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Influence of Wine pH on Changes in Color and Polyphenol Composition Induced by Micro-oxygenation

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ABSTRACT: The presence of oxygen in red wine leads to the transformation of ethanol into ethanal, which after capturing a proton will react with flavanols to start the process of forming ethyl bridges between flavanols and between flavanols and anthocyanins. Wine pH also conditions the equilibrium between the different anthocyanin structures and may thus affect anthocyanin reactivity. Consequently, the aim of this paper was to study how the pH can affect the changes induced by micro-oxygenation in two wines with different phenolic composition. The differences between micro-oxygenated wines and their controls were, in general, greater when the pH was more acidic. Specifically, the differences between micro-oxygenated wines and their corresponding controls in terms of color intensity, anthocyanin concentration, PVPP index, ethyl-linked pigments, B-type vitisins, polymeric pigments, and ethylidene-bridged flavanols were greater at lower pH. In contrast, the effects of micro-oxygenation when the pH was less acidic were much less evident and sometimes practically nonexistent. These results demonstrate for the first time that the pH of the wine has a great influence on oxygen-induced changes of color and phenolic compounds.

KEYWORDS: Wine, pH, phenolic compounds, color, micro-oxygenation

INTRODUCTION

Phenolic compounds are one of the main determinants of the quality in red wines. Anthocyanins, which are the main compounds responsible for the color of red wine, are extracted from grape skins during the maceration and fermentation processes. Other phenolic compounds present in skins and seeds are also extracted. Among these compounds, proanthocyanidins, also known as condensed tannins, are the main determinant of texture sensations such as body, bitterness, and astringency.[1−3] The molecular size and monomeric composition of proanthocyanidins seem to be related to the sensation of astringency: the greater the degree of polymerization and the greater the percentage of galloylation, the greater the sensation of astringency.[3,4]

During winemaking and aging, phenolic compounds undergo progressive structural changes, which undoubtedly influence the organoleptic characteristics of the wine. In particular, anthocyanins, which are unstable, present a high chemical reactivity, which gives rise to new more stable pigments. Different mechanisms have been proposed to explain the formation of new pigments. Some of these involve the direct condensation of anthocyanins and flavanols without the participation of oxygen,[5−7] but the most important reactions are probably those involving oxygen. During winemaking and aging, the presence of small quantities of 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 carbocations that quickly react either with another flavanol molecule or with an anthocyanin, producing ethyl-bridged flavanol−flavanol or flavanol−anthocyanin oligomers.[8] However, it has recently been shown that the compounds formed by ethyl bridges are unstable and that their cleavage can originate new compounds.[9]

On the other hand, cycloaddition reactions between anthocyanins and other small molecules can produce a new family of anthocyanin-derived pigments called pyranoanthocyanins. Specifically, the reaction with pyruvic acid or ethanal generates vitisins A and B, respectively.[10−13] In addition, the reaction between anthocyanins and vinylphenol can generate vinylphenol adducts,[14,15] and, finally, the previously formed ethylidene-bridged compounds can dissociate and generate vinylphenol adducts.[16,17]

All these reactions result in a gradual shift in the color of the wine from the initial purple-red to a reddish-brown. The astringency also diminishes, but the mechanism by which this happens it is not so clear. Theoretically, the formation of ethyl bridges should increase the degree of flavanol polymerization, which in turn should increase the astringency.[3] However, astringency decreases during aging, and this fact has been traditionally attributed to the reaction of phenolic compounds with oxygen. One possible explanation may be that the condensation reactions between anthocyanins and flavanols can diminish astringency.[4] Some authors have even suggested that the cleavage reactions of proanthocyanidins as a result of acid catalysis may cause the observed reduction in astringency.[18]

The reactivity of phenolic compounds is affected by several factors such as temperature, pH, and free SO_2 concentration,[19,20] although oxygen exposure is probably the main determinant.[21−23] In fact, the reason that wines are traditionally aged in oak barrels is because the porosity of the wood, the interstices between staves, and the bunghole allow the entry of small amounts of oxygen, which can induce all of the aforementioned reactions.[24]

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Nevertheless, oak aging is an expensive and laborious process that cannot be used for all wines. Micro-oxygenation (MOX) has therefore been proposed for reproducing, and even accelerating, the transformations of color and phenolic compounds that take place during oak aging.[25,26] The MOX technique consists of providing a controlled flow of gaseous oxygen into the wine in the form of microbubbles injected through a microdiffuser.[27]

Since its appearance in the early 1990s, MOX has become a commonly applied technique in wineries worldwide. It is believed that MOX stabilizes the color and decreases the astringency, bitterness, and herbaceous characters of wine.[28] The influence of MOX on wine quality depends on several parameters, the most important being the winemaking stage and duration of the application, the dose of the oxygen, and the composition of the initial wine.

Several publications in the literature have studied the influence of MOX on wine color, composition, and sensory attributes.[21,26,28−30] To our knowledge, however, none of these has studied the influence of wine pH on the effectiveness of MOX. The pH conditions the equilibrium between four different anthocyanin structures. The flavylium cation (red) is the main anthocyanin structure in very acidic pH conditions, but its concentration decreases progressively as the pH increases, which in turn generates the appearance of the quinoidal base (blue) by deprotonation or the hemiketal form (colorless) by dehydration and deprotonation. This hemiketal can originate the chalcone form (slightly yellow) after the overture of the heterocyclic ring.[31] Depending on the pH, anthocyanins can act as electrophiles in the flavylium form or as nucleophiles in the hemiketal form.[32] Therefore, it is quite logical that the pH can influence the reactivity of anthocyanins to oxygen. Moreover, the first step in the reactions induced by oxygen involves the formation of ethanal from ethanol.[33] After this, the ethanal must capture a proton and become a carbocation, which can react with flavanols and start the process of forming ethyl bridges.[34] Thus, it is also logical that the proton concentration must exert a non-negligible influence on this mechanism. Because the pH of wine usually ranges between 3.00 and 4.00, we have considered it interesting to study how the pH affects the oxygen-induced changes in red wine, especially when it seems that global warming is causing a general increase of wine pH.[35] In this paper, therefore, we study how the pH can affect the changes induced by MOX in two wines with different phenolic compositions.

MATERIALS AND METHODS

Chemicals. Methanol, acetonitrile, formic acid, and acetic acid were of HPLC grade and were purchased from Panreac (Barcelona, Spain). Malvidin-3-*O*-glucoside chloride, (+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-*O*-gallate were purchased from Extrasynthès (Genay, France). Phloroglucinol, L-ascorbic, 4-methylcatechol, and 4-dimethylaminocinnamaldehyde (DMACH) were purchased from Sigma (Madrid, Spain). The other chemicals were of high purity and were purchased from Panreac.

Wines. This study was carried out with two Cabernet Sauvignon wines from the 2008 vintage of the AOC Penedes. These wines were selected because of their different phenolic compositions. Wine A had a very low phenolic content, whereas wine B had a very high phenolic content. Specifically, the standard parameters of wine A at the start of the experiment were as follows: ethanol content, 12.5%; titratable acidity, 5.2 g of tartaric acid/L; volatile acidity, 0.42 g of acetic acid/L; pH, 3.5; anthocyanin content, 420 mg/L; total phenolic index (TPI), 49; and free SO_2, 20 mg/L. The standard parameters of wine B at the start of the experiment were as follows: ethanol content, 12.8%; titratable acidity, 5.9 g of tartaric acid/L; volatile acidity, 0.49 g of acetic acid/L; pH, 3.5; anthocyanin content, 1000 mg/L; TPI, 96; and 20 mg/L of free SO_2.

Argon was used during the wine-racking process to ensure that the wine received oxygen only from the micro-oxygenation. Amounts of 4500 L of both wines were divided between three stainless steel tanks of 1500 L. Two molar NaOH was added to the first tank of each wine to increase the pH to 3.9, and 2 M H_2SO_4 was added to the second tank of each wine to decrease the pH to 3.1. Finally, a similar total volume of distilled water was added to the third tank to minimize the dilution effect without altering the pH. After that, the wines were homogenized by pumping without aeration and left to stand for 24 h. Twenty-four glass bottles (750 mL), previously purged with argon, were filled with each wine and sealed with a 49 mm natural cork. The bottled wines were considered as controls. Simultaneously, each of the six different wines (wines A and B at three different pH values) was racked into three argon-purged MOX tanks for carrying out the experiment in triplicate. Oxygen concentration was immediately measured using a Clark electrode, and in all cases it was below 0.2 mg/L.

Micro-oxygenation Equipment. The multiple diffuser micro-oxygenator (VISIO 2/6-Oenodev, France) was connected to each of the 165 L MOX stainless steel tanks. These tanks were 2.5 m in height, had a diameter of 0.30 m, and were equipped with a ceramic diffuser placed 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 displacement height to guarantee their complete dissolution.

Experimental Conditions. Both the control and the micro-oxygenated wines were kept at a temperature of 16 ± 2 °C. MOX was carried out for 3 months with an oxygen flow of 15 mg/L per month. After that, 24 glass bottles (750 mL), previously purged with argon, were filled with wine from each tank and sealed with 49 mm natural corks. Analyses were done immediately after bottling and also 8 months later.

Color Parameters. The color intensity (CI) was estimated using the method described by Glories.[36] The CIELAB coordinates, lightness (L^*), chroma (C^*), and hue (H^*), were determined using the method described by Ayala et al. [37] and the data were processed with MSCV software.[38] All absorbance measurements were taken with a Helios Alpha UV−vis spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA) using 1 mm path length quartz cells.

Anthocyanin Analysis. The total anthocyanin content was determined by spectrophotometry using the method described by Niketic-Alksic and Hrazdina.[39] The PVPP index was calculated in accordance with Glories.[36]

HPLC Analyses of Anthocyanins and Derived Pigments. Reversed-phase HPLC analyses of the anthocyanins and the anthocyanin-derived pigments were carried out with an Agilent 1200 series liquid chromathograph (HPLC-DAD) and an Agilent Zorbax Eclipse XDB-C18, 4.6 × 250 mm, 5 μm column (Agilent Technologies, Santa Clara, CA), in accordance with the method described by Cano-López et al.[29] The solvents used were 4.5% of aqueous formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Elution was performed with a gradient that started at 10% B and reached 15% B at 30 min, 15.2% B at 45 min, 18% B at 60 min, 25% B at 100 min, and 25−100% B in 20 min. Chromatograms were recorded at 520 nm, and anthocyanin standard curves were made using malvidin-3-*O*-glucoside chloride.

Compounds were identified by recording their UV spectra with the diode array detector and comparing these with the UV spectra reported in the literature.[40] In addition, to confirm each peak, identity analyses were performed with the Agilent 1200 series HPLC using an Agilent 6210 time-of-flight (TOF) mass spectrometer equipped with an electrospray ionization (ESI) system. Elution was carried out under the same

Table 1. Color Parameters: Wine A[a]

parameter	pH	treatment	initial wine	after 3 months of micro-oxygenation	8 months after bottling
CI	3.1	control	10.9 ± 0.2A	10.0 ± 0.1B,α	9.0 ± 0.1C,α
		MO		11.1 ± 0.4A,*β*	10.3 ± 0.3B,*β*
	3.5	control	9.1 ± 0.1A	8.7 ± 0.1B,α	8.3 ± 0.1C,α
		MO		9.1 ± 0.1A,*β*	8.7 ± 0.1B,*β*
	3.9	control	7.9 ± 0.1A	8.3 ± 0.2B,α	7.8 ± 0.1A,α
		MO		8.3 ± 0.2B,α	8.0 ± 0.2AB,α
*L**	3.1	control	50.8 ± 0.5A	53.3 ± 0.2B,α	56.1 ± 0.2C,α
		MO		49.9 ± 1.3A,*β*	51.8 ± 1.0A,*β*
	3.5	control	55.1 ± 0.2A	56.7 ± 0.2B,α	58.6 ± 0.1C,α
		MO		56.1 ± 0.4B,α	57.6 ± 0.4C,*β*
	3.9	control	58.3 ± 0.1A	58.4 ± 0.7A,α	60.6 ± 0.1 B,α
		MO		58.9 ± 0.7A,α	60.5 ± 0.2B,α
*C**	3.1	control	57.8 ± 0.3A	54.1 ± 0.1B,α	50.1 ± 0.1C,α
		MO		53.9 ± 0.2B,α	51.4 ± 0.4C,*β*
	3.5	control	49.3 ± 0.1A	46.8 ± 0.1B,α	44.2 ± 0.2C,α
		MO		46.2 ± 0.2B,*β*	44.0 ± 0.2C,α
	3.9	control	40.4 ± 0.2A	40.1 ± 0.4A,α	40.1 ± 0.1A,α
		MO		40.1 ± 0.3AB,α	39.5 ± 0.3B,*β*
*H**	3.1	control	5.7 ± 0.2A	5.6 ± 0.2A,α	5.8 ± 0.1A,α
		MO		10.0 ± 0.6B,*β*	9.6 ± 0.3B,*β*
	3.5	control	3.8 ± 0.1A	4.2 ± 0.2B,α	6.3 ± 0.2C,α
		MO		8.3 ± 0.6B,*β*	8.9 ± 0.4B,*β*
	3.9	control	3.9 ± 0.1A	6.0 ± 0.3B,α	8.3 ± 0.1C,α
		MO		9.3 ± 0.5B,*β*	10.4 ± 0.2C,*β*

[a] All data are expressed as the average values of three replicates ± standard deviation (n = 3). Statistical analysis: two-factor ANOVA and Scheffe test (both, p = 0.05). Different letters indicate statistical differences. Latin letters (A, B, C) are used to compare the wines of the same pH throughout the time. Greek letters (α, *β*) are used to compare control and micro-oxygenation samples of the same pH at the same time.

HPLC analysis conditions as described by Cano-López.[29] The capillary voltage was 3.5 kV. Nitrogen was used both as a dry gas at a flow rate of 12 L/min at 350 °C and as a nebulizer gas at 60 psi. Spectra were recorded in positive ion mode between m/z 50 and 2400.

Analysis of Proanthocyanidins following Acid Catalysis with Phloroglucinol. Acid-catalysis cleavage in the presence of excess phloroglucinol[38] was used to analyze the monomeric composition of proanthocyanidin and its mean degree of polymerization (mDP). Ten milliliters of wine was evaporated under a low-pressure vacuum (Univapo 100 ECH, Uni Equip, Martinsried, Germany). After that, it was resuspended in 6 mL of distilled water and then applied to Set Pak Plus tC18 Environmental cartridges (Waters, Milford, MA) that had been previously activated with 10 mL of methanol and 15 mL of water. The sample was washed with 15 mL of distilled water, and then the proanthocyanidins were eluted with 12 mL of methanol, immediately evaporated under a vacuum, and then redissolved in 2 mL of methanol. Finally, 100 μL of this sample was reacted with a 100 μL phloroglucinol solution (0.2 N HCl in methanol, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid) at 50 °C for 20 min. The reaction was stopped by adding 1000 μL of 40 mM aqueous sodium acetate.[41] Reversed-phase HPLC analysis (Agilent series 1200 HPLC-DAD) was carried out in accordance with the method of Kennedy and Jones.[41] The solvents used were 1% aqueous acetic acid (solvent A) and methanol (solvent B) at a flow rate of 1 mL/min. The elution conditions were 1.0 mL/min. Elution was performed with a gradient starting at 5% B for 10 min, a linear gradient from 5 to 20% B in 20 min, and a linear gradient from 20 to 40% B in 25 min. The column was then washed with 90% B for 10 min and reequilibrated with 5% B for 5 min before the next injection. The monomers (+)-catechin, (−)-epicatechin, and (−)-epicechin-3-*O*-gallate were identified by comparing their retention times with those of the pure compounds. The phoroglucinol adducts of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, and (-)-epicatechin-3-*O*-gallate were identified by HPLC-TOF analysis. Analyses were performed with the Agilent 1200 series HPLC using an Agilent 6210 time-of-flight (TOF) mass spectrometer equipped with an electrospray ionization system (ESI). Elution was carried out under the same HPLC analysis conditions as described by Kennedy and Jones.[41] The capillary voltage was 3.5 kV. Nitrogen was used both as a dry gas at a flow rate of 12 L/min at 350 °C and as a nebulizer gas at 60 psi. Spectra were recorded in positive ion mode between m/z 50 and 2400. This assay was also carried out without the addition of phloroglucinol to measure the quantity of monomers that compose proanthocyanidins naturally present in the wines.

The number of terminal subunits was considered to be the difference between the total monomers measured in normal conditions (with phoroglucinol) and thus obtained when the analysis was performed without phloroglucinol addition. The number of extension subunits was considered as the addition of all the phloroglucinol adduct. The mean degree of polymerization (mDP) was calculated by adding terminal and extension subunits (in moles) and dividing by the terminal subunits. The total proanthocyanidin concentration was considered as the addition of all terminal and extension subunits. Because acid catalysis with phloroglucinol is not completely efficient, the real yield of the reaction was measured using a pure B2

Table 2. Color Parameters: Wine B[a]

parameter	pH	treatment	initial wine	after 3 months of micro-oxygenation	8 months after bottling
CI	3.1	control	28.1 ± 0.2A	25.8 ± 0.1B,α	20.2 ± 0.1C,α
		MO		26.9 ± 0.3B,β	22.6 ± 0.1C,β
	3.5	control	23.4 ± 0.3A	21.5 ± 0.1B,α	18.2 ± 0.1C,α
		MO		23.6 ± 0.3A,β	19.7 ± 0.3B,β
	3.9	control	20.8 ± 0.2A	19.3 ± 0.1B,α	16.6 ± 0.2C,α
		MO		20.7 ± 0.2A,β	17.7 ± 0.1B,β
L^*	3.1	control	29.6 ± 0.1A	30.0 ± 0.1B,α	33.6 ± 0.1C,α
		MO		26.9 ± 0.3B,β	30.0 ± 0.2C,β
	3.5	control	29.1 ± 0.3A	29.8 ± 0.2B,α	33.8 ± 0.1C,α
		MO		26.8 ± 0.2B,β	31.6 ± 0.3C,β
	3.9	control	28.7 ± 0.1A	30.1 ± 0.1B,α	34.8 ± 0.1C,α
		MO		28.3 ± 0.2B,β	34.0 ± 0.2C,β
C^*	3.1	control	68.1 ± 0.3A	66.1 ± 0.1B,α	64.1 ± 0.1C,α
		MO		63.3 ± 0.7B,β	62.3 ± 0.5B,β
	3.5	control	60.8 ± 0.1A	58.6 ± 0.1B,α	56.9 ± 0.1C,α
		MO		57.1 ± 0.2B,β	57.0 ± 0.2B,α
	3.9	control	55.2 ± 0.3A	52.5 ± 0.1B,α	48.8 ± 0.1C,α
		MO		52.3 ± 0.3B,α	53.1 ± 0.1C,β
H^*	3.1	control	26.9 ± 0.4A	24.5 ± 0.1B,α	20.8 ± 0.1C,α
		MO		25.4 ± 1.0B,β	22.6 ± 0.7C,β
	3.5	control	17.2 ± 0.4A	15.9 ± 0.1B,α	15.6 ± 0.1C,α
		MO		18.3 ± 1.0A,β	17.9 ± 0.8A,β
	3.9	control	9.8 ± 0.4A	10.1 ± 0.1A,α	11.6 ± 0.1B,α
		MO		14.9 ± 0.1B,β	16.1 ± 0.1B,β

[a] All data are expressed as the average values of three replicates ± standard deviation (n = 3). Statistical analysis: two-factor ANOVA and Scheffe test (both, p = 0.05). Different letters indicate statistical differences. Latin letters (A, B, C) are used to compare the wines of the same pH throughout the time. Greek letters (α, β) are used to compare control and micro-oxygenation samples of the same pH at the same time.

proanthocyanidin dimer [(−)-epicatechin-(4→8)-(−)-epicatechin]. This yield was used to calculate the total proanthocyanidin concentration.

Wine EDP Phloroglucinolysis Method. The level of ethylidene-bridged flavanols in the wine samples was determined using 2,2′-ethylidenediphloroglucinol (EDP) phloroglucinolysis in accordance with the method described by Drinkine et al.[42] Five milliliters of wine was diluted in 10 mL of water. After this, 10 mL of the sample was applied to the Set Pak Plus tC18 (5 g). Environmental cartridges (Waters) that had been previously activated with 50 mL of methanol and 50 mL of water. The sample was washed with 50 mL of distilled water and then eluted with 50 mL of methanol, immediately evaporated under vacuum, and later redissolved in 1.5 mL of methanol. Finally, 100 μL of this sample was reacted with 100 μL of phloroglucinol solution (0.2 N HCl in methanol, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid) at 50 °C for 20 min. The reaction was stopped by adding 200 μL of 400 mM aqueous sodium acetate.[42] Aqueous 4-methylcatechol (20 μL of 500 mg/L) was then added as an internal standard.

Analyses were performed with the Agilent 1200 series HPLC using an Agilent 6210 TOF mass spectrometer equipped with an ESI system and an Agilent Zorbax Eclipse XDB-C18, 4.6 × 150 mm, 5 μm column. The solvents used were 5% aqueous acetic acid (solvent A) and acetonitrile as solvent B. The sample loop was 20 μL, and the elution gradient was as follows: 10% B for 2 min, from 10 to 50% B in 8 min, from 50 to 100% B in 1 min, 100% B for 4 min, from 100 to 10% B in 1 min, and 10% B for 4 min with a 0.3 mL/min flow. The capillary voltage was 3.5 kV. Nitrogen was used both as a dry gas at a flow rate of 12 L/min at 350 °C and as a nebulizer gas at 60 psi. Spectra were recorded in positive ion mode between m/z 50 and 1100. To compare the wine samples, we used the area of the identified EDP.

Other Phenolic Compounds. The total phenolic index (TPI) was determined by measuring the absorbance at 280 nm.[19] The dimethylaminocinnamaldehyde index (DMACH index) was measured according to the method of Nagel and Glories.[43]

Statistics. All of the data are expressed as the arithmetic average ± the standard deviation from three replicates. Two- and one-factor ANOVA and a Scheffe test were carried out with SPSS software.

RESULTS AND DISCUSSION

Tables 1 (wine A) and 2 (wine B) show the wines' color characteristics. It is clear that the original wines (pH 3.5) have very different color characteristics. Wine A has lower color intensity (CI), chroma (C^*), and hue (H^*) and higher luminosity (L^*) than wine B. These data confirm that wine A is a light wine, whereas wine B is a highly concentrated wine, at least in terms of color.

As expected, CI, C^*, and L^* values of both wines were drastically influenced by the pH. Specifically, CI and C^* were higher and L^* lower when the pH was lower in both wines. H^* was also higher when the pH was more acidic in wine B, but this effect was not so clear in wine A. The changes generated by the pH in the chromatic characteristics observed in both wines can be easily explained. The lower the pH, the higher the proportion of flavylium cation and, therefore, the higher the contribution of red-colored anthocyanins.[31]

Table 3. Spectrophotometric Analysis of Anthocyanins and PVPP Index: Wine A[a]

parameter	pH	treatment	initial wine	after 3 months of micro-oxygenation	8 months after bottling
total anthocyanins (mg/L)	3.1	control	417.1 ± 3.3A	362.3 ± 13.1B,α	264.8 ± 1.0C,α
		MO		316.2 ± 4.1B,β	246.2 ± 0.5C,β
	3.5	control	415.9 ± 14.0A	377.4 ± 6.0B,α	282.3 ± 2.7C,α
		MO		343.9 ± 2.5B,β	265.2 ± 0.5C,β
	3.9	control	424.7 ± 7.1A	394.9 ± 4.5B,α	314.4 ± 2.2C,α
		MO		376.5 ± 1.0B,β	289.0 ± 2.2C,β
PVPP index	3.1	control	34.7 ± 1.3A	48.6 ± 2.3B,α	52.5 ± 1.1C,α
		MO		58.5 ± 6.8B,β	60.9 ± 0.8B,β
	3.5	control	34.5 ± 2.6A	44.0 ± 3.4B,α	45.3 ± 2.5B,α
		MO		46.2 ± 0.5B,α	53.1 ± 1.2C,β
	3.9	control	35.9 ± 4.3A	40.0 ± 1.1A,α	43.8 ± 1.1B,α
		MO		42.2 ± 2.9A,α	53.1 ± 5.8B,β

[a] All data are expressed as the average values of three replicates ± standard deviation (n = 3). Statistical analysis: two-factor ANOVA and Scheffe test (both, p = 0.05). Different letters indicate statistical differences. Latin letters (A, B, C) are used to compare the wines of the same pH throughout the time. Greek letters (α, β) are used to compare control and micro-oxygenation samples of the same pH at the same time.

Table 4. Spectrophotometric Analysis of Anthocyanins and PVPP Index: Wine B[a]

parameter	pH	treatment	initial wine	after 3 months of micro-oxygenation	8 months after bottling
total anthocyanins (mg/L)	3.1	control	1009.8 ± 10.1A	798.6 ± 4.1B,α	481.3 ± 3.7C,α
		MO		748.1 ± 6.1B,β	511.0 ± 6.6C,β
	3.5	control	981.8 ± 13.7A	796.3 ± 1.5B,α	521.5 ± 2.5C,α
		MO		741.7 ± 10.4B,β	517.4 ± 3.9C,α
	3.9	control	994.3 ± 11.3A	806.8 ± 8.6B,α	544.3 ± 6.2C,α
		MO		782.8 ± 18.7B,α	517.4 ± 3.9C,β
PVPP index	3.1	control	32.7 ± 2.1A	44.6 ± 0.3B,α	67.7 ± 0.1C,α
		MO		58.6 ± 1.6B,β	71.9 ± 2.7C,β
	3.5	control	32.0 ± 2.1A	51.5 ± 0.9B,α	62.4 ± 0.3C,α
		MO		58.9 ± 2.3B,β	66.4 ± 1.8C,β
	3.9	control	33.6 ± 1.1A	49.8 ± 1.3B,α	57.3 ± 1.5C,α
		MO		53.2 ± 3.3B,α	64.0 ± 1.4C,β

[a] All data are expressed as the average values of three replicates ± standard deviation (n = 3). Statistical analysis: two-factor ANOVA and Scheffe test (both, p = 0.05). Different letters indicate statistical differences. Latin letters (A, B, C) are used to compare the wines of the same pH throughout the time. Greek letters (α, β) are used to compare control and micro-oxygenation samples of the same pH at the same time.

CI tended to decrease over time in both wines at any pH. However, all of the MOX wines had a higher CI than their corresponding controls with the sole exception of wine A at pH 3.9. These differences were observed immediately after bottling and also after 8 months. Moreover, a clear trend was observed after 8 months of bottle storage inasmuch as the differences in CI appear to be higher when the pH is lower. Specifically, in wine A the differences in absorbance units were 1.3 at pH 3.1, 0.4 at pH 3.5, and 0.2 at pH 3.9, and those in wine B were 2.4 at pH 3.1, 1.5 at pH 3.5, and 1.1 at pH 3.9.

On the other hand, the Cielab coordinate C^* seems to decrease in all of the wines over time, but no clear trend is observed. In contrast, L^* tends to increase in all of the control wines over time, but its behavior is unclear in the micro-oxygenated wines at any pH during the first 3 months. However, after 8 months, nearly all of the micro-oxygenated wines have an L^* value lower than their corresponding controls, and this difference is greater when the pH is lower. Specifically, in wine A the differences in L^* units were 4.3 at pH 3.1, 1.0 at pH 3.5, and 0.1 at pH 3.9, and those in wine B were 3.3 at pH 3.1, 2.2 at pH 3.5, and 0.8 at pH 3.9. Finally, changes in H^* are even more complicated. In the light wine (wine A) H^* tended to increase in the MOX wines at any pH. However, in the high-phenolic content wine (wine B) the changes in H^* in the MOX wines varied according to the pH. Thus, H^* decreases at pH 3.1, is stable at pH 3.5, and increases at pH 3.9. It seems, therefore, that the pH and the polyphenol concentration affect the color changes induced by MOX.

Tables 3 (wine A) and 4 (wine B) show the results from the spectrophotometric analysis of anthocyanins and the PVPP index. These data confirm that wine A had an anthocyanin concentration much lower than that of wine B and that both wines were thus completely different at this level.

As expected, total anthocyanins decreased over time in all conditions. However, a different pattern can be observed between the controls and the MOX wines. In general, the anthocyanin concentration tended to decrease more after 3 months in the MOX wines than in the corresponding controls. After 8 months, these differences remained in wine A, but wine B behaved differently according to pH. In this high-phenolic content wine, the anthocyanin concentration in the MOX wines

Table 5. HPLC Analysis of Anthocyanins and Anthocyanin-Derived Pigments: Wine A[a]

parameter	pH	treatment	initial wine	after 3 months of micro-oxygenation	8 months after bottling
monomeric anthocyanins (mg/L)	3.1	control	158.5 ± 0.4A	106.3 ± 1.7B,α	58.9 ± 1.1C,α
		MO		65.6 ± 4.6B,*β*	32.7 ± 0.6C,*β*
	3.5	control	155.5 ± 0.3A	118.3 ± 1.2B,α	69.0 ± 2.0C,α
		MO		96.6 ± 3.4B,*β*	54.3 ± 1.3C,*β*
	3.9	control	152.9 ± 0.5A	121.8 ± 1.0B,α	85.9 ± 1.2C,α
		MO		108.7 ± 0.5B,*β*	74.4 ± 0.3C,*β*
direct adducts (mg/L)	3.1	control	1.36 ± 0.04A	1.62 ± 0.21B,α	1.69 ± 0.13B,α
		MO		1.68 ± 0.07B,α	1.45 ± 0.03C,*β*
	3.5	control	1.30 ± 0.01A	1.54 ± 0.04B,α	1.48 ± 0.12B,α
		MO		1.60 ± 0.03B,α	1.38 ± 0.03C,α
	3.9	control	1.23 ± 0.01A	1.37 ± 0.01B,α	1.35 ± 0.07B,α
		MO		1.36 ± 0.03B,α	1.38 ± 0.02B,α
ethyl-linked pigments (mg/L)	3.1	control	0.45 ± 0.07A	0.43 ± 0.03A,α	0.38 ± 0.02A,α
		MO		0.76 ± 0.06B,*β*	0.76 ± 0.08B,*β*
	3.5	control	0.45 ± 0.07A	0.44 ± 0.03A,α	0.36 ± 0.04B,α
		MO		0.42 ± 0.05A,α	0.56 ± 0.10A,*β*
	3.9	control	0.45 ± 0.05A	0.50 ± 0.05A,α	0.51 ± 0.01A,α
		MO		0.47 ± 0.04A,α	0.74 ± 0.07B,*β*
A-type vitisins (mg/L)	3.1	control	2.49 ± 0.04A	2.03 ± 0.06B,α	2.12 ± 0.03B,α
		MO		2.60 ± 0.06B,*β*	2.16 ± 0.02C,α
	3.5	control	2.47 ± 0.02A	2.06 ± 0.05B,α	2.05 ± 0.05B,α
		MO		2.62 ± 0.05B,*β*	2.12 ± 0.09C,α
	3.9	control	2.43 ± 0.04A	1.91 ± 0.05B,α	1.95 ± 0.04B,α
		MO		2.95 ± 0.04B,*β*	2.03 ± 0.12C,α
B-type vitisins (mg/L)	3.1	control	0.30 ± 0.02A	0.84 ± 0.03B,α	0.37 ± 0.04A,α
		MO		1.97 ± 0.25B,*β*	1.31 ± 0.20C,*β*
	3.5	control	0.34 ± 0.01A	0.90 ± 0.02B,α	0.27 ± 0.04C,α
		MO		0.65 ± 0.04B,*β*	0.46 ± 0.05C,*β*
	3.9	control	0.34 ± 0.04A	0.91 ± 0.09B,α	0.21 ± 0.01C,α
		MO		0.92 ± 0.02B,α	0.46 ± 0.02C,*β*
vinyl adducts (mg/L)	3.1	control	1.19 ± 0.01A	1.28 ± 0.03B,α	1.18 ± 0.19AB,α
		MO		1.69 ± 0.24B,*β*	1.58 ± 0.22B,α
	3.5	control	1.19 ± 0.04A	1.41 ± 0.05B,α	1.42 ± 0.05B,α
		MO		1.71 ± 0.08B,*β*	1.73 ± 0.07B,*β*
	3.9	control	1.18 ± 0.01A	1.65 ± 0.03B,α	1.92 ± 0.09C,α
		MO		1.96 ± 0.06B,*β*	2.07 ± 0.15B,α
polymeric peak (mg/L)	3.1	control	5.18 ± 0.29A	5.46 ± 0.31A,α	6.21 ± 0.06B,α
		MO		9.20 ± 0.85B,*β*	9.50 ± 1.00B,*β*
	3.5	control	5.05 ± 0.53A	6.22 ± 0.22B,α	6.23 ± 0.19B,α
		MO		6.36 ± 0.26B,α	6.83 ± 0.24B,*β*
	3.9	control	5.15 ± 0.19A	5.94 ± 0.15B,α	6.16 ± 0.58B,α
		MO		6.23 ± 0.24B,α	6.56 ± 0.06B,α

[a] All data are expressed as the average values of three replicates ± standard deviation (n = 3). Statistical analysis: two-factor ANOVA and Scheffe test (both, p = 0.05). Different letters indicate statistical differences. Latin letters (A, B, C) are used to compare the wines of the same pH throughout the time. Greek letters (α, β) are used to compare control and micro-oxygenation samples of the same pH at the same time.

was higher at pH 3.1, equal at pH 3.5, and lower at pH 3.9 when compared with their corresponding controls not micro-oxygenated.

The PVPP index tended to increase over time in all of the wines and did so more rapdily when MOX was applied, which would confirm that oxygen encourages the anthocyanins to

Table 6. HPLC Analysis of Anthocyanins and Anthocyanin-Derived Pigments: Wine B[a]

parameter	pH	treatment	initial wine	after 3 months of micro-oxygenation	8 months after bottling
monomeric anthocyanins (mg/L)	3.1	control	270.4 ± 10.4A	205.1 ± 27.8B,α	61.4 ± 0.1C,α
		MO		143.0 ± 6.2B,*β*	42.4 ± 2.1C,*β*
	3.5	control	270.3 ± 4.8A	195.6 ± 4.9B,α	79.3 ± 1.9C,α
		MO		156.3 ± 6.3B,*β*	61.8 ± 3.7C,*β*
	3.9	control	245.5 ± 6.1A	204.4 ± 2.4B,α	96.8 ± 0.1C,α
		MO		195.1 ± 2.2B,*β*	72.9 ± 0.1C,*β*
direct adducts (mg/L)	3.1	control	1.52 ± 0.05A	2.45 ± 0.12B,α	2.10 ± 0.10C,α
		MO		2.02 ± 0.06B,*β*	2.75 ± 0.04C,*β*
	3.5	control	1.66 ± 0.03A	2.03 ± 0.05B,α	2.41 ± 0.09C,α
		MO		1.96 ± 0.05B,α	2.87 ± 0.01C,*β*
	3.9	control	1.60 ± 0.07A	1.79 ± 0.05B,α	2.39 ± 0.02C,α
		MO		1.88 ± 0.06B,α	2.34 ± 0.08C,α
ethyl-linked pigments (mg/L)	3.1	control	1.19 ± 0.10A	1.24 ± 0.05A,α	1.62 ± 0.18B,α
		MO		2.23 ± 0.06B,*β*	1.91 ± 0.06C,*β*
	3.5	control	1.23 ± 0.10A	1.23 ± 0.06A,α	1.45 ± 0.05B,α
		MO		1.37 ± 0.15A,α	1.68 ± 0.12B,*β*
	3.9	control	1.22 ± 0.05A	1.11 ± 0.05A,α	1.42 ± 0.02 B,α
		MO		1.04 ± 0.14A,α	1.54 ± 0.04B,*β*
A-type vitisins (mg/L)	3.1	control	7.68 ± 0.01A	7.48 ± 0.05B,α	7.40 ± 0.01C,α
		MO		8.09 ± 0.04B,*β*	7.85 ± 0.05C,*β*
	3.5	control	7.60 ± 0.10A	7.42 ± 0.02B,α	7.30 ± 0.01C,α
		MO		9.91 ± 0.03B,*β*	9.38 ± 0.05C,*β*
	3.9	control	7.61 ± 0.05A	6.66 ± 0.06B,α	6.53 ± 0.01C,α
		MO		7.42 ± 0.01B,*β*	6.93 ± 0.01C,*β*
B-type vitisins (mg/L)	3.1	control	0.40 ± 0.05A	0.85 ± 0.02B,α	0.48 ± 0.01C,α
		MO		1.31 ± 0.05B,*β*	0.83 ± 0.02C,*β*
	3.5	control	0.39 ± 0.03A	0.68 ± 0.02B,α	0.28 ± 0.01C,α
		MO		0.63 ± 0.06B,α	0.33 ± 0.01C,*β*
	3.9	control	0.43 ± 0.05A	0.67 ± 0.01B,α	0.27 ± 0.01C,α
		MO		0.65 ± 0.02B,α	0.25 ± 0.01C,α
vinyl adducts (mg/L)	3.1	control	1.01 ± 0.07A	1.21 ± 0.03B,α	2.12 ± 0.08C,α
		MO		1.57 ± 0.01B,*β*	2.97 ± 0.08C,*β*
	3.5	control	1.11 ± 0.05A	1.29 ± 0.07B,α	2.05 ± 0.04C,α
		MO		1.51 ± 0.03B,*β*	1.62 ± 0.14B,*β*
	3.9	control	1.15 ± 0.06A	1.18 ± 0.05A,α	1.88 ± 0.09B,α
		MO		1.32 ± 0.07B,*β*	1.47 ± 0.09B,*β*
polymeric peak (mg/L)	3.1	control	5.99 ± 0.21A	7.38 ± 0.34B,α	7.57 ± 0.09B,α
		MO		8.39 ± 0.35B,*β*	11.23 ± 0.43C,*β*
	3.5	control	5.89 ± 0.08A	6.30 ± 0.16B,α	8.83 ± 0.01C,α
		MO		7.47 ± 0.32B,*β*	10.07 ± 0.03C,*β*
	3.9	control	5.43 ± 0.13A	6.63 ± 0.08B,α	8.58 ± 0.08C,α
		MO		8.02 ± 0.08B,*β*	9.22 ± 0.16C,*β*

[a] All data are expressed as the average values of three replicates ± standard deviation (n = 3). Statistical analysis: two-factor ANOVA and Scheffe test (both, p = 0.05). Different letters indicate statistical differences. Latin letters (A, B, C) are used to compare the wines of the same pH throughout the time. Greek letters (α, β) are used to compare control and micro-oxygenation samples of the same pH at the same time.

combine with the flavanols.[21] On the other hand, after 3 months of MOX, the PVPP index of the MOX wines compared their controls was greater when the pH was lower. Specifically, in wine A the difference between the PVVP index of MOX and control wines was 9.9 at pH 3.1, 2.2 at pH 3.5, and 2.2 at pH 3.9, and those in wine B were 14.0 at pH 3.1, 7.4 at pH 3.5, and 3.4 at pH

Table 7. Total Phenolic Compounds, Proanthocyanidins, and Related Parameters: Wine A[a]

parameter	pH	treatment	initial wine	after 3 months of micro-oxygenation	8 months after bottling
TPI	3.1	control	49.3 ± 0.2A	49.0 ± 0.1A,α	45.7 ± 0.6B,α
		MO		48.5 ± 0.9A,α	45.2 ± 0.3B,α
	3.5	control	49.1 ± 0.2A	48.8 ± 0.2A,α	45.4 ± 0.1B,α
		MO		48.4 ± 0.8A,α	45.1 ± 0.3B,α
	3.9	control	49.5 ± 0.1A	46.7 ± 0.8B,α	43.5 ± 0.2C,α
		MO		46.1 ± 0.3B,α	43.4 ± 1.1C,α
proanthocyanidins (mg/L)	3.1	control	1473 ± 21A	1423 ± 42A,α	1286 ± 27B,α
		MO		1377 ± 21B,α	1215 ± 81C,α
	3.5	control	1475 ± 25A	1429 ± 46A,α	1352 ± 21B,α
		MO		1420 ± 41A,α	1258 ± 27B,β
	3.9	control	1476 ± 37A	1423 ± 16AB,α	1397 ± 36B,α
		MO		1483 ± 70A,α	1312 ± 26B,β
DMACH index	3.1	control	38.50 ± 0.35A	35.75 ± 0.43B,α	31.83 ± 0.52C,α
		MO		29.92 ± 1.38B,β	27.83 ± 1.38B,β
	3.5	control	39.50 ± 0.10A	35.50 ± 0.66B,α	32.33 ± 0.58C,α
		MO		32.83 ± 1.81B,β	29.08 ± 0.29C,β
	3.9	control	38.13 ± 0.88A	36.42 ± 1.13A,α	33.08 ± 0.72B,α
		MO		35.58 ± 1.61B,α	32.50 ± 0.43C,α
mDP	3.1	control	4.65 ± 0.04A	4.58 ± 0.07A,α	4.52 ± 0.20A,α
		MO		4.45 ± 0.08B,α	4.58 ± 0.09AB,α
	3.5	control	4.72 ± 0.13A	4.58 ± 0.07A,α	4.85 ± 0.32A,α
		MO		4.67 ± 0.03A,α	4.71 ± 0.12A,α
	3.9	control	4.61 ± 0.01A	4.80 ± 0.06B,α	5.08 ± 0.03C,α
		MO		4.80 ± 0.13B,α	5.28 ± 0.08C,β

[a] All data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Statistical analysis: two-factor ANOVA and Scheffe test (both, $p = 0.05$). Different letters indicate statistical differences. Latin letters (A, B, C) are used to compare the wines of the same pH throughout the time. Greek letters (α, β) are used to compare control and micro-oxygenation samples of the same pH at the same time.

3.9. Although these differences were minimized after 8 months, these data suggest that MOX is more effective when the pH is lower, at least in terms of its effect on the combination of anthocyanins and flavanols.

Anthocyanins and anthocyanin-derived pigments were also analyzed by HPLC. In both wines, wine A (Table 5) and wine B (Table 6), the five anthocyanin monoglucosides (malvidin, petunidin, delphinidin, peonidin, and cyanidin) and their respective acetyl and coumaryl derivatives were detected. In general, the total monomeric anthocyanin concentration decreased over time and did so more rapidly when MOX was applied and when the pH was lower. Broadly speaking, this trend, which was observed in the five monoglucosides and also in their respective acyl derivatives, does not coincide exactly with that observed for the anthocyanins measured by spectrophotometry. This is because spectrophotometric analysis includes the contribution of other pigments, whereas the HPLC method detects only free anthocyanins.[44]

Malvidin 3-glucoside-(epi)catechin was the only detected direct adduct of anthocyanins and flavanols, and its concentration tended to increase over time in all of the wines. However, neither the pH nor micro-oxygenation seemed to exert any influence on its evolution.

Malvidin-3-glucoside-ethyl-catechin, malvidin-3-acetylglucoside-ethyl-catechin, and malvidin-3-*p*-coumaroyl-glucoside-ethyl-catechin were detected in wine samples, and all of them are grouped in Tables 5 and 6 as ethyl-bridged linked pigments. After 3 months, none of the control wines differed significantly from the original wines. Similar results were obtained with the MOX wines at pH 3.9 and 3.5. However, both MOX wines had significantly higher levels of ethyl-bridged linked pigments when the pH was more acidic (3.1). After 8 months, all of the MOX wines had small but significantly higher concentrations of these pigments than their corresponding controls. These results confirm that MOX encourages anthocyanins to combine with flavanols by means of ethyl bridges, although the differences found are more minor than expected.

Several pyroanthocyanin compounds were found in our wines. Specifically, these were type-A vitisins, which include vitisin A and A-type vitisins of peonidin-3-glucoside and malvidin 3-acetylglucoside; type-B vitisins, which include vitisin B and B-type vitisin of malvidin 3-acetylglucoside; and vinyl adducts, which include malvidin-3-glucoside-4-vinyl-catechin adduct and malvidin-3-glucoside-4-vinylphenol adduct.

After 3 months, all of the MOX wines had a small but significantly higher concentration of type-A vitisins than their respective controls without any effect from the pH being observed. After 8 months, these differences remained only in the high-phenolic content wine (B) but not in the light wine (A).

A different trend was found in the B-type vitisins because after 3 months all of the wines, both control and MOX, showed a significant increase in the concentration of these pigments. It is interesting to note that both MOX wines at pH 3.1 had

Table 8. Total Phenolic Compounds, Proanthocyanidins, and Related Parameters: Wine B[a]

parameter	pH	treatment	initial wine	after 3 months of micro-oxygenation	8 months after bottling
TPI	3.1	control	95.7 ± 1.8A	92.7 ± 0.2B,α	92.8 ± 0.7B,α
		MO		91.8 ± 0.7B,α	92.9 ± 0.8B,α
	3.5	control	95.6 ± 2.1A	91.9 ± 0.9B,α	92.7 ± 0.5B,α
		MO		91.1 ± 1.1B,α	92.5 ± 0.6B,α
	3.9	control	95.6 ± 0.5A	91.3 ± 1.2B,α	92.2 ± 0.5B,α
		MO		90.5 ± 1.2B,α	91.1 ± 0.6B,α
proanthocyanidins (mg/L)	3.1	control	2753 ± 30A	2435 ± 49B,α	2243 ± 170B,α
		MO		2560 ± 26B,β	2017 ± 152C,α
	3.5	control	2781 ± 59A	2489 ± 43B,α	2371 ± 6C,α
		MO		2579 ± 61B,α	2327 ± 5C,β
	3.9	control	2742 ± 17A	2512 ± 71B,α	2373 ± 44C,α
		MO		2634 ± 31B,β	2223 ± 234C,α
DMACH index	3.1	control	65.67 ± 1.42A	65.00 ± 1.50A,α	56.25 ± 0.71B,α
		MO		57.50 ± 0.25B,β	51.83 ± 1.18C,β
	3.5	control	67.25 ± 2.18A	65.17 ± 1.15A,α	58.00 ± 1.12B,α
		MO		60.25 ± 1.75B,β	54.92 ± 1.27C,β
	3.9	control	65.92 ± 1.13A	64.42 ± 1.04A,α	58.88 ± 1.24B,α
		MO		60.75 ± 1.64B,β	55.75 ± 1.30C,β
mDP	3.1	control	4.47 ± 0.04A	4.63 ± 0.05B,α	4.91 ± 0.01C,α
		MO		4.61 ± 0.21AB,α	4.74 ± 0.10B,β
	3.5	control	4.58 ± 0.10A	4.85 ± 0.13B,α	4.91 ± 0.04B,α
		MO		4.79 ± 0.09B,α	4.75 ± 0.20B,α
	3.9	control	4.52 ± 0.07A	4.65 ± 0.10A,α	5.24 ± 0.02B,α
		MO		4.64 ± 0.05A,α	4.90 ± 0.01B,β

[a] All data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Statistical analysis: two-factor ANOVA and Scheffe test (both, $p = 0.05$). Different letters indicate statistical differences. Latin letters (A, B, C) are used to compare the wines of the same pH throughout the time. Greek letters (α, β) are used to compare control and micro-oxygenation samples of the same pH at the same time.

significantly higher concentration of B-type vitisins than their corresponding controls and that these differences were not found at the other pH. Therefore, the very acidic pH seems to favor the cycloaddition of ethanal with anthocyanins.

After 3 months, the vinyl adduct concentration of all the MOX wines was significantly higher than in their corresponding controls, but after 8 months these differences were not so clear. In fact, some control wines had higher concentrations of these pigments after 8 months than their corresponding MOX wines. On the other hand, the pH did not appear to exert any effect on these compounds.

Some authors have suggested that the broad peak at the end of the HPLC chromatogram of the anthocyanins and derived pigments corresponds to the polymeric pigments.[29,30] This polymeric peak tends to increase its area over time in all of the wines, which suggests that the formation of polymeric structures of pigments took place both in the control and in the MOX wines. This polymeric peak tended to increase more quickly when MOX was applied to wine B at any pH, whereas this occurred only at pH 3.1 in wine A.

At 3 months, this polymeric peak was significantly higher when MOX was applied to wine B at any pH but only at pH 3.1 in wine A. Eight months later, the differences between the MOX and control wines were more clearly influenced by the pH. Specifically, at pH 3.1 the surface of the polymeric peak was around 50% higher in both MOX wines than in the controls, whereas at pH 3.5 it was around 15% higher, and at pH 3.9 it was practically nonexistent. These data suggest, therefore, that the formation of polymeric pigments in the presence of oxygen is more likely when the pH of the wine is very acidic.

Tables 7 and 8 show the total phenolic compounds and related parameters. These data confirm again that both wines are very different in their phenolic composition inasmuch as the TPI and a proanthocyanidin concentration of wine A are about half those of wine B.

As expected, no differences were found in the TPI, DMACH index, proanthocyanidin concentration, and mDP of both initial wines in terms of the pH. Nevertheless, the TPI and the proanthocyanidin concentration tended to decrease over time in all of the wines, probably as a result of the precipitation of large polymers. In general, these decreases were similar in both control and MOX wines at any pH. Some general trends were observed in the DMACH index, which also decreased in all wines over time. Specifically, the DMACH index decreased more quickly in the MOX wines than in the controls. Moreover, this decrease seemed to be more drastic when the pH was lower, especially in the less concentrated wine (A). Because DMACH reacts only with the terminal units of proanthocyanidin polymers, its decrease must be due to a decrease in the number of molecules.[43] Therefore, the observed decrease in the DMACH index may be caused either by precipitation of some of the proanthocyanidin molecules or by an increase in their degree of polymerization.

Table 9. Evaluation of Ethylidene-Bridged Flavan-3-ols[a]

parameter	pH	treatment	8 months after bottling (wine A)	8 months after bottling (wine B)
ethyl bridges (area × 10^4)	3.1	control	31.7 ± 2.2α	100.3 ± 3.7α
		MO	100.2 ± 29.7β	170.45 ± 18.5β
	3.5	control	34.1 ± 2.4α	55.8 ± 2.7α
		MO	52.5 ± 4.9β	61.0 ± 7.1α
	3.9	control	25.1 ± 0.7α	55.6 ± 7.9α
		MO	24.9 ± 2.6α	46.2 ± 2.0α

[a] All data are expressed as the average values of three replicates ± standard deviation (n = 3). Statistical analysis: one-factor ANOVA and Scheffe test (both, p = 0.05). Different letters indicate statistical differences.Greek letters (α, β) are used to compare control and micro-oxygenation samples of the same pH at the same time.

However, the mDP measured by phloroglucinolysis does not follow a pattern similar to that of the DMACH index and always maintains very similar values for all of the wines. These results indicate that MOX does not induce changes in the degree of polymerization and suggest that the decrease in DMACH might be more related to the precipitation of some of the proanthocyaninidins molecules. These results are surprising because it was thought that the MOX induced the polymerization of proanthocyaninidins, although other authors have also reported similar results.[21]

Table 9 shows the evaluation of ethyl-bridged flavanols by means of EDP phloroglucinolysis. In this instance, only wines at 8 months were analyzed because the method was not ready at the beginning of the experiment. The application of MOX did not generate significant differences in the presence of ethylidene-bridged flavanols at pH 3.9 in any of the wines. However, significant differences appeared when the pH was lower. Specifically, at pH 3.5 the presence of ethylidene-bridged flavanols was found to be greater in micro-oxygenated wine A but not in wine B, and at pH 3.1 the differences became very high in both wines. These data indicate that the formation of ethyl-bridged flavanols is highly favored in very acidic pH. Moreover, because no differences were found between the control and MOX wines at pH 3.9, these data also suggest that MOX is less effective when the pH of wines is too high.

It can be concluded that pH exerts a major effect on the evolution of color and phenolic compounds in wine during aging, especially when oxygen is added by MOX. When the pH is more acidic, the effects of MOX are clearer. The differences observed in MOX wines compared to their controls indicate that they had a more intense color and higher PVPP index, and higher concentrations of ethyl-linked pigments, B-type vitisins, polymeric pigments, and ethylidene-bridged flavanols when the pH was more acidic. In contrast, the effects of MOX when the pH is less acidic are much less evident and sometimes practically nonexistent. All of these data seem to indicate that ethyl-bridge formation between flavanol−flavanol and flavanol−anthocyanin as well as the cycloaddition with ethanal is favored at low pH.

Further studies about pH and oxygen effects on wine phenolic compounds are needed to better understand all of these phenomena and to improve the practical application of micro-oxygenation technique in wineries.

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