IMPACT OF OXYGEN ON QUALITY OF WHITE WINE

DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES
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CONTENTS

PRELIMINARY REMARKS

Parts of the work presented in this thesis have already been published or submitted to international peer reviewed journals and/or were presented on scientific conferences as oral presentations:

Full papers

1. Morozova, K., Schmidt, O. (2012) “Comparison of Methods for O_2 an CO_2 Measurement in Wine”, Mitteilungen Klosterneuburg, 62: 97-110
2. Morozova, K., Just, M., Schmidt, O., Schwack, W. (2013) “Effect of Iron and Copper on Oxidation Processes in Model Solution and Wine”, Mitteilungen Klosterneuburg (accepted).

Submitted papers

3. Morozova, K., Schmidt, O. Schwack, W. (2013) “Influence of Headspace Volume and Copper and Iron on Analytical and Sensory Changes in Bottled Riesling Wine”, European Food Research and Technology.

Oral presentations

4. Morozova, K., Schmidt, O. (2011) Einfluss von Sauerstoff auf die Weinqualität. Kellermeister-Tagung, Freiburg, 4.02.2011.
5. Morozova, K., Schmidt, O. (2011) Sauerstoffmessung im Wein: Aktuelle Messungen und Bewertung. Fortbildung Kellerwirtschaft, Staatliche Lehr- und Versuchsanstalt für Wein und Obstbau Weinsberg, Weinsberg, 15.03.2011.
6. Morozova, K., Schmidt, O. (2011) Sauerstoffaufnahme während der Weinbereitung. FDW-Tagung, Freiburg, 6.04.2011.

7. Morozova, K., Schmidt, O. (2012) Sauerstoff: Messung und Bewertung. Fortbildung Kellerwirtschaft, Staatliche Lehr- und Versuchsanstalt für Wein und Obstbau Weinsberg, Weinsberg, 1.02.2012.

8. Morozova, K., Just, M., Schmidt, O. (2012) Weinoxidation und die Rolle von Eisen und Kupfer. FDW-Tagung, Bernkastel, 27.03.2012.

9. Morozova, K., Schmidt, O. (2012) Einfluss von Sauerstoff auf die Eigenschaften von Wein – ein Überblick zu laufenden Untersuchungen. DRV/DWV-Tagung, Kitzingen, 6.12.2012

10. Morozova, K., Schmidt, O. (2013) Methodenvergleich zur O_2- und CO_2-Bestimmung im Wein. 61. Deutscher Weinbaukongress, Stuttgart, 24.04.2013.

The co-authors' contributions to the papers presented in Chapters 2-5 are specified as follows:

The work presented in this thesis was carried out under the supervision and instruction of Prof. Dr. habil. **Wolfgang Schwack** at the Institute of Food Chemistry, Hohenheim University. He functioned as an advisor with regard to the content of the work and publication of the results. Prof. Schwack proof-read all manuscripts, and was responsible for the formal aspects concerning the dissertation and the publications.

The practical experimental part in the winery of the State Institute for Viticulture, Oenology and Fruit Technology Weinsberg was carried out under the supervision of Dr. **Oliver Schmidt.** He contributed to the experimental design, supervised the cellar operations on site as long as the sensory evaluation of the experimental wines. He also proof-read all the manuscripts prior to publication.

Moriz Just carried out the experiment with small amounts of iron and copper in the model solution and Riesling wine as part of his *Master thesis*.

GENERAL INTRODUCTION

Wine market is becoming more and more competitive. A deep understanding of the market and identifying what consumers value in your product, is crucial for sustainable business success. Value arises out of a combination of product attributes for which consumers are prepared to pay extra.

Recent statistics show the effort of the German wine industry to generate greater added value, rather than to increase the volume. According to the data of the German Wine Institute the local consumers spent in 2011 some 2% more on wine than in 2010. At the same time, the overall volume of wine purchased in 2011 declined since 2008 by 2.7%. 1.5 million hectolitres of German wine were exported in 2011, a decline of 11.8% compared to the previous year. Nevertheless, there was little change in total value, which dropped by only 1.7% to 349 million euros, thanks to an increase in average price by 11.8% to 2.27 €/litre compared with the year before (Weininstitut 2013).

Consequently, the success of a wine producer depends on wine quality, which includes both chemical and sensory stability and quality of the wines. Maintenance of quality from the vineyard until bottling and assuring long shelf-life of wines are considered to be the main aims of a winemaker.

Wine oxidation is one of the major wine failures. Recently, it has been reported that up to 48% of the wines rated as faulty by judges in wine competitions exhibited off-flavours that can be linked to the erroneous management of oxygen (Ugliano et al. 2009). Oxygen has a potential to support positively, and subsequently to ruin aroma and colour of wine. The influence of oxygen depends on many factors, such as wine style, grape variety, temperature etc. Fruity and fresh character of German white and rosé wines is appreciated by consumers (Burkert 2013; Schneider 2013). Most white wines can be negatively affected already by small amounts of dissolved oxygen. Red wines, due to the richness in phenolic compounds, can resist the oxidation longer (Caillé et al. 2010; Villamor et al. 2009).

1. OXYGEN UPTAKE DURING WINEMAKING OPERATIONS

Oxygen is a colourless odourless gas present in the atmosphere at approximately 20.9% v/v. Dissolved oxygen concentration at all winemaking operations can be calculated using Henry's law for gases:

$$c=k_H p_i$$

where **c** is dissolved oxygen concentration [mol · m^{-3}], **p** – partial pressure of oxygen [Pa] and **k_H** – Henry's law constant [Pa · m^3 · mol^{-1}], which depends on the solute, the solvent and the temperature (Cohen 2007; Margalit and Crum 2004).

At 20°C and normal pressure oxygen can be dissolved in wine only up to 8,8 mg/L (Troost 1988). However, at higher pressure and lower temperature solubility increases (Blank et al. 2012; Moutounet and Mazauric 2001; Schmidt and Waidelich 2008).

Wine exposure to oxygen is possible throughout winemaking process and highly depends on cellar practices and conditions (Castellari et al. 2004; Jung 2009; Morozova 2009; Vidal et al. 2003; Vidal et al. 2004; Vidal et al. 2001). While wine is transported, contact with air is very probable and quantity of dissolved oxygen may increase. Oxygen intake more than 3 mg/L may be observed after wine transfer (Moutounet and Mazauric 2001). Dissolved oxygen concentration during wine transport also depends on the level of filling. Vidal et al. (2001) measured 0.4 mg/L in a completely full cistern, whereas the same wine transported in 80% filled cistern contained 1.4 mg/L of dissolved oxygen. Pumping may also be a source of oxygen delivering 0.1-0.2 mg/L of dissolved oxygen (Table 1) depending on the type of pump used (peristaltic of centrifuge) as long as the length and the type of tubing. Agitation effects may be reduced by decreasing pump speed (Karbowiak et al. 2009). During filtration, the greatest risk of oxygen enrichment occurs at the initial and completion phases of filtration, which may be especially dangerous for small charges (Morozova and Schmidt 2011). As mentioned in the Table 1, tartaric stabilisation may also lead to high oxygen uptake due to combined effects of mixing and cooling. Finally, dissolved oxygen measurements at bottling are ranging from 0.2 to 7 mg/L (Vidal et al. 2004). Again, oxygen uptake at the beginning of bottling was reported to be higher than in the middle of the process (Jung 2009). In addition, headspace may be a reserve of significant oxygen amounts, depending on closure type, wine filling level and bottling conditions (Vidal and Moutounet 2006). Oxygen concentration in the headspace can be calculated using ideal gas law (Karbowiak 2009):

$$p \cdot V = n \cdot R \cdot T$$

where $\boldsymbol{p}$ *is* partial pressure of oxygen in headspace [Pa], $\boldsymbol{V}$ – headspace volume [m^3], $\boldsymbol{n}$ – oxygen amount [mol], $\boldsymbol{R}$ – ideal gas constant [$8.314\ J \cdot K^{-1} \cdot mol^{-1}$] and $\boldsymbol{T}$ – absolute temperature [K].

For bottled wines, both dissolved and headspace oxygen should be taken into consideration, so total package oxygen (TPO) per bottle is usually calculated. As bottling is a source of great oxygen uptake, a good oxygen management is needed to protect fruity wines from oxidation. Total oxygen amount entering the wine may be decreased by choosing the right filling modus and by flushing empty bottles and headspaces before sealing with inert gasses (Jung 2009; Lassak 2013). After bottling, additional oxygen may be ingressing through the closure and after dissolution react with wine constituents. Oxygen transmission rates of different stoppers has been the subject of many recent studies (Dimkou et al. 2011; Dimkou et al. 2013; Godden et al. 2005; Karbowiak 2009; Lopes et al. 2007; Lopes et al. 2009; Vidal et al. 2011). Screw caps were shown to be the best barrier to limit oxygen transfer through the closure (Godden et al. 2005; Vidal et al. 2011; Vidal and Moutounet 2006).

TABLE 1: OXYGEN UPTAKE DURING WINEMAKING OPERATIONS – LITERATURE DATA AND OWN MEASUREMENTS (* - DISSOLVED OXYGEN; ** - DISSOLVED AND HEADSPACE OXYGEN)

Operation	(VALADE et al. 2006; Vidal et al. 2003; Vidal et al. 2004; Vidal et al. 2001)	(Castellari et al. 2004)	Own Measurements
Racking	-	0.37-1.3	0.2-1.5
Pumping	0.1-0.2	0.1	0.1
Plate filtration	0.2	0.04	0.2-2.2
Diatomaceous earth filtration	0.7	0.24	1.1
Cross Flow filtration	1.5	0.2	0.5-4.3
Membrane filtration	1.3		0.2-2.1
Continuous tartaric stabilisation	1.2	4	4.3-7.8
Electrodialysis	1.3	0.28	0.2-2.7
Bottling	0.38-4.3*		1.9-9.1**

2. OXYGEN CONSUMPTION

From a chemical point of view, wine is a complex fluid consisting of water, alcohols, organic acids, phenolic compounds, sugars, amino acids and minerals (Karbowiak et al. 2009). The catechol group (1,2-dihydroxybenzene) of the phenolic compounds reacts very readily with oxidants in the form of free radical reactive oxygen species to form a very stable radical anion, the semi-quinone radical. Wine is rich in substances with the catechol group and these compounds determine an antioxidant activity of wine and its role as natural preservative (Waterhouse 2002).

In a sealed bottle, headspace oxygen is absorbed by the contact surface and then dissolves into wine until the equilibrium between partial pressures in gas and liquid phases is reached, which is a function of time and concentration gradient (Höchli and Bernath 2002). After dissolution, oxygen concentration in liquid phase will decrease down to undetectable content due to chemical reactions of molecular oxygen with other wine constituents. Some researchers stated the oxygen consumption ends in a week after wine bottling (Boulton et al. 1998). Singleton et al. (1979) also observed the oxygen was reduced to below 1 mg/L in red wine after saturation in about 6 days at 30°C, mainly because of reactions with phenolics. Others claim that this process may take more time: from 10 days to three or four months (Jung 2009). Vidal and Moutounet (2006) showed that oxygen consumption rate is not linear and is higher in the first two months, when the oxygen concentration differences are greater.

Oxygen consumption rate mainly depends on the concentration of phenolic compounds available for oxidation. Thus, oxygen is consumed much faster in red wine than in white. The total capacity of oxygen absorption is 80 mg/L for white wines and 800 mg/L reds (Moutounet and Mazauric 2001). Singleton et al. (1979) indicated that white wines could absorb 10 air saturations before negative consequences of oxidation were observed. Singleton (1987) claimed that red wine could tolerate more than 30 saturations (180 mL O_2/L) before showing oxidised character. Wine ageing may also be influenced by storage temperature (Ivanova et al. 2012; Makhotkina et al. 2012), light exposure (Dias et al. 2012), bottle position (Chatonnet 2000), concentration of iron, copper and other metals (Danilewicz 2003; Danilewicz et al. 2008; Danilewicz and Wallbridge 2010) and redox state of wine (Ribereau-Gayon et al. 2006). The redox potential at inert metal electrodes in wine represents a mixed potential mainly to ethanol oxidation and reduction of protons or dissolved oxygen (Kilmartin and Zou 2001). Some researches suppose that cyclic voltammetry provides more useful information about the redox state of wine than measurements with inert electrodes (Danilewicz 2011).

3. WINE OXIDATION REACTIONS

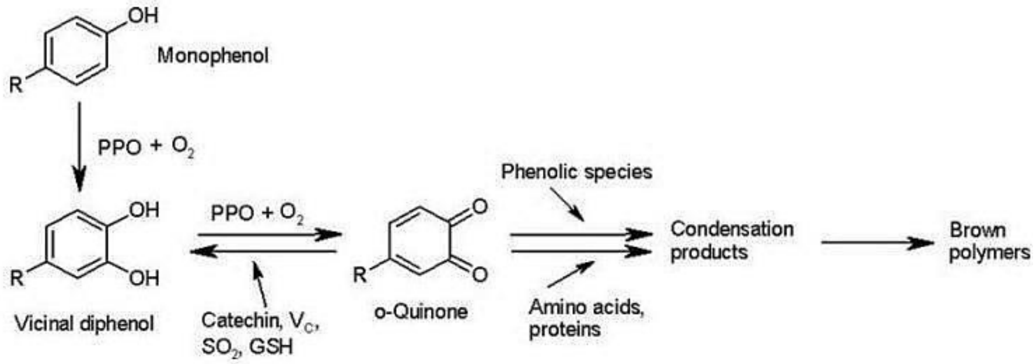
A redox system is an electron-transfer between oxidative and reductive partners (Ribereau-Gayon et al. 2006). Oxidizing agents are compounds that cause other compounds to be oxidised (to lose some of their electrons). A reducing agent, on the other hand, is a molecule or ion that causes other components to be reduced. The main reducing agents found in wines are sulphur dioxide, phenolics, ethanol and organic acids. These compounds can react or bind with oxygen and lower the overall redox potential of the system. Again, pH influences the reducing power of these agents (Zoecklein et al. 1994). Phenolics are the main substrate for oxidation reactions. This may partially explain why white wines are so much less tolerable to oxidation than red wines. The average total phenolic content in red wines is about 10 times higher than in white wines (Ribereau-Gayon et al. 2006). There are two major mechanisms of wine oxidation, namely enzymatic and nonenzymatic.

3.1. ENZYMATIC OXIDATION

The three main classes of enzymes that catalyze the oxidation of phenolic compounds are the oxidoreductases that use oxygen as electron acceptor, the monophenol monooxygenase, and the peroxidases (Oliveira et al. 2011). The oxidoreductases include catechol oxidase (also known as diphenoloxidase, phenoloxidase, polyphenoloxidase, *ortho*-diphenolase, phenolase and tyrosinase) and laccase (or *para*-diphenoloxidase), which are the main two enzymes responsible for oxidative browning during grapes processing.

Polyphenoloxidase is found in many fruits including grapes, and are responsible for the typical browning of cut fruits (and must). Polyphenoloxidase contains copper atoms in its active site and is able to oxidise o-diphenols and in some cases monophenols. The enzymatic browning (Figure 1) occurs mainly on the early stages of grape processing in presence of polyphenoloxidase and molecular oxygen (Li et al. 2008). The activity of polyphenoloxidase is inhibited by sulphur dioxide, mostly required at concentrations from 25 to 50 mg/L for complete deactivation. The enzymic browning of grape must is dependent on the content of hydroxylcinnamates such as caftaric acid and coutaric acid, and is promoted by flavanols (Cheynier et al. 1997; Cheynier et al. 2006; Cheynier et al. 1993; Guyot et al. 1995). According to Li et al. (2008) the o-quinones produced in enzymic oxidation will further undergo polymerisation and condensation reactions with other wine compounds. In the same time, o-quinones can possibly be reduced to their initial phenols by ascorbic acid, SO_2, and glutathione.

FIGURE 1: ENZYMATIC BROWNING (LI ET AL. 2008)

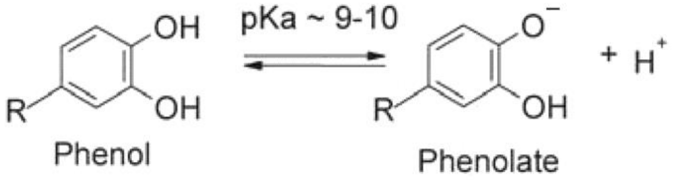


Laccase is another enzyme with a higher capability to oxidise a wider range of phenolics such as monophenols, diphenols including p-diphenol, and triphenols (Oliveira et al. 2011). This enzyme is usually carried into wines by *Botrytis cinerea* (fungus) infected grapes. Laccase is resistant to sulphur dioxide, stable and active in wine medium for a long time. Thus, minimising mouldy grapes is most preferred if possible (Zoecklein et al. 1994).

3.2. CHEMICAL OXIDATION

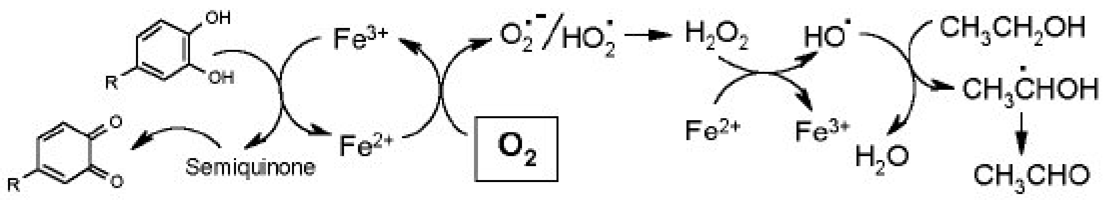
Phenolic constituents are also the main substrate for the non-enzymatic oxidations (Singleton 1987). In wine, phenolic compounds exist in equilibrium with their phenolate anion form (Figure 2). The pH controls phenol-phenolate equilibrium which sets the phenol concentration needed to undergo the reaction. Although at wine pH (3.0-4.0) the phenolate concentration is very low, at the higher pH range (4.0) the concentration is still about 10 times higher than at the lower pH range (3.0), and hence the oxidation rate of wines with high pH is much faster than at low pH (Margalit and Crum 2004). Due to high pKa values (9 to 10) only a small fraction of wine phenolics is deprotonated at pH 3-4 typical for wine. Thus, phenolic compounds are unlikely to react directly with oxygen under acidic wine conditions (Danilewicz 2003; Waterhouse and Laurie 2006).

FIGURE 2: PHENOL–PHENOLATE ANION EQUILIBRIUM (WATERHOUSE AND LAURIE 2006)



Danilewicz (2003) proposed the oxidation mechanism based on Fenton reaction (Figure 3), yielding the formation of semi-quinone radical, which is further oxidised to quinone (Elias and Waterhouse 2010; Karbowiak et al. 2009). In a Fenton reaction H_2O_2 is reduced to a more reactive hydroxyl radical ($OH^\bullet$), starting cyclic chain of radical reactions. This reaction is directly mediated by trace metals, mainly by iron and copper (Danilewicz 2003; Danilewicz 2007; Danilewicz and Wallbridge 2010). In the same reaction ferrous ion (Fe^{2+}) is transferred into ferric ion (Fe^{3+}) (Halliwell and Gutteridge 1984). The free radical (Fe^{3+}), being very powerful, will reduce the ethanol molecule to acetaldehyde.

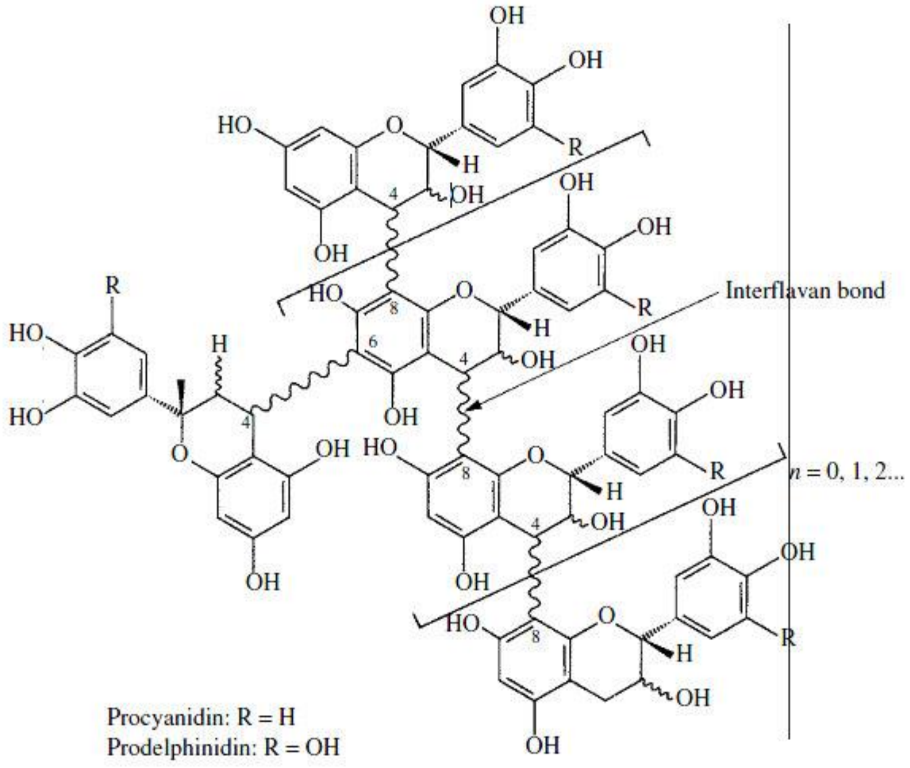
FIGURE 3: MECHANISM OF CHEMICAL OXIDATION (DANILEWICZ 2003)



Oxidation reactions are non-selective and may involve other wine compounds resulting in formation of various products, such as quinones from phenols, acetaldehyde from ethanol, or keto acids from acid oxidation (Danilewicz 2003; Waterhouse and Laurie 2006).

These reactions are the basis for further condensations of the oxidised phenols, forming a variety of phenolic polymers (Figure 4). The C-C links are the most common, O-C links (etheric) are less frequent, and the O-O (peroxy) links are rare (Boulton et al. 1998; Zoecklein et al. 1994). The oxidation rate of the polymeric products is a higher than the monomeric unit oxidation (substituted semiquinone is a higher electron donor than unsubstituted), and hence, as oxidation is continued, more polymeric oxidation reactions, slowing down the consumption of the original phenolics. This is the case in slow oxidation when competition between the different substrates is at equilibrium, for example during microoxygenation and barrel aging of red wines (Devatine et al. 2007; Durner, Ganss, et al. 2010; Durner, Weber, et al. 2010; Rayne 2007). In very fast oxidation, all units undergo changes (original and oxidised), so the distribution profile of the phenolic polymers is different than that at slow oxidation. This is partial explanation for the difference in appearance and taste of wines exposed to slow and fast oxidation (Arfelli et al. 2007; Fernández de Simón et al. 2010; Kelly and Wollan 2003; Zoecklein et al. 1994).

FIGURE 4: STRUCTURES OF CONDENSED PROANTHOCYANIDINS (RIBEREAU-GAYON ET AL. 2006)



4. IMPACT OF OXYGEN ON WINE QUALITY

Wine oxidation processes influence aroma, colour, flavour and microbiological stability of wines. The effects may vary significantly depending on type of wine, winemaking operation, oxygen amount and other conditions.

4.1. AROMA

Oxidation is often associated with the loss of fruity and floral aroma. This is mostly due to the decrease in thiolic compounds, which can be oxidised by H_2O_2 and can easily react with quinones. For example, 3-mercaptohexanol (3-MH) is formed during fermentation and plays an important role in the fruity and tropical aroma of Sauvignon blanc white wines, and red and rosé wines obtained from Cabernet Sauvignon, Merlot, and Grenache varieties. It was observed that the concentrations of this compound decreased during aging of Cabernet franc, Cabernet Sauvignon, and Merlot as a result of oxygen addition (Blanchard et al. 2004). Ugliano et al. (2011) reported a rapid decay of 3-MH in Sauvignon blanc wines sealed under closures stored in air and stored under air conditions (4 mg/L oxygen in six months). It was observed, that addition of gluthatione reduced 3-MH degradation, whereas addition of copper had an opposite effect. In some cases disappearance of thiols may, however, have a positive effect, while some of them have very strong red pepper and green fruit aromas, which can dominate in young wine of varieties like Cabernet Sauvignon and Merlot (Bouchilloux et al. 1998). Little quantities of oxygen help to reduce green herbaceous aromas in young wines (Pirdel et al. 2007).

Marais et al. (1992) found that concentration of diendiol-1, linalool, i-amyl-acetate, ethyl caproate, hexyl acetate, 2-phenethyl acetate, hexanol, 2-phenyl ethanol, and the intensity of young wine character significantly decreased with aging of Weisser Riesling wines. Another compound sensitive to oxygen is β-damascenone. This compound has a flavourful violet and ripe fruit aroma and is important for the perception of mature fruit in red wines, and can be also destroyed by oxidation (Ferreira et al. 1997).

Acetaldehyde is produced during alcoholic fermentation and as a result of oxidation processes during wine storage. It may impact an odour to the wine described as overripe, sherry-like and nutty (Zoecklein et al. 1994). However, in the experiment of Escudero et al. (2002), acetaldehyde did not seem to contribute to the oxidised aroma of wines studied.

Simpson (1978) conducted GC analysis of Riesling wines stored at 50°C in presence of air. The amounts of ethyl n-hexanoate, 1-hexanol, acetic acid, furfural, benzaldehyde, vitispirane, ethyl furoate, 2-phenethanol and 1,1,6-trimethyl-1,2-

dihydronaphthalene (TDN) increased over the storage period. Marais et al. (1992) also reported enhancement of kerosene-like character of Weisser Riesling wines during aging, which was linked to the increasing concentrations of TDN, trans-vitispirane and 2,6-dimethyl-7-octen-2,6-diol. High oxygen addition leads to the formation of off-odours, described as meat, honey, wood, rancid smell, cooked vegetables etc. (Escudero et al. 2002). Escudero et al. (2000) identified 19 compounds using gas chromatography. Four main molecules detected all along the oxidation process are: motional (potato, cereals, soup), eugenol (meat, wood), sotolon (curry, smoked, caramel) and 2,4,5-trimethyl-1,3-dioxolane (cider, herbaceous). Furfural, t-2-nonenal and benzaldehyde may also contribute to the cooked vegetable aroma of aged wines (Ferreira et al. 1997; Margalit and Crum 2004). Elevated oxygen also favours the formation of methional, that contributes to the aroma of spoiled wines (Silva Ferreira et al. 2002). Later Silva Ferreira et al. (2003) analysed Portuguese wines from Encruzado and Assario varieties, from nine different vintages, and found 3-(methylthio)propionaldehyde, phenylacetaldehyde, TDN, and sotolon to be the most important contributors to the typical aroma of an oxidation-spoiled white wine. 2-aminoacetophenone, formed via oxidative degradation of indole-3-acetic acid, is regarded as the main aroma impact compound causing an “untypical aging” (UTA) off-flavour (acacia, soapy) in *Vitis vinifera* wines (Du Toit et al. 2006; Hoenicke et al. 2002).

Although excessive wine contact with oxygen is normally undesirable, low exposure to oxygen may result in the accumulation of reductive aroma compounds, such as hydrogen sulfide (H_2S) and methanethiol (MeSH), possibly determining reductive off-flavours (Godden et al. 2001; Godden et al. 2005; Ugliano et al. 2009).

4.2. COLOUR

One of the most visible consequences of on aging is the formation of brown pigments giving brown-red hue in red wines, and yellow-brown hue in whites. Both enzymatic and non-enzymatic oxidation reactions lead to browning (Li et al. 2008).

Oxidative browning in white wines is generally undesirable (Panagiotakopoulou and Morris 1991). Phenolic compounds are the substrate for white wine browning by oxidation processes (Cilliers and Singleton 1990). The *o*-quinones with varying degrees of polymerisation formed by the phenolic oxidation are the main precursors for browning reactions in fruits. Although hydroxycinnamic acid and its derivatives are the main group of phenolic compounds found in white wine, caftaric, coutaric, ferulic and caffeic acids do not seem to play an important role in browning reactions (Du Toit et al. 2006). On the contrary, coloured pigments seem to be formed during polimerisation of flavanols, especially catechin and epicatechin (Sioumis et al. 2006). Recent studies showed, that in presence of Fe and Cu (+)-tartaric acid may be oxidised to glyoxylic acid, which can further react with flavanols, yielding yellowish xanthylium ions (Clark et al. 2003; Clark and Scollary 2003; Es-Safi et al. 2000).

White wine browning increases with increasing temperatures, pH, Fe, Cu and oxygen concentrations (Caputi Jr and Peterson 1965; Peterson and Caputi Jr 1967). Winemaking techniques influencing the amount of procyanidins, such as skin maceration, pressing or heat treatment, may consequently impact to the browning sensitivity of wine (Du Toit et al. 2006).

Oxygen induces colour changes also in red wines. During aging, the colour is changing from red-violet, in young red wines, to brown-red and brown-orange (Ribereau-Gayon et al. 2006). The violet-red colour of very young wine is caused by the almost pure content of monomeric pigments, which are in equilibrium with some small percentage of the violet anhydro-base quinoidal. In aged wines, the polymeric forms of the pigments are the major contributors to the red wine colour rather than the monomeric anthocyanins. The polymers are more stable against further oxidation, so the aging process and the colour change to brown-red is slowing down after a few years of aging. (Zoecklein et al. 1994).

Main chemical processes that induce colour changes in red wines are driven by oxygen:

1. Degradation of anthocyans. Malvidine-3-glucoside and Cyanidine-3-glucoside in contact with oxygen tend to lose their colour intensity (Lopes et al. 2007; Ribéreau-Gayon et al. 2000).
2. Polymerisation of anthocyans:
    1) Direct condensation between anthocyans and tannins in presence of oxygen (Remy et al. 2000). Resulting colourless flavenes can be oxidised to flavylium ions and further to yellow xanthylium salts (Es-Safi et al. 2000).
    2) Reactions between anthocyans and flavanols involving acetaldehyde, to give a resulting product that has a colour from violet-red to orange (Gómez-Plaza and Cano-López 2011).
    3) Formation of pyranoanthocyanins through the reaction between anthocyans and other compounds, such as pyruvic acid, vinylphenols and vinylflavanols (Du Toit et al. 2006; Gómez-Plaza and Cano-López 2011).

Influence of oxygen on red wine colour will depend on the quantity of oxygen and on the storage conditions. Formation of higher weight molecular compounds during microoxygenation stabilised the colour to SO_2 bleaching of Cabernet and Merlot wines (Rayne 2007). Wirth et al. (2010) observed, that wines stored with higher oxygen transmission rate showed higher colour intensity and higher levels of sulphite bleaching resistant pigments due to increased conversion of anthocyans to derived pigments such as pyranoanthocyanins. Caillé et al. (2010) reported intense orange hue in Grenache wines exposed to microoxygenation. The authors also observed darker colour in wines sealed under closures with higher oxygen transmission rate.

4.3. TASTE

Unripe tannins in young red wines might be perceived as harsh, bitter and very astringent (Durner, Weber, et al. 2010). The flavanol fraction of phenolic compound was reported to be responsible for these tastes (Du Toit et al. 2006). However, during wine aging, due to flavanol polymerisation, they become less reactive towards mouth proteins, resulting in wine perceived as less astringent (Nikfardjam and Dykes 2003). For this reason, red wines are usually aged in barrels before bottling (Boulton et al. 1998).

Recent studies also showed favourable effects of micro-oxygenation techniques. Micro-oxygenation (20 and 100 mg/L/month) before malolactic fermentation of Pinot Noir was shown to have positive effect on the tactile impression of wines by softening green tannins (Durner, Weber, et al. 2010). Similar influence was observed by Parpinello et al. (2012) in Cabernet Sauvignon wines (25 and 50 mL O_2/L/month). Under the influence of small quantities of oxygen soy and tobacco flavours are developed (Bertuccioli et al. 2001).

4.4. MICROBIOLOGICAL STABILITY

Oxygen is an important compound required to ensure sufficient *Saccharomyces* yeast growth, for proline degradation, and to avoid sluggish fermentations (Ingledew and Kunkee 1985; Ingledew et al. 1987). Its impact on the development of the *non-Saccharomyces* yeasts has also been shown recently (Holm Hansen et al. 2001).

There are several studies pointing out, that acetic acid bacteria survives under the relative anaerobic condition often found in wine going into a viable, but non-cultural state (Du Toit et al. 2005). Even small amounts of oxygen can lead to the significant increase in acetic acid bacteria and be the reason for wine spoilage. In case of saturation with oxygen the number of the microorganisms grows from 10^4–10^5 up to 10^8 cells/mL in several days (Du Toit and Lambrechts 2002).

As well as acetic acid bacteria, oxygen was found to make an influence on the rapid growth of *Brettanomyces* which are yeasts able to spoil wine by production of volatile phenols (Du Toit et al. 2005). These volatile molecules have unpleasant flavours of horse sweat and leather.

5. METHODS TO AVOID WINE OXIDATION

5.1. PROTECTIVE WINEMAKING

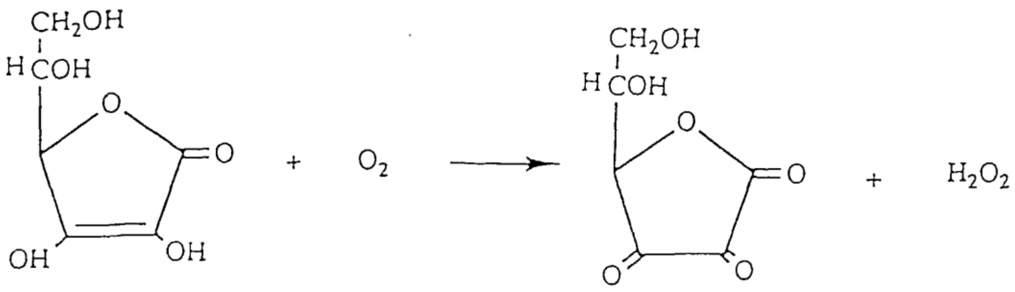
Protective winemaking strategy implements the wine protection techniques starting from grape picking until bottling and storage. It means to lower concentration of oxygen to the lowest point possible during movement or fining of delicate wines. Reductive winemaking techniques are normally used for white wine production. Marais (1998) reported positive effects of reductive processing on aroma intensities of Sauvignon blanc wines. Transfer lines, pumps, and receiving tanks are purged with inert gasses, such as nitrogen, carbon dioxide and argon, before use (Cheynier et al. 1993; Karbowiak et al. 2009). Some racking devices use nitrogen gas to pressurize the headspace above the wine to ensure protective wine transfer from barrel to barrel without pumps (Zoecklein et al. 1994). Minimisation of oxygen exposure is often coupled with the use of some chemical antioxidants (Du Toit et al. 2006).

5.2. *USE OF ANTIOXIDANTS*

Ascorbic acid

Ascorbic acid, also known as vitamin C is present in grapes, ranging from 5 to 150 mg/kg (Amerine and Ough 1974). The main antioxidant action of ascorbic acid in wine is due to the reaction with molecular oxygen. Ascorbic acid in wine undergoes oxidation to dehydroascorbic acid with the loss of two hydrogen atoms in the presence of flavones oxides. The latter is derived from oxidation of flavonoids in solutions (Zoecklein et al. 1994). It was also claimed that ascorbic acid may reduce quinones to the original state (Boulton et al. 1998), but the study of Makhotkina and Kilmartin (2009) put this in question.

FIGURE 5: OXIDATION OF ASCORBIC ACID



Although ascorbic acid is acting as an antioxidant, it may also catalyse oxidation of some wine constituents. The reaction of ascorbic acid with oxygen generates H_2O_2, which can, through oxidation with ethanol, produce acetaldehyde. The latter binds with free sulphur dioxide, making it unavailable. Thus, ascorbic acid in winemaking is generally used with sulphur dioxide (Bradshaw et al. 2011).

Ascorbic acid may also contribute to browning of white wine. Barril et al. (2008) observed intensive pigment formation in the tartaric acid based model system containing (+)-catechin and ascorbic acid. Peng et al. (1998) and Skouroumounis et al. (2005) also reported enhanced colouration in white wines containing ascorbic acid.

Sulphur dioxide

Sulphur dioxide, often abbreviated to sulphite or SO_2, is one of the major preservatives used in food industry. Sulphur dioxide and its ionic forms (bisulphite HSO_3^- or sulphite SO_3^{2-}) were reported to have various functions in wine, such as inhibition of enzymatic and non-enzymatic oxidation by reducing hydrogen peroxide or quinones, and inhibition of the growth of microorganisms (Margalit and Crum 2004). Addition of sulfur dioxide is an essential treatment in winemaking technology due to its antioxidant and antiseptic properties (Pozo-Bayon et al. 2012; Ribereau-Gayon et al. 2006). SO_2 plays an important role in scavenging H_2O_2 (Danilewicz 2003; Danilewicz 2007; Danilewicz 2011) and protecting odorous thiol compounds from oxidation (Blanchard et al. 2004). SO_2 can also react with unpleasant aromatic carbonyl compounds forming odourless bisulfite adducts (Singleton 1987).

Due to different chemical transformations that occur to sulphur dioxide in wine, its interaction as an antioxidant is not completely clear. Some researches claim that sulphur dioxide reacts directly with oxygen, and protects other components from oxidation (Clarke and Bakker 2004). However, some recent studies show that in oxygen presence sulphur dioxide can also provoke oxidation, which is prevented by polyphenols (Danilewicz 2007; Danilewicz 2011). According to stoichiometric calculation, one mg of oxygen consumes 4 mg of sulphur dioxide (Fabre 1994).

According to the recent studies, sulfites resulting from the addition of the SO_2 were related to allergic reactions by some consumers. The range of symptoms includes dermatitis, angioedema, abdominal pain, diarrhea, anaphylaxis and breathing difficulties (Vally et al. 2009; Vally and Thompson 2001; Vally and Thompson 2003). Various chemical (dimethyl dicarbonate, bacteriocines, lysozyme, colloidal silver complex) and physical (pulsed electric fields, ultrasounds, ultraviolet, high pressure) methods have been proposed recently as an alternative to sulfur dioxide in winemaking

(Izquierdo-Cañas et al. 2012; Santos et al. 2011). However, in spite of the promising results, up to now no other technique was able to replace completely the properties of SO_2.

Other antioxidants

As discussed previously, grape phenolic compounds play a role of natural antioxidants (Zoecklein et al. 1994). Control of oxygen exposure during winemaking operations may significantly help to increase the quality and shelf-life of bottled wines (Dimkou et al. 2011; Dimkou et al. 2013). Moreover, addition of enological tannins (Sonni et al. 2009), caffeic acid (Roussis et al. 2007) and glutathione (Bouzanquet et al. 2012; Roussis et al. 2012) were reported to be useful additives to prevent wine oxidation and have a potential to decrease the use sulfur dioxide concentration in wines.

6. AIMS OF STUDY

Oxidation is generally a complex process involving multiple chemical agents. Due to heterogeneous matrix effects, studies in wine even more challenging. Moreover, the effects of wine contact with oxygen may vary depending on grape variety, winemaking techniques and other factors. Thus, most of studies on the topic are focusing on interaction of chosen phenolic compounds (typically cathechine or epicathechine) with oxygen and/or metals and/or antioxidants in model solutions. Although these studies deliver valuable information about oxidation mechanisms, there is, however, limited data published on real wine experiments linking analytical data and sensory analysis. Many previous researchers were focusing on collecting oxygen uptake data during various winemaking operations (Castellari et al. 2004; Morozova 2009; Vidal et al. 2003; Vidal et al. 2004; Vidal et al. 2001). However, until now not much input has been made on the analysis of further impact of the measured amounts of oxygen on wine quality during storage.

Consequently, the main objective of this thesis was to study the impact of different oxygen additions and other factors such as temperature, sulphur dioxide, metals and ascorbic acid on white wine quality post-bottling. The goal of the study was to analyse analytical and sensory changes in wines during storage, and to develop an optimal model for gas management specifically for Riesling wines, which is one of the most important grape varieties in Germany. All unit operations were conducted in the experimental cellar of the State Institute for Viticulture, Oenology and Fruit Technology Weinsberg using common winemaking equipment. Thus, comprehensive data required for quality improvement of wine was obtained.

CHAPTER I deals with the development of a reliable and accurate measurement method for quantification of dissolved oxygen and carbon dioxide in wines. During this experiment, three different measurement techniques developed recently for O_2 and CO_2 were tested and the protocol of measurement procedure and data calculation was further used in the experiments described in CHAPTERS II-IV.

Intensive research on the effect of iron and copper addition on oxidation in wine-like model solutions has been conducted recently on laboratory scale (Danilewicz 2003; Danilewicz 2007; Danilewicz 2011; Danilewicz et al. 2008). Additional research linking model solutions experiments and the analytical and sensory analysis of real wine systems is required for further optimisation of oxygen management post-bottling. Although it is generally accepted that in an ideal case of a metal-free wine system the oxidation of phenolic compounds is unlikely to occur (Danilewicz et al. 2008), this effect should be confirmed in real wine (CHAPTER II). Furthermore, the interaction of oxygen, sulphur dioxide and average concentrations of iron and copper for Germany were investigated (CHAPTER III). A particular emphasis should be put on linking the analytical data to the advanced sensory evaluation with a panel of trained judges.

Sulphur dioxide and ascorbic acid remain the most widely used antioxidants among wine producers. In CHAPTER IV the effects of the sulphur dioxide and ascorbic acid application were examined in combination with different oxygen additions. Frequent sampling over storage time ensured significant data on the changes in monitored analytical parameters. In this chapter, again primarily attention was paid to linking the analytical changes to sensory quality of wines.

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CHAPTER I

Comparison of different methods for O_2 and CO_2 Measurement in Wine

ABSTRACT

The aim of this study was to compare several instruments for carbon dioxide and oxygen measurement in wine. For carbon dioxide determination, CarboQC, Orbisphere 3658 and agitation cylinder were used. Oxygen measurements were performed with PreSens, OxyQC and Orbisphere 3650. Sample bottles, equally treated to have the same carbon dioxide or oxygen content, were used for the measurements. Measurements were undertaken in the range from 0 to 2200 mg/L for carbon dioxide, and from 0 to 12 mg/L for dissolved oxygen. Correlation graphs and regression coefficients demonstrated that CarboQC and Orbisphere 3658 were effective for carbon dioxide determination in the whole measurement range. Agitation cylinder can also be used for measurements at normal atmospheric pressure. Oxygen measurement with PreSens, OxyQC and Orbisphere 3650 showed good reliable results. Data obtained with each of three instruments demonstrated good correlation with the initial oxygen amounts in wine samples. Consequently, any of the investigated devices may be used for dissolved oxygen measurement in wine. However, further research should is required taking in consideration limitations of the proposed method for sample preparation. Counter pressure filler is recommended for bottling of wine samples with carbon dioxide and oxygen concentrations exceeding the saturation level.

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KEYWORDS

Oxygen, carbon dioxide, wine, gases, analytical methods, luminescence, sensors

1. INTRODUCTION

Among all gases that can be dissolved in wine, oxygen and carbon dioxide can be considered the most important. Although oxygen has a much lower solubility in wine than carbon dioxide, it can be of a significant importance. Oxygen has to be considered as a very reactive chemical agent having potential to alter wine by oxidation. Different oxygen levels can have a great influence on colour, aroma, flavour and general perception of wine. The total capacity of oxygen absorption is 80 mg/L for white wines and 800 mg/L reds (Moutounet and Mazauric 2001).

The saturation value for oxygen at atmospheric pressure in water at 20°C is 9.1 mg/L (Margalit and Crum 2004). In wine these values are about 20% lower. At 20°C and normal pressure oxygen can be dissolved in wine only up to 8.8 mg/L (Troost 1988). However, at higher pressure and lower temperature more oxygen can be dissolved (Moutounet and Mazauric 2001; Schmidt and Waidelich 2008). Optimal concentrations can alter enormously for different wine styles.

In contrast to oxygen carbon dioxide has much higher solubility but a lower reactivity with wine constituents (Table 2).

TABLE 2: SOLUBILITY OF SELECTED GASES IN WATER AT 293.15 K AND PARTIAL PRESSURE 101.325 KPA (HAYNES AND LIDE 2010):

Gas	**Solubility, mole**
Nitrogen (N_2)	1.274×10^{-5}
Oxygen (O_2)	2.501×10^{-5}
Carbon dioxide (CO_2)	7.07×10^{-4}

Carbon dioxide concentration has a great impact on physical properties and sensory perception of wines. The solubility of carbon dioxide in liquids is dependent on the temperature. Dissolved carbon dioxide is reversely converted with water into carbonic acid (H_2CO_3). Carbonic acid deprotonates into bicarbonate (HCO_3^-) and carbonate ($CO3^{2-}$). The relative concentration of either CO_2, H_2CO_3, HCO_3^- or CO_3^{2-} depends on the pH of the solution. At the pH 3; which is common in wine, 99% of the carbon dioxide is present in the anhydride the gaseous form (Liger-Belair et al. 2006). The carbon dioxide solubility in wine will depend on alcohol and sugar content as well as on the total acidity and extract level.

Carbon dioxide influences the taste of beverages. It is also important for total pressure in a bottle. Carbon dioxide comes into wine from alcoholic and malolactic fermentation (El Haloui et al. 1988; Lonvaud-Funel 1995), post-fermentation processing or through direct enriched by purging into wine (Knoll 1990).

Solubility of carbon dioxide depends on temperature and it is inversely related to the concentration of alcohol. Other contributing factors include reducing sugar levels and viscosity arising from polysaccharides and phenols. At atmospheric pressure and 20°C solubility of carbon dioxide is 1500 mg/L (Zoecklein et al. 1994). Troost (1988) gives a value of 1460 mg/L.

Cellar operations also influence the concentration of carbon dioxide. Wine warm storage accelerates degassing. Racking, fining and filtration may further reduce concentration of carbon dioxide and at the same time lead to oxygen uptake (Vidal et al. 2003; Vidal et al. 2004; Vidal et al. 2001). CO_2 content can be precisely adjusted before bottling of wine using direct purging or a membrane contactor (Blank 2010).

Carbon dioxide can provide tactile sensation, magnify the sense of acidity, and enhance odour intensity. Carbon dioxide is perceptible in water at 200 mg/L, and in wine about 500 mg/L (Peynaud et al. 1996). Carbon dioxide enhances the sense of acidity, thus reinforcing tannin and bitter elements and reducing the sense of sweetness (Zoecklein et al. 1994). Optimal carbon dioxide content recommended in the literature (Blankenhorn 2002): 0.4-0.8 g/L for red wine; 0.7-1.5 g/L for white wine and 0.7-1.2 g/L for rosé wine.

Since both oxygen and carbon dioxide play an important role in winemaking, accurate measurement techniques should allow monitoring of the gases during all winemaking steps.

Available methods for oxygen measurement:

- Azide-Winkler titration method (Winkler 1888). Accurate realisation of the titration method is difficult to perform. Any oxygen uptake or loss during laboratory manipulations influences the results. The medium should be free of any compounds that may oxidise the iodide.
- Potentiometric method based on voltage difference due to chemical reaction on one of the electrodes.
- Luminescence method based on changes in light absorption and reflection properties of oxygen sensitive material.

Measurement methods for dissolved carbon dioxide include:

- Titration method (OIV-MA-AS314-01). A wine sample is cooled to 0°C. After setting up the pH level at 10-11 with NaOH the titration with sulphuric acid solution is carried out (OIV 2008).
- Manometric method. Concentration of carbon dioxide is determined by measuring temperature and pressure in a bottle.
- Agitation method
- Conductivity method
- Optical method
- Method of multiple volume expansion

The main objective of the present study was to compare the performance of available in the market instruments for measurement of dissolved oxygen and carbon dioxide in wine.

2. MATERIALS

2.1. SAMPLING DEVICES

Sampling is crucial, while the wine can lose CO_2 or take up oxygen at this step. Thus, a special technique is helpful in order to transfer the samples from the container into the measurement device. Therefore we used two devices:

- Filling Device PFD (Anton Paar Company, Austria)
- Beverage Package Sampler Orbisphere 29971-71 (Hach Lange GmbH, Germany)

Both tools are designed to transfer liquid samples from a closed container directly into the measuring chamber of a measuring instrument. They can be used with cans, glass- and PET-bottles.

2.2. MEASUREMENT DEVICES FOR CARBON DIOXIDE MEASUREMENT

CarboQC

CarboQC (Anton Paar, Austria) is a CO_2 measuring device based on method of multiple volume expansion. Technical data given by the manufacturer is presented in Table 3:

TABLE 3: TECHNICAL SPECIFICATIONS OF CARBOQC

Measurement Range	0 to 12 g/L (0 to 6 Vol.)
Temperature	-3 to 30 °C
Pressure	0 to 10 bar absolute
Repeatability	0.05 g/L (0.025 Vol.)

Orbisphere 3658

Orbisphere 3658 (Hach Lange GmbH, Germany) is an instrument which determines CO_2 concentration in liquids using conductivity method. Technical data was provided by the producer (Table 4).

TABLE 4: TECHNICAL SPECIFICATIONS OF ORBISPHERE 3658

Measurement Range	0-10 g/kg, 0-6 bar
Temperature	-5 to 35 °C
Pressure	0 to 10 bar absolute
Membrane	Hydrophobic membrane 29561A
Response time	1 min
Accuracy within ± 5 °C of calibration temperature	The greater of: ± 1% of reading; ± 0.025 g/kg or ±16 mbar

Agitation cylinder

The agitation cylinder (Schliessmann, Germany) is a simple and cheap instrument for the determination of carbon dioxide according to the method of Veitshöchheim (Jakob et al. 1997). The method is only applicable to non-carbonated beverages, such as still wine. The analysis is done manually. The whole equipment needed is a 100 mL volumetric flask with grounded neck, a plastic plug, a pipe airtight mounted in the plug, pinchcock and Teflon tip. Due to the low costs and the simple operation the agitation cylinder is widely used in the wine industry. Technical data is given in Table 5. For measurement a thermometer and conversion tables are required.

TABLE 5: TECHNICAL SPECIFICATIONS OF AGITATION CYLINDER

Measurement Range	100 to 42 mL of initial sample volume
Temperature	0 to 20 °C
Recommended pressure	0 to 1 bar
Sample volume	100 mL

2.3. *MEASUREMENT DEVICES FOR OXYGEN MEASUREMENT*

PreSens

The PreSens (Precision Sensing GmbH, Germany) instrument is an oxygen measurement device based on luminescence method. In Germany it is provided by Nomacorc Company and is known as NomaSense™. The measuring device (Fibox 3) is a stand-alone single channel fibre optic oxygen meter operating with an excitation wavelength of 505 nm. The light is transferred by a polymer optical fibre of 2 mm diameter and is connected by an optical connector to the instrument. Technical data of the device are described in Table 6.

TABLE 6: TECHNICAL SPECIFICATIONS OF FIBOX 3 LCD INSTRUMENT

Analogue output (Oxygen)	0-20% air sat. ± 0.5% air sat.
Analogue output (Temperature)	0-50 °C ± 1.5 °C
Acquisition time	Programmable (default 1 second)

Oxygen-sensitive PSt3 mini sensors were used for oxygen measurement. The sensors (spots) are made of oxygen sensitive material and can be glued with silicone directly into bottles made of clear glass. The measurement is performed without opening the bottle. Important technical data is given in Table 7.

TABLE 7: TECHNICAL SPECIFICATIONS OF OXYGEN SENSOR TYPE PST3

	Dissolved Oxygen	Gaseous and Dissolved Oxygen
Measurement Range	0-22 ppm	0-500 hPa
Limit of Detection	15 ppb dissolved oxygen	0.31 hPa
Accuracy (20°C)	± 1% at 100% air-saturation; ± 0.15% at 1% air-saturation	

OxyQC

OxyQC (Anton Paar, Austria) is an oxygen measurement instrument which uses luminescence method for determination of oxygen. The difference from the PreSens device is that oxygen measurement takes place in the chamber inside the instrument, where diodes, a sensor and the photodetector are located. Technical data is given in Table 8.

TABLE 8: TECHNICAL SPECIFICATIONS OF OXYQC

Measurement range	0 ppm to 10 ppm O_2
Pressure	0.2 - 7 ± 0.5 bar
Temperature	0 to 30 °C
Repeatability	20 ppb
Response time	<40 sec

Hach Lange Orbisphere 3650

Orbisphere 3560 (Hach Lange GmbH, Germany) is a portable oxygen measurement device using polarographic method for measurement. It can be used for dissolved oxygen measurements in liquids. Orbisphere 3650 was equipped with a 25 µm 2956A membrane. The response time for 90% of the signal at 25 C is 7.2 seconds. Technical specifications of the device are given in Table 9 (manufacturer's data):

TABLE 9: TECHNICAL DATA OF ORBISPHERE 3650

Signal Drift	<0.5% of reading between service
Temperature	-5 to 60°C
Instrument Operating Limits	-5 to 100°C
Display Units	ppm/ppb (liquid)
Maximum Display Resolution	1 ppb 0.001 ppm

2.4. GASES

Compressed nitrogen (99.98% pure) and compressed air gas (78.9% nitrogen and 21.1% oxygen) in bottles were provided by Lange & Co (Heilbronn, Germany).

2.5. WINE

White wine was produced from Müller Thurgau grapes (harvest 2010) in the experimental cellar of the State Institute for Viticulture, Oenology and Fruit Technology Weinsberg using standard winemaking techniques. Oenological parameters measured before bottling are presented in Table 10:

TABLE 10: STANDARD ENOLOGICAL ANALYSIS FOR MÜLLER THURGAU WINE USED FOR THE STUDY

Alcohol, g/L	94.9
Glycerine, g/L	4.8
Sugar, g/L	1.4
Extract, g/L	17.5
Reduced extract, g/L	7.8
pH	3.28
Titratable acidity expressed in tartaric acid, mg/L	6.0
Tartaric acid, g/L	1.9
Volatile acids, g/L	0.32
Potassium, mg/L	750
Calcium, mg/L	51
Magnesia, mg/L	96
Free SO_2, mg/L	29
Total SO_2, mg/L	82
CO_2, g/L	0.97
E 420 nm/1cm	0.05
Total phenolics, mg/L	186

3. METHODS

3.1. *CARBON DIOXIDE MEASUREMENT*

CarboQC

The wine samples in bottles were transferred to the measuring instruments by using the PFD Filling Device coupled to nitrogen gas to push the wine-sample through the system. In the experiment CarboQC and OxyQC were connected in series. The sample is taken directly from the package. A bottle is placed into the chamber of the PFD Filling System. After closing the chamber, a lever on the Filling System is pushed down, which forces a needle inside the chamber to punch the screw cap on the bottle and get inside the liquid. Then, 100 mL of beverage is forced by 6 bar nitrogen pressure into a glass measuring chamber of CarboQC. High pressure is guaranteeing no loss of gases due to sample transfer. Once the sample is in the measurement chamber, the line is closed automatically. The sample is agitated in the chamber till equilibrium between gas and liquid phases is reached. After that, total pressure of gases (CO_2+other gases present in the liquid) and temperature are measured. Then the volume of measuring chamber is expanded a second time and further agitated. Each time the total system pressure and temperature is measured after agitation. Thus the measurements of pressure and temperature provide accurate information about the CO_2 content in the beverage as well as the concentration of other gases (predominantly oxygen and nitrogen). CarboQC was checked against carbon dioxide free beverage and with a beverage with certain carbon dioxide content.

Orbisphere 3658

The liquid that is going to be measured flows through Beverage Package Sampler Orbisphere 29971-71 under pressure of nitrogen gas (1.5-2 bar) through the device at certain speed (150 mL/min is recommended), passing a hydrophobic membrane, which separates liquid from a measuring chamber with a sensor. CO_2 passes through the membrane into the CO_2-free chamber. The measuring chamber is being constantly flushed with air. The sensor determines the level of thermal conductivity corresponding to air, which is taken as a standard for “non-carbon dioxide media”. Carbon dioxide diffusing through the membrane changes thermal conductivity proportionally to the partial pressure of CO_2.

Before starting the measurements, Orbisphere 3658 was calibrated with pure CO_2 at 1 bar, 1.5 bars and 2 bars pressure using absolute pressure manometer.

Agitation cylinder

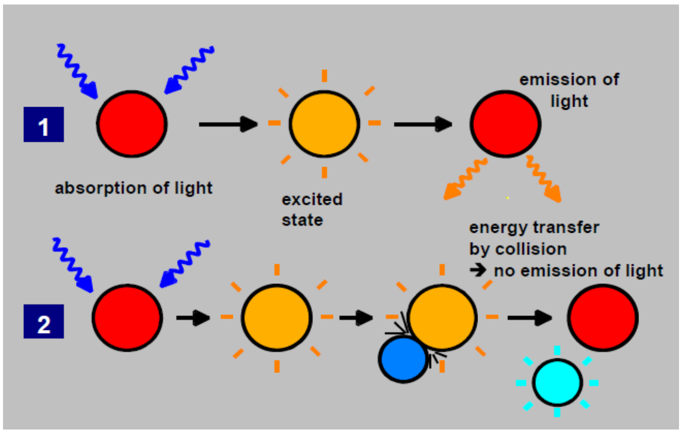
The agitation cylinder is a simple tool to manually analyse the CO_2 concentration of non-carbonated beverages. 100 mL of wine is transferred into a measuring cylinder. The transfer should be carried out very carefully in order to not lose carbon dioxide before measurement. The cylinder is closed with a closure equipped with a tube in the centre. The tube is closed with a clamp. After that the cylinder is shaken manually intensively and then stopped (CO_2 builds up a pressure). Then the clamp should be opened to let the liquid out. Shaking and opening is carried out until no more liquid is leaving the cylinder. The closure and the tube have to be removed and volume and temperature of remaining liquid is measured. The ejected volume is proportional to carbon dioxide concentration in beverage at a certain temperature. This residual volume in the cylinder is proportional to CO_2 concentration in g/L and temperature, and can be read directly from a table (Jakob et al. 1997).

3.2. OXYGEN MEASUREMENT

PreSens (NomaSense™)

The PreSens (NomaSense™) uses principle of luminescence quenching for determination of oxygen concentration. In the absence of oxygen (Figure 6.1) luminophore adsorbs light, and then emits luminescence. Oxygen deactivates luminophore (Figure 6.2) due to energy transfer, and the measurable luminescence decreases.

FIGURE 6: PRINCIPLE OF DYNAMIC QUENCHING OF LUMINESCENCE BY MOLECULAR OXYGEN (HUBER 2006)



A relation between luminescence intensity and oxygen concentration is described in Stern-Volmer equation (Figure 7):

FIGURE 7: STERN-VOLMER EQUATION

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{sv} * [O_2]$$

where: **I:** Luminescence intensity in presence of oxygen; **I_0:** Luminescence intensity in absence of oxygen; **τ:** Luminescence decay time in presence of oxygen; **τ_0**: Luminescence decay time in absence of oxygen; K_{SV}: Stern-Volmer constant (quantifies the quenching efficiency and therefore the sensitivity of the sensor); [O_2]: oxygen content.

Measuring system contains a light source (light emitting diodes - LED), a photodetector (photomultiplier - PMP), optical filters (OF), optical fibre as signal transmitter and an optical sensor.

Minisensors (oxygen sensitive spots) are calibrated by the manufacturer at certain pressure in an atmosphere with 0% and 100% of oxygen. After that, sensor spots are glued with silicon into white glass bottles. Accuracy of minisensors was checked in empty bottles at atmospheric air pressure. Then the test bottles have been filled with wine for measurement.

To determine oxygen content the fibre is pointed directly at the minisensor through the glass. Oxygen partial pressure in hPa can be read from the instrument and calculated in mg/L. Temperature compensation can be done automatically by the software, or taken into consideration at further calculations (Huber 2006).

Bottle preparation prior the test: Six clear glass 1L bottles were equipped with two mini sensors in each bottle: one for dissolved oxygen level close to the closure and the other one for dissolved oxygen concentration close to the bottom of the bottle. For the final calculations, the average value was used. All bottles were flushed with nitrogen gas prior to bottling to eliminate oxygen and carbon dioxide. Every oxygen sensor was checked in nitrogen atmosphere.

OxyQC

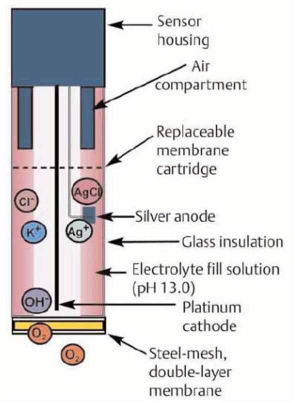
OxyQC also uses luminescence based method for oxygen measurement but is using sensors in the measuring device as described above. The oxygen measurement was carried out simultaneously with the carbon dioxide measurement. The bottles were measured directly using the PFD Filling Device which was connected in series to the

OxyQC and the CarboQC instruments. The injected gas to transfer the wine sample to the measuring instruments was pure nitrogen gas. The OxyQC and the CarboQC were switched on for twenty minutes until the first measurement was taken in order to stabilize the signal inside the measuring chamber. Accuracy of the OxyQC can be tested with air, pure nitrogen gas and Na_2SO_3 solution. In the experiment the accuracy of the instrument was tested with Na_2SO_3 solution and checked against the corresponding table provided by the manufacturer.

Orbisphere 3650

Orbisphere 3650 uses electrochemical method of oxygen measurement was, developed by Clark (Figure 8). The apparatus consists of a gold cathode, linked by potassium chloride gel. They are separated from the medium by a membrane selectively permeable to oxygen. The difference of potential established between two electrodes is modified by oxygen, circulating through the membrane (Ribereau-Gayon et al. 2006).

FIGURE 8: POLAROGRAPHIC METHOD OF OXYGEN MEASUREMENT WITH ORBISPHERE 3560



Cathode : $O_2 + 2H_2O + 4e^-$ → $4OH^-$

Anode : $Ag + KCl$ → $AgCl + e^- + K^+$

Before starting the measurement, Orbisphere 3650 was calibrated with air and adjusted according to the oxygen content in air at the measured temperature and atmospheric pressure at the present moment. Then, Orbisphere 3650 was connected to the Beverage Package Sampler Orbisphere 29971-71 (Hach Lange GmbH, Germany) and to Orbisphere 3658. After a bottle is placed in the Beverage Sampler, the gasket seals the bottle and then a hollow needle breaks the screw cap and gets into the wine. The wine is forced by nitrogen gas pressure (1.5-2 bars) through both devices. Measurement is fixed as soon as the value was stable.

3.3. SAMPLE PREPARATION

In order to obtain wine samples with different concentrations of oxygen or carbon dioxide stainless steel beverage kegs with 20 L volume were used. 20 L Kegs were filled with 10 L wine. The wine was degassed by nitrogen gas flushing for 1 hour by using a stainless frit. Wine temperature was 15°C.

After degassing the wine the headspace of the kegs was pressurized either with CO_2 or with compressed air. In both cases six different pressure levels have been applied: 0; 0.5 bar; 1 bar; 1.5 bar; 2 bar; 2.5 bar. The kegs were shaken for 10 minutes by constantly applying the adjusted pressure to allow the gases to dissolve and to reach gas equilibrium in the whole volume. Then six 1 L bottles were filled with wine from the keg one after another using a hose mounted to the keg and fed by the pressure in the keg. Then the bottles were sealed with screw caps immediately. For each pressure level two bottling sessions took place. Thus, 144 bottles were filled in total. All bottles were filled without headspace.

All bottles were shaken for five minutes and were left standing for five more minutes before measurement to reach gas equilibrium in the whole volume. After that the bottles were measured with all instruments according to the experimental protocol

(Table 11). After measurements with OxyQC and CarboQC, and after measurements with Orbispheres screw caps on bottles were destroyed in the process of sample taking and were changed for new ones. Nitrogen was used to force the wine through the measurement devices.

TABLE 11: ORDER OF MEASUREMENTS AND REQUIRED VOLUME OF WINE

1	PreSens	Non-invasive
2	OxyQC and CarboQC	~100 mL
3	Orbisphere 3650 and 3658	300-500 mL until stable value was reached
4	Cylinder	100 mL

3.4. STATISTICAL EVALUATION

Correlation tests and regression analysis was conducted with XLSTAT Software, version 2011.2.08 (Addinsoft, Germany).

4. RESULTS AND DISCUSSION

4.1. CARBON DIOXIDE

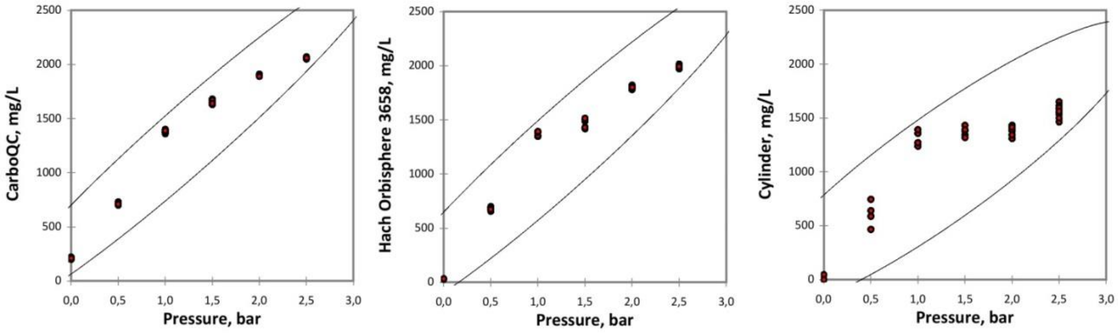
Results of CO_2 measurements with CarboQC, Orbisphere 3658 and agitation cylinder are shown in Table 12. Ideally the pressure of CO_2 applied in the keg should be equal to the pressure of CO_2, measured in wine after bottling. But in the experiment carbon dioxide was partially lost during bottling operation of wine oversaturated with CO_2 (1.5, 2.0 and 2.5 bar CO_2-pressure). These losses happen during the bottling operation and are unavoidable under normal pressure bottling. They only can be avoided by using counter pressure filler systems. However, all bottles were bottled equally, so the error due to carbon dioxide loss due to bottling can be considered equal for all samples. In addition to the loss due to the bottling we found a second CO_2 loss by the measuring technique.

TABLE 12: AVERAGE CARBON DIOXIDE CONTENT VALUES AND STANDARD DEVIATION, BASED ON MEASUREMENTS OF 12 SAMPLE BOTTLES:

CO_2 pressure, bar	Instrument	Mean CO_2, mg/L (n=12)	σ
0	CarboQC	208	6.2
	Hach Orbisphere	32	1.5
	Agitation Cylinder	8	17.5
0.5	CarboQC	718	12.2
	Hach Orbisphere	683	13.0
	Agitation Cylinder	612	75.5
1	CarboQC	1382	15.3
	Hach Orbisphere	1369	20.6
	Agitation Cylinder	1295	52.3
1.5	CarboQC	1655	20.2
	Hach Orbisphere	1465	43.8
	Agitation Cylinder	1368	32.9
2	CarboQC	1899	7.9
	Hach Orbisphere	1797	11.3
	Agitation Cylinder	1387	38.8
2.5	CarboQC	2063	6.5
	Hach Orbisphere	1988	14.0
	Agitation Cylinder	1571	55.6

There is an obvious loss of CO_2 using the agitation cylinder in wines with a CO_2 concentration higher than about 1400 mg/L. This can be explained by the sample preparation in the open volumetric flask. During filling and adjusting the volume already a loss of CO_2 takes place. In contrast to this, both other analytical methods show higher values. This can be explained by the fact, that the wine was transferred within closed systems into the measuring devices without any loss during measuring. The correlation of carbon dioxide concentrations (mg/L) measured with CarboQC, Orbisphere 3658 and Agitation Cylinder is shown in Figure 9. The regression coefficient in the full pressure range up to 2.5 bars for the agitation cylinder is at 0.811. The sealed systems CarboQC and Orbisphere 3658 have considerably higher regression coefficients of 0.941 and 0.922. Thus the results clearly confirm the earlier statement, that the agitation cylinder cannot be used for carbonated wine.

FIGURE 9: CORRELATION OF CARBON DIOXIDE CONCENTRATIONS (MG/L) MEASURED WITH CARBOQC, ORBISPHERE 3658 AND AGITATION CYLINDER IN RANGE OF CO_2 PRESSURE FROM 0 TO 2.5 BARS



In order to evaluate the CO_2 measuring methods for non-carbonated beverages, we graphed obtained data only for the CO_2 pressure up to 1.0 bar. Figure 10 shows measurement results only in the range from 0 to 1 bar CO_2 pressure applied to the kegs prior to bottling. Regression coefficients corresponding to this interval and to the whole data are given in Table 13. Results obtained with CarboQC and Orbisphere

3658 showed good correlation with the whole pressure range in keg. The agitation cylinder only can reach a satisfying regression coefficient in the interval from 0 to 1 bar CO_2 overpressure in the keg. At higher pressures the measurement with this instrument is not accurate. It can be explained with a fact that at dissolved carbon dioxide is lost during cylinder filling. However, at normal atmospheric pressure measurements with agitation cylinder were very accurate and comparable to the other techniques.

FIGURE 10: CORRELATION OF CARBON DIOXIDE CONCENTRATIONS (MG/L) MEASURED WITH CARBOQC, ORBISPHERE 3658 AND AGITATION CYLINDER IN RANGE OF CO_2 PRESSURE FROM 0 TO 1 BAR

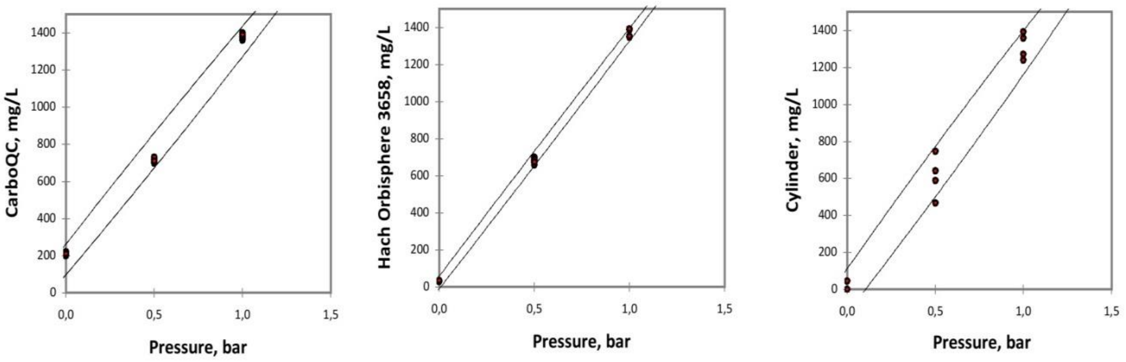


TABLE 13: REGRESSION COEFFICIENTS (R^2) FOR CO_2 MEASUREMENTS

Interval	CarboQC	Orbisphere 3658	Cylinder
0-2.5 bar	0.941	0.922	0.811
0-1.0 bar	0.994	0.999	0.989

To compare the measurement systems against each other the correlation between values obtained with the instruments were calculated for the whole CO_2 pressure range from 0 to 2.5 bar (Figure 11 and Table 14). Values obtained with CarboQC and Orbisphere 3658 strongly correlate (R^2=0.99). Regression coefficients of data obtained with these both devices against agitation cylinder are lower, although still at significant level.

FIGURE 11: CORRELATION OF CARBON DIOXIDEMEASUREMENTS WITH CARBOQC, ORBISPHERE 3658 AND CYLINDER

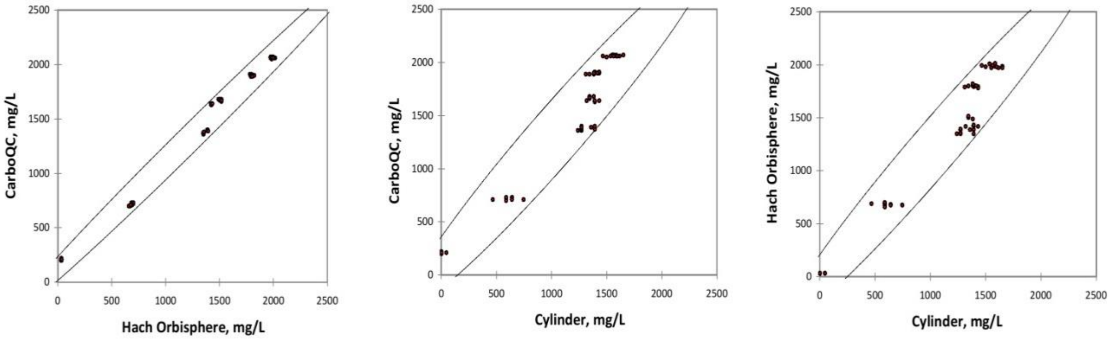


TABLE 14: REGRESSION COEFFICIENTS (R^2) FOR MEASUREMENTS TAKEN WITH THREE DIFFERENT INSTRUMENTS AGAINST ONE ANOTHER

Variables	CarboQC	Orbisphere 3658	Cylinder
CarboQC	1	0.99	0.946
Orbisphere 3658	0.99	1	0.957
Cylinder	0.946	0.957	1

4.2. OXYGEN

The oxygen concentration in the keg was adjusted with different levels of compressed air applied to the partially filled kegs. Oxygen content in air is 20.9%, so the partial pressure of oxygen is also 20.9% from the whole pressure in the keg (e.g. 1 bar will give the oxygen pressure of 209 hPa). Measurements obtained with PreSens and Orbisphere 3650 were read in hPa (partial pressure of oxygen) and then calculated in mg/L oxygen. It is not possible to get values in hPa with OxyQC. But as the instrument uses the same measurement principle and formulas as PreSens, measurements can be taken directly in mg/L and be comparable with data obtained from the other two devices.

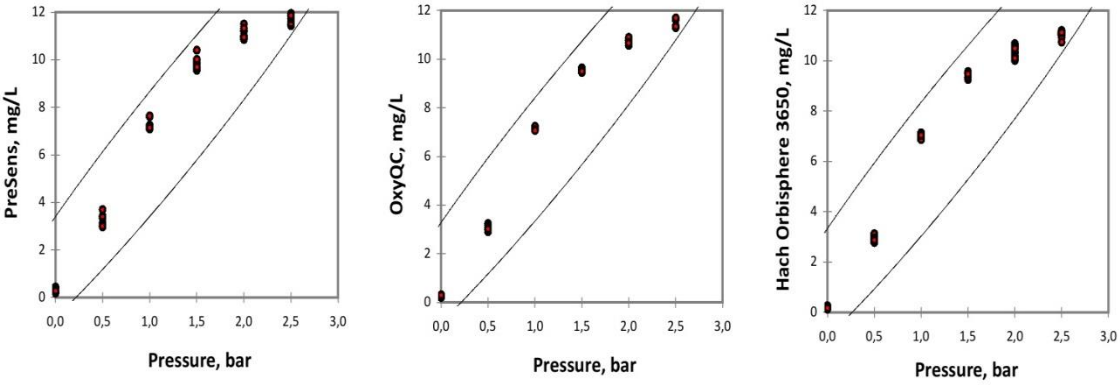
The results of the oxygen measurement and standard deviation are shown in Table 15. Results obtained with all three instruments are very close. Obviously the results of the oxygen measurement show less deviation compared to the carbon dioxide measurement.

TABLE 15: AVERAGE OXYGEN CONTENT AND STANDARD DEVIATION BASED ON 12 MEASUREMENTS

Air pressure, bar	Instrument	Mean O_2, mg/L (n=12)	σ
0	PreSens	0.28	0.06
	OxyQC	0.28	0.85
	Hach Orbisphere	0.18	1.13
0,5	PreSens	3.24	0.23
	OxyQC	3.12	0.12
	Hach Orbisphere	2.97	0.13
1	PreSens	7.25	0.18
	OxyQC	7.15	0.07
	Hach Orbisphere	6.99	0.08
1,5	PreSens	9.81	0.41
	OxyQC	9.54	0.04
	Hach Orbisphere	9.39	0.03
2	PreSens	11.07	0.61
	OxyQC	10.71	0.47
	Hach Orbisphere	10.39	0.47
2,5	PreSens	11.74	0.36
	OxyQC	11.49	0.27
	Hach Orbisphere	11.05	0.36

The correlation of the applied pressure range in the keg from 0 to 2.5 bars and the measured oxygen concentration is shown in Figure 12. Similar to the carbon dioxide the simple bottling technique without counter-pressure filler at atmospheric pressure leads to gas loss in oversaturated wine samples.

FIGURE 12: CORRELATION OF THE O_2 MEASUREMENTS WITH OXYQC, ORBISPHERE 3650 AND PRESENS IN RANGE OF AIR PRESSURE FROM 0 TO 2.5 BARS



OxyQC and PreSens, though having the same measurement principle, have showed slightly different results. This is probably due to the fact that OxyQC has isolated measurement chamber. Orbisphere 3650 showed lower measurement values compared to PreSens and OxyQC; this can be explained as calibration inaccuracy or particularity of the method. It is possible that measurement time is not sufficient for the whole amount of oxygen to diffuse through the membrane and react on the cathode. Due to limited volume of a bottle, the volume taken for analysis with Orbisphere was not exceeding 500 mL, which could be not enough to get a stable value. Another explanation could be that some amount of oxygen may be lost during the sample taking. Nevertheless, the results obtained with Orbisphere 3650 showed good repeatability, thus it can be considered a reliable instrument for oxygen measurement.

Saturation concentration of oxygen in wine is close to 8.8 mg/L. Supersaturated media may lose dissolved gases, when the overpressure is lost. This might be the reason why after reaching the saturation value of 8 mg/L the oxygen concentration in sample bottles is no more strongly correlating with compressed air pressure. This can be proved by the Table 16 that shows regression coefficients for three instruments in the range from 0 to 2.5 bars pressure and in the range from 0 to 1 bar pressure.

TABLE 16: REGRESSION COEFFICIENTS (R^2) FOR THREE INSTRUMENTS AGAINST COMPRESSED AIR PRESSURE IN KEG

Interval	PreSens	OxyQC	Orbisphere 3650
0-2.5 bars	0.935	0.936	0.926
0-1 bar	0.989	0.989	0.988

The results indicate regression coefficients in the range of pressure, not exceeding the saturation pressure, are elevated. However, the filling method used during the experiment made it possible to reach oxygen concentrations much higher, then the saturation level (up to 12 mg/L). Figure 13 and Table 17 show correlation of oxygen measurement results obtained with three instruments. Graphically explained, that all devices showed good correlation with one another. This is also proved by regression coefficients.

FIGURE 13: CORRELATION OF RESULTS OBTAINED WITH PRESENS, OXYQC AND ORBISPHERE 3650

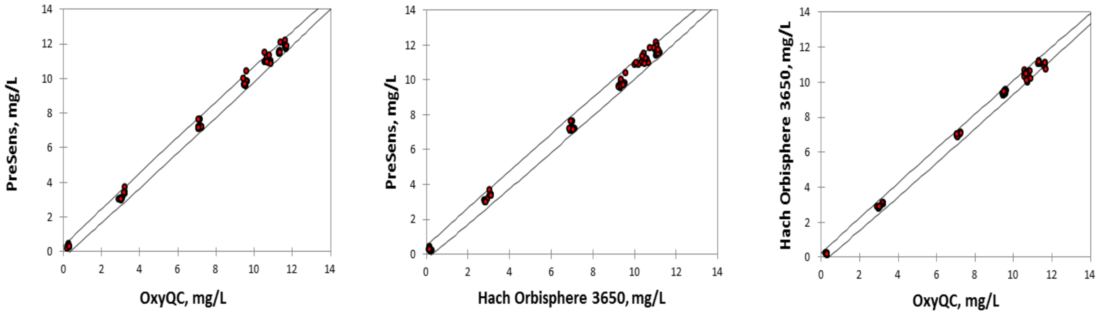


TABLE 17: REGRESSION COEFFICIENTS OBTAINED WITH THREE INSTRUMENTS AGAINST ONE ANOTHER

Variables	PreSens	OxyQC	Hach Orbisphere
PreSens	1	0.998	0.997
OxyQC	0.998	1	0.998
Hach Orbisphere	0.997	0.998	1

5. CONCLUSION

The study fulfilled its objective to compare available instruments for carbon dioxide and oxygen measurement in wine. For carbon dioxide determination CarboQC, Orbisphere 3568 and Agitation cylinder were used. Oxygen measurements were performed with PreSens, OxyQC and Orbisphere 3650. Sample bottles equally treated to have the same carbon dioxide or oxygen content were used for the measurements. Measurements were undertaken in the range from 0 to 2200 ml/L for carbon dioxide and in the range from 0 to 12 mg/L for oxygen.

Based on the data collected during carbon dioxide and oxygen measurement sessions, it was possible to build corresponding correlation graphics and to determine regression coefficients. It was shown that CarboQC and Orbisphere 3658 were effective for carbon dioxide measurement in the whole measurement range. Agitation cylinder can be also used for this purpose at pressure, not exceeding normal atmospheric pressure.

Oxygen measurement with PreSens, OxyQC and Orbisphere 3650 showed good reliable results. Results obtained with all three instruments showed good correlation against each other and the initial pressure. Consequently, any of these devices could be used for oxygen determination in wine.

However more research should be made taking in consideration limitations of the proposed method for sample preparation. Pressure filler should be used to obtain wine samples with gas concentration higher than saturation level.

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CHAPTER II

Effect of Headspace Volume and Iron and Copper Addition on Oxidation Processes in Model Solution and Riesling Wine: Chemical and Sensory Changes

ABSTRACT

Studies conducted on a model solution and a Riesling wine showed that oxygen concentration at the bottling had great impact on the evolution of the total consumed oxygen, free SO_2, and the colour of model solution and wine. In the model solution, the addition of 0.1 mg/L of iron and 0.05 mg/L of copper influenced the oxygen consumption. However, in the real wine 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, wine colour, wine browning

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1. INTRODUCTION

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). The metals belong to essential vine nutrients; they are absorbed by the root system (Zoecklein et al. 1994) and are transferred to the wine through grapes. Secondary uptake of iron and copper in the vineyard may be due to pollution, direct contact with soil or due to viticulture practices as the use of copper fungicides (Volpe et al. 2009) or pesticides (Galani-Nikolakaki et al. 2002; Kment et al. 2005) to prevent grapevine diseases.

High concentrations of heavy metals in the must are likely to be observed while processing 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 oenological 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 sulphate against H_2S off-odours may also cause excessive copper concentrations in wine (Schmidt 2001).

Concentration of 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 a metabolism catalyst and enzyme activators (Stafilov and Karadjova 2009). However, 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_2, but these reactions are thermodynamically unfavourable for oxygen as diradical (Danilewicz 2011). It is assumed that ferric iron (Fe^{3+}) 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^{3+} to Fe^{2+} (Danilewicz 2003; Danilewicz 2007; Danilewicz and Wallbridge 2010).

The oxidation reactions continue with further formation of a hydroperoxyl radical ($OOH\cdot$), which is a precursor for further reactive oxygen species (ROS) (Waterhouse and Laurie 2006). At wine pH and the presence of ferrous iron Fe^{3+}, the $OOH\cdot$ can react further to peroxyde anion and hydrogen peroxide (H_2O_2) (Danilewicz 2003; Waterhouse and Laurie 2006). In the presence of transition metals (Fe, Cu), another possible step is the formation of a hydroxyl radical ($OH\cdot$), a hydroxide ion (HO^-), and water from H_2O_2 via the Fenton reaction (Danilewicz 2003; Waterhouse and Laurie 2006), (Karbowiak et al. 2009). This $OH\cdot$ 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 were widely studied in wine-like model solutions in recent works (Danilewicz 2003; Danilewicz 2007; Danilewicz 2011). It was assumed that in absence of iron and copper the oxidation of the phenolic compounds will be minor. .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 in wine-like model solution and in real wine bottled with various headspace volumes.

2. MATERIALS AND METHODS

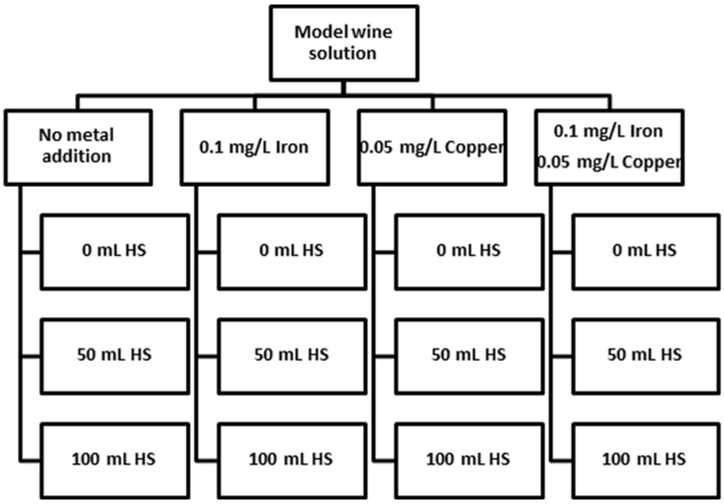
2.1. *CHEMICALS AND REAGENTS*

For the experiment the following chemicals were used: glycerol (85%, Merck, Darmstadt, Germany), (+)-tartaric acid (Merck, Darmstadt, Germany), gallic acid monohydrate (>98%, Merck, Darmstadt, Germany), potassium metabisulfite (Merck, Darmstadt, Germany), potassium ferrocyanide $K_4[Fe_2(CN)_6]$ (Begerow, Langenlonsheim, Germany), iron(II) sulphate (>99%, Merck, Darmstadt, Germany), copper(II) sulphate (>99%, Merck, Darmstadt, Germany), Titrisol 1g/L iron standard solution (FeCl3 in 15% HCl, Merck, Darmstadt, Germany), Titrisol 1g/L copper standard solution ($CuCl_2$ in H_2O, Merck, Darmstadt, Germany), LaCl3*7H2O for analysis (Merck, Darmstadt, Germany), hydrochloric acid for analysis (32%, Merck, Darmstadt, Germany), sodium hydroxide (p. a., Merck, Darmstadt, Germany). Water purification was performed using a Milli-Q™ purifier system (Millipore, Schwalbach, Germany).

2.2. *WINE-LIKE MODEL SOLUTION*

The wine-like model solution was prepared similarly to the one described in the previous work of Danilewicz (2007). For the experiment, 26 L of model solution consisting of deionized water (Millipore, Schwalbach, Germany), 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) sulphate, and copper(II) sulphate standard solutions were then added according to the scheme (Figure 14) 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.

FIGURE 14: SCHEME OF MODEL WINE EXPERIMENT AND METAL ADDITIONS



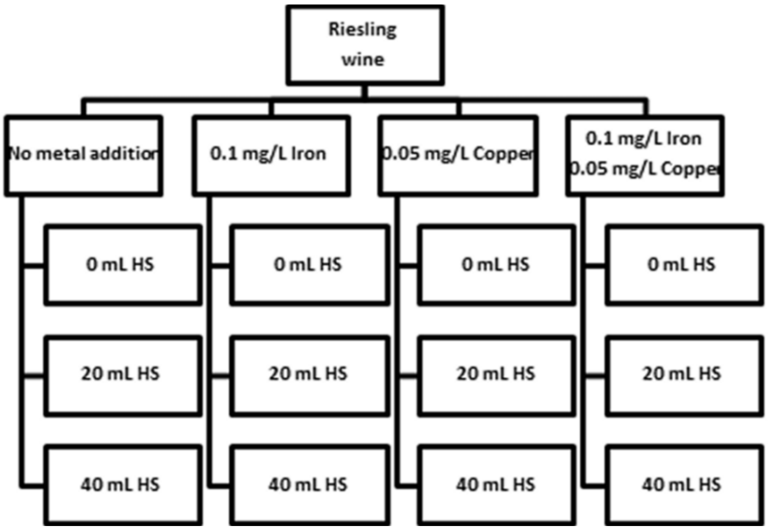
2.3. WINE EXPERIMENT

200 L of white wine from Riesling healthy hand-picked grapes (Württemberg region, vintage 2010) was produced and provided by experimental department of LVWO Weinsberg. Analytical parameters of the wine at bottling were as follows: alcohol: 12 % vol., acidity: 6.4 g/L, free SO_2: 40 mg/L, total SO_2: 100 mg/L, total phenolic content: 422 mg/L. Iron and copper content was additionally reduced with

potassium ferrocyanide (4 mg/L). This procedure is known in winemaking as “blue fining”.

Riesling wine samples were treated as the model wine solution (Figure 15), except for smaller headspace volumes. This was done to approach bottling oxygen levels commonly found in the industry. Racking and bottling were performed in 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). The bottling was performed with a manual 2-way filler system (Fjord, KHW Maschinenbau GmbH, Kaiserslautern), and the bottles were sterilized and purged with N_2 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.

FIGURE 15: SCHEME OF RIESLING WINE EXPERIMENT AND METAL ADDITIONS



2.4. 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 µL of concentrated HNO_3 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 18:

TABLE 18: 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 ω/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

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_3. Operating conditions and concentrations of calibration solutions are given in Table 19.

TABLE 19: 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 ω/min
Standard 1 (Fe)	10 mg/L
Standard 2 (Fe)	50 mg/L
Standard 3 (Fe)	250 mg/L

2.5. 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: 0-22 mg/L for dissolved oxygen and 0-500 hPa for gaseous and dissolved oxygen; response time ≤ 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 total consumed oxygen was calculated as a difference of the sum of dissolved and gaseous oxygen observed on the measurement and the sum of dissolved and gaseous oxygen observed on 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).

2.6. SULPHUR DIOXIDE MEASUREMENTS

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

2.7. COLOUR MEASUREMENTS

Colour analysis of wine and model solution was 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.

2.8. SENSORY EVALUATION

Triangle tests with 26 trained panellists were conducted in the tasting room of the LVWO Weinsberg equipped with FIZZ sensory software (Fizz Biosystemes, version 2.47) after 3 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 one different. 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 *w*as performed as described by Lawless and Heymann (2010). The 12 wines were evaluated by 2 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’, ‘oxidised’ 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. Latin square design was used to randomize the sample presentation across the panellists. The wines were served cold in clear Sensus wine tasting glasses (Zwiesel Kristallglas AG, Zwiesel, Germany). The panellists evaluated the wines and cleansed their palates with bread and bottled water.

2.9. 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, Germany).

3. RESULTS AND DISCUSSION

3.1. 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 20 and Table 21:

TABLE 20: 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

TABLE 21: 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

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.

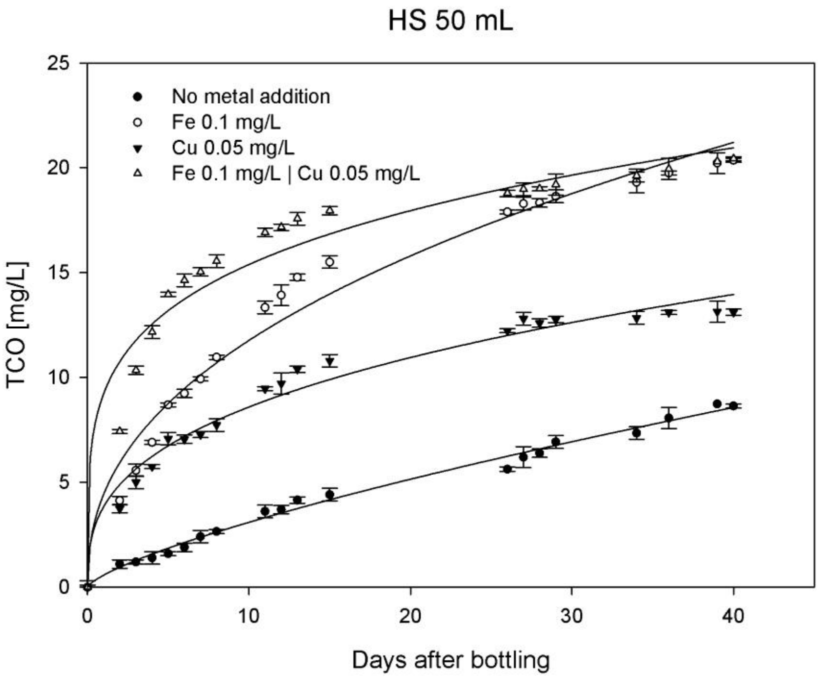
3.2. OXYGEN CONSUMPTION

Model solution

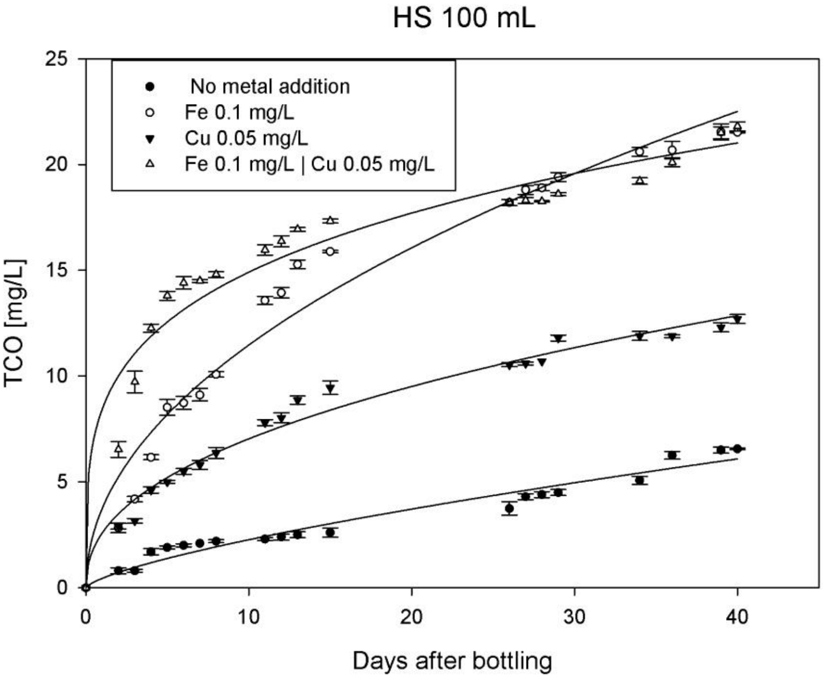
The examination of changes in total consumed oxygen in the model wine solution in six bottles (3 bottles per repetition) during 40 days after bottling presented in the Figure 16 indicates that the oxygen was consumed not linearly. Approximately 75% of the oxygen was consumed in the first 15 days. The oxygen consumption then slowed down, and continued until the whole amount was consumed.

FIGURE 16: EVOLUTION OF TOTAL CONSUMED OXYGEN IN MODEL WINE BOTTLED WITH HS 50 ML (3A) AND HS 100 ML (3B).

a



b



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 0.2-0.6 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.

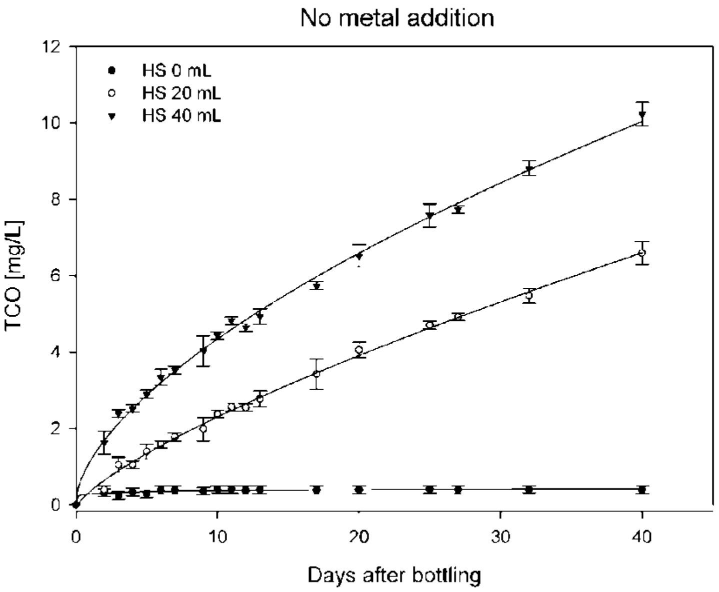
The wine samples with 50 mL (Figure 16a) and 100 mL HS (Figure 16b) and no metal addition showed a TCO increase between 5.5 and 8.6 mg/L. In presence of copper, the mean TCO level reached 11.7 mg/L and 13.1 mg/L for 50 mL HS and 100 mL HS, respectively. For the iron containing samples, the increase of 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

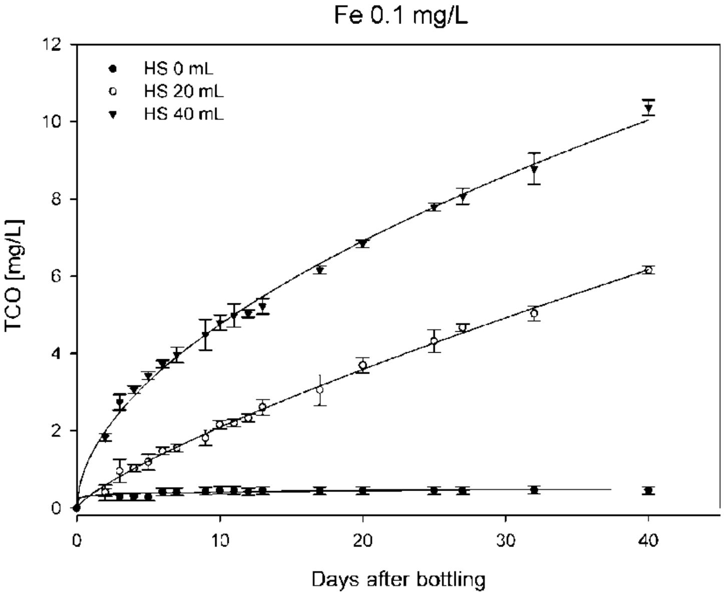
Changes in total consumed oxygen in real wine system are shown in Figure 17 (a-d). The results of the oxygen monitoring displayed clear differences according to the oxygen level at the bottling and of the HS, in particular. In the wines without HS, only 0.5-0.6 mg/L oxygen were consumed during the observation period. The consumed oxygen concentration of the wines with 20 mL HS reached 6.2-7.1 mg/L. The wines with 40 mL HS consumed 10.2-10.6 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 (Figure 16) could not be evidenced in the real wine system. No significant changes in total consumed oxygen caused by the metal addition were observed.

FIGURE 17 (A, B, C, D): TOTAL CONSUMED OXYGEN (TCO) EVOLUTION IN WINE DURING 40 DAYS STORAGE AT ROOM TEMPERATURE, MG/L

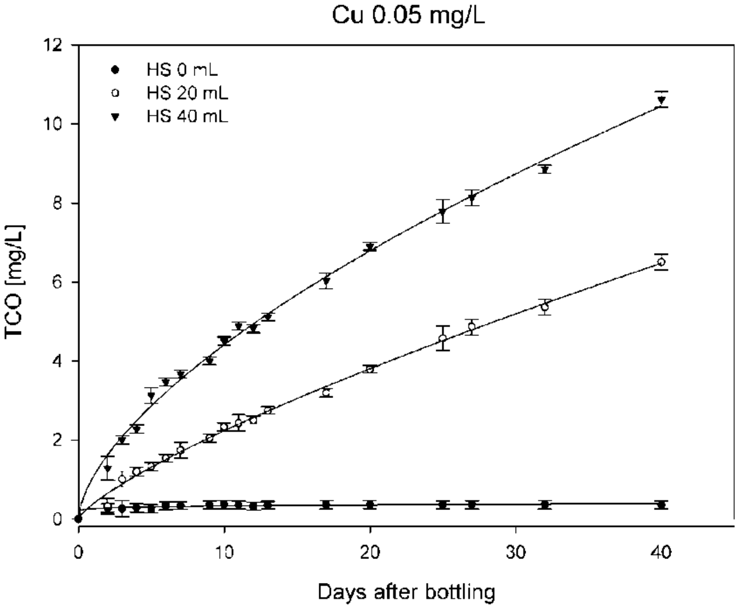
a



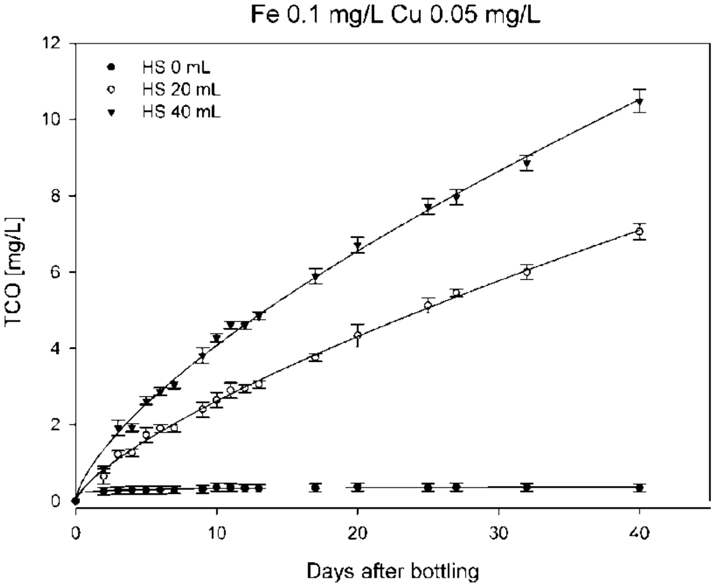
b



c



d



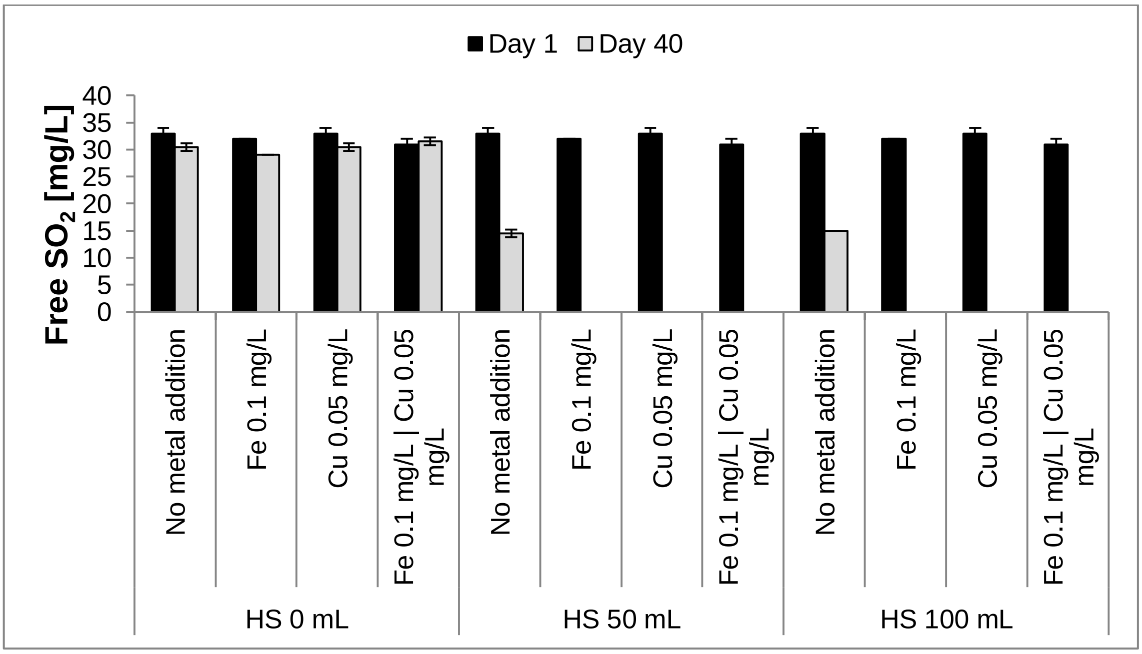
Total oxygen consumption in model wine-like solution and in real wine seems to have significant differences. In the model solution addition of iron and copper made great impact on the evolution of total consumed oxygen. In the real wine system little differences between the wines could be explained by 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).

3.3. SULPHUR DIOXIDE

Model solution

Figure 18 summarizes the impact of metal addition on free SO_2 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_2. All the other samples with addition of iron, copper or both metals showed no detectable free SO_2 after 40 days. The samples without HS (0 mL) retained about 29-32 mg/L of free SO_2 content, despite of the presence of iron and copper. Consequently, the results indicate that iron and copper addition catalyses free SO_2 degradation only when molecular oxygen is available.

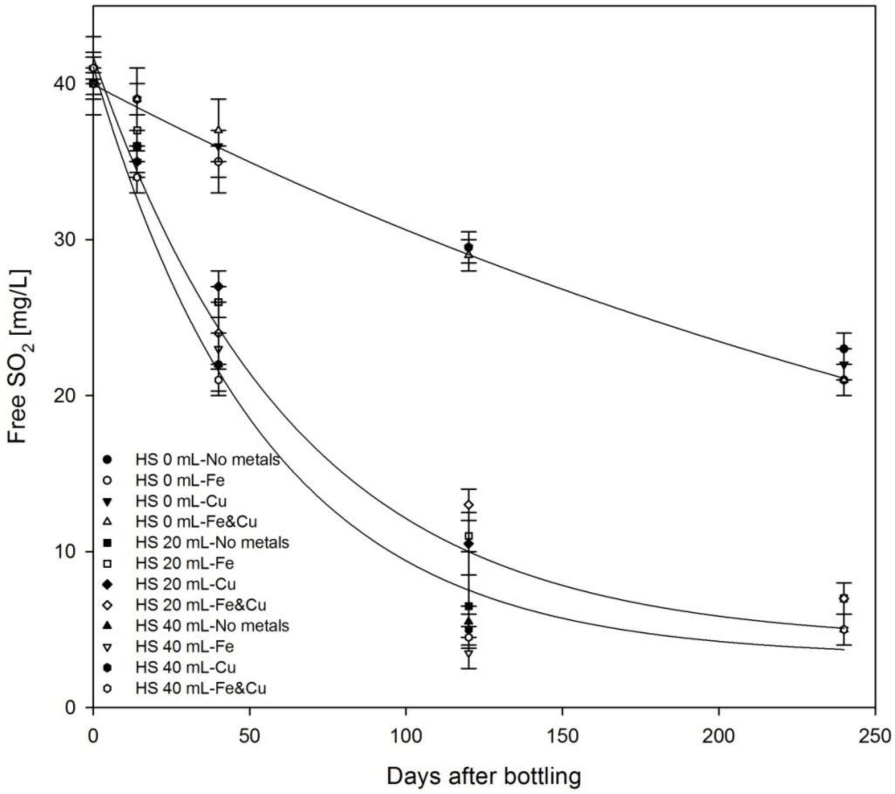
FIGURE 18: EVOLUTION OF FREE SO_2 DURING BOTTLE STORAGE OF MODEL WINE SOLUTION. ERROR BARS REPRESENT STANDARD DEVIATION.



Wine

The decay of free SO_2 during storage is shown in Figure 19. The greatest influence on the free SO_2 loss had the headspace volume. The wines without HS only lost 15 mg/L of SO_2 in nine months. Wines with 20 mL and 40 mL HS lost 33 mg/L and 35 mg/L of free SO_2, respectively, at the end of storage. However, analysis of variance showed that free SO_2 levels were not significantly different in the wines with 20 and 40 mL HS at the day 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_2 in the wines with 20 and 40 mL HS dropped by approximately 49% after 40 days, and by 86% after four months.

FIGURE 19: EVOLUTION OF FREE SO_2 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.



The results of the wines with no headspace both in model solution and in real wine measured after 40 days of storage were similar. The model wine wines with 50 mL and 100 mL HS and metal addition lost all the free SO_2, whereas the real wine with 20 mL and 40 mL HS only lost 35-44%. This is important to emphasize the impact of the oxygen content at the bottling on the SO_2 loss. The influence of the metal addition could only be observed in Riesling wine.

3.4. 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 22). However, all samples with headspace and Fe or Fe and Cu-addition showed clear colour changes on the day 40. The results of CIALAB analysis synthesized by the index ΔE showed similar results for the samples with Fe and Fe&Cu samples. The 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.

TABLE 22: COLOUR CHANGES IN MODEL WINE SOLUTION: E420 ON DAY 1, 40, CIELAB OVERALL COLOUR CHANGE (ΔE_{40D})^A: DAY 0 TO DAY 40.

	E_{420} / Day 1	**E_{420} / Day 40**	**ΔE_{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

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

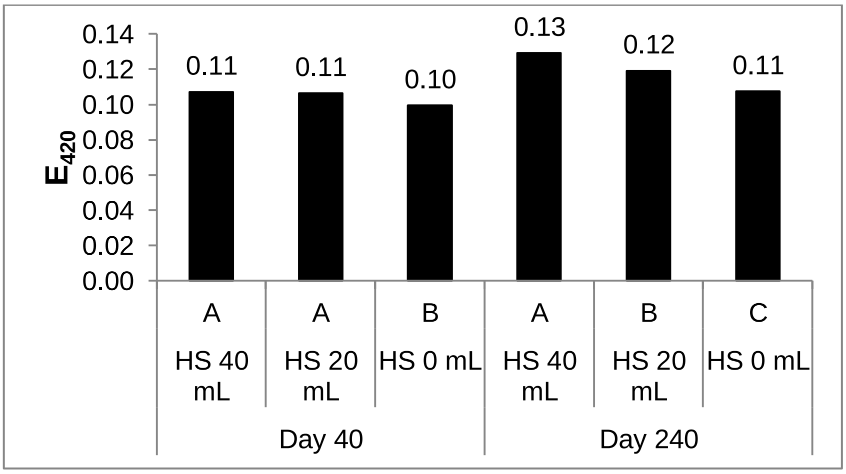
where L, a, b are the CIALAB parameters measured on the day i and j

Wine

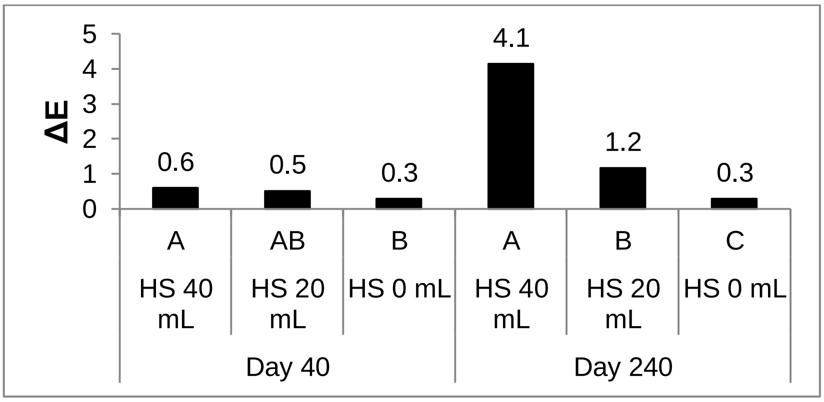
Figure 20 (a and b) shows the colour changes in the real wine after 40 and 240 days of storage. All wines without headspace (HS 0 mL) showed no significant optical colour change at the day 240 ($p<0.05$). The overall colour change (ΔE) of all wines with HS 20 and 40 mL increased after 40 days. However, no changes in absorbance at 420 nm were observed for the wines with 20 and 40 mL HS at this date.

FIGURE 20: COLOUR CHANGES IN RIESLING WINE AFTER 40 AND 240 DAYS OF STORAGE: AVERAGE VALUES OF E420 ABSORBANCE (A) AND CIELAB OVERALL COLOUR CHANGE ΔE (B).

a



b



After 240 days, the colour differences became stronger, according to the headspace volume at the bottling. All bottles with HS 20 and 40 mL showed increased values of 420 nm absorbance and ΔE. The ΔE 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_2, any influence of metals could not be observed. Two-way ANOVA analysis of results (Table 23) shows the influence of the factors ‘metal addition’ and ‘HS volume’ on measured colour parameters (E420 absorbance and ΔE). 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 23: 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 ΔE_{D40}, ΔE_{D240}: THE IMPACT OF THE METAL ADDITION AND THE HEADSPACE VOLUME (HS)

		E_{420} / Day 14	E_{420} / Day 40	E_{420} / Day 240	ΔE_{d40}	ΔE_{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

3.5. SENSORY EVALUATION

Tringle test sensory tasting was conducted 3 months after bottling. The results are summarized in Table 24. In the first 4 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 №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.

TABLE 24: RESULTS OF TRIANGLE TEST WITH LEVELS OF SIGNIFICANCE (N=26)

	Wine 1	**Wine 2**	**№ of judges**	**Correct Answers**	**Incorrect Answers**	**Significance[a]**
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.

[a] Significance levels: n.s. – not significant; * - significant at 10%; ** - significant at 5%; *** - significant at 0.1%

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 other two were conducted after nine months of storage. The results are presented in Table 25. Three-way ANOVA analysis confirmed that the headspace volume had the greatest influence with F values of 51-62 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 “oxidised”.

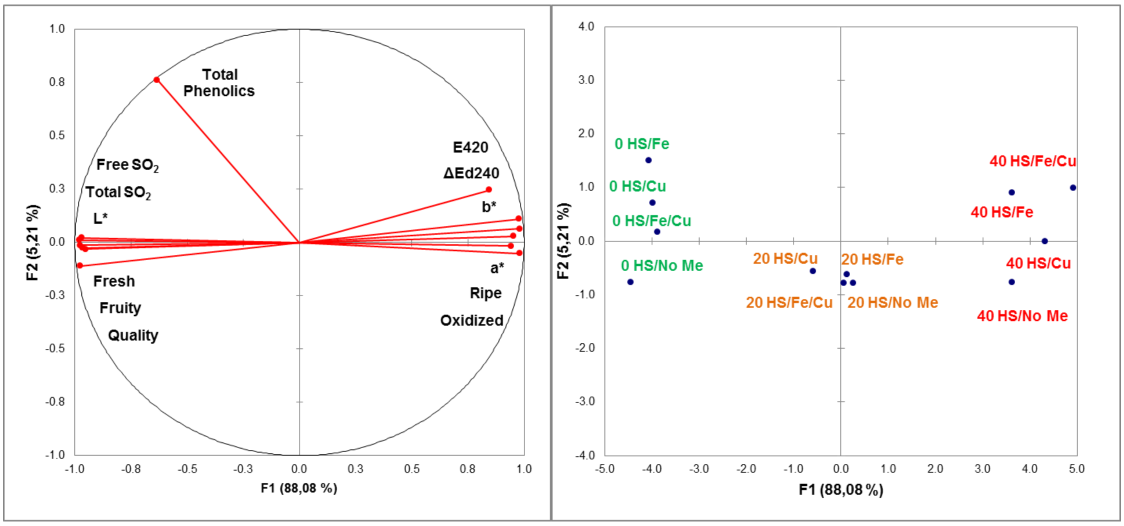
TABLE 25: MEAN INTENSITY SCORES FOR DESCRIPTIVE SENSORY ATTRIBUTES EVALUATED IN 4 DESCRIPTIVE SESSIONS

Treatment	**fruity**		**ripe**		**fresh**		**oxidised**		**Overall Quality**	
HS 0 mL| No metal addition	2.64	A[a]	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.

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 21:

FIGURE 21: 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.



The first two principal components retained 93.29% of the variance, most of which (88.08%) was accounted 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 sulphur dioxide levels and CIELAB lightness (L) values after nine months of storage. Moreover, they were 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 ‘oxidised’ aromas. The highest browning was observed in wines with 40 mL HS, which affected the overall quality.

4. CONCLUSION

In model solution the headspace volume and the metal addition contributed to significant changes in total 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 SO_2. Enhanced colour was observed in all wines, where iron was added.

The experiment with Riesling wine with similar parameters showed strong influence of the headspace volume at the bottling on wine development. The rate of oxidative browning strongly correlated with the headspace volume at the 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 completely metal-free real wine system, where the 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.

5. ABBREVIATIONS USED

HS – Headspace Volume [mL]

DO – Dissolved Oxygen [mg/L]

TCO – Total Consumed Oxygen [mg/L]

6. ACKNOWLEDGEMENTS

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CHAPTER III

The Impact of Headspace Oxygen and Copper and Iron Addition on Oxygen Consumption Rate, Sulphur Dioxide Loss, Colour and Sensory Properties of Riesling Wine

ABSTRACT

Oxygen ingress at bottling is crucial for the wine development during storage. Iron and copper are known to catalyse the oxidation processes in wines. The aim of the present study was to evaluate the impact of oxygen, and iron and copper addition on changes in analytical and sensory parameters during storage. A Riesling wine was bottled with various oxygen concentrations determined by the headspace volume in the bottle (0 mL, 10 mL and 20 mL) full with ambient air. Iron (1 mg/L) and copper (0.5 mg/L) were added to 50% of the bottles. Headspace and dissolved oxygen, free and total SO_2 and colour were monitored during three months post-bottling. Descriptive sensory evaluation took place in the end of the observation period. Fe and Cu addition had significant influence on the oxygen consumption rate, on the loss of SO_2 during storage, and on the sensory changes in wine. Initial headspace volume additionally made significant impact on the evolution of the sulphur dioxide and on the sensory profile of bottled wines.

KEYWORDS

Wine, metals, oxygen, wine oxidation, sulphur dioxide, sensory analysis

1. INTRODUCTION

Oxidation is a common spoilage in white wines. Contact with air, oxygen ingress at bottling may influence development of the bottled wine (Dimkou et al. 2011; Karbowiak et al. 2009). Modern measurement techniques allow the amount of dissolved oxygen in wine to be quantified, along with the gaseous oxygen in the headspace of the bottle (Morozova and Schmidt 2012).

Iron and copper are of major interest in wine due to their ability to form hazes or casses, such as ferric tannate casse, ferric phosphate casse, and copper casse, which cause instability and cloudiness in wine (Ribereau-Gayon et al. 2006). Iron and copper are essential nutrients for plants, but can be toxic to people in exceeding concentrations (Kostic et al. 2010). These metals are always present in wine in trace amounts, coming from grapes, soil (Almeida and Vasconcelos 2003; Pohl 2007; Taylor et al. 2003) viticulture practices (Volpe et al. 2009), or pesticides (Galani-Nikolakaki et al. 2002; Kment et al. 2005). Secondary sources of iron and copper may be oenological equipment (Eschnauer 1982) in the cellar or fining agents (Nicolini et al. 2004; Wurzinger et al. 1994). Iron and copper are minor metals found in wine in the range 0.1 - 10 mg/L (Catarino et al. 2007).

Iron and copper may contribute to the sensorial qualities of wine. High concentrations of iron are reported to give a fishy taste (Tamura et al. 2009). Copper may also change the sensory profile of wine in concentrations exceeding 0.5 mg/L (Li et al. 2008). Schmidt (2001) reported a pronounced bitter taste in wines with copper concentrations higher than 1 mg/L.

Recent studies in model wine solutions showed the key role of iron and copper in oxidation reactions occurring in wine as oxygen activators (Danilewicz 2003; Danilewicz 2007; Danilewicz 2011). As a result, hydroperoxyl radicals are formed with the following development of reactive oxygen species regarded as stronger oxidants as the molecular oxygen (Singleton 1987). Recent studies also showed that iron activates oxidative degradation of tartaric acid and the production of glyoxylic acid (Clark et al. 2011), catalysing browning reactions.

The oxidation mechanisms were recently studied in wine-like model solutions (Danilewicz 2003; Danilewicz 2007; Danilewicz 2011). It was assumed that in the absence of iron and copper the oxidation of the phenolic compounds will be minor. However, previous experiments showed great differences between the processes in model solution and wine with similar oenological parameters. Addition of 0.1 mg/L iron and 0.05 mg/L copper influenced oxygen consumption in the model solution, but this effect could not be observed in wine. Hence iron and copper may be bound by certain wine compounds (Clark et al. 2011), this may be the reason for no significant changes due to the addition of small amounts of iron and copper in previous experiments.

One of the first studies on German wines was done by Siegmund and Bächmann (1977). They determined 15 elements in 70 wines from Pfalz, Rheinhessen, Nahe and Franken regions using neutron activation analysis. The concentration of iron in the wines ranged from 0.56 to 11.4 mg/L with the mean concentration of 3.9 mg/L. However, Eschnauer and Neeb (1988) and later Henze and Bauer (2004) reported lower mean concentration of 1 mg/L for iron and 0.5 mg/L for copper in German wines, which may be due to the recent progress in viticulture techniques and oenological practices. Nikfardjam (2010) studied 84 wines from Baden Württemberg region in Germany and found average metal concentrations to be 1.6 mg/L for iron and 0.6 mg/L for copper. These concentrations were used as reference for the current study.

The aim of this study was to determine the oxygen consumption rates in the wine mediums with low and average for Germany concentrations of iron and copper. In addition, the goal of the study was to link the sensory changes to the effects on sulphur dioxide loss and wine colour, also studied by other authors (Dimkou et al. 2011; Dimkou et al. 2013; Godden et al. 2001; Godden et al. 2005; Kwiatkowski et al. 2007).

2. MATERIALS AND METHODS

2.1. CHEMICALS AND REAGENTS

Chemicals used for the experiment: potassium ferrocyanide K4[Fe2(CN)6] (Begerow, Langenlonsheim, Germany), iron(II) sulphate (>99%, Merck, Darmstadt, Germany), copper(II) sulphate (>99%, Merck), Titrisol 1g/L iron standard solution (FeCl3 in 15% HCl, Merck), Titrisol 1g/L copper standard solution (CuCl2 in H2O, Merck), LaCl3 x 7 H2O for analysis (Merck), hydrochloric acid for analysis (32%, Merck). Purified water was obtained from a Milli-Q purifier system (Millipore, Schwalbach, Germany).

2.2. WINE SAMPLES

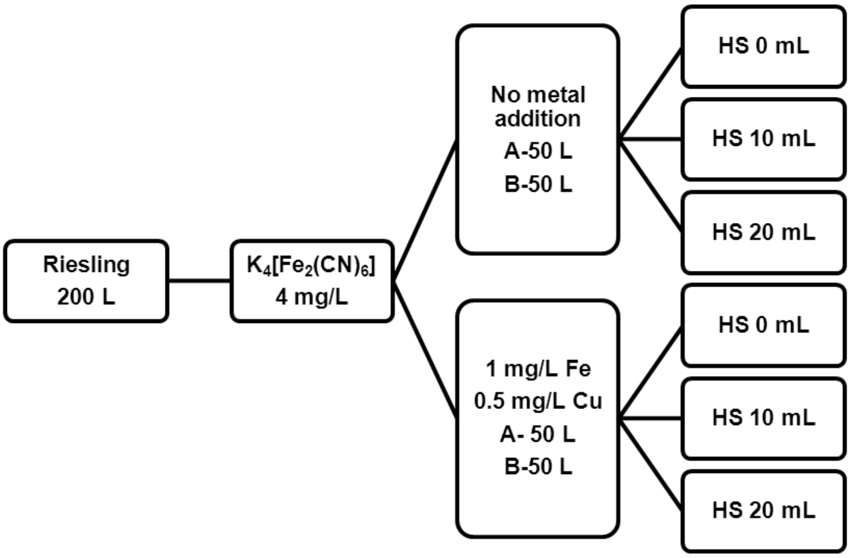
Riesling grapes were harvested in October 2010 from the vineyards of the State Institute for Viticulture, Oenology and Fruit Technology Weinsberg in the Württemberg region. The white wine (200 L) was prepared in the experimental cellar Weinsberg according to the standard procedure for small-scale winemaking: grapes were pressed directly after crushing. The clarification was done overnight, and fermentation occurred then at 15°C. After fermentation was completed, the free SO_2 content was adjusted to 50 mg/L. The wine was cold-stabilized, cross-flow filtered and stored at 15 C°. Carbon dioxide blanketing was used at all the operations to maintain the concentration of dissolved oxygen (DO) as low as possible. Analytical parameters of the wine were as follows: 12% vol of alcohol, titratable acidity: 6.4 g/L (expressed as tartaric acid), free SO_2: 50 mg/L, total SO_2: 100 mg/L, total phenolic content: 422 mg/L (expressed as

GAE). All chemical analysis was conducted in accordance with methods outlined by OIV (OIV 2008).

To reduce iron and copper content the wine was treated with potassium ferrocyanide, which is an accepted oenological technique, known as “blue fining”. The amount of potassium ferrocyanide to be used for the blue-fining was determined according to common industry procedure (Würdig et al. 1989). After addition of 4 mg/L potassium ferrocyanide, and mixing, the wine was left to stand for two days. After this period the Berlin Blue formed had all settled out, and the clear fined wine was filtered and analysed.

After readjusting SO_2 to 50 mg/L the wine was split into four CO_2-filled 50-L stainless steel kegs. Iron(II) sulphate and copper(II) sulphate were added again to two kegs to reach concentrations of 1 mg/L of Fe and 0.5 mg/L of Cu to reach the average concentrations common for Baden-Württemberg region (Nikfardjam 2010). To the other two kegs no metals were added. The experimental design is presented in the Figure 22:

FIGURE 22: SCHEME OF THE EXPERIMENTAL DESIGN



All wines were bottled into sterilized 500 mL “bordelaise” classic bottles and sealed with DEL-CAP-GD28–OSL-80 aluminium screw caps with saran tin foil supplied by Kork-Fabrik Arthur Linck GmbH (Delmenhorst, Germany). Racking and bottling were performed using nitrogen (Lange & Co. GmbH, Lippstadt, Germany) to keep the dissolved oxygen concentrations below 0.15 mg/L. This was controlled with a Hach Lange HQ40d oxygen meter (Hach Lange, Berlin, Germany). Bottling was performed with a manual two-way filler system (Fjord, Kaiserslautern, Germany) and the bottles were purged with nitrogen before processing.

The headspace volume is dependent on the type of closure: for the screw cap it is commonly between 10 and 20 mL, whereas for the bottles sealed with cork the headspace is smaller than 10 mL (Kontoudakis et al. 2008; Vidal and Moutounet 2006). In the present study, the oxygen content in the bottles was defined with different headspace (HS) volumes (0 mL, 10 mL and 20 mL of ambient air) representing typical headspace range for the industry. The experiment included six wines with two replicates each (A and B). The wine samples were then stored in upright position at 15°C.

2.3. ICP-MS AND ICP-OES ANALYSIS

Iron and copper analyses in wines were performed with ICP-MS and ICP-OES. An ELAN 6000 ICP-MS (Inductively Coupled Plasma – Mass Spectroscopy) (Perkin Elmer, Rodgau, Germany) was used for copper determination. Standard copper solutions (0.1, 0.5 and 2.5 mg/L) were used for calibration. Wines were diluted 1:5 in 50-mL volumetric flasks with water, followed by the addition of 100 µL of concentrated nitric acid to diminish matrix effects of ethanol (Taylor et al. 2003). Operating conditions were as follows: power – 1100 W; nebulizer gas flow – 0.84 L/min; lens voltage – 10.5

V. Speed of peristaltic pump for sample uptake was 25 ω/min. As internal standard, rhodium was used.

For the determination of iron, a Varian simultaneous CCD ICP-OES (Inductively Coupled Plasma - Optical Emission Spectroscopy) Vista-PRO was used. The radio frequency power was 1100 W, carrier gas flow – 1.5 L/min, and the plasma gas flow rate was increased to 15 L/min with injection speed of 30 s. The working solutions used for analyses of iron were 1:5:10 mg/L. Wines were diluted 1:10 with ultrapure water with addition of 100 µL concentrated nitric acid.

2.4. OXYGEN DETERMINATION

Dissolved and gaseous oxygen in the headspace of bottled wine were measured fluorimetrically using optodes consisting of Pst3 sensor spots, an optical fibre, and a source and receiver of optical signals (Fibox 3-Trace, PreSens GmbH, Regensburg, Germany) coupled with digital temperature sensor and a laptop with installed Fibox 3-trace Software (v3). According to the manufacturer, the sensor sensitivity is as follows: 0-22 mg/L for DO and 0-500 hPa for gaseous and dissolved oxygen; response time ≤ 30 sec (Huber 2006; Morozova and Schmidt 2012). To measure HS and DO, 2 sensor spots were fixed with silicone (RS Components, Mörfelden-Walldorf, Germany) in transparent glass bottles, six bottles for each wine. Light excitation and measurements were performed from the outside through the glass. Prior to measurements, the meter was calibrated using oxygen, carbon dioxide and ambient air. Measurements were conducted over 30 seconds until a stable reading was reached. Temperature was measured simultaneously. The sensors were periodically purged with nitrogen to check the zero point. After HS and DO measurements were taken, total consumed oxygen (TCO) rates were calculated as described in previous works (Dimkou et al. 2011) as a difference of the sum of HS and DO observed on the measurement and the sum of HS and DO observed on the initial measurement, plus the oxygen entering the bottle through the storage. In the work of Dimkou et al. (2011) measured amount of oxygen entering wine through the screw cap was 1 mg/L in 24 months, or approximately 0.05 mg/L per month. This amount was taken into account, when the calculation of TCO rates was made.

2.5. SULPHUR DIOXIDE MEASUREMENTS

For determination of free and total sulphur dioxide, the method of iodometric titration was used (OIV 2008). Wine samples were analysed at four dates, namely at 1, 30, 60 and 90 days of storage. Three independent replicates of free and total sulphur dioxide and reductants were performed using a Titrando 808 titrator (Metrohm, Herisau, Switzerland) equipped with Dosimat 805 and Tiamo Software (version 1.3), then and the mean concentration was calculated.

2.6. COLOUR MEASUREMENTS

Wine colour measurements were undertaken at room temperature soon after bottles were opened using U-3010 spectrophotometer (Hitachi, Japan) coupled with a personal computer equipped with UV Solutions software (version 2.1) was used. Density at 420 nm was measured, 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.

2.7. SENSORY EVALUATION

Four independent sensory assessments were performed to evaluate organoleptic changes in the wines during the storage period. Three sessions of descriptive sensory analysis with two replicates per treatment (HS 0, 10, 20 mL with/without iron and copper addition) were held per day. Four samples were presented per session.

The six wines were evaluated by two trained panels (n1=17, n2=15) in duplicate in the tasting lab of the LVWO Weinsberg equipped with FIZZ sensory software (Fizz Biosystemes, version 2.47). Panellists were isolated in single booths with green lighting. The panellists (27 male, 5 female) were trained in professional tasting of wines and had previously participated in white wine panels, to understand the task and to work with the software. A structured 5-point scale was used to evaluate wines in six aroma and three flavour attributes. Panellists were instructed to score the attributes from 0 (“not developed”) to 5 (“most developed”). Sensory vocabulary was built up in an evaluation session, which was carried out by the experts of the experimental cellar of LVWO Weinsberg. The list of agreed attributes necessary to describe the wines (Table 26) was for aroma: reduced, fruity (general), citrus, tropical, oxidised, UTA (untypical aging); for palate: mouthfeel, freshness and overall quality.

TABLE 26 DEFINITIONS, INCLUDING REFERENCE STANDARDS, FOR ATTRIBUTES ASSESED IN THE AROMA AND PALATE DESCRIPTIVE ANALYSIS STUDY AT 3 MONTH POST-BOTTLING

Aroma	**Description**
reduced	burnt rubber, rubbery, struck flint
fruity	intensity of fruit characters
citrus	lemon zest, soaked in white wine overnight and removed
tropical	litchi, passion fruit, peach standard: 10 mL tropical juice added to 100 mL wine
oxidised	bruised apple, sherry-like, includes aldehyde standard: white wine opened for one month
UTA (untypical aging)	standard: 2’-aminoacetophenone, 10 µL in 500 mL wine

A Latin square design was used to randomize the sample presentation across the panellists. The assessors were not aware of the number of treatments included in the study. The wines were served cold in clear tulip-shaped wine glasses. The panellists evaluated the wines and cleansed their palates with bread and bottled water.

2.8. STATISTICAL ANALYSIS

Correlation test for the free SO_2 data, analysis of variance (ANOVA) using Fisher's LSD with a 95% confidence level ($p<0.05$) and principal component analysis (PCA) of the sensory data were conducted using XLSTAT Software Version 2011.2.08 (Addinsoft, Germany).

3. RESULTS AND DISCUSSION

3.1. IRON AND COPPER

Table 27 shows the results of iron (Fe) and copper (Cu) measurements in wine samples. In the wines without metal addition, Fe content stayed below the detection limits of the used analytical methods, while Cu content did not exceed 0.1 mg/L determined by ICP-MS.

TABLE 27: IRON AND COPPER CONTENT (± S.D.) MEASURED IN THE WINES (N=3)

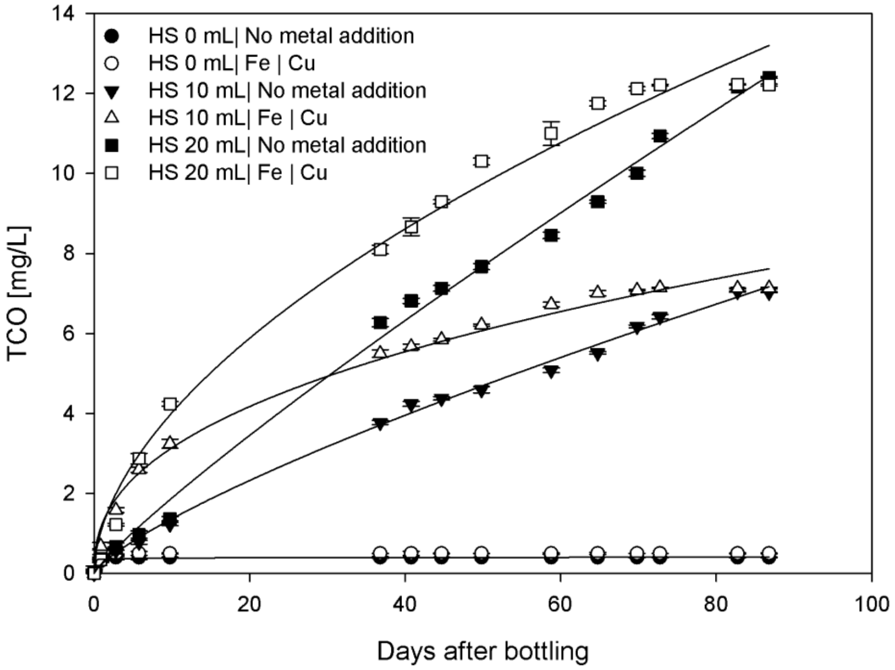
	Fe, mg/L	**Cu, mg/L**
HS 0 mL| No metal addition	<0,5	0.06±0.1
HS 10 mL| No metal addition	<0,5	0.09±0.1
HS 20 mL| No metal addition	<0,5	0.07±0.1
HS 0 mL| Fe| Cu	1.1±0.1	0.63±0.1
HS 10 mL| Fe| Cu	1.1±0.1	0.61±0.1
HS 20 mL| Fe| Cu	1.1±0.1	0.65±0.1

In the wines with iron and copper additions, the concentrations of Fe and Cu resulted in higher than expected values according to the initial experimental design, suggesting that the wine contained traces of metals even after the “blue fining” treatment. Average iron concentration determined by ICP-OES generally revealed 1.1 mg/L Fe for all wines. Average copper concentrations determined by ICP-MS were 0.61-0.65 mg/L.

2.2. OXYGEN CONSUMPTION

The development of total consumed oxygen for the different wines is shown in Figure 23. In the wines with 0 mL HS, the dissolved oxygen concentration on the day of bottling did not exceed 0.5 mg/L. This amount of oxygen was consumed by the wine in 3 days. The dissolved oxygen concentration did not change during the observed period, which is consistent with the results of Vidal et al. (2011), who reported an oxygen transmission rate (OTR) for the screw cap was close to zero. In the experiment of Dimkou et al. (2011), an OTR of 0.5 mg/L of oxygen per year was reported. This suggests that in this experiment, oxygen could have been ingressing through the closure and be consumed at the same rate.

FIGURE 23: TOTAL CONSUMED OXYGEN (TCO) EVOLUTION IN THE WINES WITH DIFFERENT HEADSPACE VOLUMES WITH/WITHOUT FE AND CU ADDITION, MG/L (SIX BOTTLES PER WINE): (●) HS 0 ML WITH NO FE AND CU ADDITION; (○) HS 0 ML WITH FE AND CU ADDITION; (▼) HS 10 ML WITH NO FE AND CU ADDITION; (△) HS 10 ML WITH FE AND CU ADDITION; (■) HS 20 ML WITH NO FE AND CU ADDITION; (□) HS 0 ML WITH FE AND CU ADDITION; ERROR BARS REPRESENT STANDARD DEVIATION; EACH DATA POINT REPRESENTS THE MEAN (±S.D.) OF SIX BOTTLES



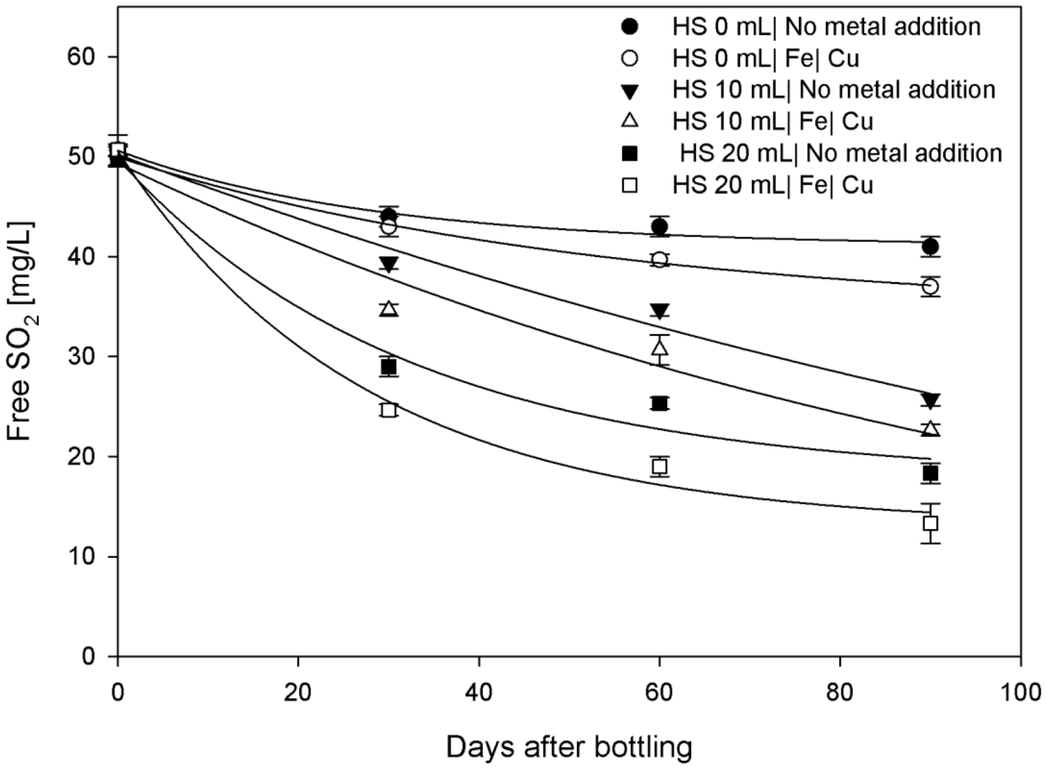
Difference in the oxygen consumption rate in wine samples with and without metal addition was observed for the wines with 10 mL and 20 mL HS. In the wines without metal addition, the oxygen consumption rate tended to be linear with a slope of about 0.08 mg and 0.14 mg per day for 10 mL HS 20 mL HS, respectively. Iron and copper addition made significant impact on the oxygen consumption rate; 45.5% (10 mL HS) and 34.7% (20 mL HS) of the total oxygen amount were consumed in 10 days after bottling in the wines with Fe and Cu addition. For the wines without Fe and Cu addition, only 17.5% (10 mL HS) and 11% (20 mL HS) were consumed. This catalytic effect was clearly observed during the whole observation period. After 65 days, 98.2% (10 mL HS) and 96.2% (20 mL HS) were consumed in the wines with Fe and Cu addition. In contrast, in the wines with no Fe and Cu addition, only 78.5% (10 mL HS) and 74.9% (20 mL HS) were consumed by that time. After 90 days of storage the whole amount of oxygen was consumed in all wine samples.

An interesting difference in dissolved oxygen rates (data not shown) was observed for the wines with the biggest headspace volume (20 mL). In the first 10 days, the concentration of dissolved oxygen in the wines without Fe and Cu addition stayed at 1.1-1.5 mg/L, opposite to the wines with Fe and Cu addition, where the dissolved oxygen concentration was below 0.5 mg/L. This may be explained through the interaction of two different processes. Firstly, the oxygen from the headspace was rapidly diffusing into the wine due to the large difference in concentrations. When dissolved in wine, the molecular oxygen should be activated to interact chemically with other wine compounds. However, due to the low iron and copper concentration in the wines without Fe and Cu addition, the molecular oxygen could not be activated and was only partially consumed. This explains higher dissolved oxygen concentrations in the wines with no Fe and Cu addition. When the difference between the oxygen concentration in the headspace and the dissolved oxygen decreased, the amount of the diffusing oxygen also decreased. Consequently, the iron and copper concentration in the wine was enough for the activation of chemical reactions. The dissolved oxygen concentration in wine decreased and became as low as for the wines with Fe and Cu addition (<0.4 mg/L).

3.2. FREE SULPHUR DIOXIDE

The evolution of the free SO_2 in the studied wines during the first 90 days of storage is shown in Figure 31. The free SO_2 concentration in the bottled wine was shown to be dependent on both the oxygen content and the concentration of Fe and Cu.

FIGURE 24: FREE SO_2 EVOLUTION DURING 90 DAYS OF STORAGE IN WINES: (●) HS 0 ML WITH NO FE AND CU ADDITION; (○) HS 0 ML WITH FE AND CU ADDITION; (▼) HS 10 ML WITH NO FE AND CU ADDITION; (△) HS 10 ML WITH FE AND CU ADDITION; (■) HS 20 ML WITH NO FE AND CU ADDITION; (□) HS 0 ML WITH FE AND CU ADDITION; ERROR BARS REPRESENT STANDARD DEVIATION; EACH DATA POINT REPRESENTS THE MEAN (±S.D.) OF THREE MEASUREMENTS



At the end of the storage period, wines with 0 mL HS lost only 19% (no Fe and Cu added) and 26% (Fe and Cu added) of free SO_2 (Table 28). In the wines with 10 mL HS, the observed loss of free SO_2 reached 49% in the wines with no metal addition and 55% in the presence of Fe and Cu. The highest losses of free SO_2 were detected in the wines with 20 mL HS: 63% for the wines with no metals added and 75% for the wines with Fe and Cu addition.

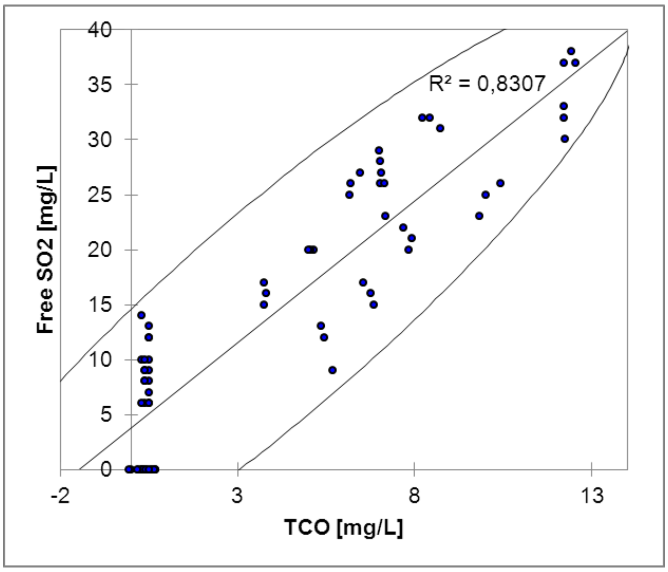
TABLE 28: AVERAGE FREE SO_2 LOSS (MG/L) IN WINES WITH VARIOUS HS VOLUMES (0 ML, 10 ML AND 20 ML) WITH/WITHOUT ADDITION OF FE AND CU AFTER 30, 60 AND 90 DAYS OF STORAGE, RESPECTIVELY

	Day 30		Day 60		Day 90	
HS 0 mL| No metal addition	7	E*	8	F	10	F
HS 0 mL| Fe| Cu	7	E	10	E	13	E
HS 10 mL| No metal addition	11	D	16	D	25	D
HS 10 mL| Fe| Cu	16	C	20	C	28	C
HS 20 mL| No metal addition	21	B	25	B	32	B
HS 20 mL| Fe| Cu	26	A	32	A	37	A

*different letters show significant differences between groups (Fischer LSD)

Pearson's test showed strong correlation between total consumed oxygen and free SO_2 loss (Figure 25) with the calculated regression coefficient of 0.8307. Data distribution over the whole experiment period allowed to determine the following equations for the free SO_2 loss (mg/L) depending on the total consumed oxygen content:

FIGURE 25: RESULTS OF PEARSON'S CORRELATION TEST BETWEEN TOTAL CONSUMED OXYGEN (MG/L) AND FREE SO_2 LOSS (MG/L), N=72



For the wines without Fe and Cu addition: $y = 2.3x + 2.75$ and for the wines with Fe and Cu addition: $y = 2.99x + 4.57$, where x is the total consumed oxygen in mg/L.

The mole ratio of the consumed free sulphur dioxide to the total consumed oxygen in the wines without Fe and Cu addition was 1.28±0.21, while in the presence of metals it was shown to be 1.9±0.22, which is close to the results shown by Danilewicz et al. (2008). However, free SO_2 loss may be dependent on other parameters, such as temperature, wine type or sulphur dioxide content in wine. The observed ratio was not constant over the storage period, which could be the subject of another study.

3.3. COLOUR

Visible colour changes could not be observed after three months (data not shown). This might be due to the fact that wines were analysed soon after the whole amount of oxygen in the bottle was consumed. Browning reactions at 15°C may take more time to occur.

3.4. SENSORY EVALUATION

To determine sensory differences between the samples, for sessions of descriptive analysis were conducted after three months of wine storage and after the oxygen was consumed. Interactions between the wine and repetition did not show any significant impact on sensory parameters, which indicated that the panel repeatability was good.

The two-way ANOVA analysis (Table 33) showed that HS volume made significant impact ($p<0.05$) on the attributes “tropical”, “fresh”, and “preference”. Metal addition influenced such positive characteristics as fruitiness, citrus aroma, freshness of wine, and quality. Negative attributes responsible for oxidised and untypical aging

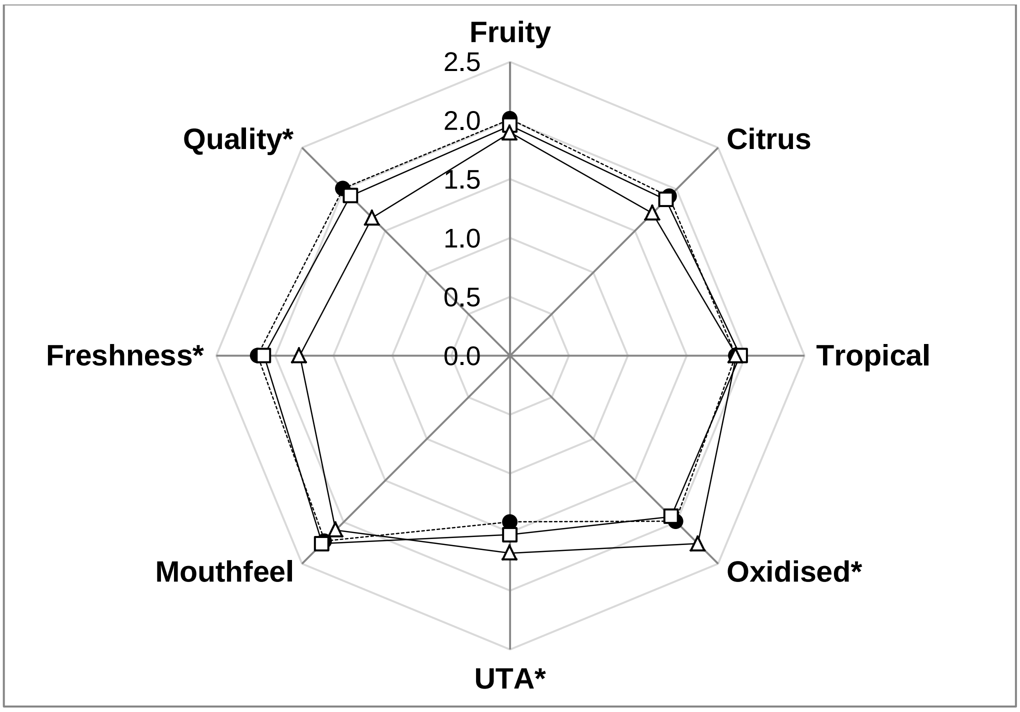
(UTA) aromas also showed to be dependent on the metal addition. The interaction of the HS volume and metal addition made significant impact on the attributes “tropical”, “UTA”, “fresh”, and general quality.

TABLE 29: TWO-WAY ANOVA ANALYSIS (F-VALUES AND PR>F) SHOWING THE INFLUENCE OF THE HS VOLUME, FE AND CU ADDITION AND THEIR INTERACTION (IN BOLD - SIGNIFICANT WITH FISCHER LSD)

		Reduced	**Fruity**	**Citrus**	**Oxidised**	**Tropical**	**UTA**	**Mouthfeel**	**Freshness**	**Quality**
Headspace Volume	F	0.122	0.563	1.905	0.060	**3.479**	1.998	2.043	**5.976**	**7.485**
	Pr > F	0.885	0.570	0.150	0.942	**0.032**	0.137	0.131	**0.003**	**0.001**
Metal Addition	F	0.202	**20.826**	**9.175**	**11.685**	2.278	**4.477**	1.526	2.480	**4.012**
	Pr > F	0.653	**<0.0001**	**0.003**	**0.001**	0.132	**0.035**	0.217	0.116	**0.046**
HS*Metals	F	0.064	1.028	1.643	0.512	**9.123**	**4.145**	0.179	**15.417**	**6.665**
	Pr > F	0.938	0.359	0.195	0.600	**0.001**	**0.017**	0.836	**<0.0001**	**0.001**

Figure 26 shows the influence of the headspace volume on the sensory perception of wines. As can be seen, the wines with 20 mL HS were scored as more oxidised, high in UTA and lower in freshness, which finally contributed to the quality ratings. Oxidised character of wines associated with higher exposure to oxygen was previously observed by other authors (Dimkou et al. 2011; Kwiatkowski et al. 2007).

FIGURE 26: RESULTS OF THE DESCRIPTIVE ANALYSIS OF SIX WINE TREATMENTS - INFLUENCE OF THE HEADSPACE VOLUME: (●) HS 0 ML; (□) HS 10 ML; (△) HS 20 ML; ATTRIBUTES MARKED WITH (*) ARE SIGNIFICANTLY DIFFERENT (P<0.05) USING FISHER'S LEAST SIGNIFICANCE DIFFERENCE METHOD



The influence of the metal addition on the sensory perception of wines is shown in Figure 27. The wines with iron and copper addition showed lower scores in fruity, citrus, tropical aromas, high in UTA and were appreciated by the panellists as lower in quality. This might be due to the binding activity of copper towards thiol compounds responsible for fresh fruity aromas in white wines. Darriet et al. (2001) showed the effect of copper spaying on the concentration of 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one. Ugliano et al. (2011) also observed decrease of 3-mercaptohexanol in wines with 0.3 mg/L copper addition.

FIGURE 27: RESULTS OF THE DESCRIPTIVE ANALYSIS OF SIX WINE TREATMENTS - INFLUENCE OF THE METAL ADDITION: (●) NO FE AND CU ADDITION; (□) FE AND CU ADDED; ATTRIBUTES MARKED WITH (*) ARE SIGNIFICANTLY DIFFERENT (P < 0.05) USING FISHER'S LEAST SIGNIFICANCE DIFFERENCE METHOD

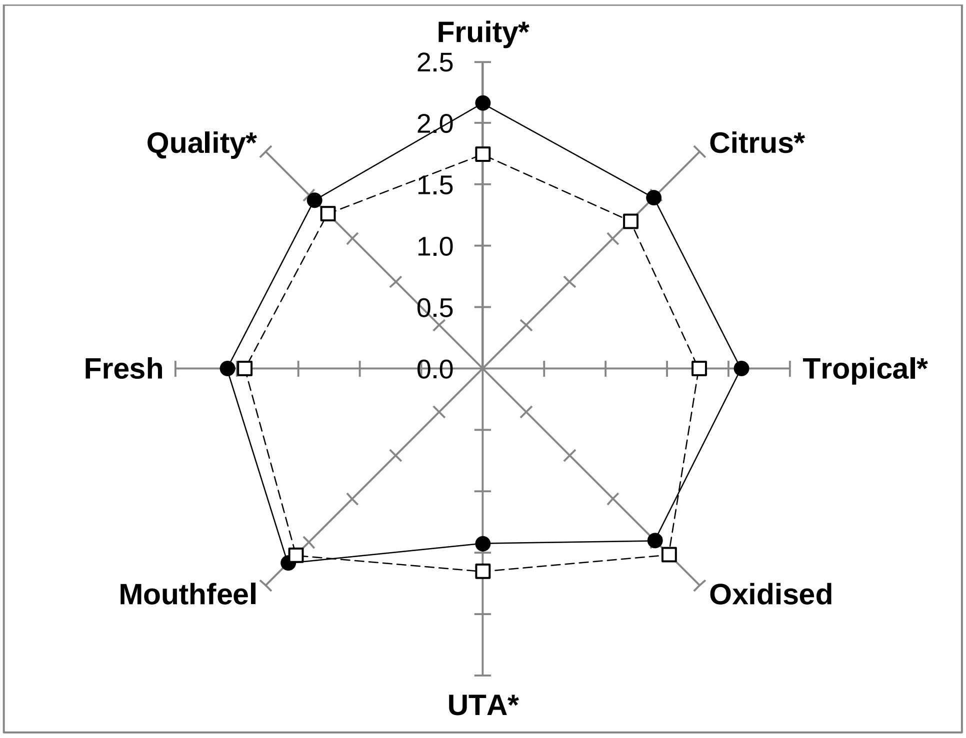
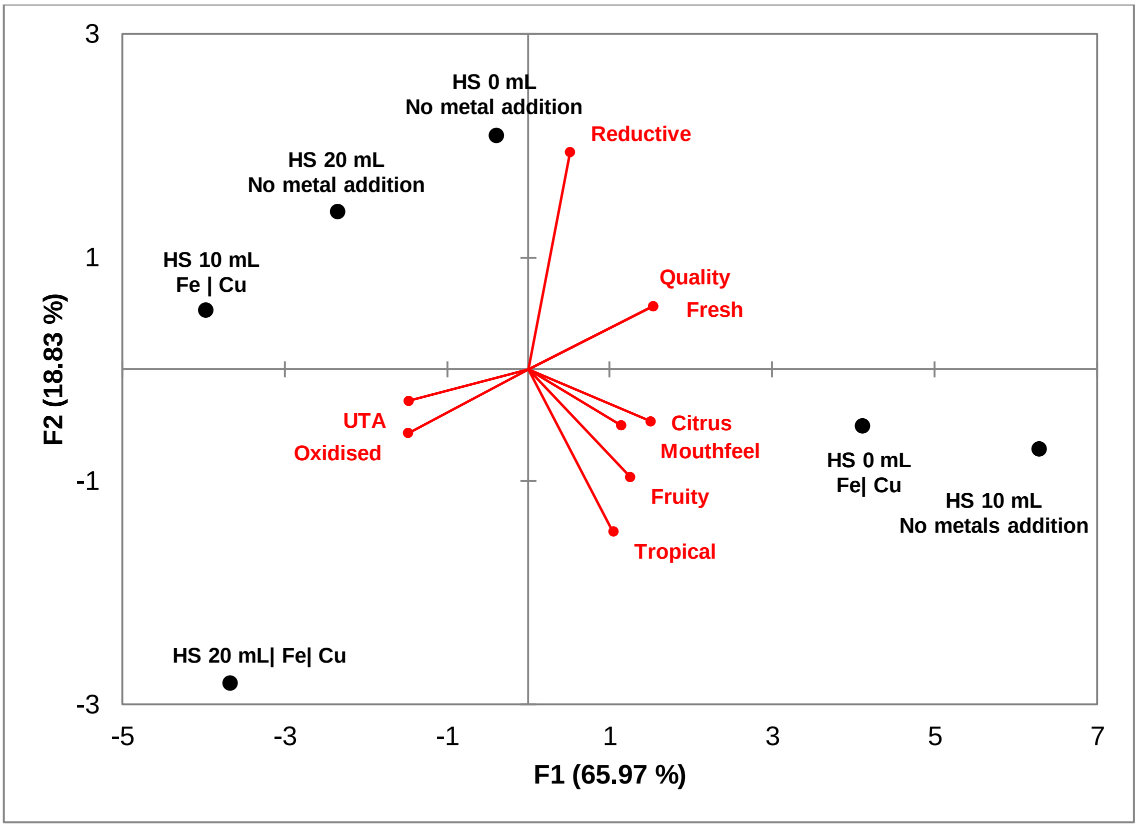


Figure 28 shows the main PCA graph that summarizes the overall sensory evaluation of the Riesling experimental wines after three months of storage. Wine bottled with minimum oxygen (0 mL headspace) and without Fe and Cu addition was appreciated by the judges as slightly reduced, although differences were not significant at the p=0.05. 'Rubbery' and 'struck flint' like aromas in Semillon wines sealed under screw cap were previously reported by Godden et al. (2001), Godden et al. (2005) and Kwiatkowski et al. (2007) also showed, that Cabernet Sauvignon red wines sealed under screw caps with HS 4 mL were rated highest in reduced aroma compared to the wines with HS 16 and 64 mL after 11.2 months of storage, suggesting that the perceived reduced character in the screw cap wines could be related to the headspace volume.

FIGURE 28: PRINCIPAL COMPONENTS ANALYSIS SUMMARIZING THE RESULTS OF DESCRIPTIVE ANALYSIS AFTER FOUR MONTHS OF STORAGE AT 15°C ON THE FIRST TWO DIMENSIONS, ACCOUNTING FOR 84,8% OF THE VARIABILITY



In contrast, the wines with 0 mL HS and iron and copper addition were scored fruitier, fresher, and higher in citrus and tropical aroma. It may be suggested that the rapid consumption of small amounts of oxygen (not more than 0.5 mg/L) may have a beneficial effect on the aroma profile of the wine.

For the wines bottled with 10 mL headspace the opposite effect could be observed. The wine without metal addition was significantly better evaluated than the wine with metal addition. Iron and copper addition catalysed the oxygen consumption and oxidation processes, and affected the aroma profile of the wine. For the wines bottled with 20 mL headspace the same trend could be shown. The wines with Fe and Cu addition were rated highest in the oxidised and untypical aging aromas. Overall, the results of this study suggest that the oxidised character could also be enhanced by higher concentrations of iron and copper.

As shown in the experiment, in the case of the Riesling wine studied, the effect of iron and copper addition was not linear. In the case of low oxygen concentration at bottling (<1.2 mg/L), higher iron and copper content may have positive effects on the sensory perception of white wine. However, if the oxygen ingress at bottling is high or in the case of closures with high oxygen transmission rates, fast consumption of molecular oxygen catalysed by iron and copper, followed by radical reactions, may ruin the fruity and varietal aromas of the wine.

4. CONCLUSION

Oxygen ingress during bottling and iron and copper concentration were shown to have great impact on white wine development.

In the wines with no metal addition, oxygen consumption after bottling tended to be linear. The addition of 1 mg/L of iron and 0.5 mg/L of copper catalysed oxygen consumption during the observation period.

Free sulphur dioxide loss was found to be proportional to the total consumed oxygen after bottling. In all wines with iron and copper addition, the free sulphur dioxide decrease was significantly higher.

Although colour changes were not observed in all wines after 90 days of storage, there were significant sensory changes. Oxygen and metals concentrations were found to make an impact on the sensory evaluation of wines. At low oxygen concentration (0 mL HS), metal addition had positive effects, eliminating reduced aromas. The opposite occurred in wines with 10 mL and 20 mL HS, Fe and Cu contributed to the development of unpleasant oxidised aromas and negatively affected the wines.

The results of the study indicate that, in the case of Riesling wine, excessive oxygen exposure due to oxygen present in the headspace of the bottle should be avoided. Moreover, iron and copper concentration also seems to make significant impact on oxygen and SO_2 consumption rates and on sensory perception of wines. These findings suggest that metals should also be taken into account, when oxygen management strategy is defined.

However, it should be noted that the results reported in the present paper represent the performance of wine samples under examination only up to three months post bottling. It is possible, that, over time, the relative performance, colour and sensory perception of the trials may change. A long-term experiment should be a subject for further research.

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CHAPTER IV

Effect of Headspace Volume, Ascorbic Acid and Sulphur Dioxide on Composition and Sensory Profile of Riesling Wine

ABSTRACT

White wine spoilage due to oxidation is a major concern during post-fermentation treatments and bottling. In this study we explored the effects of different oxygen and free SO_2 levels and ascorbic acid addition on the development of white wine. Riesling wine was bottled in 500 mL bottles with four different headspace volumes (0 mL, 10 mL, 20 mL, 30 mL), two levels of free SO_2 (50 mg/L and 70 mg/L), and with and without ascorbic acid (250 mg/L) addition. Dissolved oxygen and the oxygen in headspace were measured in the resulting 17 wines. Free and total SO_2 concentrations, ascorbic acid concentration, colour, redox potential, and antioxidative capacity were measured regularly in wine samples. After six months of storage, the wines were evaluated using sensory descriptive analysis. Both sensory and analytical results showed significant differences among the wines. Intensive wine exposure to oxygen (headspace volume) affected colour, free and total SO_2 rate, and the overall sensory quality of wine. Ascorbic acid addition had positive effect on the sensory evaluation of wines and on SO_2 levels, but combined with large headspace volumes, provoked intensive browning in wine samples.

KEYWORDS

Oxidation, ascorbic acid, sulphur dioxide, white wine

1. INTRODUCTION

Oxygen represents around 20.9 % of the atmosphere, and plays an important role in winemaking. Exposure of white wines to oxygen is normally undesirable, while it negatively affects the sensory characteristics and overall quality (Boulton et al. 1996). High oxygen additions may cause the formation of off-flavours, described as caramel, overripe fruit, rancid, honey-like, and cooked vegetables (Escudero et al. 2000; Ferreira et al. 2003).

Sulphur dioxide as an antioxidant and an inhibitor of microbiological activity is widely used in food industry and in winemaking in particular (Zoecklein et al. 1994); it protects wines from browning reactions (Boulton et al. 1996). Sulphite does not react with oxygen directly, but undergoes a chain radical reaction, and then mainly reacts with hydrogen peroxide formed during Fenton reaction, which is mainly dependent on iron and copper concentrations (Danilewicz 2007; Danilewicz and Wallbridge 2010; Elias et al. 2009). It was suggested that sulphite reacts also with quinones formed by oxidation of phenolic compounds (Danilewicz et al. 2008; Makhotkina and Kilmartin 2009). Danilewicz (2011) showed that sulphur dioxide and other nucleophiles are likely to contribute to the oxidation of phenolic compounds.

Ascorbic acid is a natural compound found in grapes in concentrations of 5-150 mg/kg (Zoecklein et al. 1994). Structurally, ascorbic acid is a L-3-ketothreo-hexuronic acid lactone containing an enediol moiety (C3-C4), which is important for its antioxidative properties (Bradshaw et al. 2011). Ascorbic acid scavenges molecular oxygen before it undergoes oxidation reactions with phenolic compounds. The reaction of ascorbic acid with molecular oxygen is thermodynamically favourable (Danilewicz 2003), although the spin restriction exists due to the triplet state of oxygen. To overcome this, interaction of transition metals or photoactive sensitizers are needed.

Ascorbic acid can also scavenge hydroxyl radicals formed in Fenton reaction and other free radicals (Bradshaw et al. 2011).

Under aerobic conditions, ascorbic acid is oxidised to dehydroascorbic acid along with hydrogen peroxide formation. Dehydroascorbic acid may convert back to ascorbic acid or may be hydrolysed to 2,3-diketogulonic acid, which undergoes further oxidation to various degradation products (Deutsch 1998). Under anaerobic conditions, dehydroascorbic acid is not formed, but ascorbic acid degrades by ring cleavage and the addition of water with furfural as a typical end product, which is reported to participate in browning reactions with wine flavonols (Es-Safi et al. 2002; Es-Safi et al. 2000).

Ascorbic acid is typically used in winemaking as an antioxidant for white wines. Skouroumounis et al. (2005) reported the addition of ascorbic acid resulted in less oxidised and more fruity aromas in Riesling and Chardonnay wines after 3 and more years of storage at 15.8°C. The authors also observed higher sulphur dioxide levels in wines, to which ascorbic acid was added at bottling, than in wines without addition.

However, it seems that ascorbic acid at low concentrations may have pro-oxidative effect in the absence of sulphur dioxide. Bradshaw et al. (2003) and Clark et al. (2008) observed enhanced coloration in model wine systems, which is probably due to metal catalysed radical reactions. Hence, ascorbic acid is commonly used together with sulphur dioxide to scavenge hydrogen peroxide and other oxidation products. Recent studies showed, that glutathione reduces *ortho*-quinones of phenolic compounds and enhances the antioxidant effect (Makhotkina and Kilmartin 2009; Sonni et al. 2011).

According to the stoichiometry of the chemical reaction, it is assumed, that one mole of free sulphur dioxide is required to scavenge hydrogen peroxide resulting from the oxidation of ascorbic acid. However, Bradshaw et al. (2004) reported a 1.7:1 ratio of consumed free SO_2 to ascorbic acid oxidised, which was higher than the stoichiometric 1:1 theoretical ratio. Barril et al. (2012) found a molar ratio of consumed free sulphur dioxide to oxygen in the presence of ascorbic acid and metal ions between 1:1 and 1.7:1. In the absence of ascorbic acid, Danilewicz et al. (2008) observed a 2:1 ratio in a wine-like model solution.

This study aimed to determine the effect of different oxygen concentrations, sulphur dioxide and ascorbic acid addition at bottling on composition and sensory properties of wine from Riesling variety.

2. MATERIALS AND METHODS

2.1. CHEMICALS AND REAGENTS

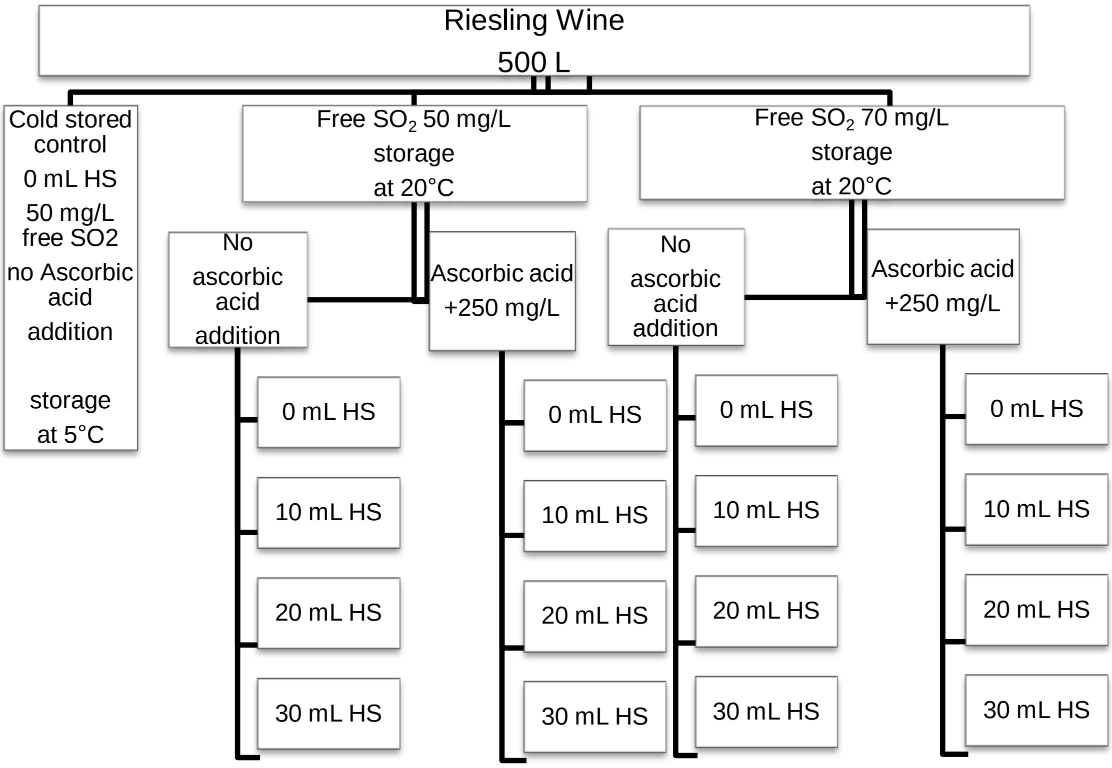
Phosphoric acid (85%), metaphosphoric acid, acetic acid (96% for analysis), methanol for analysis, ascorbic acid for analysis, potassium metabisulphite for analysis, potassium persulfate for analysis, di-potassium phosphate (anhydrous, for analysis), potassium dihydrogen phosphate for analysis, and potassium persulfate for analysis were from Merck (Darmstadt, Germany). Purified water was obtained from a Milli-Q purifier system (Millipore, Schwalbach, Germany). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (TROLOX) was from Sigma-Aldrich (Munich, Germany), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ABTS from Roche (Mannheim, Germany).

2.2. WINE SAMPLES

Wine from the 2011 vintage was made in the experimental cellar of LVWO Weinsberg, with grapes from the Württemberg region (Germany) using standard winemaking practices. The dissolved oxygen concentration in the tank prior to bottling was maintained at less than 0.2 mg/L, which was measured with HQ40d oxygen meter

(Hach Lange, Berlin, Germany). 500 L of the wine was transferred into 50 L kegs. Ascorbic acid and sulphur dioxide (as potassium metabisulphite) were added prior to bottling to generate final concentrations according to the experimental design as shown in Figure 29:

FIGURE 29: EXPERIMENTAL DESIGN SHOWING THE CONCENTRATION OF FREE SO_2, ASCORBIC ACID, AND THE HEADSPACE VOLUME IN 500 ML BOTTLES



The analysis of the main oenological parameters was performed on three bottles and the means of triplicate values are given in Table 30. The 17 sets of wine, which varied in headspace volume, ascorbic acid and free sulphur dioxide were then bottled using a manual two-way filler system (Fjord, KHW Maschinenbau, Kaiserslautern, Germany) in sterilized 500-mL “bordelaise” classic bottles and sealed under DEL-CAP GD 28–OSL-80 aluminum screw caps with saran tin foil supplied by Kork-Fabrik Arthur Linck (Delmenhorst, Germany). Racking and bottling were performed under a blanket of carbon dioxide (Lange-Gas, Lippstadt, Germany).

TABLE 30: RIESLING WINE COMPOSITION AFTER BOTTLING

	Free SO_2 50 mg/L			Free SO_2 70 mg/L	
Variable	**Cold stored control**	**No ascorbic acid added**	**Ascorbic acid added**	**No ascorbic acid added**	**Ascorbic acid added**
Reducing sugars, g/L	5.2	5.2	5.3	5.0	5.3
pH	3.2	3.2	3.2	3.2	3.2
Total phenolics, expressed in GAE mg/L	320	320	320	320	320
Total acidity (tartaric acid), g/L	5.9	5.9	5.9	5.9	5.9
Volatile acidity (acetic acid), g/L	0.5	0.5	0.45	0.5	0.45
Copper, mg/L	0.3	0.3	0.3	0.3	0.3
Iron, mg/L	0.4	0.4	0.4	0.4	0.4
Carbon dioxide, mg/L	1.1	1.1	1.1	1.1	1.1
Free SO_2, mg/L	50	50	50	67	68
Total SO_2, mg/L	149	149	151	169	168
Ascorbic acid, mg/L	ne	ne	220	ne	220

The wine with 0 HS was divided into two parts. The first part of the HS 0 bottles (further referred as “cold stored control”) were stored at 5°C in the dark and were used as a reference for analytical measurements and the sensory analysis. The chemical and sensory changes in the cold stored control were supposed to be minimal.

In the 16 other wines, oxygen concentration was set with various headspace (HS) volumes with ambient air. The bottles were stored in an upright position at constant humidity and a mean temperature of 20°C in the dark. Temperature and humidity were monitored with EBI 20-IF Temperature Data Logger (ebro Electronic, Ingolstadt, Germany). The deviation of the temperature was not more than 2°C. Measurement of gaseous and dissolved oxygen was performed twice a day during the first week after bottling. This was then repeated once every 2-3 days until all the oxygen was consumed. Analytical measurements of other parameters were performed after three and after six months of storage. Sensory evaluation took place after six months.

2.3. OXYGEN MEASUREMENTS

Dissolved and gaseous oxygen in the headspace of bottled wines were measured with the Fibox 3-Trace fiber-optic oxygen meter (PreSens, 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: 0-22 mg/L for dissolved oxygen and 0-500 hPa for gaseous and dissolved oxygen; response time ≤ 30 sec. Six bottles each fitted with two oxygen sensors 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 base of the neck of the bottle for oxygen measurement in the headspace, respectively. The manufactures 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 total consumed oxygen was calculated as a difference of the sum of dissolved and gaseous oxygen observed on the measurement and the sum of dissolved and gaseous oxygen observed on the initial measurement, plus the oxygen ingress through the closure. The oxygen transmission rate for the screw cap closure was taken from the work of Dimkou et al. (2011), namely 1 mg/L per two years, which is approximately 0.05 mg/L per month.

2.4. SULPHUR DIOXIDE MEASUREMENTS

Free and total sulphur dioxide was determined utilising the automated method, based on flow injection analysis (FIA). The analysis was conducted using FIAcompact system (Medizin- und Labortechnik Engineering GmbH Dresden, Germany) according to method accepted by OIV.

2.5. COLOUR MEASUREMENTS

Absorbance (420 nm) was measured spectrophotometrically as an indicator of oxidative browning, using a U-3010 spectrophotometer (Hitachi, Japan) coupled with a personal computer equipped with UV Solutions software (version 2.1). Cells of 10 mm path length were used for the measurements.

The wines were also analysed spectrally to obtain CIELAB colorimetric parameters (C.I.E. 1986) L*(lightness), a* (+a* red, -a* green), and b (+b* yellow, -b* blue). Overall colorimetric difference was calculated using the following formula:

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

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

2.6. REDOX POTENTIAL

The redox potential was monitored in bottled wines using a Digital pH/mV(Redox)-Meter GPHR 1400 (Greisinger Electronic, Dietenhofen, Germany) coupled with Ag/AgCl electrode filled with a recommended 3 M KCL solution, by immerging the electrode into the wine bottles for 5 min. Before measurement the electrode reading was verified with a standard GE 100 solution having a redox potential of 228 mV at 20°C. The pH was measured using a pH meter coupled with Titrando 808 (Metrohm, Herisau, Switzerland) equipped with Tiamo Software (version 1.3).

2.7. ANTIOXIDATIVE CAPACITY (TEAC)

Measurements of antioxidative capacity was performed as detailed previously by Pour Nikfardjam (2001) using the Arena 30 Photometric Analyzer (Thermo Fischer, Vantaa, Finland). Potassium persulphate was used as catalyser. Wine samples were diluted with PBS buffer solution 1:10 beforehand, according to the Folin Ciocalteu method for measuring the concentration of phenolic compounds.

2.8. ASCORBIC ACID

Ascorbic acid was determined on a Merck-Hitachi (Darmstadt, Germany) HPLC system, consisting of an L-6200 pump, an AS 2000 A autosampler, a T-6300 column thermostat, an L-4250 UV/VIS detector, and a D-7000 interface, controlled by D-7000 HSM software. Separation was performed on a Lichrocart 125-4 with Lichrospher 100 RP-18 column (5 µm particle size), equipped with a Lichrocart 4-4 (NH_2) precolumn (5 µm particle size). Solvents: A – water, B – methanol and C – 0.005 M KH_2PO_4 of pH 2.3; the gradient system is given in the Table 31. Injection volume was maintained at 20 µL; the detection wavelength at 260 nm. 40 mg of L(+)-ascorbic acid (≥99.8%) was dissolved in a 100 mL volumetric flask to make a stock solution (400 mg/L). For calibration, working solutions with concentrations of 10, 20, 40, 80, and 120 mg/L were prepared by taking 2.5, 5, 10, 20, 30 mL of stock solution, respectively, adding 2 mL of a buffer solution and filled to 100 mL with purest water in 100-mL volumetric flasks. For buffer solution 1.5 g HPO_3 and 4 mL of acetic acid were diluted with water in a 50-mL volume flask. The wines were diluted with water 1:2 in 100-mL volumetric flasks with addition of 2 mL of buffer solution, and after membrane filtration (0.2 µm) were directly injected into the HPLC system. All standard solutions (stock solution and working solutions) were prepared on the measurement day and stored cold in a refrigerator prior to injection.

TABLE 31: ELUTION GRADIENT FOR MEASUREMENT OF ASCORBIC ACID

Time, min	%A	%B	%C
0	50	50	0
5	50	50	0
20	100	0	0
40	100	0	0
60	0	0	100
90	0	0	100
120	50	50	0

2.9. SENSORY EVALUATION

Aroma and flavour attributes of the 17 wines were evaluated in four independent sessions of sensory analysis six months after bottling in the tasting lab of the LVWO Weinsberg equipped with FIZZ sensory software (Biosystèmes, Couternon, France, version 2.47). The evaluation of the wine samples was performed in isolated booths, under white lightening, with a randomized presentation order across the panellists. The panellists (n1=22; n2=23; n3=28; n4=18) had previous wine sensory experience and were additionally trained to understand the task, the vocabulary, and to work with the software. Two bottles per treatment were randomly selected and assessed. A Latin square design was used to randomize the sample presentation across the assessors. Wines were served cold in clear tulip-shaped Sensus glasses.

Unstructured scales from “low” to “high” as extreme points were used for evaluation of aroma and flavour attributes. The assessors were asked to rate the wine samples for the following aroma attributes: general fruitiness, citrus, peach, green apple, oxidised apple, honey, acetaldehyde, untypical aging and SO_2. For all aroma attributes, accept for fruitiness, the standard aromas were prepared (Table 32). “Cold stored control” wine was used as the reference wine for aroma standards. For acetaldehyde and untypical aging (UTA) attributes, three training sessions were held before each tasting as follows:

1. Three wine samples with defined concentration of acetaldehyde: no acetaldehyde, middle addition (40µL/ L) and high addition (80µL/L).
2. Three wine samples for the attribute “untypical aging” with defined concentration of 2′-aminoacetophenone (AAP): no AAP, middle addition (20µL/L) and high addition (40µL/L).
3. Four wine samples with both acetaldehyde and UTA: no acetaldehyde/no UTA, no acetaldehyde/UTA (20 µL/L of AAP), acetaldehyde/no UTA (40µL/L of acetaldehyde), acetaldehyde/UTA (40 µL/L of acetaldehyde and 20 µL/L of AAP).

TABLE 32: COMPOSITION OF SENSORY REFERENCE STANDARDS FOR THE AROMA DESCRIPTIVE ANALYSIS OF RIESLING

Aroma	**Reference standard**
Citrus	lemon zest, soaked overnight in wine and removed
Peach	canned peaches, soaked in wine
Green Apple	fresh Granny Smith apple cut in pieces, soaked in wine and removed
Oxidised Apple	bruised apple, soaked overnight in wine and removed
Honey	1 tsp of flower honey added to 1 L wine
Acetaldehyde, „Sherry“	acetaldehyde, 40 µL in 1 L wine
Untypical Aging (UTA)	2′-Aminoacetophenone, 20 µL in 1 L wine
SO_2	potassium bisulphite added to wine

Six attributes were assessed for flavour: mouthfeel, acidity, freshness, oxidation rate (negative oxidation attribute), ripeness (positive oxidation attribute) and overall quality.

Statistical analysis. Analysis of variance (ANOVA) using Fisher's least significance difference method with a 95% confidence level and PCA analysis of the data were carried out to find out the impact of the headspace volume, free SO_2 concentration, and ascorbic acid addition on the wine quality. The analysis was conducted using XLSTAT Software Version 2011.2.08 (Addinsoft, Andernach, Germany).

3. RESULTS AND DISCUSSION

3.1. OXYGEN CONSUMPTION

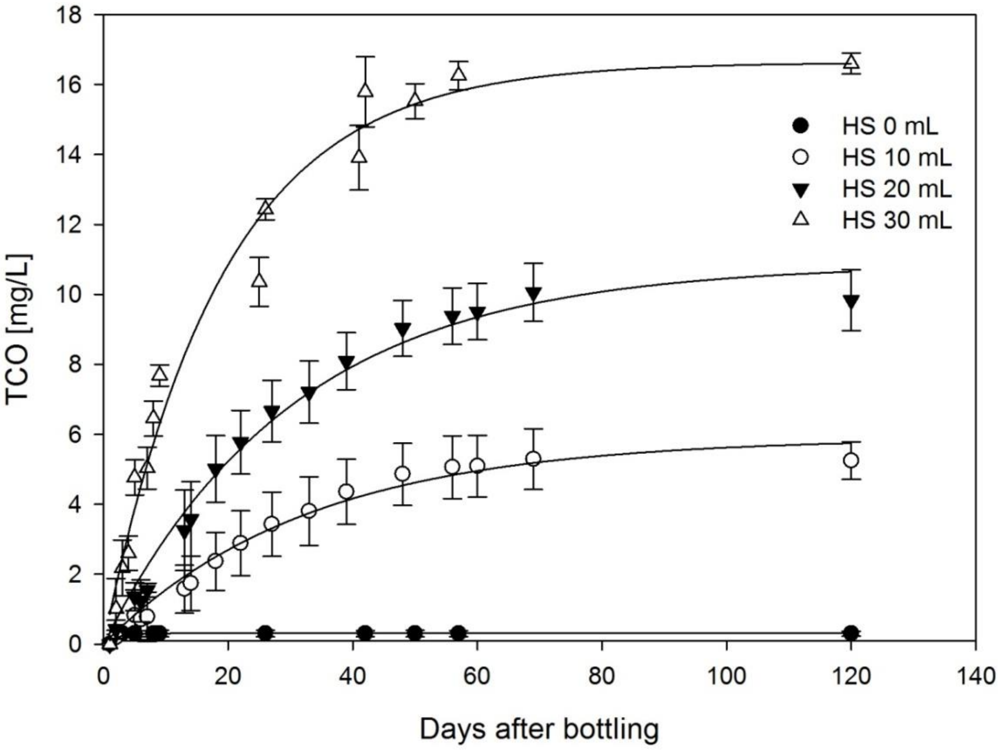
The evolution of total consumed oxygen (TCO) in the wine samples with different HS volumes (0, 10, 20, and 30 mL) in 500-mL bottles is shown in Figure 30. As the dissolved oxygen concentration generally was ≤ 0.2 mg/L, the total oxygen concentration in bottles varied due to the initial HS volume. Over time, the HS oxygen was diffusing into the wine and was consumed through chemical reactions with the wine constituents. After approximately 120 days of storage, oxygen became undetectable in all wine samples; thus the initial amount of oxygen in the bottles was fully consumed.

In the wines with 0 mL HS, the dissolved oxygen concentration after bottling was 0.3±0.1 mg/L. In this case, it was equal to the total consumed oxygen, as the bottles had no headspace with ambient air. The total amount of oxygen was completely consumed after 3 days of storage. No further increase of dissolved oxygen in these wines was observed.

In the wines with 10, 20, and 30 mL HS per 500 mL, the calculated total consumed oxygen (TCO) after 120 days of storage were 5.2±0.5, 9.8±0.8, and 16.6±0.3 mg/L, respectively. More than 55% of the initial oxygen in these bottles was consumed during the first 22 days. Recent studies showed low oxygen transmission rate (OTR) of the screw caps used, compared to other closures (Godden et al. 2005; Kwiatkowski et al. 2007; Lopes et al. 2009). Taking into consideration the screw cap OTR measured by Dimkou et al. (2011), the amount of oxygen ingressing through the closure was relatively low (~0.2 mg in four months) compared to the headspace and dissolved oxygen. However, by the stoppers with higher OTR levels, the impact could be more significant.

ANOVA analysis showed that the oxygen consumption rate well correlated with the headspace volume ($p<0.01$) On the contrary, no significant differences were observed concerning both different sulphur dioxide concentrations and ascorbic acid addition.

FIGURE 30: EVOLUTION OF TOTAL CONSUMED OXYGEN CONCENTRATION IN WINE SAMPLES WITH DIFFERENT HS VOLUMES



3.2. SULPHUR DIOXIDE

Under wine conditions, sulphur dioxide (SO_2) mainly exists in a form of bisulphite ion (Danilewicz 2003). It is known to react principally with hydrogen peroxide formed when ethanol and phenolic compounds are oxidised in the presence of iron and copper. The bisulphite may also react with quinones and reduce them to the original state (Boulton et al. 1998). Consequently, the free SO_2 concentration typically decreases during storage. The free SO_2 concentration in the treatments was shown to be dependent on both the oxygen ingress at bottling and the addition of ascorbic acid. Measurements of free sulphur dioxide showed good correlation between free SO_2 loss and the TCO (Figure 31), which is in agreement with the results previously reported by Dimkou et al. (2011).

Additionally, sulphite oxidation continued after the headspace oxygen and dissolved oxygen were fully consumed, which could be a consequence of the free radical reactions reported by Danilewicz et al. (2008).

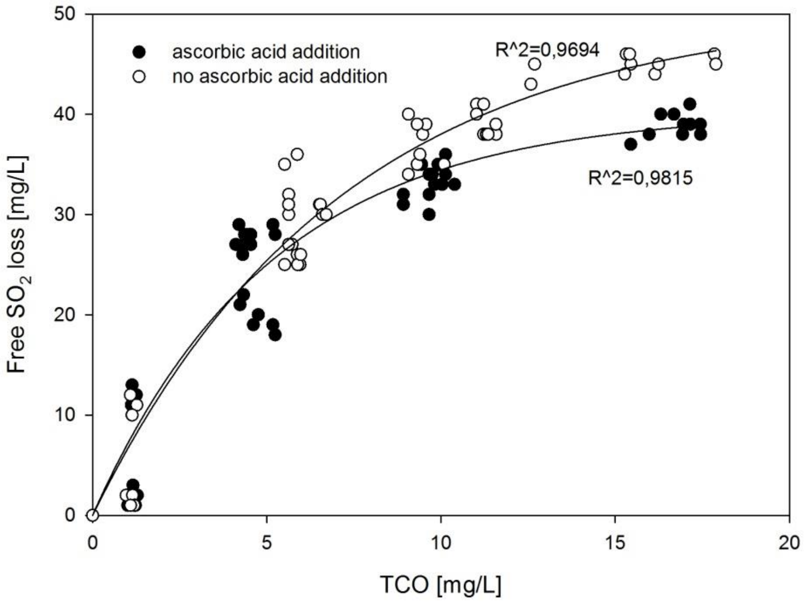
Changes in the free SO_2 concentration were also observed in the wines with HS 0 (TCO 0.3 mg/L). At the end of the observation period, free SO_2 in these wines decreased by 10 mg/L, which may be due to sulphite autoxidation (Danilewicz 2011) or oxygen ingress through the screw cap (0.3 mg in six months). In the wines enriched with ascorbic acid, loses of free SO_2 could also be a consequence of anaerobic degradation of ascorbic acid, observed by Wallington et al. (2013). The “cold stored control” wine, stored at 5°C, preserved 95% of the initial free SO_2, indicating that the oxidation processes occur very slowly at low temperatures. Consequently, storage at low temperature seems to be the key factor to extend the shelf-life of white wines.

When comparing the wines bottled with and without ascorbic acid addition, the presence of ascorbic acid obviously influenced the sulphite consumption. In the wines with relatively low TCO ($\leq$ 2 mg/L) the addition of ascorbic acid did not show a significant influence on the loss of free SO_2. However, when the TCO increased, ascorbic acid had a greater impact on the loss of SO_2 (Figure 3). The analysis of total SO_2 during storage also showed significantly lower losses in the wines with ascorbic acid addition (data not shown). The calculated mean molar reaction ratio O_2:SO_2 in the wines with no ascorbic acid addition was 1:1.2, and in presence of ascorbic acid it was observed to be as low as 1:0.8. The results indicate that the addition of ascorbic acid may significantly reduce the consumption of SO_2.

It was observed, that the O_2:SO_2 molar ratio in the wines with HS 10, 20, and 30 mL was not consistent over the storage period. In the first week, it tended to be higher than the theoretical stoichiometric 1:2 value, sometimes reaching 1:5. This may indicate that sulphite reacted not only with hydrogen peroxide, but also with quinones and the products of ascorbic acid degradation. Apparently, sulphite oxidation during the initial phase seems to be dependent on the oxygen availability in the system.

In summary, the observation of SO_2 in the studied wines showed that its loss increased with greater TCO. However, the wines with ascorbic acid addition preserved more free and total SO_2. The rate of sulphite consumption was not the same over the storage period and continued after all oxygen was consumed, which could be associated with the ingress of small amounts of oxygen through the screw cap.

FIGURE 31: FREE SULPHUR DIOXIDE LOSS IN WINE SAMPLES WITH/WITHOUT ASCORBIC ACID ADDITION DEPENDING ON TOTAL CONSUMED OXYGEN LEVEL (FIA); N=180

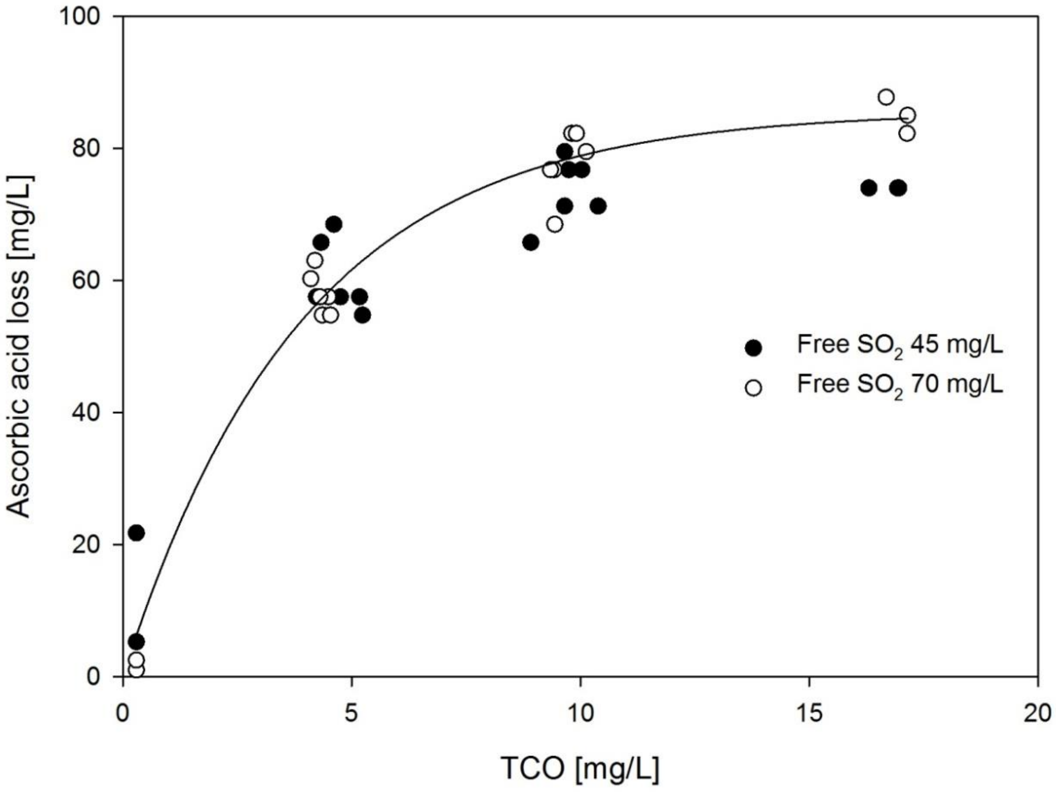


3.3. ASCORBIC ACID

The loss of ascorbic acid in the wine samples, presented in Figure 32, shows, that the increase of TCO had a significant effect on the loss of ascorbic acid. Importantly, the differences in free SO_2 did not greatly influence the ascorbic acid decay. The results obtained in the experiment indicate that higher oxygen ingress at bottling leads to higher consumption of ascorbic acid during storage. The gathered data allowed the calculation of the molar ratios of the total consumed oxygen to consumed ascorbic acid (AA). For the wines with HS 10, 20, and 30 mL in 500-mL bottles, the O_2: AA molar ratios were 1:2.39, 1:1.42, and 1:0.86, respectively.

The wine samples with 0 mL HS (0.3 mg/L TCO) also showed the ascorbic acid decay of 20.7±4 mg/L after 180 days of storage at 20°C. This indicates the process of ascorbic acid degradation by a non-oxidative mechanism, also observed by Wallington et al. (2013). At low oxygen conditions or when the total package oxygen has been consumed, the ascorbic acid may undergo degradation with formation of furfural and other products that may cause browning in white wines (Bradshaw et al. 2011). However, the non-oxidative degradation may significantly affect the wine quality only, when the wines are stored at temperatures higher than 45°C (Wallington et al. 2013), which is normally unlikely for white wine storage.

FIGURE 32: ASCORBIC ACID LOSS IN THE WINE SAMPLES BOTTLED WITH 45 MG/L AND 70 MG/L OF FREE SO_2 DEPENDING ON THE TOTAL CONSUMED OXYGEN (TCO), MG/L (N=70).



3.4. COLOUR CHANGES

The studied wine samples showed significant colour differences during the storage due to different treatments. Three-way ANOVA analysis (Table 33) showed an impact of HS volume, free SO_2 level, and ascorbic addition on colour parameters after three and six months of storage. Oxygen concentration, ascorbic acid addition, and the combination of these factors had great influence on absorbance 420 values and on the CIELAB parameters already after three months of storage. Also the combination of the factors SO_2 and HS volume was shown to influence the white wine colour.

TABLE 33: RESULTS OF A THREE-WAY ANOVA ANALYSIS OF THE COLOUR DATA OF WINE SAMPLES AFTER 90 AND 180 DAYS OF STORAGE, USING 'SO_2', 'AA' AND 'HS VOL' AS FIXED FACTORS ($P<0.05$).

Factor		**Day 90**				**Day 180**			
		E420	**L***	**a***	**b***	**E420**	**L***	**a***	**b***
SO_2	F	16.026	0.856	2.793	33.944	12.556	11.880	13.389	16.469
	P	0.000	0.359	0.100	< 0.0001	0.003	0.003	0.002	0.001
AA	F	242.706	11.613	634.994	1489.5	42.202	5.011	241.841	56.395
	P	< 0.0001	0.001	< 0.001	< 0.0001	< 0.001	0.040	< 0.001	< 0.001
HS	F	91.368	9.665	109.023	221.302	40.260	20.229	76.834	54.268
	P	< 0.0001	< 0.001	< 0.001	< 0.0001	< 0.001	< 0.001	< 0.001	< 0.001
SO_2*AA	F	8.656	0.020	0.568	2.654	0.136	4.102	1.414	0.972
	P	0.005	0.888	0.454	0.109	0.717	0.060	0.252	0.339
SO_2*HS	F	9.053	2.405	2.841	20.761	2.812	6.201	2.047	4.282
	P	< 0.0001	0.077	0.046	< 0.0001	0.073	0.005	0.148	0.021
AA*HS	F	11.274	7.195	91.172	186.303	5.588	4.856	8.162	8.394
	P	< 0.0001	0.001	< 0.001	< 0.001	0.008	0.014	0.002	0.001
SO_2*AA*HS	F	0.473	3.037	5.390	2.369	0.645	2.012	0.951	0.481
	P	0.702	0.036	0.002	0.080	0.597	0.153	0.439	0.7

Absorbance at 420 nm was measured in wine samples on the day of bottling and after 90 and 180 days after bottling. The measured value after bottling in all wine samples was 0.062±0.002. The cold stored control wine did not present colour changes over the observed storage period (0.06±0.001).

Colour differences between wine samples stored at 20°C could already be observed after 90 days of storage. The average values of measured absorbance in the wines with HS 0 and HS 10 without ascorbic acid addition was 0.063±0.002, which indicates almost no change in colour compared to the cold stored control.

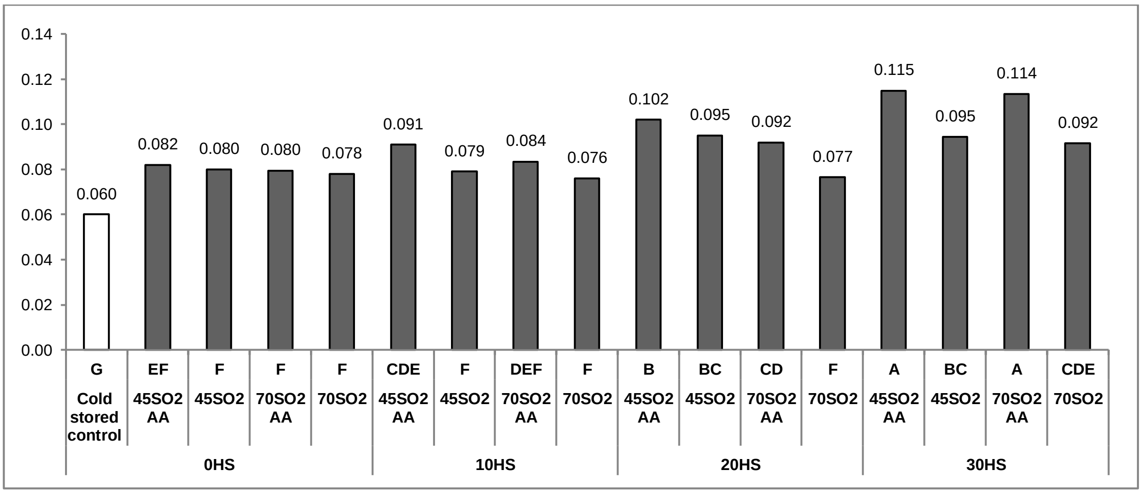
The antioxidative activity of ascorbic acid is mainly based on its ability to scavenge molecular oxygen. However, when ascorbic acid is oxidised to dehydroascorbic acid, the generated hydrogen peroxide may promote the oxidation processes in wine. This pro-oxidative effect was reported by Bradshaw et al. (2011).

Indeed, all wines with HS 20 and 30 with ascorbic acid addition showed more enhanced colouration (0.07-0.08±0.003) in comparison to those, where no ascorbic acid was added (0.068±0.003). The highest average value of 0.081±0.001 was observed in the wines with the biggest headspace volume (HS 30) and with ascorbic acid addition.

After 180 days of storage, the differences in colour greatly increased. The absorption values of the wines with HS 0 were higher compared to the cold stored control wine, which is presumably the effect of higher storage temperature (20°C). Higher oxygen ingress at bottling coupled with the addition of ascorbic acid led to intensive browning of wine (Figure 33). Higher level of free SO_2 (70 mg/L) could diminish this effect, but could not completely scavenge free radicals and stop oxidative browning. The lowest browning of wines with HS 10 and 20 were observed in the wines with 70 mg/L free SO_2 without ascorbic acid addition. This was the same as for the wines without headspace, which shows the hydrogen peroxide scavenging capacity of sulphite. Peng et al. (1998) and Chinnici et al. (2013) also observed at equal ascorbic acid addition less browning in wine samples with higher free SO_2. However, the key role seems to be the oxygen ingress at bottling.

All wines with HS 30 (16 mg/L TCO) showed intensive browning enhanced by the addition of ascorbic acid.

FIGURE 33: AVERAGE E420 VALUES IN WINE SAMPLES WITH 0, 10, 20 AND 30 ML HS INM 500 ML BOTTLES AFTER SIX MONTHS OF STORAGE (N=3): AA=ASCORBIC ACID; ANOVA: MEANS WITH DIFFERENT LETTER(S) ARE SIGNIFICANTLY DIFFERENT (P < 0.05) USING FISCHER'S LSD.



To additionally study the colour changes, CIELAB parameters were measured. Figure 34 shows the distribution of wine samples grouped according to the time of storage and addition of ascorbic acid in the L*a*b*colour plot. The axes represent the green-red (-a*+a*), blue-yellow (-b*+b*) colour hue, and lightness (L*) of measured wines. During storage the colour of the stored wines changed towards more yellowish tone. The lightness decreased, which indicates the darker coloration. Colour changes can clearly be observed after only 90 days of storage. These were caused by the addition of ascorbic acid. This is consistent with the results of Barril et al. (2009) suggesting the formation of yellow pigments by a mechanism involving xylosone, a product of ascorbic acid degradation. However, the differences between treatments increased with increasing storage time due to varying initial headspace volume. Analysis of variance confirms the great impact of ascorbic acid and high oxygen ingress on the browning of wine samples (Table 34). The increased browning was observed in the wines with larger headspace volumes and with added ascorbic acid.

FIGURE 34: 3D DIAGRAM OF CIELAB VALUES OF ALL MEASURED WINE SAMPLES WITH (AA) OR WITHOUT (NO AA) ASCORBIC ACID AT THE DAY 1, 90 AND 180 AFTER BOTTLING (N=184)

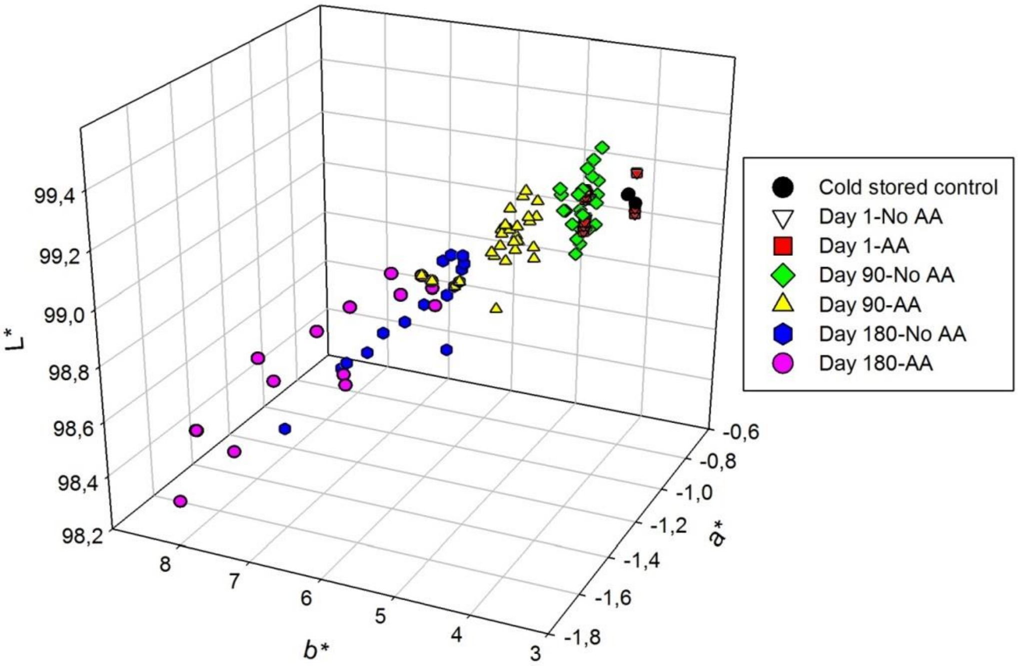


TABLE 34: MEAN CIELAB VALUES FOR WINE SAMPLES AT THE DAYS 1, 90 AND 180 AFTER BOTTLING; ANOVA (*): MEANS WITH DIFFERENT LETTER(S) ARE SIGNIFICANTLY DIFFERENT ($P < 0.05$) USING FISCHER'S LSD. OVERALL COLORIMETRIC DIFFERENCE (**ΔE_I**) WAS CALCULATED AS **ΔE_I=**$\sqrt{(L_i - L_j)^2 + (b_i - b_j)^2 + (a_i - a_j)^2}$

	Day 1						Day 90							Day 180						
Wine	L	*	a	*	b	*	L	*	a	*	b	*	ΔE_{90}	L	*	a	*	b	*	ΔE_{180}
Control	99.09	A	-0.72	A	4.04	A	99.09	A	-0.72	A	4.04	G	0.00	99.12	BCD	-0.72	A	4.16	K	0.12
0HS/45/AA	99.18	A	-0.90	A	4.42	A	99.18	ABC	-0.90	DEF	4.42	EF	0.00	99.02	AB	-1.31	C	5.73	HI	1.38
0HS/45	99.17	A	-0.89	A	4.36	A	99.17	CDEF	-0.89	B	4.36	EF	0.00	98.84	AB	-1.17	C	5.66	HI	1.37
0HS/70/AA	99.20	A	-0.91	A	4.39	A	99.19	ABCD	-0.91	DE	4.40	EF	0.01	98.94	AB	-1.28	C	5.68	HI	1.37
0HS/70	99.05	A	-0.88	A	4.43	A	99.05	ABCD	-0.88	BC	4.43	EF	0.00	98.94	CDE	-1.18	C	5.59	GH	1.20
10HS/45/AA	99.13	A	-0.87	A	4.36	A	99.13	EFG	-1.08	H	4.97	CD	0.65	98.71	BC	-1.48	E	6.47	D	2.24
10HS/45	99.13	A	-0.87	A	4.36	A	99.18	BCDE	-0.97	BCD	4.52	EF	0.20	98.90	AB	-1.23	D	5.77	G	1.48
10HS/70/AA	99.13	A	-0.87	A	4.36	A	99.12	ABC	-1.05	FG	4.81	DE	0.50	99.02	BC	-1.38	E	5.97	E	1.70
10HS/70	99.13	A	-0.87	A	4.36	A	99.26	AB	-0.98	BCD	4.22	F	0.22	99.07	A	-1.24	D	5.37	J	1.08
20HS/45/AA	99.13	A	-0.84	A	4.29	A	99.04	DEFG	-1.07	I	5.25	B	1.00	98.76	CDE	-1.59	E	7.28	B	3.10
20HS/45	99.13	A	-0.84	A	4.29	A	99.02	FG	-0.84	FG	4.60	BC	0.33	98.64	DE	-1.36	B	6.77	F	2.58
20HS/70/AA	99.13	A	-0.84	A	4.29	A	99.06	ABCD	-1.07	H	5.09	C	0.84	98.93	CD	-1.49	E	6.57	C	2.38
20HS/70	99.13	A	-0.84	A	4.29	A	99.16	AB	-0.88	CDE	4.34	EF	0.08	99.07	B	-1.26	C	5.56	I	1.34
30HS/45/AA	99.18	A	-0.90	A	4.42	A	98.94	G	-1.18	J	5.60	A	1.23	98.56	E	-1.69	F	8.01	A	3.72
30HS/45	99.17	A	-0.89	A	4.36	A	99.19	FG	-0.91	G	4.40	C	0.05	98.70	AB	-1.39	C	6.70	HI	2.44
30HS/70/AA	99.20	A	-0.91	A	4.39	A	98.99	H	-1.26	IJ	5.66	A	1.34	98.36	DE	-1.64	G	8.04	A	3.82
30HS/70	99.05	A	-0.88	A	4.43	A	99.14	DEF	-0.90	EFG	4.41	CD	0.10	98.77	BC	-1.33	C	6.39	HI	2.03

3.5. REDOX POTENTIAL AND ANTIOXIDATIVE CAPACITY

Redox potential is often applied to reversible systems that reached equilibrium, and is defined with respect to single charge carrier (Tomlinson and Kilmartin 1997). In an unstable system with many interacting compounds, like wine, the redox potential may indicate a mixed potential due to many systems. The transition of the inorganic and organic compounds from reduced state to oxidised and *vice versa* is known to be the function of the redox potential of the medium, composition of the product, and product variables, such as pH and dissolved oxygen (Nojeim et al. 1985). In our experiment, all wines were originally the same wine with the same oenological parameters and pH 3.4. Due to the fact that the measurements were performed at the same temperature and light conditions, the results can be compared.

The evolution of the oxidation reduction potential (ORP) is shown in Table 35. All wines with ascorbic acid addition showed lower values of the ORP. Peng et al. (1998) also observed a decrease of the measured redox potential with the addition of ascorbic acid. Türke et al. (2012) also showed the influence of ascorbic acid addition on the electrochemistry of pH 3.3 model wine using cyclic voltammetry.

The cold stored control wine showed the same value of the measured ORP after 180 days of storage. In the other wines the ORP values decreased. However, these results are difficult to interpret as the redox potential cannot be considered a reliable indicator of oxidation processes.

TABLE 35: MEAN VALUES OF THE OXIDATION-REDUCTION POTENTIAL (ORP) IN THE WINES AFTER BOTTLING (DAY 1) AND OVER 6 MONTHS OF STORAGE (N=3). THE VALUES IN MV WERE OBTAINED ADDING 211 MV TO GIVE VALUES VS. STANDARD HYDROGEN ELECTRODE (SHE)

	Day 1	±S.D.	Day 180	±S.D.
Cold stored control	353 b*	2	361 d	9
0HS/45/AA	304 a	2	269 a	10
0HS/45	356 b	2	287 b	8
0HS/70/AA	299 a	2	278 ab	12
0HS/70	340 ab	2	323 c	3
10HS/45/AA	303 a	2	295 b	1
10HS/45	425 c	5	370 d	3
10HS/70/AA	303 a	2	268 a	1
10HS/70	424 c	5	349 cd	1
20HS/45/AA	303 a	2	282 ab	1
20HS/45	426 c	5	357 cd	4
20HS/70/AA	303 a	2	266 a	2
20HS/70	426 c	5	349 cd	1
30HS/45/AA	304 a	1	277 ab	13
30HS/45	356 b	1	343 cd	1
30HS/70/AA	299 a	4	287 b	1
30HS/70	340 ab	4	314 c	5

*means with different letter(s) are significant ($p<0.05$)

Measured values of the antioxidative capacity (TEAC) after bottling and after six months of storage were consistent with ORP measurements (Table 36). Wines with added ascorbic acid had higher TEAC values than without. Decrease of TEAC values was observed after 180 days of storage in all wines. The cold stored control wine only showed minor changes (-0.09 mmol/L). In other wine samples, the decrease of antioxidative capacity was correlated with the total consumed oxygen. In groups with the same headspace volume, the wines with ascorbic acid addition showed greater decline of TEAC value, and the wines with higher free SO_2 decreased less.

TABLE 36: AVERAGE VALUES OF ANTIOXIDATIVE CAPACITY (TEAC) ON THE DAY 1 AND 180 (N=6) AND THE DIFFERENCE (Δ), MMOL/L

	Day 1	±S.D.	Day 180	±S.D.	Δ
Control	2.88 a*	0.01	2.79 b	0.01	-0.09
0HS/45/AA	3.64 b	0.02	3.35 e	0.07	-0.29
0HS/45	2.91 a	0.01	2.70 b	0.00	-0.21
0HS/70/AA	3.70 b	0.02	3.40 e	0.01	-0.30
0HS/70	2.88 a	0.04	2.70 b	0.01	-0.18
10HS/45/AA	3.66 b	0.03	3.16 d	0.06	-0.51
10HS/45	2.89 a	0.03	2.48 a	0.01	-0.41
10HS/70/AA	3.66 b	0.03	3.15 d	0.01	-0.51
10HS/70	2.89 a	0.03	2.56 a	0.01	-0.33
20HS/45/AA	3.68 b	0.03	3.06 cd	0.03	-0.62
20HS/45	2.89 a	0.03	2.46 a	0.01	-0.43
20HS/70/AA	3.68 b	0.03	3.08 cd	0.02	-0.60
20HS/70	2.89 a	0.03	2.55 a	0.05	-0.35
30HS/45/AA	3.64 b	0.02	2.95 c	0.07	-0.69
30HS/45	2.91 a	0.01	2.55 a	0.07	-0.36
30HS/70/AA	3.70 b	0.02	3.15 d	0.21	-0.55
30HS/70	2.88 a	0.04	2.50 a	0.01	-0.38

*means with different letter(s) are significant ($p<0.05$)

3.6. SENSORY ANALYSIS

For each analysis time point, interactions between attributes and repetition between the wines did not show any significant impact on sensory parameters, which indicates, that the panel repeatability was good.

The ANOVA analysis revealed significant differences between the "cold stored control" wine stored at 5°C and other wine samples stored at 20°C. For wines stored at

20°C, all fruit-related aroma attributes (fruitiness, citrus, peach and green apple) as well as general freshness decreased significantly ($p<0.05$), while the oxidised apple, honey, sherry and untypical aging aromas and flavours were significantly higher in these wine samples compared to the “cold stored control” wine. This is consistent with the results of other authors confirming the negative effects of storage at high temperatures on the wine quality (D’Auria et al. 2009; Dias et al. 2012; Hopfer et al. 2013). For a better understanding of the influence of the target discriminating factors, further statistical analysis is shown only for the samples stored at 20°C, to eliminate the effect of the storage temperature.

Analysis of variance (Table 37) clearly showed that the overall the sensory profile of wines was significantly influenced by the headspace volume and the addition of ascorbic acid.

The headspace volume correlates positively with all the oxidised attributes, such as oxidised apple, honey, acetaldehyde/„Sherry“, untypical aging, oxidation degree, and ripeness. Conversely, the wines with lower HS volume showed higher scores in fruity, citrus, peach, green apple, mouth feel, freshness and quality.

On average, wines with ascorbic acid addition were found to be significantly higher in the perceived intensity of fruity, citrus, peach, and green apple aromas, and significantly lower in intensities of oxidised apple, honey, and “sherry” aromas. Moreover, the wines with ascorbic acid addition were shown to be less oxidised and less ripe on the palate, fresher and generally of higher quality, compared to those with no ascorbic acid addition.

The differences in the SO_2 content were not large enough to give the wines significantly different sensory properties. However, the wines with 70 mg/L SO_2 had lower intensities of oxidised apple and honey.

TABLE 37: THE DATA PRESENTS THE RESULTS OF THREE-WAY ANOVA ANALYSIS OF THE SENSORY DATA USING 'HS VOLUME', 'ASCORBIC ACID' AND 'SO_2' AS FIXED FACTORS (N=1388). ALL INTERACTIONS OF THE THREE FACTORS WERE ADJUSTED FOR MULTIPLE TESTING USING FISHER'S LSD. VALUES THAT ARE STATISTICALLY DIFFERENT ARE MARKED IN BOLD.

	Effect of headspace volume						**Effect of ascorbic acid**				**Effect of SO_2**			
Parameter	F	Pr > F	HS 0	HS 10	HS 20	HS 30	F	Pr > F	No ascorbic acid	Ascorbic acid added	F	Pr > F	45 mg/L	70 mg/L
Fruitiness	21.1	**< 0.0001**	2.16	2.17	2.17	1.50	13.4	**< 0.0001**	1.89	2.10	0.01	0.91	1.99	2
Citrus	28.5	**< 0.0001**	1.99	1.75	1.71	1.13	44.4	**< 0.0001**	1.45	1.84	1.40	0.24	1.6	1.69
Peach	7.8	**< 0.0001**	1.68	1.64	1.50	1.26	11.7	**0.001**	1.42	1.62	0.00	0.96	1.52	1.52
Green Apple	25.2	**< 0.0001**	2.00	1.48	1.55	1.19	44.7	**< 0.0001**	1.36	1.75	1.60	0.21	1.51	1.6
Oxidised Apple	11.1	**< 0.0001**	1.70	1.98	1.96	2.32	52.7	**< 0.0001**	2.23	1.75	6.36	**0.01**	2.08	1.9
Honey	2.8	**0.041**	1.85	1.90	1.83	2.12	36.8	**< 0.0001**	2.14	1.71	3.95	**0.047**	2	1.85
Acetaldehyde „Sherry“	20.9	**< 0.0001**	1.54	1.72	1.75	2.35	86.7	**< 0.0001**	2.13	1.55	1.51	0.22	1.89	1.79
Untypical Aging	12.5	**< 0.0001**	1.54	1.83	1.83	2.24	78.4	**< 0.0001**	2.16	1.55	1.05	0.31	1.9	1.81
SO_2	26.9	**< 0.0001**	1.57	1.27	1.05	1.11	16.6	**< 0.0001**	1.16	1.34	1.99	0.16	1.21	1.29
Mouthfeel	2.4	0.067	2.30	2.35	2.39	2.18	12.1	**0.001**	2.22	2.39	0.67	0.41	2.32	2.29
Acidity	4.0	0.008	2.15	2.19	2.08	1.92	10.2	**0.001**	2.01	2.16	0.58	0.45	2.06	2.1
Freshness	19.8	**< 0.0001**	2.34	1.91	1.89	1.64	134.2	**< 0.0001**	1.61	2.28	2.32	0.13	1.9	2
Oxidation degree	36.0	**< 0.0001**	1.92	2.59	2.71	2.79	123.1	**< 0.0001**	2.83	2.17	0.13	0.72	2.52	2.48
Ripeness	15.2	**< 0.0001**	2.37	2.69	2.78	3.00	82.1	**< 0.0001**	2.97	2.45	0.06	0.80	2.72	2.7
Quality	29.1	**< 0.0001**	2.05	1.65	1.57	1.54	88.8	**< 0.0001**	1.52	1.89	0.69	0.41	1.68	1.72

The main principal component graph summarizing SO_2, colour and sensory changes in wines is shown in Figure 35. The first two principal components retained 87% of the variance, most of which (78.09%) was accounted by the F1. The first principal component was loaded mainly with the sensory attributes. The second axis (8.91%) resulted mostly from the colour changes in the examined wines.

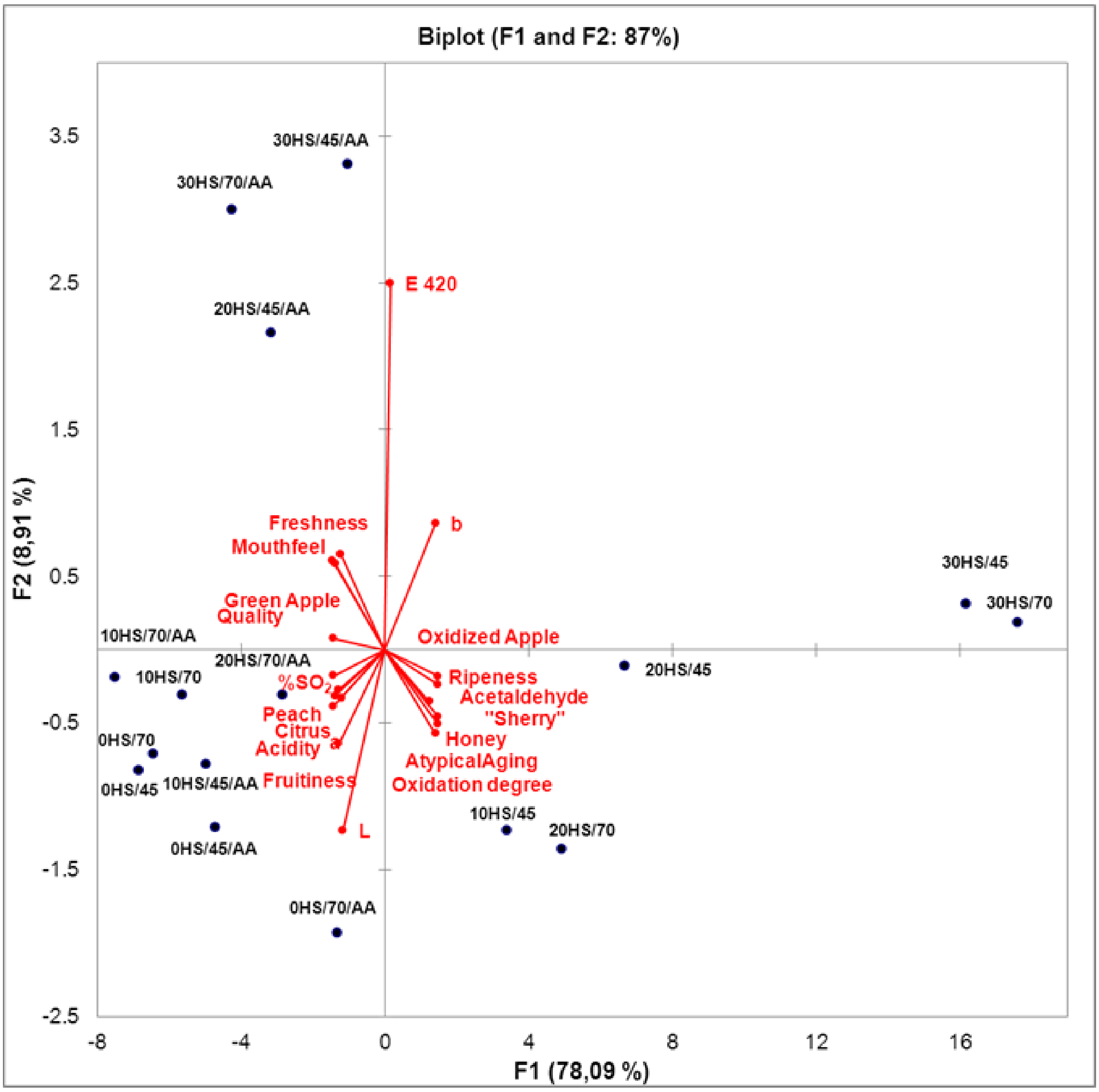
The relative positioning of the experimental wines (Figure 35) comes into agreement with the results of the analysis of variance presented in Table 37. The wines with small headspace volumes and all wines with ascorbic acid addition with high intensities of fruity, citrus, peach, green apple aromas and also with high scores in freshness, acidity and preference, are positioned on the negative side of the F1. The observed higher level of free SO_2 in these wines also contributed to their relative positioning.

On the contrary, wines without ascorbic acid addition with HS 20 (20HS/45, 20HS/70) and 30 (30HS/45, 30HS/70) were loaded on the positive side of the first principal component. Moreover, the wine with HS 10 and lower free SO_2 content (45 mg/L) was also positioned on the positive side of the F1, which shows that the amount of SO_2 seems not to be sufficient to protect the wine from negative sensory changes.

Colour changes in some wines with ascorbic acid added the second dimension to the PCA graph. The wines with ascorbic acid addition having HS 30 (30HS/45/AA, 30HS/70/AA) and the HS 20 with lower concentration of SO_2 (20HS/45/AA) showed significant colour changes and are located on the positive side of the second axis, which indicates white wine browning.

In summary, collected sensory data corresponds to the results of the chemical analysis, indicating positive effects of ascorbic acid addition on the free and total sulphur dioxide levels, sensory character and overall redox state of the Riesling wine studied.

FIGURE 35: PRINCIPAL COMPONENT ANALYSIS FOR FREE SO_2 (% SO_2: PERCENTAGE OF THE INITIAL FREE SO_2 CONTENT IN THE BOTTLE), COLOUR CHANGES AND DESCRIPTIVE SENSORY ANALYSIS OF 16 EXPERIMENTAL WINES (A AND B) AFTER 6 MONTHS OF STORAGE AT 20°C. THE WINES WERE BOTTLED WITH DIFFERENT HEADSPACE VOLUMES (0 HS, 10 HS, 20 HS AND 30 HS), VARIOUS FREE SO_2 CONCENTRATIONS (45 MG/L AND 70 MG/L) AND WITH AND WITHOUT ASCORBIC ACID (AA). THE FIGURES SHOW A PROJECTION OF SENSORY ATTRIBUTES ON PRINCIPAL COMPONENTS 1 AND 2.



4. CONCLUSION

This study demonstrates that wine exposure to oxygen at bottling plays a key role in white wine development during storage. High oxygen ingress may greatly influence the redox state of wine and affect the important quality parameters including colour, free and total SO_2, and the overall sensory quality of wine.

Free and total sulphur dioxide loss and the decline in ascorbic acid could be linked to the total consumed oxygen content. In the presence of ascorbic acid, less sulphite was consumed. The free SO_2 concentration at bottling made no significant impact on the decrease of the ascorbic acid.

Ascorbic acid addition contributed to the fruity and fresh character of the bottled wines. However, when combined with high oxygen concentration, the ascorbic acid caused enhanced white wine browning. This was scavenged in the wines with higher free SO_2 concentrations.

Based on the data shown, careful control of the oxygen ingress during bottling is crucial for white wine quality. Ascorbic acid addition seems to have positive sensory effects on the development of wines during the post bottling period. However, the possible wine browning, associated with ascorbic acid, should be taken into account. On the contrary, sulphur dioxide, in case of the Riesling wine studied, seems to be less effective to prevent negative sensory effects in wines due to excessive exposure to oxygen, but may simultaneously decrease oxidative browning in wines with ascorbic acid addition. Low oxygen ingress at bottling combined with moderate ascorbic acid and sulphur dioxide addition offer a good potential to increase the shelf-life of white wines.

5. ABBREVIATIONS USED

HS – Headspace Volume [mL]

TCO – Total Consumed Oxygen [mg/L]

AA – Ascorbic acid

ORP – Oxidation reduction potential

UTA – Untypical aging

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CONCLUDING REMARKS

Since the European wine market is becoming more and more saturated, one of the recent efforts undertaken by wine producers is to increase local wine's consumption and to promote and export in foreign markets. Recent studies show the consumer preferences towards food quality and regional products, supported by the European Union legal regulations on "designation of origin" (Gil and Sánchez 1997; Weininstitut 2013). Thus, increasing the perceived quality of wines determines above all the success of wine producers in the wine market.

The wine contact with oxygen plays a major role in development of aroma, colour and taste of wines, thereby determining the quality of wine, consumer preference and shelf-life potential. The impact of oxygen on wine quality depends on many factors, including oxygen concentration, temperature, wine type and conditions of oxygen exposure. Wine post-bottling development is complex: most red wines benefit from a small degree of oxygenation as it contributes to colour stabilisation, astringency reduction, and aroma improvement. White wines, on the contrary are less resistant to oxygen, leading to oxidative off-flavours and browning that reduce wine quality (Escudero et al. 2002; Oliveira et al. 2013).

Modern luminescence-based and conductivity-based analytical methods allow quantification of dissolved oxygen concentration and have been previously applied for measurements of oxygen ingress during winemaking operations (Morozova 2009; Vidal et al. 2003; Vidal et al. 2004; Vidal et al. 2001). In Chapter I, a comprehensive comparison of available on the market oxygen measurement instruments (PreSens, OxyQC and Orbisphere 3650) was conducted. In the experiment, the sample preparation, measurement and calculation procedures were developed for wine. The results demonstrated that each of the investigated devices is an effective tool for monitoring of dissolved oxygen in wine. A routine oxygen control along the operations may be highly recommended to wine producers aiming to improve the quality of their wines.

However, there are several limitations of the modern techniques for oxygen measurement that should also be discussed. Most of the dissolved oxygen measurement instruments available on the market initially determine the partial pressure of oxygen, which is subsequently calculated into oxygen concentration in mg/L or mole/L using gas partition coefficients, or Henry's Law constants. Henry's Law constant represents, for environmental conditions, the air-water partition coefficient for a particular chemical compound present in a dilute aqueous solution representing its relative volatility (Staudinger and Roberts 2001). It should be noted, that calculation formulas for oxygen measurement are using air-water system partition coefficients (Huber 2006) and were not specifically determined for wine, which might possibly lead to deviations. Consequently, precise definition of Henry's Law constant for oxygen specifically for wine gives a perspective for further research.

For bottled wine, both dissolved oxygen in wine and gaseous oxygen in the bottle headspace are of major importance. Since bottling line and filling conditions may lead to high oxygen ingress through the headspace followed by undesired wine oxidation (Jung 2009; Lassak 2013), headspace oxygen should also be thoroughly controlled. From three investigated devices (Chapter I), only PreSens allows direct measurement of oxygen in headspace, which is the advantage of the luminescence-based method with minisensors, compared to the other two techniques.

In Chapters II and III the kinetic of oxygen consumption rate and the corresponding development of colour, sulphur dioxide and organoleptic properties during storage, as affected by various headspace oxygen amounts and iron and copper concentrations, were analysed. The key role of iron and copper was previous reported by other authors (Clark et al. 2011; Danilewicz 2007; Danilewicz 2011; Danilewicz et al. 2008; Danilewicz and Wallbridge 2010). Up to now most research on this subject was conducted in model solutions. However, due to matrix effects, the same conditions applied to model solution and actual wine may lead to different results (Chapter II). It was observed, that small additions of iron (0.1 mg/L) and copper (0.05 mg/L), especially both, coupled with higher headspace oxygen concentrations, induced elevated oxygen and sulphur dioxide consumption rates and enhanced colour in model solution. In contrast, in Riesling wine the iron and copper additions did not contribute to any changes in the monitored parameters, whereas the final quality was determined by the headspace volume (0, 20 and 40 mL of ambient air).

Taking into account low concentrations of iron and copper, the experiment was repeated with 1 mg/L of iron and 0.5 mg/L of copper (Chapter III), which correspond to average measured amounts for 84 wines from Baden-Württemberg region (Nikfardjam 2010). Addition of iron and copper catalysed oxygen consumption, which further resulted in rapid loss of sulphur dioxide, enhanced browning and negative sensorial changes. Still, descriptive sensory analysis showed positive impact of iron and copper addition on fruity aroma of Riesling bottled with oxygen concentrations lower than 1.2 mg/L.

Although iron and copper were proved to be powerful oxidation catalysers, they can hardly be completely eliminated from wine. Concentration of iron and copper in wine depends on many factors, including soil (Almeida and Vasconcelos 2003; Pohl 2007; Taylor et al. 2003) viticulture practices (Volpe et al. 2009), pesticides (Galani-Nikolakaki et al. 2002; Kment et al. 2005), oenological equipment (Eschnauer 1982) and fining agents (Nicolini et al. 2004; Wurzinger et al. 1994). Potassium ferrocyanide treatment to remove iron and copper, known in winemaking as “blue fining”, is rarely applied in a modern winery due to possible hydrogen cyanide formation (Boulton et al. 1998). Consequently, the ideal case shown in the literature (Danilewicz 2007), when in the absence of iron and copper the reaction of oxygen with wine phenolics is not occurring is improbable in real wine. Thus, it is indispensable to find other means to control and avoid negative consequences of oxidation.

Sulphur dioxide and ascorbic acid are the antioxidants most extensively used in winemaking. While their impact on wine oxidation has been detailed studied in model wine (Barril et al. 2012; Bradshaw et al. 2003; Bradshaw et al. 2004), there is, again as in the case of catalysing metals, little data published on real wine with exact quantification of oxygen ingress. In Chapter IV, the effects of ascorbic acid addition combined with two different levels of free sulphur dioxide were investigated in wine samples bottled in 500 mL bottles with various headspace volumes full of ambient air (0, 10, 20 and 40 mL). Although sulphur dioxide was effective to reduce oxidative browning in Riesling wine, ascorbic acid demonstrated stronger antioxidative impact on sensorial qualities of the investigated wines. Additionally, positive effects of storage at 5°C, compared to 20°C, were observed on colour, SO_2 decay, and especially on sensory perception of Riesling wine. Thus, reducing oxygen ingress (<1.5 mg/L TPO) at bottling combined with moderate ascorbic acid and sulphur dioxide additions, and further storage at cool temperature will be a good strategy to obtain high quality white wines with long maintenance of fresh and fruity varietal character during storage.

Recent studies showed that sulphites resulting from the addition of the SO_2 may lead to allergic reactions with symptoms, including dermatitis, angioedema, abdominal pain, diarrhoea, anaphylaxis and breathing difficulties (Vally et al. 2009; Vally and Thompson 2001; Vally and Thompson 2003). Many researchers are focused to find an alternative to sulphur dioxide in winemaking (Izquierdo-Cañas et al. 2012; Santos et al. 2011). From oxidation point of view, oenological tannins (Sonni et al. 2009), caffeic acid (Roussis et al. 2007) and glutathione (Bouzanquet et al. 2012; Roussis et al. 2012) have a potential for application in wine production. Consequently, a broad study of the properties of these promising agents should be a subject for further research.

In summary, the present thesis provides comprehensive research results on the impact of oxygen ingress on white wine development post-bottling. Moreover, combined effects of other factors, including storage temperature, iron and copper content, sulphur dioxide and ascorbic acid were investigated. Finally, this work exclusive gives recommendations on significant quality and shelf-life improvement of white wines.

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SUMMARY

Today’s wine market is becoming more and more saturated. At given conditions, the understanding of the consumer needs and preferences determines the success of a wine producer. The value of white and rosé wines appreciated by the consumers lies in their fruity and fresh character. Wine oxidation is one of the major wine failures. Recently, it has been reported that up to 48% of the wines rated as faulty by judges in wine competitions exhibited off-flavours that can be linked to the erroneous management of oxygen.

Wine exposure to oxygen is possible throughout the whole winemaking process and depends on process conditions and applied equipment. Oxygen can be dissolved in wine up to 8.8 mg/L at normal conditions. After dissolution, oxygen concentration in liquid phase is normally decreasing to undetectable content due to chemical reactions of molecular oxygen with other wine constituents.

Wine oxidation is a complex process. The effects may vary significantly influenced by numerous factors, including a type of wine, operation, oxygen amount chemical composition of wine, pH, storage temperature, light exposure, metal content and redox state of wine. Oxygen has a potential to support positively, and subsequently to ruin aroma and colour of wine. Most white wines are negatively affected by small amounts of dissolved oxygen provoking rapid aroma loss and oxidative browning, thus decreasing attractiveness for consumers, whereas in red wines microoxygenation may help to stabilise wine colour and remove reductive off-flavours.

Up to now, most of the research on wine oxidation was focused on experiments in model solutions. Although these studies deliver valuable information about oxidation

mechanisms, there was, however, limited data published on real wine experiments linking analytical data and sensory analysis.

Consequently, the background of the present investigations was comprehensive evaluation of the effectiveness of the various factors influencing wine oxidation in real wine medium, and finally the development of innovative strategy for quality improvement and shelf-life extension of white wines.

Comparison of different methods for O_2 and CO_2 measurement in wine

The initial part of the present work was to determine a reliable quantification method for dissolved oxygen quantification in wine medium. Since dissolved carbon dioxide present in wine may influence the oxygen concentration, its amount should also be taken into account. Thus, development of dissolved carbon dioxide determination was of major importance for the further experiments described in this thesis.

The main aim of the first study was to give a review of the oxygen and carbon dioxide measurement principles and to compare several instruments for carbon dioxide and oxygen measurement in wine available in the market. For carbon dioxide determination, CarboQC, Orbisphere 3658 and agitation cylinder were used. Oxygen measurements were conducted with PreSens, OxyQC and Orbisphere 3650. Sample bottles were prepared with concentrations in the range from 0 to 2200 mg/L for carbon dioxide and from 0 to 12 mg/L for oxygen, respectively, dissolved in white wine of Müller Thurgau variety. O_2 and CO_2 measurements with six instruments were sequentially conducted at 20°C after 10 minutes shaking of trial bottles. Subsequently calculated concentrations of oxygen and carbon dioxide were used for correlation regression tests.

From the data obtained for CO_2, three investigated instruments showed good repeatable measurement results (R^2=98%) in the range from 0 to 1500 mg/L. However, in the wine samples oversaturated with CO_2 (>1500 mg/L at 20°C) CarboQC and Orbisphere 3658 showed significantly higher precision (R^2=99%), compared to the agitation cylinder, due to carbon dioxide losses during filling of the cylinder. As for oxygen data, results obtained with each of the measurement devices (PreSens, OxyQC and Orbisphere 3650) demonstrated good correlation with the initial oxygen amount in wine samples (R^2=98%) in the whole monitored range of dissolved oxygen concentrations. However, due to the flexibility of PreSens mini-sensor application inside the bottle and noninvasive measurement, this technique was found to be more advantageous, compared to the other two. Additionally, it allowed not only determination of dissolved oxygen, but also of the gaseous oxygen concentration in a bottle headspace. For these reasons, the PreSens device was chosen for further experiments.

Effect of Headspace Volume and Iron and Copper Addition on Oxidation Processes in Model Solution and Riesling Wine: Chemical and Sensory Changes

Since previous studies in model solutions published by other authors clearly showed the key role of iron and copper in oxidation reactions in wine, the main aim of this part of the thesis was to compare the effects of oxygen and iron and copper additions on oxygen consumption rate, sulphur dioxide and colour of bottled model solution and actual wine with similar properties. Model solution was prepared of deionised water, glycerol, (+)-tartaric acid, ethanol, gallic acid and potassium metabisulfite to reach a free sulphur dioxide concentration of 50 mg/L. 200 L Riesling wine (vintage 2010) was prepared using standard winemaking techniques and equipment, and was subsequently treated with potassium ferrocyanide to eliminate iron

and copper. Model solution and Riesling wine were bottled in 500 mL bottles with and without small additions of iron (0.1 mg/L) and copper (0.05 mg/L). Oxygen concentration in bottles was adjusted using various headspace volumes (0, 50 and 100 mL for model solution, and 0, 20 and 40 mL for Riesling, respectively) full of ambient air. Iron and copper concentration, total consumed oxygen, sulphur dioxide, browning rate (E420) were monitored. Additionally, sensory analysis of bottled Riesling wines (triangle tests and descriptive analysis) was conducted after 90 and 240 days of storage. The results revealed major differences between model solution and real wine. In model solution the headspace volume and the metal addition contributed to significant changes in total consumed oxygen, colour, and free sulphur dioxide. The metal addition increased the rate of the molecular oxygen consumption and resulted in elevated consumption of free SO_2. Enhanced colour was observed in all wines, where iron was added.

The experiment with Riesling wine with similar parameters showed strong influence of the headspace volume. The rate of oxidative browning and oxygen consumption rate strongly correlated with the headspace volume at the bottling. In contrast to model solution experiment, addition of small concentrations of iron and copper did not contribute to the colour and oxygen consumption rate of Riesling wine. 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 headspace volume, thus proving the significance of oxygen ingress at bottling on wine quality. Moreover, traces of metals in Riesling wines even in the wines with no iron and copper addition were sufficient to initialise oxidation processes. However, since small iron and copper additions had significant impact on model solution, further studies with Riesling wine needed to be conducted.

The Impact of Headspace Oxygen and Copper and Iron Addition on Oxygen Consumption Rate, Sulphur Dioxide Loss, Colour and Sensory Properties of Riesling Wine

For further investigation of the impact of iron and copper on white wine oxidation, Riesling wine was bottled with the addition of 1 mg/L of iron and 0.5 mg/L of copper, which correspond to average iron and copper concentrations in wines of Baden-Württemberg. Oxygen concentrations were determined, as previously reported, by the headspace volume in the bottle (0 mL, 10 mL and 20 mL) full with ambient air. In contrast to the previous experiment, addition of 1 mg/L of iron and 0.5 mg/L of copper had significant influence on the oxygen consumption rate, on the loss of free SO_2 during storage, and on the sensory changes in wine. Addition of iron and copper significantly catalysed the oxygen consumption. Free sulphur dioxide loss was found to be proportional to the total consumed oxygen after bottling. Moreover, in all wines with iron and copper addition free sulphur dioxide decay was significantly elevated compared to wines with no iron and copper addition. Although colour changes were not observed in wines after 90 days of storage, significant sensory changes were detected. Both oxygen and iron and copper addition made an impact on sensory evaluation of wines. At low oxygen concentration (0 mL headspace) metal addition had positive effect resulting in elimination reduced aromas. In contrast, for 10 mL and 20 mL headspace, wines with iron and copper addition showed lower scores in fruity, citrus, tropical aromas, and elevated scores in untypical aging and had pronounced oxidised character.

The results of the study indicate that, in the case of Riesling wine, excessive oxygen exposure due to oxygen present in the headspace of the bottle should be avoided. Moreover, iron and copper concentration also seems to make significant impact on oxygen and SO_2 consumption rates and on sensory perception of wines. These findings suggest that iron and copper concentrations should also be taken into account, when oxygen management strategy is defined.

Effect of Headspace Volume, Ascorbic Acid and Sulphur Dioxide on Composition and Sensory Profile of Riesling Wine

In the last part of the present work the effects of different oxygen and free SO_2 levels, and ascorbic acid addition on the development of white wine were investigated. Riesling wine was bottled in 500 mL bottles with four different headspace volumes (0 mL, 10 mL, 20 mL, 30 mL), two levels of free SO_2 (50 mg/L and 70 mg/L), and with and without ascorbic acid (250 mg/L) addition. Dissolved oxygen and the oxygen in headspace were measured in the resulting 17 wines. Free and total SO_2 concentrations, ascorbic acid concentration, colour, redox potential, and antioxidative capacity were measured regularly in wine samples. After six months of storage, the wines were evaluated using sensory descriptive analysis.

It was again proved that wine exposure to oxygen at bottling plays a key role in white wine development during storage. High oxygen ingress may greatly influence the redox state of wine and affect the important quality parameters including colour, free and total SO_2, and the overall sensory quality of wine. Free and total sulphur dioxide loss and the decline in ascorbic acid could be linked to the total consumed oxygen content. It was observed that in the presence of ascorbic acid less sulphite was consumed. Ascorbic acid addition also contributed to the fruity and fresh character of the bottled wines, which indicates its strong antioxidant. However, when combined with high oxygen concentration, ascorbic acid addition promoted enhanced white wine browning. This was scavenged in the wines with higher free SO_2 concentrations.

Based on the data shown, careful control of the oxygen ingress during bottling is crucial for white wine quality. Ascorbic acid addition seems to have positive sensory effects on the development of wines during the post-bottling period. However, the possible wine browning, associated with ascorbic acid, should be taken into account. On the contrary, sulphur dioxide, in case of the Riesling wine studied, seems to be less effective to prevent negative sensory effects in wines due to excessive exposure to oxygen, but may simultaneously decrease oxidative browning in wines with ascorbic acid addition.

In summary, low oxygen ingress at bottling combined with low iron and copper concentrations, moderate additions of ascorbic acid and sulphur dioxide seem to be a good oxygen management strategy and offer a good potential to improve quality and extent the shelf-life of white wines.

ZUSAMMENFASSUNG

Der Weltweinmarkt und speziell der europäische Weinmarkt wird heute oft als übersättigt angesehen. Der Erfolg einzelner Weinproduzenten hängt daher vom Verständnis und der Adaption an die Bedürfnisse der Verbraucher ab. Bei Weiß- und Roséweinen schätzen Verbraucher derzeit besonders einen fruchtigen, frischen Charakter. Oxidative Noten sind unerwünscht. Dennoch gehören sie zu den häufigsten Weinfehlern. Aus Weinwettbewerben wurde berichtet, dass bis zu 48% der fehlerhaften Weine von den Prüfern wegen Oxidationsnoten beanstandet wurden.

Sauerstoff kann vom Wein während des ganzen Weinbereitungsprozesses aufgenommen werden. Die Sauerstoffaufnahme hängt im Wesentlichen von den Prozessen und der verwendeten technologischen Ausstattung ab. Unter normalen Bedingungen kann Sauerstoff bis zu 8.8 mg/L im Wein gelöst werden. Gelöster Sauerstoff in Wein wird in der Regel durch chemische Reaktionen von molekularem Sauerstoff mit Weininhaltsstoffen bis unter die Nachweisgrenze verringert. Die vielfältigen Reaktionen der Weinoxidation sind sehr komplexe Prozesse. Ihre Auswirkungen variieren erheblich. Beeinflussende Faktoren sind Weintyp, Weinbehandlung, Sauerstoffmenge, chemische Zusammensetzung des Weines, pH-Wert, Lagertemperatur, Belichtung, Metallgehalt und Redox-Zustand des Weines. Die Wirkung von Sauerstoff ist äußerst komplex, die Weinfarbe und das Aroma kann verbessert oder gar ruiniert werden. Weißweine werden durch Sauerstoff häufig negativ verändert. Der Sauerstoff führt hier zu schnellem Aromaverlust bis hin zu oxidativer Bräunung. Gerade dies wird vom Verbraucher oft negativ bewertet. Bei Rotweinen hingegen kann Sauerstoff gezielt eingesetzt werden um die Farbe zu stabilisieren oder reduktive Aromen zu entfernen.

Die Forschung über Weinoxidation konzentriert sich derzeit auf Experimente in Modell-Lösungen. Diese Studien geben wertvolle Informationen über Mechanismen und Hintergründe der Oxidation. Jedoch befassen sich derzeit nur wenige Experimente mit echtem Wein. Bei echten Weinen bietet sich die Möglichkeit analytische und sensorische Daten miteinander zu verknüpfen.

Wichtiger Punkt der vorliegenden Arbeit ist die umfassende Bewertung verschiedener Einflußfaktoren auf die Weinalterung. Aus den Ergebnissen wurde eine Strategie für die Verbesserung der Qualität und einer Verlängerung der Haltbarkeit von Weißweinen entwickelt.

Methodenvergleich zur O_2 -und CO_2- Bestimmung im Wein

Der erste Teil der vorliegenden Arbeit besteht in der Bestimmung von zuverlässigen Methoden zur Quantifizierung des gelösten Sauerstoffs im Wein. Da das gelöste Kohlendioxid im Wein die Sauerstoffkonzentration beeinflussen kann, wurde die CO_2-Menge ebenfalls berücksichtigt. Folglich war die Entwicklung einer Methode zur Kohlendioxid-Bestimmung auch von großer Bedeutung für die weiteren Versuche, die in dieser Arbeit beschrieben sind.

Das Hauptziel der ersten Studie war es, einen Überblick zu aktuellen Sauerstoff- und Kohlendioxid-Messmethoden zu geben sowie verschiedene auf dem Markt verfügbare Instrumente für Kohlendioxid- und Sauerstoff-Messung in Wein zu vergleichen. Sauerstoff-Messungen wurden mit PreSens, OxyQC und Orbisphere 3650 durchgeführt. Zur Kohlendioxid-Bestimmung wurden CarboQC, Orbisphere 3658 und Schüttelzylinder verwendet. Probenflaschen wurden mit Konzentrationen im Bereich von 0 bis 2200 mg/L für Kohlendioxid und von 0 bis 12 mg/L für Sauerstoff abgefüllt. O_2- und CO_2- Messungen erfolgten mit den sechs Instrumenten nacheinander bei 20°C, nachdem die Flaschen für 10 Minuten geschüttelt wurden. Die anschließend berechneten Konzentrationen an Sauerstoff und Kohlendioxid wurden für die Erstellung von Korrelationen und Regression-Tests verwendet.

Die drei untersuchten Instrumente für CO_2-Bestimmung zeigten reproduzierbare Messergebnisse ($R^2 = 98\%$) im Bereich von 0 bis 1500 mg/L. Doch in den Weinproben bei dem 20°C übersättigten Bereich (>1500 mg/L) zeigten CarboQC und Orbisphere 3658 signifikant höhere Präzision ($R^2 = 99\%$) im Vergleich zum Schüttelzylinder. Dies ist logisch, da während der Befüllung des Zylinders das Kohlendioxid verloren geht. Die Ergebnisse der Sauerstoff-Messungen deuteten darauf hin, dass PreSens, sowie auch OxyQC und Orbisphere 3650 eine im gesamten überwachten Bereich der Sauerstoffkonzentrationen verlässliche und kongruente Werte liefern. Aufgrund der Flexibilität der Minisensoren, die direkt in die Flasche eingeklebt werden können, erwies sich die nicht-invasive O_2-Messung mittels PreSens als vorteilhaft. Darüber hinaus erlaubt diese Technik nicht nur die Bestimmung vom gelöstem Sauerstoff, sondern auch die Messung vom gasförmigen Sauerstoff im Kopfraum. Aus diesen Gründen wurde das PreSens-Gerät für weitere Experimente ausgewählt.

Einfluss vom Kopfraumvolumen und Eisen- und Kupfer-Zugabe auf Oxidation in Modell-Lösung und Riesling Wein: chemische und sensorische Veränderungen

In früheren veröffentlichten Studien in Modell-Lösungen wurde die wichtige Rolle von Eisen und Kupfer in Oxidationsreaktionen in Wein deutlich gezeigt. Im zweiten Teil der vorliegenden Arbeit wurden die Wirkungen von unterschiedlichen Sauerstoff-Mengen bei der Abfüllung untersucht. Des Weiteren wurde der Einfluss von Eisen- und Kupfer-Zusätzen auf die Sauerstoffverbrauchsrate, Schwefeldioxid und Farbe in Modell-Lösung und Riesling Wein geprüft. Die Modell-Lösung wurde aus hochreinem Wasser, Glycerin, (+)-Weinsäure, Ethanol, Gallussäure und

Kaliummetabisulfit zusammengestellt, außerdem wurde die SO_2-Konzentration auf 50 mg/L eingestellt. 200 L Riesling (Jahrgang 2010) wurde unter Verwendung von Standard-Techniken der Weinbereitung produziert und anschließend mit Kaliumhexacyanoferrat behandelt, um die Metalle bzw. Eisen und Kupfer zu entfernen. Modell-Lösung und Riesling wurden in 500-mL Flaschen mit und ohne Zusatz von Eisen (0.1 mg/L) und Kupfer (0.05 mg/L) abgefüllt. Die Sauerstoffkonzentration in Flaschen wurde mit Luft im Kopfraum (0, 50 und 100 mL für Modell-Lösung und 0, 20 und 40 mL für Riesling) eingestellt. Die Eisen- und Kupferkonzentrationen, Gesamtmenge an verbrauchtem Sauerstoff, Schwefeldioxid, Absorption (E420) wurden während der Lagerzeit beobachtet. Zusätzlich wurde die sensorische Bewertung von Riesling Weinen mittels Dreieckstests und deskriptiver Analyse nach 90 und 240 Tagen Lagerung durchgeführt.

Die Ergebnisse zeigten große Unterschiede zwischen der Modell-Lösung und dem Riesling Wein. In der Modell-Lösung hatte das Kopfraumvolumen und der Metall-Zusatz dazu beigetragen, dass wesentliche Änderungen in Sauerstoffverbrauchsrate, Farbe und freiem Schwefeldioxid entstanden. Der Eisen- und Kupfer-Zusatz erhöhte die Geschwindigkeit des Sauerstoff-Verbrauchs und führte zu erhöhtem Verbrauch der freien SO_2. Die intensive Farbentwicklung wurde in allen Weinen mit Eisen-Zusatz festgestellt.

Das Experiment mit Riesling mit vergleichbaren Parametern zeigte einen starken Einfluss des Kopfraumvolumens. Die Sauerstoffverbrauchsrate und die oxidative Bräunung korrelierten stark mit dem Kopfraumvolumen. Im Gegensatz zum Modell-Lösung-Experiment hatte die Zugabe von geringen Mengen an Eisen und Kupfer keinen Einfluss auf die Farbe und den Sauerstoffverbrauch im Riesling Wein. Sensorische Unterschiede zwischen den Weinen mit 0, 20 und 40 mL Kopfraum wurden bereits nach drei Monaten Lagerung bei 15°C durch die Dreieckstests nachgewiesen. Die deskriptive sensorische Analyse nach sechs und neun Monaten Lagerung bestätigte den negativen Einfluss von großem Kopfraumvolumen. Dies beweist die große Bedeutung der Sauerstoffaufnahme bei der Abfüllung auf die Weinqualität. Außerdem waren Metallmengen im Riesling Wein, auch in den Varianten ohne Eisen- und Kupferzusatz ausreichend, die Oxidationsprozesse zu initialisieren. Da die geringen Eisen- und Kupferzugaben eine erhebliche Wirkung in der Modell-Lösung und nicht im Wein hatten, waren weitere Studien mit größeren Mengen an Eisen und Kupfer in Riesling Wein erforderlich.

Einfluss von Sauerstoff im Kopfraum, Eisen- und Kupferzusatz auf Sauerstoffverbrauchsrate, Schwefeldioxidverlust, Farbe und sensorische Eigenschaften von Riesling Wein

Um die Wirkung von Eisen und Kupfer weiter auf Weißweinoxidation zu untersuchen, wurde Riesling Wein mit/ohne Zugabe von 1 mg/L Eisen und 0.5 mg/L Kupfer abgefüllt. Dies entspricht den durchschnittlichen Eisen- und Kupfer-Konzentrationen in Weinen aus Baden-Württemberg. Die Sauerstoffmengen wurden mit verschiedenen Kopfraumvolumen in den Flaschen (0, 10 und 20 mL) bestimmt. Im Gegensatz zum vorangegangenen Versuch, hatte die Zugabe von 1 mg/L Eisen und 0.5 mg/L Kupfer einen signifikanten Einfluss auf den Sauerstoffverbrauch, Verlust von freiem SO_2 während der Lagerung und auf die sensorischen Eigenschaften im Wein. Der Eisen- und Kupfer-Zusatz katalysierte deutlich den Sauerstoffverbrauch. Der Schwefeldioxid-Verlust war proportional zur Gesamtmenge an verbrauchtem Sauerstoff. Zusätzlich wurde der SO_2-Abbau in allen Varianten mit Eisen und Kupfer im Vergleich zu den Varianten ohne Eisen- und Kupferzugabe signifikant erhöht. Obwohl die Farbänderungen nach 90 Tagen Lagerung nicht festgestellt werden konnten, waren die erheblichen sensorischen Veränderungen bereits erkennbar. Der Sauerstoff, wie auch der Eisen- und Kupferzusatz hatten Einfluss auf die sensorische Bewertung der

Weine. Bei niedrigen Sauerstoffkonzentration (0 mL Kopfraum) hatten Metalle durch die Eliminierung von reduzierten Aromen eine positive Wirkung. Die Weine mit 10 und 20 mL Kopfraum und zugesetztem Eisen und Kupfer zeigten geringere Ausprägungen in Attributen wie Frucht, Citrus, tropischen Aromen und erhöhte Werte in UTA und hatten einen deutlichen oxidierten Charakter.

Die Ergebnisse der Studie zeigen, dass eine übermäßige Sauerstoffaufnahme während der Abfüllung vermieden werden muss. Außerdem scheint eine Eisen- und Kupfer-Konzentration den signifikanten Einfluss auf den O_2- und SO_2-Verbrauch, wie auch auf die sensorische Wahrnehmung der Weine zu haben. Die Ergebnisse zeigen, dass nicht nur die Sauerstoffaufnahme, sondern auch die Eisen- und Kupfer-Konzentrationen im Wein im Rahmen des Sauerstoff-Managements berücksichtigt werden müssen.

Einfluss von Kopfraumvolumen, Ascorbinsäure und Schwefeldioxid auf Zusammensetzung und sensorisches Profil von Riesling Wein

Im letzten Teil der vorliegenden Arbeit wurden die Wirkungen von Sauerstoff, freiem Schwefeldioxid und Ascorbinsäure auf die Entwicklung von Weißwein untersucht. Riesling Wein wurde in 500 mL Flaschen mit vier verschiedenen Kopfraumvolumen (0, 10, 20 und 30 mL), zwei Schwefeldioxid-Konzentrationen (50 und 70 mg/L) ohne und mit Ascorbinsäure (0, 250 mg/L) abgefüllt. Gelöster Sauerstoff, gasförmiger Sauerstoff im Kopfraum, freie und gesamte SO_2, Ascorbinsäure, Farbe, Redox-Potential und antioxidative Kapazität (TEAC) wurden regelmäßig in den resultierenden 17 Varianten während der Lagerung gemessen. Nach sechs Monaten Lagerung wurde zusätzlich die sensorische deskriptive Analyse der Weine durchgeführt.

Es wurde nochmal bewiesen, dass der Sauerstoff bei der Abfüllung eine wichtige Rolle in der Entwicklung der Weißweine während der Lagerung spielt. Eine hohe Sauerstoffzunahme hatte großen Einfluss auf den Redox-Zustand des Weines und dadurch wurden die wichtigen Parameter wie Farbe, freie und gesamte SO_2 und die gesamte sensorische Bewertung des Weines beeinflusst. Die Verluste von freiem und gesamtem Schwefeldioxid und die Abnahme der Ascorbinsäure korrelierten mit der Gesamtmenge an verbrauchtem Sauerstoff. Durch die Verwendung der Ascorbinsäure konnte der Schwefeldioxidverlust wesentlich reduziert werden. Die Ascorbinsäure trug ebenfalls zu dem fruchtigen und frischen Charakter der abgefüllten Weine bei. Die Zugabe von Ascorbinsäure kombiniert mit hoher Sauerstoffkonzentration (20 und 30 mL Kopfraum) führte zur intensiven Braunfärbung. Dieser Effekt konnte aber durch den höheren SO_2-Zusatz eliminiert werden.

Basierend auf den erhobenen Daten, ist die Kontrolle von Sauerstoffaufnahme beim Abfüllen für die Qualität von Weißwein entscheidend. Ascorbinsäure hatte eine positive sensorische Wirkung auf die Entwicklung der Weine nach der Abfüllung. Allerdings sollte die Möglichkeit der mit Ascorbinsäure verbundener Braunfärbung der Weine berücksichtigt werden. Mehr SO_2 verhindert nicht die sensorischen Auswirkungen der Oxidation.

Zusammenfassend wurde festgestellt, dass eine niedrige Sauerstoffaufnahme bei der Abfüllung kombiniert mit niedrigen Eisen- und Kupfer- Konzentrationen, moderatem Zusatz von Ascorbinsäure und Schwefeldioxid die Qualität und die Haltbarkeit von Weißweinen entscheidend verbessern konnte.

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