlett-Packard HP8452 UV-vis spectrophotometer (Palo Alto, CA), using 2-mm path length quartz cells and´a wine-like solution (pH 3.6) as a reference. The CIELAB parameters (L*, a*, b*, C^*_{ab}, and h_{ab}) were determined using the original CromaLab® software (Heredia, Álvarez, González-Miret & Ramírez, 2004), following the recommendations of the Commission Internationale de L'Eclariage (CIE, 2004): the 10° Standard Observer and the Standard Illuminant D65.

Colour differences (ΔE^*_{ab}) between two colour points in the CIELAB space are calcuated as the Euclidean distance between their locations in the three-dimensional space defined by L*, a*, and b*. Thus, mathematically, they are calculated by applying the formula:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

2.4. Statistical Analyses

For sample comparison, the data are presented as means ± standard deviation (SD) of analyses performed in triplicate. Significant differences ($p<0.05$) among the wines and for each variable were assessed by analysis of variance (ANOVA) and Tukey's honestly significant differences test.

A 2 (copigmented and non-copigmented) × 5 (colorimetric variables) repeated-measures ANOVA was carried out with cases (wines) as a random factor, in order to establish differences between the wines with and without copigmentation and the five colorimetric variables (Norman & Streiner, 1996).

Data analyses were performed using the Statistica® V 8.0 software (Statsoft, 2007).

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of the wines

Table 2 shows the mean concentration of the different pigment families during the initial and final winemaking stages of the [T], [G], [M] and [W] wines. Significant differences were seen in the total pigment contents between the [T] and the [G] and [M] wines in the initial winemaking stages but not in the final stages. The [T] wine showed the lowest total pigment content in final stages.

Regarding the total pigment content, with the mixture of grapes [M] wines with a greater pigment concentration were obtained than with the mixture of wines [W]; in turn, the total pigment content of the [M] wines was intermediate with respect to that seen for the monovarietal [G] (1001.56 mg/L) and [T] (883.36 mg/L) wines. The total pigment content in the [M] wine was significantly higher than the expected content, taking into account that only 20% of the Graciano grape variety was used in the vinification of the [M] wine (García-Marino et al., 2010). This could be due to the fact that the process pf grape co-maceration (Tempranillo + Graciano) allows the collection of [M] wines with more protected anthocyanins than wines elaborated only with the Tempranillo grape variety.

As from the initial stages, the concentration of phenolic acids in the [T] wines was significantly higher than in the [G] wines, whereas the [M] and [W] wines had intermediate concentrations between both the [T] and [G] monovarietal wines in the final stages. In the initial stage, there were no noticeable differences in the flavonol content (red wine cofactors that are of greater importance in the copigmentation phenomenon (Brouillard, Mazza, Saad, Albrecht-Gary & Cheminat, 1989; Boulton, 2001) among the wines. The flavonol content decreased in all wines as the vinification process progressed to its final stages. Differences were observed among the wines when the total content was taken into account. Thus, table 2 shows that the [M] wines had a significantly higher total flavonol concentration (229.41 mg/L) than the [W] wines (217.85 mg/L), thereby showing the same behaviour as total pigment content.

As from the initial stages the flavan-3-ol concentration of the [G] wine was significantly higher than that of the other wines studied. According to Liao, Cai & Haslam (1992), after pigments flavan-3-ols are the most abundant phenolic compound group in red wines. Nevertheless, compared with flavonols flavan-3-ols are considered ineffective copigments, with the exception of epicatechin in aqueous media since this adopts a nearly planar arrangement for easy stacking with anthocyanins, forming copigmentation complexes (Brouillard, Wigand, Dangles & Cheminat, 1991, Liao et al., 1992, Mirabel, Saucier, Guerra & Glories, 1999). In addition, since Graciano grapes have a higher content of anthocyanins and flavan-3-ols of the epicatechin type than Tempranillo (results not shown) the increased formation of these complexes -pigment-flavanol- during the early stages of winemaking could explain the higher concentration of pigments reached by the [G] wines during this period.

3.2. Colorimetric Characteristics

Table 2 shows the mean values of the colour parameters for all the [T], [G], [M] and [W] wines during the early stages (unstable wine) and final stages (stable wine). Statistically significant differences ($p<0.05$) were found among the [T], [G], [M] and [W] wines. In the final stages, when the wines were more stable, [T] showed lower chroma (C^*_{ab}) values than [G] (43.39 and 46.78 CIELAB units, respectively), which means less colour vividness. According to this result, higher lightness (L^*=55.14 CIELAB units) and hue angle (h_{ab}=5.07°) values were found in [T]. These differences were also statistically significant ($p<0.01$). All these results indicate that the Graciano cv. affords to fairly darker (lower L^* values) and more colourfulness (higher C^*_{ab}) wines. [M] and [W] showed differences for h_{ab}, with more bluish hues for the [M] wines. Both wines had significantly ($p<0.01$) lower C^*_{ab} values (43.62 and 43.41 CIELAB units respectively) than [G] and were similar to [T]. These colorimetric results are in agreement with the observed chemical behaviour, because although the total pigment concentration in the final stages did not show significant differences among the different wines (Table 2), it was observed that the content in total flavanoles (possible copigments) was significantly higher in the [G] wine than in the [T] and [W] wines.

In order to evaluate the colorimetric differences among the wines studied, the colour differences (ΔE^*_{ab}) between the [T], [G], [M] and [W] wines were determined. These were in the 1.16-6.12 CIELAB unit range (Table 3). According to Martínez, Melgosa, Pérez, Hita and Negueruela (2001), who indicated that ΔE^*_{ab} values up to 2.7 CIELAB units represent chromatic changes that can be perceived by the human eye, only the [W]/[T] pair can not be clearly detected by a non-trained human eye. This could be due to the fact that co-vinification, or coupage, [W] leads to wines that are more similar to the monovarietal type [T] than co-maceration, [M], whose behaviour seems to be somewhat closer to that of [G]. Furthermore, as shown in tables 2 and 5, during the final stages of winemaking, the [W] wines had chemical and colorimetric characteristics more similar to the [T] wine. That is, the wine present at the highest proportions (Tempranillo) has the greatest effect on the wine obtained from co-vinification, [W]. Thus, grape blending, [M], affords more different wines than wine blending [W].

3.3. Copigmentation

The colour differences between the copigmented and non-copigmented wines ($\Delta E^*_{ab[c-n]}$) are shown in Table 4. $\Delta E^*_{ab[c-n]}$ were in the 9.0-14.4 CIELAB unit range, being around 14 units in the initial stages, in which the copigmentation phenomenon is more marked (Boulton, 2001), and 9 units in the final stages.

The high values of the results obtained point to the importance of the copigmentation phenomenon in the colour of red wines during the initial stages of winemaking. In addition, in all the tests it was observed that the presence or absence of copigmentation in the same wine produced colour changes perceivable by the human eye, since in several studies it has been demonstrated that an untrained observer is able to distinguish between two colours with a ΔE^*_{ab} of 2.7 CIELAB units (Martínez et al., 2001).

In the initial stages of winemaking, where the copigmentation phenomenon is more important, the [M] wines, elaborated with a mixture of both grapes (Tempranillo and Graciano), had the highest copigmentation value (ΔE^*_{ab} = 14.44 CIELAB units). According to Schwarz et al. (2005) the formation of anthocyanins and copigmentation complexes between copigments, such as flavonols, causes an enhancement of the extraction of anthocyanins during winemaking. This could explain why the [M] wines initially had a pigment concentration greater than expected, taking into account that they had only 20% of [G]. The same authors indicated that the increase in pigment extraction could be reflected in a more intense red color, together with a bathochromic shift to purplish hues of the red colour. These results would be in agreement with the hab values shown by the [M] wines in the final stages of winemaking, which were significantly lower (bluer) than the [W] wines. The monovarietal wines elaborated from the Graciano [G] and Tempranillo grape [T] varieties had the lowest copigmentation values (ΔE^*_{ab} = 12.76 CIELAB units and ΔE^*_{ab} = 13.46 CIELAB units, respectively) (Table 4). The differences among the different wines studied were statistically significant between [M]/[G]. Therefore, the addition of 20% of the Graciano grape variety to 80% of Tempranillo grape results in higher of copigmentation values.

The colour differences ($\Delta E^*_{ab[12\text{-}5]}$) between stages 5-12 (final stages) were calculated to check the stabilization of the wines (Table 5). The [W] and [T] wines prove d to be the most stable, and the [M] wine showed a behaviour similar to that of [G]. This latter wine had a higher colour difference value, which was mainly due to changes in quantitative components (L^* and C^*_{ab}), since in this wine the qualitative component hue (h_{ab}) was the one showing the least significant change ($p<0.05$). Thus, co-maceration of the grapes afforded less stable wines than vinification with wine blending. More stable wines were obtained when 20% of the [G] wine was added to the [T] wine. That is, from the colorimetric standpoint the Graciano variety improved the behaviour of the Tempranillo variety, and in this regard it proved to be better to carry out the vinification from the blend of wines [W] rather than the blending of grapes [M], since both the colour differences ($\Delta E^*_{ab[12\text{-}5]}$) and the differences in the quantitative (lightness and chroma) and qualitative (hue) components were lower and more favourable in the [W] than in the [M] wine. After the [W] wine, [T] was the wine that showed the most stable colour ($\Delta E^*_{ab[5\text{-}12]}$ = 12.70 CIELAB units), in accordance with high copigmentation values seen during the initial stages ($\Delta E^*_{ab[c\text{-}n]}$=13.46 CIELAB units (Table 4)). [G] had the lowest copigmentation values during the initial stages ($\Delta E^*_{ab[c\text{-}n]}$=12.76 CIELAB units (Table 4)) and high colour differences were obtained in the final stages ($\Delta E^*_{ab[5\text{-}12]}$=16.84 CIELAB units (Table 5)). Therefore, it may be stated that copigmentation positively influences the later stability of wines (Boulton, 2001; Hermosín-Gutiérrez, 2005). The differences found between couples -[W]/[G], [T]/[G], [W]/[M] and [T]/[M]- were significant ($p<0.05$). Thus, with regard to the stability of the final wine, the blending of grapes affords wines that are not very stable and are similar to [G] wines, and the blending of wines leads to more stable wines, similar to [T].

Accordingly, studies of the qualitative and quantitative components of the colour differences between wines with and without the copigmentation phenomenon ($\Delta E^*_{[c\text{-}n]}$) were carried out. The (a*b*) colour diagrams of the copigmented and non-copigmented wine samples are shown in Figure 2. The distribution of the samples is different as a function of whether the copigmentation phenomenon is present or not. The samples of co-`pigmented wines are localised in the (-1°) a 10° zone of the (a*b*) diagram and show chroma values (C^*_{ab}) of 34-63 CIELAB units. The colour of the wines eith co-pigmentation displayed high chromatic intensity and a clearly violet hue. However, when the effect of co-pigmentation was removed from these wines and the dilution factor was applied in the calculation of the new chromatic parameters higher hue values (between 4° and 17°) and lower chroma values (30-59 CIELAB unidades) than the original ones were obtained, which accounts for the displacement of the samples towards the zone of more reddish hues in the (a*b*) diagram. According to the distribution of the (a*b*) samples, it may be seen that the "reconstructed" colour of the wines –i.e., those from which the effect of copigmentation was removed- had lower chromatic intensity and a loss of blue hues with respect to the colour expected theoretically from the Lambert-Beer Law. These results show that an important part of the expression of the colour of the anthocyanins is due the copigmentation phenomenon in which they are involved, which leads to changes at both qualitative and quantitative level in the colour of the wine, specifically favouring higher chromatic intensities and bluish hues. A two-level repeated measures MANOVA was applied to check whether the differences observed in the a*b* diagram were significant ($p<0.01$) for both coordinates, a* and b*, for the four wines studied ([T], [G], [M] and [W]) in the initial and final stages This indicated that the copigmentation phenomenon significantly affected ($p<0.05$) the colour of all the wines, regardless of whether they were elaborated from different varieties (Tempranillo, Graciano and mixtures thereof) or whether they were elaborated differently (co-maceration and co-vinification).

To check the difference among the wines studied, ([T], [G], [M] and [W]) as regards the a* and b* parameters, the increase between copigmented and non-copigmented wine was calculated for both parameters (Δa^* y Δb^*) in the initial stages, observing –after application of an analysis of variance (ANOVA)- that there were significant differences among the different types of wine studied, except between [M] and [T]. However, when the parameter evaluated was $\Delta E^*_{ab[c-n]}$ in the initial stages (Table 4), the differences found between [T] y [G] were not significant. Accordingly, it is clear that it is necessary to study the co-pigmentation phenomenon not only globally but also by study of the qualitative and quantitative components of the colour. In this sense, table 6 shows the values of the $\Delta L^*_{ab[c-n]}$, $\Delta C^*_{ab[c-n]}$ and $\Delta H^*_{[c-n]}$, colorimetric parameters of the copigmented and non-copigmented wines and their differences for each type of wine. Following application of the two-level repeated measure MANOVA test (copigmented and non-copigmented wines) for the colorimetric variables between the copigmented and non-copigmented wines significant differences ($p<0.01$) were consistently obtained in the four wines studied, ([T], [G], [M] and [W]) , in the initial and final stages. Table 6 shows that the difference in chroma and hue in the initial stages is significantly smaller ($p<0.05$) for wine [G] [G] ($\Delta C^*_{ab[c-n]}$=4.38 y $\Delta H^*_{[c-n]}$=2.75) as compared with [T] and [M].

The interpretation of the components of ΔE_{ab} -lightness, chroma, and especially hue differences, as the expression of qualitative observable change is very important. Therefore, tristimulus colorimetry is a good alternative in the comprehensive evaluation of the effect of the copigmentation on the wine, since in addition to confirming the importance of this process in the early stages of winemaking, through the use of the full spectrum, quantitative data are obtained that allow a visual interpretation of the changes involved to be made.

4. REFERENCES

Boulton, R.B. (1996). A method for the assessment of copigmentation in red wines. In The 47th Annual Meeting of the American Society for Enology and Viticulture, Reno, Nevada, EE.UU. Cited in *American Journal of Enology and Viticulture, 47*, 346.

Boulton, R.B. (2001). The copigmentation of anthocyanins and its role in the color of red wine. A critical review. *American Journal of Enology and Viticulture*, *52*, 67-87.

Brouillard, R., Mazza, G., Saad, Z., Albrecht-Gary, A.M., & Cheminat, A. (1989). The copigmentation reaction of anthocyanins: a microprobe for the structural study of aqueous solutions. *J. Am. Chem. Soc.*, *111*. 2604-2610.

Brouillard, R., Wigand, M.C., Dangles, O., & Cheminat, A. (1991). The pH and solvent effects on the copigmentation reaction of malvin with polyphenols, purine and pyrimidine derivatives. *Journal of the Chemical Society-Perkin Transactions II*, *2*, 1235-1241.

CIE, 2004. Colorimetry. In Publications CIE. Commission Internationale de l'Eclairage Central Bureau, Technical Report, Viena, Austria. CIE, 15.2.

Chen, C.R., & Ramaswamy, H.S. (2002). Color and Texture Change Kinetics in Ripening Bananas. *LWT - Food Science and Technology*, *35*, 415-419

Darías-Martín, J., Carrillo, M., Díaz, E., & Boulton, R.B. (2001). Enhancement of red wine colour by pre-fermentative addition of copigments. *Food Chemistry*, *73*, 217-220.

Darías-Martín, J., Martín-Luis, B., Carrillo-López, M., Lamuela-Raventós, R., Díaz-Romero, C., & Boulton, R. (2002). Effect of caffeic acid on the color of red wine. *Journal of Agricultural and Food Chemistry*, *50*, 2062-2067.

Escribano-Bailón, M.T., Gutiérrez-Fernández, Y., Rivas-Gonzalo, J.C., & Santos-Buelga, C. (1992) Characterization of procyanidins of Vitis vinifera variety

Tinta del País grape seeds. *Journal of Agricultural and Food Chemistry*, *40*, 1794-1799.

Francis, F.J., & Clydesdale, F.M. (1975). Food Colorimetry: Theory and Applications, AVI Publishing Co., Inc, Westport.

García-Marino, M., Hernández-Hierro, J.M., Rivas-Gonzalo, J.C., & Escribano-Bailón, M.T. (2010). Colour and pigment composition of red wines obtained from co-maceration of Tempranillo and Graciano varieties. *Analytica Chimica Acta*, *660*, 134-142.

García-Marino, M., Ibañez, E., Rivas-Gonzalo, J.C., & García-Moreno, C. (2006). Recovery of catechins and proanthocyanins from winery by-products using subcritical water extraction. *Analytica Chimica Acta*, *563*, 44-50.

García-Marino, M., Santos-Buelga, C., Rivas-Gonzalo, J.C., & Escribano-Bailón. M.T. (2009). Las prácticas enológicas en la evolución del contenido fenólico. *La Semana Vitivinícola*, *3248*, 6-14.

Gómez-Míguez, M., González-Manzano, S., Escribano-Bailón, M.T., Heredia, F.J., & Santos-Buelga, C. (2006). Influence of different phenolic copigments on the color of malvidin 3-glucoside. *Journal of Agricultural and Food Chemistry*, *54*, 5422-5429.

Goto, T. (1987). Structure, stability and colour variation of natural anthocyanins. In W. Herz, H. Griesebach, G.W. Kirby, & C. Tamm (Eds.), *Progess in the Chemistry of Organic Natural Products*, (pp. 113-158). New York: Springer.

Goto, T., & Kondo, T. (1991). Structure and molecular staching of anthocyanins-Flower color variation. *Angewandte Chemie International Edition in English*, *30*, 17-33.

Haslam, E. (1980). In Vino Veritas: Oligomeric procyanidins and the ageing of red wines. *Phytochemistry*, *19*, 2577-2582.

Heredia, F.J., Álvarez, C., González-Miret, M.L., & Ramirez, A. (2004). CromaLab®, análisis de color. Registro General de la Propiedad Intelectual SE-1052-04, Sevilla, España.

Hermosín-Gutiérrez, I., Sánchez-Palomo, E., & Vicario-Espinosa, A. (2005). Phenolic composition and magnitude of copigmentation in young and shortly aged red wines made from the cultivars, Cabernet Sauvignon, Cencibel, and Syrah. *Food Chemistry*, *92*, 269-283.

Hoffman, J.C., & Kanapaux, M.S. (1955). Relation of visual color rating to chlorophyll contents of snap bean pods. *Proceeding of American Society of Horticultural Science*, *66*, 339-344.

Kramer, A., & Smith, H.R. (1946). Preliminary investigations and measurement of color in canned foods. *Food Research*, *11*, 14-18.

Kunsági-Máté , S., Stampel, E., Kollár, L., & Pour Nikfardjam, M.S. (2008). The effect of the oxidation state of iron ions on the competitive complexation of malvidin by caffeic or ellagic acid. *Food Research International*, *41*, 693-696.

Liao, H., Cai, Y., & Haslam, E. (1992). Polyphenol interactions. Anthocyanins: Copigmentation and colour changes in red wines. *Journal of the Science of Food and Agriculture*, *59*, 299-305.

Mackinney, G., & West, C.A. (1940). Color changes in green vegetables. *Industrial Engineering Chemistry*, *32*, 392-396.

Martínez, J.A., Melgosa, M., Pérez, M.M., Hita, E., & Negueruela, A.I. (2001). Visual and instrumental colour evaluation in red wines. *Food Science and Technology Internacional*, *7*, 439-444.

Mazza, G., & Brouillard, R. (1987). Recent developments in the stabilization of anthocyanins in food products. *Food Chemistry*, *25*, 207-225.

Mirabel, M., Saucier, C., Guerra, C., & Glories, Y. (1999). Copigmentation in model wine solutions: occurrence and relation to wine aging. *American Journal of Enology and Viticulture*, *50*, 211-218.

Monagas, M., Bartolomé, B., & Gómez-Cordovés, C. (2006). Effect of the modifier (Graciano vs. Cabernet Sauvignon) on blends of Tempranillo wine during ageing in the bottle. I. Anthocyanins, pyranoanthocyanins and non-anthocyanin phenolics. *LWT- Food Science and Technology*, *39*, 1133-1142.

Monagas, M., Gómez-Cordovés, C., Bartolomé, B., Laureano O., & Ricardo Da Silva, J.M. (2003). Monomeric, oligomeric and polymeric flavan-3-ol composition of wines and grapes from Vitis vinifera L. cv. Graciano, Tempranillo and Cabernet Sauvignon. *Journal of Agricultural and Food Chemistry*, *51*, 6475-6481.

Moreno-Arribas, M.V., & Polo, C. (2008). *Wine chemistry and biochemistry*. New York: Springer.

Norman, G.R., & Streiner, D.L. (1996). *Bioestadística*. Madrid: Mosby/Doyma Libros S.A., Times Mirror International Publishers.

Núñez, V., Gómez-Cordovés, C., Bartolomé, B., Hong, Y.J., & Alysone Mitchell, A.E. (2006). Non-galloylated andgalloylated proanthocyanidin oligómeras in grape seeds from Vitis vinifera L. cv. Graciano, Tempranillo and Cabernet Sauvignon. *Journal of the Science of Food and Agriculture*, *86*, 915-921.

Núñez, V., Monagas, M., Gómez-Cordovés, C., & Bartolomé, B. (2004). Vitis vinifera L. cv. Graciano grapes characterized by its anthocyanin profile. *Postharvest Biology and Technology*, *31*, 69-79.

Ramaswamy, H.S., & Richards, J.F. (1980). A reflectance method to study the green-yellow changes in fruits, vegetables. *Canadian Institute of Food Science and Technology*, *13*, 107-111.

Schwarz, M., Picazo-Bacete, J.J., Winterhanlter, P., & Hermosín-Gutiérrez, I. (2005). Effect of copigments and grape cultivar on the color of red wines fermented after the addition of copigments. *J. Agric. Food Chem.*, *53*, 8372-8381.

Somers, T.C., & Evans, M.E. (1977). Spectral evaluation of young red wines: anthocyanin equilibria, total phenolic, free and molecular SO_2, “chemical age”. *Journal of the Science of Food and Agriculture*. *28*, 279-287.

StatSoft Inc. (2007). Statistica (data analysis software system), version 8.0. http://www.statsoft.com.

Walker, G.C. (1964). Color deterioration in frozen French beans (*Phaseolus vulgaris*). *Journal of Food Science, 29*, 383-388

FIGURE CAPTIONS

Figure 1. Copigmented and non-copigmented wines spectrums. a) The colour differences are not similar along the entire spectral curve b) Spectra with similar colour differences along the entire spectral curve

Figure 2. (a*b*) Colour Diagram of the copigmented and non-copigmented wines: a) All wines, b) [G] wines, c) [T] wines, d) [M] wines, e) [W] wines.