

# Effect of headspace volume and iron and copper addition on oxidation processes in model solution and Riesling wine: chemical and sensory changes

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*Studies conducted with a model solution and a Riesling wine showed that oxygen concentration at bottling had a great impact on the evolution of the total consumed oxygen (TCO), free SO<sub>2</sub>, and the colour of model solution and wine. In the model solution, the addition of 0.1 mg/l of iron and/or 0.05 mg/l of copper influenced the oxygen consumption, in the real wine, however, this effect was not observed. Sensory evaluation (triangle tests and descriptive analysis) showed significant differences between wines bottled with different headspace volumes (0, 20 and 40 ml). The presence of iron and copper had no significant impact on the sensory characteristics and colour of wines.*

**Keywords:** oxygen, wine, oxidation, iron, copper, sulfur dioxide, colour, browning

***Einfluss des Kopfraumvolumens und von Eisen- und Kupferzusatz auf die Oxidationsprozesse in Modelllösung und Wein der Sorte 'Riesling': Chemische und sensorische Veränderungen.*** Versuche mit einer Modelllösung und Riesling-Wein zeigten den großen Einfluss der Sauerstoffkonzentration bei der Abfüllung auf die Gesamtmenge an konsumiertem Sauerstoff sowie auf den SO<sub>2</sub>-Abbau und Farbunterschiede. Der Eisen- und Kupferzusatz (0,1 mg/l Fe und/oder 0,05 mg/l Cu) beeinflusste die Sauerstoffkonsumrate in der Modelllösung, im Versuch mit Riesling-Wein dagegen konnte dieser Effekt nicht bestätigt werden. Die sensorische Beurteilung der Riesling-Weine mittels Dreieckstest und deskriptiver Sensorik zeigte signifikante Unterschiede zwischen Weinen mit unterschiedlichem Kopfraumvolumen (0, 20 und 40 ml). Bei Wein hatte der Zusatz von Eisen und Kupfer keinen signifikanten Einfluss auf sensorische Eigenschaften und Farbe.

**Schlagwörter:** Sauerstoff, Wein, Oxidation, Eisen, Kupfer, Schwefeldioxid, Farbe, Bräunung

***L'influence du volume de l'espace de tête et de l'adjonction de fer et de cuivre sur les processus d'oxydation dans une solution modèle et dans du vin du cépage 'Riesling': Modifications chimiques et sensorielles.*** Les essais effectués avec une solution modèle et avec du vin Riesling ont démontré la grande influence de la concentration d'oxygène au moment de la mise en bouteille sur la quantité totale de l'oxygène consommé, sur la dégradation du SO<sub>2</sub> et sur les différences de couleur. L'adjonction de fer et de cuivre (0,1 mg/l Fe et/ou 0,05 mg/l Cu) a influé sur le taux de consommation d'oxygène de la solution modèle, mais en revanche, cet effet n'a pas pu être confirmé dans l'essai effectué avec du vin Riesling. L'appréciation sensorielle des vins Riesling au moyen du test triangulaire et par voie descriptive a fait apparaître des différences significatives entre les vins dont l'espace de tête présentait différents volumes (0, 20 et 40 ml). Quant au vin, l'adjonction de fer et de cuivre n'a eu aucune influence significative sur les caractéristiques sensorielles et sur la couleur.

**Mots clés :** oxygène, vin, oxydation, fer, cuivre, dioxyde de soufre, couleur, brunissement

Wine is a complex matrix consisting of many organic and inorganic substances, which constantly undergo changes with time. The primary source of iron and copper in wine is the soil (ALMEIDA and VASCONCELOS, 2003; POHL, 2007; TAYLOR et al., 2003). These metals belong to essential vine nutrients. They are absorbed by the root system (ZOECKLEIN et al., 1994) and transferred to the wine through grapes. A secondary uptake of iron and copper in the vineyard may occur due to pollution, direct contact with soil or due to viticultural practices, e.g. the use of copper fungicides (VOLPE et al., 2009) or pesticides (GALANI-NIKOLAKAKI et al., 2002; KMENT et al., 2005).

High concentrations of heavy metals in the must are likely to be observed during the processing of unwashed grapes (GALANI-NIKOLAKAKI et al., 2002). During fermentation, the concentrations of metal ions decrease due to biosorption by yeast cells (STAFILOV and KARADJOVA, 2009); high pH levels and high temperatures favour this process (BLACKWELL et al., 1995). Further contamination of wine with iron and copper may originate from enological equipment and fining agents (ESCHNAUER, 1982; POHL, 2007), e.g. bentonite (CATARINO et al., 2006; NICOLINI et al., 2004; WURZINGER et al., 1994). Addition of copper sulfate against H<sub>2</sub>S off-odors may also cause excessive copper concentrations in wine (SCHMIDT, 2001).

Iron and copper may influence the stability of wine (ESCHNAUER, 1982; RIBEREAU-GAYON et al., 2006). In trace amounts, iron and copper are important as metabolism catalysts and enzyme activators (STAFILOV and KARADJOVA, 2009). But at higher-than-trace levels, they may cause instability, such as ferric tannate casse, ferric phosphate casse, and copper casse (RIBEREAU-GAYON et al., 2006), change sensory characteristics, and affect the overall quality.

Iron and copper play an important role in oxidation processes in wine. For a long time, it was assumed that oxygen reacts directly with wine constituents like phenolic compounds and SO<sub>2</sub>, but these reactions are thermodynamically unfavourable for oxygen as diradical (DANILEWICZ, 2011). It is assumed that ferric iron (Fe<sup>3+</sup>) acts as a catalyst to overcome the activation energy of the electron reduction step of the oxidation process (DANILEWICZ, 2003; DANILEWICZ, 2007; DANILEWICZ, 2011; DU TOIT et al., 2006; KARBOWIAK et al., 2009; NIKFARDJAM, 2010). Copper in turn catalyses the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> (DANILEWICZ, 2003; DANILEWICZ, 2007; DANILEWICZ and WALLBRIDGE, 2010).

The oxidation reactions continue with further forma-

tion of a hydroperoxyl radical (OOH•), which is a precursor for further reactive oxygen species (ROS) (WATERHOUSE and LAURIE, 2006). At wine pH and the presence of ferric iron Fe<sup>3+</sup>, the OOH• can react further to peroxyde anion and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (DANILEWICZ, 2003; WATERHOUSE and LAURIE, 2006). In the case of presence of transition metals (Fe, Cu), another possible step is the formation of a hydroxyl radical (OH•), a hydroxide ion (HO<sup>-</sup>), and water from H<sub>2</sub>O<sub>2</sub> via the Fenton reaction (DANILEWICZ, 2003; WATERHOUSE and LAURIE, 2006; KARBOWIAK et al. 2009). This OH• seems to be very reactive towards many wine compounds (ELIAS et al., 2009). Various radicals that are formed in these reactions are able to react directly with phenolic compounds, and they are stronger oxidants than oxygen itself (ELIAS et al., 2009; SINGLETON, 1987). Iron also plays a major role in the oxidation of ascorbic acid (DU TOIT et al., 2006). The oxidation mechanisms have been widely studied in wine-like model solutions in recent research. (DANILEWICZ, 2003; DANILEWICZ, 2007; DANILEWICZ, 2011). It has been assumed that in absence of iron and copper the oxidation of the phenolic compounds will not be observed. According to previous studies (DIMKOU et al., 2011; KWIATKOWSKI et al., 2007), headspace oxygen seems to be of major importance for the quality of bottled wines. The aim of this study was to show the effects of iron and copper addition to a wine-like model solution and to real wine bottled with various headspace volumes.

## Materials and methods

### Chemicals and reagents

For the experiment the following chemicals were used: glycerol (85 %, Merck, Darmstadt, Germany), (+)-tartaric acid (Merck), gallic acid monohydrate (>98 %, Merck), potassium metabisulfite (Merck), potassium ferrocyanide K<sub>4</sub>[Fe<sub>2</sub>(CN)<sub>6</sub>] (Begerow, Langlonsheim, Germany), iron(II) sulphate (>99 %, Merck), copper(II) sulphate (>99 %, Merck), Titrisol 1 g/l iron standard solution (FeCl<sub>3</sub> in 15 % HCl, Merck), Titrisol 1g/l copper standard solution (CuCl<sub>2</sub> in H<sub>2</sub>O, Merck), LaCl<sub>3</sub>\*7H<sub>2</sub>O for analysis (Merck), hydrochloric acid for analysis (32 %, Merck), sodium hydroxide (p. a., Merck). Water purification was performed using a Milli-Q™ purifier system (Millipore, Schwalbach, Germany).

## Wine-like model solution experiment

The wine-like model solution was prepared similarly to the one described in the previous work of DANILEWICZ (2007). For the experiment, 26 litres of model solution consisting of deionized water (Millipore), 5.8 g/l glycerol, 3.8 g/l (+)-tartaric acid, and ethanol (99.6 %vol.) corresponding to 12 % v/v and 200 mg/l gallic acid were prepared. Potassium metabisulfite was added to reach a sulphur dioxide concentration of about 50 mg/l. The pH was adjusted to 3.4 with 5 M sodium hydroxide solution. The whole volume was divided into four parts, and the required volumes of iron(II) sulfate and copper(II) sulfate standard solutions were then added according to the scheme (Fig. 1) to give the required iron and copper concentrations. The wines were then bottled in 0.5 l-bottles with the dissolved oxygen concentration not exceeding 0.1 mg/l. The oxygen amount in the bottle was determined by different headspace (HS) volumes (0 ml, 50 ml and 100 ml of ambient air). Nitrogen gas (99.98 %, Lange & Co. GmbH, Lippstadt, Germany) was used to keep dissolved oxygen concentrations below 0.2 mg/l during the whole process. The bottles with model wine solution were stored in an upright position at 20 °C.

## Wine experiment

200 litres of white wine from sound, hand-picked grapes from the 'Riesling' variety (Württemberg region, vintage 2010) were produced and provided by the experimental department of the LVWO Weinsberg. Analytical parameters of the wine at bottling were as follows: alcohol: 12 %vol.; acidity: 6.4 g/l; free SO<sub>2</sub>: 40 mg/l; total SO<sub>2</sub>: 100 mg/l; total phenolic content: 422 mg/l. Iron and copper contents were additionally reduced with potassium ferrocyanide (4 mg/l). This procedure is known in winemaking as blue fining. Wine samples were treated as the model wine solution (Fig. 2), except for smaller headspace volumes. This was done to approach bottling oxygen levels commonly found in the industry. Racking and bottling were performed under reductive conditions using nitrogen to keep the dissolved oxygen content below 0.1 mg/l. This was measured and controlled with the Hach Lange HQ40d oxygen meter (Hach Lange GmbH, Berlin, Germany). Bottling was performed with a manual 2-way filler system (Fjord, KHW Maschinenbau GmbH, Kaiserslautern), and bottles were sterilized and purged with N<sub>2</sub> before processing. Then the bottles were sealed under MCA 28 aluminium screw caps (Kork-Fabrik Arthur Linck GmbH, Delmenhorst, Germany). Bottles were stored at 20 °C.

Fig. 1: Scheme of model wine experiment and metal additions

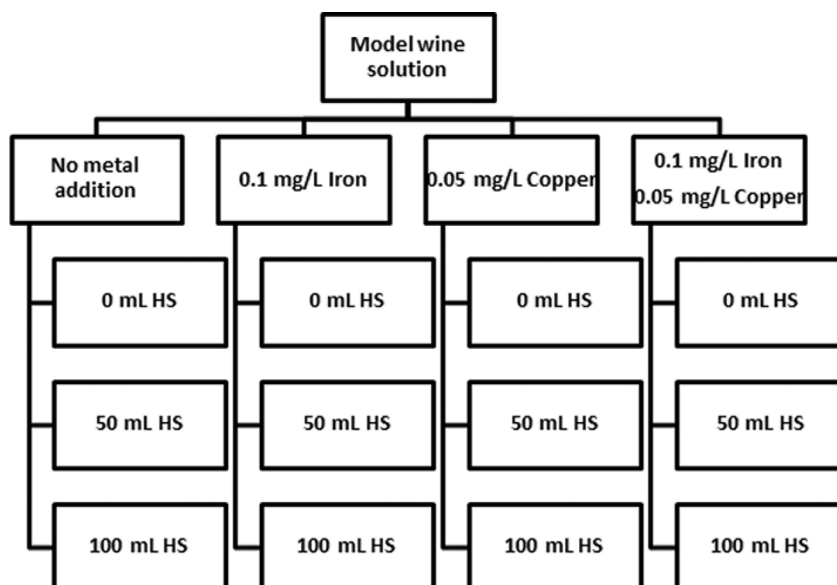


Fig. 2: Scheme of Riesling wine experiment and metal additions

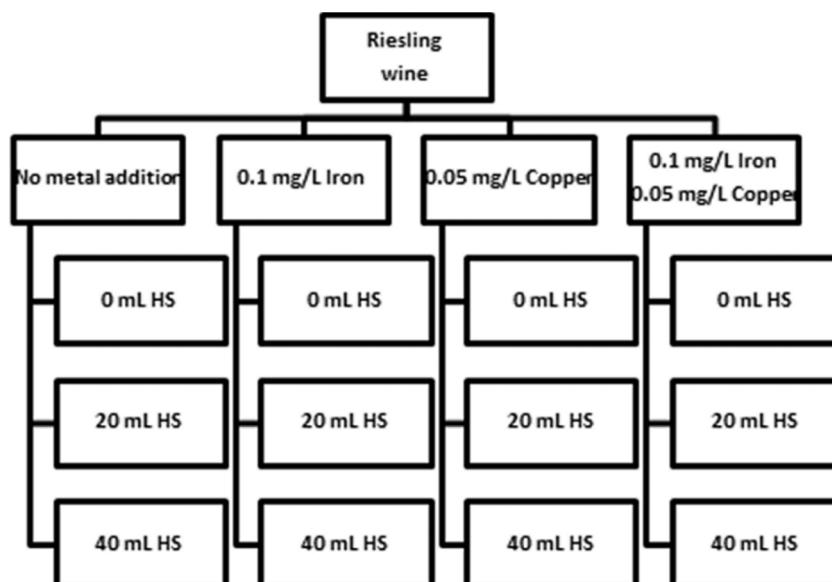


Table 1: ICP-MS operating conditions for copper measurements

Instrument parameter	Condition
Power	1100 W
Nebulizer Gas Flow	0,84 l/min
Peristaltic Pump (sample uptake)	25 $\omega$ /min
Lens Voltage	10,50 V
Standard 1 (Cu)	0.1 mg/l
Standard 2 (Cu)	0.5 mg/l
Standard 3 (Cu)	2.5 mg/l

Table 2: ICP-OES conditions and concentrations of calibration standards for measurements of iron

Instrument parameter	Condition
Power	1100 W
Carrier Gas	1,5 l/min
Plasma Gas Flow	15 l/min
Injection Speed	30 s
Peristaltic Pump Speed	20 $\omega$ /min
Standard 1 (Fe)	10 mg/l
Standard 2 (Fe)	50 mg/l
Standard 3 (Fe)	250 mg/l

## ICP-MS and ICP-OES analysis of Fe and Cu

Analyses of copper and iron were performed by the Landesanstalt für Landwirtschaftliche Chemie, University of Hohenheim, which is an accredited laboratory. For copper, an ELAN 6000 ICP-MS system (Inductively Coupled Plasma-Mass Spectroscopy) (PerkinElmer, Rodgau, Germany) was used. Wines were diluted 1:5 with ultrapure water in 50 ml-volumetric flasks with addition of 100  $\mu$ l of concentrated HNO<sub>3</sub> to diminish matrix effects and plasma instability associated with ethanol (TAYLOR et al., 2003). Rhodium was used as internal standard. Operating conditions are shown in Table 1.

Analysis of iron was conducted using a Varian simultaneous CCD ICP-OES (Inductively Coupled

Plasma-Optical Emission Spectroscopy) Vista-PRO. Wines were diluted 1:10 with addition of concentrated HNO<sub>3</sub>. Operating conditions and concentrations of calibration solutions are given in Table 2.

## Oxygen measurements

Dissolved oxygen in wine-like model solution and in wine prior to bottling was measured with HQ40d oxygen meter.

Dissolved and gaseous oxygen in the headspace of bottled wine and model solution were measured with the Fibox 3-Trace fiber-optic oxygen meter (PreSens GmbH, Regensburg, Germany) coupled with Pst3 oxygen sensors, digital temperature sensor and Fibox 3-trace Software (v3). Measurement range for Pst3

oxygen sensors given by the manufacturer: 0 to 22 mg/l for dissolved oxygen and 0 to 500 hPa for gaseous and dissolved oxygen; response time  $\leq 30$  sec (HUBER, 2006; MOROZOVA and SCHMIDT, 2012). Six bottles fitted with two oxygen sensors each were used for each modality. The sensors were glued into the bottle with silicone (RS Components, Mörfelden-Walldorf, Germany) at a height of 4 cm from the bottom of the bottle for dissolved oxygen measurement, and at 1 cm below the neck finish of the bottle for oxygen measurement in the headspace, respectively. The manufacture calibration was used. To prove the accuracy of the sensors oxygen measurements were performed in empty bottles filled with oxygen, carbon dioxide and ambient air prior to bottling.

Total consumed oxygen (TCO) rates were calculated as described by DIMKOU et al. (2011). The TCO was calculated as a difference of the sum of dissolved and gaseous oxygen observed in the measurement and the sum of dissolved and gaseous oxygen observed in the initial measurement, plus oxygen ingress through the screw cap closure. The oxygen transmission rate for the screw cap (0.61 mg/stopper/year) was taken from the review article of KARBOWIAK et al. (2009).

### Sulfur dioxide measurements

The method of iodometric titration was applied as accepted by OIV (OIV, 2008). Triplicate measurements of free and total sulfur dioxide and reductants were made using a Titrando 808 titrator (Metrohm, Herisau, Switzerland) equipped with Dosimat 805 and Tiamo Software (version 1.3).

### Colour measurements

Colour analyses of wine and model solution were conducted using a U-3010 spectrophotometer (Hitachi, Japan) coupled with a personal computer equipped with UV Solutions software (version 2.1). Spectrophotometer measurements were standardized to a 1 cm cell. The CIELAB colorimetric parameters (C.I.E., 1986)  $L^*$  (lightness),  $a^*$  (+ $a^*$  red, - $a^*$  green), and  $b^*$  (+ $b^*$  yellow, - $b^*$  blue) were calculated by the software. Overall colorimetric difference was calculated afterwards using  $L^*$ ,  $a^*$  and  $b^*$  parameters. Yellow/brown colour density (420 nm) was measured as indicator of oxidative browning.

### Sensory evaluation

The triangle test with 26 trained panellists was conducted in the tasting room of the LVWO Weinsberg equipped with FIZZ sensory software (Fizz Biosystemes, Couternon, France; version 2.47) after three months of storage. The panellists were previously trained to understand the task and to work with the software. Three samples were simultaneously presented to the panellists in a randomized order: two of them from the same formulation and a different one. The panellists were asked to taste the samples from left to the right and indicate the odd sample. Six combinations of serving order (AABB) were used to counterbalance the samples across the panel.

Descriptive analysis was performed as described by LAWLESS and HEYMANN (2010). The 12 wines were evaluated by two trained panels ( $n_1 = 18$ ,  $n_2 = 24$ ) in duplicate in the tasting room of the LVWO Weinsberg equipped with FIZZ sensory software (Fizz Biosystemes, version 2.47B). The panellists (5 female and 37 male) were students of the winemaking classes who were trained for professional tastings of wines. A structured 5-point scale was used to evaluate wines in 5 aroma and flavour parameters: 'fruity', 'ripe', 'fresh', 'oxidized' and 'overall quality'. Sensory vocabulary was built up in an evaluation session, which was carried out by the experts of the experimental cellar of LVWO Weinsberg. A Latin square design was used to randomize the sample presentation across the panellists. The wines were served cold in clear Sensus wine tasting glasses. The panellists evaluated the wines and cleansed their palates with bread and bottled water.

### Statistical analysis

Analysis of variance (ANOVA) with a 95 % confidence level and PCA analysis of the sensory data were carried out to find out the impact of the headspace volume and metal concentration on the wine quality. The analysis was conducted using XLSTAT Software Version 2011.2.08 (Addinsoft, Andernach, Germany).

## Results and Discussion

### Metal concentration

Metal levels in all wines measured with AAS were below the detection limit (data not shown). The results

of Fe and Cu measurements with ICP-OES and ICP-MS in model solutions and Riesling wines are presented in Table 3 and Table 4. Although the experimental design and metal addition concentrations were the same for model solution and wine, the resulting metal concentrations were very different. For all Riesling wines, the observed iron content was generally below the limit of detection (0.5 mg/l). This is explained by the blue fining treatment, so that the added amount of iron was still reduced below the detection limits. Despite of that we hypothesized that iron addition still could have influence on other parameters. In model solutions, the concentration of iron in the variants with iron addition was around 0.6 mg/l. The concentration of copper was slightly higher in all samples (model solution and Riesling wine), when copper was added. In addition to iron and copper, the ICP-MS analysis showed traces of manganese and zinc in all Riesling wines.

Table 3: Metal levels in model solution measured with ICP-OES and ICP-MS

	Model Solution	
	Fe, mg/l	Cu, mg/l
HS 0 ml  No metal addition	<0.5	0.060
HS 0 ml  Fe	0.60	0.047
HS 0 ml  Cu	<0.5	0.086
HS 0 ml  Fe  Cu	0.61	0.078
HS 50 ml  No metal addition	<0.5	0.060
HS 50 ml  Fe	0.63	0.047
HS 50 ml  Cu	<0.5	0.089
HS 50 ml  Fe  Cu	0.67	0.077
HS 100 ml  No metal addition	<0.5	0.057
HS 100 ml  Fe	0.62	0.049
HS 100 ml  Cu	<0.5	0.088
HS 100 ml  Fe  Cu	0.66	0.077

## Oxygen consumption

### Model solution

The examination of changes in total consumed oxygen in the model wine solution in six bottles (three bottles per repetition) during 40 days after bottling presented in Fig. 3 indicates that the oxygen was consumed not

Table 4: Metal levels in Riesling wines measured with ICP-OES and ICP-MS

	Riesling Wine	
	Fe, mg/l	Cu, mg/l
HS 0 ml  No metal addition	<0.5	0.054
HS 0 ml  Fe	<0.5	0.043
HS 0 ml  Cu	<0.5	0.060
HS 0 ml  Fe  Cu	<0.5	0.071
HS 20 ml  No metal addition	<0.5	0.047
HS 20 ml  Fe	<0.5	0.049
HS 20 ml  Cu	<0.5	0.068
HS 20 ml  Fe  Cu	<0.5	0.077
HS 40 ml  No metal addition	<0.5	0.052
HS 40 ml  Fe	<0.5	0.054
HS 40 ml  Cu	<0.5	0.062
HS 40 ml  Fe  Cu	<0.5	0.077

linearly. Approximately 75 % of the oxygen was consumed in the first 15 days. The oxygen consumption then slowed down, and continued until the whole amount was consumed.

Oxygen consumption in a bottle can be described by two parallel processes. Firstly, gaseous oxygen in the headspace (HS) and dissolved oxygen in the wine are reallocated to reach the equilibrium in partial pressures according to Fick's law. When the oxygen concentration in the HS is significantly higher than in the liquid as in our experiment, the oxygen is diffusing from the HS into the liquid. The speed of the process depends on the difference of concentrations between the two phases. When dissolved, molecular oxygen is involved in different chemical reactions with wine compounds and is consumed. The speed of the oxygen consumption depends on the redox state of wine and the concentration of oxidisable wine compounds. Thus, the dissolved oxygen is constantly decreasing, causing more gaseous oxygen to dissolve from the HS to be consumed again. This describes the higher consumption speed in the first 15 days, when the concentration difference between gaseous oxygen in HS and dissolved oxygen was higher than afterwards.

Regarding the results of the oxygen observations, it could be seen that the oxygen was consumed differently in all samples. On the day of bottling, the samples without HS contained 0.2 to 0.6 mg/l of dissolved oxygen, which was consumed in the next days (data not shown). No further increase in oxygen content was observed in these bottles. This confirms the results of other studies showing low oxygen transmission rates of screw cap compared to other types of closures (GODDEN et al., 2001; GODDEN et al., 2005). The total consumed oxygen (TCO) in the samples with a HS of 0 ml did not exceed 0.5 mg/l, and there

were no significant differences observed between the samples with and without metal addition.

The wine samples with 50 ml (Fig. 3a) and 100 ml HS (Fig. 3b) and no metal addition showed a TCO increase between 5.5 and 8.6 mg/l. In presence of copper, the mean TCO level reached 11.7 mg/l and 13.1 mg/l for 50 ml HS and 100 ml HS, respectively. For the iron containing samples, the increase of consumed oxygen was almost twice as high with 20.4 mg/l (HS 50 ml) and 21.5 mg/l (HS 100 ml). Similarly, the combination of iron and copper showed a rise of TCO of about 20.4 mg/l for the HS 50 ml and 20.8 mg/l for the HS 100 ml at the end of the observation period. Based on the data observations, even small additions of iron and copper may greatly influence the oxygen consumption rates.

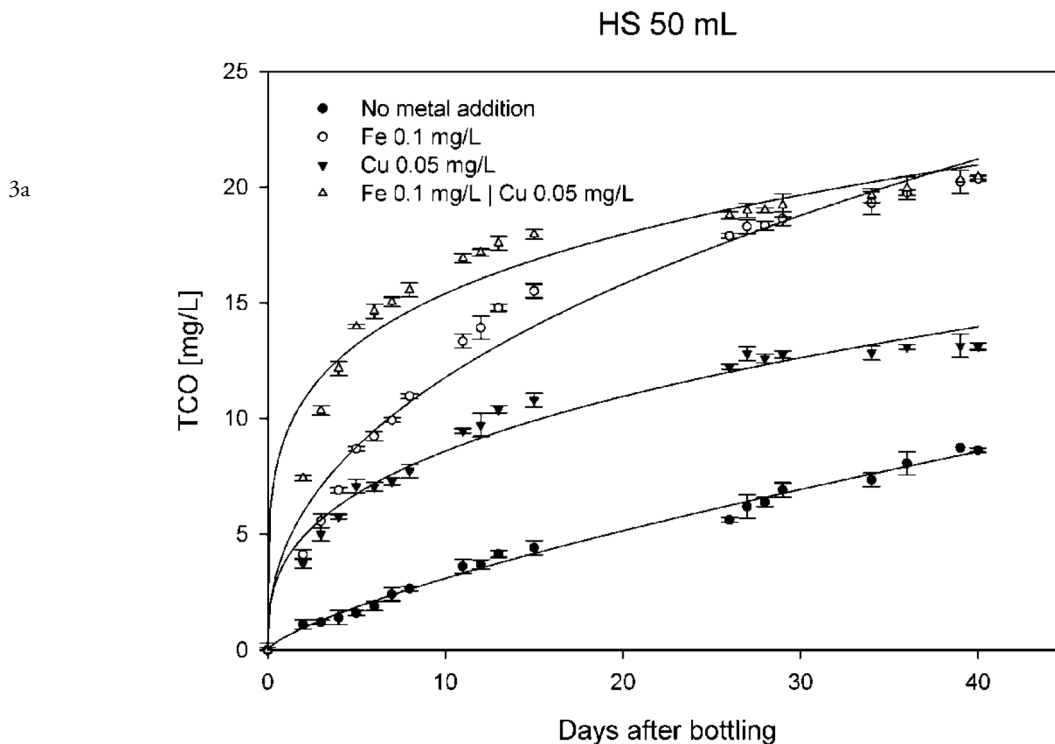
### Wine

Changes in total consumed oxygen in real wine are shown in Fig. 4 (a to d). The results of the oxygen monitoring displayed clear differences according to the oxygen level at bottling and to the HS, in particu-

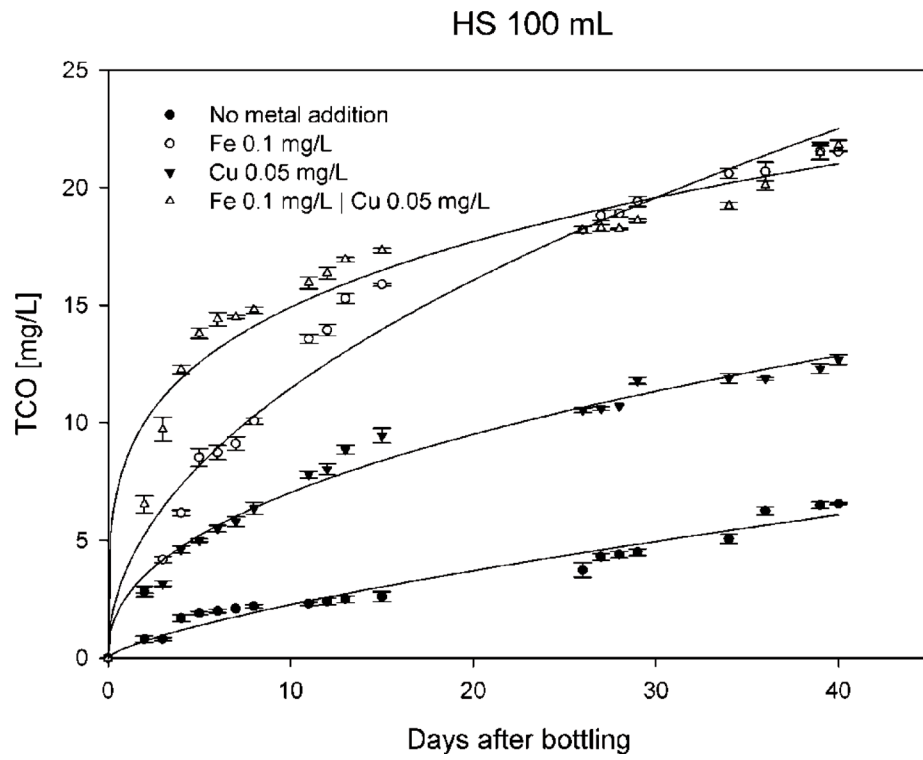
lar. In the wines without HS, only 0.5 to 0.6 mg/l oxygen were consumed during the observation period. The consumed oxygen concentration of the wines with 20 ml HS reached 6.2 to 7.1 mg/l. The wines with 40 ml HS consumed 10.2 to 10.6 mg/l until day 40. The highest oxygen consumption rate was observed during the first three days, whereafter it stabilized and turned out to be almost linear. The differences in oxygen consumption rates due to the different metal addition shown in the model wine experiment (Fig. 3) could not be evidenced in the real wine system. No significant changes in total consumed oxygen caused by the metal addition were observed.

Total oxygen consumption in model wine-like solution and in real wine seems to have significant differences. In the model solution addition of iron and copper had great impact on the evolution of total consumed oxygen. In the real wine system little differences between the wines could be explained by the fact that iron and copper could have been bound by the phenolic compounds. The results could have been also influenced by other metals present in the wine matrix (Mg, Zn).

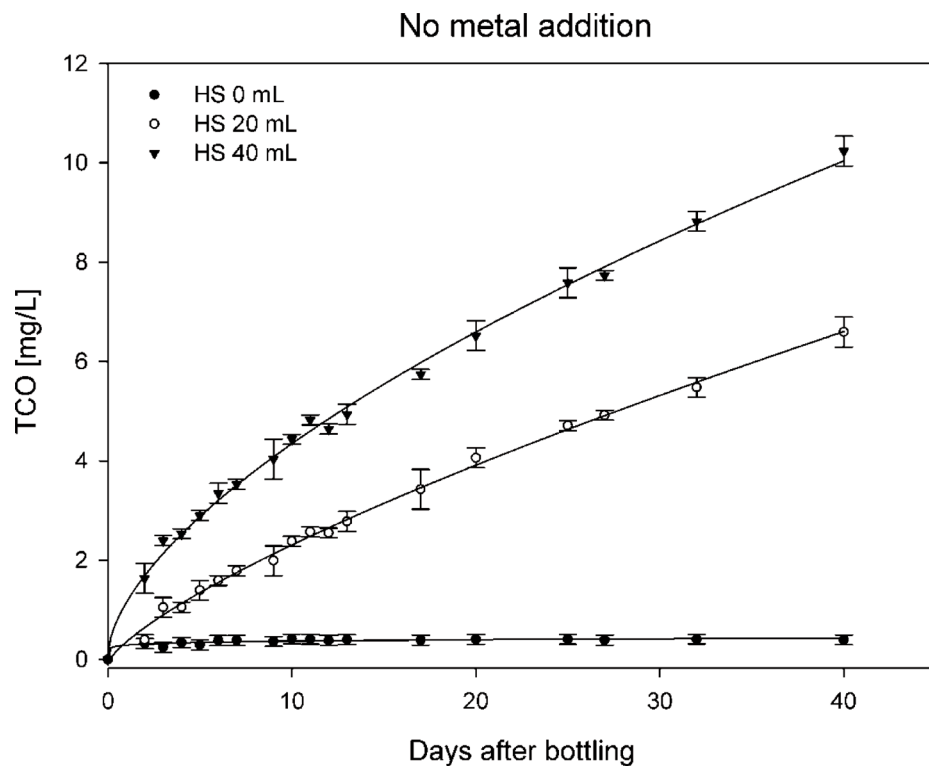
Fig. 3: Evolution of total consumed oxygen in model wine bottled with HS 50 ml (3a) and HS 100 ml (3b)



3b

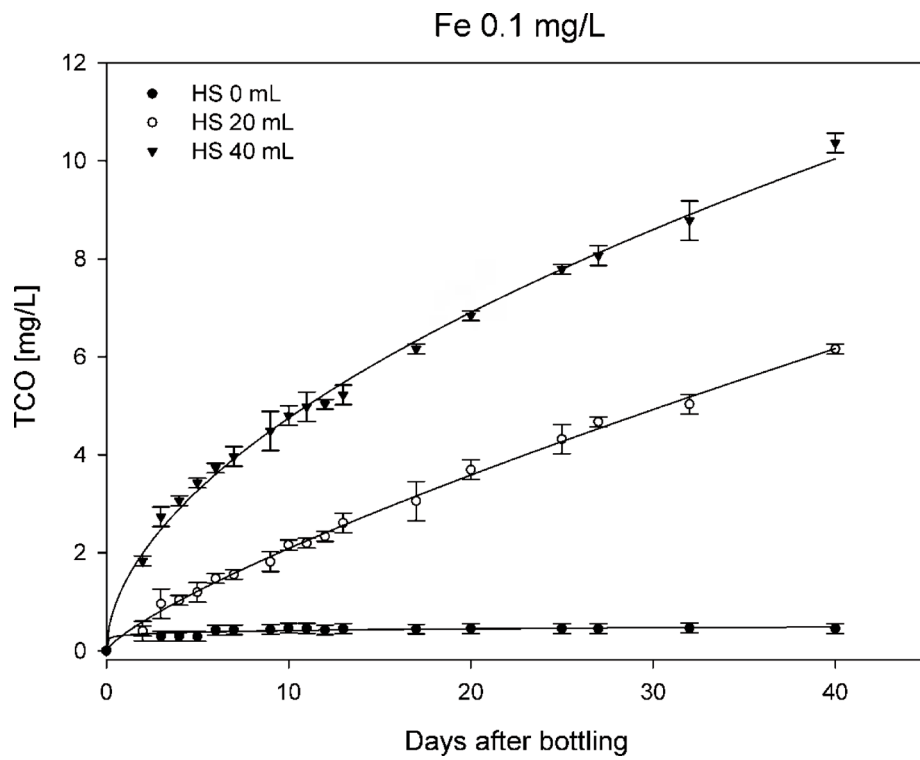


4a

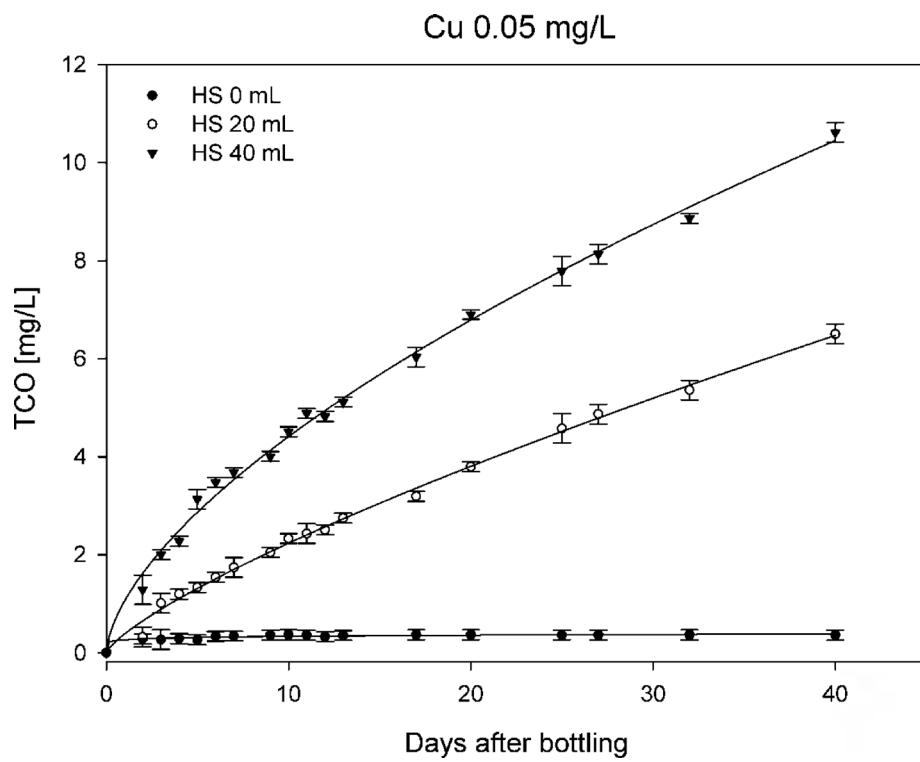




4b



4c



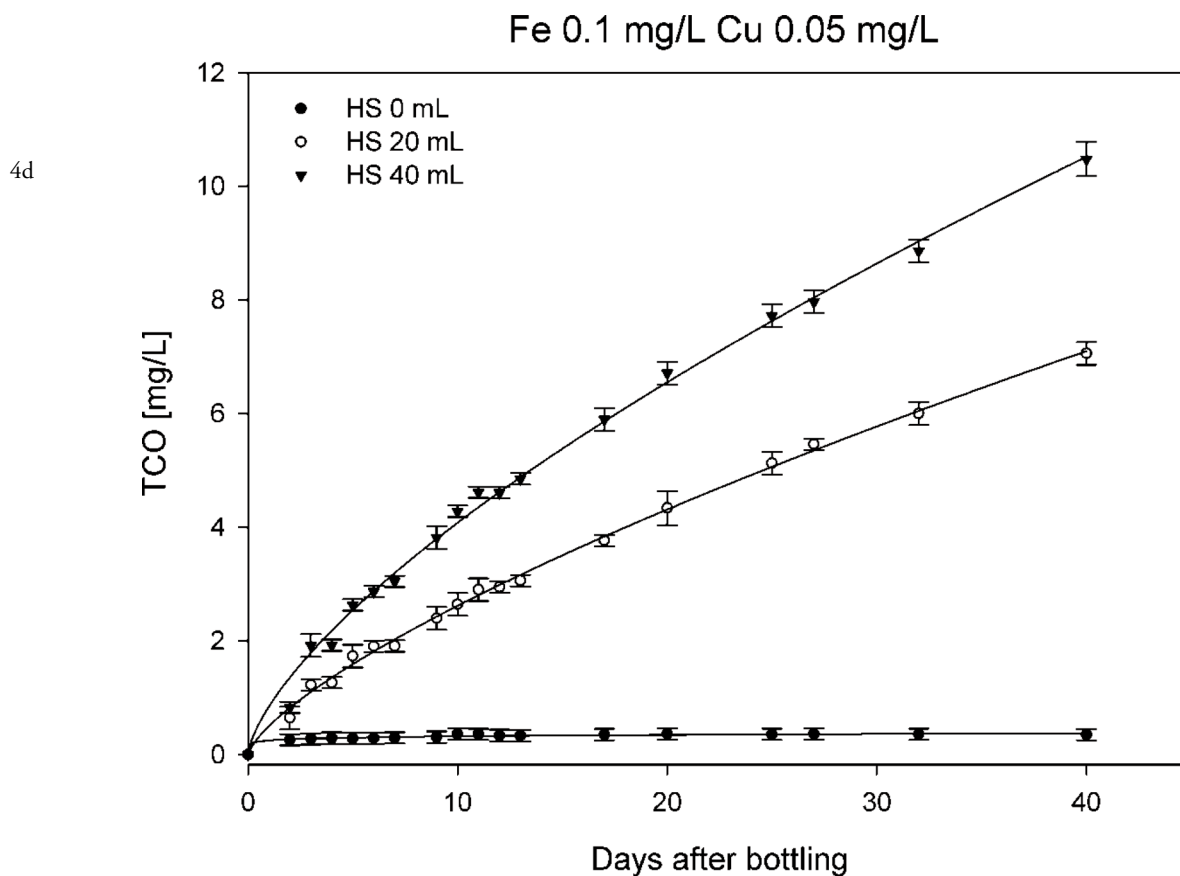


Fig. 4 (a, b, c, d): Total consumed oxygen (TCO) evolution (mg/l) in wine over 40 days of storage at room temperature

**Sulfur dioxide**

**Model solution**

Fig. 5 summarizes the impact of metal addition on free SO<sub>2</sub> levels after 40 days of storage at room temperature. The samples with no metal addition and 50 ml or 100 ml HS finally had about 15 mg/l of free SO<sub>2</sub>. All the other samples with addition of iron, copper or both metals showed no detectable free SO<sub>2</sub> after 40 days. The samples without HS (0 ml) retained about 29 to 32 mg/l of free SO<sub>2</sub> content, despite of the presence of iron and copper. Consequently, the results indicate that iron and copper addition catalyses free SO<sub>2</sub> degradation only when molecular oxygen is available.

**Wine**

The decay of free SO<sub>2</sub> during storage is shown in Fig. 6. The greatest influence on the free SO<sub>2</sub> loss had the headspace volume. The wines without HS only lost 15 mg/l of SO<sub>2</sub> in nine months. Wines with 20 ml and 40 ml HS lost 33 mg/l and 35 mg/l of free SO<sub>2</sub>, respectively, at the end of storage. However, analysis of variance showed that free SO<sub>2</sub> levels were not significantly different in the wines with 20 and 40 ml HS on days 40, 120 and 240. This may indicate that in the wines with 40 ml HS oxygen reacted with other wine compounds. Therewith, the free SO<sub>2</sub> in the wines with 20 and 40 ml HS dropped by approximately 49 % after 40 days, and by 86 % after four months. The results of the wines with no headspace both in model solution and in real wine measured after 40

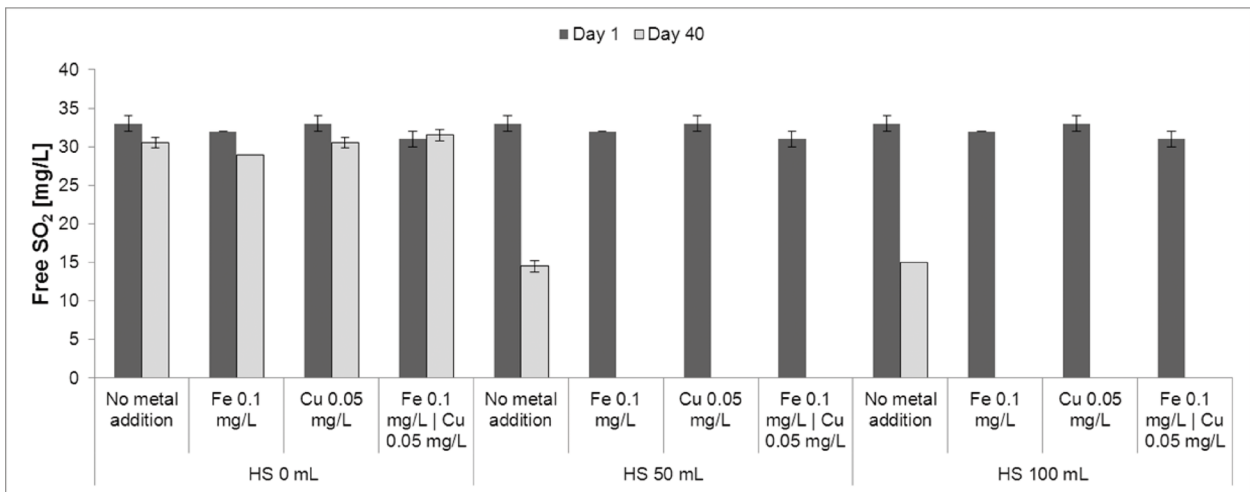


Fig. 5: Evolution of free SO<sub>2</sub> during bottle storage of model wine solution (error bars represent standard deviation)

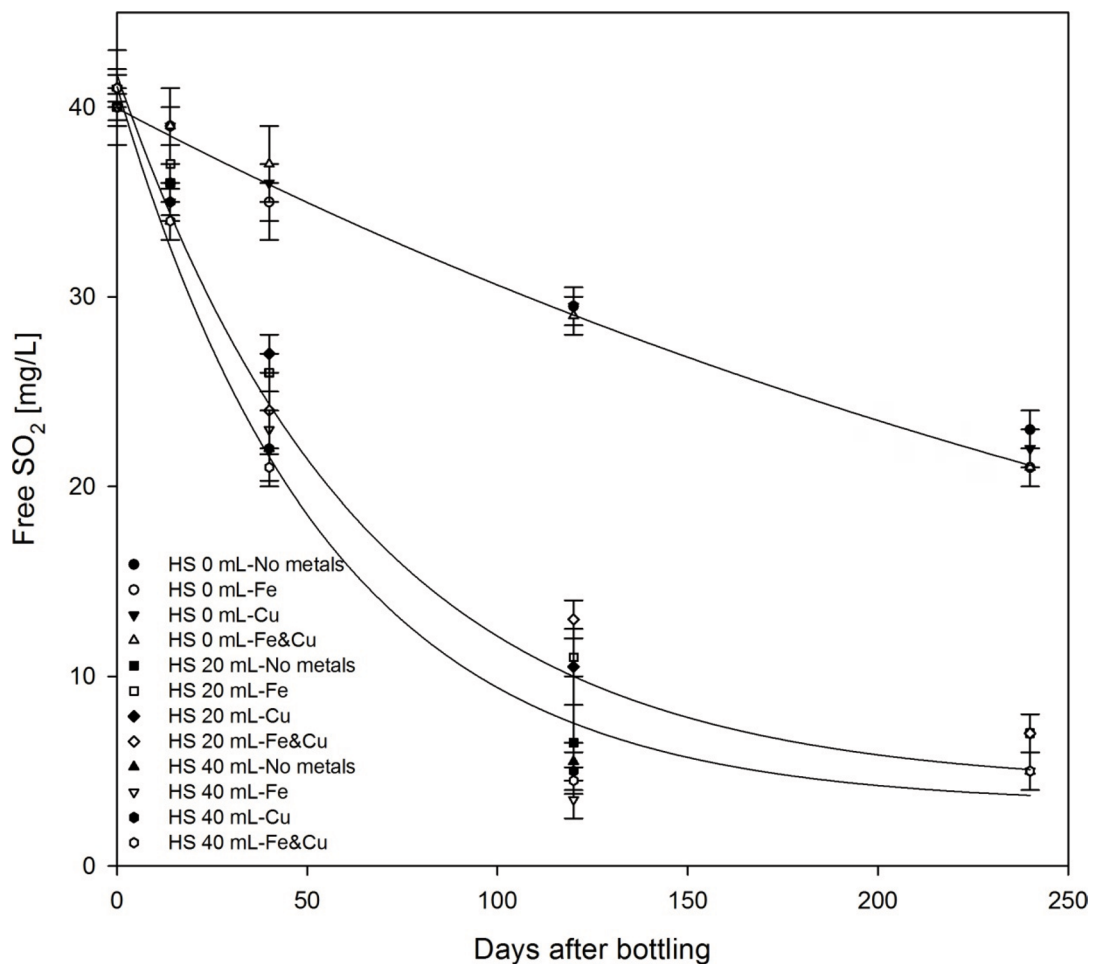


Fig. 6: Evolution of free SO<sub>2</sub> in wine bottled with different headspace volumes (0 ml, 20 ml and 40 ml) and metal addition (No metals, Fe, Cu, Fe & Cu) (error bars represent standard deviation)

days of storage were similar. The model wine wines with 50 ml and 100 ml HS and metal addition lost all the free SO<sub>2</sub>, whereas the real wine with 20 ml and 40 ml HS only lost 35 to 44 %. This is important to emphasize the impact of the oxygen content on the SO<sub>2</sub> loss at bottling. The influence of the metal addition could only be observed in model solution.

## Colour

### Model solution

The absorbance at 420 nm and colorimetric parameters were measured. The variants without headspace and all the samples with copper addition did not show any changes in light absorbance at 420 nm after the oxygen consumption was finished (Table 5). However, all samples with headspace and Fe or Fe + Cu addition showed clear colour changes on day 40. The results of CIALAB analysis synthesized by the index  $\Delta E$  showed similar results for the samples with Fe and Fe + Cu samples. A difference was observed for the samples with copper addition. The samples with Cu also showed enhanced colour compared to the samples with no metal addition. These changes could be only observed analytically, no visible colour change could be detected.

## Wine

Figure 7 shows the colour changes in the real wine after 40 and 240 days of storage. All wines without headspace (HS 0 ml) showed no significant optical colour change at the day 240 ( $p < 0.05$ ). The overall colour change ( $\Delta E$ ) of all wines with HS 20 and 40 ml increased after 40 days. However, no changes in absorbance at 420 nm were observed for the wines with 20 and 40 ml HS at this date. After 240 days, the colour differences became stronger, according to the headspace volume at bottling. All bottles with HS 20 and 40 ml showed increased values of 420 nm absorbance and  $\Delta E$ . The  $\Delta E$  values after 240 days of storage in the wines with 20 ml HS redoubled as compared to the values after 40 days of storage; the corresponding values for the HS 40 ml grew by 400 %. Again, as in the case of total consumed oxygen and SO<sub>2</sub>, any influence of metals could not be observed. Two-way ANOVA analysis of results (Table 6) shows the influence of the factors 'metal addition' and 'HS volume' on measured colour parameters (E420 absorbance and  $\Delta E$ ). The greatest impact on the colour development over the observed storage period was made by the HS volume. The influence of the metal addition was significant only at the day 240 (F value = 5.8), which is far less significant, compared to the impact of the factor HS volume (F value = 289).

Table 5: Colour changes in model wine solution: E420 on Day 1, 40, CIELAB overall colour change ( $\Delta E_{40d}$ ): Day 0 to Day 40

	E420 / Day 1	E420 / Day 40	$\Delta E_{40d}$
HS 0 ml  No metal addition	0.005 ± 0.001	0 ± 0	0.1 ± 0.10
HS 0 ml  Fe	0.016 ± 0.001	0.0115 ± 0.002	0.46 ± 0.15
HS 0 ml  Cu	0.004 ± 0	0.0015 ± 0.002	0.4 ± 0.10
HS 0 ml  Fe   Cu	0.035 ± 0.001	0.0355 ± 0.004	0.59 ± 0.13
HS 50 ml  No metal addition	0.005 ± 0.001	0 ± 0	0.11 ± 0.01
HS 50 ml  Fe	0.016 ± 0.001	0.0555 ± 0.004	2.28 ± 0.30
HS 50 ml  Cu	0.004 ± 0	0 ± 0	0.14 ± 0.10
HS 50 ml  Fe   Cu	0.035 ± 0.001	0.062 ± 0.001	1.89 ± 0.20
HS 100 ml  No metal addition	0.005 ± 0.001	0 ± 0	0.11 ± 0.05
HS 100 ml  Fe	0.016 ± 0.001	0.0705 ± 0.005	3.12 ± 0.20
HS 100 ml  Cu	0.004 ± 0	0 ± 0	0.22 ± 0.05
HS 100 ml  Fe   Cu	0.035 ± 0.001	0.076 ± 0.001	2.84 ± 0.10

$$a \Delta E_i = \sqrt{(L_i - L_j)^2 + (b_i - b_j)^2 + (a_i - a_j)^2}$$

where L, a, b are the CIALAB parameters measured on the day i and j

Table 6: Results of the 2-way ANOVA analysis for the values of E420 absorbance on the Day 14, 40 and 240 and the CIELAB overall colour change  $\Delta E_{d40}$ ,  $\Delta E_{d240}$ : the impact of the metal addition and the headspace volume (HS)

		E420 / Day 14	E420 / Day 40	E420 / Day 240	$\Delta E_{d40}$	$\Delta E_{d240}$
Metal addition	F	3,156	3,410	5,831	2,154	0,471
	Pr > F	0,107	0,094	0,033	0,195	0,713
HS volume	F	3,126	5,231	289,271	4,710	236,656
	Pr > F	0,117	0,048	< 0,0001	0,059	< 0,0001

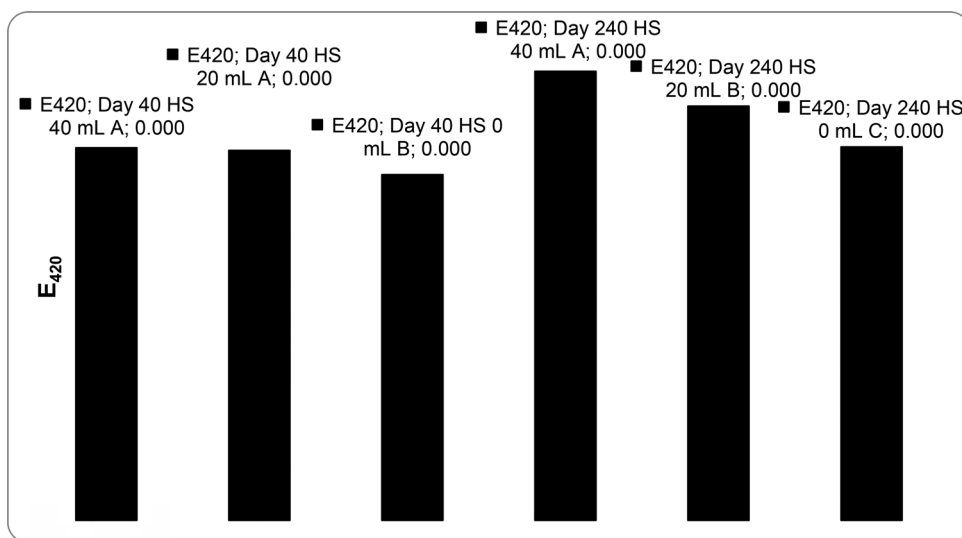


Fig. 7a

Fig. 7a,b: Colour changes in Riesling wine after 40 and 240 days of storage: average values of E420 absorbance (7a) and CIELAB overall colour change  $\Delta E$  (7b)

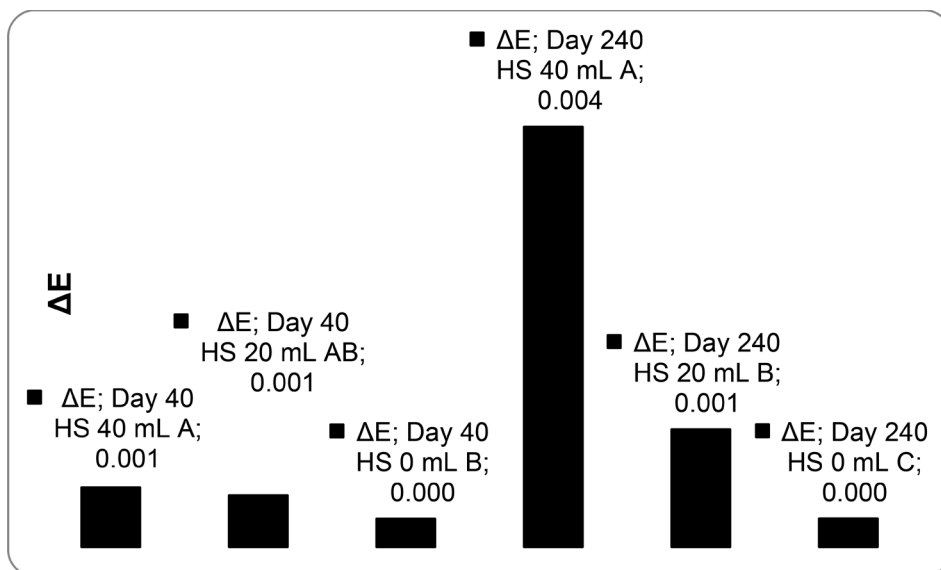


Fig. 7b

## Sensory evaluation

A triangle test was conducted three months after bottling. The results are summarized in Table 7. In the first four sessions the wines without headspace (0 ml HS) were compared to the wines with the biggest headspace (40 ml HS). These wines were proved to be significantly different from each other. Comparison of wines with the same headspace volume and different metal addition was conducted in six following sessions. There was no significant difference observed between the wines with the same HS volume with the session N° 8 as an exception, where the wine with 40 ml HS and iron addition was significantly different from the wine with 40 ml HS without metal addition. These differences could be possibly explained by the variation among sample bottles.

In order to specify the magnitude of differences between the samples found by the triangle test method, four sessions of descriptive analysis were conducted. Two sessions took place after six months of storage and two others were conducted after nine months of storage. The results are presented in Table 8. Three-way ANOVA analysis confirmed that the headspace volume had the greatest influence with F values of 51 to 62 for all the parameters, where the F values for metal addition were found to be between 0.06 for the attributes 'fruity' and 'ripe' and 0.76 for the attribute 'oxidized'.

The main PCA graph which summarizes the overall sensory and chemical evaluation of the Riesling experimental wines after nine months of storage at 15 °C is shown in Figure 8.

The first two principal components retained 93.29 % of the variance, most of which (88.08 %) was accounted for by the F1. The first principal component seems to be sufficient to explain the differences between the wines. The samples could be divided into three groups according to the HS volume.

Wines bottled with minimum oxygen (0 ml HS) are located on the negative side of the first principal component. HS 0 ml wines had higher free sulfur dioxide levels and CIELAB lightness ( $L^*$ ) values after nine months of storage. Moreover, they scored higher in 'fruity', 'fresh' and 'overall quality' parameters by the panellists. Higher HS volumes, in case of the Riesling wine studied, contributed to the oxidative spoilage of wines. The wines bottled with 20 and 40 ml HS were subject to colour changes and showed high intensities of 'ripe' and 'oxidized' aromas. The highest browning was observed in wines with 40 ml HS, which affected the overall quality.

## Conclusion

In model solution the headspace volume and the metal addition contributed to significant changes in total

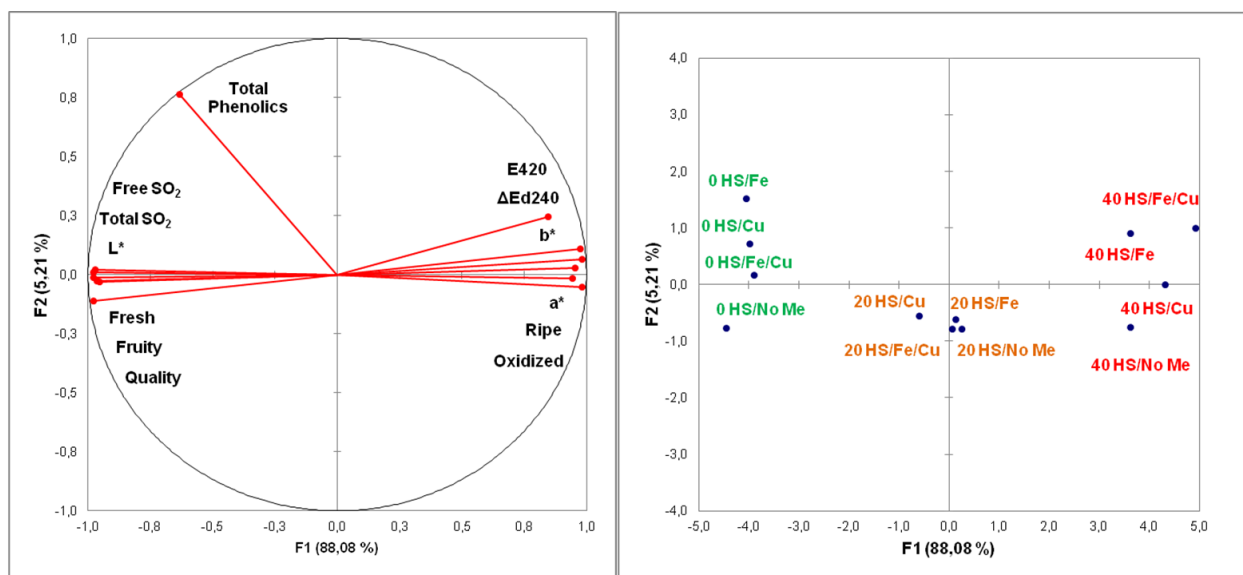


Fig. 8: Principal components analysis (PCA) showing descriptive analysis scores after 9 months of storage at 15 °C and analytical parameters on the first two dimensions, accounting for 93 % of the variability

Table 7: Results of triangle test with levels of significance (n = 26)

	Wine 1	Wine 2	№ of judges	Correct Answers	Incorrect Answers	Significance
1	HS 0 ml No metal addition	HS 40 ml No metal addition	26	13	13	*
2	HS 0 ml Fe	HS 40 ml Fe	26	14	12	**
3	HS 0 ml Cu	HS 40 ml Cu	26	18	8	***
4	HS 0 ml Fe   Cu	HS 40 ml Fe   Cu	26	19	7	***
5	HS 0 ml No metal addition	HS 0 ml Fe	26	7	19	n.s.
6	HS 0 ml No metal addition	HS 0 ml Cu	26	8	18	n.s.
7	HS 0 ml No metal addition	HS 0 ml Fe   Cu	26	11	15	n.s.
8	HS 40 ml No metal addition	HS 40 ml Fe	26	14	12	**
9	HS 40 ml No metal addition	HS 40 ml Cu	26	10	16	n.s.
10	HS 40 ml No metal addition	HS 40 ml Fe   Cu	26	10	16	n.s.

aSignificance levels: n.s. – not significant; \* - significant at 10%; \*\* - significant at 5%; \*\*\* - significant at 0.1%

Table 8: Mean intensity scores for descriptive sensory attributes evaluated in 4 descriptive sessions

Treatment	fruity		ripe		fresh		oxidized		Overall Quality	
HS 0 ml  No metal addition	2.64	Aa	2,36	A	2.38	A	2,62	A	2.10	A
HS 0 ml  Fe	2.36	AB	2,64	BC	2.21	AB	2,82	A	1.96	ABC
HS 0 ml  Cu	2.38	AB	2,62	AB	2.27	AB	2,73	A	1.98	ABC
HS 0 ml  Fe   Cu	2.45	AB	2,55	A	2.29	AB	2,71	A	2.02	AB
HS 20 ml  No metal addition	2.05	BC	2,95	CD	2.18	AB	3,28	B	1.84	ABCD
HS 20 ml  Fe	1.82	CD	3,18	DE	1.72	C	3,05	CD	1.61	DE
HS 20 ml  Cu	2.03	BC	2,97	CD	2.03	BC	2,79	BC	1.76	BCD
HS 20 ml  Fe   Cu	1.88	CD	3,12	DE	1.95	BC	2,97	BC	1.71	CD
HS 40 ml  No metal addition	1.62	DE	3,38	EF	1.37	D	3,64	CD	1.40	EF
HS 40 ml  Fe	1.39	EF	3,61	EF	1.36	D	3,63	CD	1.38	EF
HS 40 ml  Cu	1.24	F	3,76	EF	1.26	D	3,75	CD	1.32	FG
HS 40 ml  Fe   Cu	1.16	F	3,84	F	1.11	D	3,89	D	1.08	G

a Means with different letter(s) for each attribute are significantly different ( $p < 0.05$ ) using Fisher's LSD.

consumed oxygen, colour, and free sulphur dioxide. The metal addition increased the rate of the molecular oxygen consumption and resulted in higher consumption of free  $\text{SO}_2$ . Enhanced colour was observed in all model solutions, where iron was added.

The experiment with Riesling wine with similar parameters showed strong influence of the headspace volume at bottling on wine development. The rate of oxidative browning strongly correlated with the head-

space volume at bottling. Sensory analysis showed that the wines bottled with 0, 20 ml and 40 ml HS volume became significantly different already after three months of storage at 15 °C, which was proved by the triangle tests. Descriptive analysis after six and nine months of storage confirmed negative influence of bigger headspace volumes. Addition of small concentrations of iron and copper did not affect the observed parameters in Riesling wine.

In summary, the study revealed major differences between experiments with model solution and real wine. The results showed that the traces of metals in Riesling wines (even in the wines with no metal addition) were sufficient to start oxidation processes, which will be probably true for any real wine system. Thus, in production conditions it is unlikely to get an ideal case of a completely metal-free real wine system, where oxidation will not occur. Consequently, the oxygen ingress at the white wine bottling, and especially oxygen concentration in the headspace of bottles sealed under screw caps, should be minimized, which will help the winemakers to prevent the quality decrease due to oxidation spoilage.

It should be noted, that in the present study small amounts of iron and copper were added to the system. Experiments with average iron and copper concentrations with deeper insight into the analytical and sensory changes should be the subject of further research.

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### References

- ALMEIDA, C.M., and M.T. VASCONCELOS. 2003. Multielement composition of wines and their precursors including provenance soil and their potentialities as fingerprints of wine origin. *Journal of agricultural and food chemistry* 51: 4788-98.
- BLACKWELL, K.J., I. SINGLETON, and J.M. TOBIN. 1995. Metal cation uptake by yeast. A review. *Applied Microbiology and Biotechnology* 43: 579-84.
- C.I.E. 1986. Colorimetry. Commission Internationale de l'Eclairage, Wien.
- CATARINO, S., M. MADEIRA, F. MONTEIRO, A.S. CURVELO-GARCIA, and d.S.R. BRUNO. 2006. Release of contaminant elements from bentonites to wine: a contribution to achieve a test solution. *Ciência e Técnica Vitivinícola* 21: 17-31.
- DANILEWICZ, J.C. 2003. Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. *American Journal of Enology and Viticulture* 54: 73-85.
- DANILEWICZ, J.C. 2007. Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model system: Central role of iron and copper. *American Journal of Enology and Viticulture* 58: 53-60.
- DANILEWICZ, J.C. 2011. Mechanism of Autoxidation of Polyphenols and Participation of Sulfite in Wine: Key Role of Iron. *American Journal of Enology and Viticulture* 62: 319-328.
- DANILEWICZ, J.C., and P.J. WALLBRIDGE. 2010. Further Studies on the Mechanism of Interaction of Polyphenols, Oxygen, and Sulfite in Wine. *American Journal of Enology and Viticulture* 61: 166-175.
- DIMKOU, E., M. UGLIANO, J.B. DIEVAL, S. VIDAL, O. AAGAARD, D. RAUHUT, and R. JUNG. 2011. Impact of Headspace Oxygen and Closure on Sulfur Dioxide, Color, and Hydrogen Sulfide Levels in a Riesling Wine. *American Journal of Enology and Viticulture* 62: 261-269.
- DU TOIT, W.J., J. MARAIS, I.S. PRETORIUS, and M. DU TOIT. 2006. Oxygen in Must and Wine: A review. *South African Journal for Enology and Viticulture* 27: 76-94.
- ELIAS, R.J., M.L. ANDERSEN, L.H. SKIBSTED, and A.L. WATERHOUSE. 2009. Key factors affecting radical formation in wine studied by spin trapping and EPR spectroscopy. *American Journal of Enology and Viticulture* 60: 471-476.
- ESCHNAUER, H. 1982. Trace elements in must and wine: primary and secondary contents. *American Journal of Enology and Viticulture* 33: 226-30.
- GALANI-NIKOLAKAKI, S., N. KALLITHRAKAS-KONTOS, and A.A. KATSANOS. 2002. Trace element analysis of Cretan wines and wine products. *Science of the Total Environment* 285: 155-163.
- GODDEN, P., L. FRANCIS, J. FIELD, M. GISHEN, A. COULTER, P. VALENTE, P. HØJ, and E. ROBINSON. 2001. Wine bottle closures: physical characteristics and effect on composition and sensory properties of a Semillon wine 1. Performance up to 20 months post-bottling. *Australian Journal of Grape and Wine Research* 7: 64-105.
- GODDEN, P., K. LATTEY, L. FRANCIS, M. GISHEN, G. COWEY, M. HOLDSTOCK, E. ROBINSON, E. WATERS, G. SKOUROUMOUNIS, and M. SEFTON. 2005. Towards offering wine to the consumer in optimal condition—the wine, the closures and other packaging variables. A review of AWRI research examining the changes that occur in wine after bottling. *Wine Industry Journal* 20: 20-30.
- HUBER, C. 2006. Instruction Manual Fibox 3-trace v3. PreSens GmbH Josef-Engert-Str. 11 D – 93053 Regensburg Germany.
- KARBOWIAK, T., R.D. GOUGEON, J.-B. ALINC, L. BRACHAIS, F. DEBEAUFORT, A. VOILLEY, and D. CHASSAGNE. 2009. Wine Oxidation and the Role of Cork. *Critical Reviews in Food Science and Nutrition* 50: 20-52.
- KMENT, P., M. MIHALJEVIČ, V. ETTLER, O. ŠEBEK, L. STRNAD, and L. ROHLOVÁ. 2005. Differentiation of Czech wines using multielement composition – A comparison with vineyard soil. *Food Chemistry* 91: 157-165.
- KWIATKOWSKI, M.J., G.K. SKOUROUMOUNIS, K.A. LATTEY, and E.J. WATERS. 2007. The impact of closures, including screw cap with three different headspace volumes, on the composition, colour and sensory properties of a Cabernet Sauvignon wine during two years' storage. *Australian Journal of Grape and Wine Research* 13: 81-94.
- LAWLESS, H.T., and H. HEYMANN. 2010. Sensory evaluation of food: principles and practices. Springer.



- MOROZOVA, K., and O. SCHMIDT. 2012. Comparison of different methods for O<sub>2</sub> and CO<sub>2</sub> measurement in wine. *Mitteilungen Klosterneuburg* 62: 14.
- NICOLINI, G., R. LARCHER, P. PANGRAZZI, and L. BONTEMPO. 2004. Changes in the contents of micro- and trace-elements in wine due to winemaking treatments. *Vitis* 43: 41-45.
- NIKFARDJAM, M.P. 2010. Eisen und Kupfer - Starke Oxidantien im Wein. *Das Deutsche Weinmagazin* 17: 32-34.
- OIV. 2008. *Compendium of International Methods of Analysis of Wines and Musts*.
- POHL, P. 2007. What do metals tell us about wine? *Trends in Analytical Chemistry* 26: 941-949.
- RIBEREAU-GAYON, P., Y. GLORIES, A. MAUJEAN, and D. DUBOURDIEU. 2006. *Handbook of Enology - Volume 2 The Chemistry of Wine, Stabilization and Treatments 2006*. John Wiley & Sons Ltd., Chichester, England.
- SCHMIDT, O. 2001. Kupfer als Behandlungsmittel. Wiegen die Vorteile die Nachteile auf? *Rebe und Wein* 54: 20-23.
- SINGLETON, V.L. 1987. Oxygen with Phenols and Related Reactions in Musts, Wines, and Model Systems: Observations and Practical Implications. *American Journal of Enology and Viticulture* 38: 69-77.
- STAFILOV, T., and KARADJOVA, I. 2009. Atomic absorption spectrometry in wine analysis - a review. *Macedonian Journal of Chemistry and Chemical Engineering* 28: 17-31.
- TAYLOR, V.F., H.P. LONGERICH, and J.D. GREENOUGH. 2003. Multielement analysis of Canadian wines by inductively coupled plasma mass spectrometry (ICP-MS) and multivariate statistics. *Journal of agricultural and food chemistry* 51: 856-60.
- VOLPE, M.G., F. LA CARA, F. VOLPE, A. DE MATTIA, V. SERINO, F. PETITTO, C. ZAVALLONI, F. LIMONE, R. PELLECCIA, P.P. DE PRISCO, and M. DI STASIO. 2009. Heavy metal uptake in the enological food chain. *Food Chemistry* 117: 553-560.
- WATERHOUSE, A.L., and V.F. LAURIE. 2006. Oxidation of Wine Phenolics: A Critical Evaluation and Hypotheses. *American Journal of Enology and Viticulture* 57: 306-313.
- WURZINGER, A., M. NETZER, K. HEILI, and F. BANDION. 1994. Investigation and evaluation of bentonite [in enology]. *Mitteilungen Klosterneuburg* 44: 146-9.
- ZOECKLEIN, B.W., K.C. FUGELANG, B.H. GUMP, and F.S. NURY. 1994. *Wine Analysis and Production*. CHAPMAN & HALL, New York, USA.

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