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Oxygen Consumption and Development of Volatile Sulfur Compounds during Bottle Aging of Two Shiraz Wines. Influence of Pre- and Postbottling Controlled Oxygen Exposure

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ABSTRACT: The evolution of different volatile sulfur compounds (VSCs) during bottle maturation of two Shiraz wines submitted to controlled oxygen exposure prior to bottling (through micro-oxygenation, MOX) and postbottling (through the closure) was investigated. H_2S, methyl mercaptan (MeSH), and dimethyl sulfide (DMS) were found to increase during aging. Lower postbottling oxygen exposure, as obtained by different degrees of oxygen ingress through the closure, resulted in increased H_2S and methyl mercaptan. In one wine MOX increased the concentration of H_2S and methyl mercaptan during maturation. Dimethyl disulfide and DMS were not affected by any form of oxygen exposure. Overall, postbottling oxygen had a stronger influence than MOX on the evolution of VSCs. Data suggest that dimethyl disulfide was not a precursor to methyl mercaptan during bottle maturation. For the two wines studied, a consumption of oxygen of 5 mg/L over 12 months was the most effective oxygen exposure regimen to decrease accumulation of MeSH and H_2S during bottle aging.

KEYWORDS: *volatile sulfur compounds, bottle aging, methyl mercaptan, hydrogen sulfide, wine, oxygen, micro-oxygenation*

INTRODUCTION

Red wines are typically consumed after a period of aging in the bottle. The chemical transformations that take place during this period are complex and involve volatile and nonvolatile components. Overall, bottle aging of red wines, often also referred to as bottle maturation, is expected to improve aroma and mouthfeel properties. It is well accepted that oxygen plays a central role in the process of wine maturation, as it affects the type and extent of many of the chemical reactions occurring during this process.[1]

In the production of red wines, controlled oxygen addition during tank storage, known as micro-oxygenation (MOX), is increasingly of interest and being used by winemakers. MOX is expected to promote some improvements in mouthfeel properties and color stability that are commonly achieved during barrel storage.[2] Several authors have investigated the effects of MOX on wine color as well as aroma and phenolic composition,[2] but there is very limited information regarding the effects of MOX on subsequent steps of wine production, including bottle maturation. In this regard, with recent advances in the technologies applied to the production of wine closures, closures with defined oxygen transmission rate (OTR) are becoming available, providing the capacity to now also control the degree of oxygen exposure postbottling. This can have significant consequences not only for wine chemical evolution during bottle maturation[3,4] but also in the quest to deliver wines to consumers with optimal sensory characters.[5,6]

Indeed, if oxygen exposure is either too low or too high, wines can develop defects that can compromise their sensory quality. In particular, too little oxygen can result in aroma defects known as "reduction", a condition in which wines express dominant notes of egg, rotten, vegetal, and cabbage, accompanied by low intensity of fruity attributes.[7] The occurrence of this off-odor is linked to the presence of excessive concentrations of some volatile sulfur compounds (VSCs), particularly H_2S, methyl mercaptan (MeSH), and ethyl mercaptan.[7] In addition, the other VSC, dimethyl sulfide (DMS), can contribute to "reductive" notes, although its relationship with oxygen exposure remains to be established. Although it has been shown that a moderate exposure to oxygen can limit the formation of H_2S during bottle aging,[8,9] the definition of the optimal amount of oxygen remains challenging.

Indeed, the ability of a wine to develop reduction can vary with an extremely large array of variables, including grape variety and vintage, winemaking practices, and wine composition, with wine style and consumers' expectations also influencing consumer perceptions and acceptance of these characters.[9,10] Lopes et al.[8] estimated total oxygen exposure (sum of oxygen at bottling plus oxygen ingress thorough the closure) of a Sauvignon blanc wine during 2 years of bottle storage and concluded that the oxygen exposure provided by a natural cork closure allowed optimal wine aroma development, with negligible reduction. However, in the current wine market, most wines, especially whites, have a much faster turnaround time than 2 years, hence the need to better understand the development of key aroma compounds at earlier stages of bottle maturation. Moreover, different closures allow different profiles of oxygen exposure. For example, screw caps are

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typically characterized by higher exposure in the first few weeks compared to inner seal closures, due to the large headspace volume.[11] Conversely, natural cork closures should have early moderate exposure due to oxygen desorbing from the closure,[12] followed by relatively low OTR, although the latter can be highly variable within the same lot of closures.[10] Finally, synthetic closures allow a more even supply of oxygen throughout bottle maturation,[12] which, in some cases, can be fixed by the manufacturer.

Despite recent evidence suggesting that the occurrence of certain VSCs, in particular MeSH, is associated with lower consumer preference,[6] to date, there are very limited data on the pathways leading to accumulation of MeSH during wine bottle maturation. Moreover, most studies on the occurrence of reduction-related VSCs during bottle maturation of wine have been carried out in white wines,[8,9] although red wines are typically aged for longer periods in the bottle before consumption.

In this study we have investigated the influence of different forms of oxygen exposure on the evolution of several VSCs during bottle maturation of two Shiraz red wines to define oxygen regimens that can allow effective control of VSC formation during bottle maturation of a red wine. The relationships between oxidized, reduced, and esterified forms of methyl mercaptan were also studied.

MATERIALS AND METHODS

Chemicals. Reference standards of ethanethiol (EtSH, 99.7%), dimethyl sulfide (DMS, 99.8%), diethyl sulfide (DES, 99.3%), dimethyl disulfide (DMDS, 99.8%), diethyl disulfide (DEDS, 99.9%), carbon disulfide (CS2, 99.9%), and ethylmethyl sulfide (EMS, 96.0%) were of the highest purity as supplied by Sigma-Aldrich (Castle Hill, NSW, Australia). *S*-Methyl thioacetate (MeSAc, 98.8%), *S*-ethyl thioacetate (EtSAc, 99.5%), and propyl thioacetate (PrSAc, 99.7%) were of the highest purity obtainable from Lancaster Synthesis (Jomar Bioscience, Kensington, SA, Australia). The remaining chemicals listed below were of analytical reagent grade quality or better. Sodium hydrosulfide hydrate ($NaSH_3 \cdot H_2O$, 74.0%) and sodium thiomethoxide (NaSMe, 101.8%) were supplied by Sigma-Aldrich.

Wines. Shiraz wines produced in 2008 from the Barossa (SHZ1) or Virginia (SHZ2) wine regions in South Australia were obtained from two local wineries. Analytical parameters of the wine were as follows: (SHZ1) pH 3.7, residual sugars = 0.2 g/L (glucose + fructose), alcohol = 15% (v/v), volatile acidity = 0.44 g/L (as acetic acid), titratable acidity = 6.4 g/L (as tartaric acid), free SO_2 = 26 mg/L, total SO_2 = 49 mg/L, tannin = 870 mg/L (by MCP assay), anthocyanins = 460 mg/L; (SHZ 2) pH 3.5, residual sugars = 0.5 g/L (glucose + fructose), alcohol = 14.3%, volatile acidity = 0.39 g/L (as acetic acid), titratable acidity = 6.1 g/L (as tartaric acid), free SO_2 = 25 mg/L, total SO_2 = 64 mg/L, tannin = 2140 mg/L (by MCP assay), anthocyanins = 446 mg/L.

Wines were submitted to MOX at a rate of 2 mg of oxygen/L/month for 7 weeks, using 300 L "cigar-shaped" tanks (height = 3 m), fitted with a ceramic sparger and a control unit to dose oxygen. Wines not submitted to MOX were kept for the same period in similar tanks with no oxygen application.

Free sulfur dioxide levels were monitored and maintained above 25 mg/L by the addition of potassium metabisulfite. As is accepted winery practice, the wines were assessed sensorially every 7 days by at least two qualified winemakers to determine the end point of the treatment. The end point was defined as the point when differences between the treated and control wines were evident, but the control wines were not showing signs of oxidation. Table 1 shows the phenolic composition of the wines at different stages of the MOX treatment. Prior to bottling, SO_2 content of all wines was adjusted, and final values were as follows: (SHZ1 MOX) free SO_2 = 30 mg/L, total SO_2 = 67 mg/L; (SHZ1 No MOX) free SO_2 = 30 mg/L, total SO_2 = 65 mg/L; (SHZ2 MOX) free SO_2 = 30 mg/L, total SO_2 = 85 mg/L; (SHZ2 No MOX) free SO_2 = 30 mg/L, total SO_2 = 87 mg/L.

Table 1. Evolution of Wine Phenolic Composition during MOX

	0 days	21 days	49 days
SHZ1			
anthocyanin (mg/L)	446	460	441
nonbleachable pigment (au[a])	4.17	4.7	4.79
total pigment (au)	29.2	30.8	30.0
% nonbleachable pigment (au)	14.3	15.3	16.0
total phenolics (au)	73	68	67
tannin (mg/L)	2.14	1.85	1.82
SHZ2			
anthocyanin (mg/L)	460	406	401
nonbleachable pigment (au)	1.7	1.77	1.81
total pigment (au)	25.9	23.3	23.1
% nonbleachable pigment (au)	6.6	7.6	7.9
total phenolics (au)	46	43	44
tannin (mg/L)	0.87	0.77	0.8

[a]Absorbance units.

All wines were bottled under Nomacorc Classic+ synthetic closures (37 mm length, 22.5 mm diameter; Nomacorc, Zebulon, NC, USA). Three degrees of oxygen exposure during bottling were obtained by combining storage of closures in either nitrogen or air for 1 week prior to bottling with storage of bottles in either air or nitrogen for the whole length of the study. For the treatments requiring storage under nitrogen, closures or wines were kept in steel drums filled with nitrogen and sealed. Drums were periodically refilled with nitrogen to maintain the oxygen content below 10 hPa. The three different degrees of oxygen exposure (exp) obtained were as follows: low O_2 exp (closures stored in nitrogen and bottles stored in nitrogen); mid O_2 exp (closures stored in air and bottles stored in nitrogen); standard (std) O_2 exp (closures stored in air and bottles stored in air). The oxygen ingress profiles under these three conditions, measured in empty bottles (as described under Total Consumed Oxygen), are shown in Figure 1. For the bottling of each wine, empty 375 mL flint glass bottles were flushed with pure N_2 and then filled using a Framax FCS 4/1S automatic filling machine (Framax, Serravalle Pistoiese, Italy). Closures for different treatments were then applied on a Bertolaso Epsilon R corker (Bertolaso, Zimella, Italy) with the vacuum set at −15 kPa. Ten bottles, each fitted with two PreSens Pst3 oxygen sensors (Presens, Regensurg, Germany) to measure dissolved and headspace oxygen, were filled with wine and sealed across the whole bottling operation for each oxygen exposure treatment and each wine

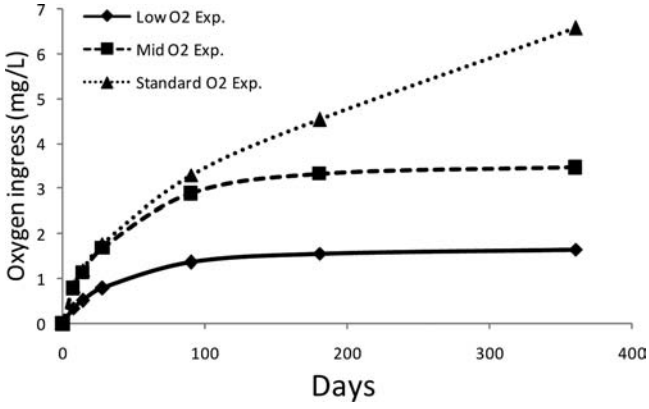

Figure 1. Oxyen ingress profiles in the bottle for the three experimental conditions (◆, low O_2 exp; ■, mid O_2 exp; ▲, std O_2 exp).

to monitor performance. These same bottles were used to monitor dissolved oxygen (DO) during storage of the wines under the different experimental conditions. All oxygen measures were carried out using a PreSens Fibox 3 trace v3 oxygen meter (Presens). Generally, dissolved oxygen values, measured 24 h after bottling, were on average 0.47 mg/L (min−max = 0.33−0.72 mg/L) and headspace oxygen was 0.67 mg/L (min−max = 0.56−0.78 mg/L).

Total Consumed Oxygen (TCO). TCO was calculated as the sum of oxygen present at bottling (headspace plus DO), plus the oxygen entering the bottle through the closure during storage, minus headspace and DO oxygen measured at each time point, the latter accounting for any residual oxygen not consumed by the wine.[11] The amount of oxygen entering the bottles under the conditions of this study was measured as described elsewhere (REF Ugliano).[9] In brief, empty bottles of the same type described above were fitted with PreSens Pst6 oxygen sensors for measurement of trace oxygen levels, placed in a corking machine, and flushed with a stream of 98% N_2 to obtain an oxygen pressure lower than 0.5 hPa. The bottle was immediately sealed with Nomacorc Classic+ closures previously equilibrated in either air or nitrogen, as described above. One hour after insertion of the closure, the oxygen pressure was measured, and then the bottles were stored in air or nitrogen, under the same conditions as the bottles filled with wine.

Chemical Analyses. Wines were analyzed 24 h after bottling and then following 3, 6, and 12 months of bottle storage. Free and total SO_2 analysis was performed according to the methods of Iland et al.[13] Determination of wine color and phenolics was carried out according to the method and calculations of Somers and Evans.[14] Tannins were quantified by MCP.[15] VSCs were analyzed by gas chromatography (GC) coupled with sulfur chemoluminescence detection (SCD), using static headspace (HS) sampling.[16] Accordingly, wine samples were cooled to 4 °C in their original containers prior to opening, and all sample handling was completed in a temperature-controlled room at 4 °C. An aliquot of wine (10 mL) was added to a 20 mL amber glass headspace vial containing 2 g of NaCl and a 3 × 8 magnetic stir bar. Internal standard solution (25 μL) was added to give known final concentrations of EMS (approximately 50 μg/L) and PrSAc (approximately 125 μg/L). Acetaldehyde (4 μL) was added to each white wine sample vial. The vial was tightly sealed with a white PTFE/blue silicone lined screw cap (Grace Davison Discovery Sciences, Baulkham Hills, NSW, Australia). The sample vials were placed into a Gerstel peltier cooled sample tray (Lasersan) at 4 °C. The vial and its contents were heated to 45 °C for 30 min with stirring at 400 rpm. A Gerstel 1.0 mL HS syringe (Lasersan) was fitted with a custom-made dual gauge cone-tip needle (0.47 mm/0.63 mm, SGE, Ringwood, VIC, Australia), and the syringe heating block was held at 60 °C. A 100 μL static HS sample was injected into the cool-on-column (COC) inlet at 10 μL/s. The syringe was purged to atmosphere with nitrogen at 10.34 kPa (BOC grade 3.5) for 3 min after injection. The gas chromatograph was fitted with a 15 m × 0.25 mm FactorFour VFWAXms fused silica capillary column, 0.50 μm film thickness (Varian, Mulgrave, VIC, Australia), connected with a fused silica universal straight connector (Grace Davison Discovery Sciences) to a 60 m × 0.25 mm VICI ValcoBond VB-5 fused silica capillary column, 0.50 μm film thickness (Chromalytic Technology, Boronia, VIC, Australia), with a 2 m × 0.53 mm retention gap. Helium (Air Liquide ultrahigh purity), linear velocity = 37 cm/s, flow rate = 2.7 mL/min in constant flow mode, was used as the carrier gas. The initial oven temperature was held at 5 °C for 5 min, increased to 150 °C at 5 °C/min, and held at this temperature for 5 min. The COC inlet (Agilent G3440A) (pressurized to 252.69 kPa) was held at 30 °C for 10 min and ramped at the same rate as the oven. The oven and COC inlet were cryogenically cooled with liquid nitrogen. An Agilent 355 SCD sulfur chemiluminescence detector coupled to the GC was used with the default SCD parameters recommended by Agilent and sulfur trap gas purifiers on all gas lines (Agilent). The detector base temperature was held at 200 °C and the dual plasma controller at 800 °C. The reagent gases were air (Air Liquide instrument grade), 60.0 sccm; hydrogen (Air Liquide ultrahigh purity), 45.0 sccm; and ozone, generated in situ from air at 41.37 kPa. All compounds were identified by means of co-injection with pure reference compounds or comparison of their retention times with those of reference standards. At each time point of the bottle aging period, three bottles were analyzed, each in duplicate.

Statistical Analyses. Analysis of variance and LSD test were carried out using XLStat.

RESULTS

Evolution of Oxygen Content. The concentrations of dissolved oxygen (DO) inside the bottle were measured over the course of the whole study. The data are shown in Table 2.

Table 2. Concentration of DO (Milligrams per Liter)[a] during Bottle Maturation under Three Regimens of Oxygen Exposure

days	low O_2 exp	mid O_2 exp	std O_2 exp	days	low O_2 exp	mid O_2 exp	std O_2 exp
		SHZ1 No MOX				SHZ2 No MOX	
0	0.69	0.57	0.72	0	0.63	0.50	0.46
7	0.06	0.1	0.07	7	0.08	0.14	0.09
14	0.06	0.05	0.02	14	0.05	0.06	0.03
28	0.05	0.03	0.03	28	0.03	0.02	0.02
90	0.04	0.02	0.02	90	0.03	0.02	0.02
180	0.025	0.02	<0.02	180	0.04	0.02	0.03
360	<0.02	<0.02	<0.02	360	<0.02	<0.02	<0.02
		MOX				MOX	
0	0.47	0.50	0.3	0	0.33	0.36	0.31
7	0.14	0.14	0.2	7	0.10	0.09	0.1
14	0.03	0.02	0.02	14	0.04	0.06	0.01
28	0.05	0.03	0.02	28	0.05	0.04	0.02
90	0.04	0.03	0.02	90	0.02	0.03	0.02
180	0.04	0.02	0.02	180	0.05	0.03	0.02
360	<0.02	<0.02	<0.02	360	0.02	<0.02	<0.02

[a]Average of five replicates. Standard deviation was in all cases below 7%.

Differences due to MOX were observed in the first 7 days after bottling, with MOX wines generally showing lower percentage oxygen consumption (values between 69 and 73% of the initial oxygen content for SHZ1 and between 44 and 58% for SHZ2) compared to no MOX wines (values between 82 and 90% for SHZ1 and of 72−87% for SHZ2). After 14 days, >90% of the initial oxygen content was consumed for all wines. After this time period, further postbottling oxygen exposure did not affect DO values, indicating that in no case wines had exhausted their capacity to consume oxygen. Because of the ability of wine to continue to consume oxygen, dissolved oxygen measurements do not indicate the total amount of oxygen entering the system or the amount of oxygen consumed. TCO was therefore calculated, which allowed evaluation of the actual degree of oxygen consumption under the different conditions (Table 3). Differences were initially minor, which was expected as initial TCO is mainly linked to consumption of readily available oxygen present in the liquid phase (DO) and in the headspace, which was not different between the treatments. By 90 days of bottle storage, however, a difference of approximately 2 mg/L of oxygen consumed was already observed across the treatments. Bottles stored in nitrogen showed virtually no variations in oxygen consumption after the first 180 days in the bottle, whereas TCO increased in wines stored in air throughout the whole storage period. After 360 days of bottle storage, a maximum difference of approximately 5 mg/L of

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Table 3. Total Consumed Oxygen (Expressed in Milligrams per Liter of Wine[a]) during Bottle Maturation under Three Regimens of Oxygen Exposure

days	low O_2 exp	mid O_2 exp	std O_2 exp	days	low O_2 exp	mid O_2 exp	std O_2 exp
		SHZ1 No MOX				SHZ2 No MOX	
0	0.0	0.0	0.0	0	0.0	0.0	0
7	1.1	1.1	1.5	7	0.8	1.0	1.2
14	1.4	1.7	2.0	14	1.1	1.6	1.8
28	1.8	2.2	2.7	28	1.4	2.2	2.4
90	2.7	3.9	4.5	90	2.2	3.9	4.3
180	2.9	4.4	5.7	180	2.5	4.4	5.5
360	2.9	4.6	7.9	360	2.6	4.6	7.6
		MOX				MOX	
0	0.0	0.0	0.0	0	0.0	0.0	0.0
7	0.8	1.0	1.4	7	0.5	0.9	1.2
14	1.2	1.6	1.9	14	0.9	1.5	1.7
28	1.6	2.2	2.6	28	1.3	2.1	2.4
90	2.5	3.9	4.4	90	2.2	3.8	4.2
180	2.7	4.5	5.6	180	2.4	4.4	5.4
360	2.7	4.7	7.7	360	2.5	4.5	7.5

[a]Average of five replicates. Standard deviation was in all cases below 7%.

oxygen was observed. TCO was not affected by Shiraz wine type or MOX ($p > 0.05$).

SO_2 and Wine Phenolic Composition. Table 4 shows the results of SO_2 analyses of the wines after 1 year of bottle aging.

Table 4. SO_2 Values of the Wines at 360 Days and Loss of SO_2[a]

exp	free SO_2 (mg/L)	total SO_2 (mg/L)	free SO_2 loss (mg/L)	total SO_2 loss (mg/L)
SHZ1 MOX low O_2	16 ± 1a	36 ± 2a	15	30
SHZ1 MOX mid O_2	15 ± 1a	34 ± 3a	15	31
SHZ1 MOX std O_2	13 ± 1b	28 ± 1b	18	38
SHZ1 no MOX low O_2	15 ± 1a	34 ± 1a	14	30
SHZ1 no MOX mid O_2	15 ± 1a	32 ± 2a	16	34
SHZ1 no MOX std O_2	12 ± 1b	27 ± 2b	19	40
SHZ2 MOX low O_2	17 ± 0a	53 ± 3a	12	33
SHZ2 MOX mid O_2	16 ± 1.5a	51 ± 5ab	15	36
SHZ2 MOX std O_2	14 ± 1b	47 ± 2b	18	39
SHZ2 no MOX low O_2	17 ± 1a	55 ± 2a	14	32
SHZ2 no MOX mid O_2	15 ± 0b	52 ± 1b	16	36
SHZ2 no MOX std O_2	12 ± 2c	46 ± 2c	18	42

[a]Different letters within each wine treatment denote statistically significant differences at $p < 0.05$.

In all cases, SO_2 values within each wine were significantly different for all treatments, confirming that the regimens of postbottling oxygen exposure introduced differences in the overall oxidative state of the wine. In the case of SHZ1, MOX resulted in slightly lower SO_2 losses compared to no MOX. This difference was not observed in SHZ2.

Major parameters related to phenolic composition of the wines are given in Table 5. Differences due to either MOX or postbottling oxygen were generally small, and the main factor influencing phenolic composition was wine. In all wines, increased oxygen exposure, either by means of MOX or postbottling oxygen, resulted in higher color density, tannins, and total phenolics. Interestingly, percent nonbleachable pigments was generally higher with increasing oxygen exposure, and this effect was magnified by MOX. Consistent with this additive effect, in SHZ1 an influence of postbottling oxygen was observed only in combination with MOX.

VSCs. Table 6 show the results of the ANOVA carried out on the total VSCs data to assess the influence of individual variables, as well as of interactions among variables. Of the four variables contributing to the experimental design, wine type showed the highest degree of significance on all of the compounds analyzed. The time factor also had a strong influence, reflecting the fact that the concentration of VSCs changes during aging. The influence of postbottling oxygen exposure was significant only for the two thiol compound H_2S and MeSH, whereas in no case did MOX alone show a significant influence on any of the VSCs measured. Interactions were in most cases significant for H_2S and MeSH, whereas other compounds varied.

Figures 2 and 3 and Table 7 show the evolution of different VSCs during bottle aging of the two Shiraz wines not submitted to MOX. TCO us also shown (Figure 2a). In the case of H_2S (Figure 2b), accumulation was observed in the first part of the bottling period for SHZ1, followed by a decline later. In the case of SHZ2, H_2S decreased in the first 90 days of storage, to increase later. The degree of oxygen exposure during bottle maturation also affected H_2S, with wines from minimum exposure always showing higher concentrations of H_2S. Over the whole period studied, SHZ1 wines showed a net increase in H_2S for both low O_2 exp and mid O_2 exp samples. In the case of SHZ2, H_2S showed a tendency to decrease in the early stages of bottle maturation, particularly in wines with mid O_2 exp and std O_2 exp. Across the whole period studied, this wine showed a net gain in H_2S only when bottles were stored under low O_2 exp. At the end of the 12 months, SHZ1 and SHZ2 had similar concentrations of H_2S.

The evolution of MeSH and DMDS is shown in Figure 2c, whereas data for MeSAc are shown in Figure 3. Although the trends were wine-dependent, the two wines studied had a propensity to accumulate MeSH during aging (time × wine interaction significant at $p < 0.001$). This trend was much stronger in the case of SHZ2, for which increases up to 3-fold were observed, compared to the 1.5-fold maximum increase of SHZ1. In SHZ1, oxygen exposure in the bottle had no statistically significant effect on MeSH; conversely, in SHZ2, oxygen exposure in the bottle had a clear influence on the final concentration of MeSH, with std O_2 exp generally resulting in lower concentrations of MeSH. Differences between low O_2 exp and mid O_2 exp were generally smaller and not in all cases significant, although in most cases the highest concentrations of MeSH were observed in wines with the lowest oxygen exposure. Only minor variations were observed in the concentrations of DMDS, which showed no obvious correlation with the evolution of MeSH.

MeSAc generally decreased in the first 180 days of bottle storage in all wines. Following this period, accumulation of MeSAc was observed in SHZ2 when stored under minimum oxygen exposure. Finally, for both wines a steady increase in DMS concentration was observed (Table 7), with SHZ1 showing higher concentration of DMS than SHZ2 at any time point. The evolution of DMS was not affected by oxygen exposure during bottle maturation.

An effect of MOX on VSCs was observed only in SHZ1 and was restricted to H_2S and MeSH (data for other compounds

Table 5. Phenolic Parameters of the Wines after 360 Days of Bottle Storage

exp	color density	hue	chemical age 1	chemical age 2	anthocyanins (mg/L)	nonbleachable pigment (au[a])	% nonbleachable pigment	total phenolics (au)	tannin (g/L)
SHZ1 MOX low O_2	14 ± 0.2	0.74 ± 0	0.58 ± 0	0.27 ± 0	204 ± 5	4.9	26.68 ± 0.1	61 ± 1	1.8 ± 0
SHZ1 MOX mid O_2	13.9 ± 0.3	0.74 ± 0	0.59 ± 0	0.27 ± 0	208 ± 4	5.01	26.64 ± 0.3	63 ± 1	1.89 ± 0
SHZ1 MOX std O_2	14.7 ± 0.2	0.75 ± 0	0.59 ± 0	0.28 ± 0	197 ± 5	5.08	27.64 ± 0.1	61 ± 1	1.84 ± 0
SHZ1 no MOX low O_2	13.6 ± 0.5	0.74 ± 0.1	0.58 ± 0	0.28 ± 0	185 ± 3	4.81	27.83 ± 0.7	56 ± 0	1.59 ± 0.5
SHZ1 no MOX mid O_2	13.4 ± 0.3	0.74 ± 0	0.58 ± 0	0.27 ± 0.1	196 ± 6	4.82	27.05 ± 0.6	59 ± 1	1.69 ± 0.3
SHZ1 no MOX std O_2	14 ± 0.3	0.75 ± 0	0.58 ± 0	0.28 ± 0	191 ± 1	4.89	27.61 ± 0.7	58 ± 1	1.69 ± 0.7
SHZ2 MOX low O_2	7.4 ± 0.2	0.74 ± 0	0.46 ± 0	0.16 ± 0	185 ± 1	2.05	16.18 ± 0.7	40 ± 0	0.77 ± 0.7
SHZ2 MOX mid O_2	7.3 ± 0.2	0.74 ± 0	0.46 ± 0	0.17 ± 0	170 ± 2	2.14	17.19 ± 0.7	39 ± 0	0.76 ± 0.2
SHZ2 MOX std O_2	7.6 ± 0.4	0.75 ± 0.1	0.47 ± 0	0.18 ± 0	170 ± 3	2.17	17.92 ± 0.4	38 ± 1	0.74 ± 0.2
SHZ2 no MOX low O_2	7.2 ± 0.2	0.73 ± 0	0.45 ± 0.1	0.16 ± 0	178 ± 2	1.98	16.24 ± 0.6	37 ± 0	0.67 ± 0.2
SHZ2 no MOX mid O_2	7.2 ± 0.3	0.73 ± 0.1	0.45 ± 0	0.17 ± 0	170 ± 2	2.01	16.51 ± 1.3	37 ± 0	0.67 ± 0.3
SHZ2 no MOX std O_2	7.4 ± 0.2	0.74 ± 0	0.46 ± 0	0.18 ± 0	170 ± 4	2.11	17.52 ± 1.2	38 ± 1	0.69 ± 0

[a]Absorbance units.

Table 6. *F* Values and Significance[a] of Different Variables for VSCs during the Course of the Study

	H_2S	MeSH	DMDS	MeSAc	DMS
MOX	1.28 ns	0.89 ns	0.17 ns	2.95 ns	0.36 ns
postbottling O_2	7.82***	13.87****	1.01 ns	2.59 ns	0.21 ns
wine	30.20****	109.13****	2.35 ns	16.97****	1583.53****
time	5.35 **	67.49****	2.44 ns	20.63****	149.61****
MOX × postbottling O_2	5.41***	16.92****	0.76 ns	2.18 ns	2.23 ns
MOX × wine	31.74****	171.28****	0.92 ns	8.55***	6241.83****
MOX × time	5.93***	100.88****	1.01 ns	10.29****	590.92****
postbottling O_2 × wine	0.92 ns	23.32****	0.39 ns	0.68 ns	1.05 ns
postbottling O_2 × time	2.94*	12.05****	1.32 ns	1.2 ns	1.72 ns
wine × time	7.79***	35.04***	1.45 ns	0.60 ns	344.73****

[a]*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ns, not significant.

and SHZ2 not shown, as effect of MOX was not significant). Figure 4 shows the differences in H_2S and MeSH concentrations during bottle maturation of SHZ1. Surprisingly, MOX resulted in a generalized increase in the concentrations of these two compounds, which was rather clear in the case of MeSH after 1 year in the bottle. An effect of postbottling oxygen was also observed, with differences compared to no MOX wines being generally larger in low O_2 exp and mid O_2 exp wines.

DISCUSSION

The goal of this study was to investigate, during bottle maturation, the relationship between different forms of controlled oxygen exposure of red wine and evolution of VSCs responsible for reductive off-odors in wine. Anecdotal evidence indicates that, although the occurrence of reduction is common across the wine industry, wines vary to a large extent in their ability to develop reduction during bottle maturation. The factors responsible for this variability have not been understood and might depend on the existence of specific precursors able to directly generate VSCs. Moreover, the composition of the wine matrix can play a key role in the accumulation of VSCs during wine maturation.[9] From this point of view, the case of red wines appears to be rather complex, due to the presence of high concentrations of phenolic compounds having very high reactivity toward both oxygen and the VSCs having a thiol group.

The experimental design used here allowed us to create, on two Shiraz wines, two stages of controlled oxygenation, taking place respectively before or after bottling. Data on TCO, SO_2 consumption, and phenolic composition during MOX and after bottle maturation confirmed that the conditions of oxygen exposure adopted resulted in changes to wine composition. With regard to wine VSCs, the results of the ANOVA (Table 6) indicated that, of the four variables contributing to the experimental design, wine type had overall the highest degree of significance. This observation supports the view that wines differ in their patterns of VSCs evolution during aging. The time factor also had a strong influence, consistent with the fact that the concentration of VSCs changes during aging (Figures 2 and 3). Although this has been long known for DMS, very few data are available for lower molecular weight VSCs such as H_2S,

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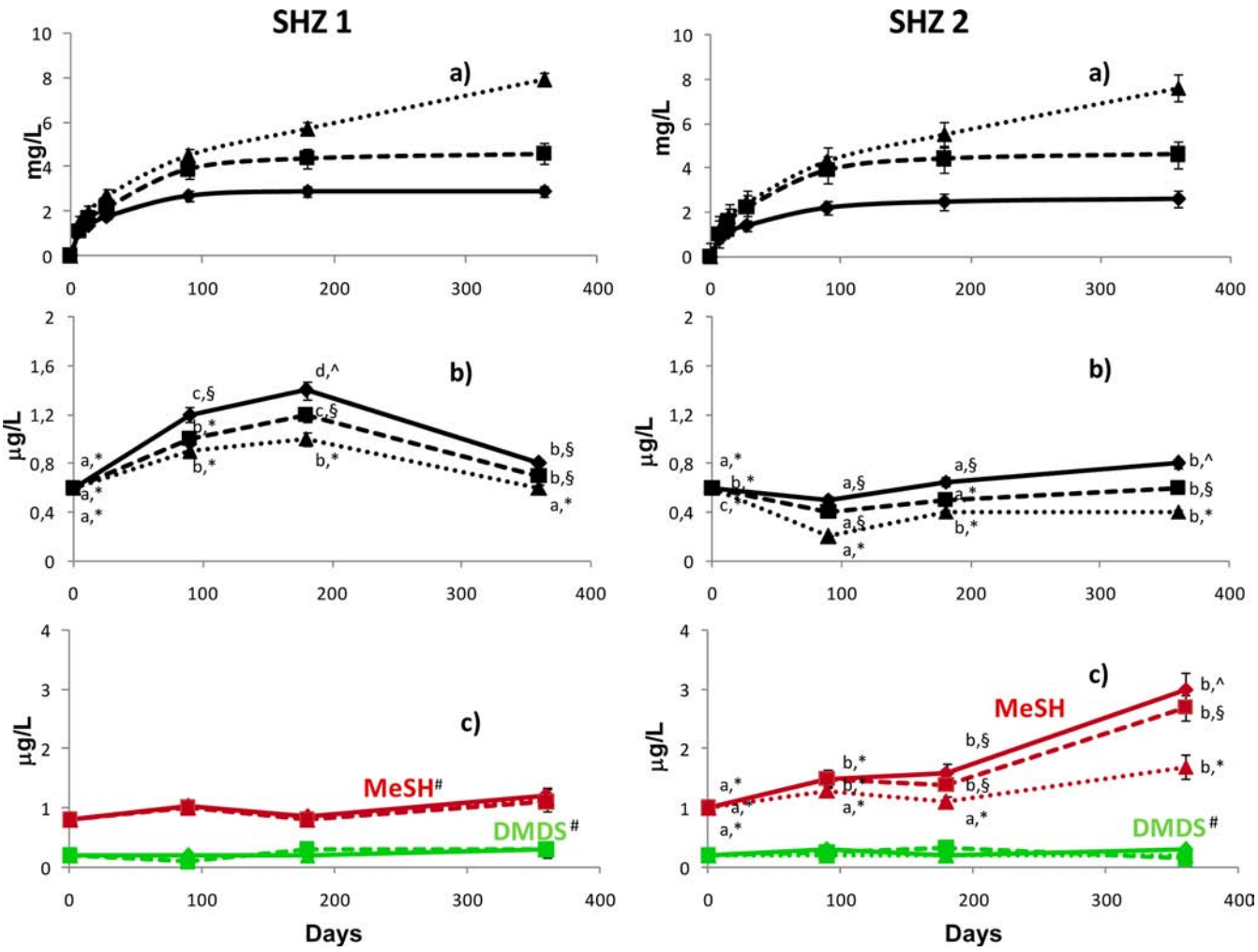

Figure 2. Evolution of (a) TCO, (b) H_2S, and (c) MeSH and DMDS during bottle maturation of no MOX wines under three regimens of oxygen exposure (◆, low O_2 exp; ■, mid O_2 exp; ▲, std O_2 exp). Within a series, different letters denote statistically significant differences ($p < 0.05$); at each time point, different symbols denote statistically significant differences ($p < 0,05$). # denotes no statistically significant difference within the series and across time points.

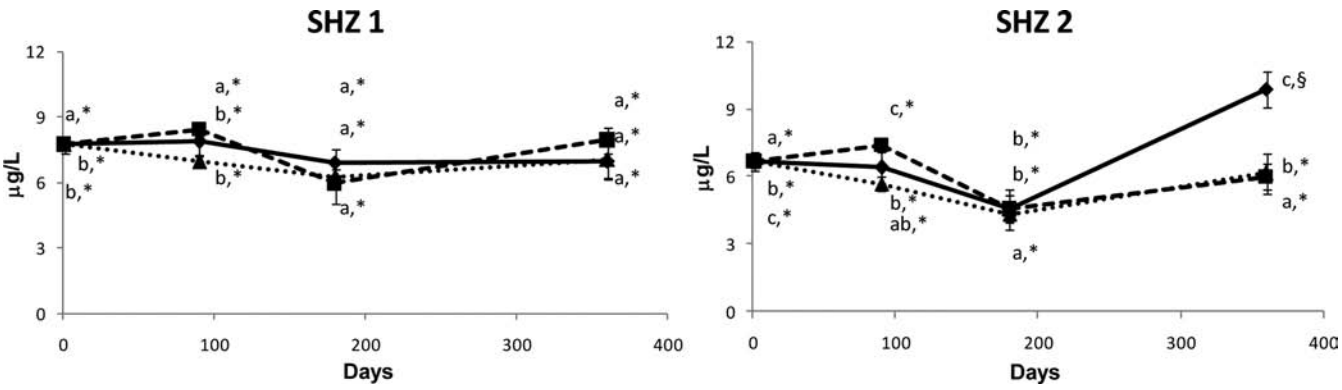

Figure 3. Evolution of MeSAc during bottle maturation of no MOX wines under three regimens of oxygen exposure (◆, low O_2 exp; ■, mid O_2 exp; ▲, std O_2 exp). Within a series, different letters denote statistically significant differences ($p < 0.05$); at each time point, different symbols denote statistically significant differences ($p < 0.05$). # denotes no statistically significant difference within the series and across time points.

MeSH, and MeSAc, which are commonly considered to be potential contributors to reductive odors.[7] Particularly, the data reported herein indicate that, in the bottle, H_2S and MeSH can accumulate to reach concentrations exceeding their odor thresholds (1.1 and 1.8 µg/L, respectively[17,18]) after a certain period of storage. For these two compounds, postbottling oxygen was found to have a highly significant influence (Table 6). Generally speaking, lowering exposure to oxygen during bottle maturation increased the wine content of these two compounds in all cases. Although this has been previously shown for white wines,[6,8,9] to our knowledge this is the first

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Table 7. Concentration of DMS (Micrograms per Liter) in Shiraz Wines without MOX during Bottle Maturation under Three Regimens of Oxygen Exposure[a]

days	low O_2 exp	mid O_2 exp	std O_2 exp
	SHZ1		
0	22.5 ± 1a	22.7 ± 1a	22.6 ± 1a
90	27.5 ± 1b	26.5 ± 2b	27.5 ± 1b
180	36 ± 2c	35 ± 2c	35 ± 3c
360	48 ± 3d	47 ± 1d	45 ± 3d
	SHZ2		
0	7.7 ± 1a	7.9 ± 1a	8.4 ± 2a
90	9.7 ± 2a	9.3 ± 0a	9.7 ± 1a
180	12. ± 0b	12.5 ± 1b	12.8 ± 0b
360	15.7 ± 1c	15.2 ± 2c	14.2 ± 2c

[a]Within each series, different values denote statistically significant differences between time points ($p < 0.05$). Within each wine, differences between treatments at each time point were never statistically significant.

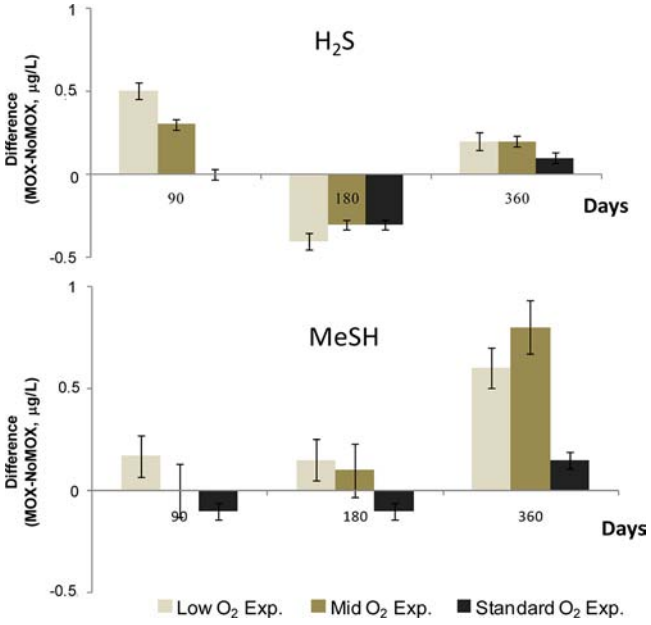

Figure 4. Effect of MOX on the evolution of H_2S and MeSH in SHZ1 wines.

time that a clear link between oxygen exposure in the bottle and wine VSCs is established for red wines.

In addition, the data presented here allow some observation regarding the relationship between key odor-active thiols such as H_2S and MeSH and other potentially related VSCs. For example, the conversion of disulfides to mercaptans under conditions of low oxygen availability has been often proposed as being responsible for mercaptan development.[19,20] However, in the wines studied here, the levels of DMDS measured were too low to account for the MeSH formed, and even under regimens of low O_2 exp, the concentration of DMDS did not decrease (postbottling oxygen not significant). Moreover, lower concentrations of MeSH were observed with increasing oxygen exposure, but reduced levels of MeSH did not result in an increase of DMDS. These results contradict the hypothesis that there is a direct equilibrium between MeSH and DMDS in wine, as suggested previously.[20] Of course, we cannot rule out the possibility that other disulfides, which were not detected with the method used here, could be involved. However, in the wine environment, it is likely that oxygen affects thiol compounds indirectly, by oxidizing wine polyphenols to form electrophiles such as quinones and procyanidin carbocations, which can in turn react with −SH groups.[21,22]

It is reasonable to assume that the ability of a wine to generate compounds such as MeSH and H_2S would therefore be determined by the precursor conversion rate but that their actual concentration at any given time during wine bottle maturation would also depend on their reactivity with the surrounding environment. In this sense, the patterns observed herein for H_2S appear quite interesting, as the two wines show opposing trends in the first half of the bottle maturation period. The marked increase observed in SHZ1 suggests that in this wine the rate of formation of H_2S was higher than the ability of the wine to consume this compound. The opposite was observed in the case of SHZ2, where H_2S initially decreased, to increase later. It was interesting to observe that H_2S showed a stronger response to TCO variations early during bottle maturation, when variations in TCO were relatively small. Conversely, later in bottle maturation, variations in H_2S across treatments were generally smaller, despite the broader TCO range applied. These patterns might reflect complex interactions between oxygen, H_2S, and other wine constituents (for example, wine phenolics), which will be discussed more in detail in the section on the effects of MOX. Interestingly, ethyl mercaptan was never detected in any of the wines, although it has been proposed that reaction of H_2S with ethanol or acetaldehyde could lead to the formation of this compound.[23]

MeSAc concentrations were found to decrease in the first half of the study, possibly due to hydrolysis of this ester at wine pH (Figure 3). However, this decrease did not translate directly into an increase in MeSH. In addition, opposite to MeSH, MeSAc was not affected by postbottling oxygen (Table 6), possibly suggesting that the link between these two compounds is less direct than previously proposed.[16] Interestingly, in SHZ2 wines from low O_2 exp, MeSAc largely increased in the second part of the bottle maturation period. The concomitant increase of MeSH in this wine could reflect esterification rather than hydrolysis. Overall, these data indicate that compounds such as DMDS and MeSAc were not major precursors to MeSH in these wines. Precursors to MeSH could include amino acids,[24] adducts resulting from addition of −SH groups to carbonyls, and agrochemicals.[23,25]

Consistent with previous findings,[26] DMS was found to increase during bottle maturation, although there was no effect of postbottling oxygen exposure. In the case of Champagne wines, DMS was found to accumulate more rapidly in wines bottled with low OTR closures, which was ascribed to the reduction of dimethyl sulfoxide.[27] Comparison of our results with those of that study are, however, difficult, due to the fact that in champagne bottles yeast lees were present, possibly resulting in high levels of enzymatic activities, including dimethyl sulfoxide reductase. Silva Ferreira et al.[28] showed that, in Port wines, addition of oxygen increased DMS formation during accelerated aging, although the concentrations of oxygen used were much higher than in the present study. Conversely, Fedrizzi et al.[29] reported that DMS can decrease under conditions of accelerated aging (30 °C with high oxygen availability). In a recent survey, wines bottled under screw cap were found to have higher concentrations of DMS compared to wines bottled with natural cork.[30] Our findings indicate that closure OTR should not affect DMS

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Table 8. TCO and VSCs Differences of Different Wine Pairs

	H_2S										
	TCO difference (mg/L)			H_2S min−max (μg/L)			significance				
	3 months	6 months	12 months	3 months	6 months	12 months	3 months	6 months	12 months		av[a]
std O_2 exp vs low O_2 exp	1.9 ± 0.2	2.9 ± 0.1	5 ± 0	0.2−1.7	0.4−1.4	0.4−1	****	ns	ns	std O_2 exp	0.6a
std O_2 exp vs mid O_2exp	0.5 ± 0.1	1.2 ± 0.2	3.1 ± 0.1	0.2−1	0.5−1.2	0.4−0.9	ns	ns	ns	mid O_2 exp	0.7a
mid O_2 exp vs low O_2 exp	1.5 ± 0.2	1.7 ± 0.2	1.9 ± 0.15	0.4−1.2	0.6−0.8	0.6−1	ns	ns	ns	low O_2 exp	0.9b
	MeSH										
	TCO difference (mg/L)			MeSH min−max (μg/L)			significance				
	3 months	6 months	12 months	3 months	6 months	12 months	3 months	6 months	12 months		av[a]
std O_2 exp vs low O_2 exp	1.9 ± 0.2	2.9 ± 0.1	5 ± 0	0.9−1.5	0.8−1.6	1 0.1−3	ns	***	****	std O_2 exp	1.2a
std O_2 exp vs mid O_2 exp	0.5 ± 0.1	1.2 ± 0.2	3.1 ± 0.1	0.7−1.4	0.8−1.2	1.1−2.7	ns	ns	****	mid O_2 exp	1.4a
nid O_2 exp vs low O_2 exp	1.5 ± 0.2	1.7 ± 0.2	1.9 ± 0.15	0.9−1.5	0.8−1.6	1.2−3	ns	ns	ns	low O_2 exp	1.6b

[a]Calculated of H_2S or MeSH data collected at all time points

development during maturation, although different closures can influence wine DMS content through scalping. A recent study has shown that DMS and MeSH act synergistically to support reduction odors in red wine. Therefore, although not directly affected by oxygen, DMS can still contribute to the higher reductive characters often observed in response to low oxygen exposure in the bottle, by supporting the contribution of MeSH.

Of the different variables tested in this study, MOX was the least effective on the evolution of VSCs (Table 6). Under the conditions of this study, during MOX the wines received a total of 3.26 mg/L of oxygen. Although this amount was in the range of TCO values that were able to affect VSCs during aging, all data collected indicated that MOX had a smaller influence on wine VSCs compared to postbottling oxygen exposure. Obviously, comparisons between MOX regimens and TCO have to be made cautiously, because whereas TCO provided a precise calculation of the amount of oxygen consumed, during MOX we were not able to evaluate whether all of the oxygen provided was consumed by the wine. With MOX, however, oxygen is not provided when compounds such as MeSH and H_2S are effectively accumulating; hence, a lower impact of this technique on their concentration is plausible. Indeed, MOX influence was observed only for SHZ1, where it resulted in increased MeSH and, to a lesser extent, also increased H_2S (Figure 4). This was somewhat surprising, as MOX is thought to decrease the concentration of reductive thiols, presumably by promoting their oxidation. However, the actual influence of MOX on perceived reductive characters in wine has not been demonstrated,[2] and the decreases in VSCs associated with MOX have been shown to be generally small.[31,32] One aspect that needs to be considered is that other studies on MOX analyzed only samples at the end of the MOX treatment, whereas here we observed the effects of MOX on the evolution of thiols during wine bottle aging. Because compounds such as H_2S and MeSH increase with time, it is likely that MOX can affect their evolution in the bottle by affecting major wine components that can in turn interact with VSCs, for example, phenolics.

One of the aims of this study was to provide a first assessment of the degrees of oxygen exposure that allow effective control of VSCs in red wine. Kwiatkowski et al.[33] observed lower reduced odors in Cabernet Sauvignon wines bottled under screw caps with increasing headspace volumes, although specific oxygen measures were not carried out. The experimental design used here allowed creation of different profiles of oxygen exposure during aging, resulting in different profiles of TCO by the wines. TCO values were then evaluated in relation to the evolution of VSCs. Comparison of the different degrees of postbottling oxygen exposure for H_2S and MeSH levels at each time point is summarized in Table 8, along with the corresponding differences in TCO. Although the number of wines studied is limited, some observations can be made.

In the case of H_2S, the effects of different degrees of oxygen exposure were significant only at 3 months of bottle storage, with increasing concentration of H_2S obtained with decreasing oxygen exposure. After this point, changes in H_2S were less affected by oxygen, although a negative correlation between oxygen exposure and H_2S concentration could still be observed (Figure 2). By comparison of this pattern with the corresponding TCO values, it can be seen that a TCO increase of at least approximately 2 mg/L (std O_2 exp vs low O_2 exp) was needed to influence H_2S concentration, although such an effect was observed only in the first 3 months. The reasons for this complex behavior require further investigation. Nevertheless, it can be postulated that, in the case of SHZ1 with no MOX, a higher degree of oxygen exposure in the first part of the bottling period, for example, by means of a more oxygen permeable closure or tailored oxygen exposure at bottling, would be needed to achieve effective control of H_2S accumulation. Nevertheless, for this wine, the data obtained from MOX indicate that relatively large additions of oxygen over a short period of time can create conditions favoring accumulations of H_2S and MeSH during bottle maturation. The behavior of SHZ2 was overall very different, highlighting once again the importance of matching wine composition and oxygen management strategies. This wine was mainly characterized by accumulation of MeSH, and the minimum

TCO value resulting in a significant effect on MeSH was 2.9 mg/L (std O_2 exp vs low O_2 exp at 6 months in SHZ2), which was achieved after 6 months of bottle storage. When the difference in TPO reached 5 mg/L, a further decrease in MeSH accumulation was observed in SHZ2 (Figure 2), which was statistically significant (Table 8). Therefore, from the point of view of limiting accumulation of reductive compounds, in the case of wines such as SHZ2 it would be ideal to allow sufficient oxygen ingress in the later stages of bottle maturation, given that MeSH appeared to develop mainly after 6 months in the bottle. Obviously, from the point of view of the global quality of wine, excessive degrees of oxygen exposure need to be considered also from the point of view of the risk of inducing wine oxidation.

In conclusion, this study provided for the first time an accurate description of the evolution of different VSCs during the aging of red wines exposed to different regimens of oxygen. Of the compounds measured, H_2S and MeSH were the most responsive to oxygen exposure. During bottle maturation, wines showed a tendency to accumulate H_2S and MeSH, which was generally greater under conditions of low oxygen exposure. The profile of H_2S accumulation was strongly wine dependent, with one of the two wines reaching above-threshold concentrations after 3−6 months, followed by a decline. MeSH increased throughout the whole storage period, with maximum values observed after 1 year of bottle maturation. At each time point, the difference in TCO needed to obtain a significant decrease in the concentration of MeSH or H_2S was assessed. In the case of H_2S, an increase in TCO of approximately 2 mg/L over 3 months was needed to achieve a significant decrease in the phase of maximum accumulation. For MeSH, a minimum increase in TCO of 3 mg/L over at least 6 months was needed, although a TCO increase of approximately 5 mg/L over 1 year gave a larger decrease. MOX had only a limited effect on VSCs, contributing to an increase in the accumulation of MeSH and H_2S in one of the two wines studied, possibly due to the influence of MOX on wine phenolic composition. Comparison between the development patterns of MeSH and DMDS showed no obvious correlation between these compounds, suggesting that, in the wines studied, DMDS did not act as a precursor to MeSH.

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Notes

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