A simple colorimetric method to measure copper in white wines

Nikolaos Kontoudakis

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Sources of copper in wine

Endogenous (natural origin)

Exogenous (human activities)

Pohl, 2007
Cu in Chardonnay grape juice and wine

- Control
- Addition 3 mg/l Cu
- Addition 6 mg/l Cu

- Grape juice
- Wine after fermentation
- Wine after cold stabilization
- Wine after bentonite treatment

X. Zhang PhD data
Importance of copper in wine quality

✓ Mechanism of glyoxylic acid-mediated polymerization of flavanols.

Fulcrand et al., 1997; Clark et al., 2003; Guo et al. 2017
Importance of copper in wine quality

✓ Cu haze in reduced conditions.
Importance of copper in wine quality

\[
\text{Cu}^{2+}_{(aq)} + \text{H}_2\text{S}_{(aq)} \leftrightarrow \text{Sulfide-bound Cu}
\]

\text{Labile Cu} \quad \text{Non labile Cu}

In model wine \(\text{Cu}_2\text{S}_2(s)\)

\(\checkmark\) \text{Cu can remove and/or induce sulfidic off-odours}

Kontoudakis et al., 2019.
The importance of copper in wine quality is highlighted. Copper can remove and/or induce sulfidic off-odours. The reaction shown is:

$$\text{Cu}^{+2(aq)} + \text{H}_2\text{S}_{(aq)} \leftrightarrow \leftrightarrow \text{Sulfide-bound Cu}$$

In model wine:

$$\text{Cu}_2\text{S}_{(s)}$$

The diagram illustrates the relationship between free H$_2$S (µg/L) and labile Cu (mg/L) in white and red wines. The data from Kontoudakis et al., 2019, shows that an increase in free H$_2$S is accompanied by an increase in labile Cu.

✓ Cu can remove and/or induce sulfidic off-odours.
Importance of copper in wine quality

Filterability of $\text{Cu}_2\text{S}$

White Wine (2:1, H2S:Cu)

- Copper can remove and/or induce sulfidic off-odours

Kontoudakis et al., 2018.
Importance of copper in wine quality

✓ Metallic taste in high levels

✓ Legal limits and toxicity
Total copper concentrations easily measured (researchers and big wineries)

Auto analyzer

ICP-OES/MS

GFAAS
Total copper concentrations measured by wineries

Colorimetric methods

Spectrophotometric method

Auto analyzer
Spectrophotometric method

Bicinchoninic acid (BCA)
potassium salt

BCA-copper complex
Spectrophotometric method

Procedure

- Blank
- Wine sample
- Wine sample + 0.1 mg/l Cu
- Wine sample + 0.3 mg/l Cu
- Wine sample + 0.5 mg/l Cu
- 10 ml wine sample
- 0.5 ml BCA solution-0.05% (w/v)-except blank
- 0.05 ml ascorbic acid-80 g/L
- 0.1 ml silver(I) nitrate-1 g/L silver(I)
- Copper and H₂O in adequate quantities

Samples were:
- Incubated at room temperature-30 min.
- Filtered (0.20 μm regenerated cellulose filters-RC)
- Measured at 563 nm with 40 mm glass cuvette
Total Cu concentration by ICP

Wines

Wine ICP
Wine ICP-silver

Cu (mg/l)

Wines

1 2 3 4 5 6 7 8 9 10 11 12
Total Cu concentration by ICP and spectrophotometric method

Cu (mg/l)

Wine ICP-silver
Wine Spectr.-silver
Wine Spectr.-silver + pH 4
Wine Spectr.
Wine Spectr.-pH 4

Wines 1 to 12
Total Cu concentration by ICP and spectrophotometric method

\[ y = 0.9247x + 0.0215 \]
\[ R^2 = 0.9336 \]

\[ y = 0.9584x + 0.011 \]
\[ R^2 = 0.977 \]
Spectrophotometric method validation parameters

- Recovery: 104 ± 9 %.
- Repeatability: average relative standard deviation (RSD) 3 ± 2 %.
- Specificity. The BCA reagent is specific for Cu in wine, with no influence from Mg, Mn, Fe, Zn and Al.
- Linearity. Linear within 0.04 to 1.0 mg/L copper.
- Stability. Samples should be filtered 30 minutes after addition BCA to wines, and then measured immediately afterwards.
- Matrix effects. Eliminated with silver(I) additions and with standard additions.
Colorimetric method-Konelab

✓ Thermo Scientific™ Konelab™ 20 Clinical Chemistry Analyser system
✓ Thermo Scientific copper test kit colorimetric reagent 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylanaline-monosodium salt (diBr-PAESA)

Colorimetric reagent 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylanaline-monosodium salt (diBr-PAESA)
Total Cu concentration by ICP and Konelab method

- Wine ICP-silver
- Wine Konelab
- Wine Konelab-silver

Wines 1 to 12 are plotted with the Cu concentration (mg/l) for each method.
Total Cu concentration by ICP vs Konelab method

$y = 0.915x + 0.0146$
$R^2 = 0.9829$

$y = 0.9341x - 0.0087$
$R^2 = 0.9935$

Cu by ICP (mg/l) vs Cu by Konelab (mg/l)

- Wine
- Wine with silver
Determination of labile copper

Procedure

✓ Same procedure like for total Cu
✓ 0.1 ml silver(I) nitrate - 1 g/L silver(I)
✓ Filtered and measured directly

2 procedures:
✓ Measure labile Cu in 7 white wines + the same wine with addition of 0.08 gr/l Cu
✓ 3 white wines during time (166h) - wines were oxidized
Reference method Stripping Potentiometry

Medium exchange stripping potentiometry with a thin mercury film on a screen printed carbon electrode

Clark et al., 2016.
Labile Cu in 7 white wines + the same wine with addition of 0.08 gr/l Cu
3 white wines during time (166h)-wines were oxidized

Possible reasons for disagreement of 2 methods:

- High titratable acidity and high concentration of organic acids
**Conclusions**

Total Cu can be measured easily in wineries with the use of colorimetric method(s).

Labile Cu potentially can be measured by colorimetric method.
Geoffrey Scollary
& Andrew Clark

Wine Australia

Mark Smith,
Paul Smith,
& Eric Wilkes
Thanks for your attention