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Evolution of 3-Mercaptohexanol, Hydrogen Sulfide, and Methyl Mercaptan during Bottle Storage of Sauvignon blanc Wines. Effect of Glutathione, Copper, Oxygen Exposure, and Closure-Derived Oxygen

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ABSTRACT: The effects of wine composition and postbottling oxygen exposure on 3-mercaptohexanol (3-MH), hydrogen sulfide (H_2S), and methyl mercaptan (MeSH) were investigated. A Sauvignon blanc wine with initial copper concentration of 0.1 mg/L was treated with copper sulfate and/or glutathione (GSH) prior to bottling to give final concentrations of 0.3 and 20 mg/L, respectively. The wines were bottled with a synthetic closure previously stored in either ambient air or nitrogen to study the effect of the oxygen normally present in the closure. Bottled wines were stored for 6 months in either air or nitrogen to study the effect of oxygen ingress through the closure. Copper addition resulted in a rapid initial decrease in 3-MH. During storage, a further decrease of 3-MH was observed, which was lower with GSH addition and lowered oxygen exposure. H_2S accumulated largely during the second 3 months of bottle storage, with the highest concentrations attained in the wines treated with GSH and copper. Lower oxygen from and through the closure promoted H_2S accumulation. The concentration of MeSH was virtually not affected by the experimental variables at 6 months, although differences were observed after 3 months of storage. The implications for wine quality are discussed.

KEYWORDS: sulfur compounds, wine, glutathione, copper, oxygen, closures

INTRODUCTION

Bottle aging is an essential component of winemaking. During its storage in the bottle, wine undergoes complex chemical changes that can affect color and aroma composition, mouthfeel, and overall perceived quality.[1] In several wine countries the regulation system imposes minimum periods of bottle aging for wines from various areas having specific regional denominations. Oxygen exposure, in the form of oxygen diffusing through the closure, is a major driver of the chemical changes occurring in wine aroma composition during bottle aging.[1] Depending on the degree of oxygen ingress through the closure, determined by closure specific oxygen transfer rate (OTR), wines can improve their aroma characteristics, as well as develop aroma defects.[1,2] Among these, the so-called "reduced" off-flavor, an aroma character often described as rotten egg, sewage, or struck flint, has been shown to negatively affect consumer acceptance.[3]

From a chemical point of view, development of reduced off-flavors during bottle aging, particularly in conjuction with the use of low OTR closures such as screw caps, appears to be linked to the accumulation of certain low molecular weight sulfur compounds such as hydrogen sulfide (H_2S) and methyl mercaptan (MeSH), characterized by odors of rotten egg, sewage, and rubber.[3,4] However, the link between use of low OTR closures and occurrence of reduced aromas is not systematic, and it has been suggested that certain wines have an intrinsic susceptibility to develop reduced off-flavors.[1,5] Moreover, although OTR appears to be a key parameter determining closure effect on wine composition, other aspects contribute to differences between types of closures. For example, the ability of different closures to adsorb specific aroma compounds[4,6] and the differences in bottle headspace volume and composition associated with different closures[7] could also play important roles. In addition, closures such as natural cork and synthetic closures are made of porous materials, and therefore they contain air and thus oxygen. Upon insertion of the closure in the bottle, part of this oxygen is released inside the bottle.[8,9] The actual contribution of this source of oxygen to the compositional changes occurring during bottle storage has not been described.

In addition to sulfides involved in reductive off-odors, the sulfur compounds often referred to as polyfunctional thiols have also been indicated as primary contributors to the aroma of many red and white wines, in particular, Sauvignon blanc.[10] 3-Mercaptohexanol (3-MH) is the most abundant polyfunctional thiol in wine, contributing to the tropical fruit aromas of Sauvignon blanc wines.[10,11] During aging, significant losses of 3-MH normally occur, particularly during the first year of storage, which can result in loss of freshness and typical tropical fruit characters.[4,12−15] However, the influence of other winemaking variables on the evolution of this powerful aroma compound during aging remains to be established. In particular, the importance of prebottling copper addition has been previously highlighted.[2] Although divalent copper is commonly added to wines to remove the low molecular weight sulfur compounds

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responsible for reduced off-flavors, copper can also bind polyfunctional thiols, including 3-MH, with potentially negative consequences on wine aroma.[2,16] Increases in the average concentration of copper in wines have been recently reported, probably due to the common practice of adding copper sulfate immediately prior to bottling to prevent formation of reduced off-flavor when low OTR closures are used.[5]

Addition of antioxidants prior to bottling is another widespread practice aimed at preserving wine against oxidative degradation. In view of the general interest in lowering the levels of SO_2 in the food industry, addition of the sulfur-containing tripeptide glutathione (GSH) to reduce SO_2 doses in wine has been suggested, as GSH exhibited increased protection toward important aroma compounds such as esters and monoterpenes compared to SO_2.[17]

In this study, we have investigated the effects of copper and GSH addition prior to bottling on the evolution of the key aroma compounds 3-MH, H_2S, and MeSH in a commercial Sauvignon blanc wine stored under different regimens of oxygen exposure. Different degrees of oxygen exposure were obtained by storing wine bottles in different atmospheres, eliminating the side effects linked to differences in closure properties (e.g., absorption of wine compounds), closure gas content, and bottle headspace volume. Synthetic closures were selected due to their consistent OTR across replicate closures.[4] In addition, by comparing the behavior of wines sealed under closures stored in air with that of wines sealed under nitrogen-conditioned closures, an assessment of the importance of closure-derived oxygen to wine aroma development was carried out for the first time.

MATERIALS AND METHODS

Chemicals. Reference standards for H_2S and MeSH ware prepared from their sodium salts, sodium hydrosulfide hydrate ($NaSH_3 \cdot xH2O$) and sodium thiomethoxide (NaSMe), respectively, which were obtained from Sigma-Aldrich (Caste Hill, NSW, Australia). The salts were dissolved in cold water (4 °C) and used immediately. 3-MH and d_{10}-3-MH were synthesized as previously described.[18]

Wines and Bottling. Sauvignon blanc wine from the 2008 vintage produced in the Adelaide Hills region was obtained from a local winery. Analytical parameters of the wine were as follows: pH 3.4, 3.4 g/L residual sugars, 13.9% (v/v) alcohol, 0.42 g/L volatile acidity (as acetic acid), 5.6 g/L titratable acidity (as tartaric acid), 41 mg/L free SO_2, 180 mg/L total SO_2, 0.1 mg/L copper, GSH < 1 mg/L. Before bottling, the wines received an addition of either no or 20 mg/L of food grade GSH (Kirkman, Lake Oswego, OR) and/or no addition of copper sulfate or an addition of copper sulfate to result in a final concentration of 0.3 mg/L of copper. This protocol gave a total of four matrix-related combinations, coded as follows: high GSH, low Cu (GSH 20 mg/L; Cu 0.1 mg/L); high GSH, high Cu (GSH 20 mg/L; Cu 0.3 mg/L); low GSH, low Cu (GSH < 1 mg/L; Cu 0.1 mg/L); and low GSH, high Cu (GSH < 1 mg/L; Cu 0.3 mg/L).

All wines were bottled under Nomacorc Premium coextruded synthetic closures (Nomacorc, Zebulon, NC). Before bottling, closures were stored at 20 °C for 1 week either in air or under nitrogen to evaluate the effects of oxygen contained in the closure on wine development. Once bottled, the wines were stored at 20 °C either in air or under nitrogen to study the effect of oxygen exposure. 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 oxygen content below 10 hPa. In total, three different closure/storage combinations were applied to all of the wines: closures stored in air and wines stored in air (A/A); closures stored in air and wines stored in nitrogen (A/N); and closures stored in nitrogen and wines stored in nitrogen (N/N). A total of 12 experimental treatments were generated (four matrix-related combinations × three closure/storage treatments). For the bottling of each wine, empty 375 mL flint glass bottles were flushed with 98% N_2 gas 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. A bottle fitted with two PreSens Pst3 oxygen sensors (Presens, Regensburg, Germany), to measure dissolved and headspace oxygen, was filled with wine and sealed after approximately every 10 bottles to monitor performance across the whole bottling operations: 5 PreSens bottles in total were filled for each wine and closure/storage combination. These same bottles were used to monitor dissolved oxygen 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. The limit of quantification of oxygen for this method was 0.02 mg/L. Generally, dissolved oxygen values, measured 24 h after bottling, were never higher than 1.12 mg/L and headspace oxygen was always below 0.95 mg/L. For each bottle, total oxygen pickup during bottling operations was between 1.32 and 1.95 mg/L.

Oxygen Ingress Measurement. A separate experiment was carried out to measure the amount of oxygen entering the bottles under the three different storage conditions of this study. For this purpose, bottles of the same type described above, fitted with PreSens Pst6 oxygen sensors for measurement of trace oxygen levels, were placed in a corking machine and flushed with a stream of 98% N_2 to obtain an oxygen pressure lower than 0.5 hPa. Once this oxygen level was achieved, the N_2 line was removed from the bottleneck, and the bottle was immediately sealed with Nomacorc Premium 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, as described above. Five replicates were used for each condition (A/A, A/N, N/N). Measures of oxygen pressure were taken every 24 h during the first week, then once a week for the following 4 weeks, and then at 3 and 6 months of storage. For each condition, the measures allowed quantification of the amount of oxygen released from the closure at bottling, as well as of the theoretical amount of oxygen entering through the closure.

Chemical Analyses. Wines were analyzed 24 h after bottling and then following 3 and 6 months of bottle storage. Wine primary chemistry analyses and CIELab measures were carried out as described by Skouroumounis et al.[19] GSH was measured as described by Du Toit.[20] 3-MH was quantified as pentafluorobenzyl derivative using a stable isotope dilution assay, by means of headspace SPME coupled with GC-MS. This analysis was carried out only 24 h after bottling and at 6 months. H_2S and MeSH were analyzed by GC coupled with atomic emission detection (AED) detection, using static headspace sampling and cool on-column injection.[21] In all cases identification of volatile compounds was carried out by mass spectra with comparison and co-injection with pure reference compounds. Triplicate samples were analyzed at each time point.

Statistical Analyses. Analysis of variance and LSD test were carried out using JMP 5.0.1 (SAS, Cary, NC). Principal component analysis (PCA) was carried out using Unscrambler 9.5 (CAMO, Oslo, Norway).

RESULTS AND DISCUSSION

Oxygen Ingress, Evolution of Dissolved Oxygen, and Wine Oxidation State. Figure 1 shows the data for oxygen ingress under the different experimental conditions. In the early stages of the experiment (days 1−50), oxygen levels inside the bottles increased quite rapidly for all treatments. In this period,

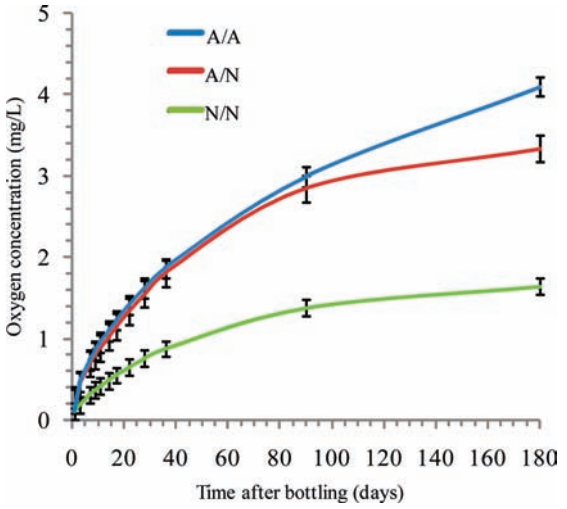

Figure 1. Effect of closure and bottle storage conditions on the ingress of oxygen into bottles filled with nitrogen over time.

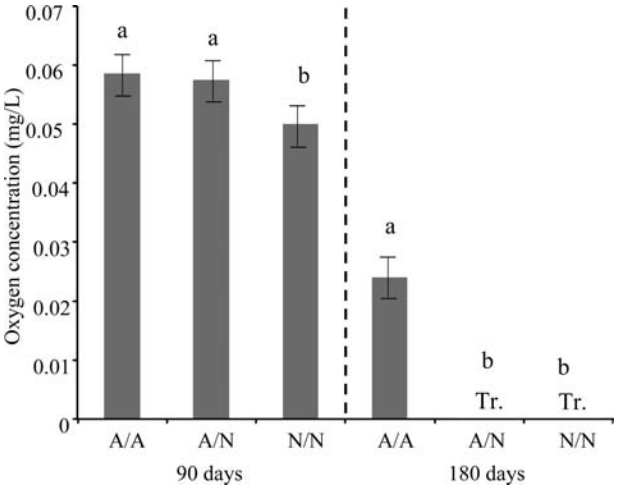

Figure 2. Effect of oxygen exposure on dissolved oxygen levels in wine at two time points. For each storage condition, data are averages of all wine treatments. The average value for each treatment was obtained from five different bottles (n = 20). Within each time point, different letters denote statistically significant values ($p < 0.01$).

samples sealed with closures previously equilibrated in nitrogen (N/N) consistently showed lower oxygen ingress, whereas no difference could be observed between samples sealed with closures equilibrated in air (A/N and A/A). It is noteworthy that an increase of oxygen could be observed in N/N bottles, indicating that 1 week of storage of the closures in N_2 did not result in complete removal of air from the closure. Nevertheless, after 50 days of storage, both A/N and A/A samples had about twice as much oxygen as the N/N treatment, clearly highlighting the contribution of oxygen migration from the closure to the total pool of oxygen present in the bottle at this stage. After about 100 days of storage, a difference in oxygen ingress became visible between the A/N and A/A treatments. Ambient oxygen needs a certain time to travel through the closure and enter the bottle as indicated by the fact that only after this point did the effect of oxygen transfer through the closure become detectable. From this point on, only the A/A samples showed further significant increase in oxygen ingress, indicating that this part of the curve represents the phase of oxygen ingress mainly associated with OTR. It is worth mentioning that, although oxygen migration from oxygen entrapped within the closures and oxygen ingress from the exterior through the closures (OTR) are linked primarily to one or the other of two distinct phases of oxygen ingress into bottles of wine, they are actually two aspects of the same technological properties of cylindrical closures. Both processes are indeed dependent on porosity, solubility of oxygen in the closure matrix, and migration coefficient of oxygen through the closure matrix. However, from merely a quantitative point of view, oxygen migrating from the closure into the bottle accounted for more than 50% of the total oxygen ingress over this time period, indicating this source of oxygen plays a key part in postbottling exposure.

Dissolved oxygen was monitored nondestructively during the course of the experiment by means of PreSens oxygen sensors applied to a set of bottles bottled and stored under the same conditions as those used for the other analytical measures. The data obtained after 3 and 6 months are shown in Figure 2 as average values for all wine matrix treatments. After 3 months of storage, differences in dissolved oxygen were small. However, further storage of the wines for an additional 3 months induced significant differences due to oxygen exposure, with samples stored under nitrogen consistently showing lower dissolved oxygen compared to samples stored in air. Dissolved oxygen during the first months postbottling is mainly linked to dissolved and headspace oxygen at bottling,[7] hence the small effect of oxygen exposure at 90 days.

Table 1. Concentration of Free SO_2 and Glutathione in Wines after 6 Months of Bottle Storage

	free SO_2[a] (mg/L)	GSH[a] (mg/L)
high GSH, high Cu A/A	20 c	3 d
high GSH, high Cu A/N	21 c	6 c
high GSH, high Cu N/N	26 a	8 b
high GSH, low Cu A/A	21 c	5 c
high GSH, low Cu A/N	23 b	8 b
high GSH, low Cu N/N	24 ab	10 a
low GSH, high Cu A/A	17 d	nd
low GSH, high Cu A/N	18 d	nd
low GSH, high Cu N/N	23 b	nd
low GSH, low Cu A/A	18 d	nd
low GSH, low Cu A/N	21 c	nd
low GSH, low Cu N/N	24 ab	nd

[a] Different letters denote statistically significant differences at $p < 0.01$. nd, not detected (samples did not receive GSH addition before bottling).

The data on SO_2 and GSH at 6 months are given in Table 1. Oxygen exposure was the variable accounting for the largest differences, with wines exposed to the lower level of oxygen, such as A/N and N/N treatments, always showing higher SO_2 and GSH concentrations and, therefore, a lower degree of oxidation. This observation is consistent with other studies in which a clear association between oxygen exposure and loss of antioxidants such as SO_2 or ascorbic acid has been observed.[4,19] Interestingly, no GSH dimer was detected in the GSH-treated samples at

Table 2. Concentration[a] of Sulfur-Containing Volatile Compounds in Sauvignon blanc Wines after 6 Months of Bottle Storage

treatment	3-MH (ng/L)	H_2S (μg/L)	MeSH (μg/L)
high GSH, high Cu A/A	507 ± 1	1.1 ± 0.2	0.5 ± 0.1
high GSH, high Cu A/N	556 ± 4	3.2 ± 0.4	0.6 ± 0.2
high GSH, high Cu N/N	676 ± 2	4.5 ± 0.4	0.7 ± 0.2
high GSH, low Cu A/A	602 ± 8	1.3 ± 0.4	0.7 ± 0.0
high GSH, low Cu A/N	663 ± 5	1.5 ± 0.1	0.6 ± 0.1
high GSH, low Cu N/N	721 ± 2	1.5 ± 0.4	0.6 ± 0.1
low GSH, high Cu A/A	241 ± 3	tr	0.3 ± 0.1
low GSH, high Cu A/N	260 ± 6	0.3 ± 0.1	0.3 ± 0.0
low GSH, high Cu N/N	341 ± 6	2.5 ± 0.0	0.4 ± 0.0
low GSH, low Cu A/A	511 ± 7	0.2 ± 0.2	0.5 ± 0.1
low GSH, low Cu A/N	568 ± 5	0.6 ± 0.2	0.6 ± 0.1
low GSH, low Cu N/N	665 ± 5	1.2 ± 0.0	0.5 ± 0.1

[a] Values are the average of three wines analyzed in duplicate. "tr" denotes value below the limit of quantification of 0.2 μg/L.

Table 3. *F* Values and Significance[a] of Different Variables for Volatile Sulfur Compounds after 6 Months of Storage

	3-MH	H_2S	MeSH
GSH	19.76***	9.72**	16.49***
copper	20.21***	5.15*	2.79 ns
oxygen exposure	2.45*	9.05***	2.43 ns
GSH × copper	66.42***	6.67***	5.73 ns
oxygen exposure × copper	6.07***	10.23***	3.12 ns
GSH × oxygen exposure	5.94***	7.1***	6.16*

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

6 months (data not shown). This suggests that, under our experimental conditions, loss of glutathione did not occur via dimerization. Most likely, with quinones forming to a higher extent under conditions of higher oxygen exposure, GSH was consumed in the reaction with quinones to form relatively stable adducts, for example, 2-*S*-glutathionyl-*trans*-caftaric acid.[22] The addition of copper caused a minor but significant decrease of final SO_2 concentrations. This observation seems in agreement with the pro-oxidant activity reported for Cu^{2+} in model wine solutions.[23]

Volatile Sulfur Compounds. The concentrations of the sulfur-containing compounds, 3-MH, H_2S, and MeSH, are reported in Table 2. 3-MH is a powerful aroma compound with an odor characteristic often described as passionfruit and is among the key odorants of Sauvignon blanc, with an odor threshold of 60 ng/L.[24] In this study, we found that all of the variables tested had the ability to significantly affect the concentration of 3-MH at 6 months (Table 3). GSH addition generally resulted in wines with increased 3-MH (significant at $p < 0.001$), in agreement with previous observations.[25] Conversely, increased copper concentration and oxygen exposure generally caused a decrease in 3-MH concentration. However, interactions between the three experimental variables studied were all significant, indicating that the chemistry of 3-MH during wine aging is complex and can be affected by multiple factors.

Analysis of the wines at bottling showed that copper addition had an immediate effect of lowering 3-MH concentration (Table 4). Therefore, to better explore the influence of individual factors as well as combinations on 3-MH evolution, net loss of 3-MH over 6 months of bottle storage was considered instead of the final 3-MH concentration (Figure 3). Addition of GSH at bottling strongly affected 3-MH losses during storage, with up to 260 ng/L less loss of 3-MH when GSH was added, indicating that antioxidant capacity of wine is a powerful modulator of 3-MH evolution during storage. Oxygen exposure also had a significant influence on 3-MH, with lower exposure resulting in lowered 3-MH losses. This effect was generally smaller than the one associated with GSH, with differences in 3-MH losses due to oxygen exposure being never larger than 170 ng/L. Other studies investigating the influence of oxygen exposure on 3-MH changes during bottle storage have concluded that increased oxygen exposure, for example, by means of the use of closure with higher OTRs, can be detrimental to 3-MH concentrations. This is due to the reactivity of 3-MH toward electrophiles generated under oxidative conditions, such as quinones.[13,23] However, at least under our experimental conditions, addition of GSH appeared to better preserve 3-MH than did a decrease in oxygen exposure, although a combination of both low oxygen exposure and GSH addition resulted in the lowest loss of 3-MH. This indicates that, whereas it is generally accepted that oxidation of aroma compounds during storage is due to too high an OTR,[4] wine composition plays a role that can be at least as important as OTR. Most likely, different wines require different OTRs to achieve optimal aroma development during aging.

In the present study the effects of GSH were studied by means of addition of a commercial food grade preparation of GSH to the wine prior to bottling, a practice that is not allowed by current wine regulation. Nevertheless, GSH is present in grapes and is produced by the yeast during fermentation,[20,26] and formulations based on inactivated yeasts, which can be used in the winery, also contain GSH [27] and are legally permitted additives to wine. Roussis et al.[17] have shown that GSH gave higher protection of wine aroma compounds compared to SO_2, increasing the stability of volatiles such as terpenes and esters.

One of the major features of the experimental design used in this study was the ability to isolate the contribution of the oxygen contained in the closure from the remaining oxygen content of the system, namely, the oxygen present in the wine and in the headspace at bottling plus the oxygen entering through the closure from the exterior environment. The A/A treatment represents the condition of maximum oxygen exposure, with the closures being filled with air and the bottles being stored in air. Conversely, in the N/N treatment the oxygen present in the closure has been largely replaced with nitrogen, and the bottles were stored in a virtually oxygen-free environment. Between these two extremes, for the A/N treatment, the same closures as A/A were used, but the storage conditions were the same as N/N. The data in Figure 3 clearly indicate that, within each matrix treatment, differences in 3-MH loss due to oxygen exposure were, in general, significant. In particular, the fact that the A/N wines were significantly different from the other two oxygen exposure levels highlights the fact that the amount of oxygen present in the closure represents an important component of the total oxygen pool, accounting for approximately half of the total 3-MH losses due to oxygen exposure. This is consistent with the data in Figure 1, which show that closure-derived oxygen is a major component of the total pool of oxygen entering in the first

Table 4. Differences in 3-MH Concentrations Due to Copper Treatment at Bottling and after 6 Months of Bottle Storage

	wines at bottling		wines at 6 months	
	concentration (ng/L)	difference[a] (ng/L)	concentration[b] (ng/L)	difference[a,b] (ng/L)
high GSH, high copper	843 ± 5	105	507−676	85−107
high GSH, low copper	948 ± 5		602−721	
low GSH, high copper	765 ± 4	232	240−340	271−325
low GSH, low copper	997 ± 2		510−665	

[a] Calculated as difference between high- and low-copper wines at each time point. [b] Range of values reflects different oxygen exposure treatments at 6 months.

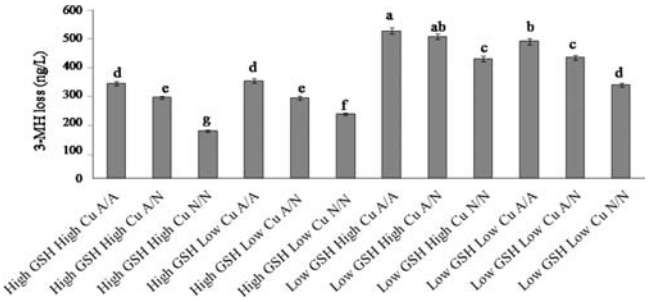

Figure 3. Effect of wine composition and oxygen exposure on the loss of 3-MH over 6 months of bottle storage. Different letters denote statistically significant differences at $p < 0.01$.

6 months. It is worth mentioning that a period of 6 months of storage between bottling and consumption is not uncommon for Sauvignon blanc wines, and in our study, the use of closures in half bottles effectively delivered the equivalent of 12 months worth of oxygen from steady state OTR in a 750 mL bottle. To our knowledge, this is the first time that the impact of closure-derived oxygen on wine composition has been described.

As for the effect of copper addition on 3-MH, the data in Table 2 indicate that copper addition can also result in a significant decrease in 3-MH final concentration, in particular, in the wines not treated with GSH, with differences that in some cases were higher than 2-fold. However, Figure 3 shows that, over 6 months, differences in 3-MH losses following bottling were relatively small, suggesting that the presence of copper only marginally increased degradation during aging. Table 4 shows a comparison of 3-MH differences 48 h after bottling and after 6 months of storage. Quantitatively, the differences observed at 6 months as a result of copper addition were in large part already present in the freshly bottled wines, indicating that the decrease of 3-MH observed after 6 months was mostly due to a relatively rapid action of copper on 3-MH at bottling rather than to its action during storage. In this study copper was added in the form of Cu^{2+}, which can directly react with −SH groups by binding them or by oxidizing them to the corresponding disulfides.[28,29] Additionally, in the presence of oxygen and other metal ions such as Fe^{2+}, Cu^{2+} can be converted to Cu^{+} and catalyze the formation of quinones,[23] which can in turn rapidly react with −SH compounds.[30,31] The higher concentrations of 3-MH in freshly bottled wines containing GSH might be therefore due to the antagonistic action of the −SH group of GSH toward the direct reaction between copper and 3-MH or toward 3-MH reaction with quinones. In any case, it is worthwhile remarking that the greatest differences in 3-MH losses associated with copper were always observed in N/N samples in comparison to the other oxygen exposure conditions (Figure 3). This suggests that the effect of copper on 3-MH during aging, albeit small, is also linked to oxygen exposure.

H_2S was detected in the experimental wines at concentrations between 0.2 and 4.5 μg/L; therefore, in some of the wines after 6 months in the bottle, this compound was present in concentrations above the reported threshold of 1.6 μg/L in white wine.[32] H_2S has been associated with the occurrence of reductive, rotten egg-like aromas in wine.[39] Similar to 3-MH, significant differences in the final concentration of H_2S were observed in response to changes in GSH, copper, or oxygen exposure (Tables 1 and 2). In general, H_2S was found to increase during time, consistent with other authors.[4] GSH-treated wines always exhibited higher H_2S concentrations than their corresponding untreated samples. Given the antioxidant capacity of GSH, this higher accumulation of H_2S could be due to the lower degree of oxidation occurring in these samples, as suggested by the SO_2 and 3-MH data. However, it has been shown that, when exposed to prolonged heating, GSH can generate H_2S.[33] The role of GSH as a precursor to H_2S during wine aging requires further investigation.

Within each set of matrix treatments, oxygen exposure determined large variations in H_2S content, with the highest H_2S concentrations generally observed in N/N wines. Figure 2 shows that at both 3 and 6 months, dissolved oxygen was significantly lower in the N/N samples. This is consistent with the generally accepted idea that extremely low oxygen exposure, such as that achieved by using screw-cap closures with tin laminate wads, can result in conditions favoring the development of unwanted reductive aromas.[4,21,34]

Surprisingly, copper addition systematically increased final H_2S concentrations, particularly when combined with low oxygen exposure. This observation is somewhat unexpected, as copper addition prior to bottling is widely carried out in the wine industry to remove reductive off-flavors related to the accumulation of H_2S and mercaptans.[5] However, metals, including copper, can also promote desulfurization of different substrates, for example, cysteine, with consequent release of H_2S, in particular, in the presence of catalysts such as pyridoxal or pyruvate.[35,36] This might explain the positive relationship observed here between copper and H_2S. Alternatively, copper−thiol complexes have been reported to effectively bind oxygen in alkaline solutions,[37] although for the GSH−copper pair this has not been proven in acidic medium such as wine. Figure 4 shows the evolution of H_2S during 6 months of storage. Wines were initially very similar in H_2S content despite copper addition, and, although in the first 3 months all wines showed an increase in H_2S, differences between treatments at 3 months were negligible. In the following 3 months of storage, differences due to

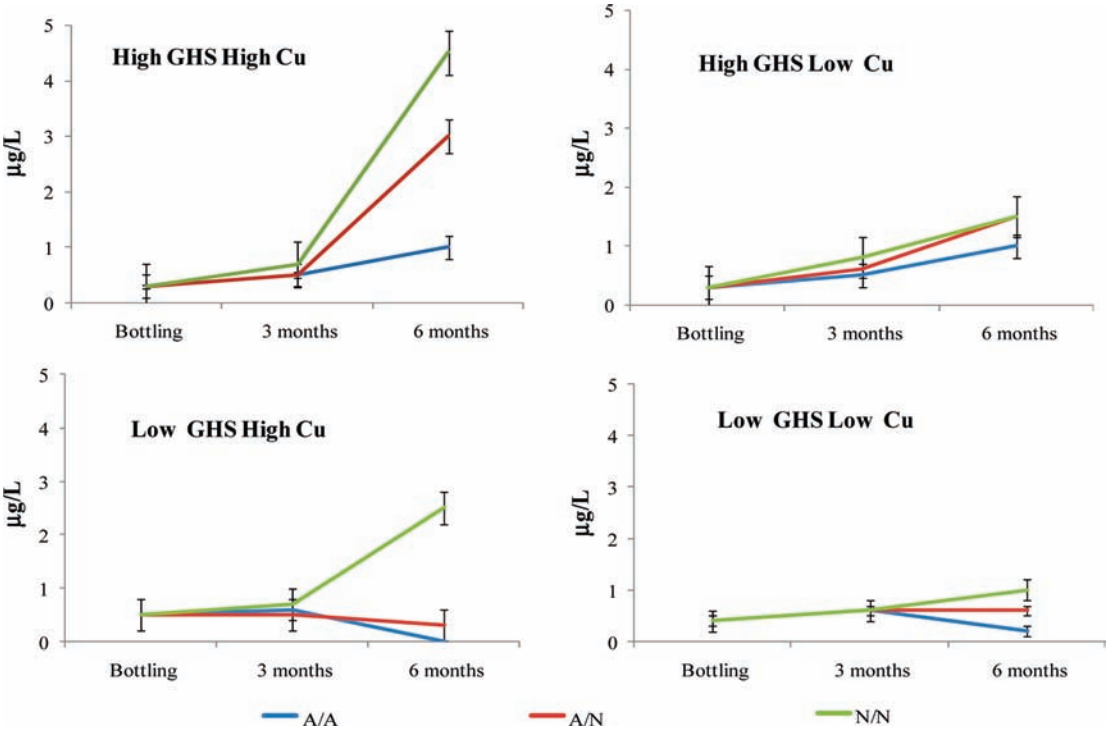

Figure 4. Evolution of H_2S concentration over 6 months of storage under different conditions for the four experimental wines.

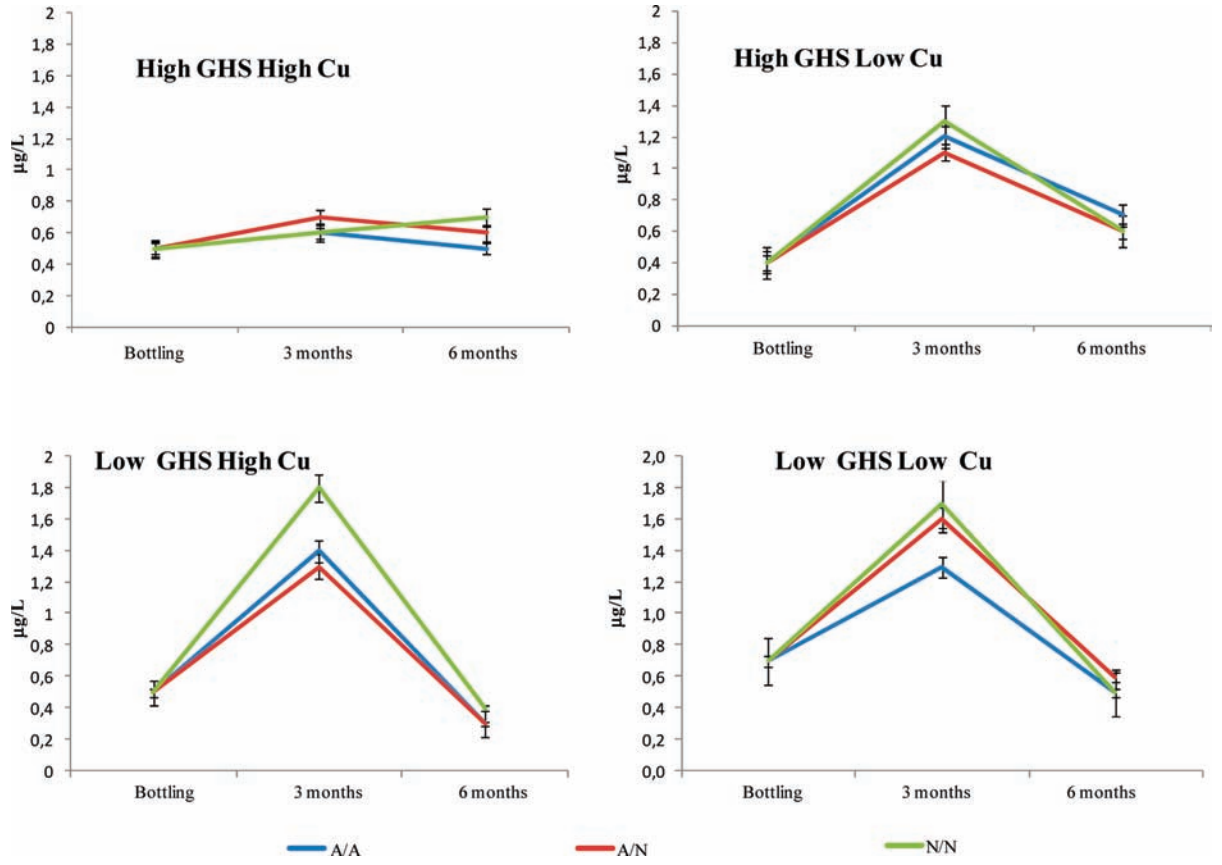

Figure 5. Evolution of MeSH concentration over 6 months of storage under different conditions for the four experimental wines.

experimental variables started to emerge, in particular, with wines stored under low oxygen exposure conditions reaching higher H_2S levels. These data suggest that wine compositional factors, such as copper and glutathione content, can determine the ability

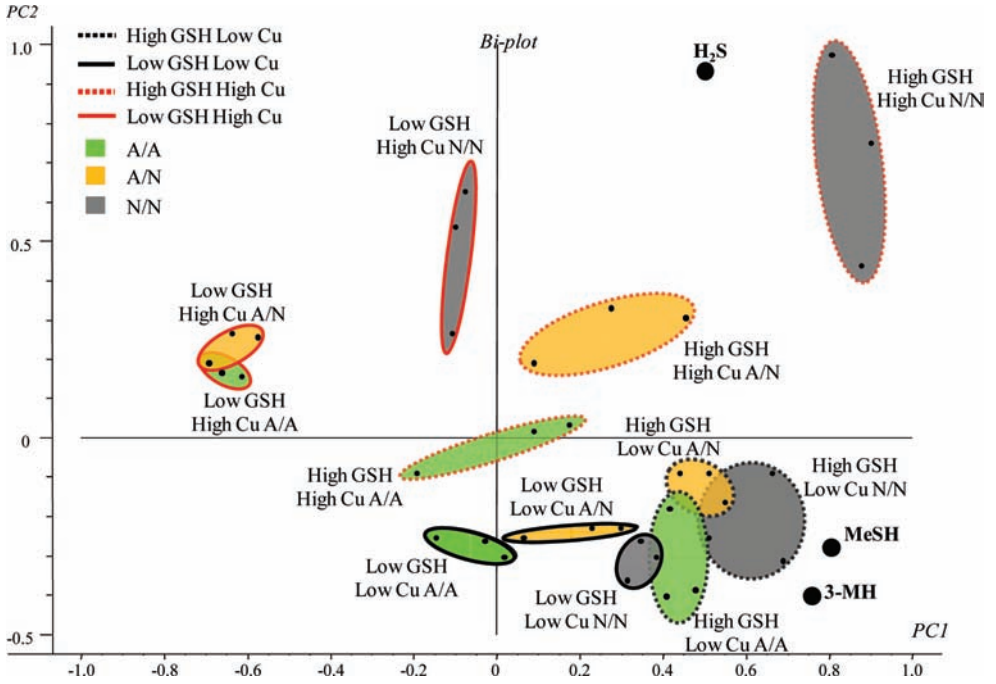

Figure 6. Biplot of principal components 1 (PC1) and 2 (PC2) for volatile sulfur compounds in the wines after 6 months under conditions of various oxygen exposure. PC1 and PC2 accounted for 70 and 25% of the variation, respectively. The data for three replicate samples are plotted and grouped; see legend for key to sample identities.

of a wine to develop H_2S during a given period of storage. At the same time, oxygen exposure is a powerful modulator of H_2S accumulation. It is worth observing that, also in the case of H_2S, the effect of the oxygen released by the closure was significant in the majority of the cases. In particular, in copper-treated wines with no GSH addition (highest theoretical oxidative conditions), closure-derived oxygen alone (A/N treatment) appeared to be responsible for most of the decrease in H_2S compared to N/N. Therefore, in addition to closure OTR, management of closure-derived oxygen represents an important option for H_2S control. The nature of the biochemical processes determining H_2S accumulation during aging remains to be established.

Of the −SH compounds studied, MeSH, also implicated in reductive and rotten egg aromas,[38] was the less responsive to the different experimental variables, with differences across wines being generally quite small (Table 2). Concentrations at 6 months were between 0.2 and 0.7 μg/L and, therefore, below the reported threshold of 3.1 μg/L in white wine.[39] In general, GSH addition resulted in a minor but significant increase in MeSH concentration at 6 months, a trend similar to that observed for H_2S. Conversely, at 6 months, MeSH was not significantly affected by copper (Table 3), in contrast with the generalized belief that increased copper doses can prevent accumulation of mercaptans during aging.[5] However, MeSH evolution during storage was different from that of H_2S, as MeSH peaked at 3 months, with maximum concentrations generally attained in the N/N samples, particularly in samples without GSH. In the following 3 months a decrease was observed, with final concentration values that were essentially similar to the initial concentration (Figure 5). The only exception to this trend was observed for the combination GSH−copper N/N, for which no peak and then decline in MeSH concentration was observed over the 6 month period. Interestingly, this wine also displayed the largest increase of H_2S in the second 3 months of storage, which resulted in the highest H_2S concentration observed in this study. It has been postulated that during aging MeSH can originate from the hydrolysis of the yeast-derived ester methylthioacetate.[40] Alternatively, under conditions of low oxygen exposure, disulfides can be reduced to their corresponding mercaptan.[41] Mercaptan concentration in wine is also affected by the occurrence of reactive species such as quinones.[41] However, under our conditions no dimethyl disulfide or methylthioacetate was detected in the wines (LOD = 0.2 and 1 μg/L, respectively). The origin of methyl mercaptan in aged wines requires further investigation.

Figure 6 shows a summary of the trends observed in this study, as obtained by PCA carried out on the concentrations of 3-MH, H_2S, and MeSH after 6 months of storage. The first two principal components explained 95% of the total variance, with principal component 1 (PC1) accounting for 70% of total variance. Separation of samples along the PC1 axis was strongly associated with addition of GSH at bottling and with oxygen exposure. 3-MH, and also H_2S and MeSH, were positively correlated with GSH addition and negatively correlated with oxygen exposure. Along the PC2 axis, which explained a further 25% of the total variance, separation of the samples based on the amount of copper added could be observed. H_2S was generally positively associated with copper additions, the opposite being true for 3-MH.

In conclusion, this work provides a first direct comparison among some compositional and technological variables that are able to affect white wine aroma development during bottle aging. GSH decreases 3-MH degradation during time, whereas copper is clearly detrimental to the concentration of this key fruity aroma compound. On the other hand, GSH, particularly in combination with copper, also induces conditions favorable to the accumulation of powerful off-odor compounds such as H_2S, and to a lesser extent, MeSH. In general, our results confirm that low oxygen exposure preserves 3-MH but also favors

accumulation of H_2S. However, the extent of this effect is strongly dependent on wine composition, which suggests that different wines could benefit from different degrees of oxygen exposure. In this sense, the amount of oxygen released from the closure into the bottle headspace following corkage affects wine aroma development during storage. Although this amount of oxygen was associated with a relatively high loss of 3-MH during storage, it also decreased accumulation of H_2S, especially under the conditions of maximum H_2S development (i.e., in the presence of GSH and high copper). Further studies are needed to confirm these findings over broader ranges of compositional characteristics and experimental conditions. Our results at this stage suggest that copper addition at bottling to remove and/or prevent reductive off-odors needs to be reassessed, especially when combined with low postbottling oxygen exposure.

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