Simplified method for the rapid quantification of copper fractions in wine

Sourced from the research article “Simplified measures of copper fractions in wine: colorimetric and filtration-based approaches” (Australian Journal of Grape and Wine Research, 2020).

Simple and inexpensive methodologies for quantifying the total Cu concentration as well as various Cu fractions in wine have been recently developed, including the application of colorimetric analysis, and/or diatomaceous earth depth filtration. Compared to other conventional Cu analysis methods, the proposed approaches are more suitable for winery laboratories for reliable and affordable quantification of total Cu and its fractions during wine production.

Copper (Cu) has been shown to impact the composition and chemistry of wine in various ways. It is often deliberately added to wine during production for ‘reductive’ off-flavour removal, as Cu(II) is believed to precipitate hydrogen sulfide that can then be removed by settling and/or filtration. However, recent research indicates that, once formed, the complexes between Cu and hydrogen sulfide can remain in wine even after filtration. Furthermore, studies in model wine systems have shown that during Cu fining, side reactions may occur between Cu, hydrogen sulfide and thiol compounds to generate polysulfanes that can release hydrogen sulfide under low oxygen conditions. In addition, Cu can also mediate oxidation reactions in wine, and therefore potentially accelerate its detrimental oxidative development.

Considering the significant effect of Cu on wine, the total Cu concentration has long been used as a potential parameter to monitor quality control during wine production. However, recent research has focussed on identifying the role that different fractions of Cu may play in wine. The particular benefit in measuring different Cu fractions is an on-going subject of research, but results to date have suggested a link between specific Cu fractions and an inhibited accumulation of reductive aroma in wine. Furthermore, measuring Cu fractions can also give an indication of the amount of Cu in the wine that is already bound to hydrogen sulfide. Consequently, the ability to measure different forms of Cu in wine is of potential value to winemakers and wine industry. However, conventional methodologies for determining total Cu concentration and Cu fractions are mainly research-orientated, requiring significant infrastructure, operation and personnel training cost and consequently are not affordable for small to medium sized wineries. To assist winery routine quality control, several approaches have been developed to enable the quantification of total Cu and Cu fractions in wine utilising equipment that is more accessible to wineries.

The Colorimetry Approach

The colorimetric technique makes use of the reaction between Cu and bicinchoninic acid (BCA) to form a purple-coloured complex (Figure 1). Quantification of Cu in samples is achieved by measuring the absorbance intensity at 563 nm with a spectrophotometer, and then converting the absorbance to concentration with a standard addition calibration curve. In order to obtain sufficient sensitivity of absorbance for typical wine Cu concentrations, a 40 mm pathlength glass cuvette is required for the analysis.

To determine total Cu concentration in white wine, the reaction between Cu in wine and the added BCA is conducted in the present of Ag(I) (silver(I)), as Ag(I) is able to release Cu from various binding agents, so that all Cu in wine can interact with BCA. Generally, a 30 min incubation time of the wine with Ag(I) and BCA is required for accurate total Cu determination. If Ag(I) addition is omitted during the analysis, the absorbance intensity can be measured either immediately after the BCA addition to wine (i.e., essentially no incubation), or after 30 min of incubation, to determine the concentration of Cu fraction I and II, respectively (Figure 2). Cu fraction I has been found to correspond to the Cu-organic acid complexes in wine, such as Cu(II)-tartarate and Cu(II)-malate, while Cu fraction II has been linked to Cu-thiol compound interactions, such as Cu-glutathionine.
Cu fraction III represents the portion of Cu in wine that cannot be sequestered by BCA itself, and is due largely to complexes formed between Cu and hydrogen sulfide in wine (i.e., sulfide-bound Cu) (Figure 2).

Direct Cu quantification in red wine with BCA is not possible, as the colour of red wine interferes the detection of Cu-BCA complex at 563 nm. Therefore, decolourisation of the red wine is necessary prior to colorimetric analysis. This is accomplished by digesting red wine with hydrogen peroxide under alkaline condition at 80 °C for at least 6 h (Figure 1B). Addition of Ag(I) to the digested red wine is not required as digestion releases all Cu from strong binding agents already4.

The Diatomaceous Earth Depth Filtration Approach

The necessity to decolourise red wine by digestion precludes the quantification of Cu fractions by colorimetric analysis. Consequently, an alternative methodology making use of diatomaceous earth depth filtration (DEDF) has been developed1. In this case, cellulose-based depth filters, with incorporated diatomaceous earth, were utilised to adsorb the sulfide-bound Cu (i.e., Cu fraction III) from the wine and leave the remaining Cu fraction – mainly Cu fraction I – in the filtrate. In red wine, there is generally a negligible amount of Cu fraction II1. After passing 100 mL of wine through the filter, held within a 45 mm membrane filter housing unit, Cu fraction I could then be determined by any routine measurement for total Cu. The DEDF approach can also be applied to white wine to distinguish Cu fraction III from a combination of Cu fraction I and II.

Accuracy of the Different Approaches

The accuracy of the colorimetric analysis for the concentration of total Cu and Cu fractions was examined by comparing the results with those obtained by inductively coupled plasma-optical emission spectrometry (ICP-OES) and stripping potentiometry electrochemical technique. The electrochemical technique is the traditional research-orientated method measuring Cu fraction I in wines. For the white wine matrix, 50 and 41 wines were measured for total Cu concentration and Cu fraction I, respectively.

Good agreement was observed for the different methodologies, as exemplified by gradients close to one in the line of best fit (Figure 3). Similar assessment was conducted for red wine as well, but Cu fraction I, obtained through DEDF, was determined by ICP-OES. This Cu fraction could also have been analysed by the digestion-colorimetric analysis approach. A total of 48 and 20 red wines were analysed for the concentrations of total Cu and Cu fraction I, respectively, and displayed good agreement between the techniques (Figure 3).

Conclusion

Industry-relevant methodologies have been developed for the measurement of the concentrations of total Cu and Cu fractions in white and red wines. These approaches1,4 have been designed for wineries with access to modest equipment and lower operator skill requirement.

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1 Clark, A. C.; Zhang, X.; Kontoudakis, N., Simplified measures of copper fractions in wine: colorimetric and filtration-based approaches. Australian Journal of Grape and Wine Research 2020, 26 (4), 399-409.
2 Kreitman, G. Y.; Danilewicz, J. C.; Jeffery, D. W.; Elias, R. J., Copper(II)-mediated hydrogen sulfide and thiol oxidation to disulfides and organic polysulfanes and their reductive cleavage in wine: Mechanistic elucidation and potential applications. Journal of Agricultural and Food Chemistry 2017, 65 (12), 2564-2571.
3 Danilewicz, J. C., Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model system: Central role of iron and copper. American Journal of Enology and Viticulture 2007, 58 (1), 53.
4 Kontoudakis, N.; Smith, M.; Smith, P. A.; Wilkes, E. N.; Clark, A. C., The colorimetric determination of copper in wine: total copper. Australian Journal of Grape and Wine Research 2020, 26 (2), 121-129.
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