



ORIGINAL RESEARCH ARTICLE

Alternative strategies for eliminating hydrogen sulfide and methanethiol from wine: results and learnings

Diego Sánchez-Gimeno¹, Eduardo Vela¹, Vicente Ferreira¹, Ignacio Ontañón^{1,*}

¹ Laboratory for Aroma Analysis and Enology (LAAE), Department of Analytical Chemistry, Faculty of Sciences, Universidad de Zaragoza, Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA), 50009 Zaragoza, Spain



*correspondence:
ionta@unizar.es

Associate editor:
Maurizio Ugliano



Received:
21 June 2023

Accepted:
10 September 2024

Published:
10 October 2024

ABSTRACT

Six different remediation procedures for removing hydrogen sulfide (H₂S) and methanethiol (MeSH) from a small selection of wines have been studied. Procedures include three modifications of classical copper fining (two precipitation adjuvants and filtration), purging wine in the reductive state with N₂, and oxidation with or without thiol-functionalised polymeric silica (SH-Sil). Free and brine-releasable (BR), H₂S, and MeSH contents of wine were determined after the treatments and after accelerated reductive ageing. Results have shown that neither the use of precipitation adjuvants nor filtration improved the results of classical copper fining. Purging wine in the reductive state with N₂ can decrease significantly the ability of wine to further accumulate H₂S and MeSH, but several cycles are required and success depends on the amounts of precursors present in the wine and on copper levels. Oxidation, regardless of the presence of SH-Sil, was the most efficient treatment to remove “reductive” off-odours in both the short and long term.

KEYWORDS: sulfur off odours, wine ageing, remediation, metal-bound H₂S, quinones



This article is published under the **Creative Commons licence** (CC BY 4.0).

Use of all or part of the content of this article must mention the authors, the year of publication, the title, the name of the journal, the volume, the pages and the DOI in compliance with the information given above.

INTRODUCTION

The development of “reductive” off-odours constitutes a recurrent problem in the wine industry, being a significant fraction of wine faults (Goode and Harrop, 2008). Furthermore, these problems have now become a bottleneck for some relevant industrial projects, such as canned wine or the use of completely anoxic stoppers (The_AWRI, 2021). The main ones responsible for “reductive” off-odours are hydrogen sulfide (H₂S) and methanethiol (MeSH), plus, eventually, a number of thiols and derivatives that can be formed from them (Franco-Luesma *et al.*, 2016; Siebert *et al.*, 2010). Both components are normal by-products of yeast metabolism (Swiegers and Pretorius, 2007) and may play a role in detoxification and signalling processes (Huang *et al.*, 2017). The production of H₂S can be sometimes very high and, often, it is not possible to attribute such overproduction to a single cause. This suggests that there are multiple non-independent causes for the development of “reductive” off-odours, including the strain of yeast, assimilable nitrogen levels of must (Jiranek *et al.*, 1995; Ugliano *et al.*, 2009; Ugliano *et al.*, 2011), the oxygenation received during fermentation (Bekker *et al.*, 2016) or the presence of residual sulfur in the grapes (Jastrzembski *et al.*, 2017).

While part of the H₂S produced during fermentation is lost by evaporation, a significant part of it remains in the wine under three different types of chemical forms. A first and generally minor part is present as free H₂S. A second fraction remains in the wine bound to metal cations, such as Cu (I), Zn (II), or Fe (II) (Franco-Luesma and Ferreira, 2014), forming a series of non-volatile complex structures. The specific composition of this fraction is poorly known, but it can be stated that the S atom in all these cases remains in its (-II) valence. The third fraction accumulates in the form of oxidised precursors (Kreitman *et al.*, 2016b). This fraction, particularly in the case of H₂S, can be very complex since an endless number of possible polysulfides, polysulfanes and polythionates (Müller *et al.*, 2022; Müller and Rauhut, 2018) involving glutathione, cysteine and other thiol-containing wine molecules can be formed (Bekker *et al.*, 2018; Kreitman *et al.*, 2017; van Leeuwen *et al.*, 2020). As it has been reported that these oxidised precursors have different stabilities, they will re-emit H₂S at different rates influenced by the presence of SO₂ (Bekker *et al.*, 2018). The possibility that metal cations, particularly copper, take part in this third fraction of oxidised precursors cannot be ruled out, nor that the presence of copper could catalyse the release of H₂S and MeSH from these precursors, as it has been suggested by some authors (Bekker *et al.*, 2018; Kreitman *et al.*, 2017).

When wine develops “reductive” off-odours during fermentation, winemakers aerate the fermenting mash. Such a process will eliminate by evaporation part of the H₂S and MeSH and will induce mild oxidation of the wine since part of the oxygen will be consumed by yeasts. Those mild oxidative conditions, similar to those of micro-oxygenation, can most likely lead to the formation of non-volatile odourless polysulfides and polysulfanes. These compounds will act as

a reservoir for “reductive” off-odours and, as soon as the wine is stored in anoxic conditions, MeSH and H₂S will be released again (Vela *et al.*, 2018). The processes ultimately responsible for the release of these molecules are not well understood, but a number of spontaneous condensation and sulfonation reactions of polyphenols in which electrons are released, are known to occur when wine is stored under anoxic conditions (Ontañón *et al.*, 2020).

Almost every additional action aimed at remediating “reductive” off-odours in the cellar involves copper fining. This is carried out by adding Cu (II) salts, such as sulfate or citrate, or diverse Cu (II)-containing formulations, including Cu (II) adsorbed by inactivated yeasts or mixtures of Cu (II) salts with oenological tannins (Vela *et al.*, 2017). These operations produce an immediate decrease in the intensity of the off-odours; not because copper can precipitate CuS, as is commonly thought, but because Cu (II) oxidises sulfides to disulfides (Kreitman *et al.*, 2016a), being itself reduced to Cu (I), which forms strong complexes with the free H₂S and thiols remaining in solution. The copper added in excess integrates the Cu fraction I (Zhang *et al.*, 2021a) which contains Cu complexed with tartaric acid. This fraction acts as a sink for the MeSH and H₂S released during wine ageing so that it is progressively transformed to Cu fractions II (Cu bonded to mercaptans) and III (Cu bonded to H₂S), respectively. Once the Cu fraction I is depleted, H₂S and MeSH will accumulate again, so that copper fining, delays but may not completely avoid, the apparition of “reductive” off-odours. Additionally, an excess of copper may induce the catalytic decomposition of methionine and cysteine to produce MeSH and H₂S (Ferreira *et al.*, 2018), and make the wine more susceptible to oxidation (Clark *et al.*, 2015a).

Several lines of research have tried to improve this situation. Some researchers focussed on understanding and controlling fermentation to limit the formation of these compounds (Jimenez-Lorenzo *et al.*, 2021; Jiranek *et al.*, 1995; Kinzurik *et al.*, 2015; Scott *et al.*, 2020; Song *et al.*, 2020; Spiropoulos *et al.*, 2000; Ugliano *et al.*, 2009; Vos and Gray, 1979; Walker *et al.*, 2021), while others studied how to remove copper and copper-bonded sulfides (Bekker *et al.*, 2016, 2021; Clark *et al.*, 2015b; Kontoudakis *et al.*, 2019; Zhang *et al.*, 2021a; Zhang *et al.*, 2021b) or the effectiveness of different strategies for remediation of “reductive” aromas in wine (Bekker *et al.*, 2021; Day *et al.*, 2015; Day *et al.*, 2021). Yet, the wine industry needs more efficient treatments.

Because of this, the main goal of the present research is to assess six different alternative strategies for the effective removal of wine H₂S and MeSH and, the effect on the tendency of wine to accumulate them. The three first strategies studied are based on copper-finishing procedures, the fourth uses inert gas, and the fifth and sixth use oxidative conditions. Beyond looking for a definitive procedure, our work aims to identify the most promising strategies and the most pressing associated challenges.

MATERIAL AND METHODS

1. Solvents and chemical standards

Ethanol was purchased from Merck (Darmstadt, Germany). Water with a resistance of 18.2 mΩ·cm at 25 °C was purified in a Milli-Q system from Millipore (Merck, Germany), and sodium hydroxide and tartaric acid were purchased from Panreac ApplyChem (Barcelona, Spain). Pure standards (> 95 %) for VSC calibration: H₂S, MeSH, were produced by the addition of water solution of sodium sulfide, Na₂S, and sodium methanethiolate, CH₃SNa (all supplied by Sigma-Aldrich, St. Louis, MO, USA) at pH 9.6. This solution was prepared daily and kept in an anoxic chamber P[box] (Jacomex, France) with Argon. The standards are stored in a desiccator to avoid hydration. Ethyl methyl sulfide (EMS) and 1-propanethiol (PrSH) were provided by Sigma-Aldrich (Steinheim, Germany). Stock solutions of EMS and PrSH were prepared in iso-octane in amber vials and were stored at -20 °C. Intermediate methanolic solutions were stored at -20 °C in amber vials with Mini-inert valves (Supelco, Ca, USA). All these solutions were prepared in the anoxic chamber.

The brine contained 350 g/L of sodium chloride NaCl (Panreac, Barcelona, Spain) in Milli-Q water. Synthetic wine was a pure water solution containing 5 g/L of tartaric acid, 12 % v/v ethanol, and pH 3.4 adjusted with diluted NaOH (0.1 m)

2. Wine samples

Five red wines, one rosé wine, and one white wine were selected: W1 (Ribera del Duero, 2017), W2 (Ribera del Duero, 2016), W3 (Ribera del Duero 2018 without SO₂ addition), W4 (rosé wine, Cariñena, 2016), W5 and W6 (La Rioja, 2017 and 2020, respectively) and W7 (white wine, Ribeiro, 2017). W5 and W6 were made in an experimental cellar with grapes spiked with micronised S. The rest of the samples were sent from different wineries to be analysed for having “reductive” off-odours or being suspects of developing them.

3. Analysis of free and BR-VSCs by GC-pFPD

These compounds were analysed following the procedure proposed by Franco-Luesma and Ferreira (Franco-Luesma and Ferreira, 2014) with some modifications. For free VSCs, 12 mL of wine and 40 µL of the internal standard solution (EMS and PrSH at 2 mg/L) were transferred to a 20 mL vial. After incubation, 1 mL of the headspace was injected and analysed in a Varian CP-3800 gas chromatograph equipped with a pulsed flame photometric detector (GC-pFPD) [Walnut Creek, CA, USA]. For the BR fraction, 0.4 mL of wine diluted with 9.6 mL of brine and 40 µL of the internal standard solution were added to a 20 mL vial. After the incubation, the extraction was carried out with an SPME fibre and then it was desorbed in the injector of the GC-pFPD.

4. Accelerated anoxic ageing assay (RA2w)

The methodology developed by Franco-Luesma and Ferreira (Franco-Luesma and Ferreira, 2016b) was carried out to

simulate and accelerate the ageing of wines under anoxic conditions. For that, samples were stored under anoxia at 50 °C during 2 weeks. Samples were prepared in the anoxic chamber by distributing three 60 mL aliquots of each wine in three 60 mL screw-capped glass tubes (Wit Deluxe, Denmark), tightly closed and double vacuum bagged, including an O₂ scavenger (AnaeroGen™ from Thermo Scientific Waltham, Massachusetts, USA) between both bags.

5. Redox potential measurements

Measurements of redox potential were carried out according to Vela *et al.* (2018) with a commercial electrode integrated by a Pt electrode, an Ag–AgCl(s) reference electrode, and a HI-98191 ORP meter (Hanna Instruments, Woonsocket, RI, USA). All measurements were carried out inside the anoxic chamber. Readings were taken by immersing the electrode into 5 mL samples and letting 35 min of equilibration.

6. Analysis of metals

Iron, copper, manganese, and zinc were determined by inductively coupled plasma-mass spectrometry (ICP-MS) as described by González *et al.* (2008). Analyses were performed in triplicate.

7. Studied remediation strategies

7.1. Copper fining with precipitation adjuvants

Two red wines (W1 and W2) were divided into four aliquots of 500 mL each: one of them was the control and the others were treated with three different treatments: 0.5 mg/L of Cu (added as CuSO₄), 0.5 mg/L of Cu and 120 mg/L of potato protein and 0.5 mg/L of Cu and 1 g/L of bentonite, respectively. All treatments were performed in duplicate.

The addition of adjuvants was carried out 24 hours after the addition of copper, allowing the formation of complexes with H₂S and thiols. Samples were stored for 7 days and were then centrifugated for 15 min at 4500 RPM and 10 °C. Free and BR-VSCs and redox potential were measured after the centrifugation. Finally, the RA2w assay was applied to the samples and the same analyses were carried out. All the experiment was performed under anoxia.

7.2. Copper fining combined with filtration

The wine W1 was spiked with 0.5 mg/L of Cu and was stored under anoxia for seven days to allow enough time for the formation of complexes with VSCs. Then, the sample was filtered inside the anoxic chamber using 0.45 µm pore size and 25 mm diameter nylon filters. Seven days later, the filtration was repeated with a 0.20 µm pore size filter. Filters were kept within the anoxic chamber at least 24 hours before the experiment to ensure they were free from oxygen. After that, free and BR-VSCs were quantified, RA2w was applied and the analyses were repeated. The copper content was measured after the second filtration. Copper was not added to the control sample, which was also not filtered. The experiment was carried out entirely in the anoxic chamber and was carried out in duplicate.

7.3. Purge with nitrogen

Two different experiments were performed. In the first one, wines W2 and W3 were used. Firstly, accelerated anoxic ageing was applied to the samples to be sure that the redox potential of wines was negative (less than -50 mV). After that, samples were purged with N_2 at 100 mL/min for 60 min. Free and BR-VSCs and redox potential were then measured. The RA2w was applied to the samples and the same measurements were carried out again. The same protocol was performed with the control samples except for the purging step. Experiments were carried out in duplicate.

Samples W4, W6, and W7 with an extreme “reductive” off-odour, were used for the second experiment. Moreover, this experiment was also carried out with W7 spiked with 100 μ g/L of copper as copper sulfate. Two cycles of reductive ageing plus purging were applied to the wine, which was then further subjected to a third RA2w. The purge was carried out with 100 mL/min N_2 for 75 min. Free and BR-VSCs and redox potential were measured after each RA2w assay or purging. The experiments were carried out in duplicate.

7.4. Strong aeration with or without polymeric thiol-functionalised silica

The experiment was carried out with the samples W1 and W5. Silica was functionalised with 3-mercaptopropyl (1 mM thiol groups) from SiliCycle (Quebec, Canada) and was added to each wine inside the anoxic chamber. After that, samples (with and without silica) were put into 500 mL glass jars outside the anoxic chamber and were saturated with air by gentle shaking, following the procedure described by Ferreira *et al.* (2015), to ensure the oxidation of VSCs and the eventual formation of disulfides, polysulfides and polysulfanes. The oxygen-saturated samples were stored for 3 days inside the anoxic chamber and then the silica was removed by centrifugation at 4500 RPM and 10 °C for 15 min. Samples were stored again inside the anoxic chamber until they consumed the remaining oxygen dissolved, which was controlled by measuring the redox potential. Once they reached negative redox potential, free and BR-VSCs were analysed. Finally, accelerated anoxic ageing was carried out and the measurements of VSCs were repeated. The experiment was performed in duplicate.

8. Statistical analysis and data treatment

Data processing, graphical representation of data, and statistical analyses were carried out with Excel 2016 (Microsoft, Washington, USA) and XLSTAT (Addinsoft, version 2019 1.1).

RESULTS AND DISCUSSION

In the present paper, several alternatives for remediating the “reductive” off-odours of wines have been studied. Three of them make use of copper and intend to eliminate part of the copper-bonded sulfhydryls using two different precipitation adjuvants, or using filtration. The fourth uses a stream of an inert gas (N_2) to purge off thiols, once the wine has become in a “reductive” state. The fifth uses strong oxidation induced

by air saturation, combined or not with the addition of polymeric silica functionalised with thiols.

Due to the difficulties linked to the work under a strict anoxic atmosphere, and the timing for some of the experiments, the work spanned nearly two years. This has made it impossible to work with exactly the same wine samples. Then, the efficiency of the different treatments was assessed by measuring free and BR forms of H_2S and methanethiol after and before accelerated reductive ageing in which samples were incubated at 50 °C in strict anoxia for 2 weeks (RA2w). As it has been demonstrated that free forms measured after RA2w correspond, approximately, to the free levels of these molecules accumulated in the wine after 1 year of anoxic ageing at room temperature (Franco-Luesma and Ferreira, 2016a), this measurement provides a reasonable estimate of the long-term efficacy of the treatment, while levels of free forms immediately after the treatment (without RA2w) assess its immediate efficacy. On the other hand, the analysis of BR-forms provides information about the fraction of H_2S and MeSH bonded to transition metals. However, this measurement is sometimes imprecise, due to the complexity of the interactions between H_2S and copper, and between copper cations and different wine constituents, such as tartaric acid, tannins, polyphenols, cysteine, or glutathione (Clark *et al.*, 2015a).

1. Copper fining with precipitation adjuvants

The first treatment consisted of the combination of copper sulfate with two different precipitation adjuvants, potato protein, and bentonite, with the aim of removing from the wine colloidal forms of copper sulfide. Some previous works have demonstrated the metal binding abilities of those materials, which reach Cu-removal efficiencies of up to 43 % for potato protein and 99 % for bentonite (Bertagnolli *et al.*, 2011; Chaves and Tit, 2011; Claus, 2020; Ulmanu *et al.*, 2003; Wang and Xiong, 2005). As detailed in the experimental section, the adjuvants were added after 24 hours of the copper treatment and were left to act for 7 days. The results of this experiment are shown in Table 1. As can be seen, the addition of copper brings about a strong decrease in the levels of free H_2S in the two wines (W1 and W2) and the three treatments. Levels of free H_2S became undetectable in all cases, except in the bentonite-treated sample of the first wine.

Levels of BR forms were also markedly affected by the copper treatments, but in this case, copper addition brought about strong increments in all cases. A similar increment of BR-forms was previously observed in one (out of three) of the cases studied by Vela *et al.* (2017). Such increases were not expected, since Cu (II) has a known oxidant character, which does not seem to be compatible with an increase in levels of reduced forms of H_2S . However, the consistency of the increments observed in the three treatments and with the two wines (plus the one in the previous work), indicates that those increments are not artefacts caused by poor analytical precision, as suggested by Vela *et al.* (2017). Instead, these results suggest that the addition of Cu (II) has quite complex effects on the distribution of the different forms of H_2S ,

TABLE 1. Copper-finishing with precipitation adjuvants. Effect of different copper treatments on free and BR forms of H₂S and MeSH (µg/L) after the treatment (Tx) and after 2 weeks of accelerated anoxic aging at 50 °C (RA2w).

W1	Moment of analysis	Untreated	Cu standard	Cu + Potato	Cu + Bentonite
Free H ₂ S	Tx	1.1 ± 0.1 ^a	< LD ^c	< LQ ^c	0.7 ± 0.0 ^b
	RA2w	13.5 ± 0.9 ^a	1.9 ± 1.9 ^b	3.4 ± 0.6 ^b	3.7 ± 0.6 ^b
BR H ₂ S	Tx	66.0 ± 8.3 ^b	138 ± 3.8 ^a	173 ± 38.6 ^a	146 ± 10.7 ^a
	RA2w	56.7 ± 1.2 ^a	45.6 ± 17.0 ^a	50.2 ± 2.2 ^a	53.4 ± 10.4 ^a
Alpha	Tx	1.7 ± 0.26 % ^a	< 0.2 % ^b	< 0.2 % ^b	0.47 ± 0.04 % ^c
	RA2w	23.8 ± 1.7 % ^a	4.2 ± 4.4 % ^b	6.8 ± 1.2 % ^b	6.9 ± 1.8 % ^b
Free MeSH	Tx	1.7 ± 0.0 ^a	1.2 ± 0.3 ^a	1.4 ± 0.2 ^a	1.4 ± 0.0 ^a
	RA2w	1.0 ± 0.2 ^a	1.0 ± 0.4 ^a	0.8 ± 0.1 ^a	0.7 ± 0.0 ^a
BR MeSH	Tx	2.7 ± 0.2 ^a	2.3 ± 0.2 ^a	3.3 ± 1.3 ^a	2.3 ± 0.2 ^a
	RA2w	2.5 ± 0.2 ^a	2.2 ± 0.2 ^a	2.4 ± 0.1 ^a	2.7 ± 0.3 ^a
Redox potential (mV)	Tx	+2.6	+0.6	-11.7	+13.0
	RA2w	-63.4	-81.7	-60.4	-78.3
Copper (µg/L)	Tx	112 ± 1.8 ^d	556 ± 8.2 ^a	337 ± 5.4 ^c	402 ± 2.1 ^b
W2	Moment of analysis	Untreated	Cu standard	Cu + Potato	Cu + Bentonite
Free H ₂ S	Tx	1.8 ± 0.6 ^a	< LD ^b	< LD ^b	< LD ^b
	RA2w	13.1 ± 0.6 ^a	2.2 ± 0.2 ^d	6.2 ± 0.8 ^b	4.2 ± 0.3 ^c
BR H ₂ S	Tx	24.6 ± 0.2 ^c	104 ± 1.1 ^a	72.1 ± 9.3 ^b	93.4 ± 0.0 ^a
	RA2w	56.0 ± 4.0 ^a	31.7 ± 4.9 ^b	46.3 ± 6.7 ^{ab}	40.5 ± 7.1 ^{ab}
Alpha	Tx	7.3 ± 2.4 % ^a	<0.2 % ^b	<0.2 % ^b	<0.2 % ^b
	RA2w	23.4 ± 2.0 % ^a	6.9 ± 1.2 % ^c	13.4 ± 2.6 % ^b	10.4 ± 2.0 % ^{bc}
Free MeSH	Tx	1.9 ± 0.2 ^a	1.4 ± 0.0 ^{ab}	1.1 ± 0.8 ^{bc}	< LQ ^c
	RA2w	2.1 ± 0.6 ^a	1.6 ± 0.7 ^a	1.4 ± 0.2 ^a	1.2 ± 0.0 ^b
BR MeSH	Tx	2.3 ± 0.1 ^a	2.0 ± 0.3 ^a	1.9 ± 0.3 ^a	1.8 ± 0.2 ^a
	RA2w	3.2 ± 0.1 ^a	2.7 ± 0.1 ^b	2.8 ± 0.1 ^b	2.8 ± 0.1 ^b
Redox potential (mV)	Tx	-2.6	+8.9	-9.6	-8.7
	RA2w	-52.2	-59.2	-75.0	-85.2
Copper (µg/L)	Tx	61.5 ± 0.3 ^d	525 ± 7.7 ^a	293 ± 6.0 ^c	339 ± 1.9 ^b

LD H₂S: 0.2 µg/L; LQ H₂S: 0.6 µg/L; LQ MeSH: 0.6 µg/L. Alpha is the ratio in percentage between free and BR forms of H₂S. Redox potential is expressed in mV vs a standard Ag/AgCl reference electrode. Copper levels were measured after the treatments. Different superscript letters denote significant differences according ANOVA test.

including the fraction of oxidised precursors. In fact, the results in the table may indicate that a significant fraction of oxidised forms of H₂S has been reduced to form HS-Cu and/or Cu₂S, which are the major chemical species measured in the BR fraction. While this thermodynamically can make sense, since Cu(I)-S(-2) bonds are very strong, the question of the chemical species providing the electrons for reducing Cu(II) to Cu(I) and S(-I) to S(-II) remains unanswered.

In any case, the result confirms that the addition of copper (II) to wine does not bring about any loss of H₂S, confirming previous results (Clark *et al.*, 2015b; Vela *et al.*, 2017). As expected, the main effect of the copper treatment is the decrease in the ratio of free to BR H₂S, designated alpha in Table 1, which simply indicates that the fraction of H₂S complexed with metals increases.

After anoxic ageing, levels of free H₂S were in all treatments much lower than those measured in the control, confirming the effectiveness of copper treatments in delaying the release of free H₂S during anoxic ageing. Levels of free H₂S accumulated were in all cases less than half of those accumulated in the control wine. It can be observed, however, that levels accumulated in the treatments with adjuvants were higher than those accumulated in the standard copper treatment, although the differences were only significant in the second wine. These results can be explained by attending to the levels of copper remaining in the wines after the treatments, as seen in the last rows of Table 1. It can be observed that both adjuvants removed significant amounts of copper, particularly potato protein, which eliminated more than 220 µg/L of copper, while bentonite removed between

150 and 190 µg/L. Smaller levels of copper, lead to higher levels of free H₂S and increased alpha, as seen in the table. This indicates that most copper removed was free copper and not copper bound to sulfur species.

Anoxic ageing also brought strong changes in the levels of BR forms, as can be seen in the table. Quite surprisingly, levels of BR forms in all copper-treated samples were much smaller than those found before RA2w and were similar (W1) or smaller (W2) than those measured in the corresponding controls. This result contrasts with the highest levels of BR forms measured before the reductive ageing and could indicate that little amounts of Cu₂S (200–250 µg/L) have precipitated, or alternatively, that they have evolved into polymeric forms no longer measurable as BR-forms.

The other clear and expected effect of anoxic ageing was the increase in the fractions of H₂S in free form. In the original wines, the ratio of free to BR forms (alpha) increased to 23 %, while in the copper-treated samples, the final levels of this ratio were in all cases below 14 %. Alpha was clearly higher in the samples treated with adjuvants, particularly in wine 2, and particularly in the potato protein, in agreement with the lower copper levels measured in these samples.

Regarding MeSH, the copper treatments had little effects, smaller than those previously reported (Vela *et al.*, 2017). In wine 1, no significant effect was observed for either the copper treatment or the filtration adjuvants. In wine two, the standard copper treatment reduced, non-significantly, levels of this thiol, while the filtration adjuvants, particularly bentonite, improved this result. Such differences were kept after reductive ageing. Results of BR forms of MeSH are

consistent with those of free forms, although reveal that the differences introduced by the adjuvants decrease after reductive ageing. This result was expected, since MeSH can be formed by metal-catalysed decomposition of methionine (Ferreira *et al.*, 2018), and this source of continuous formation gains relevance with aging (Ferreira *et al.*, 2014). The observed decrease in levels of MeSH seems to be a secondary effect of the adjuvants and may not be directly related to the observed copper removal.

It can be concluded that the effective reduction in copper levels caused by the addition of the adjuvants does not cause any significant or consistent decrease in H₂S or MeSH concentration in the wine, or in the wine's ability to accumulate them during ageing.

2. Copper fining combined with filtration

The results of this experiment are given in Table 2. As detailed in the material and methods section, the filters used were made of nylon, a material that had worked satisfactorily in a previous report to reduce copper levels in white wines (Kontoudakis *et al.*, 2019). In our case, as can be seen in the table, the filters were unable to remove any copper from the red wine used in the study. Furthermore, the treatment was unable to decrease the ability of the wine to accumulate H₂S and MeSH. In fact, the results reveal that filtration had some unexpected and undesirable effects. On the one hand, levels of free MeSH after filtration were surprisingly and significantly high. In any case, significantly higher than those found in the standard copper treatment. On the other hand, it can be also observed that filtration had apparently induced wine oxidation. This is evident in wine redox potential, which

TABLE 2. Copper fining and filtration. Effects of a double filtration through nylon filters after a standard copper fining treatment on free and BR forms of H₂S and MeSH (µg/L), after the treatment (Tx) and after 2 weeks of accelerated anoxic aging at 50 °C (RA2w).

W1	Moment of analysis	Control	Cu standard	Cu + filtration
Free H ₂ S	Tx	0.7 ± 0.0 ^a	< LD ^c	< LQ ^b
	RA2w	14.0 ± 0.4 ^a	1.9 ± 1.9 ^b	4.3 ± 0.4 ^b
BR H ₂ S	Tx	42.0 ± 5.4 ^c	138 ± 3.8 ^a	56.2 ± 2.1 ^b
	RA2w	94.6 ± 21.1 ^b	45.6 ± 17.0 ^b	218 ± 2.7 ^a
Alpha	Tx	1.7 %	< 0.2 %	< 0.4 %
	RA2w	14.7 %	4.2 %	2.0 %
Free MeSH	Tx	<LQ ^c	1.2 ± 0.3 ^b	3.9 ± 0.2 ^a
	RA2w	1.0 ± 0.0 ^a	1.0 ± 0.4 ^a	0.8 ± 0.0 ^a
BR MeSH	Tx	1.6 ± 0.1 ^b	2.3 ± 0.2 ^a	1.3 ± 0.1 ^b
	RA2w	3.9 ± 0.3 ^a	2.2 ± 0.2 ^b	3.8 ± 0.2 ^a
Redox	Tx	-10 mV	0 mV	+50 mV
Potential (mV)	RA2w	-75 mV	-82 mV	-75 mV
Copper (µg/L)	Moment of analysis	129.9 ± 0.7 ^b	586 ± 8.2 ^a	589.4 ± 3.4 ^a

LD H₂S: 0.2 µg/L; LQ H₂S: 0.6 µg/L; LQ MeSH: 0.6 µg/L. Alpha is the ratio in percentage between free and BR forms of H₂S. Redox potential is expressed in mV vs a standard Ag/AgCl reference electrode. Copper levels were measured after the treatments. Different superscript letters denote significant differences according ANOVA test.

immediately after the filtration reached a +50 mV value. Such an increase was unexpected since filtration was carried out in a completely anoxic environment, and filters were introduced into the chamber at least 24 hours after their use. Furthermore, the change in BR levels caused by filtration and the contents of BR forms in the filtered samples after reductive ageing, are compatible with those of oxidation. As can be seen, BR H₂S levels after filtration were not very dissimilar to those of the control and were much smaller than those obtained in the standard copper treatment and also than those obtained in all the other copper-treated samples in the first experiment. However, after the 2 weeks of reductive ageing, levels of BR H₂S increased to become the maxima levels registered in the present work, *i.e.*, the results suggest that filtration prevented the unknown reductive processes mentioned in the previous section, so that the number of oxidised forms increased, part of which was reduced in the accelerated reductive ageing. In any case, this result indicates that filtration does not represent any improvement in copper fining in red wines.

3. Purge with the nitrogen of wines in a reduced state

This experiment was carried out with five different wines, all of them with reductive problems. All wines were first subjected to anoxic ageing, to ensure that they had a relatively high proportion of H₂S and MeSH in free form so that purging was more effective. In the first experiment, carried out with wines 2 and 3, only one purging cycle was used. Results are presented in Table 3. Samples in the upper half of the table are the initial wines after the 1st reductive ageing (controls) and after the purging process (purged), while samples in the lower half of the table are those samples after reductive ageing. As expected, purging with nitrogen had an immediate effect the nearly complete removal of free forms of H₂S and MeSH in both wines. In wine 2, levels of free H₂S

dropped from 13 µg/L to 1.1 µg/L, while in wine 3, free H₂S dropped from an initial level of 63.3 µg/L to just 1.0 µg/L. The improvement is also seen in BR forms. BR forms of H₂S decrease -non-significantly- from 100 µg/L to 77 µg/L in wine 2 and from 83 µg/L to 27 µg/L, in wine 3. Exactly the same observations can be made about MeSH, whose levels become undetectable in both cases with the treatment, and strong and significant decreases were also observed in the BR forms. However, after 2 weeks of additional reductive ageing (2nd RA2w), purged wines developed amounts of free H₂S not significantly smaller than those found in the aged controls (after 2nd RA2w). The case of wine 3 is particularly intriguing, as despite having removed 62 µg/L of H₂S in the purge, there was hardly any impact. This suggests that the amount of precursors of H₂S that the wines can have is very large, in line with recent findings (Ferreira *et al.*, 2023) so that the amount removed by the purge is just a little fraction. It can be also concluded that one purging cycle is not enough to improve significantly the wine's tendency to accumulate reductive off-odours.

The results of the second experiment are given in Table 4. In this experiment a rosé (W4) a red (W6) and a white wine (W7), this last spiked or not with copper, were all subjected to two consecutive cycles of reductive ageing followed by purging with nitrogen. The resulting wines, after the two cycles were further aged again to assess their abilities to accumulate VSCs.

The rosé W4 contained after the 1st RA 63.3 µg/L and 133 µg/L free and BR H₂S, respectively, and the 1st purge brought those levels down to 2.8 µg/L and 37 µg/L, respectively. The one-time purged wine was then subjected to RA, after which it accumulated 37.7 µg/L and 71.8 µg/L of free and BR H₂S, respectively, which are 60 % and 54 % of that accumulated after 1st RA. The 2nd purge, brought

TABLE 3. Use of inert gas for purging VSCs, experiment 1. Effects of purging wines in reductive state (induced by a first accelerated reductive aging) with a stream of N₂ on free and BR forms of H₂S and MeSH (µg/L), after the treatment and after 2 weeks of additional accelerated anoxic aging at 50 °C (2nd RA2w).

	W2		W3	
	Control	Purged	Control	Purged
After the 1st RA2w				
Free H ₂ S	13.4 ± 0.3 ^a	1.1 ± 0.0 ^b	63.1 ± 1.1 ^a	1.0 ± 0.1 ^b
BR H ₂ S	100 ± 5.5 ^a	77.1 ± 32.8 ^a	83.2 ± 3.6 ^a	27.0 ± 0.7 ^b
Free MeSH	1.0 ± 0.0 ^a	< LD ^b	2.8 ± 0.3 ^a	< LD ^b
BR MeSH	3.4 ± 0.2 ^a	2.0 ± 0.4 ^b	4.5 ± 0.3 ^a	1.3 ± 0.1 ^b
Redox potential	-35.2	-10.6	-133.6	-50.9
After the 2nd RA2w				
Free H ₂ S	10.0 ± 0.2 ^a	8.9 ± 0.7 ^a	44.0 ± 0.8 ^a	40.4 ± 2.4 ^a
BR H ₂ S	32.1 ± 4.3 ^a	25.7 ± 2.5 ^a	47.0 ± 6.8 ^a	48.7 ± 0.0 ^a
Free MeSH	0.9 ± 0.0 ^a	0.7 ± 0.0 ^b	3.8 ± 0.1 ^a	3.2 ± 0.1 ^{bww}
BR MeSH	1.1 ± 0.0 ^a	< LQ ^b	4.5 ± 0.1 ^a	3.7 ± 0.3 ^a
Redox potential	-85.3	-84.6	-121.6	-114.0

LD H₂S: 0.2 µg/L; LQ H₂S: 0.6 µg/L; LD MeSH: 0.45 µg/L; LQ MeSH: 0.6 µg/L. Redox potential is expressed in mV vs a standard Ag/AgCl reference electrode. Different superscript letters denote significant differences according ANOVA test.

TABLE 4. Use of inert gas for purging VSCs, experiment 2. Effects of treating three wines with two consecutive cycles of accelerated reductive aging, immediately followed by purging with a stream of N₂, on free and BR forms of H₂S and MeSH (µg/L).

Wine		Initial	1st RA2w	1st purge	2nd RA2w	2nd purge	3rd RA2w
W4	Free H ₂ S	116.0 ± 3.9 ^a	63.3 ± 1.5 ^b	2.79 ± 0.88 ^e	37.7 ± 0.67 ^c	2.81 ± 0.37 ^e	22.5 ± 1.3 ^d
	BR H ₂ S	138.1 ± 4.0 ^a	133 ± 15 ^a	36.9 ± 0.6 ^c	71.8 ± 1.5 ^b	29.0 ± 0.4 ^c	65.6 ± 5.8 ^b
	Free MeSH	1.84 ± 0.01 ^b	2.83 ± 0.23 ^a	< LQ ^c	2.24 ± 0.03 ^a	0.75 ± 0.39 ^c	2.68 ± 0.06 ^a
	BR MeSH	3.7 ± 0.1 ^b	5.1 ± 0.1 ^a	2.1 ± 0.2 ^c	3.9 ± 0.8 ^{ab}	1.8 ± 0.7 ^c	3.8 ± 0.0 ^b
	Redox potential	+6	-85.7	+38.2	-73.5	+45.1	-47.7
W6	Free H ₂ S (control)			13.2 ± 0.18 ^f	32.8 ± 0.78 ^c	17.5 ± 0.32 ^e	45.6 ± 0.45 ^a
	Free H ₂ S (purged)	1.67 ± 0.06 ^g	34.1 ± 0.96 ^c	0.79 ± 0.01 ^g	27.2 ± 0.11 ^d	0.88 ± 0.01 ^g	41.1 ± 1.5 ^b
	BR H ₂ S (control)			29.8 ± 3.0 ^e	36.2 ± 0.25 ^d	29.9 ± 1.4 ^e	57.9 ± 0.05 ^b
	BR H ₂ S (purged)	23.9 ± 0.4 ^f	69.7 ± 0.7 ^a	48.6 ± 1.2 ^c	42.2 ± 2.9 ^d	39.2 ± 2.0 ^d	56.4 ± 1.3 ^b
	Free MeSH (control)			4.9 ± 0.27 ^f	7.1 ± 0.30 ^c	5.1 ± 0.25 ^{ef}	10.6 ± 0.18 ^a
	Free MeSH (purged)	2.6 ± 0.02 ^g	5.9 ± 0.04 ^d	0.17 ± 0.02 ^h	5.5 ± 0.04 ^e	0.36 ± 0.02 ^h	9.0 ± 0.04 ^b
	BR MeSH (control)			5.7 ± 1.2 ^{de}	7.9 ± 0.26 ^c	6.1 ± 0.13 ^d	13.1 ± 0.01 ^a
	BR MeSH (purged)	4.3 ± 0.36 ^{ef}	5.7 ± 0.67 ^{de}	1.8 ± 0.24 ^g	5.4 ± 0.49 ^{de}	3.0 ± 0.13 ^g	11.2 ± 0.37 ^b
	Redox potential (control)			26.3	n.m.	n.m.	-118.4
Redox potential (purged)	-14.8	-58.1	-6.3			-77.1	
W7	Free H ₂ S (control)			0.64 ± 0.02 ^{de}	1.4 ± 0.06 ^{cd}	0.98 ± 0.22 ^{cde}	17.1 ± 0.73 ^a
	Free H ₂ S (purged)	0.38 ± 0.01 ^e	1.5 ± 0.24 ^c	0.72 ± 0.16 ^{cde}	0.67 ± 0.04 ^{de}	0.90 ± 0.07 ^{cde}	5.1 ± 0.17 ^b
	BR H ₂ S (control)			30.9 ± 3.6 ^c	23.3 ± 0.26 ^c	32.2 ± 7.2 ^{bc}	60.4 ± 2.6 ^a
	BR H ₂ S (purged)	55.7 ± 20.5 ^{ab}	36.7 ± 4.6 ^{abc}	43.3 ± 7.8 ^{abc}	32.7 ± 0.75 ^{bc}	20.1 ± 1.6 ^c	19.8 ± 3.6 ^c
	Free MeSH (control)			1.7 ± 0.04 ^e	7.6 ± 0.29 ^c	5.2 ± 0.08 ^d	19.4 ± 0.8 ^a
	Free MeSH (purged)	1.3 ± 0.02 ^{ef}	7.2 ± 0.33 ^c	0.32 ± 0.06 ^g	5.5 ± 0.01 ^d	0.54 ± 0.06 ^{fg}	9.5 ± 0.02 ^b
	BR MeSH (control)			3.2 ± 0.97 ^d	6.1 ± 0.35 ^c	5.1 ± 0.63 ^{cd}	16.8 ± 0.42 ^a
	BR MeSH (purged)	2.8 ± 2.08 ^{de}	4.8 ± 0.62 ^{cd}	< LD ^a	5.1 ± 0.22 ^{cd}	3.4 ± 0.05 ^d	11.7 ± 0.59 ^b
	Redox potential (control)			79.7	n.m.	n.m.	7.1
Redox potential (purged)	3	-49.8	76.5			-23.6	
W7 (with Cu ²⁺)	Free H ₂ S (control)			0.35 ± 0.03 ^c	0.54 ± 0.002 ^c	0.30 ± 0.05 ^c	14.0 ± 0.46 ^a
	Free H ₂ S (purged)	0.38 ± 0.01 ^c	0.27 ± 0.001 ^c	0.40 ± 0.001 ^c	0.25 ± 0.02 ^c	0.38 ± 0.01 ^c	1.2 ± 0.26 ^b
	BR H ₂ S (control)			40.5 ± 4.4 ^e	60.9 ± 0.49 ^{abcde}	66.9 ± 2.7 ^{abc}	85.8 ± 1.6 ^a
	BR H ₂ S (purged)	55.7 ± 20.5 ^{bcdde}	77.6 ± 6.8 ^{ab}	41.6 ± 4.8 ^{de}	65.8 ± 8.4 ^{abcd}	47.8 ± 0.12 ^{cde}	66.0 ± 6.7 ^{abcd}
	Free MeSH (control)			0.15 ± 0.17 ⁱ	3.0 ± 0.07 ^e	3.3 ± 0.01 ^d	15.1 ± 0.04 ^a
	Free MeSH (purged)	1.3 ± 0.02 ^g	5.0 ± 0.07 ^c	< LD ⁱ	2.8 ± 0.11 ^f	0.59 ± 0.06 ^h	11.0 ± 0.06 ^b
	BR MeSH (control)			1.4 ± 0.28 ^{de}	4.4 ± 0.38 ^c	4.0 ± 0.44 ^c	15.1 ± 0.23 ^a
	BR MeSH (purged)	2.8 ± 2.08 ^{cde}	2.9 ± 0.06 ^{cde}	< LD ^a	3.6 ± 0.60 ^{cd}	3.9 ± 1.0 ^c	11.7 ± 0.32 ^b
	Redox potential (control)			84	n.m.	n.m.	-86
Redox potential (purged)	3	-59.3	91.3			-72	

LD MeSH: 0.035 µg/L; LQ MeSH: 0.12 µg/L. n.m. not measured. Redox potential is expressed in mV vs a standard Ag/AgCl reference electrode. Different superscript letters denote significant differences according ANOVA test.

down those values to 2.8 µg/L and 29 µg/L, respectively. The two-times purged wine was further subjected to RA, accumulating then 22.5 µg/L and 65.6 µg/L of free and BR H₂S, respectively, which are 60 % and 91 % of that accumulated after 2nd RA. Even if 22.5 µg/L of free H₂S is still too high a value, it represents an improvement with respect to initial values. In the case of MeSH, whose initial levels were relatively modest, the decline is not produced.

The same process was carried out with W6, a red wine that was made with grapes spiked with S powder to get a high level of VSCs and their precursors (Jastrzembski *et al.*, 2017). In this and the following cases, a control was kept throughout the process. The control suffered exactly the same operations as the wine, except for the purging processes. As can be seen, a large part of the free H₂S measured after the RA is spontaneously lost, most likely by evaporation, even in the control sample. The purging process introduces additional losses of free H₂S of 12.4 (13.2–0.79) µg/L and 16.7 (17.5–0.88) µg/L in the first and second purging processes, respectively. However, the two-times purged wine, after the 3rd RA, still accumulates 41.1 µg/L of free H₂S, an amount which is significantly, but just slightly, smaller than that accumulated by the control. A very similar result is observed for free MeSH. These results confirm that purging improves just slightly the ability of the wine to accumulate free H₂S and MeSH. The improvement cannot be appreciated in the BR fractions of both H₂S and MeSH. It should be noted, in comparison with the results obtained in W4, in which levels after RA of both free and BR H₂S, were smaller in the two-times purged wines, that in this case, final levels of all forms are maxima. This may suggest that this wine contains a higher pool of oxidised precursors of H₂S and MeSH, which is consistent with the way it was made.

The same procedure was applied to a third wine, (W7) and the same wine spiked with Cu²⁺ at 100 µg/L before the 1st RA. Both samples, W7 and W7_Cu, had particularly low levels of free H₂S after the first RA (1.5 µg/L and 0.27 µg/L, respectively), because of a previous copper finning carried out in the cellar. Under these conditions, the purging should have a limited effect on H₂S levels. However, results reveal that after the two cycles of purging, the wines develop consistently smaller levels of free and BR H₂S and MeSH than the corresponding controls. The improvements are clearly poorer in the wine containing more copper, which just confirms that copper limits, but does not prevent completely, the efficiency of the treatments. Despite the improvements, it should be noted that the problem has not been completely eradicated, since levels of free MeSH are still very high. In this case, the highest levels of free H₂S and MeSH found in the control samples after three processes of reductive ageing are particularly noteworthy. This is just the consequence that free Cu cations are no longer available, as levels of H₂S-bonded Cu have increased during ageing, as demonstrated by Zhang *et al.* (2022).

All these experiments conclude that purging a wine under reductive conditions with an inert gas has limited efficacy in solving reductive problems. Several cycles of purging

the wine in a reductive state are required to have significant effects, which can take a long time. In addition, the magnitude and relevance of the effects depend on the amounts of precursors of H₂S and MeSH present in the wine and their copper levels.

4. Strong aeration with or without polymeric thiol-functionalised silica

In the final experiments, two of the wines were subjected to an intense oxidation process during which they consumed two saturations of air. In half of the vessels, a silica functionalised with thiol was also added. The purpose of such addition was to ensure that if H₂S, during oxidation, forms major disulfides, polysulfides, or polysulfanes, most of these oxidation products remain anchored to a solid support, which should facilitate their subsequent removal by centrifugation. After three days in contact with oxygen, silica was removed and all samples were further stored in complete anoxia until they spontaneously developed negative redox potential. Their contents in free and BR forms of H₂S and MeSH after and before RA2w were analysed. Results are given in Table 5.

As can be seen, the oxidation was able to completely remove free H₂S from wine 1 and removed two-thirds from wine 5. In the presence of functionalised silica, the amount removed rose to nearly 80 %. The effects on BR forms of H₂S were also highly significant for both oxidation and the presence of silica. The small amounts of MeSH present in both wines were completely removed by oxygen.

After reductive ageing, however, all aerated samples behave similarly, regardless of the presence or absence of functionalised silica. As can be seen, all aerated samples accumulated during the anoxic ageing less than half of the free H₂S accumulated by their corresponding controls. Furthermore, the levels of free H₂S accumulated by aerated samples (between 6.3 µg/L and 7.1 µg/L in wine 1 and 7.4–7.6 µg/L in wine 5), fall within the low and safe range of H₂S accumulation, so that it can be said that the oxidation treatment was completely successful in eliminating the risk of developing “reductive” off-odours from these two red wines. This statement seems to be confirmed by the very low levels of BR H₂S and by levels of free and BR MeSH accumulated after reductive ageing, as seen in the table. These results confirm previous studies of some authors about the impact of aeration during fermentation (Bekker *et al.*, 2016; Bekker *et al.*, 2021; Day *et al.*, 2015; Day *et al.*, 2021).

The lack of effect of functionalised silica suggests that, in the present case, the oxidation induced by the wines provoked an intense formation of quinones which reacted with sulfides as nucleophiles (Nikolantonaki *et al.*, 2010; Nikolantonaki and Waterhouse, 2012), so that, basically it seems that H₂S, MeSH and, apparently, most of their forms and precursors, disappeared. It should be noted that this result is in apparent contradiction with those obtained by Vela *et al.* (2018). These authors reported that micro-oxygenation was not able to induce noticeable changes in the free or BR-forms of H₂S and MeSH, once the wine had returned to negative potentials. It can be argued, however, that micro-oxygenation

TABLE 5. Aeration as reductive remediation. Effects of oxidation, in the presence or not of thiol- functionalised silica (SH-Sil), on free and BR forms of H₂S and MeSH ($\mu\text{g/L}$), after the treatment and after 2 weeks of accelerated anoxic aging at 50 °C (3rd RA2w).

After treatment	W1			W5		
	Control	Aerated	Aerated +SH-Sil	Control	Aerated	Aerated + SH-Sil
Free H ₂ S	2.7 \pm 0.02 ^a	< LD ^c	< LQ ^b	7.0 \pm 0.2 ^a	2.2 \pm 0.1 ^b	1.4 \pm 0.3 ^c
BR H ₂ S	55.9 \pm 1.8 ^a	12.8 \pm 5.9 ^b	< LQ ^c	78.5 \pm 7.3 ^a	4.6 \pm 2.6 ^b	< LQ ^c
Free MeSH	0.51 \pm 0.01 ^a	< LD ^b	< LD ^b	0.56 \pm 0.0 ^a	< LD ^b	< LD ^b
BR MeSH	1.66 \pm 0.2 ^a	< LQ ^b	< LQ ^b	1.9 \pm 0.1 ^a	< LQ ^b	< LQ ^b
Redox potential	-15.7	+10.5	-8.9	-74.7	-55.3	-44.6
After RA2w	Control	Aerated	Aerated +SH-Sil	Control	Aerated	Aerated + SH-Sil
Free H ₂ S	15.8 \pm 1.3 ^a	6.3 \pm 0.2 ^b	7.1 \pm 1.2 ^b	20.8 \pm 0.41 ^a	7.4 \pm 1.1 ^b	7.6 \pm 0.5 ^b
BR H ₂ S	63.2 \pm 0.1 ^a	10.5 \pm 2.7 ^b	11.5 \pm 6.8 ^b	86.0 \pm 6.52 ^a	14.2 \pm 8.5 ^b	16.4 \pm 7.6 ^b
Free MeSH	1.13 \pm 0.01 ^a	0.8 \pm 0.1 ^b	0.7 \pm 0.0 ^b	0.99 \pm 0.02 ^a	< LQ ^b	< LQ ^b
BR MeSH	2.53 \pm 0.31 ^a	< LQ ^b	< LQ ^b	2.9 \pm 0.21 ^a	< LQ ^b	< LQ ^b
Redox potential	-99.7	-97.3	-93.2	-91.8	-57.2	-65.6

LD H₂S: 0.003 $\mu\text{g/L}$; LQ H₂S: 0.01 $\mu\text{g/L}$; LD MeSH: 0.035 $\mu\text{g/L}$; LQ MeSH: 0.12 $\mu\text{g/L}$. Redox potential is expressed in mV vs a standard Ag/AgCl reference electrode. Different superscript letters denote significant differences according ANOVA test.

is a mild oxidation in which O₂ levels are never very high. Under those conditions, the availability of reactive quinones would be much smaller, so that the preferential reaction of H₂S and thiols would be the formation of disulfides and polysulfides, likely by the direct reduction of the 1-HER radical formed by the Fenton-induced oxidation of ethanol (Kreitman *et al.*, 2013). Although diverse kinetic studies have demonstrated that the reaction between thiols and 1-HER is fast (de Almeida *et al.*, 2013; Lund *et al.*, 2015), results obtained here suggest that such a reaction hardly takes place if wine oxidation is very fast.

Considering the poor performance of all the different remediation strategies essayed in this paper, the result obtained in this last experiment is highly promising. However, oxidation is a double-edged knife that can promote several unpleasant changes in wine, such as browning or aroma deterioration. As the development of these detrimental changes is strongly dependent on the particular chemical composition of the wine, particularly on its polyphenolic, amino acid, and metal cation contents (Bueno *et al.*, 2018; Bueno-Aventín *et al.*, 2021), further research will be needed to assess when and how oxidation can be safely carried out as a viable remediation alternative to “reductive” off-odours.

CONCLUSION

Neither the use of precipitation adjuvants nor filtration aids can improve results obtained by classical copper fining. Even if copper can be partially removed, such removal takes place without significant reduction in the wine contents in H₂S or MeSH, or in the wine’s ability to accumulate them during aging.

Purging wine in a reductive state with inert gas can induce a significant reduction in the ability of wine to further accumulate H₂S and MeSH, but several cycles of purging are required, which may question its practical application. In addition, the overall efficiency of the process depends on the size of the pool of precursors, which, up to this date, cannot be adequately measured, and on the presence of copper.

Oxidation emerges, of all the tested strategies, as the most efficient to remove in the short and long-term, “reductive” off-odours. However, given its potential negative effects on wine quality, the development of safe procedures will require a systematic long-term research effort to understand the conditions under which H₂S and MeSH are irreversibly removed while minimising oxidation-related damage.

These conclusions have been extracted from the results obtained with a small selection of wines due to problems linked to the work under a strict anoxic atmosphere and a long observation period and although all treatments have not been tested on all wines it is evident that new strategies should be studied to remove hydrogen sulfide and methanethiol from wine, however, results of this work suggest they could be more effective if their objective is eliminating the pool of precursors.

ACKNOWLEDGEMENTS

Funding: Funded by the Spanish Ministry of Economy and Competitiveness (MINECO) (project AGL2017-87373-C3). LAAE acknowledges the continuous support of Gobierno de Aragón (T29) and the European Social Fund. D. S-G. has received a grant from the Diputación General de Aragón.

REFERENCES

- Bekker, M. Z., Day, M. P., Holt, H., Wilkes, E., & Smith, P. A. (2016). Effect of oxygen exposure during fermentation on volatile sulfur compounds in Shiraz wine and a comparison of strategies for remediation of reductive character. *Australian Journal of Grape and Wine Research*, 22(1), 24–35. <https://doi.org/10.1111/AJGW.12172>
- Bekker, M. Z., Espinase Nandorfy, D., Kulcsar, A. C., Faucon, A., Bindon, K., & Smith, P. A. (2021). Comparison of remediation strategies for decreasing 'reductive' characters in Shiraz wines. *Australian Journal of Grape and Wine Research*, 27(1), 52–65. <https://doi.org/10.1111/AJGW.12459>
- Bekker, M. Z., Kreitman, G. Y., Jeffery, D. W., & Danilewicz, J. C. (2018). Liberation of Hydrogen Sulfide from Dicysteinyl Polysulfanes in Model Wine. *Journal of Agricultural and Food Chemistry*, 66(51), 13483–13491. <https://doi.org/10.1021/acs.jafc.8b04690>
- Bertagnolli, C., Kleinübing, S. J., & da Silva, M. G. C. (2011). Preparation and characterization of a Brazilian bentonite clay for removal of copper in porous beds. *Applied Clay Science*, 53(1), 73–79. <https://doi.org/10.1016/j.clay.2011.05.002>
- Bueno, M., Marrufo-Curtido, A., Carrascón, V., Fernández-Zurbano, P., Escudero, A., & Ferreira, V. (2018). Formation and Accumulation of Acetaldehyde and Strecker Aldehydes during Red Wine Oxidation. *Frontiers in Chemistry*, 6. <https://doi.org/10.3389/fchem.2018.00020>
- Bueno-Aventín, E., Escudero, A., Fernández-Zurbano, P., & Ferreira, V. (2021). Role of Grape-Extractable Polyphenols in the Generation of Strecker Aldehydes and in the Instability of Polyfunctional Mercaptans during Model Wine Oxidation. *Journal of Agricultural and Food Chemistry*, 69(50), 15290–15300. <https://doi.org/10.1021/acs.jafc.1c05880>
- Chaves, L. H. G., & Tit, G. A. (2011). Cadmium and copper adsorption on bentonite: Effects of pH and particle size. *Revista Ciência Agronômica*, 42(2), 278–284. <https://doi.org/10.1590/S1806-66902011000200004>
- Clark, A. C., Grant-Preece, P., Cleghorn, N., & Scollary, G. R. (2015b). Copper(II) addition to white wines containing hydrogen sulfide: Residual copper concentration and activity. *Australian Journal of Grape and Wine Research*, 21(1), 30–39. <https://doi.org/10.1111/AJGW.12114/FORMAT/PDF>
- Clark, A. C., Wilkes, E. N., & Scollary, G. R. (2015a). Chemistry of copper in white wine: A review. *Australian Journal of Grape and Wine Research*, 21(3), 339–350. <https://doi.org/10.1111/ajgw.12159>
- Claus, H. (2020). How to Deal with Uninvited Guests in Wine: Copper and Copper-containing Oxidases. *Fermentation*, 6(1). <https://doi.org/10.3390/fermentation6010038>
- Day, M. P., Espinase Nandorfy, D., Bekker, M. Z., Bindon, K. A., Solomon, M., Smith, P. A., & Schmidt, S. A. (2021). Aeration of *Vitis vinifera* Shiraz fermentation and its effect on wine chemical composition and sensory attributes. *Australian Journal of Grape and Wine Research*, 27(3), 360–377. <https://doi.org/10.1111/ajgw.12490>
- Day, M. P., Schmidt, S. A., Smith, P. A., & Wilkes, E. N. (2015). Use and impact of oxygen during winemaking. *Australian Journal of Grape and Wine Research*, 21(S1), 693–704. <https://doi.org/10.1111/ajgw.12199>
- de Almeida, N. E. C., Lund, M. N., Andersen, M. L., & Cardoso, D. R. (2013). Beer Thiol-Containing Compounds and Redox Stability: Kinetic Study of 1-Hydroxyethyl Radical Scavenging Ability. *Journal of Agricultural and Food Chemistry*, 61(39), 9444–9452. <https://doi.org/10.1021/jf402159a>
- Ferreira, V., Bueno, M., Franco-Luesma, E., Culleré, L., & Fernández-Zurbano, P. (2014). Key changes in wine aroma active compounds during bottle storage of Spanish red wines under different oxygen levels. *Journal of Agricultural and Food Chemistry*, 62(41), 10015–10027. <https://doi.org/10.1021/jf503089u>
- Ferreira, V., Carrascon, V., Bueno, M., Ugliano, M., & Fernandez-Zurbano, P. (2015). Oxygen Consumption by Red Wines. Part I: Consumption Rates, Relationship with Chemical Composition, and Role of SO₂. *Journal of Agricultural and Food Chemistry*, 63(51), 10928–10937. <https://doi.org/10.1021/acs.jafc.5b02988>
- Ferreira, V., Franco-Luesma, E., Vela, E., López, R., & Hernández-Orte, P. (2018). Elusive Chemistry of Hydrogen Sulfide and Mercaptans in Wine. *Journal of Agricultural and Food Chemistry*, 66(10), 2237–2246. <https://doi.org/10.1021/acs.jafc.7b02427>
- Ferreira, V., Sánchez-Gimeno, D., & Ontañón, I. (2023). A method for the quantitative and reversible trapping of sulfidic gases from headspaces and its application to the study of wine reductive off-odors. *Food Chemistry*, 421, 136092. <https://doi.org/10.1016/j.foodchem.2023.136092>
- Franco-Luesma, E., & Ferreira, V. (2014). Quantitative analysis of free and bonded forms of volatile sulfur compounds in wine. Basic methodologies and evidences showing the existence of reversible cation-complexed forms. *Journal of Chromatography A*, 1359, 8–15. <https://doi.org/10.1016/j.chroma.2014.07.011>
- Franco-Luesma, E., & Ferreira, V. (2016a). Formation and Release of H₂S, Methanethiol, and Dimethylsulfide during the Anoxic Storage of Wines at Room Temperature. *Journal of Agricultural and Food Chemistry*, 64(32), 6317–6326. <https://doi.org/10.1021/acs.jafc.6b01638>
- Franco-Luesma, E., & Ferreira, V. (2016b). Reductive off-odors in wines: Formation and release of H₂S and methanethiol during the accelerated anoxic storage of wines. *Food Chemistry*, 199, 42–50. <https://doi.org/10.1016/j.foodchem.2015.11.111>
- Franco-Luesma, E., Sáenz-Navajas, M. P., Valentin, D., Ballester, J., Rodrigues, H., & Ferreira, V. (2016). Study of the effect of H₂S, MeSH and DMS on the sensory profile of wine model solutions by Rate-All-That-Apply (RATA). *Food Research International*, 87, 152–160. <https://doi.org/10.1016/j.foodres.2016.07.004>
- González, A., Armenta, S., Pastor, A., & de la Guardia, M. (2008). Searching the Most Appropriate Sample Pretreatment for the Elemental Analysis of Wines by Inductively Coupled Plasma-Based Techniques. *Journal of Agricultural and Food Chemistry*, 56(13), 4943–4954. <https://doi.org/10.1021/jf800286y>
- Goode, J., & Harrop, S. (2008). Wine faults and their prevalence: Data from the world's largest blind tasting. *Proceedings of the Les XX^{es} Entretiens Scientifiques Lallemand*, 7–9.
- Huang, C.-W., Walker, M. E., Fedrizzi, B., Gardner, R. C., & Jiranek, V. (2017). Hydrogen sulfide and its roles in *Saccharomyces cerevisiae* in a winemaking context. *FEMS Yeast Research*, 17(6), fox058. <https://doi.org/10.1093/femsyr/fox058>
- Jastrzebski, J. A., Allison, R. B., Friedberg, E., & Sacks, G. L. (2017). Role of Elemental Sulfur in Forming Latent Precursors of H₂S in Wine. *Journal of Agricultural and Food Chemistry*, 65(48), 10542–10549. <https://doi.org/10.1021/acs.jafc.7b04015>
- Jimenez-Lorenzo, R., Bloem, A., Farines, V., Sablayrolles, J.-M., & Camarasa, C. (2021). How to modulate the formation of negative volatile sulfur compounds during wine fermentation? *FEMS Yeast Research*, 21(5), foab038. <https://doi.org/10.1093/femsyr/foab038>

- Jiranek, V., Langridge, P., & Henschke, P. A. (1995). Regulation of hydrogen sulfide liberation in wine-producing *Saccharomyces cerevisiae* strains by assimilable nitrogen. *Applied and Environmental Microbiology*, *61*(2), 461–467. <https://doi.org/10.1128/AEM.61.2.461-467.1995>
- Kinzurik, M. I., Herbst-Johnstone, M., Gardner, R. C., & Fedrizzi, B. (2015). Evolution of Volatile Sulfur Compounds during Wine Fermentation. *Journal of Agricultural and Food Chemistry*, *63*(36), 8017–8024. <https://doi.org/10.1021/acs.jafc.5b02984>
- Kontoudakis, N., Mierczynska-Vasilev, A., Guo, A., Smith, P. A., Scollary, G. R., Wilkes, E. N., & Clark, A. C. (2019). Removal of sulfide-bound copper from white wine by membrane filtration. *Australian Journal of Grape and Wine Research*, *25*(1), 53–61. <https://doi.org/10.1111/ajgw.12360>
- Kreitman, G. Y., Danilewicz, J. C., Jeffery, D. D. W., & Elias, R. J. (2017). Copper(II)-mediated hydrogen sulfide and thiol oxidation to disulfides and organic polysulfanes and their reductive cleavage in wine: Mechanistic elucidation and potential applications. *Journal of Agricultural and Food Chemistry*, *65*(12), 2564–2571. <https://doi.org/10.1021/acs.jafc.6b05418>
- Kreitman, G. Y., Danilewicz, J. C., Jeffery, David. W., & Elias, R. J. (2016a). Reaction Mechanisms of Metals with Hydrogen Sulfide and Thiols in Model Wine. Part 1: Copper-Catalyzed Oxidation. *Journal of Agricultural and Food Chemistry*, *64*(20), 4095–4104. <https://doi.org/10.1021/ACS.JAFC.6B00641>
- Kreitman, G. Y., Danilewicz, J. C., Jeffery, David. W., & Elias, R. J. (2016b). Reaction Mechanisms of Metals with Hydrogen Sulfide and Thiols in Model Wine. Part 2: Iron- and Copper-Catalyzed Oxidation. *Journal of Agricultural and Food Chemistry*, *64*(20), 4105–4113. <https://doi.org/10.1021/ACS.JAFC.6B00642>
- Kreitman, G. Y., Laurie, V. F., & Elias, R. J. (2013). Investigation of Ethyl Radical Quenching by Phenolics and Thiols in Model Wine. *Journal of Agricultural and Food Chemistry*, *61*(3), 685–692. <https://doi.org/10.1021/jf303880g>
- Lund, M. N., Krämer, A. C., & Andersen, M. L. (2015). Antioxidative Mechanisms of Sulfite and Protein-Derived Thiols during Early Stages of Metal Induced Oxidative Reactions in Beer. *Journal of Agricultural and Food Chemistry*, *63*(37), 8254–8261. <https://doi.org/10.1021/acs.jafc.5b02617>
- Müller, N., & Rauhut, D. (2018). Recent Developments on the Origin and Nature of Reductive Sulfurous Off-Odors in Wine. *Fermentation*, *4*(3). <https://doi.org/10.3390/fermentation4030062>
- Müller, N., Rauhut, D., & Tarasov, A. (2022). Sulfane Sulfur Compounds as Source of Reappearance of Reductive Off-Odors in Wine. *Fermentation*, *8*(2). <https://doi.org/10.3390/fermentation8020053>
- Nikolantonaki, M., Chichuc, I., Teissedre, P.-L., & Darriet, P. (2010). Reactivity of volatile thiols with polyphenols in a wine-model medium: Impact of oxygen, iron, and sulfur dioxide. *Analytica Chimica Acta*, *660*(1-2), 102–109. <https://doi.org/10.1016/j.aca.2009.11.016>
- Nikolantonaki, M., & Waterhouse, A. L. (2012). A Method To Quantify Quinone Reaction Rates with Wine Relevant Nucleophiles: A Key to the Understanding of Oxidative Loss of Varietal Thiols. *Journal of Agricultural and Food Chemistry*, *60*(34), 8484–8491. <https://doi.org/10.1021/jf302017j>
- Ontañón, I., Sánchez, D., Sáez, V., Mattivi, F., Ferreira, V., & Arapitsas, P. (2020). Liquid Chromatography-Mass Spectrometry-Based Metabolomics for Understanding the Compositional Changes Induced by Oxidative or Anoxic Storage of Red Wines. *Journal of Agricultural and Food Chemistry*, *68*(47), 13367–13379. <https://doi.org/10.1021/acs.jafc.0c04118>
- Scott, W. T., Smid, E. J., Notebaart, R. A., & Block, D. E. (2020). Curation and Analysis of a *Saccharomyces cerevisiae* Genome-Scale Metabolic Model for Predicting Production of Sensory Impact Molecules under Enological Conditions. *Processes*, *8*(9). <https://doi.org/10.3390/pr8091195>
- Siebert, T. E., Solomon, M. R., Pollnitz, A. P., & Jeffery, D. W. (2010). Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection. *Journal of Agricultural and Food Chemistry*, *58*(17), 9454–9462. <https://doi.org/10.1021/jf102008r>
- Song, Y., Gibney, P., Cheng, L., Liu, S., & Peck, G. (2020). Yeast Assimilable Nitrogen Concentrations Influence Yeast Gene Expression and Hydrogen Sulfide Production During Cider Fermentation. *Frontiers in Microbiology*, *11*. <https://www.frontiersin.org/articles/10.3389/fmicb.2020.01264>
- Spiropoulos, A., Tanaka, J., Flerianos, I., & Bisson, L. F. (2000). Characterization of Hydrogen Sulfide Formation in Commercial and Natural Wine Isolates of *Saccharomyces*. *American Journal of Enology and Viticulture*, *51*(3), 233–248. <https://doi.org/10.5344/ajev.2000.51.3.233>
- Swiegers, J. H., & Pretorius, I. S. (2007). Modulation of volatile sulfur compounds by wine yeast. *Applied Microbiology and Biotechnology*, *74*(5), 954–960. <https://doi.org/10.1007/s00253-006-0828-1>
- The_AWRI. (2021, October 7). In YouTube. Extending the shelf life of canned wines. <https://www.youtube.com/watch?v=IpkefsHeT0o>
- Ugliano, M., Fedrizzi, B., Siebert, T., Travis, B., Magno, F., Versini, G., & Henschke, P. A. (2009). Effect of Nitrogen Supplementation and *Saccharomyces* Species on Hydrogen Sulfide and Other Volatile Sulfur Compounds in Shiraz Fermentation and Wine. *Journal of Agricultural and Food Chemistry*, *57*(11), 4948–4955. <https://doi.org/10.1021/jf8037693>
- Ugliano, M., Kolouchova, R., & Henschke, P. A. (2011). Occurrence of hydrogen sulfide in wine and in fermentation: Influence of yeast strain and supplementation of yeast available nitrogen. *Journal of Industrial Microbiology and Biotechnology*, *38*(3), 423–429. <https://doi.org/10.1007/s10295-010-0786-6>
- Ulmanu, M., Marañón, E., Fernández, Y., Castrillón, L., Anger, I., & Dumitriu, D. (2003). Removal of Copper and Cadmium Ions from Diluted Aqueous Solutions by Low Cost and Waste Material Adsorbents. *Water, Air, and Soil Pollution*, *142*(1), 357–373. <https://doi.org/10.1023/A:1022084721990>
- van Leeuwen, K. A., Nardin, T., Barker, D., Fedrizzi, B., Nicolini, G., & Larcher, R. (2020). A novel LC-HRMS method reveals cysteinyl and glutathionyl polysulfides in wine. *Talanta*, *218*, 121105. <https://doi.org/10.1016/j.talanta.2020.121105>
- Vela, E., Hernández-Orte, P., Franco-Luesma, E., & Ferreira, V. (2017). The effects of copper fining on the wine content in sulfur off-odors and on their evolution during accelerated anoxic storage. *Food Chemistry*, *231*, 212–221. <https://doi.org/10.1016/j.foodchem.2017.03.125>
- Vela, E., Hernandez-Orte, P., Franco-Luesma, E., & Ferreira, V. (2018). Micro-oxygenation does not eliminate hydrogen sulfide and mercaptans from wine; it simply shifts redox and complex-related equilibria to reversible oxidized species and complexed forms. *Food Chemistry*, *243*, 222–230. <https://doi.org/10.1016/j.foodchem.2017.09.122>
- Vos, P. J. A., & Gray, R. S. (1979). The Origin and Control of Hydrogen Sulfide during Fermentation of Grape Must. *American Journal of Enology and Viticulture*, *30*(3), 187–197. <https://doi.org/10.5344/ajev.1979.30.3.187>

Walker, M. E., Zhang, J., Sumbly, K. M., Lee, A., Houllès, A., Li, S., & Jiranek, V. (2021). Sulfate transport mutants affect hydrogen sulfide and sulfite production during alcoholic fermentation. *Yeast*, 38(6), 367–381. <https://doi.org/10.1002/yea.3553>

Wang, L. L., & Xiong, Y. L. (2005). Inhibition of Lipid Oxidation in Cooked Beef Patties by Hydrolyzed Potato Protein Is Related to Its Reducing and Radical Scavenging Ability. *Journal of Agricultural and Food Chemistry*, 53(23), 9186–9192. <https://doi.org/10.1021/jf051213g>

Zhang, X., Blackman, J. W., Prenzler, P. D., & Clark, A. C. (2022). Suppression of reductive characters in white wine by Cu fractions: Efficiency and duration of protection during bottle aging. *Food Chemistry*, 393, 133305. <https://doi.org/10.1016/j.foodchem.2022.133305>

Zhang, X., Bowyer, P., Scollary, G. R., Clark, A. C., & Kontoudakis, N. (2021a). Sulfide-bound copper removal from red and white wine using membrane and depth filters: Impacts of oxygen, H₂S-to-Cu ratios, diatomaceous earth and wine volume. *Food Chemistry*, 131758. <https://doi.org/10.1016/J.FOODCHEM.2021.131758>

Zhang, X., Kontoudakis, N., Wilkes, E., Scrimgeour, N., Hirlam, K., & Clark, A. C. (2021b). The removal of Cu from wine by copolymer PVI/PVP: Impact on Cu fractions and binding agents. *Food Chemistry*, 357, 129764. <https://doi.org/10.1016/J.FOODCHEM.2021.129764>