Technical Brief

Periodic Aeration of Red Wine Compared to Microoxygenation at Production Scale

V. Felipe Laurie,[1]* Sofía Salazar,[1] M. Ignacia Campos,[1]
Alejandro Cáceres-Mella,[2] and Álvaro Peña-Neira[2]

Abstract: Microoxygenation (MOX) is a winemaking technique used with the aim of enhancing certain chemical and sensory wine features. Theoretically, by infusing small volumes of oxygen in a continuous way, the quality of the product may improve and the hazards of oxygen buildup and uncontrolled oxidation are avoided. However, the effects produced by discontinued air exposure, at rates comparable to MOX, have not been reported. Therefore, the aim of this trial was to evaluate the chemical effects of an alternative oxygenation protocol, based on weekly wine aerations, compared to conventional MOX (postmalolactic fermentation). For most of the variables analyzed, the periodic aeration treatment produced effects that were equivalent to conventional MOX (e.g., a reduction in the concentration of free anthocyanins and an enhancement in polymeric pigments).

Key words: wine, oxygen, anthocyanin, color, phenol, air

Oxygen plays a crucial role during winemaking and aging, producing either beneficial or detrimental effects, depending mainly upon the opportunity and the extent of oxygen exposure. Excess oxygenation produces negative transformations ranging from off-odors to microbial spoilage (Escudero et al. 2002, Bartowsky and Henschke 2008), whereas limited oxygenation is required for a sound fermentation and wine maturation (Singleton 1987, Salmon 2006).

One technique to introduce oxygen during winemaking and aging is microoxygenation (MOX), a technology developed for the controlled addition of oxygen as would naturally occur during wine storage in barrels (Figure 1A) (Gomez-Plaza and Cano-Lopez 2011, Schmidtke et al. 2011). Some of the reported outcomes of MOX include the reactions of anthocyanins with other wine constituents, as to form more complex pigments (Atanasova et al. 2002, Cano-Lopez et al. 2006), and variations in the concentration of other wine constituents (Arapitsas et al. 2012). From a sensory standpoint, MOX has been recommended to improve the expression of fruity aromas and to reduce astringency and vegetal character (Gonzalez-Sanjose et al. 2008, Parpinello et al. 2012).

A key premise of MOX is that a continuous low-level oxygen addition should be applied in order to produce the benefits reported, avoiding oxygen buildup and uncontrolled oxidation (Schmidtke et al. 2011). Nevertheless, this idea has not been challenged. Moreover, the dissolved oxygen concentrations reported during conventional MOX have ranged from ~0 to >1 mg/L (Laurie et al. 2008, Durner et al. 2010, Nevares et al. 2010). Therefore, the aim of this trial was to evaluate the effects of an alternative oxygenation protocol, based on weekly wine aerations, compared to conventional MOX (performed postmalolactic fermentation). To the best of our knowledge, this is the first time that this type of alternative treatment has been tested in a controlled, industrial-scale study.

Materials and Methods

Wine samples and experimental setting. The study was carried out with Carménère red wine (Maule, Chile). A blend of 400,000 L was distributed in 12 tanks of ~30,000 L each (~4.4 m height), located side-by-side. To avoid oxygen pickup during the filling process, headspace blanketing with CO_2 and

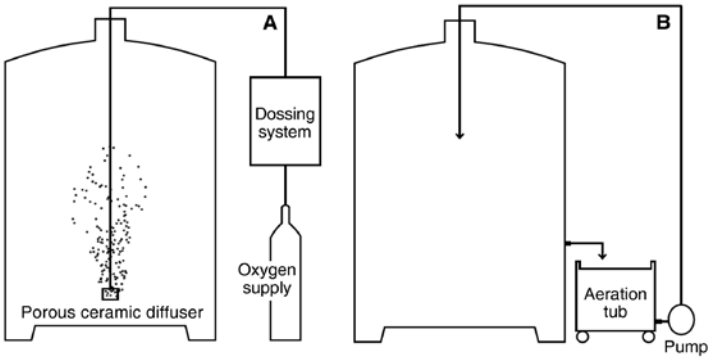

Figure 1 Schematics of the conventional microoxygenation process (**A**) and the alternative aeration system under study (**B**). Drawings not to scale.

[1]Facultad de Ciencias Agrarias, Universidad de Talca, Talca, Chile; and [2]Departmento de Agroindustria y Enología, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile.

*Corresponding author (flaurie@utalca.cl; tel: +56-71-2200214; fax: +56-71-2200212)

Acknowledgments: This research was funded by Conicyt, through Fondecyt 1110655, and had the support of Vitivinícola Patacón and Tonelería Nacional. The authors thank Matías Melnick, Juan Calderón, Felipe Ponce, and Matías Pincheira for their assistance and John Danilewicz for inspiring this work.

Manuscript submitted Sept 2013, revised Dec 2013, accepted Jan 2014

doi: 10.5344/ajev.2014.13105

nitrogen sparging (5 L nitrogen/min) was used. The following was the chemical composition of the wine at time zero: 13.3% v/v ethanol, 3.76 pH, 5.17 mg/L eq. H_2SO_4 titratable acidity (TA), 0.33 mg/L eq. acetic acid volatile acidity (VA), 22 mg/L free sulfur dioxide ($SO_{2\text{-free}}$), 29 mg/L total sulfur dioxide ($SO_{2\text{-total}}$), 1.27 g/L reducing sugars, 11.14 AU color index (sum of absorbance at 420, 520, and 620 nm), and 301 NTU for turbidity. From this point on, the wines received no extra SO_2 until the end of the trial.

The experiment encompassed four treatments, with three replications each: (1) T0 (control), a control group with no intended oxygenation or aeration; (2) T1 (MOX), a conventional microoxygenation at 5 mL oxygen per liter of wine per month (for 4 months), using a Visio-6 oxygenation system and standard microporous-ceramic diffusers (Vivelys-Chile, Requinoa, Chile) located in the center of each tank at ~10 cm above the base (Figure 1A); (3) T2 (MOX+Oak), a treatment combining MOX (as in T1) plus ~2 g/L of oak cubes (Viniblocks, American Oak, Tonelería Nacional, Colina, Chile), introduced in polypropylene-mesh bags; and (4) T3 (aeration), an aeration treatment that avoided the use of MOX equipment. The aeration involved a small volume of wine from each replication (~550 L) that was splashed-racked into a stainless-steel tub comprising a screen that helped aerating the wine. Once the wine in the tub reached ~7 mg/L of dissolved oxygen (DO), it was reintroduced into each respective tank by centrifugal pumping (15,000 L/hr) (Figure 1B). This procedure was repeated 12 times in order to incorporate approximately the same nominal amount of oxygen added with conventional MOX every week. The DO of each tub was checked with an oxo-luminescence-based meter (Fibox 3 LCD-trace v7, NomaSens, Regensburg, Germany), as explained elsewhere (Nevares et al. 2010).

The wine temperature was kept between 16 and 18°C, throughout the experiment.

Wine sampling and sampling dates. The chemical composition of the wines was monitored on 30 July (time 0), 28 Aug, 25 Sept, 25 Oct, and 22 Nov 2012, after which the treatments were stopped. A final sampling and measurement date was performed 5 days after the wines received a dose of 5 g/HL sulfur dioxide, corresponding to 4 Dec 2012. All samples were tasted on a weekly basis by the winemaking and research team without observing any defects on any of the replications.

Conventional wine analysis. Analyses of ethanol, pH, TA, VA, free and total SO_2, color index, and turbidity were performed according to official OIV methods using standard equipment (OIV 2009).

Spectrophotometric analyses of phenolic compounds. All spectrophotometric analyses were conducted in duplicate. The instruments included an Eppendorf refrigerated centrifuge (model 5427R; Hauppauge, NY), a Shimadzu UV-Vis spectrophotometer (model UV-160; Kyoto, Japan), and a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA). Total phenolics were measured with the Folin-Ciocalteu method (OIV 2009) and the absorbance values at 280 nm (Somers and Evans 1977). Iron-reactive phenolics, anthocyanins, polymeric pigments, and tannins were determined through the Harbertson–Adams assay (Harbertson et al. 2002, 2003). Finally, the proportion of color due to copigmentation, anthocyanins, and polymeric pigments at pH 3.6 were determined using the Boulton copigmentation assay (Levengood and Boulton 2004).

Chromatographic analysis of phenolics. For low molecular weight phenolics, 50 mL of wine was extracted with diethyl ether (3 × 20 mL) and ethyl acetate (3 × 20 mL). The extracts were vacuum-evaporated at 30°C, dissolved in 2 mL 50% methanol, and filtered through 0.2 µm PTFE filters. Then, 30 µL aliquots were subjected to reversed-phase HPLC-DAD (model 1200; Agilent Technologies, Santa Clara, CA) at 20°C using a Nova-Pak C18 column (4 µm particle size, 300 x 3.9 mm) (Waters, Milford, MA). The mobile phases used were (A) water/acetic acid (98:2 v/v) and (B) water/acetonitrile/acetic acid (78:20:2 v/v). The flow rates and gradient were 1.0 mL/min from 0 to 55 min (100−20% A from 0 to 55 min) and 1.2 mL/min from 55 to 90 min (20−10% A from 55 to 57 min, 10−10% A from 57 to 90 min). Chromatographic peaks were characterized by retention time and absorption spectrum (from 210 to 360 nm) of pure standards when available.

For chromatographic analysis of anthocyanin, a 2 mL aliquot of wine was filtered through a 0.2 µm nylon membrane. Then, a 100 µL aliquot was injected on a Merck–Hitachi D-7000 HPLC-DAD (Merck, Darmstadt, Germany) fitted with a LiChrospher RP-18 column (5 µm particle size, 250 x 4 mm). Separation was carried out at 25°C with flow rates of 1.1 mL/min from 0 to 22 min and 1.5 mL/min from 22 to 35 min. Mobile phases were (A) water/formic acid (90:10 v/v) and (B) acetonitrile. The gradient used was 96–85% A/4–15% B from 0 to 12 min, 85% A/15% B from 12 to 22 min, and 85–70% A/15–30% B from 22 to 35 min. The detector was set from 210 to 600 nm and quantification was carried out at 520 nm using malvidin-3-glucoside as standard (Extrasynthese, Genay, France).

Analysis of methoxypyrazines. 3-Isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) were analyzed at Centro de Aromas of Catholic University (Santiago, Chile), using an Agilent 7890A GC-MS (Agilent Technologies) as described elsewhere (Belancic and Agosin 2007). In this case, only the last set of samples (i.e., after four months of treatment) was analyzed.

Statistical analyses. The results were processed using ANOVA with StatGraphics Centurion XV (StatPoint Technologies, Warrenton, VA). Whenever significant differences were detected, Tukey–HSD test was used for mean separations.

Results and Discussion

Conventional wine analyses. The values of ethanol, pH, TA, and sugars did not vary during the course of the trial. Volatile acidity reached values between 0.39 (T0 to T2) and 0.40 ± 0.01 mg/L (T3), with no statistical differences among treatments. As reported elsewhere (Tao et al. 2007), the reduction in free sulfites was more predominant in the treated wines, with the control group (T0, 16.3 ± 1.15 mg/L) statistically different than the other treatments (T1, 10.7 ± 1.15; T2, 9.0 ± 1.00; T3, 9.7 ± 1.53 mg/L). No clear distinction was

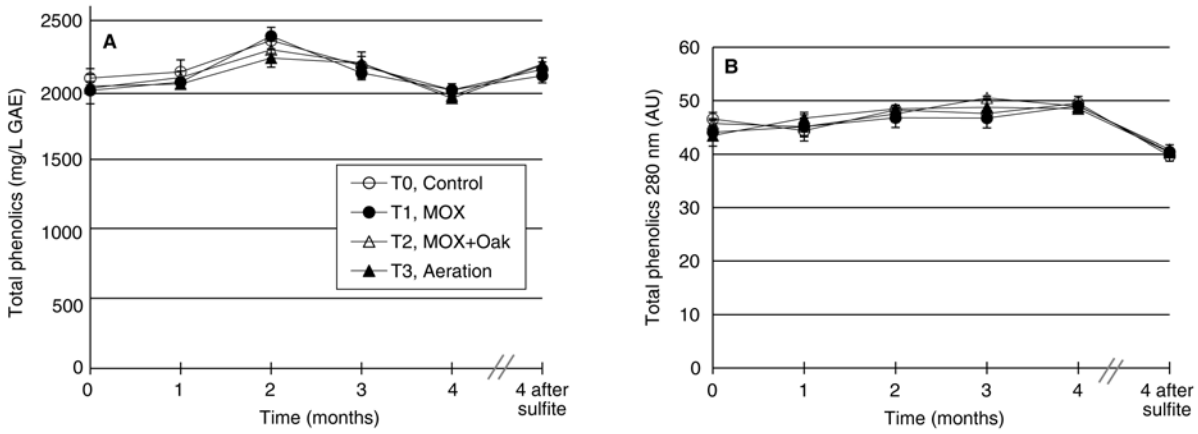

Figure 2 Evolution of total phenolics by Folin-Ciocalteu (**A**) and absorbance at 280 nm (**B**) in Carménère wine after four months of microoxygenation (5 mL/L/month) and discrete aeration, performed after malolactic fermentation.

Table 1 The effects of four months (mo) of microoxygenation (with and without oak wood) and discrete aeration treatments performed after malolactic fermentation on selected Carménère wine phenolic fractions (average values ± standard deviation).

	Treatment[b]			
Parameter[a]	**T0, Control**	**T1, MOX**	**T2, MOX+Oak**	**T3, Aeration**
Time zero				
Iron-reactive phenolics (mg/L CE)	1075.6 ± 44.7 a	991.8 ± 52.7 a	1058.0 ± 47.0 a	1052.9 ± 62.7 a
Anthocyanin (mg/L Mv3G E)	557.9 ± 16.5 a	601.8 ± 27.2 a	578.7 ± 17.9 a	583.5 ± 10.3 a
SPP (AU)	1.96 ± 0.02 a	1.65 ± 0.45 a	1.86 ± 0.17 a	1.90 ± 0.20 a
LPP (AU)	0.88 ± 0.19 a	0.48 ± 0.12 a	0.61 ± 0.10 a	0.72 ± 0.07 a
Tannin (mg/L CE)	246.1 ± 19.2 a	211.0 ± 44.6 a	245.6 ± 21.1 a	240.6 ± 25.2 a
1 mo of treatment				
Iron-reactive phenolics (mg/L CE)	973.0 ± 32.5 a	937.2 ± 12.0 a	941.1 ± 10.5 a	963.6 ± 27.0 a
Anthocyanin (mg/L Mv3G E)	551.3 ± 8.4 a	550.6 ± 17.9 a	557.7 ± 1.6 a	558.0 ± 5.2 a
SPP (AU)	2.03 ± 0.17 a	1.98 ± 0.01 a	1.99 ± 0.04 a	1.97 ± 0.04 a
LPP (AU)	0.81 ± 0.17 b	1.03 ± 0.02 ab	0.97 ± 0.04 ab	1.13 ± 0.05 a
Tannin (mg/L CE)	238.5 ± 68.1 a	270.7 ± 9.9 a	265.3 ± 7.5 a	261.7 ± 10.0 a
2 mo of treatment				
Iron-reactive phenolics (mg/L CE)	915.9 ± 185.7 a	776.7 ± 14.5 a	759.9 ± 7.3 a	779.0 ± 4.5 a
Anthocyanin (mg/L Mv3G E)	524.4 ± 5.0 a	516.1 ± 1.1 b	523.9 ± 2.3 a	517.5 ± 1.4 ab
SPP (AU)	2.14 ± 0.19 a	2.19 ± 0.03 a	2.15 ± 0.05 a	2.23 ± 0.09 a
LPP (AU)	1.03 ± 0.06 b	1.18 ± 0.10 ab	1.04 ± 0.06 b	1.34 ± 0.04 a
Tannin (mg/L CE)	295.0 ± 80.1 a	329.5 ± 19.3 a	333.6 ± 10.9 a	334.8 ± 5.7 a
3 mo of treatment				
Iron-reactive phenolics (mg/L CE)	886.1 ± 5.4 ab	862.4 ± 2.7 c	869.3 ± 15.6 bc	896.7 ± 6.5 a
Anthocyanin (mg/L Mv3G E)	507.4 ± 11.5 a	483.1 ± 11.6 ab	483.2 ± 10.6 ab	476.1 ± 5.3 b
SPP (AU)	1.91 ± 0.03 b	2.08 ± 0.04 a	2.14 ± 0.07 a	2.10 ± 0.01 a
LPP (AU)	1.14 ± 0.08 b	1.31 ± 0.05 b	1.23 ± 0.03 b	1.65 ± 0.09 a
Tannin (mg/L CE)	198.7 ± 3.0 b	218.1 ± 2.7 a	197.1 ± 6.8 b	201.8 ± 8.7 ab
4 mo of treatment				
Iron-reactive phenolics (mg/L CE)	883.8 ± 8.2 ab	861.3 ± 4.8 b	863.8 ± 32.2 ab	911.7 ± 8.4 a
Anthocyanin (mg/L Mv3G E)	481.6 ± 12.3 a	446.7 ± 7.6 ab	436.3 ± 16.8 b	436.7 ± 11.4 b
SPP (AU)	1.9 ± 0.0 b	2.1 ± 0.1 a	2.1 ± 0.1 a	2.1 ± 0.0 a
LPP (AU)	1.2 ± 0.0 c	1.4 ± 0.0 b	1.3 ± 0.1 bc	1.8 ± 0.7 a
Tannin (mg/L CE)	176.3 ± 11.3 a	181.3 ± 2.6 a	174.3 ± 3.6 a	196.2 ± 12.8 a
4 mo after sulfite addition				
Iron-reactive phenolics (mg/L CE)	892.6 ± 18.9 a	856.1 ± 19.9 ab	823.9 ± 6.7 b	879.2 ± 8.5 a
Anthocyanin (mg/L Mv3G E)	457.1 ± 1.9 a	419.1 ± 23.8 ab	406.3 ± 16.4 b	402.8 ± 18.1 b
SPP (AU)	1.9 ± 0.0 b	2.1 ± 0.3 a	2.1 ± 0.1 a	2.1 ± 0.0 a
LPP (AU)	1.1 ± 0.0 c	1.4 ± 0.1 b	1.4 ± 0.1 bc	1.8 ± 0.1 a
Tannin (mg/L CE)	149.9 ± 13.7 a	165.8 ± 20.2 a	189.1 ± 11.7 a	183.9 ± 8.0 a

[a]Iron-reactive phenolics and tannin reported as mg/L catechin equivalents (CE); anthocyanin reported as mg/L malvidin-3-glucoside equivalents (Mv3G E). SPP and LPP, small and large polymeric pigments, respectively, reported as absorbance units (AU).
[b]Different letters in the same row represent significant differences according to Tukey HSD ($p \leq 0.05$).

observed for total SO_2 (T0, 24.3 ± 1.53; T1, 26.3 ± 2.08; T2, 25.7 ± 0.58; T3, 26.3 ± 2.52 mg/L).

Spectrophotometric analyses of phenolic compounds. *Total phenolics.* Total phenolics according to Folin-Ciocalteu were within 1967 ± 24 and 2493 ±20 mg/L gallic acid equivalents (Figure 2A). An unexpected variability was observed among sampling times, regardless of the low coefficient of variation observed between replicates on each sampling date (<6% in all cases). Nonetheless, no statistical differences were observed among treatments. Correspondingly, the readings of absorbance at 280 nm (ranging from 39.7 ± 0.98 to 49.5 ± 1.28 AU) showed no statistical differences among treatments (Figure 2B). Although wine oxidation is known to reduce the concentration of phenolics due to polymerization and precipitation (Singleton 1987), these results suggest that the extent/duration of the treatments was moderate enough to delay this phenomenon.

Iron-reactive phenolics, anthocyanin, polymeric pigments, and tannin. Iron-reactive phenolics had significant differences from the third month of treatment onward (Table 1). The MOX (T1) and MOX+Oak (T2) treatments had lower average values, but the differences were statistically significant only in some cases. Anthocyanin levels decreased during the course of the trial, from average values >550 mg/L at time zero to <460 mg/L after four months (Table 1). Moreover, significant differences among treatments were observed from the second month of treatment onward. The control (T0) treatment had consistently higher average concentrations, statistically different from T2 and T3.

Small and large polymeric pigments (SPP and LPP, respectively) increased as a result of the oxygenation/aeration treatments compared to the control group (Table 1). More specifically, there were significant differences in SPP from the third month of treatment onward, and the control group (T0) had lower values than the other treatments (T1, T2, T3). For LPP, the first noticeable variation occurred after one month of treatment, in which T0 had lower LPP concentrations than the other treatments. Toward the end of the trial, all of the oxygenated and aerated treatments, particularly T3, had significantly higher LPP values than the control (T0). As previously reported elsewhere (Atanasova et al. 2002, Gomez-Plaza et al. 2000, Perez-Magarino et al. 2007), oxygen contributes to a reduction in anthocyanins and an increment of polymeric pigment.

Finally, the somewhat high variability observed for tannin analyses, combined with the strictness of the mean separation test, precluded finding significant differences (excepting the analysis at month 3).

Wine color fractions. The proportion of color due to copigmentation was significantly reduced (up to 50% less) in the wines in which oxygen or air was incorporated (T1, T2, T3) (Table 2). There were significant differences in the proportion of color due to anthocyanins at one month of treatment and at the end of the trial, and the control treatment (T0) had a

Table 2 The impact of four months (mo) of microoxygenation (with and without oak wood) and discrete aeration treatments performed after malolactic fermentation on the color parameters of Carménère wines (average values ± standard deviation).

	Treatment[a]			
Color parameter	**T0, Control**	**T1, MOX**	**T2, MOX+Oak**	**T3, Aeration**
Time zero				
Copigmentation	0.218 ± 0.032 a	0.220 ± 0.081 a	0.220 ± 0.032 a	0.303 ± 0.040 a
Δ Color	0.564 ± 0.030 a	0.572 ± 0.077 a	0.572 ± 0.032 a	0.495 ± 0.006 a
Polymeric pigments	0.218 ± 0.004 a	0.209 ± 0.004 b	0.206 ± 0.002 b	0.203 ± 0.002 b
1 mo of treatment				
Copigmentation	0.293 ± 0.019 a	0.260 ± 0.016 a	0.267 ± 0.054 a	0.218 ± 0.070 a
Δ Color	0.460 ± 0.013 b	0.475 ± 0.013 ab	0.483 ± 0.051 ab	0.537 ± 0.006 a
Polymeric pigments	0.246 ± 0.008 b	0.265 ± 0.005 a	0.250 ± 0.003 b	0.244 ± 0.004 b
2 mo of treatment				
Copigmentation	0.317 ± 0.035 a	0.309 ± 0.029 a	0.361 ± 0.019 a	0.294 ± 0.030 b
Δ Color	0.382 ± 0.033 a	0.375 ± 0.029 a	0.323 ± 0.013 a	0.390 ± 0.028 a
Polymeric pigments	0.301 ± 0.001 b	0.315 ± 0.003 a	0.316 ± 0.006 a	0.316 ± 0.003 a
3 mo of treatment				
Copigmentation	0.187 ± 0.015 a	0.123 ± 0.037 a	0.134 ± 0.008 a	0.089 ± 0.078 a
Δ Color	0.502 ± 0.011 a	0.539 ± 0.036 a	0.527 ± 0.006 a	0.584 ± 0.106 a
Polymeric pigments	0.311 ± 0.003 b	0.338 ± 0.011 a	0.339 ± 0.003 a	0.343 ± 0.002 a
4 mo of treatment				
Copigmentation	0.247 ± 0.052 a	0.149 ± 0.020 b	0.173 ± 0.043 ab	0.158 ± 0.008 ab
Δ Color	0.433 ± 0.054 a	0.488 ± 0.020 a	0.469 ± 0.036 a	0.485 ± 0.010 a
Polymeric pigments	0.321 ± 0.002 b	0.363 ± 0.005 a	0.358 ± 0.016 a	0.357 ± 0.003 a
4 mo after sulfite addition				
Copigmentation	0.276 ± 0.008 a	0.138 ± 0.020 b	0.140 ± 0.016 b	0.142 ± 0.017 b
Δ Color	0.412 ± 0.009 b	0.496 ± 0.004 a	0.496 ± 0.025 a	0.497 ± 0.027 a
Polymeric pigments	0.313 ± 0.001 b	0.366 ± 0.016 a	0.363 ± 0.015 a	0.360 ± 0.015 a

[a]Different letters in the same row represent significant differences according to Tukey HSD ($p \leq 0.05$).

smaller fraction of color due to anthocyanins than the other treatments. A significant increase in the proportion of color due to polymeric pigments was observed in the oxygenated and aerated treatments. As previously stated, oxygen exposure is expected to promote polymerization and the formation of polymeric pigments (Atanasova et al. 2002, Gomez-Plaza et al. 2000), a situation that may have contributed to lower copigmentation.

Chromatographic analyses of phenolic compounds. The phenolic compounds analyzed did not show statistical differences individually or as groups (i.e., benzoic acids, cinnamic acids, and flavanols), except for a higher concentration of protocatechuic in T2 at the end of the trial. As no statistical differences were found during the first 3 months of treatment, only the results of the final date of analyses are shown (Table 3). Some of the phenolic compounds analyzed were expected to have lower concentrations due to their incorporation into polymeric structures, but this effect was not observed.

There were significant changes in the concentrations of some of anthocyanins, which were attributable to the oxygen/air addition treatments. Most notably, there was a significant reduction in malvidin-3-glucoside concentration as a result of the MOX (T1) and aeration treatments (T3) on the final measurement dates (Table 3). This phenomenon has to do partially with the incorporation of colored compounds into larger phenolic structures, thus forming polymeric pigments. Additionally, during the four-month trial period, it was possible to observe a ~38% decrease in the concentration of malvidin-3-glucoside for the control group (T0) and a decrease of between 35 and 53% for the other treatments (T1, T2, T3).

Moreover, when the sum of anthocyanins was plotted against the different sampling points (Figure 3), it was possible to see a reduction in their concentration that was less pronounced in the control group, T0 (43% in four months), than for the other treatments (e.g., 58% reduction in four months of treatment T3).

Analysis of methoxypyrazines. The concentration of pyrazines was within the normal range of values reported for Chilean Carménère (Belancic and Agosin 2007). At the end of the trial, IBMP concentrations were between 6.63 ± 0.06 (T2) and 7.2 ± 0.14 ng/L (T0), with treatment T2 significantly less than T0 and T1 (Figure 4A). Similarly, reported IPMP values were between nodetectable (T2) and 2.15 ± 0.49 ng/L (T0). Once again, T2 was significantly lower than the other treatments (Figure 4B), suggesting an effect of the oak cubes on the reduction of pyrazines observed. Although pyrazines

Table 3 Concentration of selected low molecular weight (LMW) phenolic and anthocyanin compounds by HPLC-DAD as a result of micro-oxygenation and aeration treatments in Carménère wines; four months of treatment, after sulfite addition.

	Treatment[a]			
Compound (mg/L)	**T0, Control**	**T1, MOX**	**T2, MOX+Oak**	**T3, Aeration**
LMW phenolics				
Gallic acid	6.73 ± 1.37 a	7.07 ± 0.45 a	7.54 ± 0.59 a	8.94 ± 3.57 a
Protocatechuic acid	0.18 ± 0.03 b	0.16 ± 0.01 b	0.43 ± 0.02 a	0.16 ± 0.01 b
Syringic acid	1.89 ± 0.17 a	2.00 ± 0.10 a	2.12 ± 0.15 a	2.17 ± 0.43 a
Vanillic acid	1.09 ± 0.15 a	1.18 ± 0.07 a	1.25 ± 0.06 a	0.52 ± 0.74 a
Caffeic acid	4.06 ± 0.78 a	3.79 ± 0.32 a	3.85 ± 0.20 a	3.58 ± 0.65 a
p-Coumaric acid	1.89 ± 0.45 a	1.99 ± 0.28 a	1.90 ± 0.14 a	2.04 ± 0.30 a
(+)-Catechin	4.97 ± 0.93 a	4.55 ± 0.81 a	4.86 ± 0.29 a	4.40 ± 0.84 a
(-)-Epicatechin	3.59 ± 1.04 a	3.82 ± 0.52 a	3.78 ± 0.18 a	4.63 ± 1.64 a
Quercetin	3.81 ± 0.43 a	3.17 ± 0.95 a	2.64 ± 0.53 a	3.19 ± 1.22 a
Tryptophol	0.50 ± 0.67 a	0.55 ± 0.12 a	0.49 ± 0.07 a	1.14 ± 0.18 a
Tyrosol	32.95 ± 3.19 a	33.45 ± 1.83 a	35.58 ± 1.57 a	30.99 ± 6.90 a
Anthocyanins[b]				
Dp3Gl	35.58 ± 0.33 a	21.49 ± 0.58 b	20.09 ± 0.56 c	18.87 ± 0.21 c
Cy3Gl	15.39 ± 21.8 –	nd	nd	nd
Pt3Gl	21.48 ± 0.27 c	28.88 ± 1.74 a	25.05 ± 0.94 b	23.58 ± 0.71 bc
Po3Gl	5.08 ± 1.91 a	5.39 ± 0.33 a	3.16 ± 2.75 a	5.34 ± 0.65 a
Mv3Gl	333.97 ± 4.54 a	263.67 ± 2.61 b	296.49 ± 36.6 ab	265.42 ± 18.8 b
Dp3acGl	nd	1.89 ± 3.28 –	nd	nd
Cy3acGl	1.93 ± 2.73 a	0.57 ± 0.99 a	1.74 ± 3.01 a	2.00 ± 3.47 a
Pt3acGl	4.31 ± 6.10 a	14.94 ± 25.9 a	2.30 ± 3.98 a	2.86 ± 4.96 a
Po3acGl	3.24 ± 4.59 a	6.74 ± 6.85 a	7.42 ± 3.84 a	5.56 ± 0.90 a
Mv3acGl	101.55 ± 2.96 a	89.23 ± 3.10 a	75.07 ± 7.44 b	72.89 ± 3.24 b
Dp3cmGl	nd	nd	nd	nd
Cy3cmGl	3.30 ± 4.67 a	4.20 ± 3.80 a	1.20 ± 2.09 a	nd
Pt3cmGl	nd	1.48 ± 2.57 a	3.40 ± 3.14 a	nd
Po3cmGl	5.65 ± 1.45 a	3.28 ± 3.30 a	4.27 ± 7.40 a	nd
Mv3cmGl	49.11 ± 0.13 a	34.34 ± 3.07 b	28.30 ± 1.47 b	33.00 ± 6.86 b

[a]Different letters in the same row represent significant differences according to Tukey HSD ($p \leq 0.05$). nd: not detected.
[b]Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Po, peonidin; Mv, malvidin; Gl, glucoside; ac, acetyl; cm, coumaroyl.

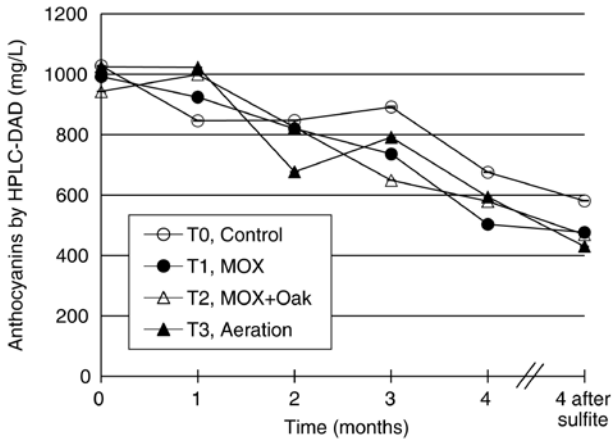

Figure 3 Evolution of anthocyanin concentration during microoxygenation and aeration treatments in Carménère wine over four months of treatment.

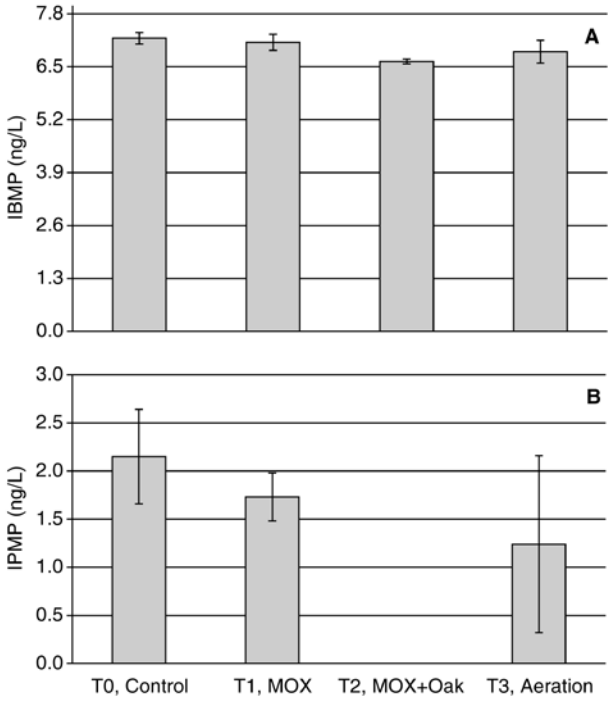

Figure 4 Concentration of 3-isobutyl-2-methoxypyrazine (IBMP) (**A**) and 3-isopropyl-2-methoxypyrazine (IPMP) (**B**) in Carménère wine after four months of microoxygenation (5 mL/L/month) and discrete aeration, performed after malolactic fermentation.

are known for their chemical stability, the sorption of volatile compounds by oak wood (Ramirez et al. 2001) and some packaging materials has been previously proposed (Blake et al. 2009). In the later example, it was also noted that the sorption level varies depending on the compounds studied.

Conclusions

The alternative aeration treatment tested was effective in producing changes in the color and phenolic composition of the treated wines, most notably in accelerating the formation of polymeric pigments. Moreover, the aeration treatment had results equivalent to the conventional MOX treatment, suggesting that well-managed discontinued aeration treatments could be used as an alternative to MOX. Furthermore, the treatment combining MOX and oak wood (T2) resulted in a rise in the concentration of protocatechuic acid and a reduction of methoxypyrazines, which should be further studied. Further evaluations of this type of alternative MOX treatment should include trials performed premalolactic fermentation and with testing of the evolution of the wine after the oxygenation treatments have ceased.

Literature Cited

Arapitsas, P., M. Scholz, U. Vrhovsek, S. Di Blasi, A. Biondi Bartolini, D. Masuero, D. Perenzoni, A. Rigo, and F. Mattivi. 2012. A metabolomic approach to the study of wine micro-oxygenation. PloS One 7(5):e37783.

Atanasova, V., H. Fulcrand, W. Cheynier, and M. Moutounet. 2002. Effect of oxygenation on polyphenol changes occurring in the course of wine-making. Anal. Chim. Acta 458:15-27.

Bartowsky, E.J., and P.A. Henschke. 2008. Acetic acid bacteria spoilage of bottled red wine–A review. Int. J. Food Microbiol. 125:60-70.

Belancic, A., and E. Agosin. 2007. Methoxypyrazines in grapes and wines of *Vitis vinifera* cv. Carmenere. Am. J. Enol. Vitic. 58:462-469.

Blake, A., Y. Kotseridis, I. Brindle, D. Inglis, M. Sears, and G.J. Pickering. 2009. Effect of closure and packaging type on 3-alkyl-2-methoxypyrazines and other impact odorants of Riesling and Cabernet Franc wines. J. Agric. Food Chem. 57:4680-4690.

Cano-López, M., F. Pardo-Minguez, J.M. López-Roca, and E. Gómez-Plaza. 2006. Effect of microoxygenation on anthocyanin and derived pigment content and chromatic characteristics of red wines. Am. J. Enol. Vitic. 57:325-331.

Durner, D., S. Ganss, and U. Fischer. 2010. Monitoring oxygen uptake and consumption during microoxygenation treatments before and after malolactic fermentation. Am. J. Enol. Vitic. 61:465-473.

Escudero, A., E. Asensio, J. Cacho, and V. Ferreira. 2002. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. Food Chem. 77:325-331.

Gomez-Plaza, E., and M. Cano-Lopez. 2011. A review on microoxygenation of red wines. Claims, benefits and the underlying chemistry. Food Chem. 125:1131-1140.

Gomez-Plaza, E., R. Gil-Munoz, J.M. Lopez-Roca, and A. Martinez. 2000. Color and phenolic compounds of a young red wine. Influence of wine-making techniques, storage temperature, and length of storage time. J. Agric. Food Chem. 48:736-741.

Gonzalez-Sanjose, M.L., M. Ortega-Heras, and S. Perez-Magarino. 2008. Microoxygenation treatment and sensory properties of young red wines. Food Sci. Tech. Int. 14:123-130.

Harbertson, J.F., J.A. Kennedy, and D.O. Adams. 2002. Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. Am. J. Enol. Vitic. 53:54-59.

Harbertson, J.F., E.A. Picciotto, and D.O. Adams. 2003. Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. Am. J. Enol. Vitic. 54:301-306.

Laurie, V.F., R. Law, W.S. Joslin, and A.L. Waterhouse. 2008. In situ measurements of dissolved oxygen during low-level oxygenation in red wines. Am. J. Enol. Vitic. 59:215-219.

Levengood, J., and R. Boulton. 2004. The variation in the color due to copigmentation in young Cabernet Sauvignon wines. *In* Red Wine Color: Revealing the Mysteries. A.L. Waterhouse and J. A. Kennedy (eds.), pp. 35-52. Am. Chemical Society, Washington DC.

Nevares, I., M. del Alamo, and C. Gonzalez-Munoz. 2010. Dissolved oxygen distribution during micro-oxygenation. Determination of representative measurement points in hydroalcoholic solution and wines. Anal. Chim. Acta 660:232-239.

OIV. 2009. Compendium of International Methods of Analyses of Wines and Musts. Organisation Internationale de la Vigne et du Vin, Paris.

Parpinello, G.P., F. Plumejeau, C. Maury, and A. Versari. 2012. Effect of micro-oxygenation on sensory characteristics and consumer preference of Cabernet Sauvignon wine. J. Sci. Food Agric. 92:1238-1244.

Perez-Magarino, S., M. Sanchez-Iglesias, M. Ortega-Heras, C. Gonzalez-Huerta, and M.L. Gonzalez-Sanjose. 2007. Colour stabilization of red wines by microoxygenation treatment before malolactic fermentation. Food Chem. 101:881-893.

Ramirez, G.R., S. Lubbers, C. Charpentier, M. Feuillat, A. Voilley, and D. Chassagne. 2001. Aroma compound sorption by oak wood in a model wine. J. Agric. Food Chem. 49:3893-3897.

Salmon, J.M. 2006. Interactions between yeast, oxygen and polyphenols during alcoholic fermentations: Practical implications. LWT-Food Sci. Technol. 39:959-965.

Schmidtke, L.M., A.C. Clark, and G.R. Scollary. 2011. Micro-oxygenation of red wine: Techniques, applications, and outcomes. Crit. Rev. Food Sci. Nutr. 51:115-131.

Singleton, V.L. 1987. Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical implications. Am. J. Enol. Vitic. 38:69-77.

Somers, T.C., and M.E. Evans. 1977. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "chemical age." J. Sci. Food Agric. 28:279-287.

Tao, J., S.I. Dykes, and P.A. Kilmartin. 2007. Effect of SO_2 on polyphenol development during red wine micro-oxygenation. J. Agric. Food Chem. 55:6104-6109.