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Cu fractions in Shiraz and Pinot noir wines during bottle aging: rates of changes and capacity for conversion

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ABSTRACT

The Cu fraction in wine associated with Cu(II)-organic acid complexes can suppress detrimental aromas attributed to hydrogen sulfide and methanethiol. However, the long-term stability of this Cu fraction (known as Cu fraction I) during bottle aging of red wine is not well understood. This study utilised Pinot noir and Shiraz wines containing 0.43 ± 0.01 and 0.56 ± 0.02 mg/L respectively of total Cu, to which 0, 0.3 or 0.6 mg/L Cu(II) was further added immediately before bottling. The bottles were then stored at 14 °C for 1 yr. Cu fraction I concentrations were measured using two independent methods: i) stripping potentiometry, and ii) ICP-OES analysis of filtrate after diatomaceous earth depth filtration. Within the first 6 months of storage, Cu fraction I was found to decrease in all wines. Using stripping potentiometry, the first-order decay rates were found to be 0.012 ± 0.001 day⁻¹ and 0.010 ± 0.001 day⁻¹ for Pinot noir and Shiraz respectively, corresponding to half-lives of 55 ± 4 and 67 ± 9 days. The decay rates for Shiraz versus Pinot noir were similar for wines with different Cu addition rates, or when rates were determined using the different analysis techniques. Both wines had a high capacity for conversion of Cu fraction I to Cu fraction III during the 1-yr storage period, with 0.4-0.8 mg/L Cu forming Cu fraction III in the Pinot noir, and 0.3-0.6 mg/L in the Shiraz. These conversion capacity ranges are higher than the typical Cu(II) additions made to wine during production. Overall, the results show that red wine has a large capacity for enabling the sulfide-binding of Cu-organic acid complexes during bottle aging and the conversion occurs at a relatively uniform rate with the concentration halving approximately every 2 months.

KEYWORDS: Cu fractions, stripping potentiometry, depth-filtration, decay rate, reductive aging, red wine, sulfide

INTRODUCTION

When a wine is first bottled, the initial phase of aging usually occurs in the presence of some oxygen. This oxygen can include oxygen dissolved in the wine and oxygen present within in the gaseous phase of the headspace; that is, between the wine surface and the closure of the bottle (Ugliano *et al.*, 2012). The sum concentration of these components of oxygen is known as total packaged oxygen. Oxygen can also permeate into the wine bottle through the closure, but the rate of ingress is generally very low (e.g., 0.0012 mg/L·day), when using screw cap closures with tin liners as the bottle closure (Lopes *et al.*, 2009). Once the total packaged oxygen has reacted with the wine components and has been depleted, a bottled wine with a screw-cap closure will undergo aging in a low-oxygen environment. For most red wines, a low-oxygen state is both suitable and beneficial for the aging of the wine. However, in certain wines, the low-oxygen conditions can lead to an accumulation of aroma compounds, such as hydrogen sulfide and methanethiol, which are normally considered detrimental (Kreitman *et al.*, 2019; Ferreira *et al.*, 2018). These aroma compounds can repress desirable *fruity/floral* aromas, and impart odours of *rotten egg*, *sewerage* and *rotten cabbage*, collectively described as ‘*reductive*’ off-aromas. Although a variety of precursors are known for the formation of these reductive aroma compounds in low-oxygen conditions, the identification of those that play an important role in the production of hydrogen sulfide during wine aging is not certain. Precursors of these reductive aroma compounds can include their oxidised forms (i.e., diorganopolysulfanes) and metal complexes, and in the case of methanethiol it can also include methyl thioacetate and disulfides (Bekker *et al.*, 2018; Kreitman *et al.*, 2019; Ferreira *et al.*, 2018).

Cu is known to influence the emergence of reductive aroma compounds during the aging of wine in low oxygen conditions (Vela *et al.*, 2017), and certain forms of Cu can repress the aroma active forms of hydrogen sulfide and methanethiol (Clark *et al.*, 2020; Zhang *et al.*, 2022).

However, legal limits exist for total Cu concentrations in wine (e.g., 1.0 mg/L maximum acceptable limit by the Organisation Internationale de la Vigne et du Vin (OIV, 2022)), and colloidal instabilities can also occur if total concentrations are too high (i.e., >0.5 mg/L in some white wines) (Rankine, 2004). Hence a balance is required between minimising total Cu concentrations and optimising protection against reductive development.

As shown in Figure 1, fractions of Cu in red and white wine can be determined using a variety of techniques (Clark *et al.*, 2020; Zhang *et al.*, 2022). In white wine, three fractions are common: Cu fraction I, attributed to dissolved Cu(II)-organic acid complexes (e.g., Cu(II)-tartrate); Cu fraction II, attributed to dissolved Cu(I)-thiol complexes (e.g., Cu(I)-glutathione); and Cu fraction III, attributed predominantly to suspended copper sulfides (e.g., Cu(I) sulfide, Cu(II) sulfide). Cu fraction III can also consist of some strongly-bound and/or aggregated Cu(I)-thiol species (Clark *et al.*, 2020). However, red wine has only been found to contain Cu fractions I and III, without any evidence of Cu fraction II. This is presumably a consequence of the lower concentrations of thiol compounds, such as glutathione (Dienes-Nagy *et al.*, 2022), in red wines compared to white wines due to the conditions being more oxidative during red wine production. Stripping potentiometry, or analysis of filtrate from diatomaceous earth depth filtration (DEDF) by inductively coupled plasma-optical emission spectroscopy (ICP-OES), can be used for the quantitation of Cu fraction I in red wine, while subtraction from total Cu concentration can allow the Cu fraction III concentration to be calculated (Clark *et al.*, 2020). The treatment of red wine with divinylbenzene (DVB) in solid phase extraction (SPE) cartridges and the measurement of the eluting wine by ICP-OES is another way of determining the Cu fraction I, albeit with some positive bias (see DVB ICP-OES in Figure 1) (Kontoudakis *et al.*, 2019). In terms of the typical distribution of Cu among the different fractions, 29 red wines that were aged in bottles for 1-4 years contained only minor amounts of Cu fraction I (i.e., around 0.03 mg/L or 11 % of total Cu), the major form being Cu fraction III (Kontoudakis *et al.*, 2019).

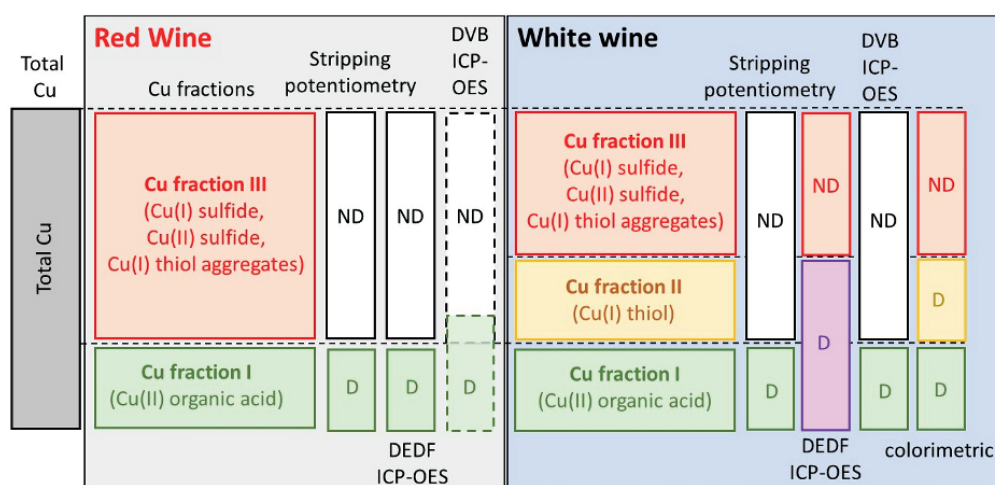


FIGURE 1. The fractions of Cu in wine and the methodologies for measurement.

D = detected; ND = not-detected; DBV = divinylbenzene; DEDF = diatomaceous earth depth filtration.

A stripping potentiometric analysis of Cu fraction I was the first to show a clear link between a specific Cu fraction and the suppression of reductive aroma compounds in bottle-aged wine (Kontoudakis *et al.*, 2019): when Cu fraction I concentrations (referred to as ‘labile Cu’ by Kontoudakis *et al.* (2019)) were above 0.02 mg/L, none of the 49 studied wines contained concentrations of free hydrogen sulfide (i.e., non-metal complexed or non-oxidised) above its aroma threshold. Furthermore, when the Cu fraction I concentrations were above 0.05 mg/L, none of the wines contained concentrations of free methanethiol above its aroma threshold. This did not mean that all the wines containing Cu fraction I below these threshold concentrations had reductive development, but that wines with a propensity to undergo reductive development could do so if the Cu fraction I was below the threshold concentrations. In white wine, Cu fractions I and II were found to transform into Cu fraction III during bottle aging, and the determination of first-order decay rates for a given white wine allowed the calculation of the time for Cu fraction I to fall below the critical concentrations of 0.02 and 0.05 mg/L (Zhang *et al.*, 2022). However, the behaviour of the Cu fractions during the aging of red wine (rates of decay, variability in change across different wines and the capacity of a red wine to convert Cu from fraction I to fraction III) is not certain.

The aim of this study was to determine the stability of Cu fraction I in red wine during bottle aging in low-oxygen conditions, as well as the rates of decay in Cu fraction I and the amount that could be converted to Cu fraction III during bottling aging. To this end, and to gain insight into variety effects, Shiraz and Pinot noir were bottled with increasing Cu concentrations and stored for 12 months at 14 °C and the periodic monitoring of Cu fraction I was carried out using two independent measurement techniques (i.e., stripping potentiometry and DEDF-ICPOES).

MATERIALS AND METHODS

1. Chemicals

Copper(II) sulfate pentahydrate was sourced from VWR (Radnor, PA, USA). An SO₂ analysis kit for Konelab analysis was provided by Thermo Fisher Scientific (Scoresby, VIC, Australia). Ultrapure water (18.2 MΩ cm) was produced from a Milli-Q Plus purification system (Merck Millipore, Bayswater, VIC, Australia).

2. Winemaking protocols and bottle aging process

Two red grape varieties, Shiraz and Pinot noir, harvested from the 2019 vintage in New South Wales and Victoria (Australia) respectively, were utilised for this study. The harvested grapes went through standard fermentation and a subsequent wine production procedure, as described by Šuklje *et al.* (2019). Two weeks prior to bottling, the addition of 0.4 mg/L Cu(II) was made to the wines using Cu(II) sulfate pentahydrate. The Cu concentrations in the wines on the day of bottling was 0.43 ± 0.01 and 0.56 ± 0.02 mg/L for Pinot noir and Shiraz respectively; other compositional details are provided in

Supplementary Table 1. Immediately prior to bottling, an extra 0.3 mg/L or 0.6 mg/L of Cu(II) was added to the 750 mL wine bottles, while no Cu addition was made to ‘0 mg/L added’ samples (i.e., the control). After filling the bottles with wine, the headspace was sparged with CO₂ (BOC Gas & Gear, Wagga Wagga, NSW, Australia) and the bottles sealed with screw cap closures. After bottling, dissolved and headspace oxygen concentrations were measured using a PSt3 oxygen sensors with a Fibrox 3 LCD trace meter (Precision Sensing GmbH, Regensburg, Germany), and the total packaged oxygen was determined as the sum of these two concentrations and expressed in terms of mg/L dissolved oxygen. The bottled wines were stored in darkness at 14 °C. Chemical analysis was performed on triplicate bottles on days 6, 49, 121, 179, 239, 312 and 372 for the Pinot noir, and days 5, 50, 120, 174, 234, 312 and 366 for the Shiraz. The concentrations of free and total sulfur dioxide, total Cu and Cu fractions were determined at each sampling time point, while the oxygen concentrations were measured more frequently until the total packaged oxygen was depleted.

3. Chemical analysis: Instrumental and methodologies

The total Cu in the wines and the total Cu concentration in the DEDF filtration were measured by ICP-OES (Varian 710, Varian, Palo Alto, CA, USA) with a high-salt nebuliser/spray chamber as described by Clark *et al.* (2020). Copper was quantified with Lutetium (Lu) as an internal standard and Cu standards were prepared in a solution of 12 % (v/v) aqueous ethanol with 1 g/L tartaric acid and 2 g/L potassium hydrogen tartrate. 10 mg/L Ag(I) was added to all the samples to enhance the detection of Cu by accelerating its dissociation from sulfide. All the standards and samples were diluted four-fold with 5% (v/v) nitric acid prior to analysis.

The electrochemical analysis of the Cu fractions was performed using medium exchange constant current stripping potentiometry with a thin-mercury-film on a DropSens 110 screen-printed carbon electrode (DropSens, Llanera, Spain) as described by Clark *et al.* (2020). The counter and reference electrodes on the 110 screen-printed platform were composed of carbon and silver respectively. Analysis was conducted with the use of a methacrylate (transparent) wall-jet flow cell (DropSens), and the passage of wine, wash and stripping solutions through the flow cell was controlled by a programmable HPLC pump as described in Clark *et al.* (2020). Stripping potentiometry was performed with a 797 VA Computrace (Metrohm, Herisau, Switzerland) and managed by the VA Computrace 797 software (v1.3). Quantitation was performed by external calibration graph generated on the same electrode as for the sample.

An analysis of Cu fractions by DEDF ICP-OES was conducted as described in Clark *et al.* (2020). Beco Sterile S80 diatomaceous earth depth filters, 45 mm in diameter and 3.5 mm in depth, were sourced from Blue H₂O filtration (Oakleigh, VIC, Australia). The filters were used within Millipore (Bedford, MA, USA) polypropylene Swinnex filter holders. The filters were preconditioned with 50 mL of 1 % (w/v) citric acid, 20 mL of 12 % (v/v) aqueous ethanol with

4 g/L tartaric acid (adjusted to pH 3.2 with NaOH), and 10 mL of sample wine using a plastic 20 mL syringe to pass solution through the filter. Afterwards, 100 mL of sample wine was passed through the filter using a 20 mL syringe, and the total Cu in the filtrate was determined by ICP-OES. All individual replicate samples were filtered with new, preconditioned filters, which were only used once and discarded after use.

Free and total sulfur dioxide were determined by a Konelab 20XT automated analyser (Thermo Fisher Scientific, Scoresby, VIC, Australia) with analysis kits supplied by the same company.

4. Statistical analysis of analytical data

All the reported data are the mean of three replicates, and all the uncertainties indicate standard deviations. The average decay rates determined for Cu fraction I (Table 1) with non-overlapping 95 % confidence intervals were considered significantly different ($p \leq 0.05$) (Payton *et al.*, 2003; Cumming *et al.*, 2007).

RESULTS

1. Oxygen and SO₂ concentrations

After bottling, the average concentrations of dissolved oxygen in the Pinot noir and Shiraz were 0.3 ± 0.2 mg/L and 0.2 ± 0.1 mg/L respectively, dropping to negligible concentrations (<0.1 mg/L) after the first time point (data not shown). Most of the total packaged oxygen (Figure 2) at bottling stemmed from the headspace oxygen content.

The total packaged oxygen decayed rapidly during storage at 14 °C, and was largely depleted by the end of 2 weeks. There was no obvious difference in oxygen decay rate with Cu treatment to the wines (Figure 2), and the Pinot noir had faster first-order oxygen decay rates than the Shiraz (i.e., 0.090 ± 0.009 versus 0.066 ± 0.007 days⁻¹).

Figure 3 shows that for most samples, the concentration of both free and total SO₂ decreased across the first two time points (days 6 and 49), after which the concentration

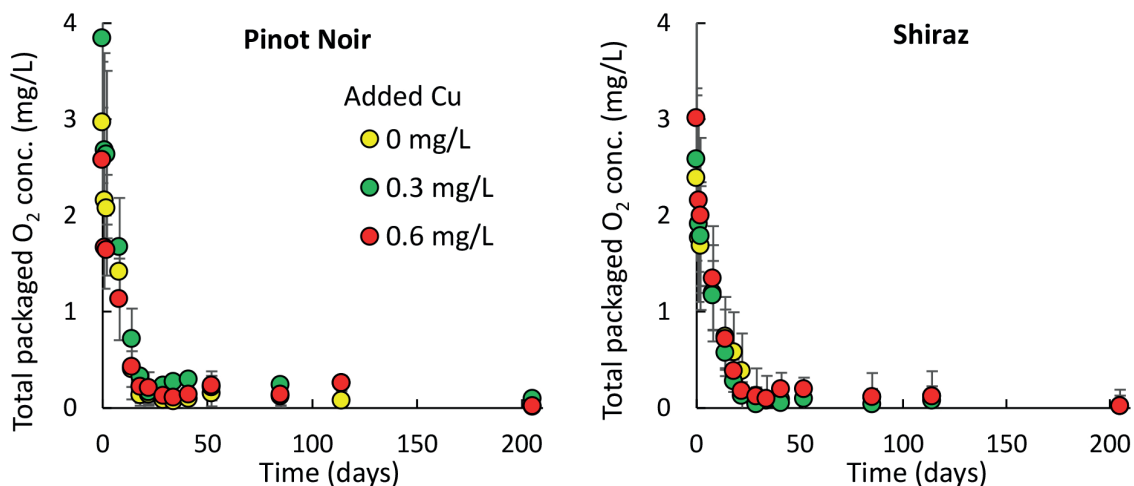


FIGURE 2. The total packaged oxygen concentration in the Pinot noir and Shiraz wines during the storage experiment.

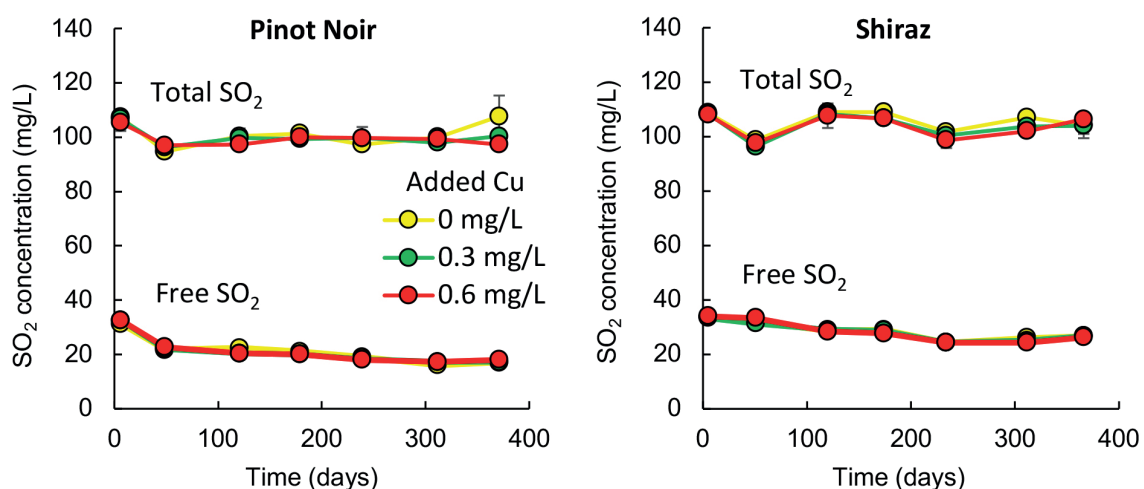


FIGURE 3. The free and total SO₂ concentrations in Pinot noir and Shiraz wines during the storage experiment.

Error bars represent the standard deviation of triplicate sample measures.

remained relatively stable. The concentrations of free and total SO₂ also remained similar, regardless of Cu-treatment, throughout the 12-month storage period.

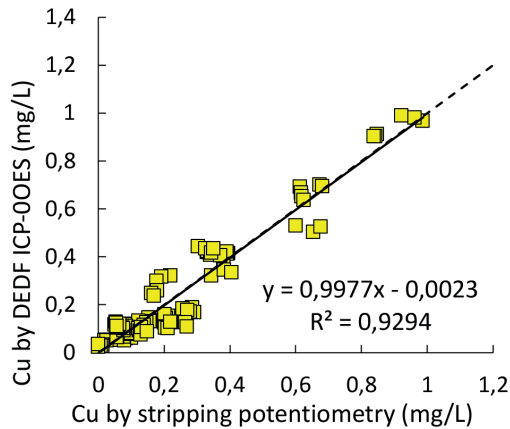


FIGURE 4. Comparison of 126 measurements of Cu fraction I: DEDF ICP-OES versus stripping potentiometry approaches.

Each data point represents a single bottle of Pinot noir or Shiraz wine analysed using both methods. The solid line is the trendline for the data, while the dashed line depicts the ideal trend (i.e., gradient = 1.0 and intercept = 0).

2. The change in Cu fractions

Cu fraction I concentrations were determined by both stripping potentiometry and DEDF ICP-OES (Figure 1), and the results obtained using these two methods during the 12-month storage experiment are compared in Figure 4. The line-of-best fit slope of 0.9977 (i.e., close to 1) and an intercept of 0.0023 (i.e., close to 0) demonstrated good agreement between the two techniques over the range of Cu fraction I concentrations measured in this investigation (i.e., 0 – 1.0 mg/L).

Figure 5 shows the changes in Cu fraction I and III concentrations during the storage experiment, as determined by the stripping potentiometry. In both the Pinot noir and Shiraz it can be seen that the Cu fraction I concentration decreased (Figures 5A and B), and the Cu fraction III concentration increased during storage (Figures 5C and D). The decrease in Cu fraction I occurred over 0-6 months for samples with 0.3 and 0.6 mg/L Cu(II) addition at bottling, and over 0-4 months for samples with 0 mg/L Cu addition (i.e., the control) at bottling (Figures 5A and B). After these periods of time the Cu fraction I reached a plateau in most samples, or slightly increased (e.g., 0.6 mg/L Shiraz sample, Figure 5B).

The decay in Cu fraction I, for the periods indicated above (i.e., 0-6 months for 0.3 and 0.6 mg/L Cu addition, and 0-4 months for 0 mg/L Cu addition), were best described by first-order decay (Supplementary Figure 2) and the rates determined are shown in Table 1. The fit of the modelled decay with the actual decay can be observed in Figures 5A and B. As expected, the Cu fraction I concentrations diverged from the first-order decay kinetics once the plateau for Cu fraction I concentrations were reached (Figures 5A and B).

During the period of first-order decay, the rates remained relatively similar across the different Cu(II) treatments and the different wines (Table 1), and when determined using the different analysis techniques of stripping potentiometry (Figures 5A and B) and DEDF ICP-OES (Supplementary Figures 2 and 3). Although the Shiraz wines showed trends of lower rates of Cu fraction I decay compared to the Pinot noir wines (Table 1), the rate differences were only significant when analysed by stripping potentiometry rather than by DEDF ICP-OES.

The half-life associated with the first-order decay rates suggested that the Cu fraction I concentration would halve approximately every 2 months.

TABLE 1. Rates constants for Cu fraction I decay and the amount of Cu fraction III produced during the storage experiment^{1,2}

Added Cu	Pinot noir Rate of Cu fraction I decay		Amount of increase in Cu fraction III ³ (mg/L)	Shiraz Rate of Cu fraction I decay		Amount of increase in Cu fraction III ³ (mg/L)
	Stripping potentiometry (day ⁻¹)	DEDF ICP-OES (day ⁻¹)		Stripping potentiometry (day ⁻¹)	DEDF ICP-OES (day ⁻¹)	
0 mg/L	0.014 ± 0.002 ^a	0.013 ± 0.004 ^a	0.36 ± 0.02	0.011 ± 0.002 ^{a,b}	0.011 ± 0.002 ^{a,b}	0.263 ± 0.003
0.3 mg/L	0.013 ± 0.001 ^a	0.013 ± 0.002 ^a	0.53 ± 0.04	0.0093 ± 0.0003 ^b	0.012 ± 0.002 ^a	0.44 ± 0.02
0.6 mg/L	0.011 ± 0.001 ^a	0.012 ± 0.002 ^a	0.80 ± 0.08	0.008 ± 0.001 ^b	0.012 ± 0.002 ^a	0.60 ± 0.03
Average	0.012 ± 0.001	0.013 ± 0.001	0.6 ± 0.2	0.010 ± 0.001	0.011 ± 0.001	0.4 ± 0.2
Half-life ⁴ (days)	55 ± 4			67 ± 9		

¹ The rate constants were calculated over 0-6 months for the 0.3 and 0.6 mg/L Cu treatments, and over 0-4 months for the 0 mg/L Cu treatments.

² The uncertainties indicated are the standard deviations, and the rate constants with different superscripts are significantly different (*p* ≤ 0.05) from one another.

³ Increase in Cu fraction III for the duration of the 12-month storage experiment as determined by stripping potentiometry analysis.

⁴ Overall average half-life for Cu fraction I concentration in the different red wines. Averaged across Cu treatments and analysis techniques for a given wine.

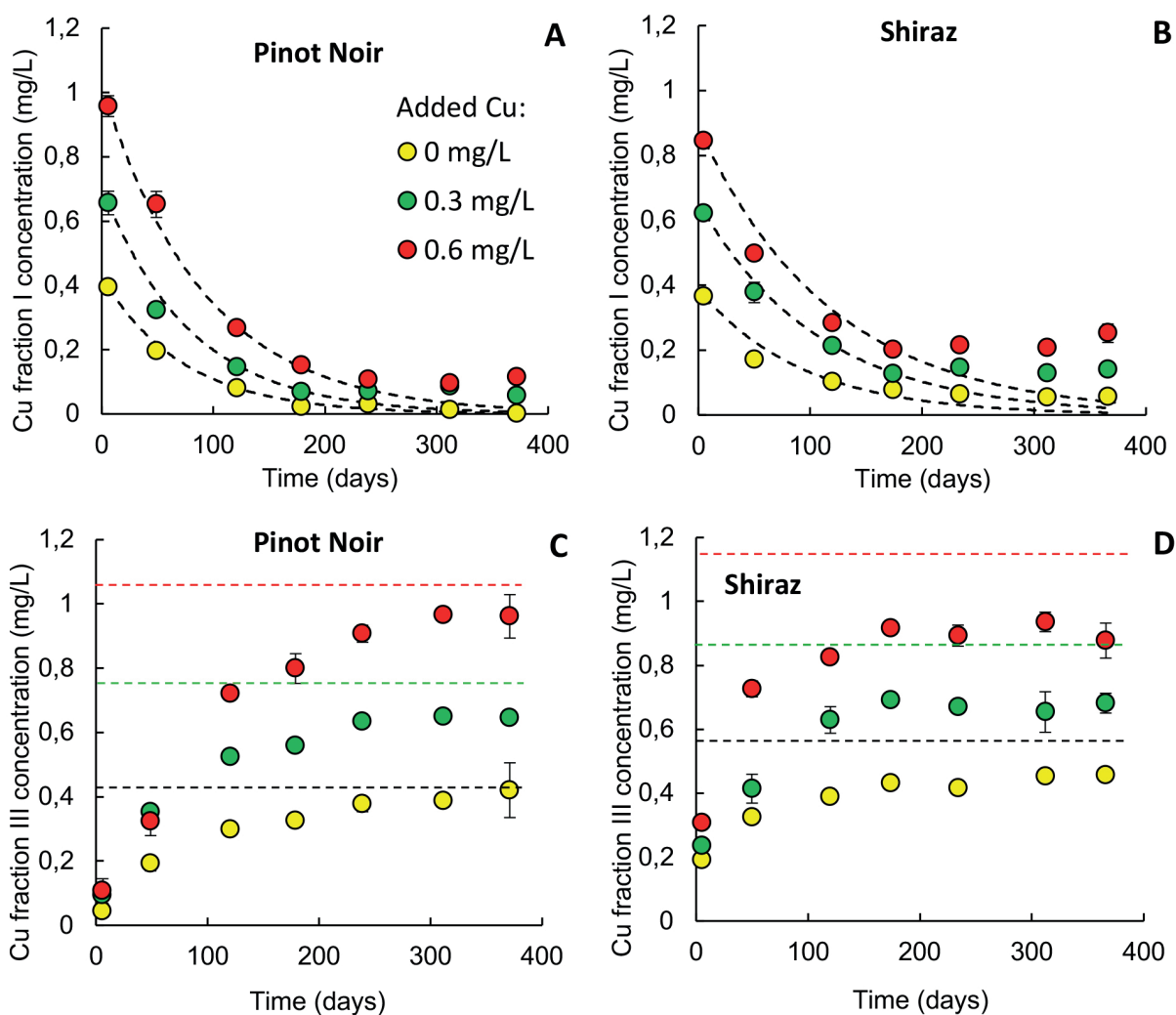


FIGURE 5. Cu fraction I (A and B) and Cu fraction III (C and D) concentrations, as determined by stripping potentiometry, in the Pinot noir (A and C) and Shiraz (B and D) wines during the storage experiment.

Error bars represent the standard deviation of triplicate sample measures. The dashed lines in panels A and B represent the modelled decay based on the first-order rate constants from Table 1, while the horizontal dashed lines in panels C and D represent the total Cu concentrations for the respective wines.

The measurements demonstrated that both red wines had the capacity to convert the majority of Cu fraction I to Cu fraction III during bottle aging (Table 1, Figure 5). Furthermore, it showed that increased Cu(II) additions at bottling led to higher amounts of residual Cu fraction I once the plateau concentrations were reached (Figure 5A,B). This was particularly evident in the Shiraz wines, in which appreciable levels of Cu fraction I remained in the 0.3 and 0.6 mg/L Cu-treated wines, but not in the 0 mg/L treatment (Figure 5B). This implied that the unknown precursors for sulfide in the Shiraz wines, utilised for the conversion of Cu fraction I to III, were depleted in the wines treated with 0.3 and 0.6 mg/L Cu(II) at bottling.

When the Cu fraction III was expressed as a percentage of total Cu (Supplementary Figure 4), the rate of increase during the first 0-6 months of the storage experiment was similar for each of the Cu treatments. This was consistent with the

similar first-order decay rates for Cu fraction I across Cu treatments. During the latter stages of the storage experiment, where the Cu fraction I concentration had reached a plateau, the percentage of Cu fraction III as a fraction of total Cu decreased in the higher Cu treatments.

DISCUSSION

Previous comparisons for the measurement of Cu fraction I by stripping potentiometry and DEDF ICP-OES have shown good agreement for wines with concentrations ranging from 0.03 to 0.20 mg/L (Clark *et al.*, 2020). Despite the samples in this study containing more than 5 times the Cu fraction I concentration (i.e., up to 1.0 mg/L), the results from Figure 4 show that the techniques still provided good agreement in the measurement of Cu fraction I. This is further evidenced by the similar first-order rate constants calculated using the different stripping potentiometry or DEDF ICP-OES data sets (Table 1).

The agreement between these two techniques confirms the negligible concentrations of Cu fraction II in the red wines, as its presence would have resulted in higher concentrations of Cu being detected by the DEDF ICP-OES method compared to stripping potentiometry (Figure 1, Clark *et al.*, 2020).

The total packaged oxygen and SO₂ concentrations (Figure 2) show that the red wines had a two-week period of aging in the presence of elevated oxygen concentrations immediately after bottling, and afterwards aging took place in low-oxygen conditions. The Cu fraction I concentration decreased in the red wines (Shiraz and Pinot noir) during bottle aging (Figures 5A and B) as previously observed for Cu fraction I in two different white wine varieties (Chardonnay and Pinot Grigio) (Zhang *et al.*, 2022). The decay rate of Cu fraction I was best described by first-order kinetics in the red wines (Table 1), which has also been found to be the case for white wines (Zhang *et al.*, 2022). However, in our previous study on white wines, the first time point after bottling did not fit the first-order decay profile and we suggested it was due to the wine containing appreciable total packaged oxygen (3.5–5 mg/L) at this time point. This is different to the red wine, for which the first time point measurement (i.e., day 6) fits the first-order decay (Supplementary Figure 3). Therefore, it is likely that the faster consumption of oxygen in red wine compared to white wine (Kontoudakis and Clark, 2020) allowed low oxygen concentrations to be reached fast enough for the rate of Cu fraction I decay to remain first-order after bottling.

The first-order rates of Cu fraction I decay in the red wines (Table 1) were of similar magnitude to previously reported rates (i.e., 0.0088 ± 0.0001 per day) in Pinot Grigio, but were significantly higher than in Chardonnay (0.0059 ± 0.0002 per days) (Zhang *et al.*, 2022). All these wines underwent storage in similar conditions of darkness and temperature (14 °C). This meant that the half-life of the Cu fraction I was around 60 days in the red wines, 80 days in the Pinot Grigio and 120 days in the Chardonnay. However, in the red wines, the Cu fraction I concentrations would decrease by half every two months immediately after bottling, while in the white wines of Zhang *et al.* (2022), the half-life trend would only be observed once the total packaged oxygen was sufficiently low in the wine (i.e., after 2 months).

The decay rates in Table 1 enabled the calculation of the time (after bottling) required for the Cu fraction I concentration to fall below critical concentrations in the red wines; that is, concentrations below which Cu fraction I did not limit hydrogen sulfide and methanethiol accumulation. For the samples without Cu(II) addition at bottling, the Shiraz was calculated as requiring 6.3 and 9.1 months to fall below a Cu fraction I concentration of 0.05 and 0.02 mg/L respectively, while the Pinot noir required 5.2 and 7.5 months respectively. Given the tendency for the measured concentrations to be higher than the modelled concentrations during the later stages of storage (i.e., at the plateau), it is likely that the times calculated for Cu fraction I depletion would underestimate the actual time required. Once the Cu fraction I concentration fell below the critical concentrations, additional time would

be required for enough reductive aroma compounds to accumulate in order to exceed their aroma thresholds in the red wine. Identifying wines prior to bottling that are at risk of developing reductive aromas during bottle aging and determining the lag period before reductive aroma emerges are the subject of future work. Already, accelerated aging experiments (i.e., using elevated wine storage temperature) have shown some links to the reductive development of wines aged at room temperature (Franco-Luesma and Ferreira, 2016). The plateauing of Cu fraction I concentrations (Figures 5A and B) suggests that the precursors in wine that enable the conversion of Cu fraction I to Cu fraction III can gradually become depleted at sufficiently high initial Cu fraction I concentrations.

Figure 5 shows that the capacity for Cu fraction I in red wine to be transformed to Cu fraction III is high when compared to the typical concentrations of Cu found in wine. For example, the average total Cu concentration of 29 red wines has been reported to be 0.3 mg/L (Kontoudakis *et al.*, 2019), which is lower than the amount of Cu transformed to Cu fraction III in all but one of the red wine treatments in Table 1 (i.e., the Shiraz without added Cu). In fact, in the red wines treated with 0.6 mg/L Cu at bottling the conversion of Cu fraction I to III was twice that of those treated with 0.3 mg/L Cu. Based on the high capacity for red wine to transform Cu fraction I to III, it is difficult to envisage Cu treatment during wine production that would comply with good practice (i.e., Cu legal limits, avoiding Cu-induced haze) and permanently maintain Cu fractions I concentrations above 0.02 mg/L. In the present study, plateau concentrations of > 0.02 mg/L for Cu fraction I were observed in the red wines (Figures 5A and B), but this was only achieved with relatively high total Cu concentrations in the wines (i.e., > 0.39 mg/L). Instead, it would appear more feasible that Cu additions be made to wine at bottling to maintain Cu fraction I concentrations at > 0.02 mg/L for a certain length of time (e.g., 4 to 6-months). However, further studies are required to determine whether red wines that have converted a significant portion of Cu fraction I to Cu fraction III (i.e., 0.1–0.2 mg/L) are still resistant to reductive aroma development once the Cu fraction I has been depleted; in other words, can a wine become ‘permanently’ resistant to reductive development once a sufficient amount of Cu fraction I has been converted to Cu fraction III, and once the most unstable reductive aroma precursors have been exhausted?

Figure 6 is a schematic summary of the transformation of the two key measurable Cu fractions in red wine. Under sufficiently high oxygen conditions, Cu fraction III can be rapidly converted to Cu fraction I, as described by Kontoudakis and Clark (2020). This is thought to be mainly due to the dissociation of copper sulfides due to oxidative mechanisms, including the reaction of sulfide/hydrogen sulfide with *ortho*-quinones (i.e., oxidised phenolic compounds) (Nikolantonaki and Waterhouse, 2012) or diorganopolysulfane production via redox cycling of Cu and/or Fe (Kreitman *et al.*, 2017). In fact, the diorganopolysulfanes are examples of an ‘oxidation product’ that could also act as precursors to Cu fraction III

formation due to the production of hydrogen sulfide in low oxygen conditions (Figure 6). It should be noted that the presence of 2.5–4 mg/L total packaged oxygen just after the bottling of the Shiraz and Pinot noir wines (Figure 2) was not enough to cause any measurable oxidative increase in Cu fraction I concentration.

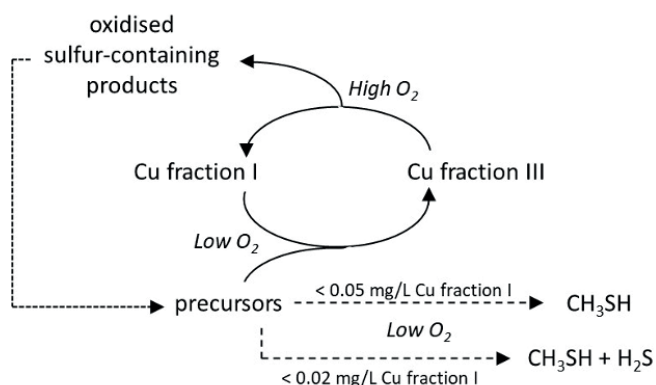


FIGURE 6. The transition between Cu fraction I and Cu fraction III in red wine, and the potential accumulation of methanethiol (CH₃SH) and hydrogen sulfide (H₂S) under conditions of low Cu fraction I concentration.

In low oxygen conditions, Cu fraction I present at bottling can readily source sulfide from precursors and undergo first-order decay to form Cu fraction III (Figure 6). Cu fraction II, which is common in white wine but has not been detected in red wine (Clark *et al.*, 2020), is not essential for the conversion of Cu fraction I to Cu fraction III. The concentration of the precursors that enable the transition of Cu fraction I to III was sufficiently high in the studied red wines for the binding of most of the Cu in the wine. In addition, the first-order decay of Cu fraction I demonstrated lower stability for precursors in the presence of higher concentrations of Cu fraction I. Identifying the key precursor/s involved in the transition between Cu fractions (as shown in Figure 6) will still be necessary in further studies, but it is presently known that diorganopolysulfanes contribute to the release of a certain amount of hydrogen sulfide during wine aging in low oxygen conditions. Once Cu fraction I concentration falls below the critical concentrations of 0.02 and 0.05 mg/L (Kontoudakis *et al.*, 2019), the accumulation of the reductive aroma compounds can presumably occur from a slow degradation of precursors in a mechanism that is independent of Cu fraction I. As already mentioned, the lag period between loss of Cu fraction I and the accumulation of the reductive aroma compounds requires further investigation.

CONCLUSIONS

This study demonstrated that Cu fraction I, the key form of Cu that enables protection against reductive red wine development, decreased during the bottle aging of Shiraz and Pinot noir wines. This loss of Cu fraction I was found to fit first-order decay kinetics and by determining the associated

rate constants the half-life of this form of Cu was calculated to be approximately 2 months. The concentrations of Cu fraction I converted to Cu fraction III during the bottle aging of the red wine treatments was greater than the typical total Cu concentrations found in most red wines. This study provides key insights into the behaviour of Cu fractions during the bottle aging of red wine.

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