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Influence of Micro-Oxygenation Treatment before Oak Aging on Phenolic Compounds Composition, Astringency, and Color of Red Wine

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Micro-oxygenation is usually applied to red wines as a cheaper alternative to oak aging. It has been suggested, however, that micro-oxygenation can also be used to complement oak aging in order to improve the quality of very astringent and herbaceous red wines. In this paper we study how applying the micro-oxygenation technique before oak aging affects the composition and quality of astringent red wines. When this technique is applied prior to oak aging, the wines have a slightly less intense red color and significantly higher levels of combined and free anthocyanins and ethyl-bridged anthocyanin−flavanol pigments. On the other hand, no differences in other newly formed pigments are found. Applying micro-oxygenation before oak aging does not affect the total proanthocyanidin concentration, but it produces wines with a slightly (though significantly) higher mean degree of proanthocyanidin polymerization and a drastically lower astringency. These wines also present a clearer impact of wood aromas.

KEYWORDS: Wine; phenolic compounds; micro-oxygenation; oak aging

INTRODUCTION

High-quality red wines are traditionally stored for a long time in oak barrels to improve their sensorial attributes. Clearly, wood provides many substances that enhance the intensity and complexity of wine flavor (*1*). However, oak aging also leads to color stabilization, lower astringency, and the disappearance of excess vegetative notes. These latter transformations seem to be associated with small quantities of oxygen that penetrate the porosity of the wood, the interstices between staves, and the bunghole (*2*).

The dissolved oxygen leads to the formation of ethanal from ethanol. The ethanal can in turn react with flavanols to induce the formation of a very reactive carbocation that quickly reacts either with another flavanol molecule or with an anthocyanin, producing ethyl-bridged flavanol−flavanol and flavanol−anthocyanin oligomers (*3*). More recently, it has been reported that ethanal also participates in the formation of new pigments such as vitisin B and other pyranoanthocyanins (*4*, *5*).

According to these data, oak aging should increase both the proanthocyanidin degree of polymerization and anthocyanin−flavanol combinations. Some authors have suggested that these processes are responsible for the color stabilization and lower astringency observed during oak aging (*3*−*6*). However, it has recently been shown that the compounds formed by ethyl bridges are unstable (*7*).

Oak aging is an expensive and laborious process that cannot be used for all wines. Micro-oxygenation has therefore been proposed for reproducing, and even accelerating, the transformations of color and phenolic compounds that take place during oak aging (*8*).

The technique involves providing a controlled flow of oxygen to the wine. The amount of oxygen must be sufficient to produce a high enough ethanal concentration for inducing the polymerization and combination reactions (*9*, *10*). Too great an oxygen flow may be unsuitable, however, as this can lead to the oxidation of aromas, the precipitation of high-molecular-weight polymers, and browning.

The use of micro-oxygenation to stabilize the color and decrease the astringency and herbaceous characters of wine has proved highly successful (*11*), and the technique is now used in wineries around the word as a cheaper alternative to oak aging. Nevertheless, the micro-oxygenation provides neither the flavors nor ellagic tannins that it gives the contact with the oak wood.

Some winemakers also recommend using micro-oxygenation as a complementary technique to oak aging. Some astringent

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and herbaceous wines can retain these negative characteristics even after a long period of oak aging (*12*). In such cases, applying micro-oxygenation before oak aging could help to reduce these negative sensory attributes (*13*).

Some publications in the literature have studied the influence of micro-oxygenation on wine color, composition, and sensory attributes (*10*−*14*). To our knowledge, however, none of these have studied the application of this technique before oak aging for the treatment of very astringent wines.

In this paper, therefore, we study how applying a micro-oxygenation treatment prior to oak aging affects the phenolic composition, astringency, and color of a very astringent red wine.

MATERIALS AND METHODS

Chemicals. HPLC-grade methanol, acetonitrile, and formic acid were purchased from Merck (Mollet del Vallés, Spain). Epicatechin, ovoalbumin, tannic acid, and 4-dimethylaminocinnamaldehyde (DMACH) were purchased from Sigma (Madrid, Spain). The other chemicals were of high purity and purchased from Panreac (Barcelona, Spain).

Wines. This study was carried out with a very astringent red wine from the *Vitis vinifera* cv Cabernet sauvignon from the 2003 crop. The wine (1500 L) was obtained from the experimental cellar of the Faculty of Enology of the Rovira i Virgili University in Constantí (Tarragona, Spain). Grapes were harvested when they were not completely ripened. Winemaking was carried out trying to extract a high proanthocyanidin concentration in order to obtain a very astringent wine. The length of the maceration was 21 days. Two "rack and return" by day were carried out during the first 4 days of fermentation. After that, a daily pump over was done until the end of maceration. Press wine was added to the mixture to enhance astringency and herbaceous characters.

The analytical characteristics of the wine at the start of the experiment were as follows: ethanol content, 12.8%; titratable acidity, 5.2 g of tartaric acid/L; volatile acidity, 0.52 g of acetic acid/L; pH, 3.74; residual sugars, 1.96 g/L; sugar free extract, 24.6 g/L; free SO_2, 23 mg/L; total SO_2, 60 mg/L.

Oak Barrels. Six new American oak barrels each with a capacity of 225 L were used. The cooperage (Torné, Villafranca del Penedès, Spain) guaranteed the maximum homogeneity between casks.

Micro-Oxygenation Equipment. The multiple diffuser micro-oxygenator (VISIO 2/6-Oenodev, France) was connected to three stainless steel tanks of 275 L. These tanks were 2.2 m in height, had a diameter of 0.4 m, and were equipped with a ceramic diffuser placed at 10 cm above the bottom of the tank. These dimensions were necessary so that the oxygen bubbles produced during micro-oxygenation would have a sufficient height of displacement to guarantee their complete dissolution.

Experimental Conditions. All wines were kept at a temperature of 16 ± 2 °C. Around 675 L of the wine was distributed in three oak barrels and stored for 8 months (control wine). The wines were then racked individually to three 200 L stainless steel tanks and stored for 3 months to make the total time of conservation in both experimental conditions equal. These experimental conditions (i.e., first, oak aging and afterward, stainless steel conservation) were chosen as control conditions in order to better reproduce the typical procedure of oak aging in commercial cellars.

Simultaneously, 825 L of the same wine was stored in the three micro-oxygenation tanks (275 L in each tank). These wines (MO wine) were kept for 3 months with an oxygen flow of 3 mg/L per month. The wines were then racked individually to three barrels without any previous step and stored there for 8 months.

Free sulfur dioxide was analyzed every 15 days by the OIV method (*15*) and corrected to be kept at 25−30 mg/L (0.29−0.35 mg/L of molecular sulfur dioxide). After the experiment, sulfur dioxide was added to maintain free sulfur dioxide concentration at 25 mg/L. The wines were immediately bottled in 750 mL bottles and closed with 38 mm × 21.5 mm synthetic stoppers (Supreme Corq, Supreme Corq, LL; WA). Just before the chemical analyses, the wines were centrifuged (10 000*g*; 10 min).

Color Parameters. The components yellow (A420 nm), red (A520 nm), and blue (A420 nm) and the color intensity (CI) were estimated using the method described by Glories (*16*). The CIELAB parameters, lightness (L*), chroma (C*), and hue (H*) were determined according to Ayala et al. (*17*).

Anthocyanin Analysis. Total anthocyanins were determined using the method described by Niketic-Alksic and Hrazdina (*18*). Free and combined anthocyanins were calculated using the PVPP index (*16*). The anthocyanin fraction contributing to wine color was calculated using the ionization index (*19*). The percentage of color due to copigmentation was calculated according to Boulton (*20*).

HPLC Analyses of Anthocyanins and Derived Pigments. Analyses were performed in a Hewlett-Packard 1100 series liquid chromatograph. Separation was achieved on a 5 μm AQUA C18 150 mm × 4.6 mm column (Phenomenex, Torrance, CA) thermostated at 35 °C. No precolumn was used for the HPLC analysis of the pigment profiles. Even if the chromatogram baselines improve with the use of a precolumn, some retention of complex pigments may occur. The solvents used were (A) 0.1% trifluoroacetic acid in water and (B) HPLC-grade acetonitrile. The following gradient was established: isocratic 10% B for 5 min, 10−15% B for 15 min, isocratic 15% B for 5 min, 15−18% B for 5 min, and 18−35% B over 20 min. A flow rate of 0.5 mL/min was used. Double on-line detection was carried out in a diode array spectrophotometer (DAS), using 520 nm as the preferred wavelength, in a mass spectrometer (MS) connected to the HPLC system via the DAS cell outlet. The mass spectrometer was a Finnigan LCQ (San Jose, CA) equipped with an ESI source and an ion trap mass analyzer, which were controlled by the LCQ navigator software. Nitrogen was used as both auxiliary and sheath gas at flow rates of 6 and 1.2 L/min, respectively. The capillary voltage was 4 V, and the capillary temperature was 195 °C. Spectra were recorded in positive ion mode between *m*/*z* 150 and 1500. The MS detector was programmed to perform a series of three consecutive scans: a full scan, a zoom scan of the most abundant ion in the first scan, and an MS−MS scan of the most abundant ion, using a normalized collision energy of 45%. The compounds were identified from their elution characteristics, UV−vis spectra, molecular ion, and MS^2 fragmentation patterns by comparison with previous data (*21*). Pigment quantification was obtained from the areas of their chromatographic peaks recorded at 520 nm and comparison with calibration curves obtained with an external standard of malvidin 3-glucoside (Extrasynthese, Lyon, France).

Other Phenolic Compounds. The contents of phenolic compounds were determined by measuring the absorbance at 280 nm (*19*). Total proanthocyanidins were estimated according to Ribéreau-Gayon and Stonestreet (*22*). Dimethylaminocinnamaldehyde index (DMACH index) was measured according to Nagel and Glories (*23*).

Mean Degree of Polymerization (mDP) and Percent of Monomers. The proanthocyanidin mDP and the percentage of monomers were measured according to Souquet et al. (*24*). A volume of 4 mL of wine was evaporated on Genevac apparatus and then resolubilized in 4 mL of water with 2% acetic acid. A volume of 2 mL of wine was eluted on SPE cartridges waters tc18 (500 mg) which were previously preconditioned with 5 mL of water following with 10 mL of methanol and then with 10 mL of the first solvent, water−2% acetic acid. After rinsing the cartridge with 5 mL of water the components of interest were eluted with 8 mL of methanol. This fraction was evaporated. A volume of 100 μL of 0.1 N HCl in methanol containing 50 g/L of phloroglucinol and 10 g/L of ascorbic acid reacted with this fraction at 50 °C for 20 min and then was combined with 100 μL of aqueous sodium acetate (200 mM).

The proanthocyanidin composition of the wine was determined by phloroglucinolisis after isolating and calibrating each of the reaction products.

Phloroglucinol adducts were analyzed by HPLC. The column was a C18 (Atlantis dc 18 water 4.6 mm × 250 mm; Waters Corporation, Milford, MA) that exhibited enhanced retention of polar compounds. The method used a binary gradient with mobile phases containing 2% formic acid in water (phase A), 1% v/v aqueous acetic acid (Phase A), and 2% formic acid in acetonitrile/water (80/20) (phase B). Eluting peaks were monitored at 280 nm. The elution conditions were 1.0 mL/min; 0% B for 5 min, a linear gradient from 0 to 20% B in 55 min.

The column was then washed with 90% B for 10 min and reequilibrated with 0% B for 5 min before the next injection.

The LC−MS analyses in positive mode were performed on the same column to identify the components of the reaction.

The mDP was calculated by adding up all the units (lower and upper units) and dividing by the sum of the terminal units in mol. The percentage of each monomer in the polymer was calculated by dividing each monomer by the all monomers and expressing this as a percentage (*24*−*26*).

Astringency. Astringency was estimated using ovoalbumin as a precipitation agent and tannic acid solutions as standards in accordance with the previously described method (*27*).

Sensory Analysis. All the wines were tasted by a group of 10 expert enologists from the Rovira i Virgili University 6 weeks after bottling. A previous sensory season has been developed to homogenize the criteria. Two sensory trials were conducted. The first of these was a triangular test between the control and the micro-oxygenated wine in which the tasters were only asked to recognize the different glass. The second was a descriptive trial in which each expert evaluated each wine for 10 sensorial attributes: five olfactory (fruit, green pepper, toasting, spices, and coffee) and five gustatory (body, mouth feel, persistence, bitterness, and astringency), on a scale from 1 to 10. The values indicate the intensity of the sensation for each attribute.

Statistics. For color and chemical analysis, all the data are expressed as the arithmetic average ± the standard deviation from three replicates for each experimental condition. For the descriptive sensorial analysis, all the data are expressed as the arithmetic average from the 10 expert tasters. In this case, the standard deviation is not included in order to avoid excessively complicating the graphs. Statistical comparisons between values were established with Student's *t*-test using Statview (SAS Institute Inc., Cary, NC, version 4.01). In the triangle test, statistically significant differences between wines were determined by considering a p value of less than 0.05 (*28*).

RESULTS AND DISCUSSION

As we explained above, this work was performed to compare the application of a micro-oxygenation treatment before oak aging with a process that uses oak aging only. The treatment for the experimental group was clear (3 months in stainless steel with micro-oxygenation prior to 8 months of oak aging). However, the treatment for the control group was not so obvious. If the control wine had spent only 8 months of oak aging, it would be not comparable with the micro-oxygenated wine because the time of evolution was not the same. Therefore, we decided to maintain the wine for 3 months in stainless steel tanks to equalize the time of storage. The scientific logic would suggest storing the control wine for 3 months in stainless steel tanks before oak aging to reproduce exactly the conditions of the micro-oxygenated wine. However, these conditions are typically not used in wineries. Nowadays, the wine cellars put the wine in casks as early as possible, even before malolactic fermentation. The reason for proceeding this way is that winemakers consider this practice to be good to enhance wine quality, and it also allows the producer to obtain a stabilized and aromatized wine before (*19*, *29*). For this reason, we decide to first perform oak aging and afterward store the wine in stainless steel tanks, to better reproduce the typical conditions of commercial cellars.

Table 1 shows the color parameters of the wine at the beginning of the experiment and after the application of both experimental conditions. In general, the changes in color during aging were as expected. Both the MO and control wines presented a lower color intensity (CI) and a higher hue (H*) and luminosity (L*) than their initial values. However, the chroma (C*) was almost not modified.

When we compared the color parameters of the wine that was micro-oxygenated before oak aging (MO wine) with those of the wine that was only oak aged (control wine) we found some statistically significant differences. The values of CI and the three color components (A420-yellow, A520-red, and A620-blue) were significantly lower in the MO wine. On the other hand, chroma (C*), hue (H*), and especially luminosity (L*) significantly increased when micro-oxygenation was applied before oak aging. Micro-oxygenation before oak aging therefore seems to produce wines with a slightly less intense color and slightly more evolved to yellowish nuances. Although no information is published about the effect of micro-oxygenating wines before oak aging, these results agree in general terms with the observed effect of oxygen on the evolution of wine color (*30*, *31*).

Table 1. Color Parameters[a]

	initial wine	control wine	MO wine
A420	0.460	0.488 ± 0.023 b	0.465 ± 0.010 a
A520	0.925	0.847 ± 0.040 b	0.791 ± 0.023 a
A620	0.232	0.218 ± 0.011 b	0.177 ± 0.006 a
CI	16.17	15.53 ± 0.74 b	14.33 ± 0.38 a
C*	54.30	53.05 ± 0.09 a	54.62 ± 0.60 b
L*	37.00	38.90 ± 1.90 a	42.73 ± 0.93 b
H*	7.95	14.53 ± 0.17 a	16.33 ± 0.21 b

[a] All data are expressed as the average of three replicates ± standard deviations ($n = 3$). CI, color intensity; C*, chroma; L*, luminosity; H*, hue. Statistical analysis: one-factor ANOVA and Scheffe's test (both $p = 0.05$). Different letters indicate statistical differences.

Table 2. Spectrophotometric Analysis of Anthocyanins and Related Parameters[a]

	initial wine	control wine	MO wine
free anthocyanins (mg/L)	269	130 ± 16 a	178 ± 16 b
combined anthocyanins (mg/L)	145	194 ± 2 a	210 ± 10 b
total anthocyanins (mg/L)	414	322 ± 13 a	389 ± 9 b
ionization index (%)	35.3	51.7 ± 2.3 a	48.9 ± 4.5 a
copigmentation index (%)	14.8	11.6 ± 1.8 a	10.6 ± 2.2 a

[a] All data are expressed as the average of three replicates ± standard deviations ($n = 3$). Statistical analysis: one-factor ANOVA and Scheffe's test (both $p = 0.05$). Different letters indicate statistical differences.

Table 2 shows the results from the spectrophotometric analysis of anthocyanins and other related parameters. Total and free anthocyanins decreased during the aging period, whereas the combined anthocyanins increased.

Simultaneously, the contribution of anthocyanins to wine color (ionization index) increased and the percentage of the color due to the copigmentation process decreased. Both changes were probably due to the augmentation of the combination of anthocyanins with proanthocyanidins. These results agree with those published by other authors (*19*, *20*, *32*).

Some statistically significant differences were found between MO and control wines regarding anthocyanins. As expected, the concentration of combined anthocyanins in MO wines was significantly higher than in control wines. This indicates that color is more stable in the MO wines than in control wines (*6*). Unexpectedly, however, the MO wine also had a higher free anthocyanin concentration than the control wine. These results were confirmed by the HPLC analysis of anthocyanins (**Table 3**).

The total anthocyanin concentrations determined by HPLC (*33*) were much lower than those obtained by spectrophotometry (*19*). This could be logical as spectrophotometric analysis is

Table 3. HPLC Analysis of Anthocyanins and Anthocyanin-Derived Pigments[a]

	control wine	MO wine
anthocyanidin-3-monoglycosides (mg/L)	28.68 ± 4.04 a	60.38 ± 7.71 b
acetylated anthocyanins (mg/L)	5.70 ± 0.44 a	11.00 ± 1.61 b
p-coumaroyl anthocyanins (mg/L)	4.17 ± 0.26 a	6.00 ± 0.57 b
total anthocyanins (mg/L)	37.34 ± 4.73 a	77.38 ± 9.73 b
vitisin A (mg/L)	6.74 ± 0.08 a	6.71 ± 0.17 a
vitisin B (mg/L)	2.06 ± 0.07 a	1.90 ± 0.10 a
vinylcatechin–malvidine (mg/L)	4.14 ± 0.13 a	4.29 ± 0.04 a
vinylphenol–malvidine (mg/L)	1.57 ± 0.03 a	1.59 ± 0.04 a
catechin–ethyl-malvidine (mg/L)	4.29 ± 0.07 a	4.54 ± 0.10 b

[a] All data are expressed as the average of three replicates ± standard deviations ($n = 3$). Statistical analysis: one-factor ANOVA and Scheffe's test (both $p = 0.05$). Different letters indicate statistical differences.

Table 4. Total Phenolic Compounds, Proanthocyanidins, and Related Parameters[a]

	initial wine	control wine	MO wine
A280 nm	78.0	75.6 ± 1.5 a	76.7 ± 0.3 a
proanthocyanidins (g/L)	4.50	4.12 ± 0.05 a	4.08 ± 0.01 a
DMACH index	46.7	31.3 ± 1.5 a	32.8 ± 1.4 a
astringency (g/L)	0.707	0.596 ± 0.024 b	0.383 ± 0.025 a
mDP		4.53 ± 0.11 a	4.77 ± 0.01 b
(+)-catechin (%)		15.94 ± 0.24 a	15.92 ± 0.05 a
(–)-epicatechin (%)		60.19 ± 0.23 a	60.42 ± 0.27 a
(–)-epigalocatechin (%)		21.17 ± 0.15 a	21.03 ± 0.33 a
(–)-epicatechin gallate (%)		2.71 ± 0.13 a	2.65 ± 0.02 a

[a] All data are expressed as the average of three replicates ± standard deviations ($n = 3$). DMACH, dimethylaminocinnamaldehyde; mDP, mean degree of polymerization. Statistical analysis: one-factor ANOVA and Scheffe's test (both $p = 0.05$). Different letters indicate statistical differences.

known to overestimate the total anthocyanin concentration because in its measurement it includes the contribution from other pigments (*34*), whereas only free anthocyanins are detected by the HPLC method (*33*). With the PVPP index we can indirectly quantify both free anthocyanins and, by subtraction, the combined ones (*16*). In any case, the free anthocyanin values obtained by this method were still higher than those obtained by HPLC, probably because the different way used to express the results in each case, which makes them not directly comparable.

Despite the different values obtained by HPLC and spectrophotometric methods, the anthocyanin concentrations showed the same tend. The MO wine had significantly higher levels of anthocyanidin-3-glycosides and acetylated and *p*-coumaroyl anthocyanins than the control wine.

These results are surprising. Micro-oxygenation favors the combination of anthocyanins and flavanols via the formation of ethyl bridges (*30*, *35*). A higher combined anthocyanin concentration in MO wines could be therefore completely logical, but simultaneously, a lower free anthocyanin concentration should be expected. A possible explanation for the higher free anthocyanin concentration in MO wine may be the instability of anthocyanin−ethyl-flavanol compounds, which may undergo cleavage of the ethyl bridge and allow free anthocyanins and structural rearrangements (*7*). Theoretically, in the first 3 months of aging, the formation of these compounds in the MO wine must be higher than in the control wines because of the micro-oxygenation process and the subsequent availability of acetaldehyde (*35*−*37*). In further stages, a degradation of the ethyl-linked oligomers would take place to again releasing free anthocyanins.

Table 3 also shows the concentration of the anthocyanin-derived pigments. No important differences were found between the composition of the control wine and the MO wine. The levels of pyranoanthocyanins such as vitisin A, vitisin B, and vinylcatechin− and vinylphenol−anthocyanin adducts were almost identical. We should point out that, as pyruvic acid and vinylphenol (precursors of vitisin A and vinylphenol−anthocyanins) are products of the metabolism of microorganisms (*38*), we should expect that vitisin A and vinylphenol−anthocyanin adducts to be present at similar levels in both wines. Microorganisms also produce ethanal, but this compound may also derive from the oxidation of ethanol. We should therefore expect that the MO wine have higher levels of ethyl-linked products and, possibly, vitisin B. The origin of vinylcatechin−anthocyanin adducts is more debatable. It has been suggested that vinylcatechin can derive either from the reaction between catechin and acetaldehyde or from the degradation of catechin−ethyl-anthocyanin adducts (*39*) and that vinylcatechin later reacts with the anthocyanin to yield the derived pigment. Higher levels of this type of pigments would therefore be expected in MO wines. However, no important differences were found in the concentrations of vitisin B or vinylcatechin−anthocyanin adducts in these wines. We should bear in mind that both wines were maintained in new oak barrels for 8 months and that oak aging also implies a certain micro-oxygenation. Also, most of these anthocyanin-derived pigments are probably produced during the fermentation process (*38*). This may be why no major differences were found between the anthocyanin-derived pigments in the MO wine and the control wine.

It has been reported that catechin−ethyl-anthocyanin adducts are produced during red wine micro-oxygenation (*40*). Our results agree with this, though the differences between the wines are very slight and just a minor but significant increase of 6% was detected in the levels of catechin−ethyl-malvidin in the MO wine with respect to that of the control wine (see **Table 3**). This small difference could be due to instability of the ethyl-bridged compounds and their tendency to increase in size in the presence of available ethanal (*7*, *9*, *41*).

Table 4 shows the results for total phenolic compounds, proanthocyanidins, and other related measurements. Generally, proanthocyanidin concentration decreases throughout aging in both wines. This decrease may be explained by the precipitation of some of the polymers formed during the aging period (*19*). This increase in the degree of polymerization during wine aging is supported by the decrease in the DMACH index. The DMACH reacts with the catechin moieties in the proanthocyanidin ends, so its reactivity diminishes as the degree of polymerization increases. As we expected (*42*), the increase in the degree of polymerization and proanthocyanidin precipitation throughout aging was accompanied by a decrease in astringency, measured by chemical analysis. On the other hand, despite the decrease in proanthocyanidin concentration, the differences in total phenolic compounds (A280 nm) were only slight. This is probably because of the precipitation of the proanthocyanidins is compensated by the dissolution of other phenolic compounds from the wood of the casks (*1*, *6*, *43*).

No statistically significant differences were found between the proanthocyanidin concentrations, A280 nm, or DMACH indexes of the control wine and the MO wine. However, the MO wine was 35% less astringent than control wine. This supports the suitability of using micro-oxygenation to treat very astringent wines before oak aging.

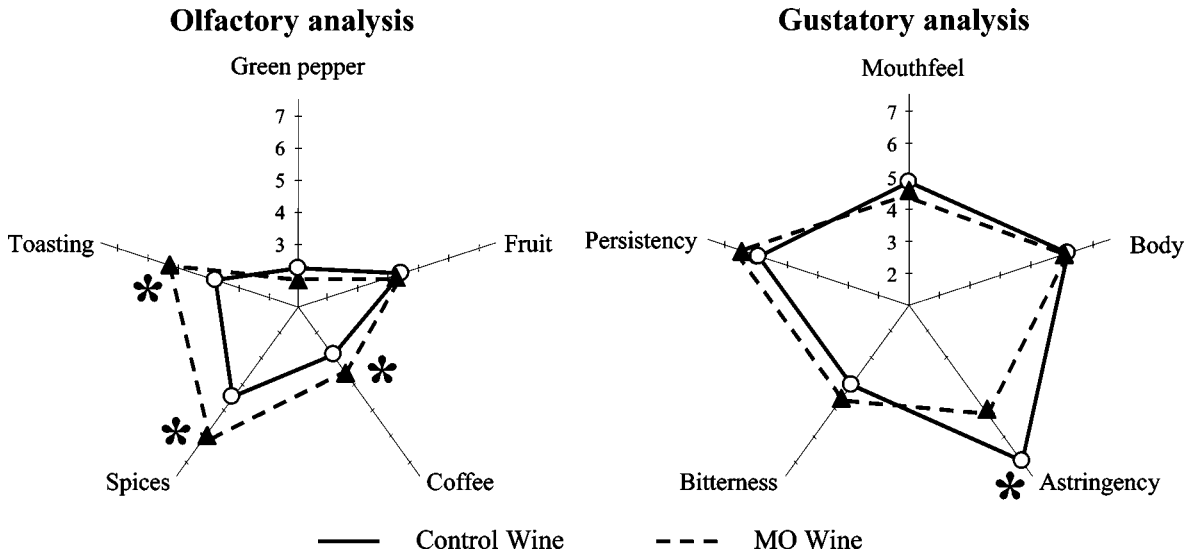

Figure 1. Sensory analysis of control and micro-oxygenation wines. All data are the arithmetical average corresponding to the results of 10 tasters. The ∗ indicates the existence of statistically significant differences.

Table 4 also shows the mDP of the proanthocyanidins and their monomeric composition. Though the differences are small (5%), the mDP of MO wine is significantly higher than that of the control wine which confirms that, as expected, micro-oxygenation favors the polymerization of proanthocyanidins (*10*, *12*, *35*, *37*). The increase in the mDP was not detected in the DMACH index. As shown above, this index is only an indirect measurement of the polymerization degree and so is less precise than using acid cleavage in the presence of phloroglucinol (*43*−*46*). On the other hand, no significant differences were found between the monomeric compositions of the proanthocyanidins of these wines.

Some authors have reported that the size of the proanthocyanidin molecule is related to astringency: the greater the mDP, the greater the astringency (*47*, *48*). In our case, the MO wines present a slightly, but significantly, higher mDP, but they are much less astringent. The reason for this apparent contradiction may be related to the fact that many other factors influence wine astringency. Some authors have suggested that the combination of proanthocyanidins and anthocyanins may be responsible for the decrease in astringency during oak aging (*49*, *50*). Our results indicate that MO wines have a significantly higher concentration of catechin−ethyl-malvidin and combined anthocyanins than control wine. Although these differences are small, they may partially explain the lower astringency of the MO wines.

On the other hand, the sensory comparison of the two wines by the triangular test indicates that 7 out of 10 tasters differentiated between the two wines. According to the statistical tables (*28*), these results are statistically significant ($p < 0.05$) and confirm that micro-oxygenation before oak aging produces a different wine.

Figure 1 shows the results of the descriptive sensory analysis of the two wines. The MO wine had significantly higher levels of toasting, spices, and coffee aromas and a significant lower astringency than the control wine. No differences were found in the other sensorial attributes. The higher intensity of some of the olfactory attributes could be due to the fact that MO wine was more recently extracted from wood than control wine or because micro-oxygenation before oak aging accentuates these aromatic notes. Similarly, the observed sensory astringency had the same tendency as the astringency determined by chemical analysis, which confirms micro-oxygenation before oak aging may help to diminish this disagreeable sensation.

We can conclude that applying micro-oxygenation before oak aging presents the disadvantage of producing wines with a slightly less intense red color and somewhat more evolved toward yellowish nuances. However, this technique has the advantages that the wines obtained have a higher anthocyanin concentration, a clearer impact of oak aromas, and especially, a much lower astringency. Applying micro-oxygenation before oak aging is therefore mainly advised for very astringent wines such as press wines or wines obtained from not well-ripened grapes.

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