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Effect of Red Grapes Co-winemaking in Polyphenols and Color of Wines

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The red grapes co-winemaking effect on phenolic fraction and wine color has been studied for the first time, where Monastrell was comacerated and cofermentated with Cabernet Sauvignon and Merlot. Changes in the relative abundance of anthocyanins were observed as well as hyperchromic shifts at 530 and 620 nm; these effects remain constant after aging. Co-winemaking also favored copigmentation, giving way to more stable anthocyanins and facilitating their polymerization. With regard to color evolution, the mixture of Monastrell with Merlot grapes was more appropriate than with Cabernet Sauvignon for aging wines in oak barrels. The extent of copigmentation was more important in young wines than in aged wines. This is mainly due to the self-anthocyanin monomer reactions in the case of young wines, whereas in aged wines copigmentation is mainly due to the reaction between the anthocyanins and other polyphenolic cofactors. Discriminant analysis showed the possibility of differentiating wines according to the aging time and the type of wine, with color parameters (color intensity, OD 620 nm, and OD 520 nm) being the most important discrimination variables in the first case and petunidin-3-glucoside and peonidin-3-glucoside contents in the second case.

KEYWORDS: Co-winemaking; polyphenols; color; grape; red wine

INTRODUCTION

Color is one of the most important characteristics for defining the quality of wines, and the compounds responsible for the color of young wines are polyphenols, specifically anthocyanins. These compounds, which are responsible for the red and purple colors, are influenced by pH and medium composition, and they participate in different reactions during the winemaking process, which affect their stability. After fermentation, the anthocyanin content decreases, as they are submitted to degradation, oxidation, bleaching by SO_2, complexation with metals, polymerization with flavan-3-ols, and copigmentation reactions (*1*). Thus, their color is changed during bottle or barrel aging (*2*−*5*). Color stability is directly related to wine quality, so the application of enological techniques that improves this factor is of major interest. Any reaction that prevents anthocyanin bleaching, by development complexes that either prevent its oxidation or help its polymerization, will maintain the desirable color. The origin of these reactions in red wines has been discussed by several authors. They (*6*−*10*) suggest that copigmentation of anthocyanins is the first step toward the formation of more stable polymeric pigments. Molecular associations among anthocyanins (self-association) or with other molecules called cofactors may take place (*9*). These associations generate a hydrophobic site that involves a higher number of anthocyanins taking part in wine color (*10*, *11*), thus resulting in an increase in this quality. Anthocyanins may associate with several substances, some of them acting as strong copigments—caffeic acid (*12*−*14*) and ferulic acid (*12*)—and others acting as weak copigments—(−)-epicatechin and (+)-catechin (*1*).

Some grape varieties can be rich in certain cofactors while other grapes are deficient. In addition, they do not all have the same anthocyanin and polyphenolic contents. Therefore, some varieties could benefit in the presence of others that might have an excess of these compounds. This complementary effect and consequently a higher color stability can be achieved with the co-winemaking of different grape varieties that implies both comaceration and cofermentation steps. This process has also been called cofermentation, although this last term does not always mean that all grapes have been macerated together.

Traditionally, co-winemaking has been performed in places (Côte du Rhone, France; Chianti, in Italy; North of Rioja, Spain) where different varieties have a similar ripening schedule, although today's technology and refrigeration allow comaceration of grapes that ripen at different times. Co-winemaking is not commonly used since monovarietal wines are produced first and later mixed by "coupages". However, the ability to form additional copigmentation reactions during maceration would give rise to more intensively colored wines than would occur simply by blending the individual wines that had been macerated

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separately, as suggested by Boulton (*9*). However, no other bibliographic reference has been found concerning this topic.

Potential benefits of this type of vinification include more complexity, better texture, increased aromatic character, better color and color stability, plus enhanced aging ability, although the improvements depend on the ability of some of the grapes in the mixture to benefit from additional molecules and the ability of other grapes to provide those molecules. However, if this practice is carried out inadequately, it may damage wine quality due to anthocyanin dilution or pigment adsorption to the skins and pulp (*15*).

The Monastrell variety, also known as Mourvedre, is ranked third in terms of Spanish area grown and is becoming increasingly popular worldwide. It produces high quality wines, mainly rosé and young red, although it has recently been proven that this variety may give high quality aged red wines (*16*) if adequate maceration has taken place. The red wines made from Monastrell have a low total phenol content, thus their color changes quickly. For this reason, the maceration of Monastrell grapes with varieties of high total phenolic content could be of interest, where suitable aging would improve color by increasing the wood phenols. Among the varieties that can be used for comaceration and cofermentation with Monastrell, both Cabernet Sauvignon and Merlot are good options due to the fact that there are traditional red wine grapes as well as its high total phenol content (*17*, *18*).

The purpose of this work is therefore to study the co-winemaking effect of red varieties on Monastrell wine quality, especially on phenolic content and color stability, after fermentation and after 9 months of maturation in oak barrels.

MATERIALS AND METHODS

Vineyards representative of each variety, Monastrell, Cabernet Sauvignon, and Merlot, with similar periods of ripeness and yields of less than 8000 kg/ha, were selected in the Jumilla region (Murcia, Spain). These varieties were picked at optimum ripening under optimum sanitary conditions.

Wines were produced in duplicate with the following mixtures of grapes: 80% Monastrell + 20% Cabernet Sauvignon, 60% Monastrell + 40% Cabernet Sauvignon, 80% Monastrell + 20% Merlot, 60% Monastrell + 40% Merlot, and 100% Monastrell. Each grape mixture was crushed and destemmed together and separated in different batches, so sample homogenization was assured. Maceration was carried out at the same time as the fermentation, which took placed in 14 days. The cap was punched down twice a day. Immediately after cuvaison, a dried active yeast specific for red wine was added;the selected strain was *Sacharomyces cerevisae* r.f. *cerevisiae* (Fermol Rouge AEB, Brescia, Italy). The amount of yeast added was 20 g/hL, and it was used according to the commercial specifications. Fermentation was at 28 °C, and 500 L of each wine was obtained. Wines were analyzed after alcoholic fermentation (time 0) and before 9 months of aging in new French Allier oak barrels with medium toasting (Magreñan S. L., Logroño, La Rioja, Spain).

During aging in barrels, the redox potential of wines was measured periodically with an Oenox 100 m (Medi-Oeno, Bourdeaux, France) in order to determine the influence of oxygen on the results.

The following parameters were analyzed in triplicate for each wine: total phenolic compounds, total anthocyanin content, and their fractionation into monomer, red and brown polymers, tannins, individual anthocyanins, low molecular weight phenolic compounds, chromatic characteristics, and copigmentation. Total phenolic compounds were evaluated by the Folin−Ciocalteu index (*19*), total anthocyanin content and their fractionation were analyzed by the Ribéreau-Gayon et al. (*20*) method, and tannins were analyzed according to Montedoro and Fantozzi (*21*). For these analyses, a Lambda 3B Spectrometer (Perkin-Elmer, Norwalk, United States) was used.

Individual anthocyanins were isolated from wines by C_{18} cartridges (Waters, Milford, MA), previously conditioned with 2 mL of methanol (Merck, Darmstadt, Germany), 5 mL of water (milliQ), 2 mL of methanol, and 5 mL of water. Two milliliters of wine was applied to each cartridge and eluted with 8 mL of a 16% acetonitrile (Merck) solution adjusted to pH 2 with formic acid (Panreac, Barcelona, Spain) and then concentrated in a rotavapor (VV 201 1 Heidolph, Schwabach, Germany) to dryness. The resulting fractions were analyzed by high-performance liquid chromatography (HPLC), according to Johnston and Morris (*22*), and an Agilent 1100 chromatograph system with a diode array detector (Palo Alto, CA) with a Nova-Pack C_{18} 4 μm analytical column (150 mm × 3.9 mm i.d.) was used. The mobile phases were as follows: (A) acidify water (10% formic acid) and (B) acetonitrile. All solvents were of chromatographic grade. The gradient profile was 98% A (1 min), 94% A (4 min), to 86% A (20 min). Chromatograms were recorded at 520 nm. Compounds were identified by comparing their spectra with those published by Hebrero et al. (*23*), and the quantification was made using malvidin-3-glucoside chloride (Extrasynthése, Genay, France) as external standard according to the method proposed by Cacho et al. (*24*).

The low molecular weight phenolic compounds [gallic acid, (+)-catechin, vanillic acid, caffeic acid, (−)-epicatechin, ferulic acid, syringic acid, *p*-coumaric acid, and *trans-*/*cis*-resveratrol] were analyzed following the HPLC method described by Castellari et al. (*25*). Compounds were identified by comparing their spectra at 280 and 320 nm with their standards (Fluka Chemica, Buchs, Switzerland). An Agilent 1100 HPLC system with a diode array detector with an Inertsil ODS2 5 μm analytical column (250 mm × 4 mm i.d.) was used. Solvents were (A) methanol−water (5:95 v/v) and (B) methanol−water (50:50 v/v) and the gradient profile was as follows: 97% A (5 min), 30% A (85 min), 0% A (35 min), to 40% A (3 min). For quantification, a five point calibration curve for each compound was used. All solvents used were of chromatographic grade.

The chromatic characteristics measured were color intensity according to Glories (*26*) and CIELAB color parameters (*27*), which include L^* (lightness), a^* (red-green coordinate), b^* (yellow-blue coordinate), C^* (chroma), and H^* (hue). A Lambda 3B Spectrometer (Perkin-Elmer) was used for scanning between 380 and 780 nm at 5 nm intervals with 1 mm quartz cells. The parameters were provided by the Color of Wines-2001 software from Perkin-Elmer Hispania (Madrid, Spain). Chromatic differences were calculated according to Ayala et al. (*28*), using the expression $\Delta E = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2}$.

The contribution of copigmentation to total wine color (% copigmentation) and the degree of anthocyanin polymerization (% polymerization) were determined following the method proposed by Boulton (*9*, *29*). Wine samples were centrifuged at 3000 rpm for 10 min and then adjusted to pH 3.6 using formic acid. The total wine color at pH 3.6 was assumed to be A^{acet}, which is the result of the addition of 20 μL of 10% acetaldehyde to 2 mL of wine sample, kept for 45 min. The polymeric pigment wine color was assumed as A^{SO2}, which is the result of the addition of 160 μL of a 5% SO_2 solution to 2 mL of wine sample. Wine color with no copigmentation effect was considered to be A^{dil}, which is the result of the dilution of wine sample 1:20 with a synthetic dilution wine (12% alcohol and 5 g/L of tartaric acid in water, adjusted to pH 3.6). All measurements were carried out at 520 nm, using water as blank. The following calculations were made: % copigmentation = $[(A^{\text{acet}} - A^{\text{dil}})/A^{\text{acet}}] \times 100$; % polymerization = $(A^{\text{SO2}}/A^{\text{acet}}) \times 100$.

Statistical Analysis. Significant differences among wines and sampling times for each of the parameters analyzed were assessed with a one-way analysis of variance using SPSS Version 11.5 statistical package for Windows (SPSS, Chicago, IL). Discriminant analyses were performed with SPSS to establish the relationship between the parameters measured, the type of wine (Monastrell, Monastrell/Cabernet Sauvignon, Monastrell/Merlot), and the sampling times (T0, 9 months).

RESULTS AND DISCUSSION

In preliminary studies, it was observed that monovarietal Monastrell wines have a lower phenolic content than other grape varieties cultivated in the Jumilla D. O. area (*17*, *18*). Besides

Table 1. Phenolic Fraction of Wines after Fermentation[a]

	Mo ($n = 6$)	Mo-CS (80:20) ($n = 6$)	Mo-CS (60:40) ($n = 6$)	Mo-Me (80:20) ($n = 6$)	Mo-Me (60:40) ($n = 6$)
polyphenols					
Folin–Ciocalteu index	2124.98 a	2251.24 b,c	2206.55 b	2275.08 c	2243.79 b,c
% anthocyanin monomers	70.59 a	78.75 c	78.75 c	75.41 b	73.98 b
% anthocyanin red polymers	17.44 b	15.78 a	14.50 a	14.58 a	15.44 a
total anthocyanin (mg/L)	485.16 a	541.50 b	597.36 c	521.30 a,b	502.39 a
tannins (mg gallic acid/L)	1313.24 a	1394.22 b	1360.87 b	1406.61 b	1399.26 b
% individual anthocyanins					
delphinidin-3-glucoside	5.57 b	4.84 a	4.96 a	6.47 c	6.59 c
cyanidin-3-glucoside	2.98 b	1.82 a	1.45 a	2.09 a	1.51 a
petunidin-3-glucoside	10.46 b	9.78 a	8.42 a	10.90 b	10.47 b
peonidin-3-glucoside	13.19 c	8.80 a	8.46 a	10.54 b	9.44 b
malvidin-3-glucoside	67.80 a	75.95 c	75.59 c	69.71 b	72.24 b
low molecular weight compounds (mg/L)					
gallic acid	24.04 a	29.64 b	29.97 b	32.62 b	31.56 b
(+)-catechin	41.52 a	52.70 b	53.29 b	63.58 b	63.26 b
vanillic acid	1.48 a	2.20 a,b	1.38 a,b	3.05 b	2.42 b
caffeic acid	1.73 a,b	1.67 a	1.51 a	2.02 b	1.95 b
(–)-epicatechin	36.01 a	44.09 b	46.16 b	55.68 c	61.78 c
ferullic acid	1.41 b	1.11 a	1.21 a	1.31 a	0.92 a
syringic acid	3.28 a	3.31 a	4.21 a	4.79 a	3.99 a
p-coumaric acid	0.46 a	0.75 b	0.90 b	0.50 a	0.42 a
trans-resveratrol	1.46 a	1.23 a	1.43 a	1.52 a	1.14 a
cis-resveratrol	0.20 a	0.23 a	0.21 a	0.38 b	0.41 b

[a] Mo, Monastrell; Mo-CS, co-winemaking of Monastrell with Cabernet Sauvignon; Mo-Me, co-winemaking of Monastrell with Merlot; proportions 80:20 and 60:40 have 80 and 60% of Monastrell grapes, respectively; Folin–Ciocalteu index (mg gallic acid/L). Different superscript letters between columns indicate significant differences ($p < 0.05$).

it was shown that to increase this phenolic content it was convenient to blend it with other grape varieties, among which Cabernet Sauvignon and Merlot were the most adequate. As well, a higher Monastrell proportion was recommended to maintain the authenticity of their wines (*30*, *31*), more specifically 80:20 and 60:40, proportions that were used for this research.

The results of classic analysis on all of the wines after the alcoholic fermentation step were similar: alcohol, 13.85−13.92%; tritrable acidity, 5.93−6.16 g/L (as tartaric acid); volatile acidity, 0.29 g/L (as acetic acid); pH 3.66−3.73; and between 10.00 and 12.25 g/L free SO_2 and 22 g/L total SO_2. Wines obtained by co-winemaking of Monastrell with Cabernet Sauvignon grapes showed the highest pH and, thus, the lowest total acidity values. The wine that showed the highest alcohol degree was the Monastrell wine.

The phenolic composition of wines after the alcoholic fermentation step is shown in **Table 1**. The monovarietal wine as compared with the ones obtained by blending grapes, independently of the proportion used, had the lowest content of total anthocyanins, percentage of anthocyanin monomers, Folin−Ciocalteu index, and tannins, as other authors have shown (*17*, *18*, *31*, *32*). However, these wines had a higher percentage of red polymers. The tannin/anthocyanin ratio has been used by different authors as an index for wine quality (*4*, *33*). The best T/A ratio for wines that are going to be submitted to oak barrel aging are between 1 and 4 (*11*, *34*). The T/A ratio was maintained within this interval in all wines, although those resulting from the addition of Cabernet Sauvignon grapes to Monastrell grapes (T/A 2.71) caused a decrement [T/A (80:20) 2.57; T/A (60:40) 2.28], while the addition of Merlot produced an slightly increment in the proportion 60:40 (T/A 2.79) or it does not improve it when the proportion 80:20 (T/A 2.70) was used. At this stage, it seems that the co-winemaking between Monastrell and Cabernet Sauvignon grapes will better support the aging effect. No significant differences were observed between the different grape proportions, as it was also observed in a previous paper (*35*).

The individual anthocyanin content may be used as a color stability marker for young wines, with malvidin-3-glucoside being the most abundant. Monastrell wines showed the lowest proportion of this anthocyanin and the relative abundance of the other anthocyanins varied with the grape variety used on blending but not with the proportion. Thus, in Monastrell wines, the second most abundant anthocyanin was peonidin-3-glucoside, but for the wines from co-winemaking, this was petunidin-3-glucoside since Cabernet Sauvignon and Merlot monovarietal wines have more petunidin-3-glucoside than peonidin-3-glucoside (*36*). Therefore, the addition of either of the two complementary grape varieties to Monastrell produced a significant decrease in cyanidin-3-glucoside and peonidin-3-glucoside, along with an increase in malvidin-3-glucoside. Moreover, when Merlot was used, the delphinidin-3-glucoside percentage increased significantly whereas petunidin-3-glucoside percentage did not change, while both of them decreased when Cabernet Sauvignon was used. Wines that showed the higher percentage of monomeric anthocyanins, from the Monastrell/Cabernet Sauvignon grapes, also had the higher malvidin-3-glucoside content (average value of 155.65 mg/L), followed by Monastrell/Merlot (average value of 144.24 mg/L) and Monastrell wines (average value of 118.34 mg/L).

The low molecular weight polyphenol content of wines after fermentation was analyzed as they are also color dependent. No significant differences between wines in relation to syringic acid and *trans*-resveratrol contents were detected. The concentration of gallic acid, (+)-catechin, and (−)-epicatechin increased significantly when Monastrell co-winemaking was carried out, while ferullic acid content decreased. Monastrell/Cabernet Sauvignon wines had significantly higher contents of *p*-coumaric acid than Monastrell wines while Monastrell/Merlot wines had significantly higher contents of vanillic acid and *cis*-resveratrol than Monastrell wines. In all wines, the most abundant

Table 2. Color Parameters of Wines after Fermentation[a]

	Mo ($n = 6$)	Mo-CS (80:20) ($n = 6$)	Mo-CS (60:40) ($n = 6$)	Mo-Me (80:20) ($n = 6$)	Mo-Me (60:40) ($n = 6$)
color intensity	13.88 a	14.10 b	14.25 b	14.40 c	14.38 c
OD 520 nm	7.86 a	7.92 a	7.81 a	8.38 b	8.23 b
OD 620 nm	1.58 a	1.70 c	1.70 c	1.63 b	1.61 b
H^*	19.92 b	17.58 a	17.92 a	19.43 b	19.58 b
C^*	39.40 b	35.61 a	36.18 a	38.99 b	39.19 b
L^*	7.57 b	6.22 a	6.51 a	7.55 b	7.90 b
ΔE^*ab		4.48 b	3.46 b	0.42 a	0.45 a

[a] Mo, Monastrell; Mo-CS, co-winemaking of Monastrell with Cabernet Sauvignon; Mo-Me, co-winemaking of Monastrell with Merlot; proportions 80:20 and 60:40 have 80 and 60% of Monastrell grapes, respectively; OD 520, 520 nm optic density; OD 620, 620 nm optic density; ΔE^*ab, chromatic differences in reference to Monastrell. Different superscript letters between columns indicate significant differences ($p < 0.05$).

Table 3. Contribution of Copigmentation and Polymerization to the Color of Wines after Fermentation[a]

	Mo ($n = 6$)	Mo-CS (80:20) ($n = 6$)	Mo-CS (60:40) ($n = 6$)	Mo-Me (80:20) ($n = 6$)	Mo-Me (60:40) ($n = 6$)
% copigmentation	28.68 a	38.66 d	36.86 c	34.18 c	31.38 b
Δcolor	59.34 a	98.88 d	87.10 c	75.15 b	70.51 b
% polymerization	22.79 a	21.75 a	22.53 a	21.41 a	22.21 a

[a] Mo, Monastrell; Mo-CS, co-winemaking of Monastrell with Cabernet Sauvignon; Mo-Me, co-winemaking of Monastrell with Merlot; proportions 80:20 and 60:40 have 80 and 60% of Monastrell grapes, respectively. Different superscript letters between columns indicate significant differences ($p < 0.05$).

compound was (+)-catechin, followed by (−)-epicatechin and gallic acid, these being the most abundant compounds in grape seeds (*37*).

Color parameters of wines after fermentation are shown in **Table 2**. Co-winemaking wines had a higher color intensity than Monastrell wine, emphasizing those with Merlot with the highest values. The addition of Cabernet Sauvignon grapes did not change the absorbance measured at 520 nm but did significantly increase the absorbance measured at 620 nm, which indicates that these wines were more bluish independently of the proportions used. Merlot mixture wines increased both optical densities, so these wines were bluer and more reddish. Parameters C^* (chroma), H^* (hue), and L^* (lightness) did not show significant changes when any of the two Merlot proportions was used. However, these parameters decreased with Cabernet Sauvignon, so these last wines were less reddish, less yellow, and with a darker color than the Monastrell wine. It was observed that chromatic differences (ΔE^*ab) between Monastrell/Cabernet Sauvignon and Monastrell wine are significantly higher than those between Monastrell/Merlot and Monastrell wines, which indicates that the color of Monastrell wines scarcely changes when co-winemaking when Merlot is used. When Cabernet Sauvignon is used, the difference in color is important, producing a wine color that is less red, less yellow, bluer, and darker than Monastrell.

The maximum wavelengths observed for all wines were at 530 and 620 nm. Co-winemaking produced a hyperchromic shift at 530 and 620 nm in both proportions of grapes, although the effect was much more important in Monastrell/Merlot wines at 530 nm, which shows a higher contribution to red color, while the contribution to blue color was higher in Monastrell/Cabernet Sauvignon wines (620 nm).

Table 3 shows the fraction of color due to copigmentation in reference to the total color (% copigmentation), the color enhancement due to copigmentation of free anthocyanin, whose value corresponds to that calculated by Boulton (*29*) as Δcolor $= [(A^{\mathrm{acet}} - A^{\mathrm{dil}})/(A^{\mathrm{dil}} - A^{\mathrm{SO2}})] \times 100$, and the fraction of color due to polymeric pigments (% polymerization). As can be seen, most wine color was due to copigmentation (up to 38.66% in 80:20 Monastrell/Cabernet Sauvignon wines), and the fraction of color due to polymerization was lower (21.75%). The higher extent of copigmentation was also observed for co-winemaking wines, especially Monastrell/Cabernet Sauvignon being the best proportion 80:20. However, the color due to the polymeric pigment was similar in all of them. According to the results obtained from the analysis of low molecular weight polyphenols, the copigmentation increment in co-winemaking wines cannot be attributed to a higher content in these compounds, some of these, such as caffeic acid and ferulic acid, being indicated by several authors as important cofactors (*9*, *38*). However, copigmentation between anthocyanins (self-association) may also take place (*39*−*42*), so the higher copigmentation in co-winemaking wines must be attributed to the free anthocyanin increment provided by Cabernet Sauvignon and Merlot. The highest percentage of anthocyanin red polymers was observed in Monastrell wines, but this did not result in a color increase due to polymerization. As it is indicated in the literature (*43*, *44*), ethanol may have a negative influence on copigmentation, an effect that may contribute as well to the high copigmentation in Monastrell/Cabernet Sauvignon wines, as these have the lowest alcoholic degree.

The effect of aging on wines after 9 months in 225 L French oak barrels was also studied. In this case, the redox potential of wines was measured periodically and no significant differences were observed among barrels, so the effect of oxygen will not be considered in the discussion of these results.

First of all, the classic wine analysis was carried out, observing the evident changes due to aging. Results showed that volatile acidity increased to values between 0.59 and 0.66 g/L (as acetic acid); total acidity decreased to values between 4.69 and 4.77 g/L (as tartaric acid); the pH values were similar, between 3.70 and 3.75; alcohol changed between 13.66 and 14.24%, and SO_2 increased to values between 30.5 and 34.5 g/L free SO_2 and between 40 and 53.5 g/L total SO_2 because periodical corrections were made.

It is known that wine extracts wood phenols (*45*−*47*) and parameters such as the Folin−Ciocalteu index and tannin content should increase. Nevertheless, in relation to young wines (**Table 1**), the Folin−Ciocalteu index and tannin content were seen to decrease in co-winemaking wines after 9 months of aging (**Table 4**), probably due to the polyphenol polymerization reaction that takes place (*48*). However, in Monastrell wines, the wood phenol extraction reactions were more predominant than polymerization ones (the Folin−Ciocalteu index and tannin content increased), as phenolic compounds present in wood do not polymerize with natural wine polyphenols (*49*, *50*). As was expected, total anthocyanin and monomer percentages declined basically during aging due to the polymerization reactions; therefore, the percentage of red polymers increased. At 9 months of aging, the lowest Folin−Ciocalteu index, tannin content, and red polymer percentage were obtained for Monastrell/Cabernet Sauvignon wines, even though they had the highest total anthocyanin content and the highest anthocyanin monomer percentage. Monastrell and Monastrell/Merlot wines had a similar content of total anthocyanin, red polymer percentage, tannin content, and Folin−Ciocalteu index, but the anthocyanin monomer percentage was lower in Merlot wines. The T/A ratio

Table 4. Phenolic Fraction of Wines after 9 Months of Aging in French Oak Barrels[a]

	Mo ($n = 6$)	Mo-CS (80:20) ($n = 6$)	Mo-CS (60:40) ($n = 6$)	Mo-Me (80:20) ($n = 6$)	Mo-Me (60:40) ($n = 6$)
		polyphenols			
Folin–Ciocalteu index	2249.50 b	2157.8 a	2126.36 a	2245.29 b	2247.64 b
% anthocyanin monomers	65.84 b	73.17 c	72.90 c	65.14 b	63.11 a
% anthocyanin red polymers	21.55 b	18.20 a	18.34 a	22.33 b	22.45 b
total anthocyanin (mg/L)	342.73 a	380.18 c	419.29 d	361.73 b	339.36 a
tannins (mg gallic acid/L)	1410.46 b	1318.43 a	1297.79 a	1391.94 b,c	1386.44 b,c
		% individual anthocyanins			
delphinidin-3-glucoside	6.16 b	5.14 a	5.68 a	6.94 c	7.95 c
cyanidin-3-glucoside	3.04 c	1.47 a	1.48 a	2.08 b	1.88 b
petunidin-3-glucoside	9.94 b	8.57 a	8.14 a	10.07 b	10.28 b
peonidin-3-glucoside	10.17 c	6.90 a	6.06 a	8.45 b	7.62 b
malvidin-3-glucoside	70.69 a	77.63 c	78.93 c	72.46 b	72.27 b
		low molecular weight compounds (mg/L)			
gallic acid	37.89 a	38.23 a	34.34 a	39.59 b	42.88 b
(+)-catechin	36.85 a	40.02 a	38.55 a	44.03 b	51.21 b
vanillic acid	1.86 a	2.11 a	1.75 a	1.89 a	2.18 a
caffeic acid	2.43 b	2.15 a	1.73 a	1.88 a	2.18 a
(–)-epicatechin	48.0 a	52.17 a	47.45 a	56.53 b	68.81 b
ferullic acid	0.62 a	0.91 a	0.60 a	0.71 a	0.75 a
syringic acid	7.33 b	7.14 a	5.96 a	7.34 b	8.32 b
p-coumaric acid	0.60 a	1.12 b	1.07 b	1.00 b	0.90 b
trans-resveratrol	1.30 c	0.79 a	0.75 a	1.08 b	1.02 b
cis-resveratrol	0.39 b	0.29 a	0.25 a	0.51 c	0.44 c

[a] Mo, Monastrell; Mo-CS, co-winemaking of Monastrell with Cabernet Sauvignon; Mo-Me, co-winemaking of Monastrell with Merlot; proportions 80:20 and 60:40 have 80 and 60% of Monastrell grapes, respectively; Folin–Ciocalteu index (mg gallic acid/L). Different superscript letters between columns indicate significant differences ($p < 0.05$).

Table 5. Color Parameters of Wines after 9 Months of Aging in French Oak Barrels[a]

chromatic characteristics	Mo ($n = 6$)	Mo-CS (80:20) ($n = 6$)	Mo-CS (60:40) ($n = 6$)	Mo-Me (80:20) ($n = 6$)	Mo-Me (60:40) ($n = 6$)
color intensity	10.60 a	11.03 b	11.05 b	11.42 c	11.54 c
OD 520 nm	5.40 a	5.60 b	5.71 b	5.83 c	5.85 c
OD 620 nm	1.40 a	1.50 b	1.59 b	1.56 b	1.46 b
H^*	23.76 b	20.93 a	20.68 a	21.68 a	22.73 a
C^*	44.77 c	40.02 a	39.67 a	42.00 b	42.71 b
L^*	10.59 c	8.47 a	8.51 a	9.30 b	9.60 b
ΔE^*ab		5.47 b	5.64 b	3.15 a	1.92 a

[a] Mo, Monastrell; Mo-CS, co-winemaking of Monastrell with Cabernet Sauvignon; Mo-Me, co-winemaking of Monastrell with Merlot; proportions 80:20 and 60:40 have 80 and 60% of Monastrell grapes, respectively; OD 520, 520 nm optic density; OD 620, 620 nm optic density; ΔE^*ab, chromatic differences in reference to Monastrell. Different superscript letters between columns indicate significant differences ($p < 0.05$).

was almost two units higher in aging wines than in young ones due to the decrease in anthocyanin. Monastrell wines showed the highest value (T/A 4.12) in aging wines, followed by Monastrell/Merlot wines [T/A (80:20) 3.85; T/A (60:40) 4.08] and Monastrell/Cabernet Sauvignon wines [T/A (80:20) 3.47; T/A (60:40) 3.10]. This fact suggests that co-winemaking wines, especially both wines made with Cabernet Sauvignon and the Merlot one (80:20), can be submitted to longer aging since they do not exceed 4, which is the limit value for the of T/A ratio in aging wines but not in Monastrell wine.

After aging, the order of abundance of anthocyanins was the same as was found in young wines. Malvidin-3-glucoside, delphinidin-3-glucoside, and cyanidin-3-glucoside percentages increased, whereas petunidin-3-glucoside and peonidin-3-glucoside decreased. This could indicate that these last anthocyanins took a greater part in the polymerization reactions. Wines made with Cabernet Sauvignon showed the highest percentage and concentration of malvidin-3-glucoside (average value of 132.06 mg/L in contrast to 95.56 mg/L in Monastrell wine and 113.55 mg/L in Monastrell Merlot wines).

Because of aging, a significant increase in gallic acid and syringic acid content was observed, whereas ferulic acid content decreases in all wines. Gallic acid content increased as it is an important constituent of wood, as well as syringic acid, which also increased (*46*, *51*). Although ferulic acid is also a significant constituent of oak wood (*51*), it suffered a decrement while aging possibly due to its significant participation as a cofactor in copigmentation reactions (*8*). Wines made with Cabernet Sauvignon showed significantly lower contents in several of these compounds than those elaborated with Merlot. The most abundant compounds in all of the wines were the same as those in young wines, that is to say (−)-epicatechin, (+)-catechin, and gallic acid, although in this case (−)-epicatechin is more abundant than (+)-catechin. These facts confirm that (+)-catechin has a higher polymerization ability than (−)-epicatechin during aging. Resveratrol and its derivates, whose beneficial effects for human health have been demonstrated (*52*, *53*), were more abundant in Monastrell wines whereas the lowest content was found in Monastrell/Cabernet Sauvignon wines.

The color parameters of aged wines (**Table 5**), such as color intensity, OD 520, and OD 620, decreased in relation to young wines, the lowest values being for Monastrell wine. Parameters C^* and H^* rose during the aging; thus, wines had a redder and more yellow color. Lightness (L^*) rose also, which confirms that aging in barrels clarifies wine. The Monastrell wine showed the highest values of C^*, H^*, and L^*. Chromatic differences of co-winemaking wines in relation to Monastrell wines (ΔE^*ab) were higher than in young wines, and they indicated as before that wines elaborated with Cabernet Sauvignon had the most different colors, independently of the proportion used.

Chromatic differences for each type of wine after aging as compared to after fermentation are indicative of color stability. Thus, the highest chromatic differences were shown by Mo-

Table 6. Contribution of Copigmentation and Polymerization to the Color of Wines after 9 Months of Aging in French Oak Barrels[a]

	Mo ($n = 6$)	Mo-CS (80:20) ($n = 6$)	Mo-CS (60:40) ($n = 6$)	Mo-Me (80:20) ($n = 6$)	Mo-Me (60:40) ($n = 6$)
% copigmentation	18.51 b	14.69 a	14.44 a	17.25 b	19.57 b
Δcolor	42.86 b	31.61 a	32.08 a	41.59 b	49.10 b
% polymerization	38.26 a	39.76 b	39.43 b	39.68 b	40.33 b

[a] Mo, Monastrell; Mo-CS, co-winemaking of Monastrell with Cabernet Sauvignon; Mo-Me, co-winemaking of Monastrell with Merlot; proportions 80:20 and 60:40 have 80 and 60% of Monastrell grapes, respectively. Different superscript letters between columns indicate significant differences ($p < 0.05$).

nastrell wines (6.82), followed by Monastrell/Cabernet Sauvignon wines (average 5.17) and Monastrell/Merlot wines (average 4.59), which indicates that co-winemaking wines, above all Monastrell/Merlot, have a greater color stability than Monastrell wines. For this reason, the low stability of Monastrell wines during aging due to their low polyphenolic initial content is confirmed (*17*, *18*, *54*, *55*).

After aging, the hyperchromic shift was higher at 530 and 425 nm and smaller at 620 nm, resulting in wines that are redder and yellowish but not as bluish as young ones. **Table 6** shows the contribution of copigmentation and polymerization to the color of aging wines. The fraction of color due to copigmentation decreased considerably as a result of aging, although this fraction increased due to polymerization. The most important changes in these parameters were shown by Monastrell/Cabernet Sauvignon wines, which presented the highest copigmentation in young wines. According to several authors (*6*−*10*), it was observed that anthocyanin copigmentation is a previous step to their participation in formation of more stable polymerized complexes. The color enhancement due to copigmentation of free anthocyanin decreased, specially in Monastrell/Cabernet Sauvignon wines, in which the medium decrement observed with regard to young wines was more than twice that of the other two types of wine. Therefore, contrary to what occurred in young wines, the lowest values of this parameter were found in Monastrell/Cabernet Sauvignon wines. This fact probably explains that although these wines have the highest percentage of anthocyanin monomers, they do not have the highest copigmentation since free anthocyanin does not contribute to copigmentation in such an important way. Monastrell and Monastrell/Merlot wines showed the highest percentage of color due to copigmentation and color enhancement due to copigmentation of free anthocyanin. Monastrell wines presented the highest caffeic acid content, which is a strong cofactor according to several authors (*12*−*14*), and they showed in general the highest polyphenolic content. Monastrell/Merlot wines had the highest amount of low molecular weight polyphenols and one of the highest total polyphenolic contents; therefore, wines with the highest cofactor contents now showed the highest copigmentation. These results permit us to conclude that in young wines from co-winemaking, copigmentation is more important among anthocyanin monomers (self-association), whereas in aging wines the extent of copigmentation is notably lower than in young wines and it takes place with the intervention of other polyphenolic cofactors. Monastrell/Cabernet Sauvignon aged wines showed the lowest copigmentation, which may be influenced by its high alcoholic degree.

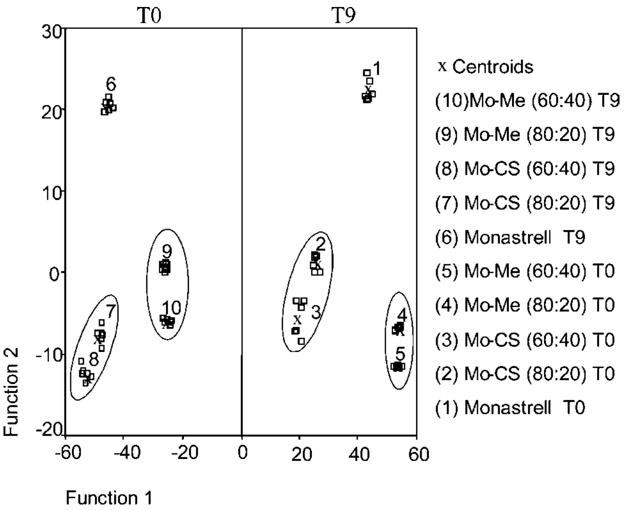

Figure 1. Discriminant plot of the different wines when the aged time and the type of wine were used as different variables.

Wines were clearly separated by two canonic discriminating functions: the first, which correctly separated young wines from aging ones, thus explaining 88.3% of the variance; and the second, which separated wines according to their types, hereby explaining 94.4% of the accumulated variance (**Figure 1**), and no significant differences have been observed between the proportions used. Color parameters (color intensity, OD 620 nm, and OD 520 nm) were observed as being the variables that contributed most to the differentiation according to aging time, whereas petunidin-3-glucoside and peonidin-3-glucoside were the variables that contributed most to the differentiation with regard to wine type. This information concurs with the results previously shown.

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