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ARTICLE

Effect of Micro-Oxygenation and Wood Type on the Phenolic Composition and Color of an Aged Red Wine

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Many studies have recently been published focused on the effects of micro-oxygenation on the quality of wines, its application modes, and doses, etc. However, there are still few scientific papers on how previously micro-oxygenated wines perform during storage or barrel aging. This study focused on the evolution of the phenolic composition, especially of anthocyanins, and color, together with astringency and tannins, during micro-oxygenation before barrel aging. In addition, to evaluate whether wine evolution during aging depends on barrel type, wines were aged in four different oak barrel types. Tempranillo wines, some micro-oxygenated before malolactic fermentation and others not, were aged for 12 months in American, French, Central European, and Spanish oak, following wine evolution during that period. The study was carried out for two consecutive vintages. Results showed that all wines evolved similarly; therefore, the micro-oxygenation treatment neither accelerated nor delayed the typical changes of aging. Slightly different evolutions were detected according to the barrel wood type, whether or not the wine was micro-oxygenated. The varied evolutions must therefore be associated with the differences from each oak type (structure, grain and density, composition, etc.).

KEYWORDS: Micro-oxygenation; aging; red wine; phenolic compounds; color; anthocyanins; oak

INTRODUCTION

Aging of a red wine in wood has significant effects on its quality and favorably modifies certain sensory characteristics, essentially associated with color, but others such as aroma, flavor, bitterness, astringency, etc. Many factors determine the physical−chemical and sensory characteristics of the wine. Some factors are associated with the wine, such as alcohol content, pH, structure, etc. (*1*), but others are associated with the barrel, such as its origin and wood type, porosity, level of toasting, age or months of use, etc. Several prior studies have indicated the influence of some of these factors on phenolic composition and color in aged wines (*2*−*4*).

In general, most of the published works which have studied the effect of wood on wine composition or characteristics have focused on American or French oak barrels. The number of studies with other types of oak barrels is still relatively limited. These studies generally show that regardless of barrel type used, the wine changes during barrel aging stem partly from incorporating new substances released from the wood and partly from oxidation, condensation, and polymerization reactions of wine components, mediated by the oxygen passing through the staves (*5*−*15*).

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Understanding the role of oxygen played in all these reactions led to the development of micro-oxygenation, a technique that consists of adding small controlled doses of oxygen to wine, as happens during barrel aging. This helps induce the changes that take place during oxidative aging in tank-aged wines, such as wine structuring and color stabilization (*16*, *17*).

Studies on micro-oxygenation applications indicate that it can be performed at any time during the winemaking process. However, the best results are achieved when oxygen is added at the end of alcohol fermentation and before beginning malolactic fermentation (*16*, *18*−*20*).

Many articles have been published on the effect of micro-oxygenation on several red wine characteristics. However, very few of them have studied the evolution of wines after applying micro-oxygenation during prolonged storage or during barrel aging (*21*−*26*).

For this reason, we considered it interesting to study the evolution of wines treated with micro-oxygenation during their aging in wood. This study focused on the evolution of phenolic composition, especially anthocyanins and color, since these are the parameters that change the most, together with astringency and tannins, during the micro-oxygenation treatment applied to structure wines before barrel aging (*16*−*27*). To evaluate whether wine evolution during aging depends on the type of barrel they are aged in, wines were aged in four different types of oak barrels.

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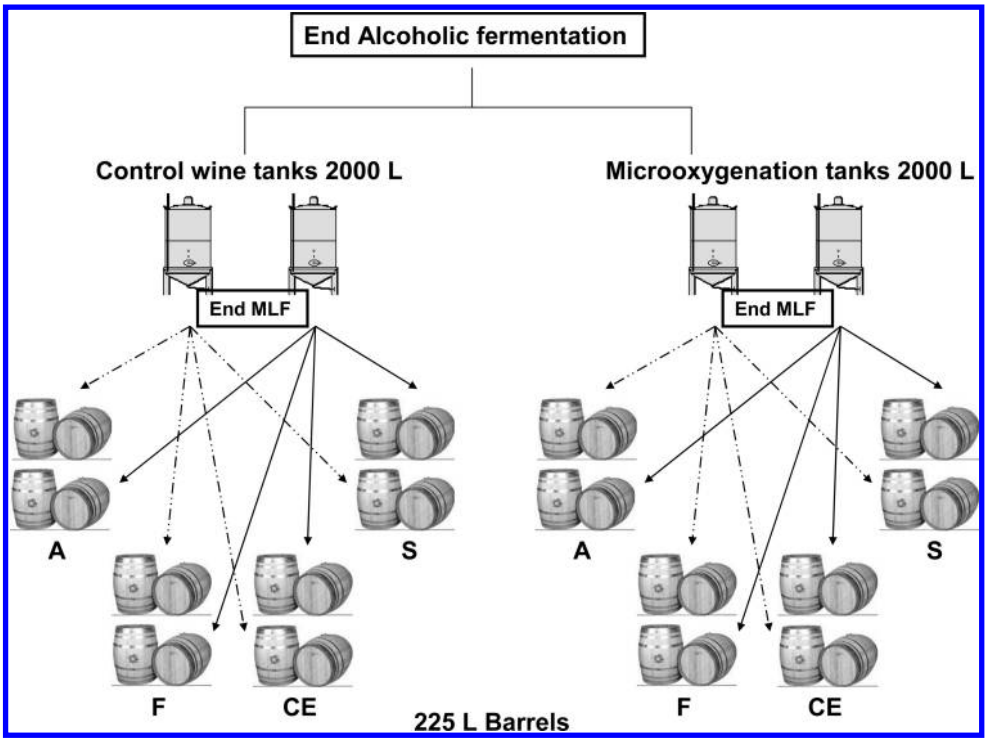

Figure 1. Scheme of the experimental design: MLF, malolactic fermentation; A, American oak; F, French oak; CE, Central European oak; S, Spanish oak.

Table 1. Characteristics of the Oaks Used to Make the Barrels

	type of oak			
characteristics	American oak	French oak	Central European oak	Spanish oak
species	*Quercus alba*	*Quercus petraea*	*Quercus petraea*	*Quercus robur/Quercus petraea*
origins	EE UU (Kentucky, Missouri, Minnesota)	France (Lorraine- Vosges)	Poland	Spain (Galicia and Navarra)
grain	medium	fine	fine-medium	fine
cut process	sawing	splitting	splitting	splitting
toasting degree	medium-high	medium-high	medium-high	medium-high
seasoning process	open air	open air	open air	open air

Table 2. Enological Parameters of Control (T) and Micro-Oxygenated Wines (MO) of the First and Second Vintage, and Aged for Twelve Months (12 mB) in Each Type of Wood[a]

first vintage			pH	TA	F-SO2	T-SO2	Alc. level	VA	T	K
Initial wine	0 mB	T	3.70	4.6	27	63	13.6	0.34	1.7	1405
		MO	3.76	4.5	24	60	13.7	0.49	1.1	1405
American oak	12 mB	T	3.59	4.8	25	57	13.7	0.77	1.4	1295
		MO	3.57	4.8	21	52	13.7	0.76	1.2	1250
French oak	12 mB	T	3.64	4.7	25	56	13.5	0.68	1.3	1240
		MO	3.56	4.8	23	55	13.5	0.69	1.3	1180
Central European oak	12 mB	T	3.60	4.6	25	56	13.5	0.68	1.3	1195
		MO	3.58	4.7	23	55	13.5	0.67	1.3	1150
Spanish oak	12 mB	T	3.61	4.6	22	57	13.4	0.65	1.3	1365
second vintage			pH	TA	F-SO2	T-SO2	Alc. level	VA	T	K
Initial wine	0 mB	T	3.65	4.9	19	36	14.6	0.53	1.6	1325
		MO	3.65	4.8	22	24	14.7	0.59	1.5	1350
American oak	12 mB	T	3.60	4.5	16	26	14.6	0.64	1.2	1185
		MO	3.65	4.7	13	26	14.7	0.73	1.0	1240
French oak	12 mB	T	3.60	4.6	13	26	14.6	0.66	0.9	970
		MO	3.65	4.7	18	26	14.4	0.74	0.7	1100
Central-European oak	12 mB	T	3.62	4.7	19	24	14.4	0.66	0.8	1080
		MO	3.64	4.6	16	26	14.4	0.68	0.8	1115
Spanish oak	12 mB	T	3.62	4.6	16	26	14.2	0.65	0.8	1080
		MO	3.66	4.5	10	16	14.4	0.66	0.7	1105

[a] 0 mB: 0 months of aging. No statistically significant differences were found between the parameters in each treatment at $\alpha = 0.05$: TA, total acidity (g/L of tartaric acid); F-SO2, free SO_2 (mg/L); T-SO2: total SO_2 (mg/L); Alc. level: alcohol level; VA, volatile acidity (g/L of acetic acid); T, tartaric acid (g/L); K, potassium (mg/L).

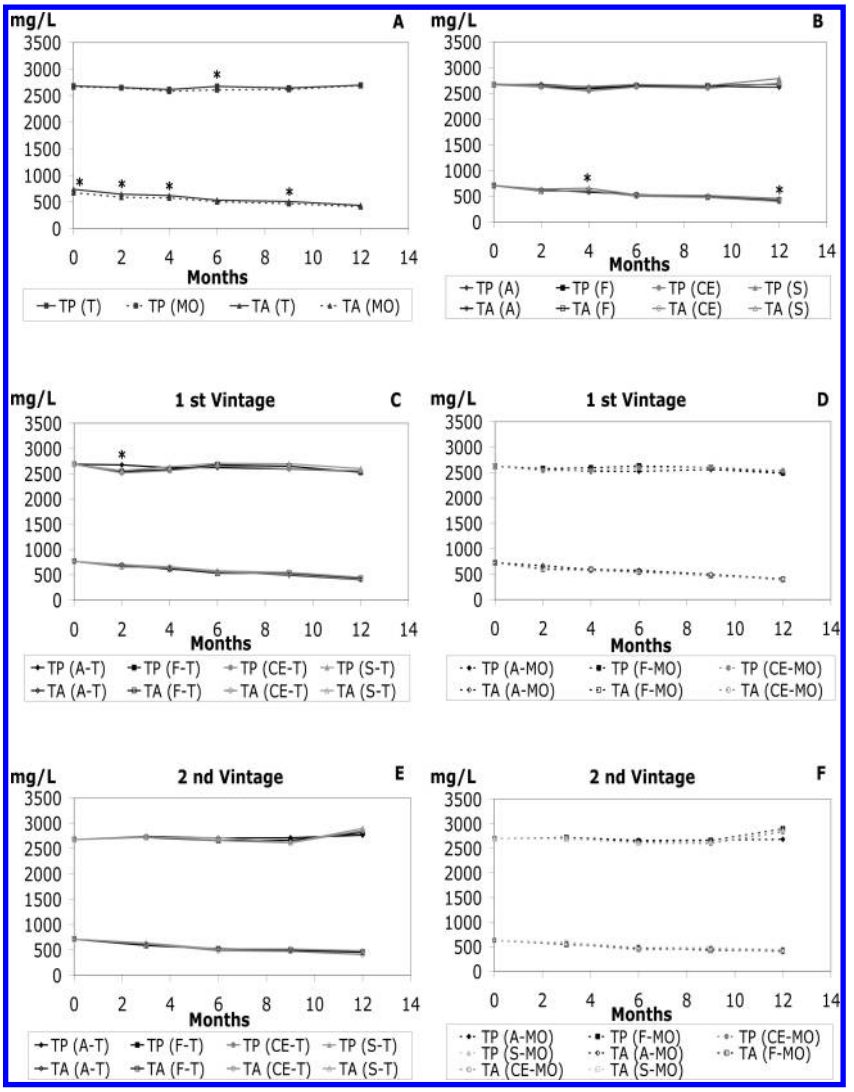

Figure 2. Evolution of total polyphenols (TP) and total anthocyanins (TA). (**A**) Mean values of control (T) versus micro-oxygenated wines (MO). (**B**) Mean values of wines aged in each wood type. (**C**−**F**) Mean values of control (T) and micro-oxygenated wines (MO) aged in each oak type: American (A), French (F), Central European (CE), and Spanish (S). The asterisk indicates that statistically significant differences were detected with the LSD test for $\alpha = 0.05$.

MATERIALS AND METHODS

Wine Production and Aging. During two consecutive vintages, wines were produced with *Vitis vinifera* grapes of the Tinta de Toro variety, synonym of Tempranillo, adapted to the edaphoclimatic conditions of the Designation of Origin Toro, located in the Autonomous Community of Castilla y León in the North of Spain. Wines were produced in the winery of the Estación Enológica (Enological Center), following the traditional vinification process for reds. The experimental design is shown in **Figure 1**. Following alcohol fermentation, when reducing sugars reached 1−2 g/L, wines were decanted and split: one part was kept in two 2000 L tanks without micro-oxygenation, becoming the control wines; the other one was put in two 2000 L (3-m high tanks) for micro-oxygenation before malolactic fermentation. The micro-oxygenation equipment used was a modular five-head VisiO2 micro-oxygenator (Oenodev, France). Oxygen was provided through a diffuser composed of a porous ceramic membrane, and the amount of oxygen was established according to the initial characteristics of the wines, such as the presence of herbaceous and reduced aromas, green tannins, astringency, etc. (*27*). The total amount of oxygen added to the wines from the first vintage was 36 mL/L/month and from the second vintage 41 mL/L/month. The treatment lasted 20 days in both vintages. These wines were called the micro-oxygenated wines. Once the micro-oxygenation process was completed, both the control and the micro-oxygenated wines underwent malolactic fermentation in a tank and were then aged in new barrels with a medium-high toasting level for 12 months. Two American, French, Central European, and Spanish oak barrels were used for each replicate and treatment (show **Figure 1**). It is important to note that during the first vintage only the control wines were aged in Spanish oak barrels since this type of oak is not usually used to obtain barrels. Therefore, in the first vintage we could only get barrels for aging the control wines. Then, the number of replicates for each treatment and oak is $n = 4$, except for the control wines aged in Spanish oak barrels of the first vintage where $n = 2$. The characteristics of the oaks used to make the barrels are given in **Table 1**.

Samples were taken of the control and the micro-oxygenated wines before putting them in barrels, and these became the initial samples. Next, samples were taken from the control and the micro-oxygenated wines periodically throughout the 12 months of barrel aging.

The wines transferred to the barrels were very clean, and since all of them were new, no racking was carried out during the 12 months of aging. Furthermore, the wines were tasted by winemakers monthly in order to detect the appearance of off-flavors (ethylphenol, sulfur compounds, acetic acid, etc.), and no off-flavors were detected during aging in any of the wines studied. The sampling and the filling processes were carried out through the small opening on top of the barrels using an aspiration system, and they took only a few minutes, minimizing the entrance of oxygen into the barrel. These processes were the same for all of the treatments and replicates.

Reagents. Gallic acid and catechin were purchased from Sigma-Aldrich (St. Louis, MO, USA), and malvidin-3-glucoside was from

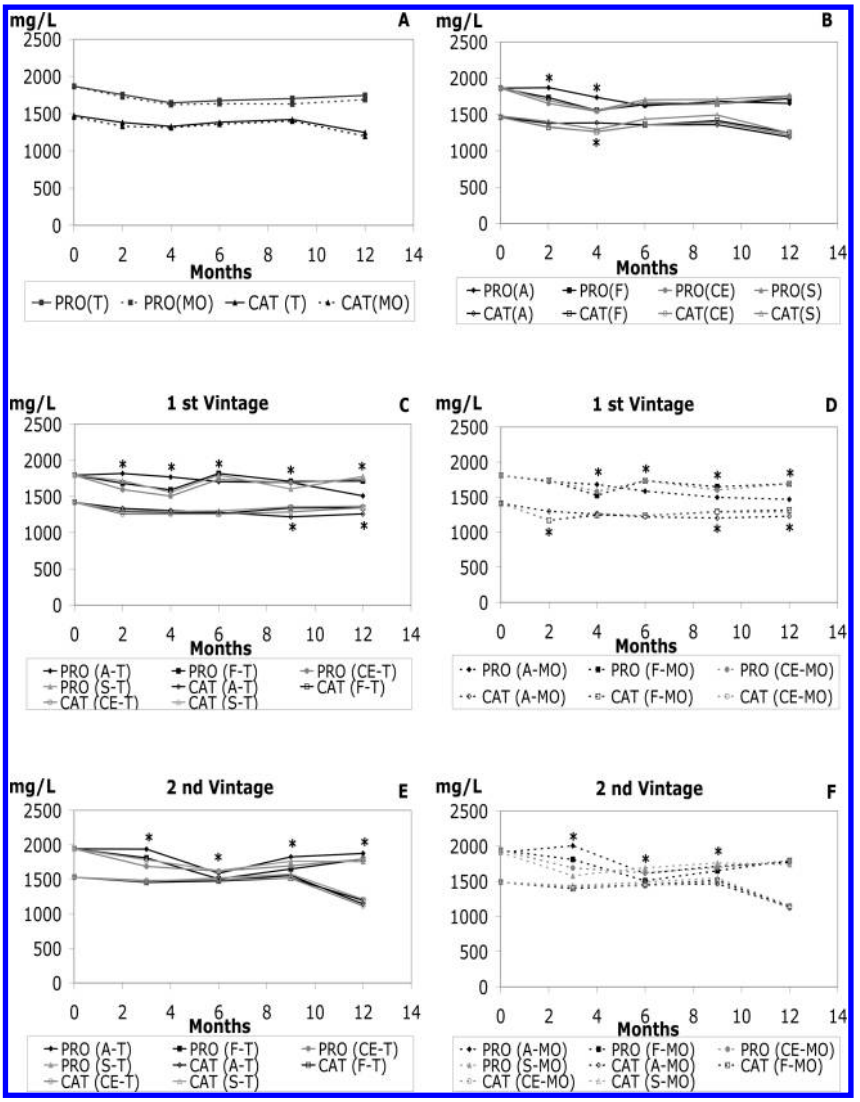

Figure 3. Evolution of proanthocyanidins (PRO) and catechins (CAT). (**A**) Mean values of control (T) versus micro-oxygenated wines (MO). (**B**) Mean values of wines aged in each wood type. (**C**−**F**) Mean values of control (T) and micro-oxygenated wines (MO) aged in each oak type: American (A), French (F), Central European (CE), and Spanish (S). The asterisk indicates that statistically significant differences were detected with the LSD test for $\alpha = 0.05$.

Extrasynthèse (Lyon, France). Milli-Q water, formic acid (Merck, Darmstadt, Germany), and methanol (Lab-Scan, Dublin, Ireland) were used in high-performance liquid chromatography (HPLC) analyses.

Analytical Methods. Total acidity, pH, alcohol content, potassium, free and total sulfur dioxide, volatile acidity, and tartaric acid were analyzed by means of official analysis methods (*28*).

Several groups of phenolic compounds were assessed: total polyphenols determined by reaction to Folin−Ciocalteu reagent (Panreac, Barcelona, Spain) were expressed as mg/L of gallic acid, and total anthocyanins were expressed as mg/L of malvidin-3-glucoside (*29*); polymeric anthocyanins (*30*) and catechins expressed as mg/L of D-catechin (*31*); and proanthocyanidins expressed as mg/L of cyanidin chloride (*32*).

The detailed analysis of anthocyanin compounds was performed with a high performance liquid chromatography system (HPLC), Agilent Technologies LC Series 1100, equipped with an automatic injector and a diode array detector (DAD). Wine was filtered through a 0.45 μm PVDF membrane and injected directly. Separation was conducted with a reverse phase Spherisorb C18 column, 150 mm × 4.6 mm (internal diameter); particle size of 5 μm, at room temperature and protected by a precolumn of the same material as the column. The method was based on the one described by Pérez-Magariño and González-Sanjosé (*33*), optimizing chromatographic conditions. The eluents used were water/formic acid (90:10, v/v) (eluent A) and water/methanol/formic acid (40:50:10) (eluent B). A flow of 0.7 mL/min was applied with the following linear gradient: 30−75% B, from 0 to 23 min; from 75 to 100% B, from 23 to 28 min; followed by methanol washing from 28 to 35 min and recovery of initial conditions. Detection in Scan mode (260−600 nm) was conducted, and peaks were quantified according to their response at 530 nm, expressed in equivalents of malvidin-3-glucoside.

Peaks were identified on the basis of data obtained from analyzing the wines in a HPLC (Agilent Technologies LC Series 1100) equipped with a quadropolar mass detector. The parameters of the mass detector used were those established by Revilla et al. (*34*). Twenty-two compounds were identified that were grouped as glucoside anthocyanins: delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside; as acetic anthocyanins, delphinidin-3-(6-acetyl)-glucoside, cyanidin-3-(6-acetyl)-glucoside, petunidin-3-(6-acetyl)-glucoside, peonidin-3-(6-acetyl)-glucoside, and malvidin-3-(6-acetyl)-glucoside; as cinnamic anthocyanins, delphinidin-3-(6-*p*-cumaril)-glucoside, cyanidin-3-(6-*p*-cumaril)-glucoside, petunidin-3-(6-*p*-cumaril)-glucoside, malvidin-3-(6-*p*-cumaril)-glucoside, and malvidin-3-(6-cafeil)-glucoside; as pyruvic derivatives, petunidin-3-glucoside pyruvate and malvidin-3-glucoside pyruvate (Vitisin A); and as Vitisin B and condensation derivatives, malvidin-3-glucoside-(epi)catechin, peonidin-3-glucoside-(epi)catechin, malvidin-3-glucoside-ethyl-(epi)catechin, and malvidin-3-glucoside-4-vinyl-phenol.

Colorimetric red wine analyses were conducted with classic Glories (*35*) and Sudraud (*36*) parameters: color intensity, percentage of red, blue, and tonality; and CIELab (L^*, a^*, b^*) parameters, according to OIV (*37*) recommendations. We also calculated the CIELab color difference, which

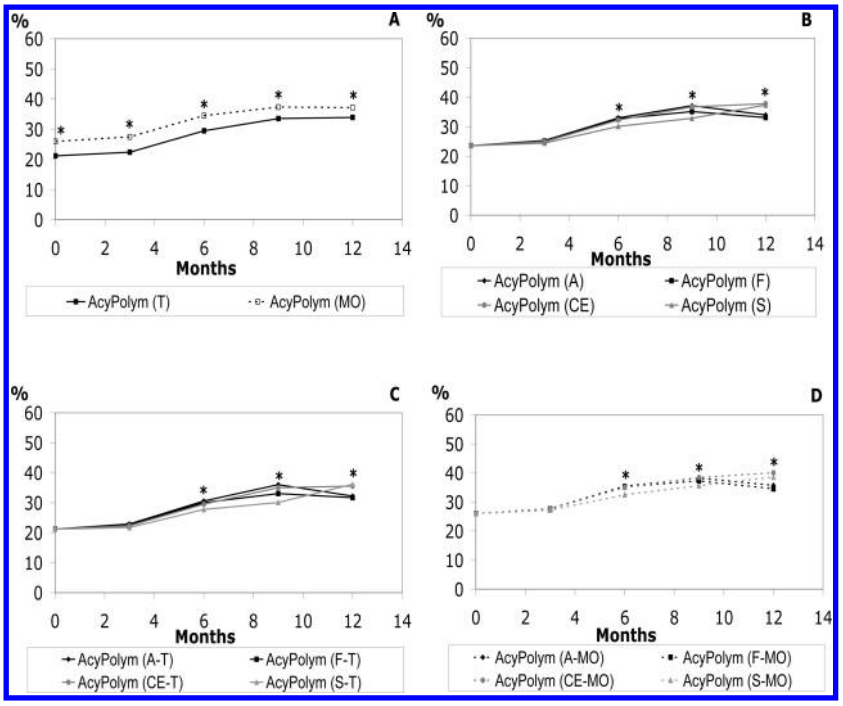

Figure 4. Evolution of polymeric anthocyanin percentage (AcyPolym). (**A**) Mean values of control (T) versus micro-oxygenated wines (MO). (**B**) Mean values of wines aged in each wood type. (**C**−**D**) Mean values of control (T) and micro-oxygenated wines (MO) aged in each oak type: American (A), French (F), Central European (CE), and Spanish (S). The asterisk indicates that statistically significant differences were detected with the LSD test for $\alpha = 0.05$.

numerically quantifies the color perception difference between two samples (*38*):

$$\Delta E^*a,b = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Spectrophotometric measurements were carried out with a UV−vis Shimadzu UV-1700 pharmaSpec (China) spectrophotometer, with the wines previously centrifuged and in 1-mm quartz cuvettes. All analyses were carried out in duplicate.

Sensory Analysis. A quantitative sensory descriptive analysis or profiling was chosen to carry out the sensory valuation of the wines. The methodology and profile established in a previous work (*20*) were applied. Seven-point structured scales were used. The tasting panel comprised 10 expert judges, with at least 7 of them participating in each tasting session. For this study, of all parameters evaluated in the descriptive profile, we considered only those corresponding to chromatic characteristics (color intensity, blue−violet, reds or garnets, and oranges hues) and the feeling of astringency (astringency, green, hard, smooth, and dry tannins) since these are ones the most closely linked to the physically−chemically studied parameters. The types of tannins selected correspond to the parameters previously defined as green tannins, negative sensation including an intense astringent sensation with intense acid feel and strong green or herbaceous notes; as hard tannins, intense astringent sensations, still unpleasant with intense rough notes but less aggressive than green tannins; as smooth tannins, a positive sensation including pleasant astringency, smooth sensation that fills the mouth; and as dry tannins, a negative sensation including excessive dryness with the lack of mouth lubrication (*20*).

Statistical Analysis. The data of the micro-oxygenated wines were compared with those of the control wines, independently of the oak barrel in which they were aged and of the vintage, with $n = 32$, except for the first vintage in micro-oxygenated wines, where $n = 28$. We also compared the wines aged in each barrel type with each other, independently of the treatments and the vintage, with $n = 16$, except for the Spanish oak wood, where $n = 12$. The effects of factors such as barrel type and micro-oxygenation treatment were valuated by applying the variance analysis (ANOVA). The LSD (least significant difference) test determined statistically significant differences between the means. Confidence intervals of 95% were used. All statistical analyses were conducted with the statistical package Statgraphics Plus 4.0 (1999 Manugistics, Inc., USA).

RESULTS AND DISCUSSION

Classic enological parameters were analyzed to control their influence on the evolution of wines during barrel aging, a fact observed in previous works (*1*). The results were very similar for all wine samples and the two vintages, as summarized in **Table 2**. These results indicate that the evolution of enological parameters was similar in both control and micro-oxygenated wines and that they were the same for all barrel types and for the two vintages studied. These results agree with other studies published on micro-oxygenation, which indicated that this technique did not cause changes in these parameters if micro-oxygenation treatment is applied correctly (*16*, *17*, *24*−*27*). Therefore, it can be said that micro-oxygenation does not modify the classic enological parameters. The results of the analyses carried out are summarized in graphs.

Results showed that the evolution of the overall phenolic composition (total polyphenols and total anthocyanins) was similarly in all wines studied (**Figure 2**). In general, the level of total polyphenols remained constant, while, as usual, the level of total anthocyanins decreased gradually (*3*, *9*, *13*). The results obtained also showed that the previous treatment of micro-oxygenation did not influence the evolution of these parameters during aging (**Figure 2A**), which are in agreement with previously published papers (*18*−*21*). Any influence of the type of wood (**Figure 2B**) or of the vintage (**Figure 2C** to **F**) was detected.

Results confirmed phenomena previously observed in other studies related to the effect of micro-oxygenation on the phenolic fraction. Thus, comparing mean values of total anthocyanins for all control wines versus all of the micro-oxygenated wines, the latter showed significantly lower contents than the control wines (**Figure 2A**), in line with the results described by other authors (*18*, *19*, *21*, *27*), regarding the decrease caused by micro-oxygenation on the total value of anthocyanins. We also observed that the differences gradually disappeared with aging, also coinciding with prior results; this shows that the effects of the micro-oxygenation treatment decrease as wines age under oxidative conditions.

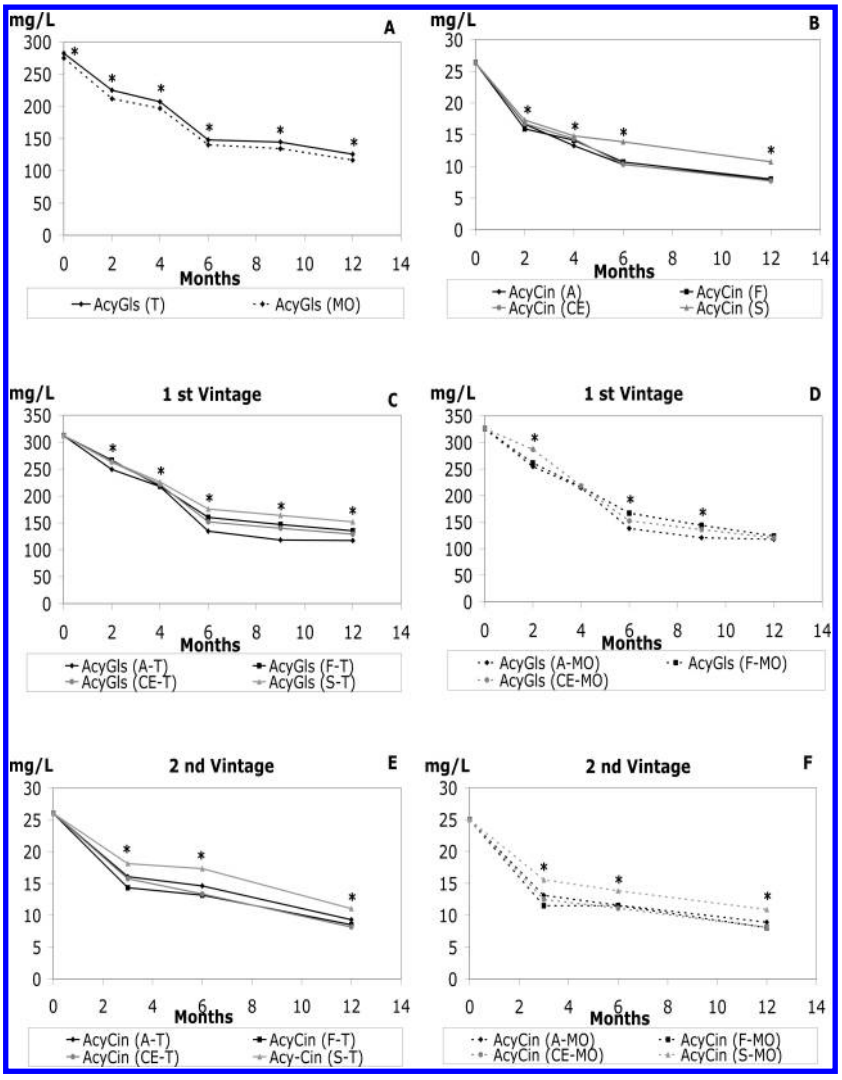

Figure 5. Evolution of glucoside anthocyanins (AcyGls) and cinnamic anthocyanins (AcyCin). (**A**) Mean values of control (T) versus micro-oxygenated wines (MO). (**B**) Mean values of wines aged in each wood type. (**C**−**F**) Mean values of control (T) and micro-oxygenated wines (MO) aged in each oak type: American (A), French (F), Central European (CE), and Spanish (S). The asterisk indicates that statistically significant differences were detected with the LSD test for $\alpha = 0.05$.

Evolution of other phenolic families studied, catechins and proanthocyanidins (**Figure 3**), showed somewhat different results from those described for total polyphenols and total anthocyanins. In general, these parameters evolved in a similar manner whether the wines had been micro-oxygenated or not, with no significant differences noted in the overall mean values in either wine type. However, slight differences in the evolution of both families according to their vintage and barrel type were found, more notably for proanthocyanidins. Vintage differences are common with wines, but in this case, they are probably also due to barrel heterogeneity. The most remarkable outcome was the effect of oak type (**Figure 3B**), although this was only temporary. The proanthocyanidin content in wines aged in American oak initially evolved differently from the rest of the wines, showing significantly higher values up to the sixth month of aging. This could be due to lower oxidation and/or polymerization of these compounds. Overall, the levels of catechins and proanthocyanidins showed a slightly downward trend.

The results previously described for anthocyanins and flavanols (catechins and proanthocyanidins) were similar to those observed for polymeric anthocyanins (**Figure 4A**). Thus, the mean percentage of polymeric anthocyanins in the micro-oxygenated wines was initially statistically higher than that in the control wines. These results, together with the lower content of total anthocyanins in micro-oxygenated wines, coincide with those found by Castellari et al. (*18*, *22*), Atanasova et al. (*10*), Cano-López et al. (*39*), and Pérez-Magariño et al. (*27*), showing that micro-oxygenation favors the formation of new polymeric pigments through the condensation of anthocyanins with other wine compounds.

Micro-oxygenated and control wines evolved more or less parallelly, although initial differences became quantitatively smaller with aging. The increase in polymeric anthocyanins both in the control and in the micro-oxygenated wines demonstrates that these compounds are also formed due to the oxygen provided through the wood pores during barrel aging. However, formation of these new pigments slows down with aging. It is also important to consider the transformation of the new pigments into other more complex ones.

Again, significant differences were found among the evolution of wines aged in each barrel type (**Figure 4B**). However, in this case they were more evident after the sixth month of aging. In general, maximum percentage of polymeric anthocyanins ranged

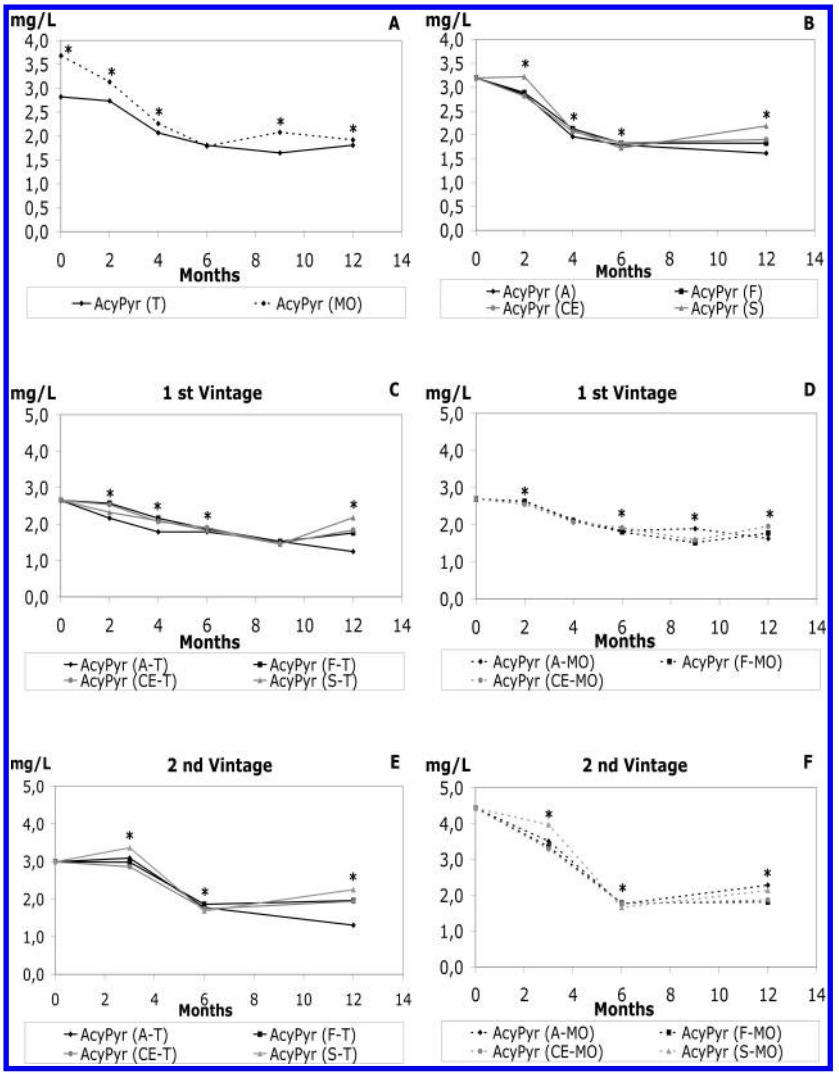

Figure 6. Evolution of pyruvic anthocyanins (AcyPyr). (**A**) Mean values of control (T) versus micro-oxygenated wines (MO). (**B**) Mean values of wines aged in each wood type. (**C**−**F**) Mean values of control (T) and micro-oxygenated wines (MO) aged in each oak type: American (A), French (F), Central European (CE), and Spanish (S). The asterisk indicates that statistically significant differences were detected with the LSD test for $\alpha = 0.05$.

between 35 and 40%, and wines aged in Spanish oak attained that level more slowly. Spanish oak wines maintained lower values than wines aged in the other oaks for up to nine months, but achieved higher values after 12 months of aging. This effect was independent of the fact of whether or not the wine was micro-oxygenated (**Figure 4C** and **D**); therefore, it could be associated to a barrel characteristics. It was remarkable that the percentage of these pigments remained constant during the last three months of aging in wines aged in Central European oak, while wines aged in French and American oaks showed lower levels at 12 months compared to that at 9. Once more, these results were found both in micro-oxygenated and control wines. This fact evidences that wood type seems to influence the rate of formation and transformation of wine anthocyanin pigments, and this effect is independent of previous color stabilization through micro-oxygenation treatment. Furthermore, the effect produced by wood is in addition to that from micro-oxygenation, and both effects are positive for the wine. These phenomena could be associated with barrel porosity, which modulates oxygen passage, and with the release of compounds from the wood (tannins, flavonols, etc.), modulating the medium's redox potential. Both oxygen and redox potential have a significant impact on the development of anthocyanin transformation reactions.

The evolution of monomeric or free anthocyanin levels (glucoside and acetic or cinnamic anthocyanins) showed results similar to those discussed so far. As the three groups of anthocyanins evolved in a similar manner in all cases, the evolution of only some of them is shown (**Figure 5**). Because of the initial effect of micro-oxygenation and the parallel evolution independent of this treatment, the levels of monomeric anthocyanins were always significantly greater in control wines (**Figure 5A**).

The evolution of the level of these pigments was significantly different according to the type of oak. These findings appeared repeatedly in both vintages and independent of micro-oxygenation treatment (**Figures 5B**−**F**). In general, wines aged in Spanish oak barrels showed statistically higher contents of monomeric anthocyanins. This is due to the fact that, in the first months of aging, the levels of these compounds in these wines dropped more slowly than in the others. These results agree with the previously described slower rate of new pigment formation shown by lower polymeric anthocyanin values found in wines aged in Spanish oak in the first nine months of aging. This could be associated with less intense polymerization reactions in these wines, probably due to lower oxidative conditions.

The level of pyruvic pigments evolved similarly in all of the micro-oxygenated and control wines (**Figure 6**), showing a

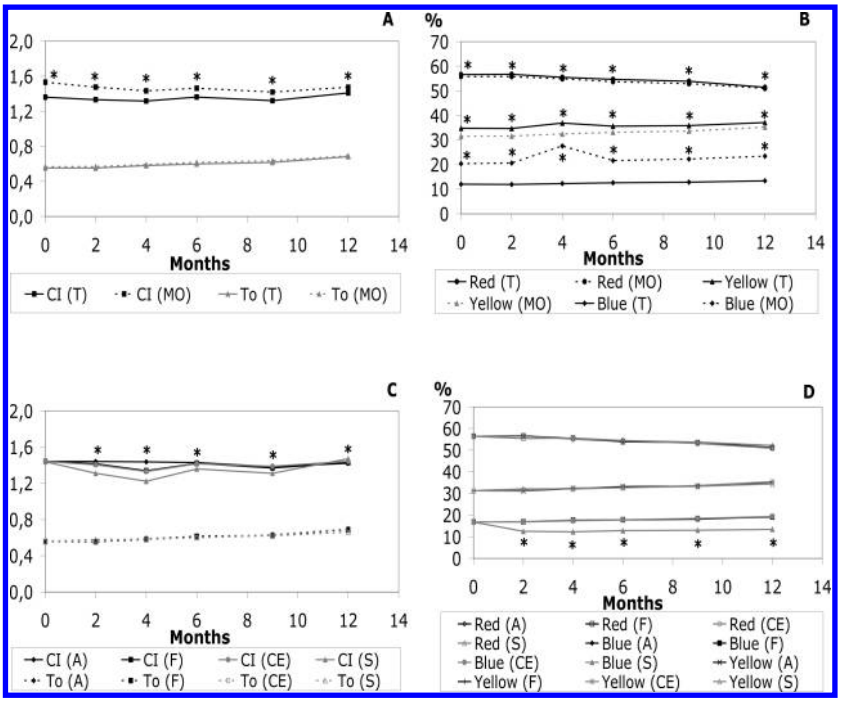

Figure 7. Evolution of color intensity (CI in absorbance units), tonality (To), and percentages of red, yellow, and blue. (**A**−**B**) Mean values of control (T) versus micro-oxygenated wines (MO). (**C**−**D**) Mean values of wines aged in each wood type: American (A), French (F), Central European (CE), and Spanish (S). The asterisk indicates that statistically significant differences were detected with the LSD test for $\alpha = 0.05$.

Table 3. Mean Values of Chromatics Differences ($\triangle E^*$) in CIELab Units ± Standard Deviation between the Control and Micro-Oxygenated Wines Aged in Each Type of Wood[a]

oaks	vintages	0 mB	6 mB	12 mB
American	first	7.44 ± 0.10	0.69 ± 0.06	1.33 ± 0.07
	second	3.30 ± 0.01	2.78 ± 0.08	1.27 ± 0.06
French	first	7.44 ± 0.10	2.13 ± 0.02	1.52 ± 0.02
	second	3.30 ± 0.01	2.99 ± 0.01	2.45 ± 0.05
Central European	first	7.44 ± 0.10	2.76 ± 0.06	1.19 ± 0.04
	second	3.30 ± 0.01	4.04 ± 0.07	1.47 ± 0.07
Spanish	first	7.44 ± 0.10		
	second	3.30 ± 0.01	3.82 ± 0.02	2.48 ± 0.06

[a] 0 mB, 0 months of aging; 6 mB, six months of aging; 12 mB, twelve months of aging.

Table 4. Mean Values of Chromatics Differences ($\triangle E^*$) in CIELab Units ± Standard Deviation between Each Pair of Wines Aged in Different Woods and of Each Vintage[a]

oaks	wines	vintages	6 mB	12 mB
American−French	T	first	1.53 ± 0.07	1.11 ± 0.08
		second	0.30 ± 0.04	0.88 ± 0.09
	MO	first	1.45 ± 0.04	1.10 ± 0.02
		second	0.29 ± 0.02	1.71 ± 0.02
American−Central European	T	first	2.14 ± 0.05	1.51 ± 0.05
		second	3.19 ± 0.02	1.53 ± 0.07
	MO	first	1.99 ± 0.04	0.59 ± 0.05
		second	2.93 ± 0.05	1.64 ± 0.08
American−Spanish	T	first	2.79 ± 0.03	1.92 ± 0.02
		second	4.44 ± 0.01	2.56 ± 0.06
	MO	first		
		second	3.73 ± 0.04	2.69 ± 0.09
French−Central European	T	first	0.65 ± 0.06	0.93 ± 0.08
		second	2.95 ± 0.02	1.57 ± 0.04
	MO	first	1.05 ± 0.03	0.85 ± 0.01
		second	2.73 ± 0.02	0.31 ± 0.05
French−Spanish	T	first	1.34 ± 0.09	1.24 ± 0.08
		second	4.22 ± 0.03	1.94 ± 0.01
	MO	first		
		second	3.68 ± 0.02	1.69 ± 0.02
Central European−Spanish	T	first	1.01 ± 0.02	2.07 ± 0.01
		second	2.24 ± 0.02	1.75 ± 0.01
	MO	first		
		second	2.38 ± 0.05	1.94 ± 0.09

[a] T, control wine; MO, micro-oxygenated wine; 6 mB, six months of aging; 12 mB, twelve months of aging.

downward trend regardless of wood type and prior structuring (micro-oxygenation). However, qualitative and some quantitative differences were observed between the two vintages studied.

In general, the drops were sharper during initial aging in micro-oxygenated wines (**Figure 6A**). This was especially notable in the second vintage wines (**Figure 6F**), in which micro-oxygenation led to the formation of pyruvic derivatives, leading to wines with a high content of these pigments. In spite of this, micro-oxygenated wines showed higher levels than their respective control (nonmicro-oxygenated) wines, and overall differences were generally significant. These results agree with those obtained by Cano-López et al. (*25*) who also observed a drop in pyranoanthocyanins during aging, especially pyruvic adducts, and a higher concentration of these compounds in micro-oxygenated wines. However, these results contrast with those previously described by Pérez-Magariño and González-Sanjosé (*33*) and Alcalde-Eon et al. (*40*), who found an increase in pyranoanthocyanin levels during the first year of barrel aging. The differences in these studies could be due to different medium conditions (redox potential, level of pyruvic acid or acetaldehyde, etc.) that modify the balance between cyclo-addition reactions leading to pyranoanthocyanins and disappearance reactions mainly due to their incorporation into more complex structures such as portisins (*15*, *41*−*43*).

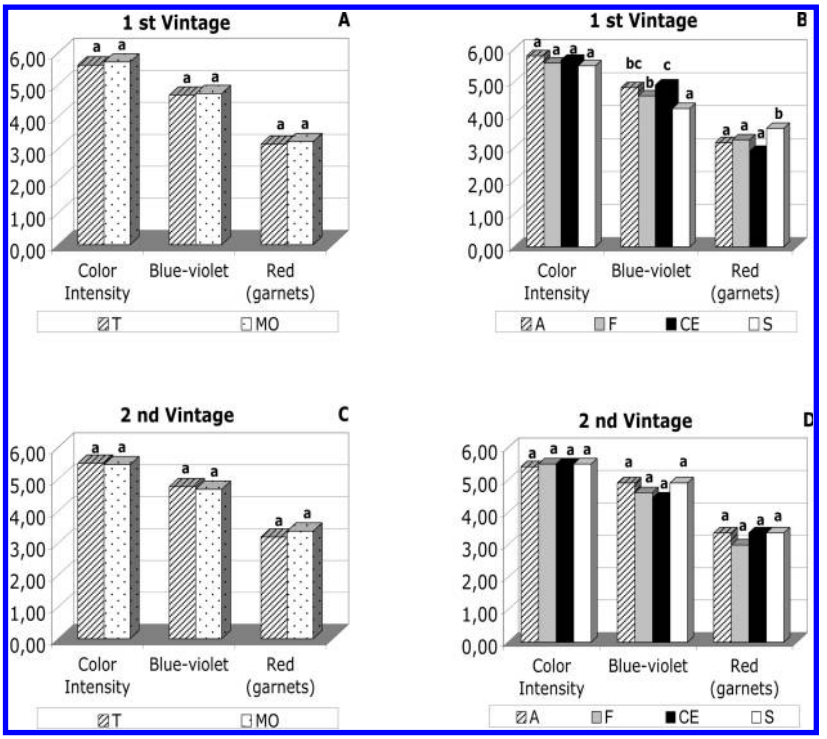

Figure 8. Mean values of sensory chromatic characteristics, color intensity, blue−violet hues, and reds (garnets) of wines aged for 12 months. (**A** and **C**) Mean values of control (T) versus micro-oxygenated wines (MO). (**B** and **D**) Mean values of wines aged in each wood type: American (A), French (F), Central European (CE), and Spanish (S). Values with the same letter in each parameter indicate that no statistically significant differences were detected with the LSD test for $\alpha = 0.05$.

The loss of anthocyanin compounds detected in this study was less than that of monomeric anthocyanins described earlier. These results coincide with those of Mateus and Freitas (*44*), who indicated that in Port wines aged in oak barrels for one year, the main monoglucoside anthocyanins dropped 80−90%, while pyruvic derivatives decreased 15−25%. These authors stated that this was because the latter compounds are more stable.

Significant differences were noted in the content of pyruvic anthocyanins in wines aged in different oak types, which were more notable in control than in micro-oxygenated wines. In general, wines aged in Spanish oak had higher concentrations of these compounds than those aged in other oaks (**Figure 6B**), and in most cases, wines aged in American oak barrels had the lowest pyruvic anthocyanin levels after a year of aging (**Figure 6C−E**).

The relationship between phenolic compounds and wine color is well known; therefore, it was to be expected that the changes described in phenolic composition would be reflected in changes in the color of the wines studied, as clearly shown in the results of chromatic parameters (**Figure 7**). All of the micro-oxygenated wines showed significantly higher values of color intensity and percentage of blue, and a lower percentage of red and yellow than the control wines (**Figure 7A** and **B**). These data agree with those already described for the greater anthocyanin drop, together with higher percentages of polymeric anthocyanins and greater contents of pyruvic derivatives (*45*, *46*).

No significant differences were found in the tonality values, which shows that micro-oxygenated wines did not oxidize chromatically any more than the control wines.

The results demonstrate that the stability and improved color caused by micro-oxygenation previously described by several authors (*16*, *17*, *20−25*) were maintained during the first year of barrel aging. However, quantitative differences were found to be smaller over time. When analyzing the data by the barrel-type factor, statistically significant differences were found between the color intensity values and the percentage of blue in the wines aged in each barrel type. The major differences were observed in wines aged in Spanish oak, in which both parameters were statistically lower (**Figure 7C** and **D**). These results are partially different from those obtained by Fernández de Simón et al. (*3*), who found that, after 12 months of aging in barrels, the color intensity values of wines aged in Spanish oak barrels were similar to those of wines aged in French oak and lower than those of wines aged in American oak.

The CIELab parameters showed an evolution similar to that of the previously mentioned Glories parameters. These results were expected given the good correlation between the two types of parameters (*47*). To avoid repetitions, these results are not shown, but the chromatic differences between the control and the micro-oxygenated wines for each barrel type and the two vintages are presented (**Table 3**). It is important to remember that this parameter is calculated on the basis of the a^*, b^*, and L^* values marking the difference between the potential or theoretically perceptible color during tasting, when the wine is viewed through the glass, with limits set by values higher or equal to 3 CIELab units (*48*). Considering this limit, the results pointed out that initially there were differences between the control and the micro-oxygenated wines, which were reduced after 12 months of aging. The loss of differences between the two types of wines (control and micro-oxygenated) is assumed to be caused by the fact that both wines are subject to micro-oxygenation through wood during barrel aging and that the chromatic characteristics therefore tend to become more similar.

In general, no color differences were found between the wines aged in different woods (**Table 4**), regardless of oak type, micro-oxygenation treatment, or vintage. However, in the second vintage, some color differences were detected between the wines

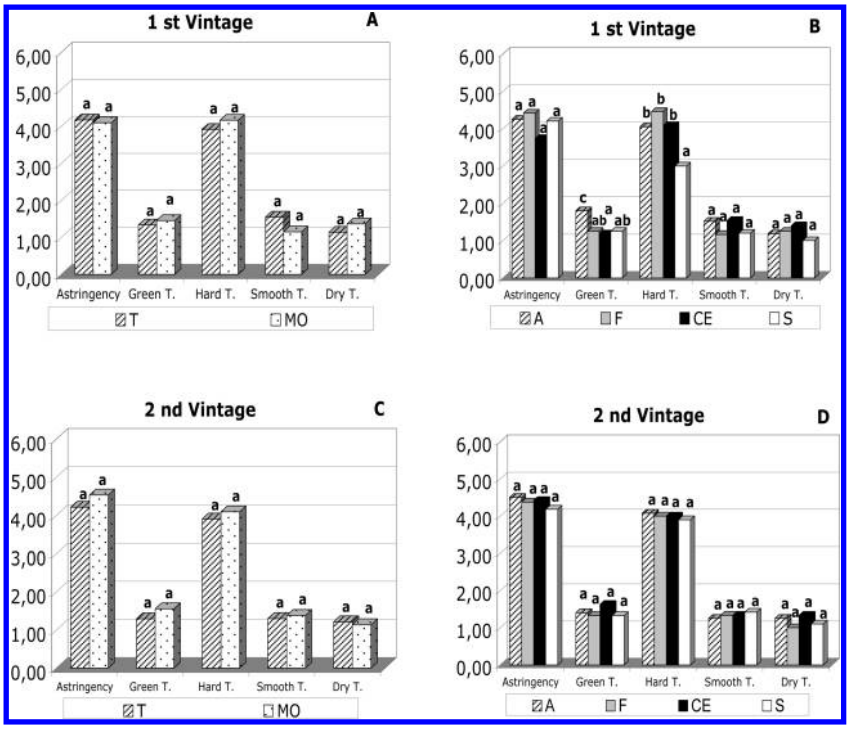

Figure 9. Mean values of astringency, green, hard, smooth, and dry tannins of wines aged for 12 months. (**A** and **C**) Mean values of control (T) versus micro-oxygenated wines (MO). (**B** and **D**) Mean values of wines aged in each wood type: American (A), French (F), Central European (CE), and Spanish (S). Values with the same letter in each parameter indicate that no statistically significant differences were detected with the LSD test for $\alpha = 0.05$.

that were aged for six months. These differences were found between the wines aged in Spanish oak and those aged in French and American oaks. It is important to note that Spanish oak-aged wines also showed the greatest color differences according to Glories' parameters, color intensity and percentage of blue.

The scores of the sensory analysis of the wines aged for 12 months showed generally no differences in the values of color intensity, blue−violets, and reds among the different wines, neither by micro-oxygenation treatment nor by oak type (**Figure 8A** and **B**), in either of the two vintages.

The assessment of the judges showed small differences in the results corresponding to the wines from the two vintages. The judges gave the control wines from the first vintage, aged in Spanish oaks, lower scores in blue−violet hues and higher in reds (garnets) than the rest of the wines aged in other oaks (**Figure 8B**). This agrees with the instrumental color analysis. However, for the second vintage, the tasters gave no significantly different scores to any of the wines by barrel type or micro-oxygenation treatment (**Figure 8C** and **D**).

The tasters found no significant differences among the different wines for the parameters on tannicity. In general, no significant differences were noted in the assessment of tannic sensations between the control and the micro-oxygenated wines of both vintages (**Figure 9A** and **C**). The exception was the results related to hard tannins between the different woods used. For the first vintage, the wines aged in American, French, and Central European oaks were assessed as having the higher hard tannin content, whereas the wines aged in Spanish oak received the lowest scores in this parameter (**Figure 9B**). Likewise, wines in American oak had the highest green tannin content. However, the wines had similar scores for the second vintage (**Figure 9D**).

These results demonstrate that the previously described tannin content differences in young micro-oxygenated and nonmicro-oxygenated wines (*16*, *20*) clearly decrease with aging. Furthermore, these differences eventually disappear.

In summary, it can be said that the micro-oxygenated wines studied evolved in a parallel and similar manner to those not micro-oxygenated. Therefore, the positive chromatic effects of micro-oxygenation remained notable during the studied aging period. Thus, it can be concluded that the color intensification and violet hues produced by micro-oxygenation are maintained at least during the first year of aging.

Evolution of total phenolic composition was independent of the type of wood in which the wine was aged. However, the wood type did affect evolution of the anthocyanin fraction. Of the four types of oak studied, Spanish oak produced the most notable changes, slowing down the transformation of monomeric anthocyanins into more complex and stable structures such as pyranoanthocyanins and polymeric pigments. This demonstrates that wood type influences the evolution of wine color, and it can be concluded that this fact is independent of whether the wine has been previously micro-oxygenated. These results pointed out that interactions between micro-oxygenation and type of oak barrel were not detected.

Finally, at a sensory level, it was difficult to detect differences in color and astringency either in wines previously structured (micro-oxygenated) or not, or between wines aged in different types of oak barrels after the sixth month of aging. In fact, differences were completely undetectable after 12 months of aging. These data seem to indicate that the positive sensory effects of micro-oxygenation on color and astringency are masked or lost after six months of aging.

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