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Evolution of Phenolic Compounds and Astringency during Aging of Red Wine: Effect of Oxygen Exposure before and after Bottling

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ABSTRACT: The aim of this study was to evaluate the effect of oxygen exposure of red wine, before (micro-oxygenation) and after (nano-oxygenation) bottling, on the phenolic composition and astringency of wine. The astringency was evaluated by sensory analysis and by a method based on the SDS-PAGE of salivary proteins after reaction of saliva with wine (SPI, saliva precipitation index). Micro-oxygenation caused a stabilization of color, but this effect disappeared after long aging. For the wine with the lower pH a decrease of wine astringency and SPI was observed 42 months after micro-oxygenation. Oxygen ingress through the closure postbottling was positively correlated with the decrease of SPI. Therefore, the astringency and reactivity of wines toward salivary proteins of a bottled red wine can be modulated by controlled oxygen exposure during aging. For both experiments the effect of oxygen exposure depended on wine composition.

KEYWORDS: *wine, micro-oxygenation, nano-oxygenation, astringency, phenolic compounds*

INTRODUCTION

Generally, high-quality red wines require a period of aging in the bottle, after which they are ready to be consumed. During this time modifications of the sensory properties of the wine occur, such as the decrease of astringency[1] and the stabilization of color from purple red to tawny. Numerous regulations for specific appellations of high-quality red wine impose a minimum period of bottle aging to achieve the specific sensory attributes that are requested by the appellation. However, wine aging implies a significant financial cost for the industry, so it is important to understand the timing, the factors, and the mechanisms of astringency and color changes during bottle aging. Astringency is a tactile sensation mainly elicited by the precipitation of salivary proteins, which reduces mouth lubrication.[2] Wine components responsible for astringency include phenolics such as proanthocyanidins (PAs) or wood tannins (ellagitannins and gallotannins) that react with salivary proteins, causing their complexation and subsequent precipitation on the mouth epithelium.[3] The color of red wine is due to anthocyanins and derived copigments. Whereas the main reactions involved in color stabilization are known,[4−7] the impact of reactions occurring during aging and involving PAs and anthocyanins on the decrease of wine astringency are still not well established.[1,8,9] In the past, this phenomenon was attributed to the formation of greater polymeric structures.[10] Nowadays, the reduction of the average molecular weight of PAs rather than their increase is believed to decrease wine astringency.[1]

Many of the complex reactions involving phenolics are affected by oxygen exposure of wine, and a moderate uptake of oxygen during aging can accelerate and/or trigger specific reactions influencing sensory properties.[8,11−14] For this reason, the aging in oak barrels of red wines is a widely used practice. In fact, the ingress of small amounts of oxygen through the wood and between wood staves results in a great number of chemical reactions involving wine and wood phenolics enhancing the decrease of wine astringency and the stabilization of color.[15,16] As this practice, as well as bottle aging, is expensive and implies a significant financial cost in the wines' final price, micro-oxygenation (MOX) has been proposed. It consists of adding a continuous oxygen flow to a tank, simulating the oxygen uptake occurring during wood aging. Despite extensive research in this area,[17−20] very few studies have evaluated the fate of micro-oxygenated wines after a long period of bottle aging.[21,22]

Besides the winemaking process, wine can be further exposed to oxygen during aging in the bottle, depending on the oxygen permeability of the closure. Because of the extremely low rates of oxygen ingress through a closure, this form of oxygen exposure has been referred to as nano-oxygenation.[21] Oxygen transmission rates (OTR) of wine closures may vary widely depending on closure type and strongly influence the evolution of white and red wines during bottle aging.[21,23−27] Additionally, the oxygen present at bottling, often referred to as total package oxygen (TPO), together with the oxygen released from the closure upon insertion in the bottleneck also contributes to oxygen exposure in the bottle.[28,29]

Despite the fact that oxygen exposure has been linked to a great number of reactions by phenolics involved in astringency decrease, a clear correlation between chemical transformation of phenolics and their sensory properties has never been reported. This can be due to the fact that the sensorial activity

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of wine tannins is not easy to predict because it is influenced by their chemical nature, quantity, and the inner balance with the other compounds present in wine.[2,30−34] For all of these reasons, during recent years several analytical methods have been developed to predict wine astringency by evaluating the ability of wine to form insoluble complexes with human saliva.[35−37] In this work, the saliva precipitation index (SPI), based on the precipitation of selected salivary proteins after reaction with wine polyphenols,[38] was utilized to objectively evaluate changes in astringency as a function of the oxygen uptake before and after bottling. The sensory rating of astringency, the polyphenolic composition, and chromatic characteristics were also evaluated.

MATERIALS AND METHODS

Wines. *Micro-oxygenation Trials.* Two Aglianico red wines were prepared according to the traditional winemaking protocol from the 2006 vintage. Wines were produced by Cantina del Taburno winery in agreement with the standard procedure used for the production of Aglianico del Taburno DOC wine. The base parameters (mean ± standard deviation) of wine A at the start of the experiment were as follows: ethanol content, 13.60 ± 0.30% v/v; pH, 3.64 ± 0.01; titratable acidity, 4.60 ± 0.09 g/L expressed as tartaric acid; volatile acidity, 0.41 ± 0.06 g/L expressed as acetic acid; free SO_2, 28 mg/L; total SO_2, 64 mg/L; mol SO_2, 0.67 mg/L. The standard parameters of wine B were as follows: ethanol content, 13.06 ± 0.40% v/v; pH, 3.46 ± 0.03; titratable acidity, 5.17 ± 0.30 g/L expressed as tartaric acid; volatile acidity, 0.26 ± 0.10 g/L expressed as acetic acid; free SO_2, 26 mg/L; total SO_2, 70 mg/L; mol SO_2, 0.78 mg/L. They were chosen because of their different pH value. For both wines two micro-oxygenation treatments (MO*X*) were applied. Each wine was transferred from the initial tank into six 50 hL tanks (3 m high). Micro-oxygenation was performed on four tanks with a Microdue system (Enologica Vason, Pedemonte, Verona, Italy). Oxygen was provided through a diffuser composed of a porous ceramic membrane. Two tanks were denoted MO1 and two others as MO2, whereas two tanks were the control wines. Treatment MO1 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C). Treatment MO2 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C) followed by the application of 1.5 mL/L of O_2 for another 8 weeks (wine temperature was 15.5 °C). Three months after MO*X* treatment, each wine was bottled in 750 mL glass bottles previously flushed with 98% N_2 gas and sealed with a 44 mm natural cork. Analyses were carried out at bottling and after 42 months of aging in the bottle.

Nano-oxygenation Trials. For the study of the influence of the closure OTR on wine phenolic composition and astringency, two red wines were used. Wine 1 was a blend consisting of 40% Cabernet Franc, 40% Merlot, and 20% Blaufrankisch. Wine 2 was a Montepulciano. All bottles were sealed using Nomacorc coextruded synthetic closures (Nomacorc SA, Thimister Clermont, Belgium), and three distinct levels of oxygen exposure in the bottles were obtained by using closures with different oxygen ingress profiles: Select 300 ($W1_{low}$ and $W2_{low}$), Select 500 ($W1_{medium}$ and $W2_{medium}$), and Select 700 ($W1_{high}$ and $W2_{high}$). The values of total oxygen exposure (TOE) in the bottle for each wine are the sum of closure contribution and TPO. Closure contribution is intended as the combination of oxygen ingress through the closures (OTR) and the amount of oxygen released from the closure following insertion in the bottleneck. These values were provided by the manufacturer and were calculated using the procedure described by Dieval et al.[39] Nominal OTR values for the three closures, measured in 100% oxygen, were 0.011 mL/day for Select 300, 0.017 mL/day for Select 500, and 0.021 mL/day for and Select 700. TPO at bottling was measured by means of oxoluminenscence, using a Nomasense oxygen analyzer (Nomacorc SA). Measurements were taken approximately 1 h after bottling, and results are given in Table 1. Analyses were carried out 10 months after bottling. At the time of color and SPI analyses, free SO_2 values of the wines were as follows: $W1_{low}$, 22 mg/L; $W1_{medium}$, 22 mg/L; $W1_{high}$, 20 mg/L; $W2_{low}$, 19; $W2_{medium}$, 17 mg/L; $W2_{high}$, 15 mg/L.

Table 1. Wine Codes, Total Package Oxygen (TPO) at Bottling, Closure Contribution, and Total Oxygen Exposure (TOE) for the Two Wines and the Three Closures Used

	TPO[a] at bottling[b] (mg/L)	closure contribution[b,c] (mg/L)	TOE[b] (mg/L)
$W1_{low}$	9.8	3.2	13
$W1_{medium}$	9.8	4.0	13.8
$W1_{high}$	9.8	4.5	14.3
$W2_{low}$	6.5	3.2	9.7
$W2_{medium}$	6.5	4.0	10.5
$W2_{high}$	6.5	4.5	11

[a]TPO is the sum of dissolved and headspace oxygen after bottling. [b]The variation coefficient of data is 10%. [c]Values at 12 months of bottle storage include oxygen release from the closure and OTR.

Standard Chemical Analyses and Spectrophotometric Measurements. Standard chemical analyses (alcoholic strength by volume, titratable acidity, pH, volatile acidity, free and total SO_2, and total polyphenols (Folin−Ciocalteu)) were measured according to the *OIV Compendium of International Methods of Wine and Must Analysis*.[40] Condensed tannins (proanthocyanidins, PAs) were evaluated as described by Ribéreau-Gayon and Stonestreet.[41] Total anthocyanins and SO_2 bleaching anthocyanins were determined according to the method of Ribéreau-Gayon and Stonestreet.[42] Vanillin reactive flavans (VRF) were determined according to the method of Di Stefano and Guidoni.[43] Color intensity (CI) and hue were evaluated according to Glories methods.[44] A Shimadzu UV-1800 (Kyoto, Japan) UV spectrophotometer was used; 10 mm plastic cuvettes were used. Photometric accuracy was of ±0.002 Abs, and photometric repeatability was < ±0.001 Abs. All analyses were carried out in triplicate.

HPLC Analysis of Anthocyanins. HPLC separation of anthocyanins was carried out according to the *OIV Compendium of International Methods of Wine and Must Analysis*.[40] Analyses were performed in a HPLC Shimadzu LC10 ADVP apparatus (Shimadzu Italy, Milan, Italy), consisting of an SCL-10AVP system controller, two LC-10ADVP pumps, an SPD-M 10 AVP detector, and an injection system Rheodyne model 7725 (Rheodyne, Cotati, CA, USA) equipped with a 20 μL loop. A Waters Spherisorb column (250 × 4.6 mm, 4 μm particle diameter) with precolumn was used. Twenty microliters of wine or calibration standards was injected onto the column. All of the samples were filtered through 0.45 μm Durapore membrane filters (Millipore, Ireland) into glass vials and immediately injected into the HPLC system. The HPLC solvents were solvent A, water/formic acid/acetonitrile (87:10:3) v/v, and solvent B, water/formic acid/acetonitrile (40:10:50) v/v. The following gradient was established: zero-time conditions were 94% A and 6% B; after 15 min, the pumps were adjusted to 70% A and 30% B, at 30 min to 50% A and 50% B, at 35 min to 40% A and 60% B, and at 41 min (end of analysis), to 94% A and 6% B. This zero-time solvent mixture was followed by a 10 min equilibrium period prior to injection of the next sample. A flow rate of 0.80 mL/min was used. Detection was carried out by monitoring the absorbance signals at 518 nm. For calibration the external standard method was used: the calibration curve was plotted for the malvidin-3-monoglucoside (Extrasynthese, Lyon, France) on the basis of peak area. The calibration curve was obtained by injecting five solutions (in triplicate) containing increasing concentrations of malvidin-3-monoglucoside. The anthocyanins concentrations were expressed as milligrams per liter of malvidin-3-monoglucoside. Calibration curve was characterized by a determination coefficient (R^2) = 0.996. The analyses were carried out in triplicate.

Human Saliva. Whole human saliva was obtained by mixing saliva samples collected from six nonsmoking volunteers (three males and three females). The resulting saliva was centrifuged at 10000*g* for 10 min at 4 °C, and the supernatant (referred as HS) was used for the analysis. The binding assays were performed by mixing 100 μL of HS and 50 μL of wines into microcentrifuge tubes maintained at 25 °C for 5 min. The sodium dodecyl sulfate−polyacrylamide gel

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Table 2. Chromatic Characteristics and Phenolic Compounds (Mean Value ± Standard Deviation) of Non-micro-oxygenated (Control) and Micro-oxygenated (MO1 and MO2) Aglianico Red Wine (Wine A): 3 Months after Microoxygenation Treatment (MO*X*) and after 42 Months of Storage in Bottle[a]

parameter	treatment	3 months after micro-oxygenation	42 months after bottling
CI	control	8.23 ± 0.40 a	8.14 ± 0.08 a
	MO1	13.43 ± 0.71 c	8.14 ± 0.10 a s
	MO2	12.00 ± 0.85 b	8.10 ± 0.01 a s
hue	control	0.54 ± 0.07 a	1.01 ± 0.01 a s
	MO1	0.51 ± 0.01 a	1.00 ± 0.03 a s
	MO2	0.50 ± 0.03 a	0.97 ± 0.02 a s
total anth[b] (mg/L)	control	434.33 ± 9.71 a	42.31 ± 0.47 a s
	MO1	515.00 ± 7.55 c	42.31 ± 0.47 a s
	MO2	486.33 ± 14.84 b	41.77 ± 0.47 a s
SO_2 dec anth (mg/L)	control	132.67 ± 4.04 b	0.79 ± 0.13 a s
	MO1	134.33 ± 3.21 b	0.90 ± 0.06 a s
	MO2	87.33 ± 3.06 a	1.35 ± 0.98 a s
Dp3glc	control	5.72 ± 0.29 b	3.02 ± 0.04 c s
	MO1	4.68 ± 0.06 a	2.93 ± 0.10 b s
	MO2	4.92 ± 0.49 a	1.47 ± 0.04 a s
Cy3glc	control	0.68 ± 0.25 a	0.23 ± 0.17 a
	MO1	0.49 ± 0.09 a	0.73 ± 0.67 a
	MO2	0.46 ± 0.01 a	tr
Pt3glc	control	7.47 ± 0.21 a	0.38 ± 0.03 a s
	MO1	7.29 ± 0.08 a	0.76 ± 0.48 a s
	MO2	7.59 ± 0.32 a	0.57 ± 0.11 a s
Pn3glc	control	5.41 ± 0.13 a	0.44 ± 0.05 a s
	MO1	5.40 ± 0.14 a	1.28 ± 0.50 a s
	MO2	5.78 ± 0.16 a	0.60 ± 0.13 a s
Mv3glc	control	75.56 ± 0.45 a	3.15 ± 0.15 b s
	MO1	75.33 ± 0.04 a	3.78 ± 0.56 b s
	MO2	80.98 ± 1.47 a	2.03 ± 0.16 a s
Pn3acglc	control	1.47 ± 0.07 b	0.28 ± 0.20 a s
	MO1	1.27 ± 0.03 a	0.14 ± 0.08 a s
	MO2	1.30 ± 0.08 a	tr
Mv3acglc	control	4.89 ± 0.03 b	2.20 ± 0.13 a s
	MO1	4.75 ± 0.03 a	2.06 ± 0.54 a s
	MO2	5.17 ± 0.09 c	1.60 ± 0.35 a s
Pn3 cmglc	control	1.31 ± 0.06 a	tr
	MO1	1.57 ± 0.18 b	tr
	MO2	1.46 ± 0.07 a	tr
Mv3 cmglc	control	9.93 ± 0.14 a	0.42 ± 0.19 a s
	MO1	9.88 ± 0.20 a	0.82 ± 0.59 a s
	MO2	10.73 ± 1.00 a	0.68 ± 0.36 a s
total mon anth	control	112.46 ± 0.34 a	10.13 ± 0.40 b s
	MO1	110.66 ± 0.62 a	12.45 ± 2.78 b s
	MO2	118.39 ± 3.52 a	6.96 ± 0.60 a s

[a]Different letters and "s" indicate statistical differences ($p < 0.05$). Lower case letters (a, b) are used to compare control and micro-oxygenation samples at the same time. "s" is used to indicate significant differences between the wines of the same micro-oxygenation level throughout the time. Treatment MO1 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C). Treatment MO2 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C) followed by the application of 1.5 mL/L of O_2 for another 8 weeks (wine temperature was 15.5 °C). [b]Anthocyanins were evaluated spectrophotometrically. Dp3glc, delphinidin 3-glucoside; Cy3glc, cyanidin 3-monoglucoside; Pt3glc, petunidin 3-monoglucoside; Pn3glc, peonidin 3-monoglucoside; Mv3glc, malvidin 3-glucoside; Dp3acglc, delphinidin 3-(6^{II}-acetyl)glucoside; Cy3acglc, cyanidin 3-(6^{II}-acetyl)glucoside; Pn3acglc, peonidin 3-(6^{II}-acetyl)glucoside; Mv3acglc, malvidin 3-(6^{II}-acetyl)-glucoside; Pn3 cmglc, peonidin 3-(6^{II}-coumaroyl)glucoside; Mv3 cmglc, malvidin 3-(6^{II}-coumaroyl)glucoside. All monomeric anthocyanins are expressed as mg/L of Mv3glc.

electrophoresis (SDS-PAGE) analyses were performed on the resulting supernatant (S).

SDS-PAGE. Electrophoresis was performed on a Bio-Rad Protean II xi Cell electrophoresis apparatus (Bio-Rad, Milano, Italy) using a PowerPac 1000 Bio-Rad power supply set at 150 V/gel for the stacking gel and at 180 V/gel for the resolving gel. Samples (S) and fining proteins were mixed with an equal volume of 2× electrophoresis sample buffer (0.125 M Tris-HCl, 4% SDS; 20% v/v glycerol, 0.2 M DTT, 0.02% bromophenol blue, pH 6.8) and were heated at 95 °C for 4 min. Samples were successively processed by SDS-PAGE using 14% acrylamide resolving gels. The stacking gel was 5% acrylamide (Bio-Rad). Both gels were fixed with a mixture of ethanol, acetic acid, and deionized water (40:10:50) for 1 h. After washing in water for 5 min, the gels were stained with Coomassie Brilliant Blue R250 staining solution (Bio-Rad no. 161-0436). The destain step was performed by incubation in the destain solution Coomassie Blue R250 (Bio-Rad no. 161-0438).

SPI Determination. The SPI was determined as reported by Rinaldi et al.[38] The calibration curve was obtained by the density reduction of two protein bands selected from the pool of salivary proteins because they were better correlated with sensory analysis. The densitometric analysis of proteins was performed before and after the interaction of saliva with five standard solutions containing tannic acid (2−10 g/L in water) (Extrasynthése). The percentage of the optical density reduction was correlated with the sensory evaluation for astringency of the same tannic solutions. The correlation coefficient (R^2) was 0.978. SPI detection limit was 5, corresponding to 0.7 g/L of tannic acid. The reproducibility of the method used was tested by seven replicate analyses of a sample of red wine. The coefficient of variation (CV) of 4.8% demonstrated the good reproducibility of the analysis.

Sensory Analysis. *Selection and Training Sessions.* Twenty-four subjects were recruited from the University of Naples "Federico II" (Corso di Laurea in Viticoltura ed Enologia) to participate in the sensory sessions. All of them had a long experience as wine tasters, but with different backgrounds: 6 were aroma researchers, 7 were wine-makers, and 11 were enology students. Panelists were trained to differentiate astringency from bitterness and sourness using 3.0 g/L tannic acid, 0.25 g/L caffeine monohydrate, and 4.0 g/L tartaric acid as examples of astringency, bitterness, and sourness, respectively. Eighteen panelists indicated an ability to discriminate among these taste stimuli. In the following sessions, selected panelists were familiarized with astringency rating. They were asked to evaluate overall astringency of different concentrations (from 0.1 to 5.0 g/L) of commercial tannin (Biotan, Laffort, Bordeaux, France) on a 9-point scale (denoted absent, very weak, weak, weak moderate, moderate, moderate strong, strong, very strong, and extremely strong) first in water and then in wine solution. In each session five unknown samples (10 mL)

Table 3. Chromatic Characteristics and Phenolic Compounds (Mean Value ± Standard Deviation) of Non-micro-oxygenated (Control) and Micro-oxygenated (MO1 and MO2) Aglianico Red Wine (Wine B): 3 Months after Micro-oxygenation Treatment (MO*X*) and after 42 Months of Storage in Bottle[a]

parameter	treatment	3 months after micro-oxygenation	42 months after bottling
CI	control	7.93 ± 0.25 a	8.63 ± 0.60 a
	MO1	9.97 ± 0.40 c	8.21 ± 0.09 a s
	MO2	8.07 ± 0.50 b	8.39 ± 0.04 a
hue	control	0.42 ± 0.03 a	1.11 ± 0.00 a s
	MO1	0.57 ± 0.03 b	1.22 ± 0.00 a s
	MO2	0.58 ± 0.05 b	1.12 ± 0.00 a s
total anth[b] (mg/L)	control	369.67 ± 73.70 a	45.01 ± 0.47 b s
	MO1	466.33 ± 8.74 b	36.38 ± 0.00 a s
	MO2	393.33 ± 16.26 ab	42.04 ± 0.00 b s
SO_2 dec anth (mg/L)	control	102.33 ± 1.53 b	1.16 ± 0.21 b s
	MO1	139.33 ± 3.21 c	0.79 ± 0.06 a s
	MO2	70.00 ± 3.00 a	0.86 ± 0.08 a s
Dp3glc	control	9.03 ± 0.15 b	3.19 ± 0.17 b s
	MO1	8.17 ± 0.54 a	1.52 ± 0.45 a s
	MO2	9.17 ± 0.04 b	3.06 ± 0.07 b s
Cy3glc	control	0.59 ± 0.03 c	tr
	MO1	0.40 ± 0.02 a	tr
	MO2	0.44 ± 0.01 b	tr
Pt3glc	control	11.85 ± 0.04 ab	0.31 ± 0.04 a s
	MO1	11.31 ± 0.17 ab	0.51 ± 0.34 a s
	MO2	12.45 ± 0.51 b	0.27 ± 0.01 a s
Pn3glc	control	5.97 ± 0.53 a	0.14 ± 0.05 a s
	MO1	5.11 ± 0.20 a	0.22 ± 0.09 ab s
	MO2	5.99 ± 0.49 a	0.31 ± 0.02 b s
Mv3glc	control	126.18 ± 1.11 a	3.16 ± 0.25 b s
	MO1	119.67 ± 1.76 a	1.25 ± 0.12 a s
	MO2	135.18 ± 7.03 b	3.37 ± 0.11 b s
Pn3acglc	control	1.34 ± 0.08 ab	0.21 ± 0.06 b s
	MO1	1.40 ± 0.04 b	tr
	MO2	1.25 ± 0.09 a	0.13 ± 0.08 a s
Mv3acglc	control	7.77 ± 0.05 a	2.14 ± 0.24 b s
	MO1	7.42 ± 0.13 a	0.88 ± 0.42 a s
	MO2	7.74 ± 0.46 a	1.67 ± 0.19 b s
Pn3 cmglc	control	1.78 ± 0.16 ab	tr
	MO1	1.51 ± 0.23 a	tr
	MO2	1.95 ± 0.07 b	tr
Mv3 cmglc	control	14.637 ± 0.041 b	0.35 ± 0.06 b s
	MO1	13.232 ± 0.507 a	0.22 ± 0.09 a s
	MO2	15.286 ± 0.161 c	0.31 ± 0.05 ab s
total mon anth	control	179.13 ± 1.48 b	9.44 ± 0.60 b s
	MO1	168.22 ± 3.55 a	4.61 ± 0.70 a s
	MO2	189.46 ± 8.31 b	9.08 ± 0.36 b s

[a]Different letters and "s" indicate statistical differences ($p < 0.05$). Lower case letters (a, b) are used to compare control and micro-oxygenation samples at the same time. "s" is used to indicate significant differences between the wines of the same micro-oxygenation level throughout the time. Treatment MO1 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C). Treatment MO2 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C) followed by the application of 1.5 mL/L of O_2 for another 8 weeks (wine temperature was 15.5 °C). [b]Anthocyanins were evaluated spectrophotometrically. Dp3glc, delphinidin 3-glucoside; Cy3glc, cyanidin 3-monoglucoside; Pt3glc, petunidin 3-monoglucoside; Pn3glc, peonidin 3-monoglucoside; Mv3glc, malvidin 3-glucoside; Dp3acglc, delphinidin 3-(6^{II}-acetyl)glucoside; Cy3acglc, cyanidin 3-(6^{II}-acetyl)glucoside; Pn3acglc, peonidin 3-(6^{II}-acetyl)glucoside; Mv3acglc, malvidin 3-(6^{II}-acetyl)-glucoside; Pn3 cmglc, peonidin 3-(6^{II}-coumaroyl)glucoside; Mv3 cmglc, malvidin 3-(6^{II}-coumaroyl)glucoside. All monomeric anthocyanins are expressed as mg/L of Mv3glc.

were presented in balanced random order at room temperature (18 ± 2 °C) in black tulip-shaped glasses coded with three-digit random numbers. The assessors were instructed to pour the whole sample in their mouth, hold it for 8 s, expectorate, and rate the perceived overall astringency using the 9-point scale described before. Judges waited for 4 min before rinsing with deionized water for 10 s twice and then waited at least 30 s before the next sample. Each sample was evaluated within 5 min. Astringency was expressed as the maximum of intensity perceived. The data obtained were used for assessing the reliability and consistency of the panelists, which were considered to be acceptable ($p < 0.05$ for reproducibility of scores of replicate samples). The accuracy with rating was monitored by the use of standards during each tasting session, consisting of three CT wine solutions (very weak astringency = 0.1 g/L; moderate astringency = 2.5 g/L; extremely strong astringency = 5.0 g/L) to provide reference for three points on the 9-point scale. The evaluations of panel performance were based only on a one-way random model under the assumption that panelists are homogeneous. The tendency toward consistency in the repeated measurements of the sample was referred to as the reliability. The reliability coefficient was used for assessing the performance of the panel.[45]

Sensory Evaluation Sessions. At the beginning of each session, panelists tasted the standard reference solutions for astringency, which consisted of three commercial tannin wine solutions (very weak astringency = 0.1 g/L; moderate astringency = 2.5 g/L; extremely strong astringency = 5.0 g/L) representing the intensity of the sensation on the 9-point scale. The same procedure and conditions as used in the training session were applied for red wine evaluation. During the eight tasting sessions three experimental wines were evaluated in duplicate. The assessors were instructed as described under Selection and Training Sessions.

Statistical Analysis. All of the data are expressed as the arithmetic average ± standard deviation of three replicates. Analysis of variance was carried out on phenolic compound and sensory data. Fisher's least significant differences (LSD) procedure was used to discriminate among the means of the variables. Elaborations were carried out using Statgraphics Plus-PC (Manugistics, Inc.).

RESULTS AND DISCUSSION

Effect of Micro-oxygenation on Changes in Phenolic Composition, Color, SPI, and Astringency during Aging. The effect of micro-oxygenation on chromatic characteristics and pigments of wines A and B are reported in Tables 2 and 3. For both wines an increase of color intensity and of the content of total anthocyanins was observed 3 months after the MO1 treatment. A significant increase in the intensity of color just

Table 4. Phenolic Compounds of Non-micro-oxygenated (Control) and Micro-oxygenated (MO1 and MO2) Aglianico Red Wine (Wine A): 3 Months after Micro-oxygenation Treatment (MO*X*) and after 42 Months of Storage in Bottle[a]

parameter	treatment	3 months after micro-oxygenation	42 months after bottling
total polyphenols	control	2176 ± 67 a	2110 ± 60 b
	MO1	2364 ± 78 b	2103 ± 112 ab
	MO2	2748 ± 55 c	2957 ± 58 a s
proanthocyanidins (PAs) (mg/L)	control	2986 ± 113 a	2792 ± 120 a
	MO1	3183 ± 77 a	2780 ± 148 a s
	MO2	2865 ± 194 a	2775 ± 318 a
vanillin reactive flavans (VRF) (mg/L)	control	1446 ± 11 b	925 ± 20 c s
	MO1	1539 ± 41 c	871 ± 31 b s
	MO2	1011 ± 57 a	784 ± 21 a s
VRF/PAs	control	0.485 ± 0.016 b	0.328 ± 0.015 ab s
	MO1	0.489 ± 0.023 b	0.303 ± 0.007 a s
	MO2	0.354 ± 0.032 a	0.356 ± 0.016 b

[a]Treatment MO1 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C). Treatment MO2 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C) followed by the application of 1.5 mL/L of O_2 for another 8 weeks (wine temperature was 15.5 °C). Different letters and "s" indicate statistical differences ($p < 0.05$). Lower case letters (a, b) are used to compare control and micro-oxygenation samples at the same time. "s" is used to indicate significant differences between the wines of the same micro-oxygenation level throughout the time.

after the micro-oxygenation treatment has been previously reported,[18,46,47] which was due to the formation of new pigments such as those deriving from the combination of anthocyanins and flavanols via the formation of ethyl bridges.[48] The higher values of total anthocyanins in MO wines confirmed this hypotesis. Increased levels of micro-oxygenation (MO2) resulted in a significant decrease of SO_2 decolorable anthocyanins. Therefore, the higher the levels of oxygen, the higher the formation of new anthocyanin-derived pigments stable to pH changes and bisulfite bleaching.[14] After 42 months, all wines showed an increase of hue and a decrease of total and SO_2 decolorable anthocyanins. However, the effect of micro-oxygenation on CI and hue for both wines and for total and SO_2 decolorable anthocyanins for wine A was no longer detected. Several authors observed that during aging in barrels and/or bottles for several months, the differences between MO wines and respective control ones were minimized,[22,46] and our results suggest that, with time, this trend is enhanced to such an extent as to cancel the differences. With regard to monomeric anthocyanins, no clear trend has been observed 3 months after MO*X*: delphinidin 3-glucoside and peonidin 3-glucoside decrease for wine A but, because of the increase of malvidin 3-*p*-acetylglucoside in MO2 and peonidin 3-*p*-coumarylglucoside in MO1, no significant effect of MO*X* on the content of total monomeric anthocyanins was detected; for wine B a lower content of delphinidin 3-glucoside, cyanidin 3-glucoside, and malvidin 3-*p*-coumarylglucoside was observed when a MO1 level was applied; in contrast, higher values of malvidin 3-glucoside and malvidin 3-*p*-coumarylglucoside were detected when the level of treatment was MO2. The decrease of monomeric anthocyanins with MO*X* is in agreement with reported results[18,22,47] and can be related to the involvement of these molecules in the oxygen-activated reactions between anthocyanins and flavanols. These reactions determine the formation of anthocyanin−ethyl flavanol compounds, which are unstable and may undergo cleavage of the ethyl bridge with consequent liberation of monomeric anthocyanins.[14] This latter phenomenon could explain the increase of several monomeric anthocyanins observed in our and previous studies.[17] In agreement with the literature[49,50] the content of total monomeric anthocyanins in bottle decreased over time.

Three months after the treatments, the content of total phenolics increases with MO*X* level for both wines (Tables 4 and 5). Because different phenolic classes possess slightly different chemical properties,[51] the data observed may be due to the formation of phenolics with a higher reactivity toward the Folin−Ciocalteu reagent used for the analysis. In contrast, the aged MO*X* wines showed a lower content of total phenolics with respect to control ones, indicating that phenolic compounds changed or rearranged over time, giving less reactive compounds.

In the case of wine A, no statistically significant difference in PAs was detected at both dates of sampling. According to Vrhovsek et al.,[52] the Bate−Smith reaction used to determine the tannins provides an estimation of high proanthocyanidins corresponding to ≥5 units; therefore, no variation in this kind of molecule was observed. Despite the same initial content of PAs of the two wines, wine B showed a decrease of PAs when the higher level of micro-oxygenation was applied. This might be due to the fact that PAs can have different reactivities toward oxygen and oxygen-derived compounds depending on the monomers constituting the polymers as well as the polymerization degree.[53] Variations in polymerization degree of tannic molecules are also suggested by the finding that changes in VRFs (corresponding to phenolic polymers of 2−4 units) are detected with MO*X* and time for both wines. For wine A a decrease was always observed when MO2 level was applied; for wine B a decrease of VRF occurred always when MO1 level was applied. Because vanillin reacts only with terminal units of tannic molecules, its decrease may be caused either by precipitation of tannins or by an increase of their polymerization degree. With regard to the polymerization degree, different behaviors were reported: some researchers thought that MO induced the polymerization of PAs,[19] but recently no variation of degree of polymerization of proanthocyanidins was detected.[22] It is also not clear if the changes of the polymerization degree of proanthocyanidins[1,9] or the combination of

Table 5. Phenolic Compounds of Non-micro-oxygenated (Control) and Micro-oxygenated (MO1 and MO2) Aglianico Red Wine (Wine B): 3 Months after Micro-oxygenation Treatment (MOX) and after 42 Months of Storage in Bottle[a]

parameter	treatment	3 months after micro-oxygenation	42 months after bottling
total polyphenols	control	2233 ± 49 a	2580 ± 10 c s
	MO1	2529 ± 53 b	2200 ± 61 a s
	MO2	2898 ± 25 c	2320 ± 56 b s
proanthocyanidins (PAs) (mg/L)	control	2893 ± 162 b	3588 ± 118 b s
	MO1	2945 ± 163 b	3122 ± 63 a
	MO2	2634 ± 102 a	3298 ± 88 a s
vanillin reactive flavans (VRF) (mg/L)	control	1294 ± 12 b	1174 ± 15 b s
	MO1	1268 ± 11 a	947 ± 13 a s
	MO2	1197 ± 35 a	1173 ± 24 b
VRF/PAs	control	0.448 ± 0.023 b	0.331 ± 0.013 b s
	MO1	0.431 ± 0.021 a	0.314 ± 0.014 b s
	MO2	0.455 ± 0.008 b	0.285 ± 0.029 a s

[a]Treatment MO1 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C). Treatment MO2 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C) followed by the application of 1.5 mL/L of O_2 for another 8 weeks (wine temperature was 15.5 °C). Different letters and "s" indicate statistical differences ($p < 0.05$). Lower case letters (a, b) are used to compare control and micro-oxygenation samples at the same time. "s" is used to indicate significant differences between the wines of the same micro-oxygenation level throughout the time.

proanthocyanidins and anthocyanidins is responsible for the decrease of astringency of wine. Therefore, only on the basis of these analyses, the effect of micro-oxygenation and bottle aging on wine astringency is not predictable.

In this study, concerning sensory analysis, the variations occurring in phenolic composition 3 months after MOX are not enough to cause a significant variation of the astringency (Figure 1). For both wines a decrease of astringency was detected after 42 months of aging in bottle but, at this time, in the case of wine A no effect of MOX was observed, whereas, for wine B, a significant decrease of astringency was detected with increasing MOX levels. This result is confirmed by data on SPI (Table 6),

Table 6. SPI (Mean Value ± Standard Deviation Expressed as Grams per Liter of Tannic Acid) of Non-micro-oxygenated (Control) and Micro-oxygenated (MO1 and MO2) Aglianico Red Wines (Wines A and B) after 42 Months of Storage in Bottle[a]

	wine A	wine B
control	2.59 ± 0.53 a	4.24 ± 0.03 c
MO1	2.86 ± 0.64 a	3.71 ± 0.19 b
MO2	2.89 ± 0.58 a	3.41 ± 0.15 a

[a]Treatment MO1 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C). Treatment MO2 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C) followed by the application of 1.5 mL/L of O_2 for another 8 weeks (wine temperature was 15.5 °C). Different letters indicate statistical differences ($p < 0.05$). Lower case letters (a, b) are used to compare control and micro-oxygenation samples for the same wine.

which gives a direct measure of the reactivity of whole wine phenolics with salivary proteins. In the literature, both a significant decrease[17] and no effect[21] on the astringency with micro-oxygenation of wines have been reported. This is the first time that a significant effect was observed with time, and it happens only for the wine with the lower pH. Therefore, in agreement with recent findings[22] showing that pH exerts a major effect on the evolution of phenolic compounds during aging, our data seem to show that this effect can result in a variation of wine reactivity toward salivary proteins and, then, in wine astringency. However, this result can be also due to (i) differences in phenolic composition between the two wines; (ii) the direct effect of wine pH on astringency perception;[34] and (iii) the lower content of molecular SO_2 protecting wine components from oxidation.

Effect of OTR on Changes in Phenolic Composition, Color, SPI, and Astringency. To evaluate the effect of nano-oxygenation, two red wines with different levels of total package oxygen TPO at bottling (9.8 mg/L = W1 and 6.5 mg/L = W2)

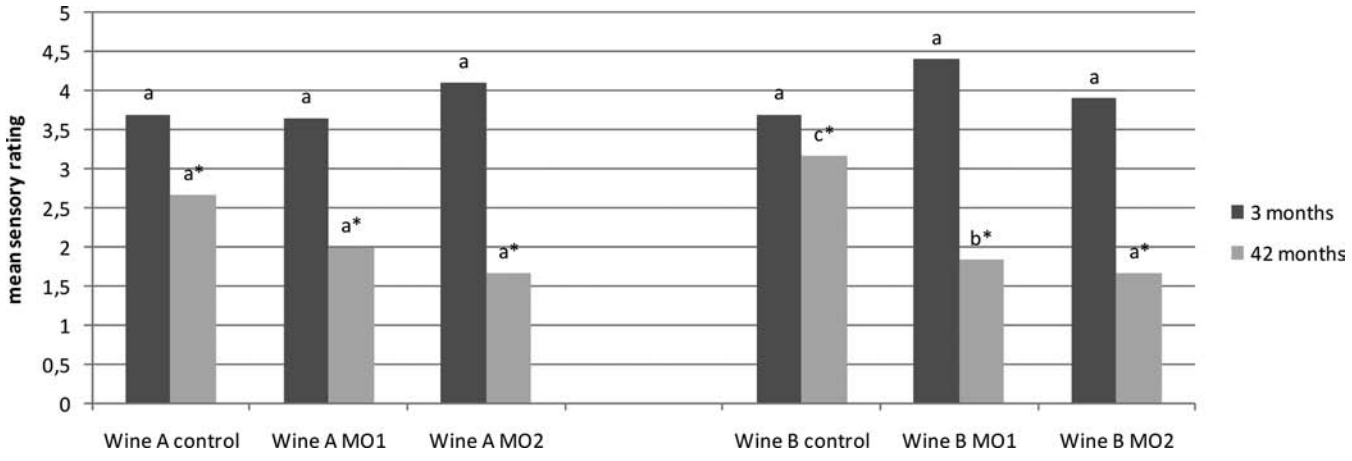

Figure 1. Mean sensory rating of astringency of experimental wines A and B evaluated throughout the time. Control: non-micro-oxygenated wine. Treatment MO1 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C). Treatment MO2 consisted of the application of 2 mL/L of O_2 for 8 weeks (wine temperature was 11.5 °C) followed by the application of 1.5 mL/L of O_2 for another 8 weeks (wine temperature was 15.5 °C). Different letters and * indicate statistical differences ($p < 0.05$). Lower case letters (a, b) are used to compare control and micro-oxygenation samples at the same time. * designation is used to indicate significant differences between the wines of the same micro-oxygenation level throughout the time.

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Table 7. Chromatic Characteristics and Total and Monomeric Anthocyanins of Wine 1 Closed with Synthetic Closures at Low, Medium, and High Oxygen Transfer Rates (OTR)[a]

	color intensity	hue	total anth[b](g/L)	Dp3glc	Cy3glc	Pt3glc	Pn3glc	Mv3glc	Mv3acglc	Mv3 cmglc	total mon anth
$W1_{low}$	5.75 ± 0.09 ab	0.87 ± 0.00 a	100.46 ± 0.77 a	1.13 ± 0.25 b	5.65 ± 1.21 b	2.61 ± 0.46 b	2.88 ± 0.22 c	33.04 ± 0.95 c	1.55 ± 0.20 b	0.48 ± 0.16 b	47.34 ± 1.84 c
$W1_{medium}$	5.80 ± 0.04 b	0.88 ± 0.01 a	99.65 ± 1.02 a	0.84 ± 0.14 a	3.93 ± 0.68 a	1.52 ± 0.28 a	1.73 ± 0.26 a	17.10 ± 0.76 a	0.86 ± 0.11 a	0.26 ± 0.02 a	26.24 ± 1.20 a
$W1_{high}$	5.73 ± 0.02 a	0.88 ± 0.02 a	100.05 ± 0.77 a	0.70 ± 0.03 a	3.46 ± 0.28 a	1.67 ± 0.13 a	2.44 ± 0.28 b	19.14 ± 1.27 b	1.68 ± 0.27 b	0.24 ± 0.03 a	29.32 ± 1.41 b

[a]Different letters indicate statistical differences ($p < 0.05$). Lower case letters (a, b, c) are used to compare wines aged with closures at different OTR. [b]Anthocyanins were evaluated spectrophotometrically. Dp3glc, delphinidin 3-glucoside; Cy3glc, cyanidin 3-monoglucoside; Pt3glc, petunidin 3-monoglucoside; Pn3glc, peonidin 3-monoglucoside; Mv3glc, malvidin 3-glucoside; Dp3acglc, delphinidin 3-(6^{II}-acetyl)glucoside; Cy3acglc, cyanidin 3-(6^{II}-acetyl)glucoside; Pn3acglc , peonidin 3-(6^{II}-acetyl)glucoside; Mv3acglc, malvidin 3-(6^{II}-acetyl)glucoside; Pn3 cmglc, peonidin 3-(6^{II}-coumaroyl)glucoside; Mv3 cmglc, malvidin 3-(6^{II}-coumaroyl)glucoside. All monomeric anthocyanins are expressed as mg/L of Mv3glc.

Table 8. Chromatic Characteristics and Total and Monomeric Anthocyanins of Wine 2 Closed with Synthetic Closures at Low, Medium, and High Oxygen Transfer Rates (OTR)[a]

	color intensity	hue	total anth[b] (g/L)	Dp3glc	Cy3glc	Pt3glc	Pn3glc	Mv3glc	Mv3acglc	Mv3 cmglc	total mon anth
$W2_{low}$	13.92 ± 0.13 a	0.78 ± 0.00 a	319.90 ± 3.99 a	2.95 ± 0.61 a	10.97 ± 0.64 a	28.56 ± 1.46 a	44.58 ± 1.88 b	217.69 ± 6.13 a	48.45 ± 1.00 b	2.38 ± 0.10 a	355.59 ± 3.83 a
$W2_{medium}$	13.73 ± 0.14 a	0.77 ± 0.00 a	324.21 ± 3.52 a	3.50 ± 0.12 a	11.49 ± 0.29 a	26.93 ± 0.38 a	38.47 ± 1.13 a	202.01 ± 11.84 a	41.53 ± 1.05 a	2.57 ± 0.28 a	326.51 ± 11.97 a
$W2_{high}$	13.98 ± 0.12 a	0.77 ± 0.00 a	319.63 ± 4.60 a	3.64 ± 0.12 a	11.18 ± 0.55 a	33.14 ± 1.41 b	52.32 ± 1.13 c	221.55 ± 4.59 a	52.39 ± 1.30 c	2.36 ± 0.29 a	367.25 ± 6.25 a

[a]Different letters indicate statistical differences ($p < 0.05$). Lower case letters (a, b, c) are used to compare wines aged with closures at different OTR. [b]Anthocyanins were evaluated spectrophotometrically. Dp3glc, delphinidin 3-glucoside; Cy3glc, cyanidin 3-monoglucoside; Pt3glc, petunidin 3-monoglucoside; Pn3glc, peonidin 3-monoglucoside; Mv3glc, malvidin 3-glucoside; Dp3acglc, delphinidin 3-(6^{II}-acetyl)glucoside; Cy3acglc, cyanidin 3-(6^{II}-acetyl)glucoside; Pn3acglc, peonidin 3-(6^{II}-acetyl)glucoside; Mv3acglc, malvidin 3-(6^{II}-acetyl)glucoside; Pn3 cmglc, peonidin 3-(6^{II}-coumaroyl)glucoside; Mv3 cmglc, malvidin 3-(6^{II}-coumaroyl)glucoside. All monomeric anthocyanins are expressed as mg/L of Mv3glc.

were sealed with different closures and analyzed after 10 months of storage in bottle. Three increasing oxygen transfer rate (OTR) conditions (W_{low}, W_{medium}, and W_{high}), ensured by using synthetic closures with controlled oxygen permeability, were compared (Table 1).

ANOVA was performed, separately for W1 and W2, on color data (Tables 7 and 8), polyphenol data (Table 9), SPI (Table 10), and sensory data (Figure 2).

The color intensity of $W1_{low}$ did not differ from that of $W1_{high}$, whereas $W1_{medium}$ and $W1_{high}$ are similar to each other (Table 7). This result seems in contrast with data reported by Caillé et al.,[21] which showed a positive correlation between the color intensity of red Grenache wine and OTR. The different trend may depend on the differences in TPO of closures used in the two studies. However, also wine type can affect the evolution of color intensity during bottle aging.

For W1, a significant loss of total monomeric anthocyanins of $W1_{medium}$ and $W1_{high}$ with respect to $W1_{low}$ was observed (Table 7). The last two samples were similar to each other. This trend is observed for the five main grape native anthocyanins, namely, delphynidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, malvidin 3-glucoside, and malvidin 3-*p*-coumarylglucoside. The highest percentage of loss of monomeric anthocyanins (comprised between 38 and 44% of total monomeric anthocyanins) with respect to that observed in the literature[26,27] can be related to the highest oxygen exposure of W1. Further studies aimed to examine the relationship between OTR, the antioxidant power of wine, and time of storage can better elucidate these phenomena.

No differences among closures for color intensity and total monomeric anthocyanins were detected for W2 wines (Table 8). The fact that these wines were characterized by lower total oxygen exposure (6.5 vs 9.8 mg/L) and higher content of phenolic compounds (3400 vs 2300 mg/L) acting as oxygen quenching compounds could explain this result.

In Table 9, a significant effect of OTR on total phenolics content of W1 wines is shown, whereas no differences were observed among W2 wines. As each phenolic compound had different reactivity toward the Folin−Ciocalteu reagent,[51] our results indicate that the OTR of closures can determine a variation in the chemical nature of wine phenolics. In fact, whereas no variation in PAs was detected, a significant loss of VRF of $W1_{high}$ and $W1_{medium}$ with respect to $W1_{low}$ was detected. Because vanillin reacts with free carbons C6 and C8 of the A ring of flavanols, the decrease of low molecular weight proanthocyanidins reactive toward vanillin (VRF) is consistent with the minor presence of nucleophile sites on flavanol molecules due to the significant effect of the OTR of closures. The fact that this phenomenon occurs only for W1 and not for W2 (Table 9) indicates that both TOE and the native composition in phenolics of red wine are determining factors in the development of condensation and polymerization reactions of tannins.

The SDS-PAGE analysis of human saliva (HS) after the interaction with experimental wines was performed to determine the SPI values of bottled wines (Table 10). For W1 wine the SPI was significantly lower when the bottles were sealed with closures at high OTR. A significant decrease of SPI was detected also for W2 wines, and the loss of phenolics reactive toward salivary proteins was positively correlated with OTR. These results are partially confirmed by the sensory rating of astringency: in agreement with SPI data, $W1_{high}$ was less astringent than $W1_{low}$, whereas, in contrast with SPI data, $W1_{medium}$ did not differ from $W1_{high}$ (Figure 2). For W2 no differences

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Table 9. Phenolic Compounds (Mean Value ± Standard Deviation) of W1 and W2 Red Wines Closed with Synthetic Closures at Low, Medium, and High Oxygen Transfer Rates (OTR)[a]

	total phenolics (mg/L)	proanthocyanidins (PAs) (g/L)	vanillin reactive flavanes (VRF) (mg/L)	VRF/PAs
$W1_{low}$	2278 ± 13 a	2420 ± 30 a	761 ± 23 b	0.314 ± 0.008 b
$W1_{medium}$	2373 ± 33 b	2415 ± 46 a	704 ± 4 a	0.291 ± 0.007 a
$W1_{high}$	2393 ± 43 b	2428 ± 35 a	720 ± 26 a	0.297 ± 0.013 a
$W2_{low}$	3437 ± 58 a	1112 ± 20 a	3990 ± 53 a	0.279 ± 0.007 a
$W2_{medium}$	3373 ± 49 a	1115 ± 20 a	3959 ± 46 a	0.282 ± 0.002 a
$W2_{high}$	3350 ± 61 a	1156 ± 33 a	4192 ± 92 b	0.276 ± 0.005 a

[a]Different letters indicate statistical differences ($p < 0.05$). Lower case letters (a, b, c) are used to compare wines aged with closures at different OTR.

Table 10. SPI of W1 and W2 Red Wines Closed with Synthetic Closures at Low, Medium, and High Oxygen Transfer Rates (OTR)[a]

	SPI (g/L tannic acid)
$W1_{low}$	2.21 ± 0.05 b
$W1_{medium}$	2.27 ± 0.09 b
$W1_{high}$	2.07 ± 0.08 a
$W2_{low}$	2.71 ± 0.08 c
$W2_{medium}$	2.44 ± 0.10 b
$W2_{high}$	2.30 ± 0.06 a

[a]Different letters indicate statistical differences ($p < 0.05$). Lower case letters (a, b, c) are used to compare wines aged with closures at different OTR.

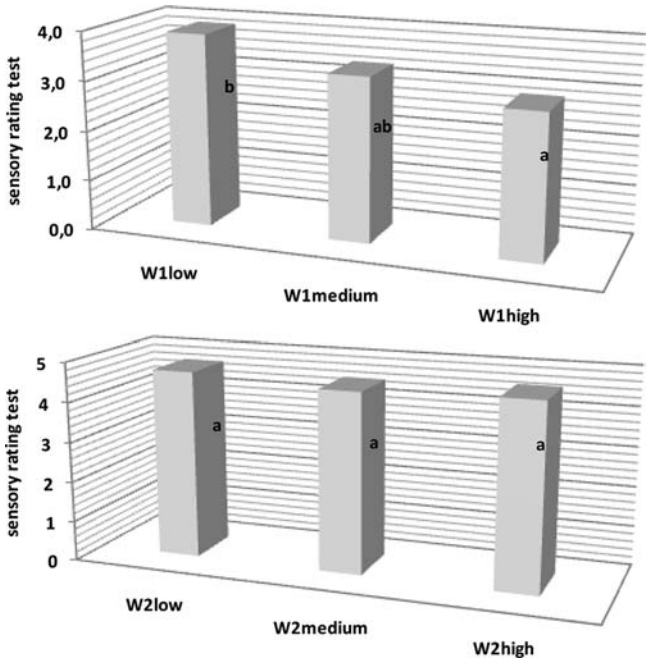

Figure 2. Mean sensory rating of astringency of experimental wines 1 and 2 closed with synthetic closures at low, medium, and high oxygen transfer rates (OTR). Different letters indicate statistical differences ($p < 0.05$).

among wines were detected. The discrepancy between sensory analysis and SPI can be due to different reasons such as the interference of wine components, the changes occurring in phenolic stimuli, and, the sensitivity of the two analytical methods used. Concerning the interference of wine components such as tartaric acid, ethanol, fructose, and mannoproteins, a recent study showed that they affect both the sensory perception of astringency and SPI, and this effect depends on wine phenolics.[34] However, the effects detected were not the same for the two methods considered. It is therefore likely that oxygen exposure can determine changes in wine phenolics detectable only by means of SPI, this analysis being more sensitive than sensory analysis to slight changes in binding reactivity of tannins.

In conclusion, the oxygen exposure of red wine before and after bottling affects the evolution of phenolics and astringency during aging in the bottle. This is the first time that a direct effect of (i) the addition of micro quantities of oxygen to wine before bottling and (ii) the oxygen permeating toward closures on the reactivity of wine phenolics toward salivary proteins has been shown. These effects are a function of aging time, wine initial composition (pH and phenolic composition), and oxygen level. Therefore, before applying MO and choosing a bottle closure, one must consider the expected consumption date of the bottled wine, the oxygen present at bottling, and the wine composition.

Further studies about the influence of each wine compound on the evolution of wine astringency and reactivity toward salivary proteins during aging in the bottle are needed to improve the use of both micro-oxygenation and closures at specific OTR in the wine industry.

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