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Removal of sulfide-bound Cu from white wine by membrane filtration

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Abstract

Background and Aims: Sulfide-bound Cu in wine may act as a potential source of hydrogen sulfide. The aim of this study was to understand how the white wine matrix can influence the filterability of sulfide-bound Cu.

Method and Results: Sulfide-bound Cu was formed in situ with copper(II) sulfate and sodium sulfide addition to white wine and model wines. The amount of subsequent Cu passing through membrane filters was measured by flame atomic absorption spectroscopy or inductively coupled plasma with optical emission spectroscopy. Nanoparticle tracking analysis was utilised to measure the size of particles generated after copper(II) and sulfide addition. The majority of the particles were around or below 0.2 μm, and polyethersulfone and nylon membranes remove up to 40–90 % of sulfide-bound Cu from white wine. The regenerated cellulose, teflon and glass fibre membranes removed minimal sulfide-bound Cu.

Conclusions: Membrane filtration removed sulfide-bound Cu by adsorption rather than by particle size discrimination. Polysaccharides and proteins were the components of white wine that most inhibited adsorption.

Significance of the Study: The addition of copper(II) to wine with hydrogen sulfide results in products that cannot be removed by filtration on the basis of their particle size, but instead may be partly removed by adsorption onto membrane filters to an extent impacted by wine composition and the filtration medium.

Keywords: *copper sulfide, membrane filtration, nylon , polyethersulfone (PES), polysaccharide, teflon (PTFE), white wine*

Introduction

Recent work has shown that sulfide appears to interact more strongly with copper (Cu) in wine than other likely copper-complexing species (Clark et al. 2016, Kontoudakis et al. 2017). Electrochemical and solid phase extraction techniques showed that a single measured fraction of Cu dominated ten white wines, and after assaying likely complexing agents, only hydrogen sulfide in the model wine system was able to generate the same fractions of Cu as detected in the white wines. This inferred that either the major form of Cu in white wine is that involving sulfide binding or that an unknown complexing agent in wine was also capable of impacting the classification of Cu by the speciation techniques adopted. Coupled with this is evidence that in low oxygen conditions during bottle aging, the total copper concentration in wine has been linked to an increase in the hydrogen sulfide concentration, as well as other volatile sulfur compounds, a process contributing to the reductive development of wine (Ugliano et al. 2011, Viviers et al. 2013, Ferreira et al. 2017). The hydrogen sulfide can be sensed by the consumer as an off-aroma (rotten egg) and has a detection threshold of 1.6 μg/L (Siebert et al. 2009). A portion of the hydrogen sulfide that accumulates in wine during reductive aging has been attributed to release of sulfide from sulfide-bound Cu in low oxygen conditions, although to date, the exact mechanisms of release remain to be established (Smith et al. 2015, Ferreira et al. 2017).

Copper sulfide formation is a complex process with various stoichiometric and non-stoichiometric compounds possible. For solid salts these include covellite (CuS), yarrowite (Cu_9S_8), spionkopite ($Cu_{1.4}S$), anilite ($Cu_{1.75}S$), digenite ($Cu_{1.8}S$), djurlite ($Cu_{1.95}S$) and chalcocite (Cu_2S), and thus ranging from a S to Cu ratio of 1:1 to 1:2 (Nelwamondo et al. 2012). The difficulty in assigning a simple oxidation state to either copper or sulfur is complicated by the inherent ready polarisability of the sulfur electron cloud that in essence results in the Cu–S bond being more covalent than ionic (Buluggiu et al. 1972). To add to the complexity of copper sulfide chemistry is its ability to exist as 'nanoparticles' that may be dispersed within solution. A nanoparticle is generally used to indicate a particle of 1–100 nm size that behaves as a whole unit in terms of its transport and properties (Zaman et al. 2014). The definition is, however, complicated by nanoparticles having a size distribution, which in some cases may be considerably large and partially outside the 1–100 nm range. Also, there is a tendency for the nanoparticle term to be also used for larger particles (100–500 nm) if they exhibit specific behaviour (i.e. transparency or turbidity in solution) which are different from those of the corresponding bulk materials (European Commission 2010). Copper sulfide nanoparticles are composed of Cu(II) [or Cu(I)] and S^{2-} units and a study has estimated that a particle size distribution between 3 and 26 nm arises from 10^4 to 10^6 CuS units (Ciglene ki et al. 2005). Copper sulfide can also potentially exist as clusters (Luther et al. 2002). Clusters are defined as a polynuclear complexes with exact chemical composition and structure, and are generally small enough to behave as a

dissolved species (Luther and Rickard 2005). It is recognised that there can be overlap in the dimensions of clusters and nanoparticles, such that nanoparticles can also be small enough to be indistinguishable from dissolved species (Luther and Rickard 2005). As a result, in the general scientific literature, there is often no clear discrimination between the terms 'cluster' and 'nanoparticle' (Schmid and Fenske 2010), and generally the points of difference are the less precise characterisation and the existence of a size distribution for nanoparticles compared to clusters.

The reaction between copper(II) and excess hydrogen sulfide in a model wine resulted in copper(II) reduction and hydrogen sulfide oxidation, and it was proposed to form a copper(I) sulfide complex at a ~1.4:1 mole ratio of hydrogen sulfide to copper(II) (Kreitman et al. 2016). The higher S:Cu ratio here is surprising, given that the more common ratio of S:Cu is between 1:1 and 1:2 (Nelwamondo et al. 2012). Kreitman et al. (2016) appear to have based their interpretation on a study that examined the mechanism of copper sulfide formation in an aqueous system at pH 8 where copper sulfide clusters, composed of neutral six-membered Cu_3S_3 rings, were proposed to form during the titration of sulfide with copper(II) (Luther et al. 2002). These rings were proposed to evolve to more complex polynuclear nanoclusters, $[Cu_4S_5]^{4-}$ and $[Cu_4S_6]^{4-}$, each containing Cu(II). This cluster formation, however, has been disputed and data supporting the existence of these CuS clusters, and their corresponding stoichiometry, were instead attributed to artefacts in electrochemical and laser ablation mass spectrometry analysis (Ciglene ki et al. 2005). Instead, Ciglenecki

et al. (2005) proposed that no evidence exists to prove that such transient CuS cluster species have a lifetime sufficiently long to make them significant components of dissolved copper(I) sulfide during copper(II) and sulfide titration. Instead they proposed the initial formation of a brown sol (solution of dispersion of fine particles) of $Cu_2S.S^0(s)$ which transforms gradually to a green sol of CuS(s). Ciglenecki et al. (2005) did indicate, however, that nanomolar Cu concentration in more complex environmental samples, containing natural surfactants, may be more conducive to slow CuS growth and subsequent cluster stabilisation.

The matrix of wine provides particular complexity to CuS formation with the presence of selected proteins or amino acids, which in synthetic media have been shown to act as capping agents on copper(I) sulfide nanoparticles (Nelwamondo et al. 2012, Kreitman et al. 2016). The capping agents are able to bind strongly to the nanoparticle surface and act as natural surfactants, with a polar group that confers stability in aqueous conditions to enable solubility of the copper sulfide. In a model wine, hydrogen sulfide was shown to bind more strongly to copper(II) than to cysteine, but the presence of cysteine was able to induce less visible precipitate, hence potentially acting as a capping agent in the model wine conditions (Kreitman et al. 2016). It is apparent from this review of published work that the formation of CuS can be influenced by a range of compounds that may be present in wine and thus affect the exact composition of the final copper sulfide species. For terminology purposes, the

copper sulfide dispersed in wine and model wine used in this study will be referred to as sulfide-bound Cu, appreciating that it is most likely a complex mixture of stoichiometric forms, and could exist as clusters and/or nanoparticles. In terms of the physical behaviour of sulfide-bound Cu in wine or model wine, it does not readily settle, and only presents the slightest gold/brown tint to these solutions when at high concentration, that is barely evident with addition of 0.25 mg/L hydrogen sulfide and 1.0 mg/L copper(II) (Clark et al. 2015).

Sulfide-bound Cu as a potential source of hydrogen sulfide in wine is likely to be significant in terms of mass balance. For example, if 0.60 mg/L Cu is present in wine in the form of copper(I) sulfide, which is postulated to have a mole ratio of hydrogen sulfide to copper of ~1.4 : 1, then this is a potential source of 0.45 mg/L hydrogen sulfide, which is well above its aroma threshold of 0.0016 mg/L. Although release of all sulfide from Cu is unlikely to occur, even in low oxygen conditions, a 1 % release of sulfide from copper(I) sulfide would result in the formation of 0.005 mg/L hydrogen sulfide, which is three times the aroma threshold. Of course, such a calculation does not take into account other thiol compounds, for example capping agents, that may be incorporated into or upon the surface of the sulfide-bound Cu material.

The ability to remove sulfide-bound Cu particles by filtration has been investigated utilising cellulose-based membranes (Clark et al. 2015), and in comparison to filtration media used in wine production, cellulose is utilised in depth filtration media

rather than membranes (Bowyer et al. 2013). Other filtration media used in wine production include nylon or polyethersulfone membranes (Bowyer et al. 2013), with pore sizes of 0.45 μm or greater, but their efficiency for sulfide-bound Cu removal is unknown. Clark et al. (2015) showed that sulfide-bound Cu could be removed from a 12%(v/v) aqueous ethanol tartaric acid (pH 3.2) solution with a 0.45 or 0.2 μm pore size regenerated cellulose (RC) membrane filter. This was not the case, however, for white wine where the sulfide-bound Cu concentration was largely not impacted by membrane filtration as determined by flame atomic absorption spectroscopy (FAAS) before and after filtration. Clark et al. (2015) proposed that the presence of more complexing agents for copper(II) in white wine compared to the model wine were inducing the formation of smaller sulfide-bound Cu particles that were able to pass through the RC filter. Work by Bekker et al. (2016), demonstrated that copper(I) sulfide particles in model wine provided average size ranging from 90–134 nm, and a size distribution from 20–300 nm, depending on the concentration and ratio of addition of sodium sulfide and copper(II). However, the particle size of sulfide-bound Cu in wine was not measured.

This study aimed to understand the relative filterability of sulfide-bound Cu in model wines compared to that in white wine. The main white wine components impacting filterability were assessed and five membranes compared for their ability to

remove sulfide-bound Cu. Further, the particle size of sulfide-bound Cu formed both in model wine and in white wine was determined using nanoparticle tracking analysis.

Materials and methods

Quantification of copper

For samples prepared at a concentration of 1.0 mg/L or above, the copper concentration was determined by FAAS using a Varian SpectrAA 50B with a Cu hollow cathode lamp (S & J Juniper, Harlow, England; operating wavelength of 324.8 nm). In order to aid the dissociation of sulfide-bound Cu prior to analysis, samples were adjusted to 10.0 mg/L silver(I) by the addition of 0.1 mL of 1000 mg/L silver(I) (prepared from silver nitrate) to 10.0 mL of sample. The addition of silver(I) was found to improve the recovery of copper and the repeatability of its measurement by FAAS in solutions where copper was predominantly in a sulfide bound form. For example, after the addition of sodium sulfide and Cu(II) to four different model wine systems (Control, or Control with addition of protein, white wine phenolic substances, or polysaccharides) at a mole ratio of 2 : 1 for hydrogen sulfide to Cu [1.0 mg/L Cu(II)], the average recovery of Cu was 98 ± 10 % and 66 ± 20 % and for samples with and without silver(I), respectively. Other than the addition of silver(I), the samples were analysed directly by FAAS and quantified by the use of an external calibration graph. The copper(II)

calibration standards for FAAS were prepared in the model wine and silver(I) also added to the standards at the same rate as the samples.

For samples prepared at a concentration of 0.30 mg/L, the copper concentration was determined by inductively coupled plasma with optical emission spectroscopy (ICPOES). The instrumental and associated settings were identical to those described in Rousseva et al. (2016). Samples were diluted fourfold with 5.0 %(v/v) nitric acid prior to analysis, and quantification was afforded by an external calibration graph generated from copper(II) standards in the model wine, also diluted fourfold with 5.0 %(v/v) nitric acid.

Model and white wines

A model wine solution was prepared as described previously (Clark et al. 2003) and consisted of 12% (v/v) aqueous ethanol buffered to pH 3.2 with 11 mmol/L potassium hydrogen tartrate and 7 mmol/L tartaric acid. The white wine utilised for the model wine / wine mixtures (Figures 1,2) was a blend of Vermentino and Fiano (2016) and had a pH of 3.30, 20 mg/L free SO_2, 120 mg/L total SO_2 with a copper concentration of 0.16 mg/L. The white wine utilised for the assessment of membrane filtration media, was an equal blend between four separate bottles of white wine that were all low in copper concentration (< 0.07 mg/L); the blend had a free and total SO_2 concentration of 22 and 127 mg/L, respectively, and was adjusted to a pH of 3.2 prior to use. The white

wines blended were a Chardonnay (2014), Verdelho (2013), Riesling (2013) and Moscato (2013), and all were 3–4 years old when used. All white wines, immediately after opening their bottle, were either used directly or blended, as indicated. Once opened, white wines were used without further protection against oxygen during sample preparation, sodium sulfide and copper(II) addition, and during filtration. All operations were conducted, however, within an hour of opening the bottle of wine in order to minimise oxidation.

In situ production of sulfide-bound Cu in the model wine, white wine and mixtures

A stock solution of copper(II) ($CuSO_4.5H_2O$) was prepared in the model wine [500 mg/L copper(II)] and diluted fivefold with model wine to provide 100 mg/L copper(II). The stock of hydrogen sulfide was prepared with sodium sulfide (Na_2S; Sigma-Aldrich, St Louis, MO, USA; > 97 % purity) in water at 4°C (1.06 g/L Na_2S, 13.6 mmol/L) and it was prepared immediately prior to usage.

Model wines or white wines were placed in 100 mL volumetric flasks followed by addition of hydrogen sulfide (as sodium sulfide), single inversion to mix, then addition of copper(II), and triple inversion to mix. Hydrogen sulfide and copper(II) were added at a 2:1 ratio, with either hydrogen sulfide at 31.4 μmol/L and copper(II) at 15.7 μmol/L (1.0 mg/L), or 62.8 μmol/L and 31.4 μmol/L (2.0 mg/L), respectively. Where indicated in the text, a hydrogen sulfide to copper(II) ratio of 5:1 was also

utilised [at 1.0 mg/L copper(II)], as well as samples at the ratio of 2:1 but with lower copper(II) (0.3 mg/L, 4.72 μmol/L) and hydrogen sulfide (9.44 μmol/L) concentration. All solutions were prepared in triplicate. Unless otherwise indicated, filtration was conducted with a 0.20 or 0.45 μm regenerated cellulose membrane syringe filter of internal diameter 26 mm, and 10.0 mL of sample was passed through the filter. Non-filtered and filtered samples were analysed for copper by either FAAS or ICPOES. In the data presented in subsequent figures, the amount of copper eluting through the filter is expressed as a proportion of the total copper originally added to the solution (Figure S1).

To assess the behaviour of sulfide-bound Cu in model wine/white wine blends the following solutions were prepared in triplicate: (i) 100%(v/v) model wine; (ii) 99%(v/v) model wine and 1%(v/v) white wine; (iii) 95%(v/v) model wine and 5%(v/v) white wine; and (iv) 90%(v/v) model wine and 10%(v/v) white wine. The order of addition of sodium sulfide and copper(II), the mixing of solutions and filtration was as described in the previous section. The remaining unfiltered samples (80 mL), were then diluted twofold so that they all contained a composition of 95%(v/v) model wine and 5%(v/v) white wine after dilution. Consequently, 10.0 mL samples were taken for either direct analysis (i.e. unfiltered) for copper by FAAS, or filtered through a 0.20 or 0.45 μm RC membrane syringe filter (26 mm i.d.) and then analysed by FAAS.

Model wines to survey the impact of wine components on sulfide-bound Cu filterability

All samples were prepared as 12%(v/v) aqueous ethanol solutions and were adjusted to a final pH of 3.2 with either 10%(w/v) sodium hydroxide or 10 mol/L sulfuric acid. The organic acids examined were tartaric acid, malic acid, succinic acid and lactic acid and all were used at a concentration of 2 g/L. Additionally, 300 mg/L caffeic acid, 2 g/L glucose, 1 mg/L iron(II), 40 mg/L SO_2 or 200 mg/L ascorbic acid were added to the (2 g/L) tartaric acid model wine system.

The components extracted from white wine were polysaccharides, protein and phenolic substances. Details on the method of extraction of these components from wine have been described previously, along with a detailed analysis of their composition (Kontoudakis et al. 2017). A summary of the composition can be found in Table S1. The wine components to be assessed were added at a typical wine-like concentration (Ferreira et al. 2001, De Beer et al. 2005, Gawel et al. 2016), with the white wine protein at 50 mg/L, white wine polysaccharide at 200 mg/L and the white wine phenolic substances at 200 mg/L.

Assessment of membrane filtration media

All membrane filters were sourced from Phenomenex (Lane Cove, NSW, Australia), and were of the following composition: RC, nylon, polyethersulfone (PES),

polytetrafluoroethylene (teflon, PTFE) and glass fibre (GF) (borosilicate). Apart from the GF membrane, the membranes were utilised at both 0.20 and 0.45 μm pore size. The GF membrane had a pore size of 1.2 μm (the lowest available pore size for this type of filter). All membrane filters were used as syringe filters with a diameter of 25–28 mm, average surface area of 5.5 cm^2 and thickness of 0.14 mm. During the survey of the different membrane types and pore sizes, samples were prepared in 250 mL volumes in triplicate, and 10 mL aliquots of sample were collected and either left unfiltered, or filtered via the various 0.45 μm or 0.20 μm syringe filters, and all solutions placed in a corresponding 15 mL centrifuge tube for later analysis by FAAS or ICPOES. Collection of the unfiltered sample and the filtered samples was all conducted within an hour of preparing the original sulfide-bound Cu solutions.

Particle size measurements

Model wine, white wine, sulfide-bound Cu in model wine and sulfide-bound Cu in white wine were analysed for particle size using a nanoparticle tracking analysis (NTA) system. For the time-course experiment, a Sauvignon Blanc wine was transferred to three separate Duran reagent bottles, wrapped in aluminium foil to limit light exposure, and each bottle was sampled on preparation, and then after 1, 2, 3, 5, 6, 21 and 28 h. Samples were prepared, stored and sampled in an anaerobic hood. Ten replicate measurements were performed on each replicate sample at each time point.

Nanoparticle tracking analysis was carried out with a NanoSight NS300 instrument (Malvern Instruments, Malvern, England), consisting of a conventional optical microscope, CCD camera, a sample chamber with a laser light source and a syringe pump. The samples were injected into the sample chamber with a sterile 1 mL syringe. A video clip of the nanoparticles submitted to their natural Brownian motion was captured over 60 s at 22.0°C and analysed by the analytical software version 2.3. Analysis was performed with a constant and controlled sample flow. The samples were measured with manual shutter and gain adjustments. As recommended for polydispersed samples the 'extended dynamic range mode' was used. Post-acquisition NTA settings were optimised and kept constant between samples, and each video was then analysed to give mean particle size together with an estimate of the concentration.

Results and discussion

In this study, copper(II) was added to white wine containing hydrogen sulfide to mimic the copper fining of wine. The mole ratio of hydrogen sulfide to Cu in the wine (2:1) was higher than would be typically encountered during wine production, but selected to maximise the proportion of copper in the sulfide-bound form, and hence allow study of its interaction with filtration media. After the addition of copper(II), the samples were filtered within 1 h to ensure minimal time for sample oxidation and thereby maximise sulfide-bound copper prior to filtration. As a result, a white wine prepared with added hydrogen sulfide and Cu in the manner described above was analysed by electrochemistry (Clark et al. 2016) which showed that all Cu remained in a sulfide

bound state (i.e. electrochemically inactive) after filtration (Figure S2). As the FAAS and ICPOES techniques used to detect copper in subsequent samples of this study did not provide information on the form of copper present at the time of measurement, the detected copper will be referred to as Cu.

Impact of white wine on the filterability of sulfide-bound Cu in a model wine system

Previous research has shown a clear difference in sulfide-bound Cu filterability in white wine and model wine (Clark et al. 2015). Therefore, it was decided to assess the proportion of white wine in a model wine system that would begin to inhibit the removal of the sulfide-bound Cu by filtration. Although membrane filters of pore size less than 0.45 µm are rarely used in wine production (Bowyer et al. 2013), both 0.45 and 0.20 µm pore sizes were investigated to allow insights into the impact of filter pore size and also to allow more direct comparison to previous studies (Clark et al. 2015). It was originally envisaged that perhaps a different proportion of white wine in the model wine may also enable control over the size of sulfide-bound Cu particles formed in situ. Figure 1 shows the results of forming sulfide-bound Cu in solutions with a different proportion of white wine to model wine, and the subsequent filtration of the solutions. On the figures, a value close to 100% means that the Cu is largely all passing through the filter, whilst a value close to 0% means that most Cu is retained on the filter (Figure S1). From the results in Figure 1, it is evident that as little as 1%(v/v) white wine in model wine largely prevented sulfide-bound Cu (with copper at a concentration of 2.0

mg/L) removal by filtration with an RC membrane filter of either 0.45 or 0.20 μm pore size. This suggested that either the interfering agent in white wine was at high concentration or extremely potent at low concentration to afford an effect despite the white wine being diluted 100-fold.

A portion of each of the unfiltered samples in Figure 1 was diluted twofold with model wine and white wine such that they all had a final composition of 5%(v/v) white wine and a sulfide-bound Cu concentration equivalent to 1.0 mg/L copper. The samples were then taken and filtered in the same manner as for Figure 1 and the resulting filtrates measured for Cu. The results in Figure 2 show that even the model wine with pre-formed sulfide-bound Cu, after adjustment to 5%(v/v) white wine, no longer allowed sulfide-bound Cu removal by filtration. Intriguingly, this meant that the active white wine component could impact the filterability of the sulfide-bound Cu after formation of the suspended solid. Therefore, if filtration was more successful at removing sulfide-bound Cu in the model wine than the white wine due to particle size, then addition of the white wine to a concentration of 5%(v/v) was altering the size of the sulfide-bound Cu particles to be less than 0.2 μm. An alternative explanation was that the filtration material was removing sulfide-bound Cu particles from the model wine by an adsorption process rather than size discrimination and that the white wine matrix hindered this adsorption. The ability of polymer membranes to adsorb

nanoparticles is well established. For example, cellulose acetate membranes have been used to support iron nanoparticles in waste water treatment (Wu et al. 2005).

Particle size measurements of sulfide-bound Cu in white wine and model wine systems

To differentiate between the two possible mechanisms for action of the filtration membrane on sulfide-bound Cu filtration, nanoparticle tracking measurements were made (Figure 3). Preliminary measurements showed the addition of copper(II) to white wine, without addition of any hydrogen sulfide, resulted in negligible change in particle size or concentration within the wine (Figure S3). In contrast, a dramatic increase in the particle concentration was observed with the addition of both hydrogen sulfide and copper(II) (Figure S3), implying that the sulfide-bound form of Cu was critical for the formation of the particles. The results in Figure 3a show that the sulfide-bound Cu particles formed in the model wine system are in fact smaller than those found in the white wine system, which is the opposite result expected if filtration due to size was responsible for the results in Figure 1. The analysis of the particles in all solutions of Figure 3a was conducted 10 min after their formation. The particles in the model wine were well below 0.2 μm, whilst for white wine the majority of the particles was just below 0.2 μm, but a portion (~<20%) were found to fall within the 0.2–0.3 μm range. These results demonstrate that the removal of sulfide-bound Cu from the model wine by the 0.2 μm pore size RC filter was not due to a size-related mechanism. Instead it is

more likely that the sulfide-bound Cu particles within the model wine matrix were adsorbed on the RC medium.

As shown in Figure 3b, the average size of the sulfide-bound Cu in the model wine and Viogner wine was 102 ± 2 and 211 ± 3 nm in diameter, respectively. If it is assumed the particles were only due to Cu and sulfide, relative to particles with radii of 3–26 nm (or 6–52 nm diameter), with an estimated 10^4–10^6 CuS units suggested by Ciglene ki et al. (2005), the copper(I) sulfide in both model and white wine systems would be in significant excess of 10^6 CuS units in their particles with radii of 51–105 nm. The particle size measurements, however, tell nothing of the composition of the particles and hence they could also include surface adsorbing wine components that contribute to the overall size of the particles, which would result in much smaller numbers of CuS units. Further work could investigate a lower concentration of Cu and sulfide addition to wine, along with lower ratio of hydrogen sulfide to Cu, which would be closer to the Cu fining conditions found in wine. Assessment should be made if such conditions have any substantial effect on particles sizes and consequent adsorption behaviour onto filtration material.

The size of the sulfide-bound Cu particles in Sauvignon Blanc wine was monitored over time to establish the initial particle concentration (particles/mL) and size that could be detected, along with any changes over time. Figure 3c shows that at

'time 0' the sulfide-bound Cu formed in the wine is 175 ± 1 nm, indicating that this particle size is obtained within the 10 min after addition of copper(II) and sodium sulfide to the wine. Figure 3d shows that there is a general increase in sulfide-bound Cu particle size from 175 ± 1 nm to 240 ± 2 nm in diameter over a 28 h period, and that a plateau in particle size occurs from around 20 h. This change in size is coupled with a decrease in particle concentration (Figure 3c). Consequently, there is gradual conversion of smaller nanoparticles initially formed to larger particles until a mean size around 200–240 nm is reached.

Identification of white wine components able to influence sulfide-bound Cu adsorption on RC filter membranes

A survey was conducted to assess those components of white wine that were impacting the adsorption of sulfide-bound Cu on the RC filtration membrane. Initially components found in white wine in the g/L concentration range were surveyed, apart from those already present in the model wine system. The main compounds that inhibit and/or promote wine oxidation were also surveyed, including SO_2, ascorbic acid, iron(II) and caffeic acid as a model phenolic substance. In all cases, the filtration of the model wine with an added component, each containing sulfide-bound Cu (at 1.0 mg/L copper), resulted in less than 10% of the Cu passing through the filter (data not shown) despite the presence of the individual wine components. This was the case with RC filters at both 0.45 and 0.20 μm pore size. These results clearly indicated that none of the

components surveyed were by themselves responsible for inhibiting the adsorption of sulfide-bound Cu onto filtration membranes in a white wine matrix; that is, the single component addition to model wine could not replicate the results of white wine filtration.

Consequently, it was decided to assess white wine polysaccharide and protein macromolecules, previously extracted from white wine, as well as a generalised group of white wine phenolic substances (Table S1). The addition of white wine protein or polysaccharide to the model wine with sulfide-bound Cu (equivalent to 1.0 mg/L copper) allowed essentially 100% of the Cu to pass through the filter, compared to 4 ± 1% in the absence of either macromolecule confirming that these two macromolecules were able to prevent the adsorption of sulfide-bound Cu on the RC filtration media. The mechanism is most likely due to the macromolecule preferentially adsorbing onto the filtration media in place of sulfide-bound Cu and/or the sulfide-bound Cu interacting with the macromolecule preferentially to the filtration media. Polysaccharides and proteins are well known to adsorb onto filtration media (Susanto and Ulbricht 2007, Ulbricht et al. 2009) as well as interacting with copper sulfide particles. As an example of the latter, macromolecules can act as 'spacers' in colloidal and nanoparticle suspensions where their role is to either keep the nanoparticle surfaces apart or hold them together (Cabane et al. 1989). The physical chemistry behind these two roles is strongly dependent on the specific surface chemistry of the macromolecules and the

nanoparticles. For example, in biological settings, the interaction of proteins with nanoparticles is known to induce or inhibit protein aggregation due to the ability of the nanoparticle surface to generate dense layers of proteins surrounding this surface (Zaman et al. 2014). Furthermore, copper casse induced in white wines at a high copper concentration has been attributed to copper sulfide formation and its consequent interaction with wine protein to induce flocculation and a haze (Peterson et al. 1958). Both proteins and polysaccharides are also used in the production of copper sulfide nanoparticles where their role is to act as growth scaffolds and stabilisers. In this manner, amylose and xylan polysaccharides, bovine serum albumin and viral-based proteins have been used to produce well defined copper sulfide nanoparticles (Li et al. 2013, Zaman et al. 2013, Han et al. 2016, Huang et al. 2017).

The white wine phenolic substances allowed 31 ± 5% of the sulfide-bound Cu to pass through the RC membrane filter. Consequently, it was able to prevent some adsorption of sulfide-bound Cu onto the filtration material but not as efficiently as the macromolecules surveyed. Given that caffeic acid alone showed no ability to prevent adsorption, it suggests that the combined phenolic substances present in white wine may be required to exert a significant effect. At the low pH of wine, the interaction of phenolic substances with sulfide-bound Cu would be expected to be less than that of acidic polysaccharides and/or thiol-containing proteins.

Impact of membrane composition and pore size on sulfide-bound Cu removal

Several membrane types (Figure 4) were assessed for their activity in adsorbing sulfide-bound Cu from white wine, the model wine, and the model wine with added white wine polysaccharides. All solutions had the added copper(II) concentration of 1.0 or 0.3 mg/L and hydrogen sulfide to copper(II) ratios of 2:1 (as per the previous experiments). The white wine experiments were also conducted at the higher ratio of 5:1 [hydrogen sulfide to copper(II)] to assess if there was any impact of increasing hydrogen sulfide concentration on the filterability of sulfide-bound Cu. In the model wine systems, the ratio of 2:1 was known to bind the majority of added copper(II) but in white wines this was not entirely certain (Clark et al. 2015, 2016, Kreitman et al. 2016). In white wine a larger potential suite of components competing with copper(II) for hydrogen sulfide would be present, and therefore a larger ratio of hydrogen sulfide to copper(II) may be necessary in white wine for complete binding of copper(II).

The results showed that the RC, nylon and PES membranes could all remove the sulfide-bound Cu from the model wine, regardless of the pore size (i.e. 0.2 or 0.45 μm) and the concentration of copper(II) utilised to prepare the copper(I) sulfide (1.0 or 0.3 mg/L). The PTFE (0.2 and 0.45 μm) and the GF (1.2 μm) membrane filters, however, had minimal impact on the model wine solutions of sulfide-bound Cu prepared at high copper concentration. Both had increased activity for the sample with less added

copper(II), with the PTFE and GF membranes allowing 10–30% (depending on pore size) and 70%, respectively, of sulfide-bound Cu to pass through the filter.

On addition of the white wine polysaccharide to the model wine system, the RC membrane allowed all sulfide-bound Cu to pass through the filter in the samples regardless of the pore size or sulfide-bound Cu concentration. The PES permitted 60–70% sulfide-bound Cu to pass through the filter, while nylon allowed the least amount (~30 %), again largely independent of pore size or sulfide-bound Cu concentration. On repeating the experiment in a white wine, the results were similar to that of the model wine with polysaccharide added (Figure 4, see the blue and red bars). A difference between the performance of the filtration media in the two solution matrices was the ability of the PES membrane to remove more sulfide-bound Cu in the white wine compared to the model wine with added polysaccharide. The PES activity in the white wine was comparable to the results obtained with nylon at pore sizes of 0.2 μm, but PES was significantly more efficient at removing sulfide-bound Cu at 0.45 μm for the higher sulfide-bound Cu concentration. At low sulfide-bound Cu concentration there was no significant difference between the nylon and PES membrane filters for the amount of Cu passing through the filter in the white wine tested. A further five white wines, with added hydrogen sulfide and Cu (mole ratio 2:1), were assessed with the same membrane filters (Figure S4) and showed similar results to that in Figure 4. The main difference was a wine-dependent variability in the action of PTFE for sulfide-

bound Cu removal but this was more apparent when the sulfide-bound Cu was present at the higher concentration (i.e. 1.0 mg/L Cu) and less pronounced at the more wine-relevant concentration (0.3 mg/L Cu).

The high adsorption efficiency of PES and nylon for sulfide-bound Cu is most likely due to increased hydrophobic and/or polar activity of these media compared to RC, teflon and GF. Indeed, both nylon and PES have hydrocarbon and aryl backbone regions conducive to hydrophobic interactions, and both have carbon–oxygen double bonds inducing a dipole and subsequent negative polarity towards the oxygen atoms. Nylon also contains an amide bound hydrogen atom, capable of hydrogen bonding, but this does not appear critical for the adsorption of sulfide-bound Cu in wine as it is not present in PES. Indeed, the hydrogen bonding capability of nylon means that it can adsorb more phenolic substances from wine than PES (Bowyer et al. 2013), and this may lead to steric hindrance of the sites that would otherwise lead to adsorption of sulfide-bound Cu. Alternatively, the decreased ability of PES to interact with wine components of negative character may explain its slight improved adsorption of sulfide-bound Cu in wine compared to nylon. It is yet to be determined, however, if the main interaction of the filtration media with sulfide-bound Cu in white wine is directly with exposed surface CuS units, or if it is with another wine component complexed to the surface of the sulfide-bound Cu species.

It was also noted that the back pressure encountered when using the filters, based on the physical force required to depress the syringe plunger, was in the order of PTFE >> nylon > RC > PES > GF. This was for a given pore size (0.45 or 0.2 μm), apart from the GF filter which was only utilised with a higher pore size (1.2 μm). This order of filtration media is consistent with the relative flux of aqueous solutions or wine through filtration media for a given pore size (Riedel 1996, Bowyer et al. 2013, Corning 2018).Therefore, based on this relative back pressure and efficiency to remove sulfide-bound Cu in the presence of macromolecules, PES would appear to have advantages over the other membrane media.

Conclusion

The removal of sulfide-bound Cu by membrane filtration is hindered by white wine protein and polysaccharide macromolecules. This was attributed to the process of sulfide-bound Cu removal being a consequence of adsorption by the filter medium, a mechanism that is inhibited by macromolecules. This macromolecule interference in sulfide-bound Cu removal was most apparent for RC media, whilst nylon and PES were able to still remove significant amounts of sulfide-bound Cu. In contrast, PTFE led to limited removal of sulfide-bound Cu, apart from lower metal concentration in the model wine and in some white wines at high sulfide-bound Cu concentration.

Further work is required on a larger scale to provide a sense of adsorption capacity of a membrane filter for a given volume of wine. In this study, 10 mL of wine was passed over a membrane filter of 5.5 cm^2 area and 0.14 mm thickness. Depth filters with increased adsorption capability are likely to have increased efficiency in removal of sulfide-bound Cu than membrane filters. Also, confirmation is required that a concomitant decrease in total hydrogen sulfide is achieved in conjunction with the removal of hydrogen sulfide from the wine. Finally, assessment of the filterability of sulfide-bound Cu in red wine is necessary.

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Figure legends

Figure 1. Proportion of total copper (2.0 mg/L), present as sulfide-bound Cu, eluting through the filter (0.45 or 0.2 μm pore size regenerated cellulose membrane filters). The model wine samples contained 0 ( ), 1 ( ), 5 ( ) and 10 ( ) % (v/v) white wine. All had sulfide-bound Cu formed in situ with addition of 4.8 mg/L sodium sulfide and 2.0 mg/L copper(II) (mole ratio of 2:1 for hydrogen sulfide to copper(II)).

Figure 2. Proportion of total Cu (1.0 mg/L), present as sulfide-bound Cu, eluting through the filter (0.45 or 0.2 μm pore size regenerated cellulose membrane filters). Samples were all the non-filtered samples in Figure 1, containing 0 ( ), 1 ( ), 5 ( ) and 10 ( ) %(v/v) white wine in model wine, that were then diluted twofold to achieve white wine composition of 5% (v/v) and subsequently filtered. The sulfide-bound Cu present prior to twofold dilution and filtration was equivalent to that formed by 4.8 mg/L sodium sulfide and 2.0 mg/L copper(II) (mole ratio of 2:1 for hydrogen sulfide to copper(II)).

Figure 3. (a) Particle size measurements for sulfide-bound Cu (CuS) prepared by addition of 4.8 mg/L sodium sulfide and 2.0 mg/L copper(II) to a Viognier wine (——), model wine (MW) (——) or water (——) (all measured 10 min after sample preparation); (b) mean sizes of samples mentioned above;(c) particle concentration in Sauvignon Blanc wine 0 (——), 1 (——), 2 (——), 3 (——), 4 (——), 5 (——), 21 (——) and

28 h(——) ; and (d) particle size in white wine over 28 h. Also shown in (a) are particle sizes in the model wine (——) and water without sulfide-bound Cu present (——).

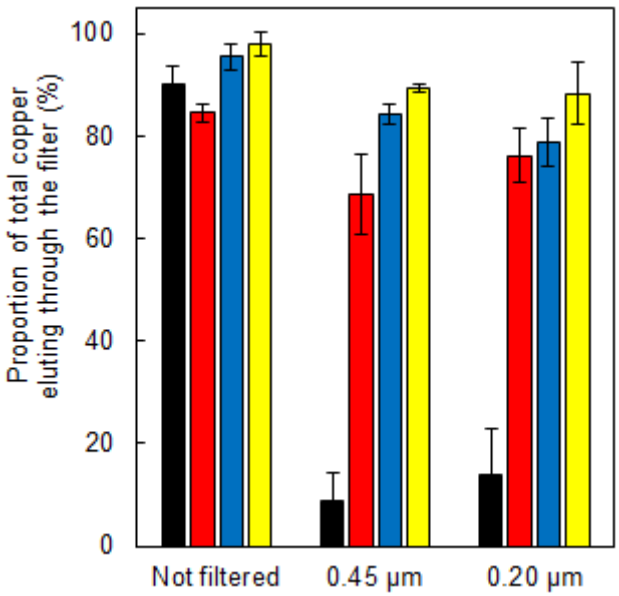
Figure 4. Impact of membrane type on the amount of Cu eluting through the filter at (a) high (1.0 mg/L copper) and (b) low (0.3 mg/L copper) concentration of sulfide-bound Cu. The sulfide-bound Cu was prepared at hydrogen sulfide to copper(II) ratios of 2:1 in the model wine ( ), model wine with added polysaccharide ( ) and white wine ( ), as well as at 5:1 in the white wine ( ).

Figure S1. The membrane filtration of samples, and the depiction of filtered Cu as a proportion of total Cu in the sample.

Figure S2. The determination of non-sulfide bound Cu (i.e. electrochemically active Cu) in a white wine by medium exchange constant current stripping potentiometry after filtration through 0.20 µm regenerated cellulose membrane. Prior to filtration the white wine had either (a) 0 mg/L or (b) 1.1 mg/L addition of hydrogen sulfide, followed by a 1.0 mg/L addition of Cu to both. The total Cu in both filtered solutions was 0.95 mg/L and 0.80 mg/L, respectively. The non-sulfide bound Cu in (a) was determined as 0.8 ± 0.1 mg/L, and (b) as being less than the limit of detection for the analysis conditions employed (< 0.04 mg/L, or < 5 % of the total Cu). Conditions for the analysis are as described within Clark et al. (2016), including a deposition time of 60 s and quantification via an external calibration graph generated in model wine. The white wine was the same as that used in Figure 4, which is detailed in the Methods section.

Figure S3. The particle size in White wine (Viognier) with no addition (——), or addition of 2 mg/L of copper(II) (——), or both 2.1 mg/L of hydrogen sulfide and 2 mg/L of copper(II) (mole ratio of 2:1 for hydrogen sulfide to Cu) (——).

Figure S4. The impact of membrane filter type on the amount of Cu eluting through the filter at (a) high (1.0 mg/L added copper) and (b) low (0.3 mg/L added copper) concentration of sulfide-bound Cu. The sulfide-bound Cu was prepared at hydrogen sulfide to copper(II) ratios of 2:1 in five different white wines. The following are the variety, vintage, free sulfur dioxide (mg/L), total sulfur dioxide (mg/L), pH, titratable acidity (g/L tartaric acid equivalents) and ethanol concentration (%(v/v), respectively: W1 Chardonnay ( ), 2015, 25, 131, 3.37, 6.09, 14.5; W2 Semillon Sauvignon Blanc ( ), 2017, 32, 149, 3.16, 5.70, 11.0; W3 Sauvignon Blanc ( ), 2017, 25, 146, 3.29, 6.39, 12.0; W4 Semillon Sauvignon Blanc ( ), 2017, 34, 148, 3.16, 5.81, 11.0; and W5 Chardonnay ( ), 2017, 29, 148, 3.25, 6.61, 12.5.



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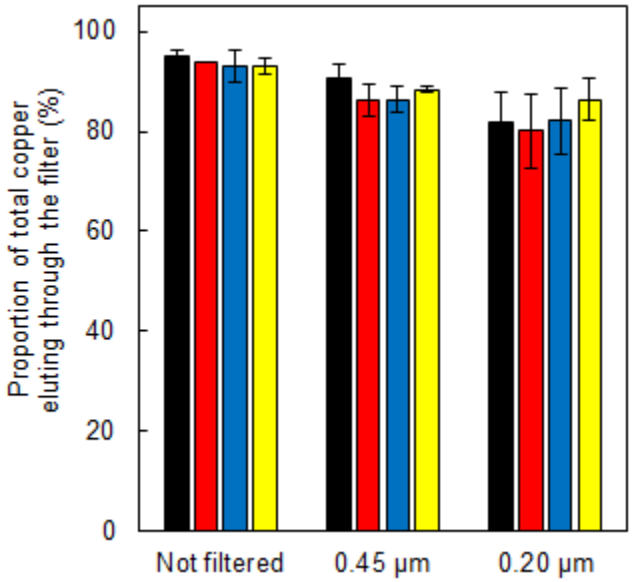

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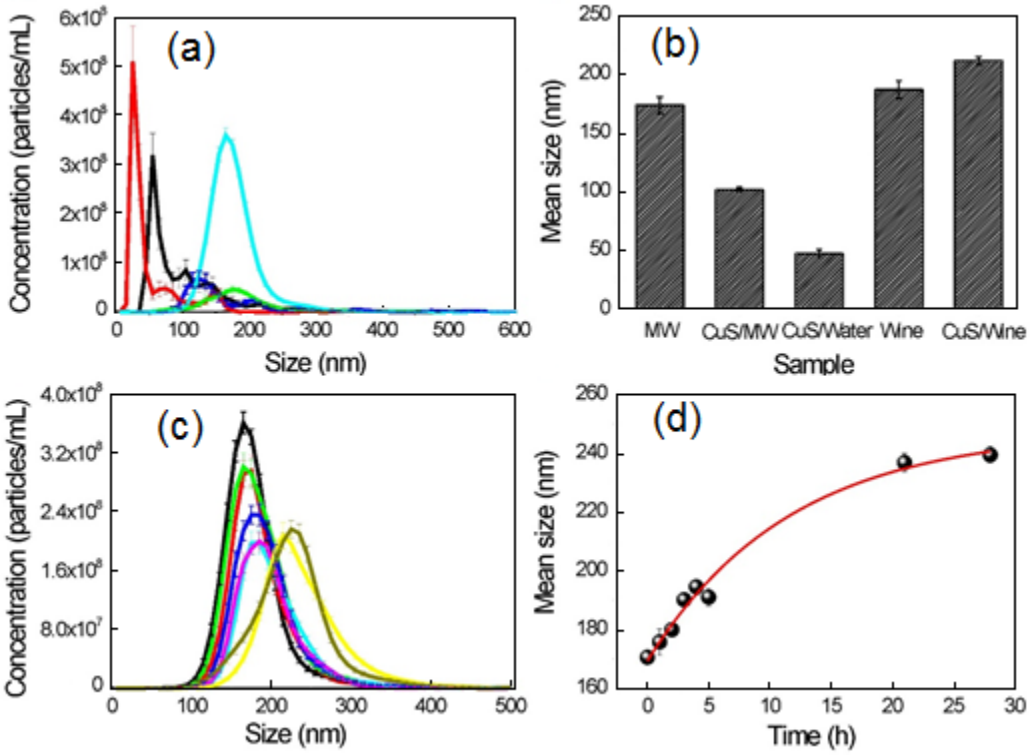

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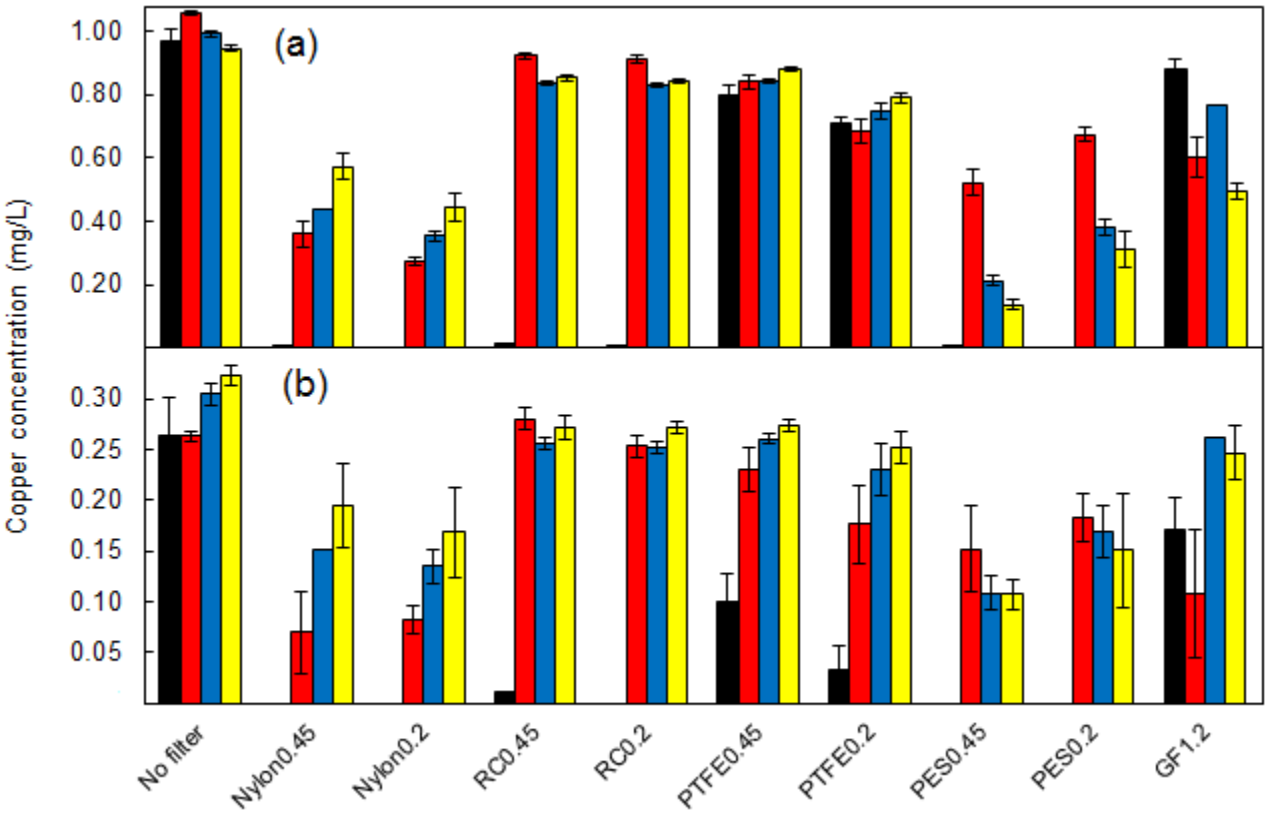

Fig4.tif

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