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Effect of glass swirling on the removal of 'reductive' off-odours caused by H_2S in wine

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ABSTRACT

'Reductive' off-odours, particularly those associated with hydrogen sulfide (H_2S), can greatly affect the sensory quality of wine by masking desirable fruity aromas. This study investigated the effect of glass swirling on the volatilisation of free H_2S and its subsequent influence on aroma perception in model and Chenin blanc wine. Sensory analysis and chemical measurements were conducted to evaluate the changes in 'reductive' off-odours and 'fruity' attributes following glass swirling and the exposure of wine to ambient air conditions after swirling. H_2S analyses, conducted under ambient and in an anaerobic environment, revealed that a decrease in H_2S concentrations after swirling was mainly due to volatilisation and not oxidation. Varietal thiol concentrations, however, were not significantly influenced by these treatments. High H_2S concentrations were closely associated with increased 'reductive' off-odours and diminished 'fruity' characteristics. Swirling for 30 seconds significantly decreased the perception of 'reductive' aromas while enhancing 'fruity' attributes such as 'pineapple', 'passionfruit', and 'peach/apricot'. Overall, leaving the glass open post-swirling did further improve the aroma perception of the fruity characters; however, this effect was strongly influenced by the concentration of H_2S and the wine matrix.

KEYWORDS: wine glass swirling, reductive off-odours, Chenin blanc, hydrogen sulfide

INTRODUCTION

'Reductive' volatile sulfur compounds (VSCs), primarily hydrogen sulfide (H_2S) and methanethiol (MeSH), can cause off-odours associated with 'rotten egg', 'cabbage', and 'cooked vegetable', respectively (Franco-Luesma *et al.*, 2016; Siebert *et al.*, 2010). These compounds often appear during fermentation as byproducts of yeast metabolism (Kreitman *et al.*, 2019; Smith *et al.*, 2015; Swiegers *et al.*, 2005). Oxygen-limiting winemaking practices (Bekker *et al.*, 2016a; Bekker *et al.*, 2018; Smith *et al.*, 2015) or reductive bottle storage conditions (Franco-Luesma & Ferreira, 2014; Franco-Luesma & Ferreira, 2016; Ugliano *et al.*, 2011) may promote the formation of these unwanted aromas after bottling and during storage.

Winemakers often utilise reductive winemaking and bottling conditions to prevent oxidation and to preserve desirable varietal-thiols, such as 3-sulfanylhexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHA) in wine (Danilewicz, 2012; Nikolantonaki & Waterhouse, 2012). These varietal thiols have very low sensory thresholds (4.2 and 60 ng/L for 3SHA and 3SH, respectively) and can be sensitive to oxidation (Coetzee & Du Toit, 2015) and volatilisation. The preservation of these key varietal thiols is imperative in wines such as Sauvignon blanc and Chenin blanc, where these compounds contribute to the desirable tropical aromas of 'passionfruit', 'grapefruit', and 'guava' (Lund *et al.*, 2009; Wilson, 2017; Coetzee & Du Toit, 2012). However, similar reductive conditions that protect these varietal thiols can also promote the formation of 'reductive' VSCs, leading to unwanted off-odours (Ugliano *et al.*, 2011).

Bottled wines stored for extended periods are often described as 'closed' or displaying age-related 'reductivity' (Franco-Luesma & Ferreira, 2016; Franco-Luesma *et al.*, 2016). In such cases, sommeliers and other wine experts anecdotally recommend aerating the wine by swirling the glass. The orbital shaking or the action of swirling the glass prior to the sensory evaluation of a wine is done to increase the surface area of the wine in contact with air, which is believed to aid in the evaporation/volatilisation of unwanted aroma compounds and increase oxygen pick-up (Paola *et al.*, 2018), and hence facilitate oxidative reactions of VSC in the wine. These oxidation processes of VSC in wine are slow, especially when antioxidants such as sulfur dioxide (SO_2) are present.

Quinones that are formed through the oxidation of phenolic compounds in wines react rapidly with H_2S (Nikolantonaki & Waterhouse, 2012). However, varietal thiols and H_2S also compete with SO_2 (which is more concentrated in wines) for reaction with quinones (Bekker *et al.*, 2016b). Furthermore, the direct oxidation of 'reductive' compounds might also occur (Kreitman *et al.*, 2017; Kreitman *et al.*, 2019). The alternative hypothesis suggests that 'reductive' compounds volatilise during the swirling process of wine in a glass. These compounds are highly volatile due to their low boiling points (López *et al.*, 2007), especially when compared to other wine constituents (Davis & Qian, 2019). Hence, swirling 'reductive' wine in a glass could enhance the evaporation of these compounds, allowing them to escape, which subsequently decreases their direct and indirect effects on the perception of the wine's fruity character (Franco-Luesma *et al.*, 2016).

Limited studies have investigated the effects of H_2S and MeSH on the sensory characteristics of wine (Franco-Luesma *et al.*, 2016; Pavelescu *et al.*, 2021; Zhang *et al.*, 2022). A knowledge gap thus exists regarding the effects of moderate and high free H_2S concentrations in wines containing varietal thiols on the overall aroma character. Swirling such wines in a glass before sensory evaluation is often employed by wine consumers to lower the suppressive effects of H_2S on the 'tropical' and 'fruity' aromas. However, to the best of our knowledge, the mechanism of decreasing levels of 'reductive' off-odours in wine by glass swirling has not yet been scientifically demonstrated.

This study aimed to investigate the effects of the sensory suppression of low and high free H_2S concentrations on the 'fruity' and 'tropical' aromas in a varietal thiol-containing model and Chenin blanc white wine. Furthermore, the chemical and sensory effects of wine glass swirling were quantified in the wines containing free H_2S and varietal thiols. Finally, the possible volatilisation or oxidation causes of the loss of free H_2S in the swirled wine samples were investigated and examined.

MATERIALS AND METHODS

1. Experimental design

1.1. Varietal thiol and hydrogen sulfide combinations

Model wines were prepared by adding two different concentrations of 3SH and 3SHA, creating a low (T: 600 ng/L + 42 ng/L) or high (hT: 1,500 ng/L + 125 ng/L) varietal thiol treatment. These concentrations were based on the previously reported levels in South African Sauvignon blancs and Chenin blancs (Coetzee *et al.*, 2018). Each model wine was further supplemented with low (H_2S: 4 µg/L) or higher (hH_2S: 8 µg/L) H_2S concentrations, resulting in four treatment combinations (Table S1), which were combinations of low varietal (T) and high varietal (hT) thiol additions, as well as low H_2S (H_2S) and high H_2S (hH_2S) additions.

In Chenin blanc (CH) wine, the natural concentrations of varietal thiols were not modified, but were similar to the (hT) treatments of the model wine. H_2S was added at 10 µg/L (H_2S) and 20 µg/L (hH_2S) (Table S2). These concentrations were previously reported in the higher concentration range in commercial wines investigated by Siebert *et al.* (2010). The natural H_2S concentration in this wine was below the level of detection of the 4LL gas-detection tubes used (refer to section 2.5.2.).

1.2. Glass swirling and serving treatments for sensory evaluation

Each treatment was served in ISO-standard wine glasses at 20 ± 2 °C, using 25 mL per sample, covered with Petri dish lids. Wines were presented to 10 trained panellists within two minutes after the wines were poured. The following glass swirling treatments were performed (refer to Tables 1 and 2).

- (T) and (T + H_2S): poured and covered for 15 minutes prior to sensory evaluation.
- (T + H_2S + S): poured, swirled in an uncovered glass for 30 seconds and then covered for 15 minutes prior to sensory evaluation.
- (T + H_2S + S + O): poured, swirled in an uncovered glass for 30 seconds, left open for 15 minutes, and then covered just before sensory analysis.

The swirling was performed manually at approximately 1.5 to 2 swirls per second for 30 seconds. The 15-minute waiting period ensured that the H_2S present in the solution reached equilibrium in the headspace of the glass (Franco-Luesma *et al.*, 2016). The same protocol was followed for the Chenin blanc wines (Tables S1 and S2).

2. Model wine and real wine preparation

2.1. Model wine

Model wine was prepared using Milli-Q water, 5 g/L tartaric acid (Brenn-O-Kem, South Africa), 12.5 % ethanol (96 % ethanol, Protea Chemicals, South Africa), and adjusted to pH 3.5 using sodium hydroxide (NaOH, Sigma-Aldrich, USA). The model wine was prepared and stored in 10 L Cornelius stainless steel kegs (Safer, Italy) and sparged with nitrogen gas (Afrox, South Africa) until the dissolved oxygen levels were lower than 0.3 mg/L. Oxygen concentrations were measured using a NomaSense O_2 P300 meter (Normacorc, Germany).

The model wine was transferred from the kegs into argon-flushed 440 mL brown bottles (Consol, South Africa) under complete oxygen exclusion (N_2 pressure gas), sealed with crown caps (BeerLab, South Africa) and cooled to 4 °C before the addition of the varietal thiol compounds.

2.2. Chenin blanc wine

A 2022 commercial Chenin blanc white wine was sourced from the Breedekloof Wine Valley (Worcester, South Africa), stored in 20 L stainless steel kegs (Safer, Italy), and sparged with nitrogen gas to achieve dissolved oxygen levels of lower than 0.3 mg/L. The wine was then bottled using the same conditions and bottles as the model wines. Bottled samples were cooled to 4 °C before H_2S additions. The wine's composition was as follows: glucose 0.00 g/L, fructose 0.9 g/L, total acidity 5.3 g/L, pH 3.48, ethanol 12.4 % (analysed with WineScan FT 120 instrument, FOSS Analytical, Hillerød, Denmark, as described in Nieuwoudt *et al.* (2006)), and free SO_2 39 mg/L and total SO_2 101 mg/L (Ripper method as described in Walls *et al.* (2022), using a R2009 Metrohm 862 Compact Titrosampler, Herisau, Switzerland).

TABLE 1. Experimental design for model wine aroma evaluation.

	No H_2S addition, no swirling and glass closed	H_2S addition, no swirling and glass closed	H_2S addition, swirled + glass closed	H_2S addition, swirled + glass uncovered
Treatment 1	Low varietal thiols (T)	Low H_2S (T + H_2S)	T + H_2S + S	T + H_2S + S + O
Treatment 2	Low varietal thiols (T)	High H_2S (T + hH_2S)	T + hH_2S + S	T + hH_2S + S + O
Treatment 3	High varietal thiols (hT)	Low H_2S (hT + H_2S)	hT + H_2S + S	hT + H_2S + S + O
Treatment 4	High varietal thiols (hT)	High H_2S (hT + hH_2S)	hT + hH_2S + S	hT + hH_2S + S + O

Note: Model wines were treated with different concentrations of varietal thiols (low: T, high: hT) and hydrogen sulfide (low: H_2S, high: hH_2S). Wines were either covered after swirling (S) or left open after swirling (S + O).

TABLE 2. Experimental design for Chenin blanc wine aroma evaluation.

	No H_2S addition, no swirling and glass closed	H_2S addition, no swirling and glass closed	H_2S addition, swirled + closed	H_2S addition swirled + uncovered
Treatment 1	CH	Low H_2S (CH + H_2S)	CH + H_2S + S	CH + H_2S + S + O
Treatment 2	CH	High H_2S (CH + hH_2S)	CH + hH_2S + S	CH + hH_2S + S + O

Note: Chenin blanc wines with naturally higher varietal thiol concentrations were treated with low (H_2S) and high (hH_2S) hydrogen sulfide levels. Wines were either covered after swirling (S) or left open after swirling (S + O).

2.3. Chemical compounds and additions

2.3.1. Addition of varietal thiols to model wine

The model wine bottles were placed in a custom-made glove box (Figure S1) filled with argon (O_2 < 1 %) and spiked with 3SH + 3SHA (Interchim, France). The 3SH and 3SHA stock solutions were stored at –80 °C, and their concentrations were confirmed using Ellman's reagent.

After the varietal thiol addition, the bottles were instantly sealed with new crown caps under a blanket of argon in the anoxic glove box (O_2 < 1 %) and stored at 4 °C. The selected bottles were then opened and spiked with H_2S in the same anoxic glove box when required, using freshly prepared 50 mg/L H_2S stock solution (details below). The bottles were immediately resealed under argon, using new crown caps in the anoxic glove box.

2.3.2. Hydrogen sulfide (H_2S) stock solution preparation and additions

The H_2S stock (50 mg/L) was prepared using food-grade ammonium sulfide solution ($(NH_4)_2S$, 20 %), Sigma-Aldrich, South Africa). Glassware was deactivated (deionised) using Extran MA 05 (Merck, South Africa), rinsed, and oven-dried (90 °C, 8 hours). Milli-Q water (4 °C) was degassed, and the ammonium sulfide liquid was added in an inflatable anoxic bag (Erlab's Captair Pyramid glove bag, Labotec, South Africa), which was flushed with argon gas (99.9 %, Afrox, South Africa) until O_2 < 1 %. The H_2S stock solution was aliquoted into 20 mL PTFE-sealed vials (Separation Scientific, South Africa) and used immediately.

Before opening, the control wine bottles (model wines with added varietal thiols and Chenin blanc wines) and H_2S stock solution were placed in the custom-made anoxic glove box, then flushed with argon (10 L/min for 30 minutes), reaching O_2 < 1 % before these were opened and H_2S treatments added. The bottles were sealed instantly in the anoxic chamber after H_2S additions.

2.4. Sensory analysis

A trained sensory panel (10 females, aged 30–63) with prior sensory experience in evaluating varietal thiols and 'reductive' aromas participated in this study. During training, the panel was instructed not to swirl the glass, but to smell the wine sample for a maximum of 40 seconds, followed by a one-minute break.

To familiarise the panel with the smell of the model wine matrix and prevent them from focusing on the smell of alcohol, a marked glass containing only model wine was presented to every panellist at the start of each sensory training session.

The first two two-hour sensory training sessions were dedicated to generating descriptors of the sensory attributes present in the wine samples. An extra two-hour session was used to narrow down the number of attributes used. The panel generated a total of 15 sensory attributes to describe the aromas present in the model wine. The reference standards used to train the sensory panel are listed in Table S3.

The 15 sensory attributes that were generated were used to construct a CATA (check-all-that-apply) sensory lexicon. The CATA analysis was performed in triplicate over two separate days. The purpose of the CATA analysis was to assess whether the differences between the treatments were qualitative or quantitative. Samples were presented with three-digit codes and randomised within each flight using a Williams Latin square design.

After CATA analysis, six hours of sensory training were done prior to the descriptive analysis (DA) over three separate days to calibrate the panel in terms of intensity rating consensus and line scale usage. The panellists were asked to rate each attribute selected during the CATA analysis on a scale from 0 to 100. The DA testing sessions for the model wine-based trial were conducted in triplicate, resulting in one replicate of a treatment being evaluated each day.

Prior to the DA sessions for the Chenin blanc-based trial, the panel received four hours of training. The DA testing sessions for the Chenin blanc trial were also done in triplicate over three separate days. The sensory data were captured using the Compusense cloud software package (Compusense Inc., Guelph, ON, Canada). All sensory data was collected using Compusense Cloud (Compusense, Canada).

2.5. Chemical analyses

Chemical analyses of the model wine and Chenin blanc treatments were performed after swirling to elucidate the chemical mechanisms behind the sensory observations. Sampling was first conducted under ambient air conditions, mimicking those used for sensory analysis, and then repeated in the argon-flushed custom-made anoxic glove box (O_2 < 1 %, as measured with NomaSense O_2 P300 meter (Normacorc, Germany)).

2.5.1. Varietal thiols

Sampling of treated wines followed the exact timing and swirling treatments as prior to sensory analysis. The equivalent of 20 mL was collected from wine samples in ISO glasses, immediately sealed in the glass shell vials (flushed with argon), and then stored for analysis. The extraction of varietal thiol from the model wine and the real Chenin blanc wine was done as described by Mafata *et al.* (2018). Briefly, wine (20 mL) was derivatised with an excess of 4,4′-dithiodipyridine (Sigma-Aldrich (Louisville, MO, USA) and extracted using solid-phase extraction, using (SPE) cartridges (Supelclean ENVI-18 SPE) from Supelco (Bellefonte, PA, USA). The resulting sample was analysed using a Waters Acquity UltraPerformance Convergence Chromatograph (UPC2) using a Waters Viridis BEH 2EP Column (130 Å, 1.7 µm, 3 mm × 100 mm; Waters, MA, USA). Standards for 3SH and 3SHA were purchased from Sigma-Aldrich (Louisville, MO, USA).

2.5.2. Hydrogen sulfide (H_2S)

Model and Chenin blanc wines were treated with the same high H_2S concentrations (20 µg/L) for the chemical analysis using 4LL gas-detection tubes (GDT) (GASTEC Corporation, Japan) due to the quantification limit (LOQ) of H_2S GDT being 3.5 µg/L, and the authors wanted to ensure that the swirling effects on H_2S concentrations were measurable.

Schott glass bottles (250 mL) were used to perform the experiment instead of ISO glasses to avoid losing H_2S in the sample transfer. Another reason was that during the adapted GDT-H_2S analysis (Allison *et al.*, 2022), the glass vessel used should have withstood the pressure introduced by sparging with N_2 (Allison *et al.*, 2022; Chen *et al.*, 2017). Importantly, in this experiment, the ratio of headspace to liquid in the Schott glass bottles and the ISO glass was designed to be similar.

Each wine sample (50 mL) was added to each Schott bottle container and covered immediately (Figure S2). The treatments were then done as for the sensory analysis (Tables S1 and S2) in three repeats: "control", "glass closed", "swirled + closed", and "swirled + uncovered". The swirling was conducted under both ambient and in the anoxic chamber filled with argon (O_2 < 1 %) to evaluate the effect of oxygen on H_2S loss.

2.5.3. Setup of hydrogen sulfide gas-detection tube analysis

The H_2S GDT sparging setup was adapted and slightly simplified from the H_2S GDT sparging setup described by Chen *et al.* (2017) and later improved by Allison *et al.* (2022) to achieve simultaneous and faster sample analysis.

A 250 mL Schott bottle was sealed with a repurposed fermentation barrel bung (Figure S2). The central hole of the bung served as the nitrogen inlet, and the H_2S GDT outlet was inserted in the side hole (Figure S2). The polyvinylchloride (PVC) tubing connected the nitrogen gas source to a manifold containing 12 outlets, ensuring simultaneous sparging of wine samples (30 minutes) (Figure S3).

Previous work demonstrated that SO_2 significantly interfered with the quantification of H_2S when using GDT (Allison *et al.*, 2022). Therefore, an alternative method for preventing SO_2 interference was developed, expanding on the previously suggested use of SO_2 GDT described by Allison *et al.* (2022), as pre-treatment. The amount of acetaldehyde (ACS reagent ≥ 99.5 %, Sigma-Aldrich) needed to bind with SO_2 was calculated and injected through the N_2 sparging inlet. This allowed for the binding of free SO_2 while preventing H_2S from escaping. This targeted method of using acetaldehyde addition to bind with SO_2 successfully prevented the interference of SO_2 in the H_2S GDT readings. The recovery of H_2S after acetaldehyde addition to model wine containing SO_2 was the same as the samples where only H_2S was added without SO_2 and acetaldehyde (Table S4).

2.6. Statistical analyses

Chi-square analysis and correspondence analysis were performed on the contingency table created from the attribute citation frequencies of the CATA data. Mixed-model analysis of variance (ANOVA) was performed on the intensity scores captured during the DA sensory evaluation sessions, with 'judge' and 'repeat' as random factors and 'treatment' as fixed factors. Fisher's post-hoc test was used to determine pairwise comparisons when a significant ANOVA result was found ($p < 0.05$). Analyses were done using the R package "lme4". Graphs were produced in Statistica (TIBCO Statistica® 14.1.0). Principal component analysis was performed on the correlation matrix of the significant sensory attribute intensity scores averaged over all the panellists. One-way ANOVA with 'treatment' as a factor was performed on the chemical data. Levene's test was done to test for homogeneity of variance. Normal probability plots were investigated to check for normality and found to be acceptable. Fisher's LSD post-hoc test was performed when a significant ANOVA result was found ($p < 0.05$).

RESULTS AND DISCUSSION

1. Chemical analysis

1.1. Varietal thiols

Varietal thiols 3SH and 3SHA combinations were added to model wine at concentrations of 600 ng/L + 42 ng/L or 1,500 ng/L + 125 ng/L, respectively. Maintaining an approximately 1:1 odour active values ratio (OAVs) (Benkwitz *et al.*, 2012). Moreover, these concentrations fall within the range of concentrations found in South African Sauvignon blanc and Chenin blanc wines (Coetzee *et al.*, 2018; Wilson, 2017).

In the Chenin blanc control samples ("CH"), the detected concentrations of 3SH and 3SHA were 1,552 ng/L and 326 ng/L, respectively. When higher thiol concentrations ("hT") were added to the model wine, the resulting concentrations were 1,256 ng/L and 91 ng/L, respectively. The swirling of the glass prior to 3SH and 3SHA quantification was done in ambient air. There were no significant differences in 3SH and 3SHA concentrations when both model and Chenin blanc wines were swirled and left open post-swirling (Figures S4 and S5). This demonstrates that oxidation or volatilisation did not markedly affect thiol concentrations under these conditions.

1.2. Hydrogen sulfide

In both the model (results not shown) and the Chenin blanc wines (Figure 1), free H_2S concentrations declined in a similar fashion when wines were swirled, and the glasses were left uncovered in ambient air. To determine whether the loss of H_2S was due to oxidation or volatilisation, the experiment was repeated in the anoxic chamber (argon environment). The observed H_2S losses followed a similar pattern in both the model wine and the Chenin blanc wine, respectively (refer to Figure 1 for the Chenin blanc data).

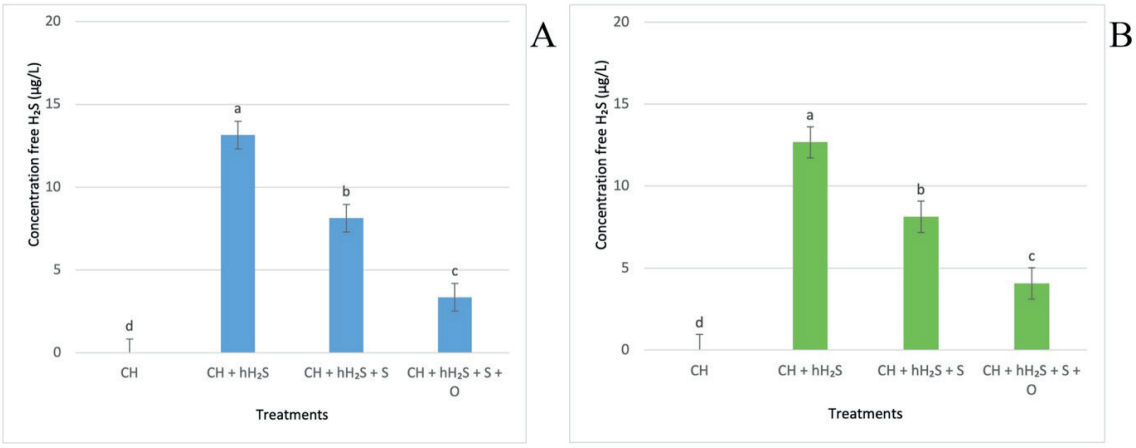


FIGURE 1. Concentrations (µg/L) of high hydrogen sulfide (hH_2S) added to the Chenin blanc with naturally high varietal thiol concentrations (CH). The swirling treatments were done in ambient air (a) and under anoxic argon (Ar) atmosphere (b). The treated Chenin blanc wines (hT + hH_2S) were swirled for 30 seconds in Schott bottles and covered for 15 minutes (hT + hH_2S + S); or swirled for 30 seconds in Schott bottles and left uncovered for 15 minutes. Vertical bars indicate 0.95 confidence intervals. Different letters indicate a significant difference ($p < 0.05$).

This suggests that the loss of H_2S was due to volatilisation rather than oxidation.

In the model wine, free H_2S decreased by 70 % in the ambient air and 72 % in the anoxic chamber, confirming that volatilisation was the primary reason for the loss of free H_2S in the model wine. These results align with the previous findings by Franco-Luesma *et al.* (2016), who reported 70 % of free H_2S being present in the headspace of the wine glass after 15 minutes of equilibration time in ambient air.

In Chenin blanc wine, swirling resulted in a 38 % decrease of free H_2S in ambient air and 36 % in the anoxic chamber (Figure 1). When the wine was left open and exposed to oxygen, free H_2S declined by 97 % in ambient air and 86 % in the anoxic chamber. The more significant loss in ambient air suggests that oxidation contributed to H_2S depletion, though volatilisation remains the dominant mechanism. However, trace metal ions in real wine may facilitate oxidation in the absence of molecular oxygen (Kreitman *et al.*, 2017), complicating direct comparisons between model and real wine systems. Similarly, the presence of trace metals, as well as other wine compounds naturally present in wine that can bind free H_2S, also inherently complicates the direct comparison between the model and real wine systems.

The findings of this study suggest that while oxidation may play a minor role, volatilisation is the primary driver of H_2S loss when wine is swirled either in a glass or in a decanter and exposed to air.

2. Sensory analysis

2.1. Effects of glass swirling in model wine

In the model wine, the free H_2S and swirling treatments were associated with different effects on the sensory perception of the 'fruity' character of the model wine, depending on the concentrations of the varietal thiols. Principal component analysis (PCA) biplots showed some consistent patterns (Figures 2 and 3) in all these treatments. The control wines that contained only varietal thiols ("T" or "hT") were associated with 'fruity' attributes. In contrast, all the model wines treated with H_2S and not swirled were associated with 'reductive' attributes, positioned on the opposite side of the biplots. The swirling treatments occupied intermediate positions, with samples that were only swirled ("S") clustered closer to the 'reductive' H_2S-treated samples, while those that were swirled and left open ("S + O") were associated with more 'fruity' attributes.

In model wines containing low varietal-thiol concentrations ("T"), the addition of low H_2S levels increased 'boiled egg' and 'sulfur/reductive' off-odours compared to the control (Figure 4). High H_2S concentrations in the low varietal-thiol model wine suppressed the 'passionfruit' sensory attribute (Figure 5). Interestingly, swirling appeared to restore the 'passionfruit' attribute to the same levels as in the control samples before a significant decrease in 'reductive' off-odours occurred. However, the decrease in 'boiled egg' and 'sulfur/reductive' off-odours required the additional step of leaving the glass open after swirling (Figure 5).

The PCA biplot in Figure 3A indicates how the grouping of "hT", "hT + H_2S + S" and "hT + H_2S + S + O" was opposite to that of "hT + H_2S". These results highlight the suppressive effects of H_2S on the 'fruity' attributes and that the negative effects of H_2S were easily removed by a 30-second glass swirling in model wines with high varietal-thiol concentrations and low H_2S concentrations. In model wines with high varietal-thiol concentrations ("hT"), low H_2S concentrations did not decrease the 'passionfruit' aroma (Figure 6). The PCA biplot in Figure 3A and the DA analysis in Figure 6 graphically illustrate the association between 'boiled egg' off-odour and the "T + H_2S" treatment. However, covering the glasses after swirling only decreased the 'boiled egg' off-odour significantly, while increasing the 'passionfruit' and 'grass/fresh green' attributes compared

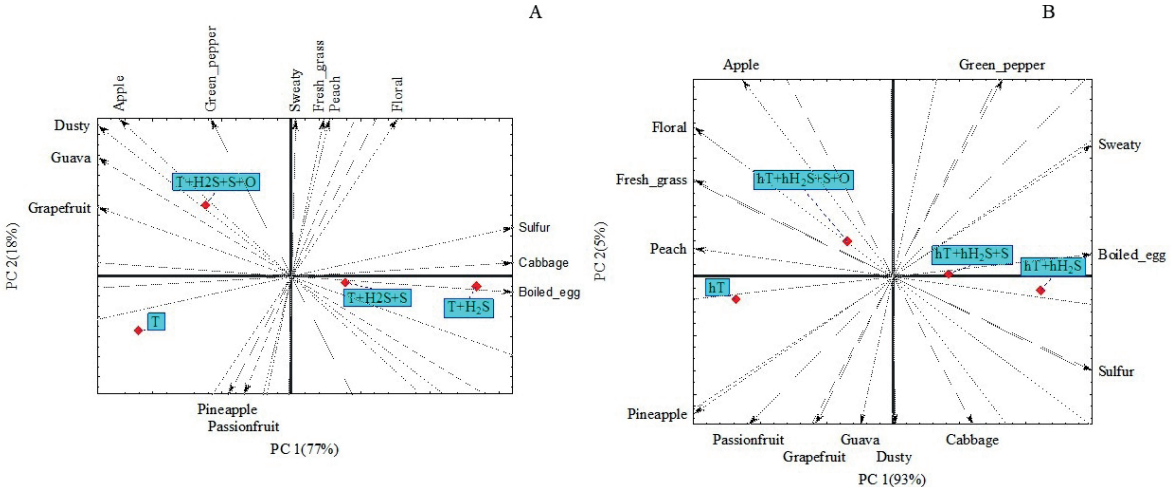


FIGURE 2. PCA sensory biplot of the low varietal-thiol (T) and low hydrogen sulfide (A) or high hydrogen sulfide (B) treatments in model wine (MW). The treated model wines (T + H_2S) were swirled for 30 seconds in ISO wine glasses and covered for 15 minutes (T + H_2S + S); or swirled for 30 seconds in ISO wine glasses and left uncovered for 15 minutes (T + H_2S + S + O), prior to sensory evaluation by the trained sensory panel.

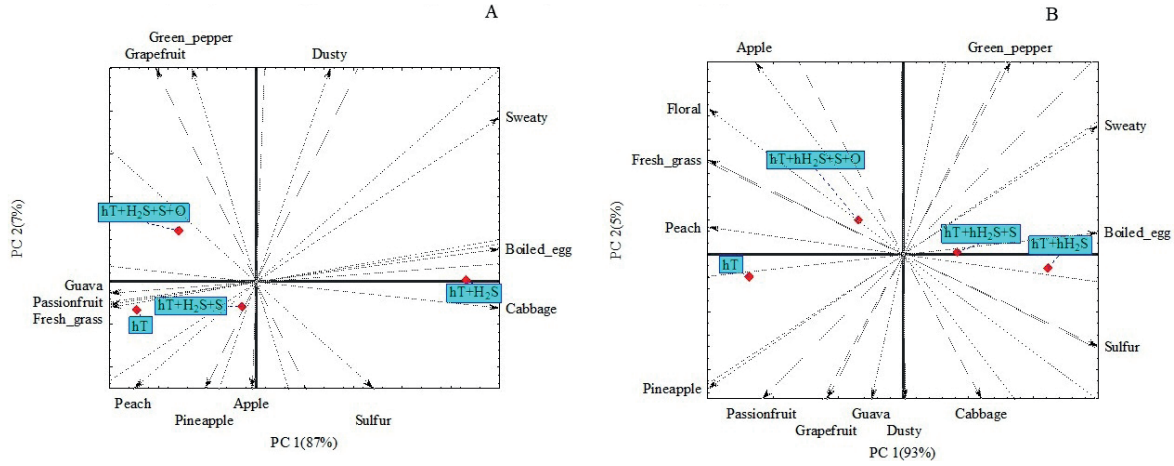


FIGURE 3. PCA sensory biplot of the high varietal-thiol (hT) and low hydrogen sulfide (A) or high hydrogen sulfide (B) treatments in model wine (MW). The treated model wines (T + H_2S) were swirled for 30 seconds in ISO wine glasses and covered for 15 minutes (T + H_2S + S); or swirled for 30 seconds in ISO wine glasses and left uncovered for 15 minutes (T + H_2S + S + O), prior to sensory evaluation by the trained sensory panel.

to the "hT + H_2S" treatment. In contrast to the previous treatments in which low varietal-thiol concentrations were present (Figures 4 and 5), swirling with the lid covered lowered 'boiled egg' associated with lower H_2S concentrations; while leaving the glass open significantly decreased the 'sulfur/reductive' and 'cabbage' off-odours compared to the "hT + H_2S" treatment (Figure 6). It has been reported that high thiol levels can contribute to 'sweaty' aromas in certain wines, which may lead to it being repulsive (Coetzee & Du Toit, 2012), which can explain the detection of 'reductive' associated descriptors in the "hT" wines (Figure 6).

The associations of fruity attributes, such as 'passionfruit', 'pineapple', 'peach', and 'apple', were less pronounced in the swirling treatment of the high varietal-thiol, high-H_2S model wine (Figure 3B) compared to the high varietal-thiol, low-H_2S model wine (Figure 3A). The marked suppression of multiple 'fruity' attributes by high concentrations of H_2S ("hT + hH_2S") (Figure 7) is consistent with previous studies that demonstrate the masking effects of VSCs associated with 'reductive' aromas. Franco-Luesma *et al.* (2016) reported that 'banana' and 'yellow fruit' aromas were diminished in model wines with elevated H_2S. Similarly, a survey of 104 white wines by Zhang *et al.* (2024) showed that total and free MeSH, free H_2S, and phenylmethanethiol (PMT) were negatively associated with overall fruit aroma. In another study, where 71 commercial Chardonnay wines were surveyed, Nandorfy *et al.* (2023) found that MeSH, ethanethiol, 2-furanmethanethiol (2FMT), and PMT were strongly and negatively correlated with 'fruity' attributes, likely due to perceptual masking. Notably, 2FMT and PMT suppressed descriptors such as 'peach', 'apple/pear', and 'floral' aromas. Previous studies have also shown that methional, a compound that imparts 'potato'/'cooked potato' aroma attributes, can suppress 3SH-derived fruity notes (Coetzee *et al.*, 2015).

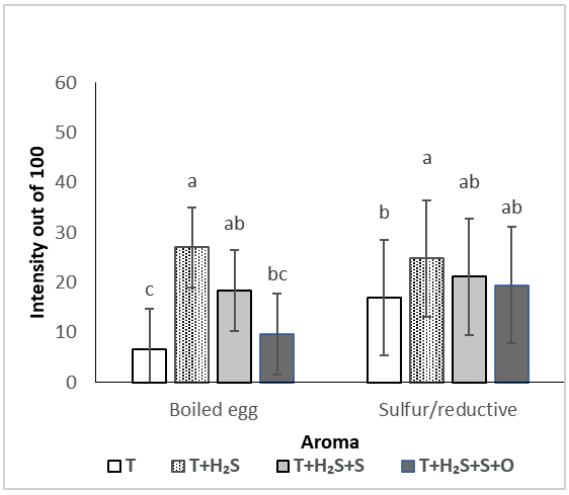


FIGURE 4. Results of descriptive analysis (DA) performed on the low varietal-thiol (T) and low hydrogen sulfide (H_2S) treatments in the model wine. The treated model wines (T + H_2S) were swirled for 30 seconds in ISO wine glasses and covered for 15 minutes (T + H_2S + S); or swirled for 30 seconds in ISO wine glasses and left uncovered for 15 minutes (T + H_2S + S + O), prior to sensory evaluation by the trained sensory panel. Only the significantly different sensory attributes are shown. Vertical bars indicate 0.95 confidence intervals. Different letters indicate a significant difference ($p < 0.05$).

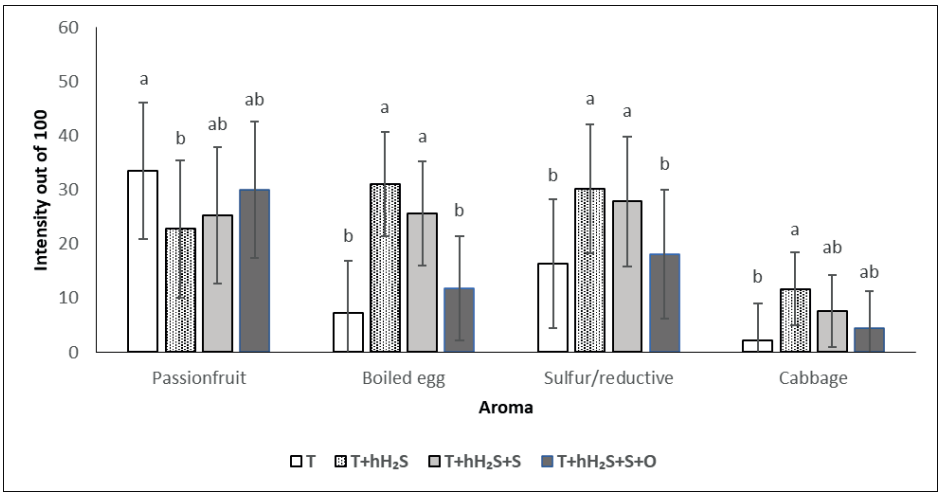


FIGURE 5. Results of descriptive analysis (DA) performed on the low varietal-thiol (T) and high hydrogen sulfide (hH_2S) treatments in the model wine. The treated model wines (T + hH_2S) were swirled for 30 seconds in ISO wine glasses and covered for 15 minutes (T + hH_2S + S); or swirled for 30 seconds in ISO wine glasses and left uncovered for 15 minutes (T + hH_2S + S + O), prior to sensory evaluation by the trained sensory panel. Only the significantly different sensory attributes are shown. Vertical bars indicate 0.95 confidence intervals. Different letters indicate a significant difference ($p < 0.05$).

For high varietal-thiol and high H_2S treatments, swirling alone did not significantly decrease the 'boiled egg' and 'reductive' off-odours (Figure 7). Only when the wine glass was left open post-swirling did the 'boiled egg' and 'sulfur/reductive' off-odours decrease to a level that was indistinguishable from the control, "hT" (Figure 7). These results further support the role that volatilisation plays in the removal of H_2S when wine glasses or decanters are swirled or left for a period before the wine is consumed. The comparable loss of free H_2S after swirling in both ambient air and an anoxic atmosphere, as mentioned earlier, also supports these results. However, the 'fruity' and 'reductive' aroma characters were not significantly different between the "hT + hH_2S + S + O" and "hT + hH_2S + S" treatments (Figure 7). This suggests that, other than glass swirling, additional exposure to air is also required to decrease the masking effect of H_2S on the fruity attributes (Figure 7) through the loss of H_2S through volatilisation and the subsequent decrease in the perception of 'reductive' aromas. The PCA biplot in Figure 3B further illustrates this, as "hT + hH_2S + S" clustered closely with "hT + hH_2S," indicating that swirling alone was insufficient to decrease 'reductive' off-odours in high-H_2S, high varietal-thiol treatments. This emphasises the importance of volatilisation in mitigating 'reductive' sensory defects associated with higher H_2S concentrations.

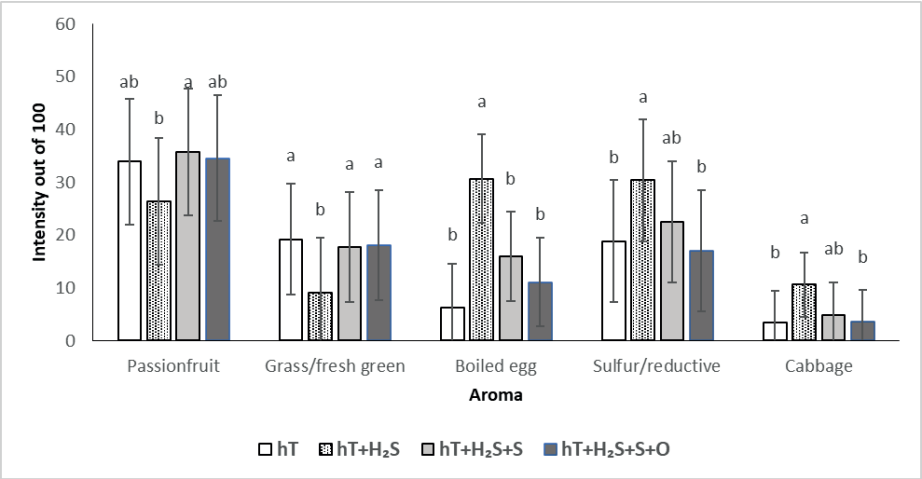


FIGURE 6. Results of descriptive analysis (DA) performed on the high varietal-thiol (hT) and low hydrogen sulfide (H_2S) treatments in the model wine. The treated model wines (hT + H_2S) were swirled for 30 seconds in ISO wine glasses and covered for 15 minutes (hT + H_2S + S); or swirled for 30 seconds in ISO wine glasses and left uncovered for 15 minutes (hT + H_2S + S + O), prior to sensory evaluation by the trained sensory panel. Only the significantly different sensory attributes are shown. Vertical bars indicate 0.95 confidence intervals. Different letters indicate a significant difference ($p < 0.05$).

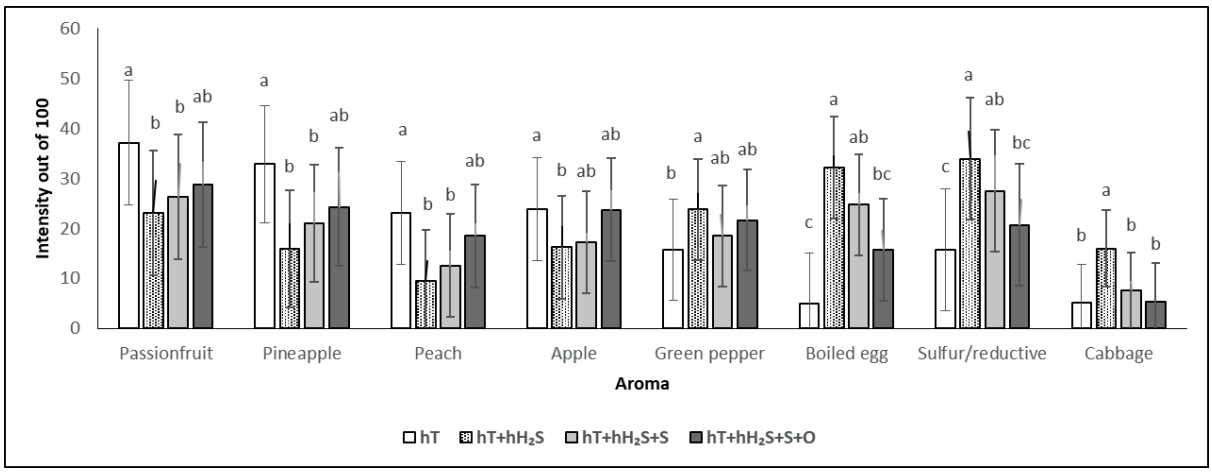


FIGURE 7. Results of descriptive analysis (DA) performed on the high varietal-thiol (hT) and high hydrogen sulfide (hH_2S) treatments in the model wine. The treated model wines (hT + hH_2S) were swirled for 30 seconds in ISO wine glasses and covered for 15 minutes (hT + hH_2S + S); or swirled for 30 seconds in ISO wine glasses and left uncovered for 15 minutes (hT + hH_2S + S + O), prior to sensory evaluation by the trained sensory panel. Only the significantly different sensory attributes are shown. Vertical bars indicate 0.95 confidence intervals. Different letters indicate a significant difference ($p < 0.05$).

2.2. Effects of glass swirling in Chenin blanc

The results of the swirling treatments in Chenin blanc wine had a similar sensory impact as was measured in the model wine; however, the effects on the sensory attributes were less pronounced. The PCA biplots, showing how various treatments are positioned relative to one another, with hydrogen sulfide (H_2S)-treated wines and control wines (CH) situated apart. (Figures 8A and 8B).

2.2.1. Low H_2S in Chenin blanc

No significant differences were observed between the "CH" and "CH + H_2S + S" treatments for sensory attributes 'peach/apricot' and 'boiled egg' (Figure 9), indicating that a 30 seconds swirling of the glass effectively decreased the perception of 'boiled egg' off-odour associated with low concentrations of H_2S in the Chenin blanc wine. While the 'peach/apricot' aroma was suppressed in the "CH + H_2S" treatment, its intensity was notably higher in the "CH + H_2S + S" treatment, which involved swirling the glass for 30 seconds and then covering it. Compared to "CH", the effect was more pronounced when the glass was covered post-swirling compared to when it was left open, suggesting that volatilisation and subsequent headspace equilibration may influence the release of the volatile compounds associated with desirable 'fruity' aromas. When the glasses were closed and the volatile compounds protected from volatilisation, it allowed the 'fruity' aromas to accumulate

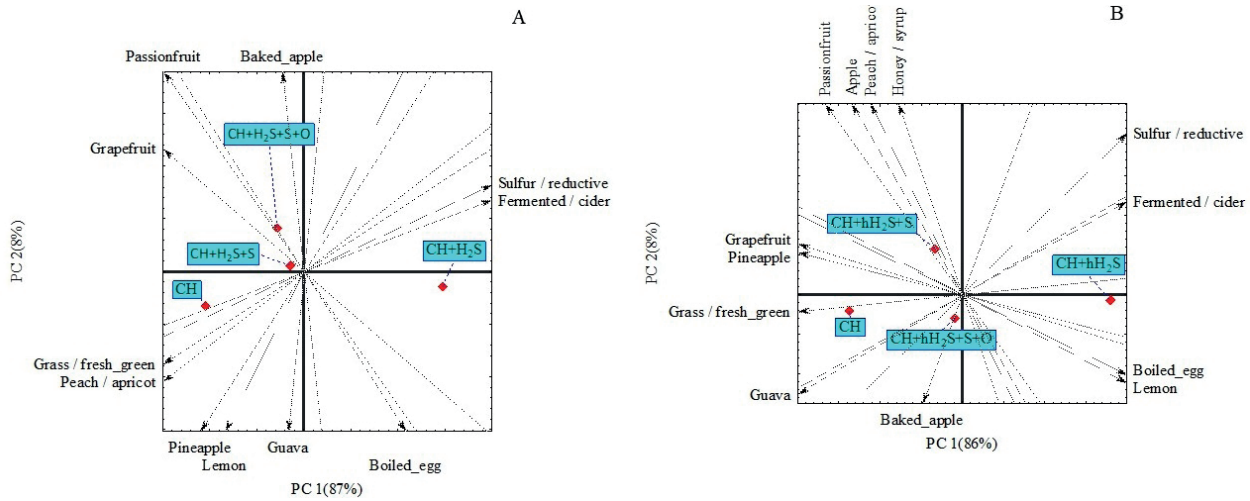


FIGURE 8. PCA sensory biplot of the Chenin blanc wine with added low hydrogen sulfide (A) and high hydrogen sulphide (B). The treated Chenin blanc wines (CH + H_2S) were swirled for 30 seconds in ISO wine glasses and covered for 15 minutes (CH + H_2S + S); or swirled for 30 seconds in ISO wine glasses and left uncovered for 15 minutes (CH + H_2S + S + O), prior to sensory evaluation by the trained sensory panel.

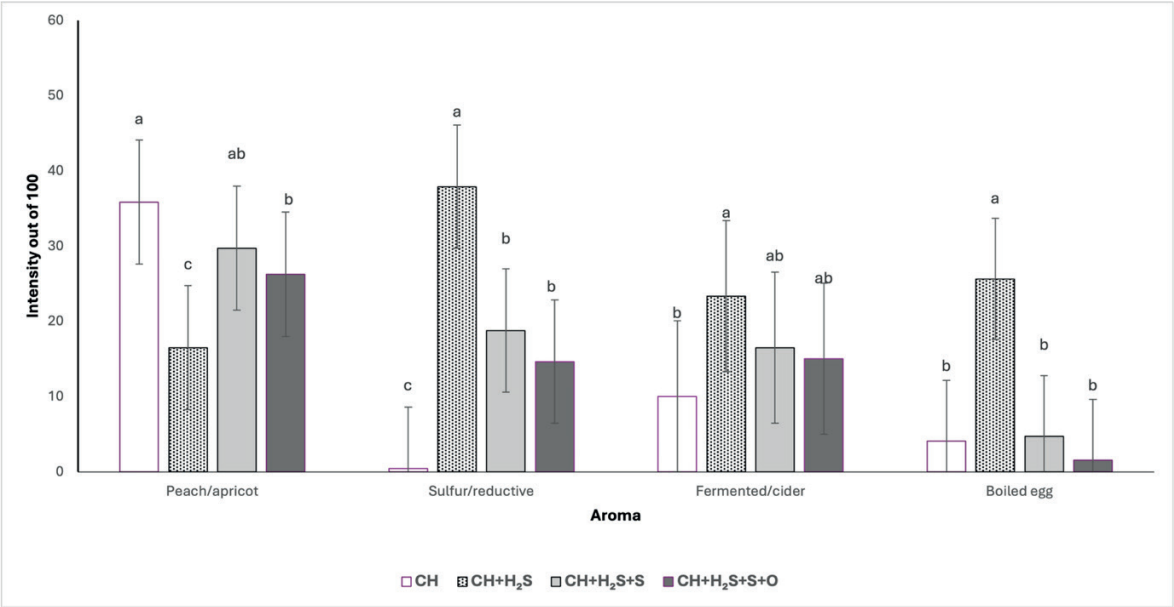


FIGURE 9. Results of descriptive analysis (DA) performed on the Chenin blanc (CH) treated with low hydrogen sulfide (H_2S). The treated Chenin blanc wines (CH + H_2S) were swirled for 30 seconds in ISO wine glasses and covered for 15 minutes (CH + H_2S + S); or swirled for 30 seconds in ISO wine glasses and left uncovered for 15 minutes (CH + H_2S + S + O), prior to sensory evaluation by the trained sensory panel. Only the significantly different sensory attributes are shown. Vertical bars indicate 0.95 confidence intervals. Different letters indicate a significant difference ($p < 0.05$).

and reach equilibrium between the headspace and the liquid phase while protecting against the loss of the 'peach/apricot' aromas through volatilisation. However, when the glass was left open for 15 minutes post-swirling, the 'fruity' aromas may have been lost through volatilisation, which decreased their aroma intensity. This possibly resulted in a lower intensity of 'peach/apricot' aroma for the glasses that were left open after swirling when compared to the control, "CH" (Figure 9). The observed effect of equilibration time on increasing the aromatic intensity of the sensory attributes of the wine is in agreement with the findings of Hirson *et al.* (2012), who demonstrated that, when a wine is left to equilibrate, the volatile compounds increase in concentration over time, which improves the sensory perception. However, differences between the glasses that were swirled and covered and left open compared to "CH" did not reflect in the 'sulfur/reductive' and 'boiled egg' aromas. This might be due to the higher volatility of H_2S compared to fruity esters, where only swirling is sufficient to significantly reduce these compounds compared to the control.

The positioning of the two swirling treatments, "CH + H_2S + S" and "CH + H_2S + S + O", closer to the "CH" treatment and further away from "CH + H_2S" indicates that lower H_2S concentrations and their corresponding 'reductive' off-odours were effectively decreased by the swirling of the wine glass alone (Figure 8A). Notably, the effects of headspace equilibration and swirling observed in real Chenin blanc wines closely mirror those seen in high-varietal-thiol model systems (Figure 3A), thereby confirming the reproducibility of this phenomenon across diverse wine matrices.

The minimal difference between the "CH + H_2S + S" and "CH + H_2S + S + O" treatments suggests that the majority of H_2S volatilisation occurred during the initial swirling process, with little additional loss occurring when the glass was left open. This is consistent with previous results in the Chenin blanc wines, where a significant decrease in free H_2S concentrations was observed within 30 seconds of swirling (Figure 1). H_2S is highly volatile with a boiling point of –60.3 °C, which is rapidly lost when it is present in unsealed vessels.

These results suggest that in Chenin blanc wine with low H_2S concentrations, a 30-second swirling treatment effectively decreases 'reductive' off-odours while restoring suppressed 'fruity' attributes, particularly when the glass is subsequently covered to allow volatile equilibrium.

2.2.2. Higher H_2S in Chenin blanc

Chenin blanc wines containing higher concentrations of H_2S were more closely associated with 'reductive' off-odours and negatively associated with 'fruity' aromas (Figure 8B), as was seen in wines treated with lower H_2S concentrations. Sensory evaluation revealed that wines with high H_2S exhibited more widespread suppression of the 'fruity' aromas than those with lower H_2S levels. Five key aroma attributes: 'pineapple', 'passionfruit', 'peach/apricot, 'grapefruit', and 'apple' were significantly suppressed in "CH + hH_2S" (Figure 10), whereas only the 'peach/apricot' attribute was significantly suppressed by the low H_2S treatment (Figure 9). This stronger suppression effect of high H_2S concentrations on the 'fruity' aromas in wine highlights the intensity of H_2S-derived 'reductive' off-odours relative to other aroma-active compounds present in the wine.

The "CH + hH_2S + S" treatment is situated closer to the "CH" in Figure 8B on the PCA graph compared to the "CH + hH_2S + S + O" treatment. Swirling alone was highly effective in mitigating the suppressive effects of high H_2S concentrations on the perception of 'fruity' aromas. The "CH + hH_2S + S" treatment showed a significant increase in 'fruity' attributes of 'pineapple', 'passionfruit', 'peach/apricot', and 'apple' compared to "CH + hH_2S" (Figure 10). However, these 'fruity' attribute improvements were not observed in "CH + hH_2S + S + O" compared to "CH + hH_2S",

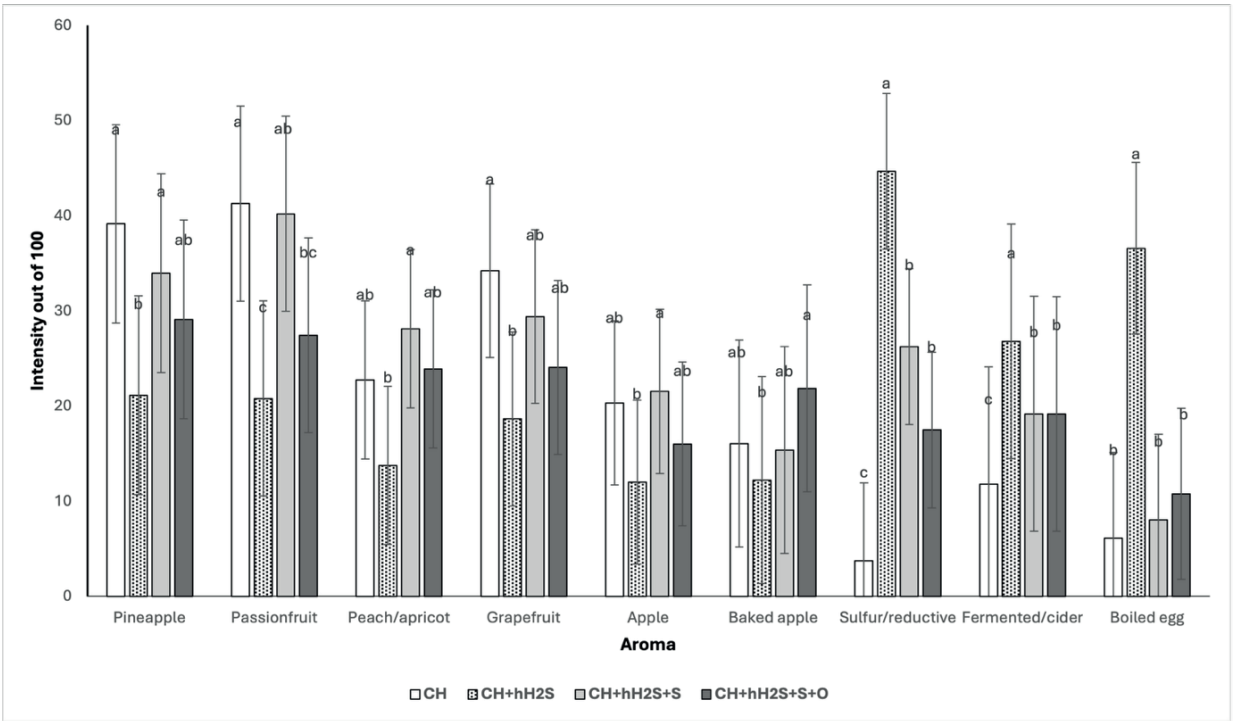


FIGURE 10. Results of descriptive analysis (DA) performed on the Chenin blanc (CH) treated with high hydrogen sulfide (hH_2S). The treated Chenin blanc wines (CH + H_2S) were swirled for 30 seconds in ISO wine glasses and covered for 15 minutes (CH + H_2S + S); or swirled for 30 seconds in ISO wine glasses and left uncovered for 15 minutes (CH + H_2S + S + O), prior to sensory evaluation by the trained sensory panel. Only the significantly different sensory attributes are shown. Vertical bars indicate 0.95 confidence intervals. Different letters indicate a significant difference ($p < 0.05$).

showing that leaving the glass open post-swirling did not further enhance 'fruity' aroma perception. These results suggest that the majority of H_2S loss occurred within the first 30 seconds of swirling, and additional volatilisation from an open glass did not contribute further to decreasing 'reductive' off-odours (Figure 10). This is somewhat contradictory with our results in model wine (Figure 7), where the "CH + hH_2S + S + O" treatments led to more significant decreases in 'boiled egg' and 'sulfur/reductive' compared to "CH + hH_2S", which further emphasise the need to validate sensory outcomes from model wine in real wine matrices.

Interestingly, leaving the glass open post-swirling led to a decrease in the 'passionfruit' attribute compared to the control, "CH". Moreover, the intensity of most of the 'fruity' attributes ('pineapple', 'passionfruit', 'peach/apricot', and 'apple') in "CH + hH_2S + S + O" were not significantly different from those of "CH + hH_2S" (Figure 10). A similar pattern was observed in low H_2S treatment, where the 'peach/apricot' attributes were more pronounced in "CH + H_2S + S" than in "CH + H_2S + S + O" (Figure 9). These results suggest that while swirling effectively decreased free H_2S levels, keeping the glass covered after swirling allows the headspace to equilibrate with the wine, enhancing the perception of fruity aromas. On the other hand, leaving the glass open likely causes continued volatilisation of aroma compounds, preventing their accumulation in the headspace and decreasing the 'fruity' aroma attributes.

Although chemical analysis showed a significant decrease in free H_2S concentrations in "CH + hH_2S + S + O" compared to "CH + hH_2S + S" (Figure 1), the sensory data did not reflect a corresponding decrease in 'reductive' off-odours (Figure 10). This suggests a potential masking effect from the complex composition of aroma-active compounds in Chenin blanc, which may obscure the impact of individual volatile compounds, or the possible involvement of other sulfur-containing compounds, which can also contribute to the 'reductive' aroma (Franco-Luesma *et al.*, 2016). Similar masking and suppressive interactions have been reported in previous studies, such as the suppression of 3SH-related 'tropical' aromas by methional (Coetzee *et al.*, 2015), the suppression of ester-derived sensory attributes by high varietal thiol concentrations (Iobbi *et al.*, 2023; King *et al.*, 2011), and the suppression of fruity characters by VSCs (Franco-Luesma *et al.*, 2016; Nandorfy *et al.*, 2023; Zhang *et al.*, 2024). Changes in free H_2S concentrations in wines might also not necessarily result in corresponding changes in the intensity of specific sensory attributes, which might be due to differences in headspace concentrations or wine matrix effects, of which the latter has been shown for varietal thiols (Wilson *et al.*, 2019). In addition, recent work has highlighted the role of copper speciation in shaping the equilibrium between free and bound sulfhydryl compounds in wine. Zhang *et al.* (2024) demonstrated that specific Cu fractions can suppress free sulfhydryl concentrations and their associated 'reductive' aromas, suggesting that Cu–thiol interactions may attenuate the sensory expression of H_2S even when measurable chemical concentrations are present. The current study investigated concentrations of H_2S higher than the reported sensory threshold of H_2S (1.1–1.6 µg/L; Siebert *et al.*, 2010) and more research is required to assess its effect at peri-threshold and threshold values on varietal thiols in the context of matrix effects and Cu-related equilibria.

Another possible explanation for the decreased perception of 'fruity' attributes when leaving the glass open is the oxidative loss of esters and terpenes. However, such oxidation reactions typically occur at very slow rates and are highly unlikely. Moreover, varietal thiols, which are highly sensitive to oxidative degradation, showed no significant loss under these conditions (Figures S4 and S5). Additionally, the presence of free SO_2 in the real Chenin blanc wine likely provided antioxidant protection, preventing rapid oxidation-related aroma loss (Bekker *et al.*, 2016b; Nikolantonaki *et al.*, 2014).

CONCLUSION

This study is the first to use targeted addition experiments to demonstrate that free H_2S can suppress varietal thiol-derived tropical and fruity aromas, advancing our understanding beyond correlative survey data. It highlighted the significant role of glass swirling in decreasing concentrations of 'reductive' off-odours in Chenin blanc wine, especially those caused by free H_2S. Swirling wine for 30 seconds effectively decreased 'reductive' off-odours intensity caused by H_2S, while restoring key fruity aromas, demonstrating that volatilisation of free H_2S occurs primarily within this short time frame. The obtained results also suggest that keeping the glass covered post-swirling allows volatile aroma compounds to equilibrate in the headspace, enhancing the perception of 'fruity' attributes in Chenin blanc wines. In contrast, leaving the glass open post-swirling led to a decline in some fruity attributes in real wine, likely due to the continued volatilisation of key aroma compounds. These results were not always reflected in the model wines, where leaving the glass open after swirling resulted in further decreases in reductive aromas, depending on the initial varietal-thiol and H_2S concentrations. Lastly, the decrease in 'reductive' aromas due to H_2S by glass swirling seems to be mostly due to volatilisation and not oxidation. These results contribute to a better understanding of the relationship between swirling of wine glasses and the effect of "opening up" of wine aromas, a practice commonly employed by wine consumers and experts.

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