Justus-Liebig-University Giessen
Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management
and
Hochschule Geisenheim University
Center of Wine Science and Beverage Processing Technology
Institute of Enology

Bottling Process and Closure Choice Influence Oxygen Levels in Wine and Wine Post-Bottling Development

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Evdokia Dimkou
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2nd Supervisor: Professor Dr. Sylvia Schnell

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Contents

List of abbreviations and symbols

A_{420}, abs_{420}	Absorption at 420 nm
BPAA	bis-9,10-anthracene-4-trimethylphenylammonium dichloride
CIE	Commission Internationale de l'Eclairage
Co-ex	Coextruded
DO	Dissolved oxygen
FSO_2	Free sulfur dioxide
HS	Headspace
HSO	Headspace oxygen
OTR	Oxygen transmission (or transfer) rate
SC	Screw cap
TCO	Total consumed oxygen
TDN	1,1,6-trimethyl- 1,2-dihydronaphthalene
TSO_2	Total sulfur dioxide

1. Introduction

Oxygen is a very common and very reactive element that can change physical and chemical properties of the material it reacts with. Its function in food production, packaging and storage is mostly negative due to the oxidative and microbial spoilage occurring under oxygen exposure (Elstner 1990). Yet oxygen´s role in winemaking is rather diverse: it can oxidize young wines kept in tanks with ullage but it can also stimulate yeast activity during fermentation or benefit red wine color by means of microoxygenation (Jones et al. 2004). Oxygen´s positive and negative impact on wine quality continues to the very last steps of wine production, namely at bottling and during bottle storage, where wine ageing and post bottling development occur. Too much oxygen in the bottle makes wines lose freshness and fruitiness during storage, while by inadequate amounts some wines become reduced with aromas such as rubber and struck flint (Gibson 2005). Furthermore oxygen levels in bottled wine effect sulfur dioxide (SO_2) levels and thus wines shelf life (Reeves 2009).

The question arise how much oxygen is needed for young wines to maintain quality and develop as expected in the bottle, without developing oxidative or reductive flavors. Since bottling and bottle storage are the last steps of wine production where oxygen uptake can occur, controlled oxygen exposure is needed. The variety of wine styles and the compositional variation within a given style make recipes of adequate oxygen exposure useless. Therefore successful oxygen management at and post bottling is possible only when deep knowledge on the impact of bottling and storage on oxygen levels in wine and wine quality is achieved. This study deals with the oxygen uptake at bottling and during storage and investigates its impact on wine´s post bottling development.

1.1 Oxygen in bottled wine

A newly sealed wine bottle contains dissolved oxygen in the wine and gaseous oxygen in the headspace. During storage, oxygen enters the package through the closure depending on closure´s permeability properties. The following chapters offer an overview of these different sources of oxygen in bottled wines, while in a subsequent section the methods of measuring oxygen are summarized.

1.1.1 Dissolved oxygen

Every time a liquid comes in contact with air, oxygen is diffusing in both directions until its partial pressure in both phases equalizes. The maximal concentration of oxygen in this liquid will depend on the nature of the liquid and its temperature (Moutounet and Vidal 2005). For wine, values between 6

and 9 mg/L have been given from different authors at 20°C (Du Toit et al. 2006, Pfeifer 2000, Moutounet and Mazauric 2001, Singleton 1987, Schneider 2005a).

During winemaking air contact of the wine occurs. Table 1 shows several examples of oxygen uptake after cellar operations measured by different authors. Data show that bottle filling can increase wine´s dissolved oxygen (DO) from less than 2 up to 4 mg/L. This discrepancy among the authors can be explained by many reasons such as differences in wine temperature, bottling systems, measuring methods etc. Table 2 for instance, shows oxygen uptake at bottling under different bottling systems (McClellan 1990). Using long filling tubes minimizes oxygen uptake to 0.5 mg/L because turbulence and therefore extensive air incorporation in wine is prevented. Moreover, evacuating the bottle before filling by means of N_2, CO_2 or Ar keeps DO levels in wine after filling under 0.5 mg/L.

Table 1: Oxygen uptake during various winemaking operations measured by different authors in mg/L.

	Castellari et al. 2004	Schneider 2005	Moutounet et al. 2001	Pfeifer 2000	Ribereau-Gayon 2000	Vidal et al. 2001, 2003 und 2004
Pumping	0.1		0.3		2	< 0,7
Diatomaceous earth filtr.	< 0.6	4			7	< 2
Plate filtration	< 0.1				4	< 0,5
Cross-flow filtration	< 0.4	5				< 2
Racking			3-8	< 2		
Racking, running-in bottom	< 1				3	
Racking running in top	< 1				5	
Centrifugation	< 2	5			8	< 2
Filling (bottles)	< 2	2	< 4		3	< 4
Filling (Bag in Box)	< 0.1					< 0.7
Barrique storage per year		30			20-45	
Wine stabilization (-5°C)	2-9					2.4

Reproduced from Friedel 2007

Table 2: Oxygen uptake during bottling with different systems in mg/L.

Filling system	O_2 increase [mg/L]*
Vacuum filler, 40 mbar	1.3
Normal pressure filler, short tube	2
Normal pressure filler, long tube	0.5
Pressure filler, 1.5 bar (air, short tube)	3
Pressure filler 1,5 bar (evacuation of bottle and filling height correction with CO_2, short tube)	< 0.5

McClellan 1990

Besides bottling systems, wine temperature at bottling will also influence oxygen uptake. Several authors have proved that oxygen solubility increase up to 20 mg/L by decreasing wine temperature at 12°C, while increasing temperature at 50°C drops solubility at 0.6 mg/L (Müller-Späth 1977).

However, higher temperatures accelerate oxidation reactions, which means that increasing temperature is not a recommended way of decreasing DO.

Another important aspect of oxygen uptake at bottling is that DO increase is much higher in the beginning and at the end of the process, rather than in the middle (Friedel 2007). That is because the first liters of wine that flow through the system come in contact with the air (or water) residing in the system before bottling starts. When bottling procedure comes to the end, the wine in the filler becomes less while the space above it bigger. Thus DO of the wine in the filler increase. Therefore differences in DO can be also due to measurement time point.

Finally, the DO measurement method used (see 1.2.1) can be responsible for the differences found in DO increase at bottling by different authors.

1.1.2 Headspace oxygen

Although DO increase is often considered as the main impact of bottling on oxygen levels in wine, bottle headspace represents also a significant oxygen pool for bottled wine. As already mentioned, when a liquid comes in contact with a gas, oxygen will move in both directions until its partial pressure in both phases equalizes. That means that headspace oxygen (HSO) will slowly dissolute into the wine and increase DO.

Depending on the kind of the closure used and the headspace volume applied HSO will vary. Normally, bottles sealed with screw caps have up to three times larger headspaces than bottles sealed with cylinder closures, such as natural or synthetic corks (Reeves 2009). Kontoudakis et al. (2008) measured headspace volume under cylindrical closures between 3 and 7 mL and under screw caps 14 mL. Schneider (2005) estimates that a 4 mL headspace under a cylindrical closure would contain 1.5 mg/L oxygen, whereas the same bottle sealed with a screw cap would contain due to its larger headspace approximately 5 mg/L oxygen.

However sealing with cylindrical closure generates an overpressure in the headspace, while sealing with screw cap not. Kontoudakis et al. (2008) measured the overpressure in headspaces of wine bottles and found values between 17 and 140 kPa. A vacuum is often applied to the bottles with cylindrical closures to ensure that headspace pressure is kept to about ±20 kPa (Reeves 2009). O´Brian et al. (2009) calculated that 10 mL headspace with 15 kPa would increase wine´s DO in a 750 mL bottle by 3.3 mg/L oxygen.

The use of gases such as CO_2, N or Ag in order to replace the air in the headspace offers an opportunity to minimize HSO (Müller-Späth 1977, Du Toit et al. 2006, O´Brian et al. 2009, Reeves 2009). Headspace flushing is a common operation that can reduce HSO to 0.2-7% (Reeves 2009). A 3.5 mL headspace would then increase DO in a 750 mL bottle only by 0.5 mg/L. However, under screw caps with 8 mL headspace, the DO increase would be three times greater (Stelzer 2005).

In compare to DO, there are much less studies that investigate the levels of HSO in wine bottles. This is mainly due to the difficulties of measuring gas oxygen in a closed space such as the bottle headspace (see 1.2.2). Vilacha und Uhlig (1984) suggested a method to calculate HSO in bier bottles by measuring DO right after shaking the bottle for several minutes. A second measurement in another, not shacked bottle would provide the HSO value. Vidal and Moutounet (2006) measured the total package oxygen in 0.75 L wine bottles with 5-18 mL headspace volume and found 1.4-6.4 mg oxygen of which 38-75% represents HSO. Gibson (2005) quotes DO increase due to HSO approximately 1 and 3 mg/L in bottles closed with corks and screw caps respectively.

1.1.3 Closure´s oxygen transfer rate

Once a bottle is sealed, a third source of oxygen emerges: the oxygen that enters the bottle through the closure. In horizontally stored bottles this oxygen will dissolve directly into the wine, whereas in upright stored bottles it will first reach the headspace and then will dissolve into the wine. Oxygen transfer rate (OTR) describe how fast oxygen moves through the closure . OTR values are usually expressed in mL oxygen per day per closure with air on one side and inert gas on the other (Reeves 2009).

Table 3 gives a literature overview of OTR values for a variety of wine closures. Differences are obvious between diverse closure systems. However, noticeable are also differences among researchers within the same type of closure which may trace back to the different measuring methods used (see also 1.2.3) or to the storage position. Of all closures natural corks appear to have the highest variability. That is because cork, as a natural product, shows low homogeneity. Therefore oxygen permeability varies sometimes even from cork to cork within a single parcel.

Differences in OTR can also rise from different bottle storage position. Lopes et al. (2006) found small differences in oxygen ingress between bottles stored upright und bottles stored horizontally, while Gibson (2005) advise that OTR data extracted from measuring methods applied on dry corks, should not be used to predict wet natural cork performances and found out that upright storage results in more rapid wine development.

Table 3: OTR values for wine bottle closures measured by different authors in mL/day.

Closure	OTR	Method (see 1.2)	Bottle storage	Reference
Natural cork	0.002	SO2 loss	horizontal	Casey 1994
Natural cork	0.018	MoCon	vertical	Godden et al. 2005
Natural cork	0.013	MoCon		Hart and Kleinig 2005
Natural cork	0.080	Not given		Silva et al. 2003
Natural cork	0.004 - 0.192	MoCon		Gibson 2005
Natural cork	0.002 - 0.006	Indigo carmine	horizontal	Lopes et al. 2005
Natural cork	0.001 - 0.004	Indigo carmine	vertical	Lopes et al. 2005
Natural cork	0.006	Colorimetric		Ortiz et al. 2004
Synthetic a	0.030 - 0.038	Not given	horizontal	Silva et al. 2003
Synthetic b	0.031	MoCon		Gibson 2005
Synthetic c	0.006	Indigo carmine	horizontal	Lopes et al. 2005
Screw cap	0.0003 - 0.0007	Indigo carmine	horizontal	Lopes et al. 2005
Screw cap	0.0005	Not given		Godden et al. 2005
Screw cap Saran	0.001	Not given		Peck 2005
Screw cap PVC	0.004	Not given		Peck 2005

Reproduced from Reeves 2009

An important aspect that should also be discussed here is the behavior of cylindrical cellular structure closures, such as natural and synthetic corks after bottling. During sealing, these closures are being compressed in order to fit in the bottle neck. This results in an increase of the internal gas pressure up to 70% (Reeves 2009). Consequently, part of the oxygen residing in the pores of the closure itself will move towards the closure´s ends. At the outside end of the closure oxygen will escape to the surrounding until internal closure pressure equals that of the atmosphere. At the inside end the situation is more complex, depending on headspace pressure and composition. In any case increased oxygen diffusion from the closure into the bottle during the first weeks occurs out of the cells of the cork and not through the cork (Reeves 2009). Therefore we believe that the findings of Lopes et al. (2006), about oxygen diffusion through the closure occurring much more intensive during the first month of bottle storage than in the time period after that, could be due to this phenomenon.

Yet as the time goes by, pressure in the closure decrease. However, driven by a concentration difference between the two ends of the closure, oxygen will keep diffusing from the closure into the headspace. As oxygen removal out of the headspace continues due to its dissolution into and consumption by the wine (upright bottles), a steady state situation in oxygen ingress will be reached after a time period depending on closure characteristics, storage position and temperature (Reeves 2009). Skouroumounis und Waters (2007) identified three stages of DO increase, which reflect the different phases of oxygen ingress through and out of the closure as well as the HSO contribution (figure 1).

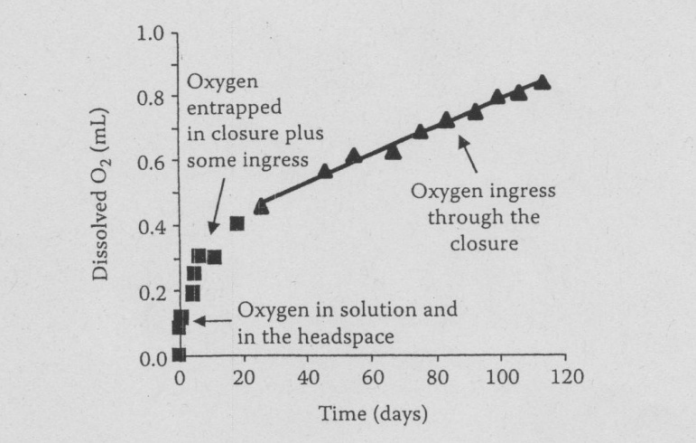


Figure 1: Oxygen Ingress into a 375 mL bottle sealed with a synthetic closure (Skouroumounis und Waters 2007 in Reeves 2009)

1.2 Measuring oxygen

The following chapters offer an overview of the different methods available for determination of DO, HSO as well as OTR. Methods that are suitable for more than one measurement (e.g. DO as well as HSO) are mentioned in more than one chapter.

1.2.1 Measuring dissolved oxygen

Colorimetric methods

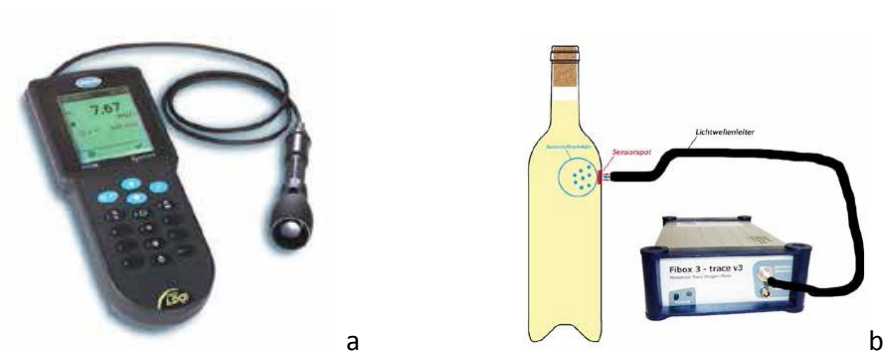
In 1933 Ribéreau-Gayon developed a method to measure DO in wine using indigo carmine as an indicator, which changes its color to red via oxidation. Miedeaner (2002) developed this method for bier industry and used it to measure DO also in sealed bottles (Friedel 2007). An ampoule containing the dye was put into the bottles at bottling and was destroyed later by shaking the bottle. Kielhöfer and Würdig (1962) developed the dithionite colorimetric method. However this was almost completely replaced from the electrochemical methods described below. Lopes et al. (2005) used a version of this method to measure oxygen ingress through closures (see 1.2.3).

Electrochemical methods

Traditionally DO has been measured by means of electrochemical systems based on Clark's electrode (Moutounet and Vidal 2005). An example of this method is the WTW system (Wissenschaftlich Technische Werkstätten GmbH), which uses a galvanic amperometric sensor (Cellox 325). Although different systems of this technology are available, they are all sensitive to other chemicals and also consume oxygen during measurement (Nevares and del Alamo 2007). Particularly detrimental for its use in the wine industry is the interference of the carbon dioxide in the measurement.

Optical methods (Quenching)

Other alternatives to measure DO can be found in the market as well. Most of them are based on the principle of luminescence quenching of the photoluminescence systems with different solutions and kind of sensors (Nevares and del Alamo 2007). During this measurement, oxygen deactivates a luminophore, a material that glows after light exposure. The oxygen concentration is therefore related to the intensity of the light produced by the luminophore. Apart from the luminophore (sensor), an optical system includes a light source and a light detector. By systems such as Hach LDO (Hach Lange, Berlin, Germany), the sensor, the light source and the light detector are included in one device (internal luminophore), whereas in systems such as Nomasense (Nomacorc SA, Belgium) - previously known as PreSens (PreSens Precision Sensing GmbH, Germany), OxySense (OxySense, Dallas USA) and MoconOpTech-O2Platinum (MoCon, Minneapolis, USA) the sensor is separated from the detector (remote luminophore, figure 2). This allows a non-invasive measurement of oxygen in sealed bottles. Oxygen sensor spots are glued inside bottles, in the wine or in the headspace region, providing indications for DO and HSO concentrations on an incorporated display or a notebook. The bottles should be colorless with walls thinner than 10 mm (Jung and Schüßler 2012).



a b

Figure 2: Photoluminescence devices based on internal (a) and remote (b) luminophore.

The PreSens producer offers two types of sensors, the Pst3 and Pst6 with measuring range from 0 to 100 and from 0 to 4.2% oxygen respectively. Furthermore it offers the possibility to measure oxygen in tanks or other containers without using the sensor spots, but by replacing the optic fiber with a dipping probe. For these reasons and mainly because of the possibility for non-destructive measurements, the PreSens technology was used in this study to measure DO and HSO as well as oxygen ingress through the closures (see 1.2.2 and 1.2.3).

1.2.2 Measuring headspace oxygen

Optical methods (Quenching)

In contrast to DO only few methods that measure gaseous oxygen in the headspace of bottles are available. Many authors just calculate the HSO using mathematical formulas including the volume, the temperature and the composition of the headspace. As described in the chapters above, the non-destructive optical methods, such asNomasense, OxySens and MoconOpTech-O2Platinum are suitable for measuring gaseous HSO as well. In this study, HSO was measured using the PreSens technology. Oxygen sensors were glued inside wine bottles in the headspace region and the HSO values were transmitted via optic wire on a display. However, while DO can be directly measured in mg/L, HSO is measured in hPa (partial pressure in the headspace which is analogous to the oxygen concentration) and then converted in mg using the ideal gas formula:

$p*V=n*R*T$

p = partial pressure of oxygen in the headspace in hPa
V = headspace volume in cm^3
n = amount of substance in mmol
R = gas constant 83.14 hPacm^3/mmol*K*
T = absolute temperature in K (°C + 273.15)

The amount of substance (n) can be replaced using following formula: $n=m/Mw$

n = amount of substance in mmol
m = mass in mg
Mw = molecular weight of oxygen (32 mg/mmol)

HSO can be then expressed in mg, in mg/L headspace or in mg/L wine. In this study often HSO is expressed in mg/L wine.

1.2.3 Measuring closure´s oxygen transfer rate

Wine parameters

The first methods used to measure OTR were based on changes in wine parameters such as SO_2 (Casey 1994) and A_{420} (Skouroumounis et al. 2005a/b). However correlations between oxygen ingress and changes of wine parameters were not always strong enough. This is due to the fact that wine parameters can be influenced from wine composition or antioxidants such as ascorbic acid (Reeves 2009).

Colorimetric methods

Other common methods for OTR measurements are colorimetric instruments such as MoCon Ox-Tran (MoCon, Minneapolis, USA) or changes in optical properties of oxygen-sensitive materials such as BPAA (bis-9,10-anthracene-4-trimethylphenylammonium dichloride) dye used by Skouroumounis and Waters (2007) or indigo carmine used by Lopes et al. (2005). These methods have also been criticized. MoCon instrument for not reflecting normal cork application (bottles stored horizontally), since it uses dry corks with gas on both sides (Gibson 2005, Lopes et al. 2006) and indigo carmine because the dye solution does not correspond the wine composition (Jung and Schüßler 2012). However an overview of these three methods is given below:

MoCon Ox-Tran is the most recognized measuring method for OTR of closures worldwide (Jung and Schüßler 2012). However its application is quit complicated because the bottle neck including the closure of sealed bottles must be cut and glued on a special plate. The new headspace which emerges has an oxygen sensor connected to the MoCon device. The oxygen that enters this headspace through the closure is being transported to the MoCon device by means of another gas after the initial oxygen of the headspace as well as the oxygen incorporated in the closure itself is removed (see 1.1.3). Consequently the actual measurement of OTR begins one to three months later (Jung and Schüßler 2012). The oxygen measurement in the MoCon device occurs colorimetric by means of special dyes.

The **indigo carmine** method measures oxygen ingress in a package in the region between 0.25 and 2.5 mL (Jung and Schüßler 2012). Control bottles contain an indigo carmine solution which changes its color from yellow to indigo when oxygen contact occurs. This color change can be measured by a color scan device which then translates the results in oxygen ingress (Lopes et al. 2005).

The **BPAA** method is based on the indigo carmine method. BPAA, which normally absorbs light in visible spectrum, is added to the solution. BPAA loses this property when reacts with oxygen. Consequently color change can be translated in oxygen in wine.

Optical methods (Quenching)

In this study OTR measurements were done using again the PreSens technology, as described from Nygaard et al. (2009) and O´Brian et al. (2009). Empty wine bottles were purged with Nitrogen until oxygen content reached zero. Bottles were then sealed with different closures. The increase of the oxygen concentration in the bottles due to oxygen ingress through the closure was monitored over time using sensors glued inside the bottles.

1.3 Oxygen and wine quality

Many studies in the literature have been dealing with the influence of oxygen in bottled wine on wine quality (Wildenradt and Singleton 1974, Müller-Späth 1977, Singleton et al. 1979, Casey 1996, Silva Ferreira et al. 2003a/b, Godden et al. 2002/2005, Hart and Kleinig 2005, Skouroumounis et al. 2005a/b, Braikowich et al. 2005, Kwiatkowski et al. 2007, Lopes et al. 2009, Caillé et al. 2010, Wirth et al. 2010, Ugliano et al. 2011). Most of them are focusing on the effects of oxygen on SO_2 and shelf life, brown coloration as well as aroma and sensory properties. Summarizing these studies we can say that wines with higher oxygen exposure show after a storage period lower levels of SO_2, more browning and higher oxidized characters compared to wines with lower oxygen exposure, which get higher scores in citrus and fruity aroma but also in unwanted reductive characters. However, it is not always clear if these effects are mainly due to DO, HSO or closure OTR.

Nevertheless these findings relate to the fact that oxygen in wine reacts with color, aroma and taste compounds during oxidation reactions (Du Toit et al. 2006). The predominant substrates for oxidation in wine are phenolic molecules (Singleton 1987). Due to the fundamental difference in the phenolic composition of red and white wines, it is necessary to distinguish between those two. Since this study deals with Riesling wines, following chapters are giving an overview on the role of DO, HSO and closure OTR mainly on white wine quality parameters such as shelf life, color and aroma.

1.3.1 Sulfur dioxide and wine shelf life

SO_2 is used throughout winemaking due to its antioxidant, anti-enzymatic and antimicrobial properties. Before bottling, SO_2 is added to the wine to ensure its shelf life. When added to finished wine, SO_2 undertakes following functions (Kettern 1985, Casey 2003):

a) it binds carbonyl compounds derived from the fermentation, such as acetaldehyde and chromophoric carbonyl groups, protecting the wine from their undesirable sensory properties
b) it acts as an antioxidant by reacting with oxidants derived from the contact of the wine with oxygen, protecting that way other wine compounds, e.g. aroma compounds from being oxidized
c) it inhibits the activity of microorganisms, e.g. acid bacteria, as well as enzymes such as phenol oxidases - if any still active in wine - protecting that way wine from bacterial spoilage and enzymatic oxidation.

As a result of the first function mentioned above, SO_2 in wine exists in two forms: the bound or fixed SO_2 (on wine compounds) and the surplus or free SO_2, which acts as an antioxidant and inhibitor. The bound SO_2 includes varying levels of binding. Casey (1996) showed actually three stages of SO_2: the free SO_2, which is the first that is lost during bottle storage due to oxidation; the labile, which is bound but it replenishes the free when it is lost; and the permanently bound. Initially the free SO_2 may decrease with no effect on the labile, but when free SO_2 reaches a limit, labile SO_2 starts to dissociate (Reeves 2009). When the entire surplus SO_2 is lost, any further oxidation gradually releases the carbonyls and their undesirable sensory effects (Casey 2003). The amount of permanently bound SO_2 and the limit of free SO_2 under which labile SO_2 starts to dissociate depend on wine type and composition. Godden et al. (2001) suggest as critical free SO_2 concentration in the region between 10 and 15 mg/L. When free SO_2 falls below this level, symptoms of oxidation begin to appear.

Several studies have showed that closure´s OTR is mainly responsible for SO_2 decline and post bottling oxidation of wine (Godden et al. 2002, Skouroumounis et al. 2005a/b, Hart and Kleinig 2005, Godden et al. 2005, Braikowich et al. 2005, Lopes et al. 2006, Kwiatkowski et al. 2007, Lopes et al. 2009). However Casey 2003 suggested that SO_2 decline post-bottling occurs mainly due to the incorporation of air and oxidants before and during bottling and to a much lesser extent due to the oxygen ingress into the bottle through the closure (figure 3). However, because SO_2 oxidation includes several steps, it will take some time for the oxidation symptoms to appear. Therefore the author believes, that even if oxidation symptoms start to become noticeable several months after bottling, the real reason is the oxygen at and before bottling rather than the closure. Casey 2009 respond to the belief, that SO_2 decline post-bottling is much higher than the maximum DO in wine could cause, with the counter argument, that air contact before and during bottling can raise the total amount of oxygen in wine above the saturation limit (8 mg/L at 20°, more at lower temperatures) in following ways:

a) formation of oxidants due to air contact in the days before bottling (0-6 mg/L)
b) emulsification of air and wine during filling (1-2 mg/L)
c) compressed air in the headspace when the cork is driven into the bottle without effective vacuum application (3-6 mg/L)

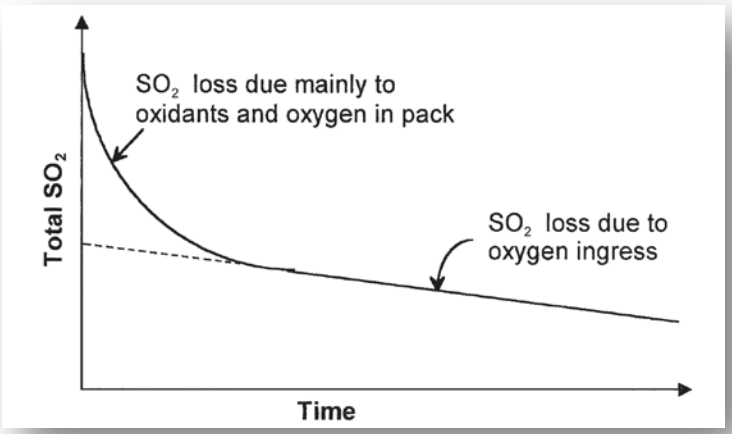


Figure 3: A notional representation of SO_2 decline post-bottling (Casey 2002).

In wine, SO_2 is hydrated and exists mainly as the bisulfite ion (HSO3-). It has long been proposed that two SO_2 molecules react with one molecule oxygen to produce two sulfate ions. Consequently, it was envisaged that, by reacting with oxygen, SO_2 protected vulnerable wine constituents from oxidation (Ribéreau-Gayon et al. 2000, Clarke and Bakker 2004). Danielwicz et al. (2008) however, explained that as the reaction rate of oxygen with SO_2 is quite slow relative to what can occur in wine, the main antioxidant action of SO_2 is to react with hydrogen peroxide produced as a result of polyphenol oxidation (Boulton et al. 1996). Fe (II) catalyzes the reduction of hydrogen peroxide to produce hydroxyl radicals, which are highly reactive and will oxidize ethanol to acetaldehyde (Danilewicz 2003, Waterhouse and Laurie 2006).

The interaction of SO_2 with oxygen is in fact quite complex (figure 4). It involves chain reactions, initiated by Fe (III), which oxidize bisulfite to sulfite radical. This radical reacts rapidly with oxygen, producing the highly oxidizing peroxomonosulfate radicals, which by reacting with bisulfite produce sulfate and regenerate sulfite radicals to continue the chain process (Brandt et al. 1994, Brandt and van Eldik 1995, Connick et al. 1995). Catechols are known to block this reaction, presumably by scavenging intermediate peroxomonosulfate radicals. Danilewicz (2007) believe that the direct interaction of oxygen and bisulfite is therefore very unlikely to occur to a significant degree in wine because of the radical scavenging activity of polyphenols. Moreover they have demonstrated that the rate of SO_2 oxidation is dependent on catechol concentration.

The studies of Danilewicz (2008) have proved that the rate of reaction of oxygen and SO_2 in model wine is dependent on the concentration of the catechol. The author is therefore convinced that SO_2 does not simply react with oxygen to protect vulnerable polyphenols from oxidation as has long been assumed, but that the autoxidation of SO_2 is a radical chain reaction, which is blocked by radical

scavenging polyphenols and hence the direct interaction of oxygen and SO_2 should not occur in wine conditions.

Furthermore they have shown that a quinone is produced on oxidation of a catechol in these model wine conditions and that the O_2/SO_2 molar reaction ratio of 1:2 indicates that SO_2 reacts not only with hydrogen peroxide but also with this quinone. In real wine it is possible that polyphenols could compete with bisulfite for quinones, depending on SO_2 concentration. This has implications in the deliberate exposure of wine to oxygen such as in microoxygenation and barrel aging when SO_2 concentration could affect the results (Danilewicz 2008).

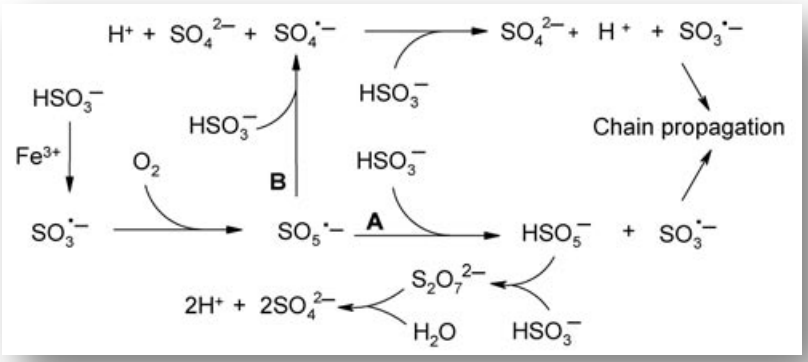


Figure 4: Radical chain reaction involved in bisulfite oxidation (Danielwicz 2008).

1.3.2 Oxygen and wine color

The chemical interpretation of white wine color has always been a little-known field. Phenolic compounds such as benzoic and cinnamic acids, catechins, procyanidins and flavonols are involved (Ribereau-Gayon et al. 2006). Besides the phenolic fraction, Myers and Singleton (1979) identified also a 'non-phenolic' fraction consisting mainly of polysaccharides and protein compounds. These two fractions participate in the color of dry white wines (measured at 420 nm) by 50% each. This proportion changes when the wine is oxidized, chemical or enzymatic. The phenolic fraction is then responsible for most of the color (Ribereau-Gayon et al. 2006).

Among the phenolic components identified, derivatives of quercetin, caffeic acid and *p*coumaric acid are all more-or-less intensely yellow-colored. Tannins are also yellow and their color varies according to the oxidation level of the medium. Oxidation of dry white wine produces browning, due to modifications in tannins and highly oxidizable caffeic acid derivatives (Cheynier et al., 1990). The other compounds are relatively unaffected by oxidation, especially the non-phenolic protein and glucide fractions.

White wine color ranges between light yellow or green and deep yellow or brownish hew (Du Toit et al. 2006). Brown color, measured at 420 nm, is an indication for oxidation and therefore unwanted. Although it can be induced by enzymatic oxidation, enzymes are in wine no longer active because of their precipitation during alcoholic fermentation and the alcohol inhibition in the finished product. Hence, browning in white wine is a chemical process that is slower than enzymatic oxidation (Du Toit et al. 2006).

Non enzymatic browning in white wines can occur according to three mechanisms. The first is the oxidation of phenolic molecules to their corresponding quinones, in varying degrees of polymerization, producing a yellow-brown coloration. This oxidation reaction is influenced by the copper and iron concentrations (Du Toit et al. 2006). The second mechanism is the oxidation of tartaric acid to glyoxylic acid, which leads to the condensation of phenolic molecules due to the glyoxylic acid acting as a bridge between phenolic molecules. Varying degrees of polymerization of the latter contributes to the yellow-brown spectrum. Finally, acetaldehyde, produced during oxidation, can increase the yellow color by inducing the condensation of phenolic molecules (Es-Safi et al. 1999c, Lopez-Toledano et al. 2004, Monagas et al. 2005).

Besides absorption at 420 nm, white wine color can be described using a three dimensional system called CIELab. CIE is an abbreviation for the International Commission on Illumination based on the French title (Commission Internationale de l'Eclairage). CIELab is a color measurement system of CIE which is based on a three-dimensional color space (ETS Laboratories 2009). CIELab values describe the coordinates of a specific color in a three dimensional space. There are three axes: L* describing light to dark, b* for blue to yellow, and a* for red to green. The system was developed to represent color in a manner that is consistent with human vision and proportional to perceived color differences. ΔE represents the total color difference between the two samples and values greater than one indicate color difference that can be seen by human eye.

Several studies illustrate the impact of bottling and closure on color of white wine. Godden et al. (2002) have shown that a Semillon wine sealed with screw caps had lower browning compared to the same wine sealed with synthetic closures, natural and technical corks. Lopes et al. (2009) assessed browner color in Sauvignon Blanc wine sealed with synthetic closures than in the same wine in glass ampoules or under screw caps. However, the authors concluded that these differences were apart from the different OTR also due to differences in DO at bottling as well as eventual differences in the HSO of the different treatments. Skouroumounis et al. (2005a/b) did studies on Riesling and Chardonnay wine and showed that different closures and bottling practices caused differences in color after some months of storage. Riesling was in this study more resistant to color changes than Chardonnay. Kwiatkowski et al. (2007), who tested the impact of different headspace volumes by

screw cap on a Cabernet Sauvignon wine, found that already after 12 months wines under large headspace volume had darker color than those under small headspace.

1.3.3 Oxygen and wine aroma

There are about 600 to 800 compounds that contribute to wine aroma (Rapp 1998). These can originate from the grapes (methoxypiyrazines, terpens etc.), from the yeasts (higher alcohols, fatty acids, esters, aldehydes and ketones) or from the oak in case of barrel ageing. These compounds can change their concentration or even be lost or converted to other compounds over time in the bottle (Reeves 2009). New compounds that appear during bottle ageing are known as “bottle bouquet” and include TDN (1,1,6-trimethyl-1,2 dihydronaphthalene) methional, sotolon, eugenol and phenylacetaldehyde with aromas reminding an kerosene, cooked vegetables, roasted and baked products, maple syrop, honey and nuts.

Oxygen exposure influences wine aroma in different ways. Although the aroma complexity of some wines may increase with a little oxygen contact, the majority of white wines loses their fruitiness and increases their oxidation character even by small additions of oxygen (Du Toit et al. 2006). Higher additions of oxygen can lead to the formation of unwanted off-flavors, with oxidized white wines being described as caramel, overripe fruit, crushed apple, acetaldehyde, woody, rancid, farm-feed, honey-like and cooked vegetables (Escudero et al. 2002, Silva Ferreira et al., 2003b). Odorants formed during oxidation include acetaldehyde, octenol, furfural, benzaldehyde etc. The oxidative aroma formation of white wine dependents on parameters such as oxygen concentration, wine pH, storage conditions, SO_2 concentration, phenolic composition and ascorbic acid concentration (Du Toit et al. 2006). The floral aroma of white wine seems to degrade faster at higher temperatures, with O_2 additions, and with lower pH values increasing this trend. SO_2 additions decrease this degradation. At lower temperatures (15°C) however, degradation proceeds faster at pH 4 than pH 3, and the addition of oxygen has an even more dramatic effect, with the floral aroma almost disappearing after a single saturation (Du Toit et al. 2006). The formation of linalool oxide is enhanced by high temperatures and low pH values. Aromatic degradation can occur before any color change can be identified (Singleton et al. 1979, Boulton et al. 1996, Silva Ferreira et al. 2003a).

Du Toit et al. (2006) give a literature summary around the aroma of oxidized Riesling wines: Simpson (1978) studied the effects of oxygen addition and enhanced ageing at 50°C for 28 days on the composition of Riesling wine. The concentrations of some aroma compounds, such as ethyl n-hexanoate, hexyl acetate, acetic acid, ethyl n-octanoate, vitispirane, 1-hexanol, ethyl furonate and ethyl lactate did not differ significantly between the treatments. However, benzaldehyde, diethyl

succinate and 1,1,6-trimethyl- 1,2-dihydronaphthalene (TDN), increased from 0, 3.8, 0.066 mg/L to 0.18, 4.4 and 0.09 mg/L, respectively. The concentration of 2-phenylethanol was lower in the oxidized wine. Enhanced ageing under anaerobic conditions increased ethyl n-octanoate, vitispirane, ethyl furonate, ethyl n-decanoate, TDN and 2-phenethanol concentrations. Marais *et al*. (1992) found that TDN, trans-vitispirane, 2,6-dimethyl-7-octen-2,6-diol and trans- 1,8-terpin concentrations, and the intensity of the bottle-aged kerosene-like character, increased significantly with ageing in Riesling wines. However, decreases were observed in diendiol-1, linalool, isoamyl acetate, ethyl caproate, hexyl acetate, 2-phenethyl acetate, hexanol, 2-phenyl ethanol, and in the intensity of young wine character, with higher storage temperatures accelerating these changes. This study clearly showed that lower storage temperatures (15°C) were more favorable for the sensory development of Riesling during ageing.

However, inadequate oxygen can also alter wine aroma. Sulfur compounds, such as hydrogen sulfide (H_2S) and its products, can be produced by hydrolysis or unaerobic during bottle storage (Reeves 2009). Some of these compounds may play a positive role in the aroma complexity but in general they are considered as a fault, described as *struck flint*, *rubbery*, *rotten egg* etc. (Godden et al. 2001, Skouroumounis et al. 2005). Figure 5 shows how this aroma compounds in wine increase under low oxygen exposure (Ugliano et al. 2009).

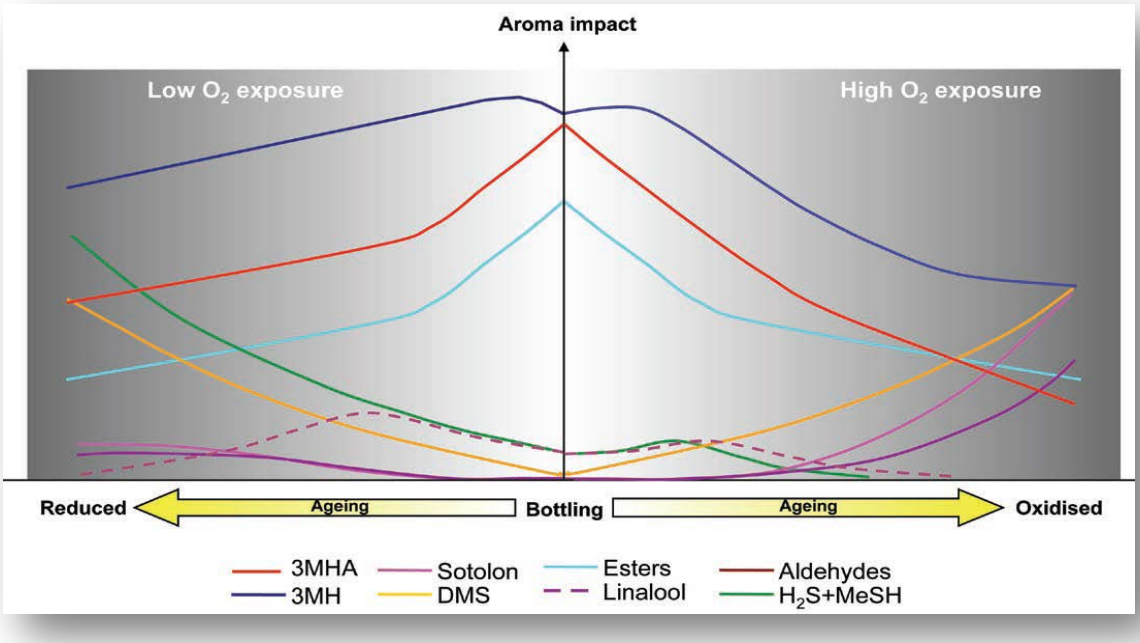


Figure 5: *Theoretical representation of the evolution of the impact of some aroma compounds during ageing of wine under different oxygen regimes (Ugliano et al. 2009).*

Godden et al. (2002) have shown that a Semillon wine sealed with screw cap demonstrated, after 3 years of storage higher scores in citrus and fruity aroma but also in unwanted reductive character than the same wine under natural and synthetic corks. That indicates that aroma development post-bottling can be influenced from closure permeability with closure with low OTR resulting to more fruity but also more reductive aromas. Also Hart and Kleinig (2005) found higher reduced characters in wines were under screw caps and oxidized under synthetic closures. Skouroumounis et al. (2005a/b) did studies on Riesling and Chardonnay wines and showed too that screw cap closure ROTE resulted to reduced whereas synthetic closure to oxidized characters. Lopes et al. (2009) assessed higher oxidized aroma in Sauvignon Blanc wine sealed with synthetic closures than in the same wine in glass ampoules or under screw caps, which nevertheless developed reduced characters. The authors concluded that these differences were apart from the different OTR´s also due to differences in DO at bottling as well as eventual differences in the HSO of the different treatments.

1.4 Objectives of the study

The underlying objective of this work was to investigate the impact of oxygen exposure at bottling as well as during storage on wine´s post bottling development. The specific aims were as follow:

a. Monitoring of the evolution of DO, HSO and closure OTR during bottle storage.
b. Evaluation of the impact of HSO and closure OTR on SO_2 levels, color, aroma and sensory properties of Riesling during bottle storage.
c. Evaluation of the impact of DO at bottling and closure OTR on SO_2 levels and sensory properties of Riesling during bottle storage.

The following chapters deal with the aims of the study mentioned above as follows: chapters 2.1 and 2.2 emerge out of the first bottling trial which examines the impact of HSO and closure OTR on SO_2 levels, color, aroma and sensory properties of the wine post-bottling (specific aim b). Chapter 2.1 additionally investigates the evolution of DO, HSO and closure OTR in the bottle during storage (specific aim a). Chapter 2.3 emerges out of a second bottling trial which deals with the impact of DO at bottling on SO_2 levels and sensory properties of wine post-bottling (specific aim c). Chapters 2.1 and 2.3 have been published in American Journal of Enology and Viticulture, while 2.2 contains mainly unpublished data.

2. Impact of oxygen exposure at and post-bottling on wine post-bottling development

2.1 Impact of headspace oxygen and closure on sulfur dioxide, color and hydrogen sulfide levels in a Riesling wine

EVDOKIA DIMKOU,[1*] MAURIZIO UGLIANO,[2] JEAN-BAPTISTE DIÉVAL,[2] STÉPHANE VIDAL,[2] OLAV AAGAARD,[2] DORIS RAUHUT[3] AND RAINER JUNG[1]

[1]*Geisenheim Research Center, Section of Enology and Wine Technology, Blaubachstraße 19, 65366 Geisenheim, Germany*

[2]*Nomacorc Oxygen Management Research Center, 2260 Route du Grès, 84100 Orange,*

[3]*Geisenheim Research Center, Section of Microbiology and Biotechnology, Von-Lade-Straße 1, 65366 Geisenheim, Germany*

**Corresponding author, email* Dimkou@fa-gm.de

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Impact of Headspace Oxygen and Closure on Sulfur Dioxide, Color, and Hydrogen Sulfide Levels in a Riesling Wine

Evdokia Dimkou,[1]* Maurizio Ugliano,[2] Jean Baptiste Dieval,[2] Stéphane Vidal,[2] Olav Aagaard,[2] Doris Rauhut,[3] and Rainer Jung[1]

Abstract: Following an experimental design replicating typical winery conditions, a Riesling wine was bottled with different headspace oxygen levels and sealed with either a coextruded closure or a screwcap to investigate the impact of headspace oxygen and closure oxygen transfer rate on wine evolution. Using luminescence technology, dissolved oxygen and headspace oxygen, as well as oxygen ingress through the closure, were monitored during 24 months of bottle storage. Under typical winery conditions, headspace oxygen introduced at bottling was found to be a major component of oxygen in bottled wine. Headspace oxygen at bottling influenced loss of sulfur dioxide during bottle storage, being the main cause of sulfur dioxide decline during the first four months after bottling in 375 mL bottles. The loss of sulfur dioxide was not correlated with the evolution of dissolved oxygen, but with the total amount of oxygen consumed by the wine. After 24 months in the bottle, color differences due to different headspace oxygen and closure oxygen transfer rate were generally minor. Conversely, differences in closure oxygen transfer rate were responsible for significant differences in the final concentration of the off-odor compound hydrogen sulfide, with screwcap generally associated with higher levels of this compound. Even if less significantly, the amount of oxygen present in the headspace at bottling also had an effect on final hydrogen sulfide, with higher concentrations observed in wines bottled with lower headspace oxygen.

Key words: wine bottling, headspace oxygen, dissolved oxygen, oxygen ingress, closure OTR, oxygen consumption

Oxygen is a key reactant that can change chemical and sensory properties of wine (Ribereau-Gayon 1933, Wildenradt and Singleton 1974, Du Toit et al. 2006). In bottled wine, oxygen derives from the bottling process or enters the package during storage. During bottling operations, contact of the wine with air (e.g., during bottle filling) can result in increased levels of dissolved oxygen (DO) (Kielhöfer and Würdig 1962, Perscheid and Zürn 1978, Kettern 1985, Schneider 2005). Additionally, gaseous oxygen present in bottle headspace (HS) is another major source of oxygen, which can vary depending on HS volume and HS management technology, such as evacuation or inerting (Müller-Späth 1977, Kettern 1985, Schneider 2005). Subsequently, during storage, oxygen ingress through the closure, as determined by the oxygen transfer rate (OTR), is responsible for additional oxygen uptake (Perscheid and Zürn 1978, Schneider 2005, Müller-Späth 1977, Skouroumounis et al. 2005a, Lopes et al. 2006).

Several studies have investigated the role of oxygen ingress through the closure on wine development. The general conclusion, which is now widely accepted in the wine industry, was that after a certain time of storage, wines bottled with closures with different OTR will exhibit different chemical and sensory characteristics (Skouroumounis et al. 2005a, 2005b, Lopes et al. 2006, Godden et al. 2002, Hart and Kleinig 2005, Lopes et al. 2009, O'Brien et al. 2009). In particular, after three years of storage, a Semillon wine sealed with screwcap retained higher sulfur dioxide (SO_2) and showed lower browning compared to the same wine sealed under synthetic closures, natural corks, and technical corks (Godden et al. 2002). In addition, wines that retained higher concentrations of SO_2 also had higher scores in citrus and fruity aroma. However, wines also had unwanted reductive character. Similar conclusions were proposed later for Riesling and Chardonnay wines (Skouroumounis et al. 2005a, 2005b) and for red wines (Hart and Kleinig 2005). More recently, it has been suggested that an optimal degree of oxygen exposure should be identified in order to prevent formation of reductive off-flavors such as hydrogen sulfide (H_2S) without incurring an excessive loss of fruitiness due to oxidation (Lopes et al. 2009, O'Brien et al. 2009).

Only a limited number of studies have investigated the contribution of different levels of oxygen at bottling, such as DO and particularly HS oxygen. It was suggested that, in addition to OTR, variations in DO at bottling as well as in HS oxygen could be responsible for chemical and sensory differences following bottle storage (Lopes et al. 2009). However, different HS volumes, and therefore levels of HS oxygen, had no influence on the reduced characters of a Semillon wine 24 months postbottling (Godden et al. 2005). Brajkovich et al. (2005) also did not find significant differences in aroma and SO_2 concentrations between different HS volumes in

[1]Geisenheim Research Center, Section of Enology and Wine Technology, Blaubachstraße 19, 65366 Geisenheim, Germany; [2]Nomacorc Oxygen Management Research Center, 2260 Route du Grès, 84100 Orange, France; and [3]Geisenheim Research Center, Section of Microbiology and Biotechnology, Von-Lade-Straße 1, 65366 Geisenheim, Germany.

*Corresponding author (email: dimkou@fa-gm.de)

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Sauvignon blanc wine. In a study on the impact of different HS volumes on a Cabernet Sauvignon wine sealed by screwcap, after 12 months wines bottled with higher HS volume had lower SO_2 concentrations and darker color than those with lower HS volume (Kwiatkowski et al. 2007). However, these were extreme situations, with 4 mL and 64 mL HS volume, respectively.

In the above-mentioned studies HS oxygen was not measured, so a comprehensive rationalization of the different results is not possible. In addition, HS oxygen was generally adjusted by changing wine volume in the bottle, while in the winery HS oxygen is often adjusted by applying a flush of inert gas to remove oxygen. In this sense, the impact of HS oxygen on wine evolution is unknown and the question remains open as to whether HS volume and HS composition are equivalent in terms of wine development, given that changes in the concentration of oxygen in the headspace can affect oxygen diffusion in the wine. In common industry practice, screwcaps are bottled with a larger headspace and cylindrical closures are bottled with a smaller headspace. In addition, no study has been carried out to date to investigate whether oxygen at bottling is equivalent to oxygen delivered during storage through the closure and whether different types of closures require different types of HS management.

The aim of this study was to investigate the influence of HS volume and composition on wine development during bottle storage and to study the effects of combining different HS oxygen at bottling with closures with different OTR. An experimental design replicating typical winery conditions was adopted. Oxygen evolution inside the bottles was accurately measured using a luminescence-based technology (Dieval et al. 2009, Nygaard et al. 2009) in a first attempt to describe the correlation existing between oxygen consumption and wine chemical changes during bottle storage.

Materials and Methods

Wine. Approximately 1000 L of Riesling wine (Rheingau region, vintage 2007) was vinificated at Geisenheim Research Center. The fermentation took place in stainless-steel tanks between 18 and 22°C and bentonite fining was performed four months later. The wine was stored in a tank with no ullage until bottling. Analytical parameters of the wine at bottling were as follows: 12.7% alcohol, 9.7 g/L sugar, pH 3.33, 7.1 g/L acidity, 54 mg/L free SO_2, 135 mg/L total SO_2, and 0.3 mg/L DO.

Bottles and closures. A coextruded (Co) synthetic closure (Nomacorc Classic, 43 x 22 mm; Nomacorc SA, Thimister Clermont, Belgium) and a screwcap (Sc) closure (CSI 5SE, 28 x 15 mm, PVC-free; Alcoa, Kelkheim, Germany) were used. The bottles were colorless, Saint Gobain 0.375 L glass (375 mL at 52 mm for the cylindrical closure finish and at 28 mm for the screwcap finish).

Oxygen measurements. Dissolved oxygen of the wine prior to bottling was measured in the tank with a Fibox 3-Trace fiber-optic oxygen meter coupled to an oxygen dipping probe (PreSens GmbH, Regensburg, Germany). Dissolved oxygen and HS oxygen were measured with the Fibox 3-Trace fiber-optic oxygen meter coupled to Pst3 oxygen sensors (linearity range from the manufacturer: 0 to 50% oxygen). Five bottles fitted with two oxygen sensors each were used for each modality. The sensors were glued inside the bottle with silicone (RS Components, Mörfelden-Walldorf, Germany) at a height of 8 cm from the bottom of the bottle for DO measurement and in the headspace for HS oxygen measurement. These bottles were equally distributed in the bottling line during bottling (approximately one bottle with sensors every 30 bottles). Manufacturer calibration was used for all the sensors, with no further calibration. All oxygen measurements in the bottle were taken right after bottling, weekly during the first two months of storage, and at 4, 8, 10, 14, and 24 months after bottling (without shaking of the bottles).

Bottling. The bottling set up is shown (Figure 1). Two HS volumes, 6 mL (HS6) and 18 mL (HS18), were applied for each closure. For each HS volume, oxygen content was adjusted by CO_2 flushing, with the highest HS oxygen obtained with no flushing, and therefore the headspace consisted of ambient air. Medium (Med) and High levels of HS oxygen were obtained for each closure. For the coextruded closure, an extra set of wines was prepared with lower HS oxygen (Low) to study the effect of high inerting on coextruded closures. The HS oxygen was measured in hPa, converted into mg in headspace, and then to potential mg/L in wine, taking into account the wine volume (376 mL for the cylindrical closure HS6, 365 mL for the cylindrical closure HS18, and 379 and 367 mL for the screwcap, respectively). The bottling line included a plate filter (Seitz Enzinger Noll, Worms, Germany), a 100 L filler tank, and a three-head manual filler (KTM-Troxler, Ettenheim-Münchweier, Germany), a semiautomatic corking machine with vacuum (GAI 4040, Prospero Equipment Corp., Pleasantville, NY), an industrial corker with CO_2 flushing (Seitz Enzinger Noll), and a semiautomatic screwcap machine (VAW Aluminium AG, Grevenbroich Germany). The bottles were rinsed with SO_2 and sterile water directly before bottling (Stroh GmbH, Schloßböckelheim, Germany).

The filling level was adjusted manually using a silicone stopper fitted with two adjustable tubes. One tube was adjusted to the desired filling height, the other was set shorter, to be in the headspace. After each bottle was filled, the silicon stopper was held for some seconds on the top of the bottle neck closing the headspace, so that the first tube was immersed in the wine as deep as the desired filling height. By

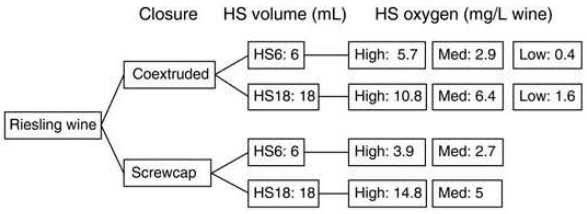

Figure 1 Headspace (HS) volume and HS oxygen expressed in mg/L wine at bottling (results are means of five replicates per treatment).

applying a gas pressure through the second tube, excess wine was pushed upward and removed from the bottle neck via the second tube, which was connected to a vacuum pump for increased efficiency. The gas pressure was obtained by air for the High HS oxygen, whereas CO_2 (purity 100%; Air Liquid, Düsseldorf, Germany) was used for the Med oxygen level to obtain increased removal of HS oxygen. For the Low oxygen level (only cylindrical closures), filling height was adjusted by CO_2 pressure and residual oxygen was removed by flushing the headspace directly with CO_2. Bottles were then sealed with one of the two closure types. In no case was vacuum applied at closure insertion. All bottles were stored upright in the storage room of the cellar in Geisenheim Research Institute at 14 to 16°C and 55% humidity.

Following bottling, DO was 1.08 ± 0.15 mg/L, confirming consistency across the different wines. Given that DO before bottling was 0.3 mg/L, DO increase due to bottling was ~0.8 mg/L, which is consistent with other studies (Kielhöfer and Würdig 1962, Perscheid and Zürn 1978, Lopes et al. 2009) and indicates a well-controlled process.

Determination of total consumed oxygen. Total consumed oxygen (TCO) at each time point was calculated as the sum of oxygen present at bottling (HS plus DO), plus the oxygen entering the bottle through the closure during storage, minus HS and DO oxygen measured at each time point, the latter accounting for any residual oxygen not consumed by the wine. The result obtained was the total amount of oxygen consumed by the wine. In order to quantify oxygen ingress through the closure during time, 10 bottles for cylindrical closure and 10 for screwcap were fitted with one Pst6 oxygen sensor each (linearity range 0 to 4.2% oxygen) at 8 cm from the bottom. These empty bottles were filled with pure nitrogen (purity 99.8%; Air Liquid) until complete removal of the oxygen present and then were directly sealed with one of the closures used. Measurements of oxygen were taken at the time points previously indicated to quantify oxygen ingress through the closure in the course of time.

Sulfur dioxide measurements. Duplicate measurement of free and total SO_2 was carried out on three bottles from each treatment right after bottling, weekly during the first month of storage, and after 4, 10, 14, and 24 months of storage. The measurements were carried out by flow injection analysis with a FIAstar 5000 Analyzer (Foss, Rellingen, Germany). Calibration of the analyzer was done before each set of measurements using a set of 10 known solutions (0, 10, 20, 30, 50, 100, 120, 150, 180, and 200 mg/L SO_2).

Color measurements. The CIELab parameters L*, a*, and b* were calculated using the ASTM software (Standards on Color and Appearance Measurement, West Conshohocken, PA) after scanning samples of three bottles per treatment (same bottles used for SO_2 analysis) in a CADAS 200 spectrophotometer (1 cm cuvette; Hach Lange GmbH, Düsseldorf, Germany).

Hydrogen sulfide. Hydrogen sulfide (H_2S) was analyzed after 24 months of storage by gas chromatography (GC) coupled with a pulsed flame photometric detector (PFPD), using static headspace sampling. A 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) was used, equipped with a MPS 2 headspace sampler (Gerstel, Mülheim an der Ruhr, Germany), a CIS-4 cooled injection system (Gerstel), and a 5380 PFPD (OI Analytical, College Station, TX). Chromatographic separations were performed on a SPB-1 sulfur column (30 m x 0.32 mm i.d., 4 µm film thickness; Supelco, Sigma-Aldrich, Munich, Germany). Analytical conditions were as follows: injector temperature program, -100°C, 12°C/sec until 40°C for 1 min, then 12°C/sec until 180°C for 8 min; oven temperature program, 29°C for 7 min, 10°C/min until 180°C for 10.5 min. Helium was used as carrier gas. Detector temperature was 250°C. Analyses of the samples were carried out in duplicate. More details on sample preparation and analytical parameters can be found elsewhere (Rauhut et al. 1998, 2005, Irmler et al. 2008).

Statistical analyses. Analysis of variance and Tukey tests (0.05 significance) were carried out using SPSS 15.0 (IBM SPSS, Chicago, IL). Regression analysis and Pearson's correlation test (0.05 significance) were conducted with XLSTAT 2010 software (Addinsoft Deutschland, Andernach, Germany).

Results and Discussion

Headspace oxygen. Headspace oxygen data at bottling (Figure 1) showed that different levels of HS oxygen were created by CO_2 flushing of the headspace. Differences in HS oxygen between the modalities with same closure and HS volume were statistically significant ($p < 0.05$). Although similar values of HS oxygen were obtained by different combinations of HS volumes and degree of HS inerting (e.g., Co/HS6/High and Co/HS18/Med), HS volume has to be consistently considered in conjunction with expansion of the wine inside the bottle. Indeed, too small of HS volumes could increase the risk of wine leakage. Therefore inerting a larger headspace could be a safer solution for HS oxygen management than reducing HS volume. In addition, high inerting a small headspace (Co/HS6/Low) was overall the most effective approach to reduce HS oxygen levels (Figure 1).

The HS volumes of 6 mL and 18 mL represent typical industry settings for cylindrical and screwcap closures, respectively (Paine 1991, Schneider 2005). However, as 375 mL bottles were used here, the levels of oxygen contained in these headspaces (expressed in mg/L wine) would be 50% lower if 750 mL bottles were used. When the values are calculated for 750 mL bottles, final values of 0.2, 1.4, and 3 mg/L wine in bottles with cylindrical closure (HS6), and 2.5 and 7.3 mg/L wine in screwcap bottles (HS18) were obtained for the three inerting levels. It can be concluded that the range of HS oxygen concentrations in this study is similar to that found in other studies (Vidal et al. 2004, O'Brien 2009). Therefore, although obtained in an experimental setup, the observations of this study provide meaningful indications regarding bottling management in large-scale winemaking.

Headspace oxygen decreased during storage in all treatments because of its dissolution into the wine and consequent consumption through different chemical reactions (Figure 2A, B). The time required for HS oxygen to become undetectable varied between two weeks and less than eight months depending on initial concentration and closure type; the two

extremes were Co/HS6/Low and Sc/HS18/High with 0.4 mg/L wine and 14.5 mg/L wine initial HS oxygen, respectively. However, in the majority of the cases, HS oxygen was completely consumed within one to three months.

Dissolved oxygen. Starting from a DO after bottling of 1.08 ± 0.15 mg/L across all modalities, a decrease in DO was observed over the entire experiment (Figure 2C, D), with complete consumption occurring within one to eight months, depending on initial HS oxygen. In no case did DO increase again once it reached zero (data not shown). Changes in DO reflect the net balance between dissolution of oxygen from the headspace into the wine and consumption by the wine itself. Therefore, in general, the latter was higher than the former during the timeframe of the study. In samples with lower HS oxygen, DO started decreasing immediately after bottling, whereas in samples with higher HS oxygen, an early transient increase in DO was observed. This suggests that, during the early stages of bottles storage, in these samples the rate of oxygen dissolution into the wine was higher than consumption. In general, the magnitude of this DO increase was proportional to the concentration of oxygen in the headspace. Among the concentrations of HS oxygen tested, a critical concentration of 3.9 mg/L wine was identified as the threshold value of HS oxygen causing this transient DO increase (Sc/HS6/High). Below this value of initial HS oxygen, no DO increase was observed with a HS oxygen of 2.9 mg/L wine (Co/HS6/Med), suggesting that for this HS oxygen the rate of consumption was higher than that of dissolution. Therefore, it is likely that, in terms of DO increase, the actual critical HS oxygen was between 2.9 m and 3.9 mg/L wine for the particular wine tested and the size of the bottles used in this study.

Although an increase in DO was observed for both closures, there was a difference between coextruded closures and screwcaps. Coextruded closures with high and medium initial HS oxygen showed an increase of DO during the first two days after bottling (Figure 2C), after which DO started to decrease because of oxygen consumption. Conversely, in screwcap samples, DO initially decreased and only after ~10 days postbottling was a decrease observed. This difference could be partly due to extra pressure generated in the headspace by cylindrical closures, which could have accelerated the dissolution of oxygen into wine (Kielhöfer and Würdig 1962). In addition, the differences in wine–headspace contact surfaces have to be considered. In the case of cylindrical closures, HS18 resulted in a filling height below the bottle shoulder, and therefore in a larger wine–headspace contact surface, which probably favored rapid dissolution of oxygen in the wine. In the case of coextruded closures, high HS treatments were only introduced for comparison (as they are not commercially realistic), whereas both HS volumes are in theory possible for screwcap closures.

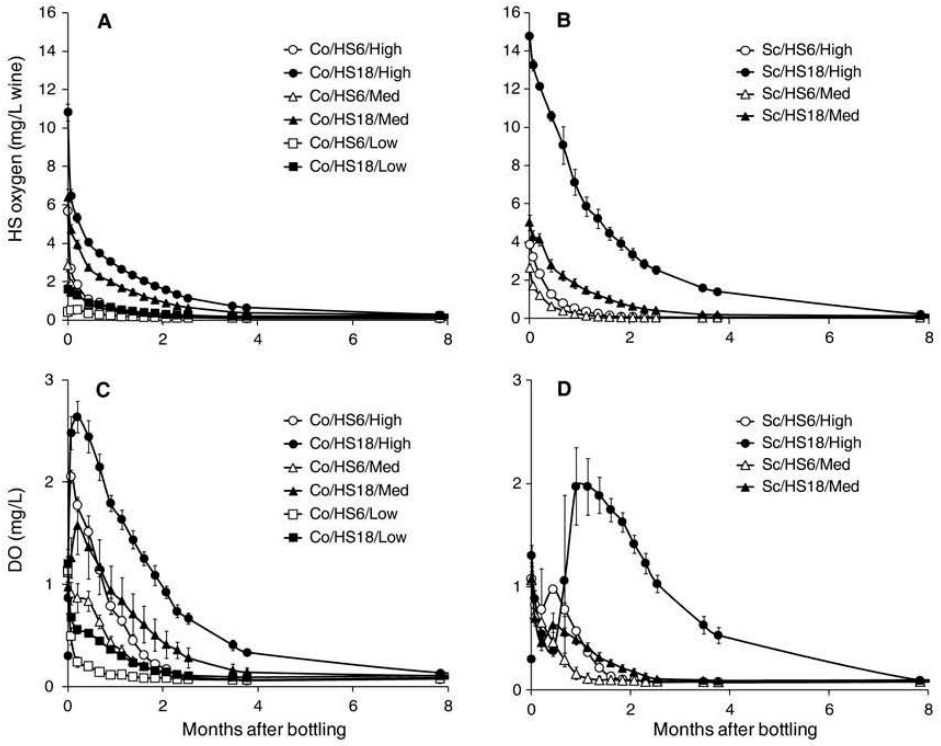

Figure 2 Evolution of headspace (HS) oxygen (A, coextruded; B, screwcap; expressed in mg oxygen per L wine) and dissolved oxygen (DO) (C, coextruded; D, screwcap; •, tank measurement before bottling) during bottle storage.

Another interesting difference between the two types of closures is that, despite similar values of initial HS oxygen (for example Co/HS6/Med and Sc/HS6/Med), complete consumption of initial DO was longer with cylindrical closures. Elsewhere, a faster DO drop in white wine was measured under screwcap than under natural cork (Perscheid and Zürn 1978). These findings reflect the fact that, due to their porous nature, cylindrical closures contain air, and therefore oxygen (Jung and Zürn 2000, Lopes et al. 2007, Ugliano et al. 2011), which is in part released gradually into the headspace following closure insertion in the bottle. In addition, ingress of oxygen through the closure, although limited at this stage, was higher for the coextruded closures used here.

Free and total SO_2. The evolution of free SO_2 in the different treatments during storage was determined (Figure 3). Similar profiles were obtained for total SO_2 (data not shown). Consistent with other studies (Godden et al. 2005, Brajkovich et al. 2005, Kwiatkowski et al. 2007, Lopes et al. 2009), a rapid decrease in free SO_2 was observed early in the study. Differences between treatments became significant in the first week ($p < 0.05$), and wines with higher HS oxygen showed a more rapid decline of free SO_2. Differences reached their maximum at about four months, and remained mostly unchanged for the following 20 months of bottle storage. At 10 months, the evolution of SO_2 was not significantly affected by initial HS oxygen ($p < 0.05$), while closure OTR started to be the main source of differences between the modalities (OTR data presented later). In general, ~55% of the global free SO_2 decrease under coextruded bottles and 80% under screwcap bottles occurred during the first four months, confirming that the greatest loss of free SO_2 during bottle storage is associated with the oxygen present at bottling, which is largely HS oxygen. Therefore, management of HS oxygen at bottling allows great control of SO_2 decline during bottle storage and, consequently, during wine shelf life.

Following the first four months of storage, wines sealed with screwcaps showed virtually no further loss of free SO_2, consistent with the very low OTR of this type of closure. Conversely, a further decline was observed for cylindrical closures, given their permeability to oxygen (Figure 4). During the first four months, when free SO_2 evolution was mainly dependent on initial headspace, losses of free SO_2 were consistently smaller when lower HS oxygen (and smaller HS volume) was applied. In the following 20 months, initial HS oxygen had a minor impact on the decline of free SO_2, while closure OTR was the main modulator of SO_2 concentration. From these results it is clear that management of both HS oxygen at bottling and OTR offer the potential to control, to a certain extent, the decline of free SO_2 during bottle

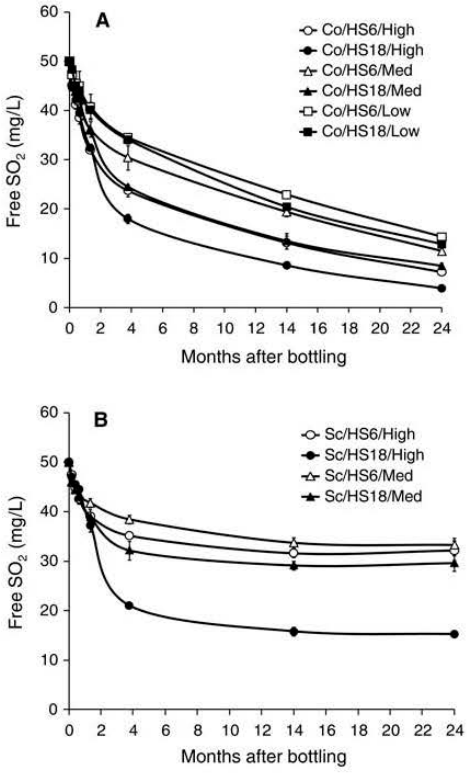

Figure 3 Evolution of free SO_2 during bottle storage (A, coextruded; B, screwcap).

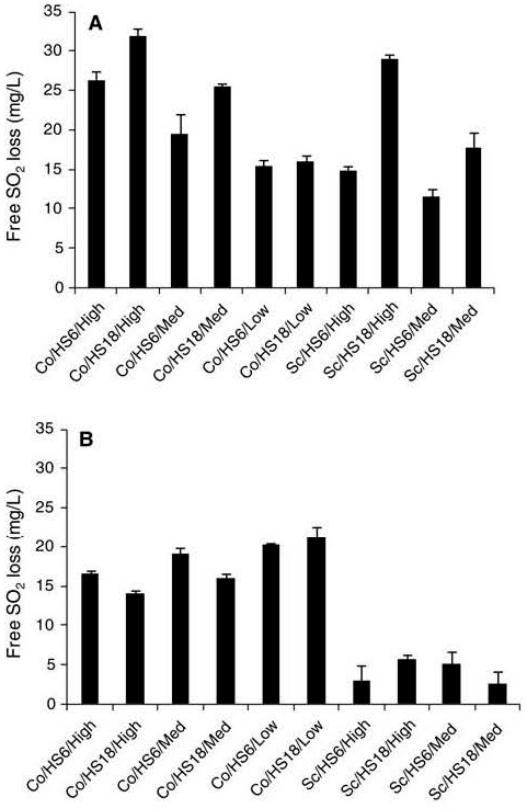

Figure 4 Free SO_2 loss during the first four months (A) and between months 4 and 24 (B) of bottle storage.

storage. However, when the HS contained higher oxygen, sealing with extremely low OTR closures such as screwcap did not prevent significant loss of SO_2 early in wine life (Sc/HS18/High; Figure 3). On the contrary, removal of oxygen by inert gas flushing significantly reduced initial SO_2 loss, even for closures with higher OTR (Co/HS6/Low). In this study, CO_2 inerting of the headspace reduced the loss of free SO_2 up to 17 mg/L in the first four months of storage (Co/HS18/Low vs. Co/HS18/High). At this point it should be emphasized that the wine volume in the bottles was half the volume typically obtained in practice (375 instead of 750 mL), whereas the HS volume, the HS oxygen, and the oxygen ingress through the closure were realistic for 750 mL bottles. To that effect, the time of storage in these smaller bottles corresponds to twice as much as in larger bottles.

Another interesting observation was that, in the first four months, the greatest improvement in free SO_2 loss was observed when inerting was applied to screwcap closures with larger headspace, which is the typical industry setting for this type of closure. This highlights the importance of management of HS oxygen for screwcap closures and, in general, in situations where larger HS volumes are used, as they involve higher levels of oxygen.

Correlation between SO_2 evolution and oxygen. A Pearson's correlation test was carried out to explore the correlation between SO_2 loss and decline of DO (Table 1). A correlation coefficient higher than 0.7 could only be obtained for the first two weeks of bottle storage, while at four months the correlation was already lower. Although our data is consistent with the empirical observation that, at least in the presence of high concentrations of available oxygen (i.e., HS oxygen or DO), a rapid decline of SO_2 is observed (Brajkovich et al. 2005, Kwiatkowski et al. 2007, Lopes et al. 2009), the direct reaction between oxygen and SO_2 is extremely slow under wine conditions (Waterhouse et al. 2006). Conversely, SO_2 loss is linked to oxygen through reaction of SO_2 with the products of wine oxidation, in particular hydrogen peroxide (Danilewicz et al. 2008). Additionally, the DO measurement only reflects the oxygen that is present in the wine at any given time. In a highly reactive environment such as wine, it reflects the occurrence of any excess oxygen that the wine has not yet consumed at that time point. Therefore, once DO reaches a value of zero, consumption of oxygen is still taking place, but cannot be quantified, explaining why a good correlation between SO_2 loss and DO was observed only at the early time points.

In order to overcome this limitation, total consumed oxygen (TCO) was calculated as the sum of the oxygen present at bottling (HS oxygen plus DO), plus the oxygen entering the bottle through the closure during storage, minus DO and HS oxygen measured at each time point. Oxygen ingress through the closure was measured by quantifying oxygen ingress in bottles filled with nitrogen, sealed with the closures used in the study, and stored under the same conditions as the experimental wines. In total, during 24 months, 13 ± 1 mg/L oxygen was delivered by the synthetic closure and 1 ± 0.1 mg/L by the screwcap closure. The amount of oxygen entering the bottles sealed with the closure with higher OTR (13 mg/L) is similar to the initial HS oxygen in a screwcap bottle with large headspace (Sc/HS18/high, 14.8 mg/L wine), a setting that is commonly used in the industry for a closure with lower OTR. Clearly, bottling can be a key component of the total oxygen in the bottle.

The TCO obtained 24 months after bottling ranged from 5 to 25 mg/L (Figure 5). Headspace oxygen right after bottling ranged from 0.4 to 14.5 mg/L wine, indicating the contribution of the HS oxygen reservoir to total consumed oxygen. Correlation analysis confirmed that TCO was well correlated with SO_2 decline throughout bottle storage (Table 1). Calculation of TCO can therefore provide a valuable tool to predict SO_2 loss during bottle storage and, therefore, to estimate the shelf life of wine.

Table 1 Correlations between dissolved oxygen (DO) and total consumed oxygen (TCO) and free SO_2 decrease at different time points (days and months) of bottle storage (Pearson, 0.05 significance).

	12 days	4 mo	10 mo	14 mo	24 mo
DO	0.787	0.613	0.178	0.296	0.000
TCO	0.806	0.979	0.973	0.987	0.970

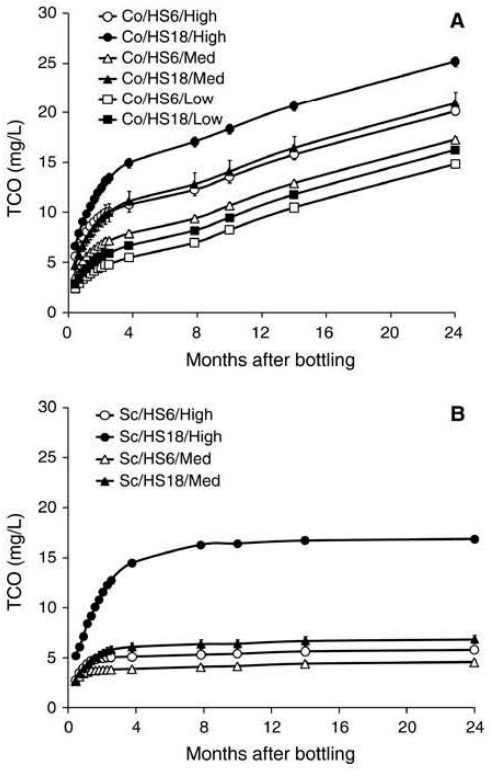

Figure 5 Evolution of total consumed oxygen (TCO) during bottle storage (A, coextruded; B, screwcap).

Color measurements. Wine absorbance at 420 nm (abs_{420}) is commonly used to assess the degree of wine color oxidation. After 24 months of storage, absorbance values were consistently less than 0.097 (Table 2), indicating that the degree of oxidation in the wines was relatively low, although several statistically significant differences were observed. Data collected at different times during storage showed that HS oxygen only affected color development during the first four months ($p < 0.05$). From the subsequent time point (10 months), differences in color development were due to OTR ($p < 0.05$). CIELab analysis coupled with calculation of ΔE_{ab} was carried out to investigate the probability of sensorially relevant differences. The results confirmed that, in general, color variations among the different experimental wines were not likely to be detected by human eye, as ΔE_{ab} was less than 1.0 (data not shown). These results differ from others (Skouroumonis et al. 2005a), which showed that differences in oxygen exposure during storage resulted in significant color differences. Considering that, after 24 months, several wines already had free SO_2 less than 10 mg/L, the lack of major color differences observed here is quite interesting, as it has been suggested that below this level there is a high risk of advanced color oxidation (Godden et al. 2001). Our data indicate that this value needs to be considered carefully, suggesting that generalizations are not possible. Wine content of phenolic compounds is highly variable, depending on grape variety, region, vintage, and winemaking technology. The only case in which, after 24 months, ΔE_{ab} greater than 1.0 was observed was for the pair Co/HS18/High and Sc/HS18/Med, which had a TCO difference of ~15 mg/L oxygen.

Correlation between TCO and abs_{420} was quite low (Pearson correlation 0.568), opposite of that observed for SO_2, suggesting that not only TCO at a specific time point but also timing of oxygen exposure can affect wine color development. For example, in the screwcap treatments, oxygen exposure occurred mainly in the early stages.

Hydrogen sulfide. Hydrogen sulfide (H_2S) was measured in the wines after 24 months of storage by gas chromatography coupled with pulsed flame photometric detection (GC-PFPD) (Figure 6). Type of closure significantly affected final H_2S concentration and in general was the experimental variable introducing the greatest changes in H_2S after 24 months of bottle storage (Table 3). Samples under screwcap closures were consistently characterized by increased H_2S, with values up to 200% higher than coextruded closures (Figure 6), consistent with previous findings highlighting an association between low oxygen exposure during storage and higher levels of H_2S (Lopes et al. 2009). Once all the oxygen present at bottling was consumed, the wine environment became highly reductive under screwcap, favoring accumulation of H_2S. Conversely, coextruded closures provided a moderate supply of oxygen throughout storage, decreasing H_2S accumulation over time. Among the other variables, HS composition at bottling also affected H_2S, with increasing HS oxygen consistently resulting in lower H_2S (Figure 6, Table 3), with Sc/HS6/High as the only exception. Interactions between HS oxygen and type of closure were also significant, although to a lesser extent. Thus, within each type of closure, the pattern of H_2S accumulation over time can be affected by initial HS oxygen.

Finally, HS volume was the experimental variable with the least effect on final H_2S, and in some case larger headspace resulted in slightly higher H_2S concentrations. High transient levels of H_2S can trigger side reactions between H_2S

Table 2 ANOVA analysis and Tukey tests (0.05 significance) for wine color absorbance (abs_{420}) after 24 months of bottle storage.

Treatment	abs_{420}[a]
Sc/HS18/Med	0.0782 a
Sc/HS6/High	0.0825 ab
Sc/HS6/Med	0.0839 abc
Co/HS6/Low	0.0842 abc
Sc/HS18/High	0.0862 bcd
Co/HS6/High	0.0865 bcd
Co/HS18/Low	0.0891 cd
Co/HS6/Med	0.0902 cd
Co/HS18/Med	0.0914 de
Co/HS18/High	0.0975 e

[a]Same letters indicate no significant difference between the values (significance 0.05).

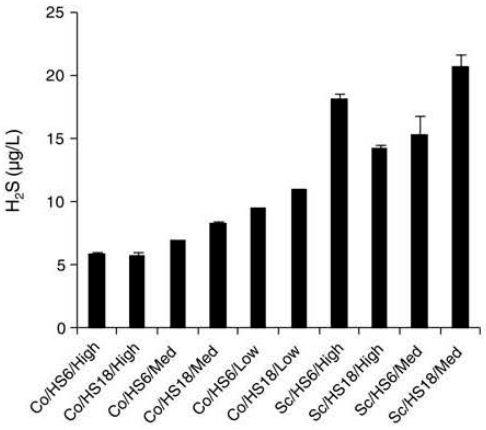

Figure 6 Concentration of hydrogen sulfide (H_2S) in the wines after 24 months of bottle storage.

Table 3 F values and significance of the different factors influencing H_2S concentration in wine after 24 months of bottle storage.

Variable	F	Signf[a]
HS oxygen	37	***
Closure	1082	***
HS volume	4	*
HS oxygen x closure	19	**

[a]*, **, and *** indicate significance at $p < 0.05$, 0.01, and 0.001, respectively.

and other wine components, resulting in a net loss of H_2S (Marchand 2002). However, in our study, that could explain the differences observed within each treatment between HS6 and HS18, but not the overall pattern of H_2S in the final wines. In any case, the results of this study provide evidence that management of oxygen at bottling has the potential to affect wine aroma development during storage. Hydrogen sulfide has been associated with off-flavors of rotten egg, often referred to as "reduced" aromas. An odor threshold of 1.6 µg/L has been proposed for H_2S in white wine (Siebert et al. 2009), indicating that in the wines studied here H_2S was present in concentrations higher than its threshold, therefore potentially contributing to the aroma characteristics of the wines.

Conclusion

Management of HS oxygen and closure selection can affect the evolution of different wine components during aging, including SO_2 and H_2S. The decline of SO_2 during storage is directly linked to the amount of oxygen consumed by the wine. Therefore, during the first four months in 375 mL bottles, SO_2 loss is closely dependent on oxygen present in the headspace, while after 10 months in the bottle, oxygen entering through the closure becomes the main factor affecting SO_2 decline. The combination of different forms of headspace management at bottling, coupled with closure selection, offers potential for tailoring SO_2 addition and wine shelf life according to different needs. Data collected here on H_2S also indicate that headspace management can affect compositional parameters that are potentially linked to wine sensory quality, although the effect of closure type on final H_2S appeared to be stronger. Further studies are needed to fully elucidate the implications of HS management at bottling on wine aroma composition and sensory properties, in particular with regard to the balance between reduced and oxidized aromas.

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2.2 Impact of headspace oxygen on aroma composition and sensory properties of a Riesling wine

Abstract

A Riesling wine was bottled under different headspace volumes and headspace oxygen levels via CO_2 flushing. The impact of headspace on sulfur dioxide losses and color evolution has been discussed in previous publication (Dimkou et al. 2011). This article examines the influence of headspace oxygen and volume on aroma composition and sensory properties of Riesling. Analytical aroma data at 24 months post-bottling showed that small headspace volume was related to higher concentrations of fruity aroma compounds, such as ethyl decanoate and ethyl octanoate (described as grape and fruity aroma respectively). Within treatments with same headspace volume, headspace flushing with CO_2 had an opposite effect as treatments with low headspace oxygen demonstrated lower concentrations of these compounds. Low headspace oxygen resulted also to slightly lower concentrations of cis-linalooxide, a compound contributing to the floral aroma of white wines. Finally, wines bottled with low headspace oxygen had 24 months after storage higher concentrations in sulfur compounds such as H_2S and DMS, compounds typically responsible for reduced aromas. Headspace volume had here a diverse effect as large volume resulted to higher H_2S but lower DMS concentrations. A sensory descriptive analysis at 14 and 24 months post-bottling showed that the analytical differences between the test wines were not great enough to be perceived from the panelists, as wines did not differ in terms of *reductive, fruity,* or *flowery.* However, the different headspace treatments did had an impact on sensory evolution of bottled wines as treatments bottled under high headspace oxygen obtained higher ratings for the attribute *oxidative* than those bottled under low headspace oxygen. Combining low headspace oxygen with small headspace volume offered the best possibility to protect wine from oxidation up to 24 months of storage.

Introduction

Wine bottling and its impact on wine quality has been concerning researchers for many years (Kielhöfer and Würdig 1962, Dimkou et al. 2011). The most important aspect of bottling in these studies is the oxygen exposure of the wine as oxygen can alter its chemical and sensory properties (Ribereau-Gayon 1933, Wildenradt and Singleton 1973, Du Toit et al. 2006). Oxygen exposure at bottling occurs when wine comes in contact with air (e.g. at filling) and dissolved oxygen (DO) increases (Kielhöfer and Würdig 1962, Perscheid and Zürn 1978, Kettern 1985, Schneider 2005). However the bottle headspace is responsible for further oxygen uptake in wine, especially when no

headspace management technology, such as evacuation or inerting has been applied (Müller-Späth 1977, Kettern 1985, Schneider 2005).

Although several studies deal with the consequences of DO increase during bottling on wine´s post-bottling development, the contribution of headspace oxygen (HSO) on sensory and aroma evolution of bottled wines has barely been investigated. Lopes et al. (2009) suggested that, in addition to closure´s permeability to oxygen, variations in DO and HSO at bottling could also be responsible for chemical and sensory differences following bottle storage. However, according to Godden et al. (2005) and Brajkovich et al. (2005), different headspace volumes and therefore levels of HSO, had no influence on wine evolution post-bottling. On the contrary, Kwiatkowski et al. 2007 found that 12 months after bottling wines bottled with high headspace volume had darker color than those with lower headspace volume which indicates that headspace could play a role on the sensory evolution in the bottle.

Our study investigates the influence of headspace volume and composition on post-bottling development of a Riesling wine over 24 months of bottle storage. The impact of headspace management on sulfur dioxide (SO_2) and color as well as the evolution of HSO after bottling has been reported in Dimkou et al. (2011). This article discusses further the impact of headspace management on sensory properties and aroma composition of a Riesling in terms of volatile sulfur compounds and fermentation by-products, such as higher alcohols and esters.

Materials and Methods

Wine

Approximately 1000 L of Riesling wine (Rheingau region, vintage 2007) was vinificated at Geisenheim Research Center. The fermentation took place in stainless-steel tanks between 18 and 22°C utilizing common winemaking practices for wines of these types and bentonite fining was performed four months later. The wine was stored in a tank with no ullage until bottling. Analytical parameters of the wine at bottling were as follows: 12.7% alcohol, 9.7 g/L sugar, pH 3.33, 7.1 g/L acidity, 54 mg/L free SO2, 135 mg/L total SO2, and 0.3 mg/L DO.

Closures, bottles and bottling

The closure used was a co-extruded (Co) synthetic closure Nomacorc Classic (43x22 mm, Nomacorc SA, Belgium) and the bottles were colorless Saint Gobain 0,375 L bottles. For full details of the bottling process and chemical analysis followed see Dimkou et al. (2011). Briefly, the bottling set up is shown in figure 1. Two headspace volumes, 6 mL (HS6) and 18 mL (HS18) were applied. For each headspace volume, HSO concentration was adjusted by means of carbon dioxide (CO_2) flushing, with

the lowest HSO consisting exclusively of CO_2 and the highest of air (no flushing). Oxygen measurements were carried out with the Fibox 3-Trace fiber-optic oxygen meter (PreSens GmbH, Regensburg, Germany) as described in Dimkou et al. 2011. The HSO was measured in hPa, converted into mg in the headspace, and then to potential mg/L in wine, taking into account the wine volume (376 mL for the HS6 and 365 mL for the HS18 respectively). Following bottling, DO was 1.08 ± 0.15 mg/L, confirming consistency across the different wines. Given that DO before bottling was 0.3 mg/L, DO increase due to bottling was approximately 0.8 mg/L, which is consisted with other studies (Kielhöfer and Würdig 1962, Perscheid and Zürn 1978, Lopes et al. 2009) and indicates a well controlled process. All bottles were stored upright in the storage room of the cellar in Geisenheim Research Center at 14-16°C and 55% humidity.

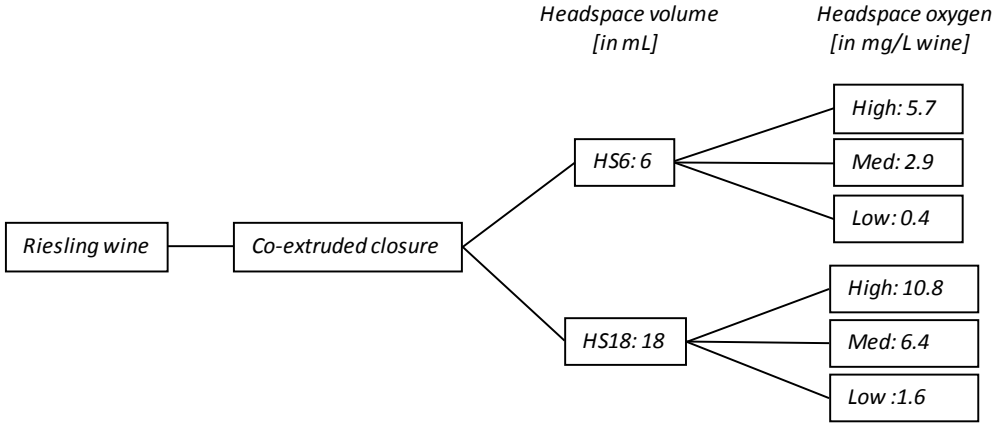

Figure 1: Headspace volumes (in mL) and headspace oxygen levels (in mg/L wine) of the different wine treatments at bottling (results are means of five replicates per treatment).

Analysis of volatile fermenting by-products

Volatile aroma compounds were analyzed at 24 months of bottle storage via Gas Chromatography - Mass Spectrometry (GCeMS) analysis. GCeMS was performed using a GC Hewlett Packard (HP) 5890 Series II (Agilent, Santa Clara, USA), coupled to a 5972 HP Mass Selective Detector (Agilent). A CIS 3 cooled injection system (Gerstel GmbH, Mülheim, Germany) was adjusted to the GC. Compounds were separated on a Varian VF-5MS column (Palo Alto, USA) with dimensions 60m-0.32mm-1 mm. The analysis method of Rapp et al. (1994) was modified as follows: injection was splitless (1 min) with the injector start temperature of 30C and then increased to 230°C at 12°C/min, and held for 4 min. The initial oven temperature was 40°C for 5 min, then increased to 125°C at 3°C/min, further increased to 200°C at 6°C/min and held for 14.2 min. Helium was used as carrier gas at a constant flow rate (1 mL/min). The mass spectrometer was set to scan mode, covering a mass-to-charge ratio range (m/z) from 35 to 250 atomic mass units (amu). The temperature of the MS was set to 180°C.

Analysis of volatile sulfur compounds

Volatile sulfur compounds were analyzed 24 months post-bottling by means of gas chromatography (GC) coupled with a pulsed flame photometric detector (PFPD), using static headspace sampling. A GC 6890 (Agilent Technologies, Santa Clara, USA) gas chromatograph equipped with a headspace MPS 2 sampler (Gerstel, Mülheim an der Ruhr, Germany), a cooled injection System CIS-4 (Gerstel, Mülheim an der Ruhr, Germany) and a 5380 PFPD (OI Analytical, College Station, Texas, USA) were used. Chromatographic separations were performed on a SPB-1 Sulfur column (30 m x 0.32 mm I. D., 4 µm film thickness (Supelco, Sigma-Aldrich, Munich, Germany)). Analytical conditions were as follows: injector temperature program -100 °C, 12 °C/sec until 40 °C for 1 min, then 12 °C/sec until 180 °C for 8 min; oven temperature program: 29 °C for 7 min, 10 °C/min until 180 °C for 10.5 min. Helium was used as carrier gas. Detector temperature was 250 °C. Analyses of the samples were carried out in duplicate. More details on sample preparation and analytical parameters can be taken from Rauhut et al. 1998, Rauhut et al. 2005 and Irmler et al. 2008.

Sensory Descriptive Analysis

Four treatments representing the most extreme bottling conditions were selected to be tested in the descriptive analysis at 14 and 24 months post-bottling: Co/HS6/Low, Co/HS18/Low, Co/HS6/High and Co/HS18/High. Panels of 17 and 15 assessors respectively, all staff of the Geisenheim Research Center or master students of the University Of Applied Science Of Wiesbaden in Geisenheim with previous experience in wine tasting, were convened for this study. Both descriptive analyses were carried out in four sessions each. A list of aroma attributes was generated by the panel during the first session, while during the second session the panelists were trained on these attributes using aroma references (table 1). Four mouth descriptors - CO_2 perception, sour, sweet and body - were also rated but no references were provided during formal sessions. Additionally, panelists had to rate for hedonic *liking*, e.g. their overall impression for each wine. The accession of the wines took place during the third and fourth session. Twelve wines (two replicates of each treatment) were tested per session. Wines were assessed monadically and randomly in a Latin Square Design. They were served at 15±1°C in white wine sensory glasses (Schott Zwiesel, Zwiesel, Germany) and were tasted within 1 h after pouring. Each attribute was rated on a 9 cm unstructured line scale. The preparation of panel sheets and the statistical data processing were done using the software FIZZ (Version 4.46A, Biosystemes, Couternon, France).

Table 1: Attributes selected by the panelists to describe the wines as well as reference standards used for the training.

Descriptive analysis at 14 months		Descriptive analysis at 24 months	
Attribute	Reference standard (in 100 mL wine)	Attribute	Reference standard (in 100 mL wine)
Citrus	Approx. 2 cm^3 lemon skin	Citrus	Approx. 2 cm^3 lemon and grapefruit skin
Apple	0.1 mL natural aroma type extract	Apple	0.1 mL natural aroma type extract
Peach	0.1 mL natural aroma type extract	Peach	0.1 mL natural aroma type extract
Pineapple	Approx. 2 cm^3 pieces of pineapple	Tropical	Approx. 2 cm^3 pineapple and mango pieces
Flower	0.05 mL linalool	Flower	0.05 mL 2-phenylethanol
Pepper	4 broken peppercorns	Oxidative	Wine opened for 48 hours
Oxidative	Wine opened for 48 hours	Reductive	0.3 mL dimethyl disulfide
Reductive	0.3 mL dimethyl disulfide		

Data analysis

Data was subjected to a 2-factors variance analysis (ANOVA) followed by Fisher's Least Significant Difference (LSD) tests (P = 0.05), using SPSS 15.0 (IBM SPSS, New York, USA) and software XLSTAT 2010 (Addinsoft Deutschland, Andernach, Germany). Principal Component Analysis (PCA) models were carried out to obtain a more comprehensible overview of the results.

Results and Discussion

Volatile fermenting by-products

Aroma fermenting by-products were measured at 24 months post-bottling by means of GCeMS. Among the 26 compounds analyzed, only ethyl decanoate and ethyl octanoate presented considerable differences between the treatments. Ethyl decanoate has a threshold of 200 µg/L whereas ethyl octanoate a threshold of 5 µg/L and they have been described as grape and fruity aroma respectively (Francis and Newton 2005). Since ethyl octanoate was present in the wines in concentrations above its threshold (figure 2a), it is clear that this compound could contribute to sensory differences between the wines. Ethyl decanoate was located below its threshold (figure 2b), but it could also contribute to aroma characteristics of the wines via interactions with other aroma compounds (Laska and Hudson 1991). In general, the concentrations measured in this Riesling wine after 24 months of bottles storage were in line with those reported by other authors for these specific aroma compounds in white wines (Francis and Newton 2005, Knoll et al. 2011).

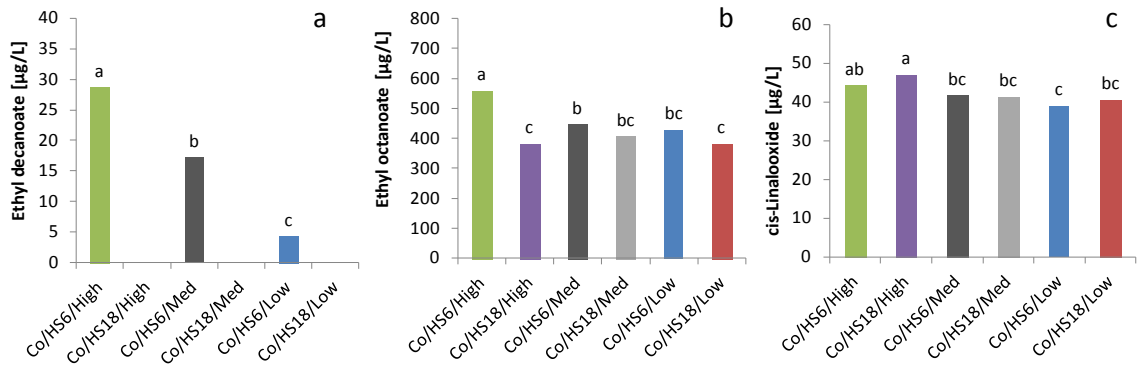

Figure 2: Concentration of aroma compounds in the different wines after 24 months of bottle storage.

HSO appeared to influence these aroma compounds positive as wines with higher HSO were characterized by higher concentrations. This trend was more pronounced in ethyl decanoate as Low HSO treatments were significantly lower than Med HSO treatments, which were significantly lower than High HSO treatments. However, it was surprising that this ester was not detected in treatments with large headspace volume, indicating that the accumulation pattern of this compound is more complex than just being benefited from higher oxygen exposure. Ethyl octanoate demonstrated a similar trend where higher HSO had a positive effect, whereas larger headspace volume a negative. These results indicate that oxygen exposure at bottling in form of high HSO favors these compounds, while large headspace volume not.

Similar to the above mentioned esters, cis-Linalooxide appeared to be higher in High HSO bottles at 24 months of storage (figure 2c). However, headspace volume did not seem to play a role in the concentration of this compound. Again oxygen exposure at bottling seems to favors cis-Linalooxide, which is related to sweet and floral aromas (Wang et al. 1994).

Volatile sulfur compounds

Volatile sulfur compounds were analyzed 24 months post-bottling by means of GC coupled with a PFPD. Among the ten compounds measured, only hydrogen sulfide (H_2S) and dimethyl sulfide (DMS) were detected in the test wines (figure 3). These compounds have been associated with wine´s reductive character, as H_2S has been described with rotten egg and sewage-like odor (Clarke and Bakker 2004) and DMS with cabbage and sulfur (Francis and Newton 2005). In our study, H_2S varied between 6 and 22 µg/L (figure 3a). A wide range of aroma threshold values has been suggested for H_2S. Siebert et al. 2009 have proposed an aroma threshold of 1.6 µg/L for white wine indicating that H_2S could contribute to the aroma characteristics of the wines tested. However, DMS was detected in concentrations lower than 10 µg/L (figure 3b), which is the aroma threshold for DMS proposed by Guth (1997). Nevertheless it could also be contribute to aroma characteristics of the wines via interactions with other aroma compounds (Laska and Hudson 1991).

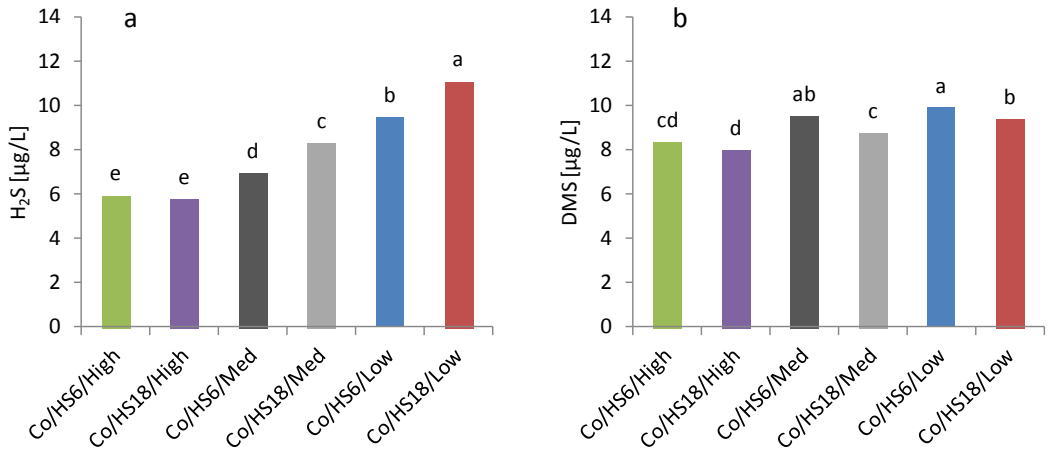

Figure 3: Concentration of volatile sulfur compounds in the different wines after 24 months of bottle storage (graphic A modified from Dimkou et al. 2011).

Headspace composition at bottling affected the concentration of sulfur compounds, as increasing HSO resulted in lower concentrations of these compounds. All High treatments had in all cases significantly lower concentrations of H_2S and DMS than Low treatments, while Med treatments were located between those two. This indicates that oxygen at bottling has the potential to influence wine aroma development during storage. Previous findings have associated low oxygen exposure during storage with higher levels of H_2S (Lopes et al. 2009) and DMS (Vasserot et al. 2001). In our study low oxygen exposure at bottling favored the accumulation of these compounds at 24 months post-bottling.

Headspace volume also affected final concentration of sulfur compounds. Even if not always significant, H_2S was higher in large headspace volume treatments than in small headspace volume treatments with the same headspace composition indicating that although higher oxygen exposure at bottling in form of higher HSO eliminates this compound, headspace volume benefits the accumulation of H_2S. This is an indication that the accumulation pattern of H_2S is more complex than just being inhibited by oxygen exposure as HSO affected it negative and headspace volume positive. On the other hand, DMS was always lower in treatments with larger headspace volume than in small headspace volume treatments with the same headspace composition, although not always significant. This indicates that DMS is clearly favored from low oxygen exposure, both in form of HSO and headspace volume.

Sensory Descriptive Analysis

Figure 4 gives the scores of the attributes showing significant differences between the wines at the descriptive analysis at 14 and 24 months after bottling. The results imply that different wines have occurred after bottling one single wine with different headspaces. It is known that different grades of oxygen exposure post-bottling due to closures with different oxygen permeability results to wines

with different chemical and sensory characteristics (Skouroumounis et al. 2005a, 2005b, Lopes et al. 2006, Godden et al. 2002, Hart and Kleinig 2005, Lopes et al. 2009, O'Brien et al. 2009). However, in our study, different grades of oxygen exposure at bottling due to different headspaces influenced too the sensory evolution of the bottled wines even under the same closure.

Among the treatments tested Co/HS18/High (largest headspace volume and highest HSO level) got in both tastings the highest scores for *oxidative* and the lowest scores for *citrus* and hedonic *liking* indicating that bottling conditions can influence sensory characteristics of wines already at 14 months post-bottling in 0.375 L bottles. Combination of large headspace volume and high oxygen concentration in the headspaces resulted to excessive oxidative character as well as low *citrus* and *liking* scores. Therefore, the oxidative character of bottled wines is not just a matter of closure, like previous studies of Godden et al. 2002 and Lopes et al. 2009 have shown, but also a matter of bottling and particularly headspace treatment.

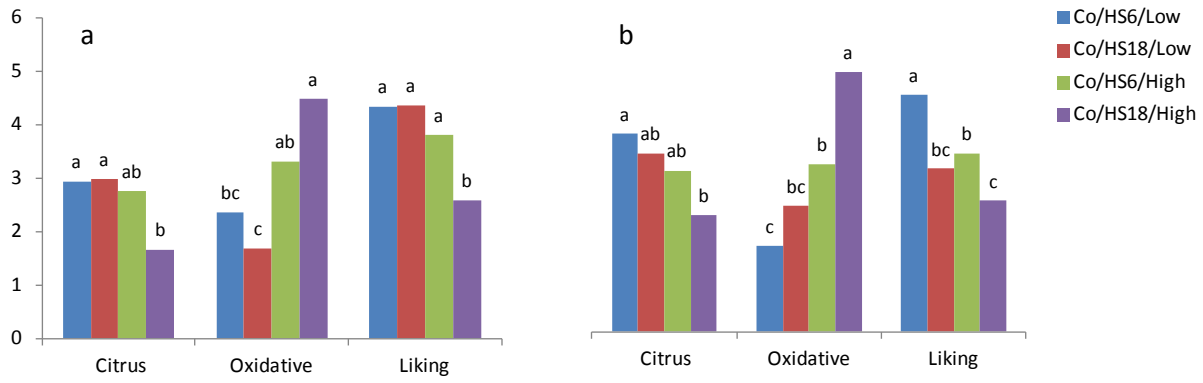

Figure 4: Ratings for the attributes showing significant differences between the treatments at the Sensory Descriptive Analysis at 14 (a) and 24 (b) months post-bottling.

The next most similar wine to Co/HS18/High in terms of sensory properties was the treatment Co/HS6/High (same HSO level as before but smaller headspace volume). Although at 14 months Co/HS6/High appeared to be statistically as much *oxidative* as Co/HS18/High, it got higher scores for liking which indicates that keeping headspace volume small offers a possibility to protect wines from being negatively perceived. Additionally Co/HS6/High appeared to be less oxidative than Co/HS18/High at 24 months post-bottling. That means that keeping headspace volume small, even without managing HSO level, offers a possibility to protect wines direct against oxidation, at least on the long term. In other words Co/HS6/High appeared somewhat oxidized at 14 months but did not oxidize further at 24 months like Co/HS18/High did. This is in agreement with Kwiatkowski et al. (2007) who found that large headspace volumes result to more oxidized wines after 24 months of storage. However, headspace volume has to be consistently considered in conjunction with expansion of the wine inside the bottle. Indeed, too small headspace volumes could increase the risk

of wine leakage. Therefore inerting a larger headspace could be a safer solution for HSO management than reducing headspace volume.

The next observation that can be made on figure 4 is that Co/HS18/Low got the lowest scores for oxidation, at least at 14 months of storage. This indicates that managing levels of HSO can successfully prevent wine oxidation, even under a large headspace volume. This could also be interesting for bottles sealed with screw caps, since headspace volume under this kind of closures is on average three times greater than under cylindrical closures (Reeves 2009). Furthermore Co/HS18/Low got high ratings for *citrus* and *liking*, indicating that managing headspace composition offers a possibility to avoid negative sensory development of wines post-bottling.

An even longer protection against oxidation up to 24 months post-bottling was provided by adjusting also a smaller headspace volume: Co/HS6/Low was least *oxidative* at 24 months, demonstrating at the same time the highest scores in *citrus* and hedonic *liking*. These results confirm that managing headspace – in volume and composition – offers the possibility to protect wines from excessive oxidation and ensure a positive post-bottling sensory evolution of wines.

Considering the aroma analysis in relation to the sensory analysis we conclude that the analytical differences of the wines in terms of volatile fermenting by-products and sulfur compounds were not great enough to be perceived by the panelists as wines did not differ significantly in the attributes *reductive*, *fruity* or *flowery*. However we cannot exclude the possibility that these analytical differences between the wines played a role on hedonic liking scores. On the other hand, wines differ from each other sensorial in the attributes *citrus*, *oxidative* and hedonic *liking* but these differences were not detected analytically. Apparently more analytical compounds should have been included to the analysis in order to determine this kind of sensorial differences.

Summary

Figure 5 summarizes the sensory and analytical data of the wines at 24 months in a PCA plot. The first two principal components accounted for more than 90% of the total variance, with PC1 accounting for 66% and PC2 for 26% of the total variance. Along PC1 a separation was observed based on headspace composition. Wines with High HSO were situated on the right side of the plot, associated with the attribute *oxidative* and the aroma compound cis-Linalooxide, while wines with Low HSO were on the left side, related to H_2S and DMS as well as *reductive*, *citrus* and CO_2 mouth perception. These results confirm that HSO is an important factor influencing sensory attributes and aroma composition of bottled wine. Within each headspace treatment, headspace volume also accounted for a significant degree of sensory differentiation across the wines. Under High HSO, a large

headspace volume resulted to even more pronounced oxidized character, while small headspace volume was characterized from higher ester concentration. Under Low HSO, large headspace volume was more associated to H_2S and DMS, while small headspace volume to *citrus* and CO_2 mouth perception.

Conclusion

HSO level and headspace volume are two bottling factors difficult to separate. In our study HSO appeared to favor some positive aroma compounds such as cis-Linalooxide, ethyl decanoate and ethyl octanoate and to eliminate some negative ones such as H_2S and DMS. Headspace volume had often the opposite effect as larger volume favored H_2S and eliminated DMS and the esters. In terms of sensory evolution, the Riesling wine studied here developed a moderate oxidative character and high citrus notes at 14 months of storage when a small headspace volume and no headspace flushing were applied. However, low HSO (CO_2 flushing) protected wine from oxidation much more efficiently even under large headspace volume, at least for the first 14 months. This protection lasted more than 24 months when small headspace volume was additionally applied. Therefore CO_2 flushing combined with small headspace volume offered the best possibility to protect the wine from oxidation.

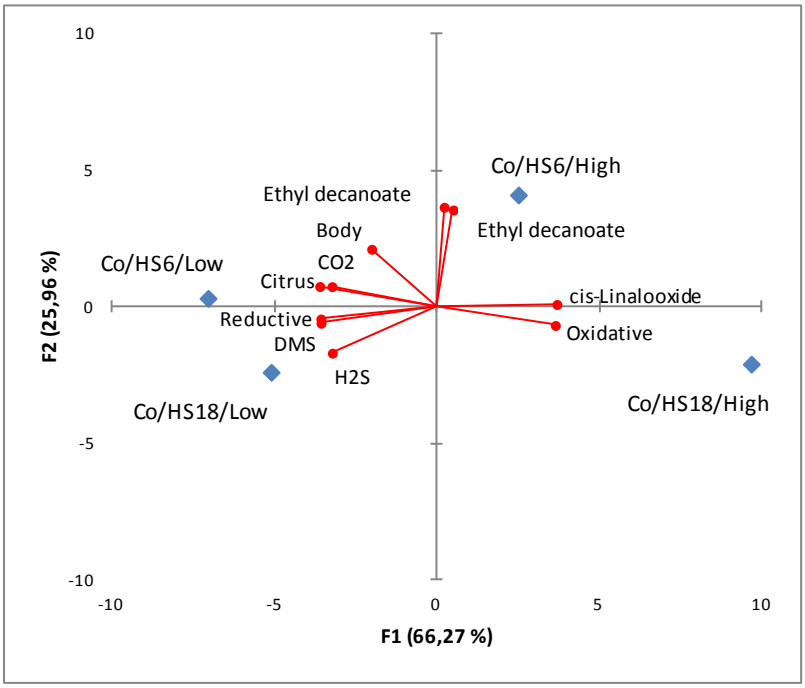

Figure 5: Principal Component Analysis for the sensorial and analytical data at 24 months post-bottling.

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2.3 Impact of dissolved oxygen at bottling on sulfur dioxide and sensory properties of a Riesling wine

EVDOKIA DIMKOU,[1*] MAURIZIO UGLIANO,[2] JEAN-BAPTISTE DIÉVAL,[2] STÉPHANE VIDAL[2] AND RAINER JUNG[1]

[1]*Hochschule Geisenheim University, Center for Wine Science &Beverage Processing Technology, Institute of Enology, Von-Lade-Str. 1, 65366 Geisenheim, Germany*

[2]*Nomacorc Oxygen Management Research Center, 2260 Route du Grès, 84100 Orange,*

**Corresponding author, email* Dimkou@fa-gm.de

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Impact of Dissolved Oxygen at Bottling on Sulfur Dioxide and Sensory Properties of a Riesling Wine

Evdokia Dimkou,[1]* Maurizio Ugliano,[2] Jean-Baptiste Diéval,[2]
Stéphane Vidal,[2] and Rainer Jung[1]

Abstract: A Riesling wine was bottled with different levels of dissolved oxygen and sealed with two different coextruded closures and one screwcap closure in order to investigate the impact of dissolved oxygen and of closure oxygen transfer rate on wine evolution. Dissolved oxygen introduced at bottling influenced sulfur dioxide decline during bottle storage, especially during the first three months. However, the loss of sulfur dioxide was more strongly correlated with the total amount of oxygen consumed by the wine rather than with the evolution of dissolved oxygen. Closure oxygen transfer rate also influenced sulfur dioxide loss, and this effect became greater with time in the bottle. Although closure was an important factor influencing sensory attributes of bottled wine, within each closure, dissolved oxygen accounted for significant differences across the wines. Wines bottled with high dissolved oxygen showed significantly higher ratings for oxidation, confirming the influence of dissolved oxygen management on the evolution of wine over time.

Key words: wine bottling, dissolved oxygen, oxidative, Riesling

The contact of wine with air is common during the wine-making process, leading to the dissolution of oxygen into the wine. The oxygen introduced in the wine is consumed in several chemical reactions, resulting in major changes in the chemical composition of the wine (Ribereau-Gayon 1933, Kielhöfer and Würdig 1962, Wildenradt and Singleton 1974, Ribereau-Gayon et al. 2006, Du Toit et al. 2006, Karbowiak et al. 2009). Among these, wine exposure to oxygen results in a loss of sulfur dioxide (SO_2), the main wine antioxidant, which is consumed in reactions with many of the chemical species resulting from the oxidation of wine phenolics, including as hydrogen peroxide and quinones (Waterhouse and Laurie 2006). Sensory characteristics of the wine are also likely to change in response to oxygen exposure. In white wine, a moderate oxygen exposure is viewed as potentially favorable to avoid reductive off-odors, while excessive oxygen exposure can result in loss of fresh and fruity aromas as well as oxidative browning (Skouroumounis et al. 2005, Lopes et al. 2009, Ugliano et al. 2009, 2011). SO_2 is provided at different steps of wine processing and at bottling to ensure protection of the wine against oxidative spoilage.

In consideration of growing concerns about the health implications of SO_2 addition to foods and beverages, a better understanding of the potential of improved oxygen management to wine quality appears to be necessary. Several wine-making operations, such as filtration, pumping, and racking, are known to potentially promote incorporation of atmospheric oxygen into the wine, with consequent increase of wine dissolved oxygen (DO) (Kielhöfer and Würdig 1962, Perscheid and Zürn 1978, Kettern 1985, Schneider 2005, Vidal and Moutounet 2006). Bottling has been indicated by several authors as a set of operations potentially resulting in a large increase in wine DO (Vidal et al. 2001, Valade et al. 2006, Karbowiak et al. 2009, O'Brien and Colby 2009). With the recent introduction in the wine industry of devices allowing relatively rapid measure (and consequent management) of DO during bottling, there is a growing interest in the influence of oxygen at bottling on subsequent wine development in the bottle. We have recently shown that the management of the oxygen present in bottle headspace (HSO) can significantly contribute to optimizing wine shelf life and aroma development during bottle storage (Dimkou et al. 2011). In addition, it has been previously shown that the oxygen transmission rate (OTR) of the closure also has a primary role in determining the degree of oxygen exposure in bottled wine (Lopes et al. 2009, Caillé et al. 2010, Wirth et al. 2010, Ugliano et al. 2011, Dimkou et al. 2011). To date, very little information is available regarding the importance of DO to wine bottle development.

In this study, a Riesling wine was bottled with three different DO levels, using closures with three different oxygen transmission rates. Analytical and sensory characteristics of the wines were determined at different time points to assess the contribution of DO to wine bottle development under different closures.

Materials and Methods

Wine. The wine used in this experiment was produced during the 2008 vintage from Riesling grapes grown in the

[1]Hochschule Geisenheim University, Institute for Enology, Von-Lade-Str. 1, 65366 Geisenheim, Germany; and [2]Nomacorc Oxygen Management Research Center, 2260 Route du Grès, 84100 Orange, France.

*Corresponding author (email: Dimkou@fa-gm.de)

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Rheingau region in Geisenheim. The wine was produced at the Hochschule Geisenheim University in a stainless-steel tank between 18 and 22°C, using typical winemaking practices for wines of these types. The wine was stored in a stainless tank without ullage. Before bottling the wine in September 2009, analytical components were 12% v/v alcohol, pH 3.3, 4.3 g/L residual sugar, 7.7 g/L titratable acidity, 45 mg/L free SO_2 (FSO_2), and 160 mg/L total SO_2 (TSO_2).

Closures and bottles. Three closures were used: two 43 x 22 mm co-extruded (Co-ex1 and Co-ex2) closures (Nomacorc SA, Thimister Clermont, Belgium) allowing different oxygen ingress and a screwcap closure (SC) (Alcoa CSI 5SE short cap, 28 x 15 mm with a PVC-free sealing compound; Alcoa, Kelkheim, Germany). Oxygen ingress through the three closures during the study was quantified by monitoring oxygen increase in bottles filled with nitrogen and sealed with the different closures using a Fibox 3 Trace fiber-optic oxygen meter coupled with PSt6 oxygen sensors (linearity range given from the manufacture: 0 to 4.2% v/v oxygen) (PreSens GmbH, Regensburg, Germany), as described in Dimkou et al. 2011. Ten bottles for each cylindrical closure and 10 bottles for the screwcap closure were fitted with one PSt6 oxygen sensor, each at 8 cm from the bottom. These empty bottles were filled with pure nitrogen (N_2, purity 99.8%, Air Liquide, Düsseldorf, Germany) until complete removal of the oxygen present and then were directly sealed with one of the closures. Measurements of oxygen were taken at different time points to quantify oxygen ingress through the closure in the course of time, using the Fibox 3 Trace fiber-optic oxygen meter (see oxygen measurements below). All bottles were clear glass Saint Gobain 0.375 L (375 mL at 52 mm from the top for the cylindrical closure and at 28 mm from the top for the screwcap closure).

Bottling. The bottling line included a plate filter (Seitz Enzinger Noll, Worms, Germany) with EK Pall/SeitzSchenk depth filter sheets (40 x 40 cm, Pall Corporations, Dreieich, Germany), a semiautomatic gravity filling machine prototype (Perrier, Le Cheylard, France), a semiautomatic corking machine with vacuum (GAI 4040, Prospero Equipment, Pleasantville, NY), and a semiautomatic screwcap machine (VAW Aluminium AG, Grevenbroich, Germany) with 2000 Newton sealing force. The bottles were rinsed with SO_2 (13 g/L) and sterile water (Stroh GmbH, Schloßböckelheim, Germany). According to published measurements (Münster 2012), remaining SO_2 in the bottle after rinsing can add 3 mg/L SO_2 to bottled wine (in 0.375 bottles) on average.

Approximately 450 L Riesling wine was bottled with three levels of DO (Low, Med, and High) and was sealed with three different closures each (Figure 1). Initial wine DO in the tank was 2.4 mg/L due to pumping and filtering the wine the day before. Since this value was relatively high, bottling started with the wine untreated, which was considered as the Med modality. Bottle inertion was done by the Perrier filling machine with vacuum (90 kPa) and N_2 injection (3 sec at 15 kPa, purity 99.8%, Air Liquide). Before sealing the bottle headspace was manually purged with carbon dioxide (CO_2, purity 100%; Air Liquide) by holding a tube flushing CO_2 obliquely above the headspace for 5 to 10 seconds. After bottling Med DO bottles, the 300 L wine left in the tank was sparged with N_2 (purity 99.8%; Air Liquide) using 5 frits flushing N_2 sunk in the middle of the wine volume for more than 30 min (until the DO reached 0.9 mg/L) to produce the Low DO wine. Subsequently, CO_2 (+ pressure/flow rate, purity 100%; Air Liquide) was introduced in the wine for 15 min through the same frits in order to replace some of the CO_2 lost. Before bottling the Low DO bottles, ~10 L wine was left out of the system to replace the wine in the filter. Bottle evacuation and purging at filling as well as manual headspace purging at sealing were applied. After finishing the Low DO bottles, air was introduced in the tank using the same frits as before until DO in the tank was 4.5 mg/L in order to create the High DO wine. Approximately 10 L wine was removed from the system. Bottle evacuation and purging at filling was in this case inactivated. However, bottle headspace was treated with CO_2 as in the other two treatments.

DO and HSO measurements were taken within 30 min after bottling each treatment. DO in all Low DO bottles was 1.0 mg/L, and HSO was 0.5, 1.2, and 0.2 mg/L wine in Co-ex1, Co-ex2, and SC, respectively (HSO was measured in hPa, converted into mg in headspace, and then to potential mg/L in wine, taking in account the wine volume [375 mL]). DO in all Med DO bottles was 2.5 mg/L, and HSO was 0.8, 1.2, and 0.5 mg/L in Co-ex1, Co-ex2, and SC, respectively. And DO in all High DO bottles was ~5 mg/L, and HSO was 0.9, 1.6, and 0.4 mg/L wine in Co-ex1, Co-ex2, and SC, respectively. All bottles were stored upright in the storage room of the cellar in Geisenheim Research Institute at 14 to 16°C and 55% humidity.

Oxygen measurements. DO before bottling (in the tank) as well as DO and headspace oxygen (HSO) at bottling and postbottling were measured with the Fibox 3 Trace fiber-optic

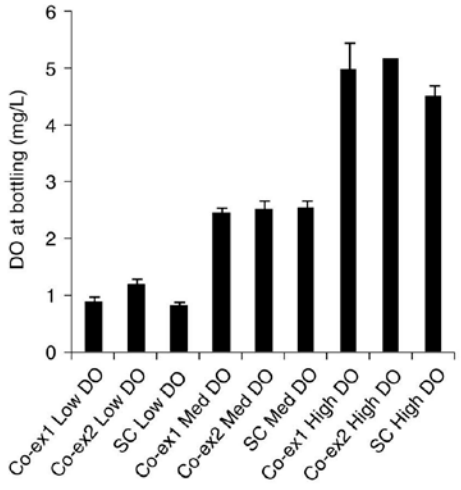

Figure 1 Dissolved oxygen measured immediately after bottling. Error bars represent standard deviations (n = 5).

oxygen meter (PreSens) coupled with an oxygen dipping probe or with PSt3 oxygen sensors (linearity range, 0 to 50% v/v oxygen), respectively, as described in Dimkou et al. 2011. Five bottles fitted with two oxygen sensors each were used for each modality. The sensors were glued inside the bottle using a silicone rubber compound flowable fluid (RS Components, Northants, UK) at a height of 8 cm from the bottom of the bottle for DO measurement and in the headspace for HSO measurement. These bottles were equally distributed in the bottling line during bottling. Manufacturer calibration was used for all the sensors, with no further calibration. Oxygen measurements in the bottle were taken within 30 min after bottling, weekly during the first month of storage, and at 1.5, 3, 6, and 12 months after bottling without shaking the bottles before measurement.

Sulfur dioxide measurements. Three bottles per treatment were used for each FSO_2 and TSO_2 measurement. Each bottle was measured in duplicate. Measurements were taken within 3 hours after bottling, weekly during the first month of storage, and at 1.5, 3, 6, and 12 months after bottling. The measurements were carried out using a FIAstar 5000 Analyzer (Foss Analytical, Rellingen, Germany), a multichannel flow-injection analyzer for the automatic, simultaneous determination of FSO_2 and TSO_2 in red and white wines, according to manufacturer guidelines. The analyzer uses flow-injection technology (an automated method in which a sample is injected into a continuous flow of a carrier solution that mixes with other continuously flowing solutions before reaching a detector) coupled with a digital dual wavelength photometer with automatic background correction as detector. Calibration of the analyzer was done before each set of measurements using a set of 10 known solutions (0, 10, 20, 30, 50, 100, 120, 150, 180, and 200 mg/L SO_2). They were prepared immediately before analysis using a 1 g/L SO_2 stock solution, which was prepared using water, sodium metabisulfite ($Na_2S_2O_5$), and ethanol within 5 days before analysis, according to manufacturer guidelines.

Sensory analysis. A panel of 65 students of the University of Applied Science of Wiesbaden in Geisenheim (enology and viticulture, fourth semester) was trained to carry out a descriptive sensory analysis of the wines 15 months after bottling. Because of limited time availability, students were separated in three groups of approximately 20 assessors, each group testing a different closure. The first group assessed Coex1 Low, Med, and High DO; the second group assessed Coex2 Low, Med, and High DO; and the third group assessed SC Low, Med, and High DO. The descriptive analysis was conducted in four sessions. A list of aroma attributes was generated by the panel during the first session, while during the second session the panelists were trained on these attributes using aroma references (Table 1). All attributes were rated on 9 cm unstructured line scales. The accession of the wines took place during the third and fourth sessions. The wines were served at 15 ± 1°C in ISO standard wine glasses (Schott Zwiesel, Zwiesel, Germany) and were tasted within 1 hr after pouring, both monadically and randomly in a Latin square design. The preparation of panel sheets and the statistical data processing were done using the FIZZ software (ver. 4.46A, Biosystèmes, Couternon, France).

Statistical analyses. Data analysis was performed using XLSTAT 2010 (Addinsoft Deutschland, Andernach, Germany). A correlation analysis (Pearson, 0.05 significance) and an analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) means comparison tests (p = 0.05) were performed for the FSO_2 data, testing for correlations of FSO_2 losses with initial DO and consumed oxygen as well as the effect of closure and DO at bottling on FSO_2 drop. ANOVA was performed for the sensory data, testing for the effect of wine type, panelist, and replicate. Factorial ANOVAs, testing for the effects of initial DO and closure, were used for each sensory attribute for the wine sensory descriptive analysis data set, controlling for the effects of session and panelist, that is, treating panelist and session as a random effect. When there was a significant effect from an ANOVA, a Fisher's LSD means comparison test (p = 0.05) was performed. A principal component analysis (PCA, type: Pearson [n]) was performed on the means for the sensory data using XLSTAT 2010.

Results and Discussion

Oxygen ingress during storage. The evolution of total oxygen ingress through the closures into empty bottles purged with N_2, as described in Dimkou et al. 2011, is shown (Figure 2).

Table 1 Attributes selected by panelists to describe wines and reference standards used for training.

Attribute	Reference standard (in 100 mL wine)
Citrus	~3 cm^3 lemon, orange, and grapefruit peel
Hay	~5 g hay
Peach	0.1 mL natural aroma-type extract
Pear	0.1 mL natural aroma-type extract
Honey	1 teaspoon honey
Oxidative	Wine open for 48 hr
Phenolic	0.2 mL phenol (C_6H_6O)

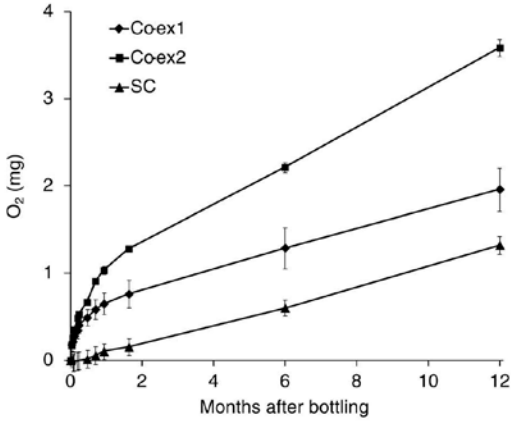

Figure 2 Total oxygen ingress into empty nitrogen-purged bottles, sealed with different closures. Error bars represent standard deviations (n = 5).

The Co-ex2 closure allowed the highest oxygen ingress, which was approximately twice that of the Co-ex1 closure. The SC closure was characterized by the lowest oxygen ingress. However, it was surprising to observe that after approximately two months of storage, SC and Co-ex1 exhibited the same slope, indicating that the main differences between these two closures was due to oxygen ingress in the first two months after bottling, which is due to the release of oxygen from the closure upon application to the bottle (Ugliano et al. 2011). This component is obviously higher in the case of cylindrical closures, but the similar slope observed between SC and Co-ex1 was unexpected, as it is normally accepted that screwcaps have much lower OTR compared to other closures. One report has noted that microscopic creases in the surface of the liner can result in much higher OTR values (Crochiere 2007). Fabre and Riedo (2008) also observed that imperfections linked to bottle manufacturing could result in imperfect glass-liner seal, with consequent higher OTR and even wine leaks; infrared spectroscopy enabled them to demonstrate that such imperfections are not linked to glass composition, but to imperfect glass surface. It is possible that the higher oxygen ingress observed here for screwcaps was linked to an imperfect seal and therefore oxygen leakage, although replicate bottles did not show the inconsistency in oxygen ingress values typically associated with leakage.

Dissolved and headspace oxygen. Immediately after bottling, DO in Low and Med DO bottles was only 0.1 mg/L higher than it was in the tank immediately before bottling each treatment, indicating that the bottle evacuation and N_2 purging applied was very efficient. In High DO bottles, where evacuation and purging was inactivated, DO was 0.5 mg/L higher than in the tank. In all treatments, DO started to decrease immediately after bottling and was consumed in the majority of the treatments within two and four months of bottle storage (Figure 3). At higher OTR (SC<Co-ex1<Co-ex2), DO decline was slower due to oxygen ingress through the closure. The rate of oxygen consumption was similar to that observed previously in Riesling wine (Dimkou et al. 2011).

Initial HSO at bottling varied between 0.5 and 0.8 mg/L wine in the Co-ex1 bottles, between 1.2 and 1.5 mg/L wine in Co-ex2 bottles, and between 0.2 and 0.5 mg/L wine in SC bottles (Figure 4). Although all headspaces were treated similarly, a variation in HSO values arose depending on the type of the closure. This inconsistency was mainly due to the manual inerting that was applied. SC demonstrated the lowest HSO because the inerting of the headspace was easier than by the cylindrical closures, where the corker did not permit as much as efficient inerting. The higher HSO under the cylindrical closure with the higher oxygen permeability

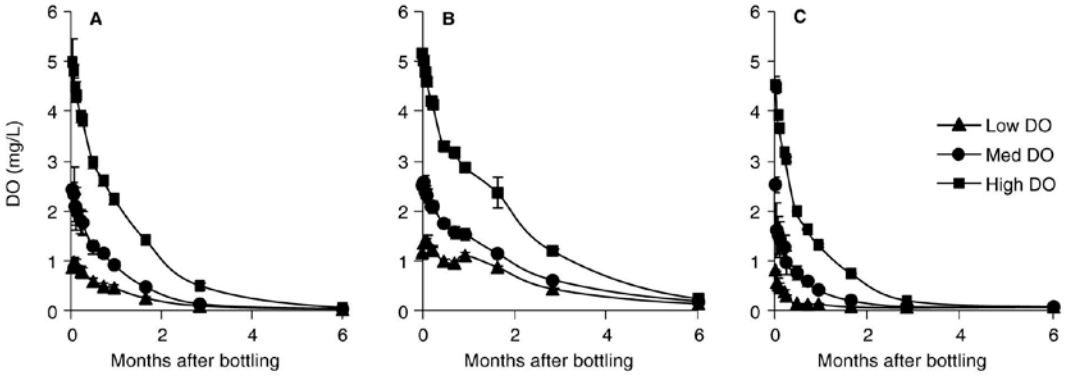

Figure 3 Evolution of dissolved oxygen during bottle storage under Co-ex1 (**A**), Co-ex2 (**B**), and SC (**C**). Error bars represent standard deviations (n=5).

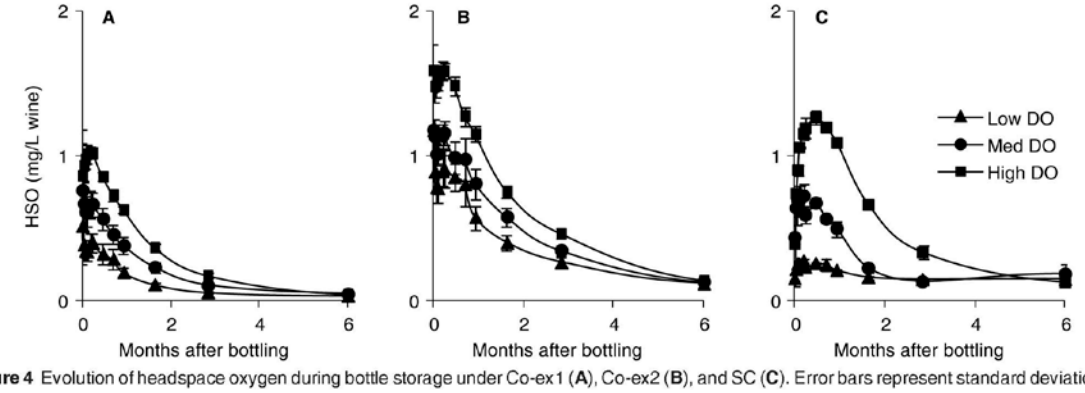

Figure 4 Evolution of headspace oxygen during bottle storage under Co-ex1 (**A**), Co-ex2 (**B**), and SC (**C**). Error bars represent standard deviations (n = 5).

(Co-ex2) can be traced back to differences on elastic recovery between the two closures.

HSO decreased during storage in all treatments due to oxygen consumption by the wine and consequent diffusion from the headspace into the wine. However, High DO treatments as well as all SC treatments showed an increase of HSO during the first week of storage, followed by a constant decrease in the second week until the end of the storage period. This could be due to oxygen initially migrating from wine into the headspace due to the difference in partial pressure of oxygen between the liquid and gas phase. In the majority of the cases, HSO was completely consumed within two and four months, in agreement with a previous study (Dimkou et al. 2011).

Free and total sulfur dioxide. In agreement with previous observations (Brajkovich et al. 2005, Kwiatkowski et al. 2007, Lopes et al. 2009, Dimkou et al. 2011), FSO_2 decreased during bottle storage, with a rapid decline in the first three months followed by a slower decline after 3, 6, and 12 months of storage (Figure 5). The extent of FSO_2 decline was affected by DO at bottling and by closure type. After three months of storage, FSO_2 losses were in most cases significantly affected by initial DO, with greater SO_2 loss occurring by higher initial DO. Comparing High and Low DO samples with the same closure indicates that, for the range of DO values tested, initial DO can account for up to 8 mg/L SO_2 lost. The range of DO at bottling applied in this study (0.5, 2.5, and 5 mg/L) is similar to that reported by other authors (Müller-Späth 1977, Vidal et al. 2001, Valade et al. 2007, Lopes et al. 2009) suggesting that such range could be representative of actual situations. These results clearly indicate that management of DO at bottling is crucial to SO_2 consumption postbottling, especially in the short term. Closure OTR also influenced FSO_2 loss, and this effect became progressively more evident with time in the bottle. Comparing Co-ex2 and SC treatments with the same DO level at 6 or 12 months, we observe that closure can account for up to 10 mg/L FSO_2 loss. In total, SC and Co-ex1 treatments (except Co-ex1 High DO) showed significantly lower FSO_2 losses at 6 and 12 months compared to Co-ex2, regardless of the DO level.

A correlation analysis was conducted to examine the relationship between FSO_2 loss and DO (Table 2). FSO_2 decline was initially well correlated with DO, but this correlation weakened over time, as the loss of FSO_2 continues after DO is completely consumed. Direct reaction between oxygen and SO_2 is extremely slow under wine conditions (Waterhouse and Laurie 2006), so that SO_2 loss is linked to oxygen through reaction of SO_2 with the products of wine oxidation, in particular hydrogen peroxide (Danilewicz et al. 2008). DO measurement only reflects the oxygen that is present in the wine at any given time. Therefore, when an excess of oxygen (HSO or DO) is present, such as in the weeks following bottling, consumption of such excess can be monitored and compared with the corresponding loss of FSO_2. In agreement with previously reported observations, in this phase a positive correlation between the evolution of FSO_2 and DO was observed (Brajkovich et al. 2005, Kwiatkowski et al. 2007, Lopes et al. 2009, Dimkou et al. 2011). However, once initial excess oxygen is consumed, oxygen ingress in the bottle is only due to oxygen permeability of the closure, occurring therefore at a very low rate. Under these conditions, oxygen consumption by the wine is typically faster than oxygen ingress through the closure (Dimkou et al. 2011), so that no detectable oxygen will accumulate in the wine. Nevertheless, consumption of this oxygen also contributes to SO_2 loss, which explains why a good correlation between and DO and loss of FSO_2 was observed only at the early time points.

More accurate prediction of FSO_2 loss can be obtained using the total consumed oxygen (TCO), which was well correlated with FSO_2 loss throughout the entire storage period.

Table 2 Correlations between free SO_2 decrease and dissolved oxygen (DO) and as well as total consumed oxygen (TCO) at different time points of bottle storage (Pearson, 0.05 significance).

	Days after bottling					
	21	28	49	85	180	360
DO (mg/L)	0.931	0.898	0.932	0.936	0.750	0.554
TCO (mg/L)	0.858	0.854	0.912	0.931	0.988	0.987

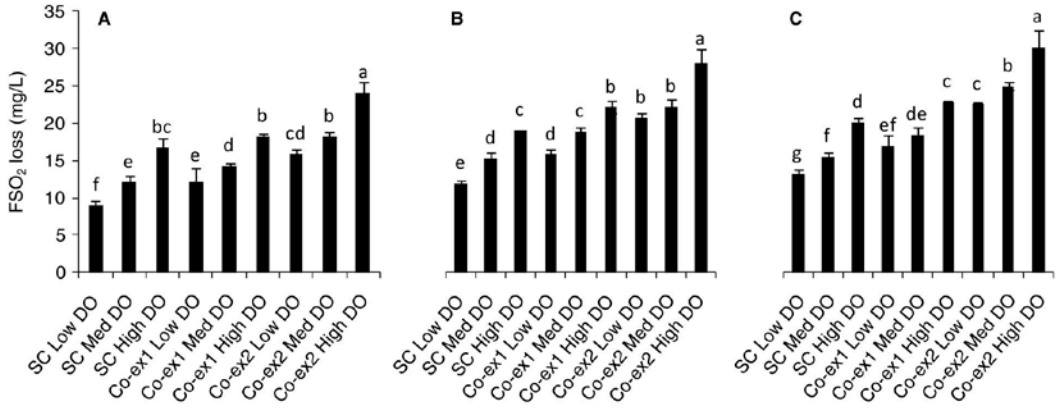

Figure 5 Free SO_2 loss after 3 (**A**), 6 (**B**), and 12 (**C**) months of bottle storage. Error bars represent standard deviations (n = 3).

TCO is calculated as the sum of the oxygen present at bottling (HSO plus DO), plus the oxygen entering the bottle through the closure during storage, minus DO and HSO measured at each time point. We have previously reported a similar observation for HSO (Dimkou et al. 2011). Those findings and the ones reported herein indicate clearly that, in addition to closure OTR, both DO and HSO at bottling represent a key component of any oxygen management strategy aimed at improving wine shelf life.

Sensory analysis. Sensory data after 15 months of bottle storage was plotted using principal component analysis (PCA) (Figure 6). The first two principal components (PC) accounted for over 70% of the total variance, with PC1 accounting for 47% and PC2 for 26% of the total variance. A separation was observed along PC1 based on the type of closure. Wines sealed with closure Co-ex2 are situated on the top left quadrant, related to attributes oxidative and phenolic. Closure Co-ex1 is in the top right quadrant, where mainly fruity attributes such as citrus and pear prevail. Finally, SC closures are plotted toward the bottom of the graph, partly

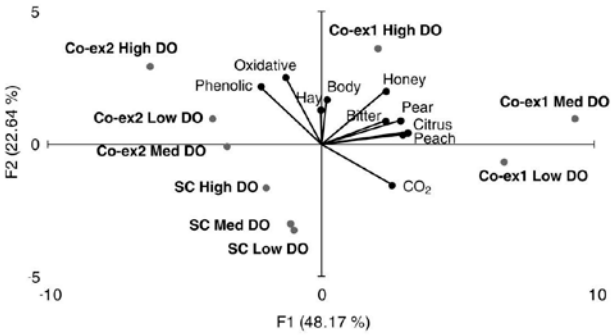

Figure 6 Principal component analysis for the sensory data at 15 months postbottling.

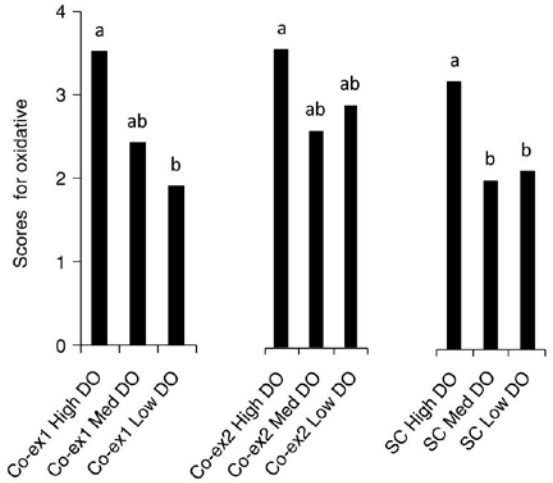

Figure 7 Scores for the attribute oxidative given by the panelist at the descriptive analysis at 15 months postbottling on a 9 cm line scale.

related to CO_2 perception in the mouth. Although observed in 375 mL bottles within 15 months, these results confirm that closure is an important factor influencing sensory attributes of bottled wine, as observed in other studies (Godden et al. 2002, Skouroumounis et al. 2005, Hart and Kleinig 2005, Kwiatkowski et al. 2007, Lopes et al. 2006, 2009, O'Brien and Colby 2009). However, within each closure, DO at bottling also accounted for a significant degree of sensory differentiation across the wines, as higher DO values were more closely associated with developed characters (such as oxidation). Overall, the trend observed suggested that, in the case of the Riesling wine studied, Co-ex2, the closure with the highest OTR, resulted in excessive wine development, which was further exacerbated by high DO. This observation indicates that, in the case of Co-ex2, the higher amount of oxygen entering through the closure over 15 months reduced the influence of DO at bottling, determining an overall dominance of oxidized attributes. Conversely, for the other two closures, the interaction between closure OTR and initial DO was more complex and allowed overall the development of wines with different aroma characteristics. For example, Co-ex1 High DO was situated nearer to honey and hay attributes, while Co-ex1 Med and Low DO were plotted toward fruity attributes. Within SC closures, Med and Low DO were characterized by higher CO_2 than High DO.

After 15 months of bottle aging, wines bottled with High DO had higher ratings for oxidation (Figure 7), confirming the influence of DO management on the evolution of wine over time. Differences were significant mainly between High and Low DO treatments, whereas under the lower OTR of the SC closures, differences were significant also between High and Med DO treatments. On the contrary, differences between the wines sealed with higher OTR closure (Co-ex2) were not significant. These results confirm that sensory differences linked to DO at bottling are more likely to be observed in the presence of lower OTR, while higher OTR could mask them. However, as we have shown previously (Dimkou et al. 2011), all sources of oxygen—DO, HSO, and OTR—should be taken in account as they are involved in the total oxygen consumed in wine (TCO). Most studies on the influence of oxygen on the aroma evolution of white wine have focused on oxidative spoilage, identifying some of the key aroma compounds involved in this defect (Ferreira et al. 1998, Escudero et al. 2000, 2002, Silva Ferreira et al. 2002, 2003a, 2003b). The sensory differences observed in this study indicate that, while excessive oxygen exposure has to be avoided, modulation of oxygen exposure levels in the moderate range allowed expression of different sensory attributes. The identification of optimal range of oxygen exposure for different wine styles appears worthy of further investigation, also in view of recent observations highlighting the existence of different segments of consumer preferences in response to different levels of oxygen exposure (O'Brien et al. 2009).

Conclusion

This study investigated the significance of DO values at bottling on the postbottling evolution of wine under closures

with different oxygen permeability. DO value at bottling is a major contributor to FSO_2 decline postbottling, so that difference in wine SO_2 residual content could still be seen after 12 months of bottle storage in consequence of initial differences in DO. These data indicate that careful management of oxygen at bottling can significantly contribute to a more rationalized use of SO_2 in winemaking, potentially contributing to reducing SO_2 doses in bottled wine. Oxygen ingress through the closure is also key to wine evolution, and after 15 months in 375 mL bottles, wine sensory profile was mainly affected by the type of closure. However, even after this period of aging, the intensity of certain aroma descriptors, in particular the rating of the oxidized attribute, was significantly affected by initial DO. These results demonstrate that wine chemical and sensory evolution during bottle maturation is affected not only by closure selection but also by process conditions at the time of bottling. While from a chemical point of view (such as the effect of FSO_2 decline) the consequences of differences in initial DO can be seen relatively soon, from a sensory point of view the influence of DO will manifest after a certain period of time, also depending on the OTR of the closure. In the course of this study a higher than expected OTR was observed for screwcap closures, suggesting an imperfect seal between glass and closure. Further investigations on this aspect are needed.

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3. Discussion

Oxygen and its impact on wine quality have been concerning researchers for many years (Du Toit et al. 2006). In 1873 Luis Pasteur was the first to state that oxygen is the best friend and the worst enemy of wine (Friedel 2007). Since then many studies have been dealing with questions like how much oxygen uptake occurs during wine making, what are the critical steps throughout the wine production and what are the consequences on wine´s chemical and sensory properties (Ribereau-Gayon et al. 2000).

Several studies have investigated the process of bottling in terms of oxygen uptake and its impact on wine quality (Kielhöfer and Würdig 1962, Kettern 1985, Godden et al. 2002, Hart and Kleinig 2005, Vidal and Moutounet 2006, Crochiere 2007, Kondudakis et al. 2008, Lopes et al. 2009). There is now a general conclusion that oxygen uptake at bottling may cause early oxidation in bottled wine. However, most of these studies focus on the increase of dissolved oxygen during bottling process disregarding the oxygen might be trapped in the headspace at sealing. Therefore the contribution of headspace oxygen in the total oxygen uptake at bottling is not known. In general, and mainly due to the difficulty of oxygen measurement in closed bottles, there is a lack of comprehension about how much oxygen uptake at bottling precisely occurs, in which form (dissolved or in the headspace) and how does this behave after sealing the bottle and throughout storage. As a consequence, it is not exactly known how dissolved and headspace oxygen influence wine quality post-bottling.

In addition to that, the development of numerous alternative wine closures in the last decades have introduced a further aspect of oxygen uptake, namely the oxygen ingress through the closure (or between closure and glass surface) which varies depending on oxygen permeability. Several studies have shown that sealing a single wine with different closures will result in wines with different profiles after a certain time of storage (Godden et al. 2002, Skouroumounis et al. 2005a, Kwiatkowski et al. 2007, Lopes et al. 2009). However, the closure type influences the volume of the headspace but also its content through its impact on the partial pressure in the headspace. Thus the question arise to what extend differences in bottled wines can be traced back to the different closures or rather to variations on the headspace properties. Furthermore, the different potential combinations of headspace oxygen levels, dissolved oxygen at bottling, and closure type have never been investigated.

The objective of this study was to investigate the impact of oxygen uptake at bottling – dissolved and in the headspace – as well as the impact of oxygen ingress through the closure during storage on wine´s post bottling development. The specific aims were to measure dissolve and headspace oxygen

at bottling with precision, to monitor their evolution direct after sealing and during bottle storage as well as to evaluate the impact of dissolved and headspace oxygen on wine quality in terms of SO_2 losses (shelf life), color, aroma and sensory. Additionally we were able to investigate the effects of possible combinations of bottling treatments (diverse HSO and DO levels) with different closures. Accordingly, this discussion was arranged in three parts:

a. Monitoring dissolved oxygen, headspace oxygen and oxygen transmission rate post-bottling
b. Impact of headspace oxygen and closure on wine properties post-bottling
c. Impact of dissolved oxygen at bottling and closure on wine properties post-bottling

Monitoring dissolved oxygen, headspace oxygen and oxygen transmission rate

Different levels of headspace oxygen (HSO), headspace volume and dissolved oxygen (DO) at bottling as well as several types of closures were tested in this study in order to evaluate their impact on wine aging. Using a Nomasense device we were able to measure DO and HSO precisely in a nondestructive manner and thus to monitor their evolution in the same wine bottles throughout storage. Using empty bottles purged with Nitrogen, we were able to monitor the oxygen transmission rate (OTR) through the different closures used in the study.

The results of DO measurements showed that during bottle storage DO decreased constantly in all treatments and was completely consumed between one and eight months. In the majority of the bottles it became not detectable within two and four months depending on initial HSO and closure type (co-extruded vs. screw cap). Under high HSO, DO decline was slower as oxygen molecules dissolute from the headspace into the wine. Changes in DO reflect the net balance between dissolution and oxygen consumption by the wine itself. Therefore, in general, the latter was higher than the former throughout storage period. However, while in samples with lower HSO DO started decreasing immediately after bottling, an early transient increase in DO was observed in the cases where HSO was high. This suggests that, in these samples the rate of oxygen dissolution into the wine was higher than consumption during the early stages of bottle storage. In general, the magnitude of this DO increase was proportional to the concentration of oxygen in the headspace.

Although this initial DO increase was observed in both co-extruded and screw cap bottles, a difference existed between those two. Co-extruded closures with high and medium initial HSO showed an increase of DO during the first 2 days after bottling, after which DO started to decrease due to oxygen consumption. Conversely, in screw cap samples, DO initially decreased, and only after approximately 10 days post-bottling an abrupt increase followed by the constant decrease was observed. This difference could be partly traced back to the overpressure which is generated in the

headspace by the application of cylindrical closures, which accelerates the dissolution of oxygen into the wine (Kielhöfer and Würdig 1962). In addition, the wine-headspace contact surface has to be considered as in some cases of cylindrical closures, application of large headspace volumes resulted in a filling height below the bottle shoulder, and therefore in a larger wine-headspace contact surface, which probably favored rapid dissolution of oxygen in the wine.

Complete consumption of initial DO took longer in the case of cylindrical (co-extruded) closures, in spite of similar values of initial HSO. Likewise, Perscheid and Zürn (1978) measured a faster DO drop in white wine under screw cap than under nature cork. This reflects the fact that, due to their porous nature, cylindrical closures contain air, and therefore oxygen (Jung and Zürn 2000, Lopes et al. 2007, Ugliano et al. 2011), which is in part released gradually into the headspace following closure insertion in the bottle. In addition, ingress of oxygen trough the closure, although limited at this stage, was higher for the co-extruded closures used here. As a result DO decline in wine was slower under cylindrical closures.

Measurements of HSO indicated that HSO at bottling varied from 0.2 up to 14.5 mg/L wine depending on the headspace treatment applied (different levels of CO_2 flushing vs. no flushing) and closure used (different types of co-extruded vs. screw cap). This means that HSO represents from an almost insignificant up to a very important source of oxygen in bottled wines. Although low values of HSO could be obtained by different combinations of headspace volumes and degree of inerting, the choice of headspace volume has to be considered in relation to wine expansion inside the bottle. Indeed, too small headspace volumes could increase the risk of wine leakage. Therefore inerting a bigger headspace could be a safer solution for HSO management than reducing headspace volume. In addition, high inerting a small headspace was overall the most effective approach to reduce HSO levels.

HSO decreased during storage in all bottles due to its dissolution into the wine and consequent consumption through different chemical reactions. The time required for HSO to become undetectable varied between two weeks and less than eight months depending on initial concentration but also on closure type. The higher the initial HSO was the longer the time period needed for its complete dissolution. Also under co-extruded closures HSO decline took longer compared to screw caps due to higher oxygen permeability of the cylindrical closure. While HSO migrates from headspace into the wine, further oxygen enters from closure into the headspace. Therefore HSO decline is slower in bottles sealed with cylindrical closures. HSO was completely consumed within one and four months in the majority of the cases. (Notice that all bottles were stored upright and the headspace was arranged between closure and wine surface).

However in some cases HSO increased during the first week of storage, while it decreased constantly as described above only in the second week until the end of the storage period. This occurred in bottles with high DO and low HSO and could be explained due to oxygen migration from wine into the headspace due to the difference in partial pressure.

Regarding the oxygen entering the bottle through the closure, two phases were observed for cylindrical closures: a rapid exponential increase in the first weeks followed by a linear curve with smaller slope until the end of storage period. Skouroumounis und Waters (2007) identified similar stages of DO increase, which reflect the different phases of oxygen ingress. The first phase represents the release of the oxygen residing in the pores of the closure itself which starts moving towards closure´s ends (headspace and environment) due to the high gas pressure in the closure after it has been compressed in order to fit the bottle neck. Concentration gradients, responsible for the flow of oxygen molecules occur along the closure and change during time depending on factors such as oxygen partial pressure in the headspace, in the environment and in the closure itself (Reeves 2009). Depending on the type (oxygen permeability) of the co-extruded closure, this phase lasted in our study from one to two months and delivered 0.7 to 1.3 mg oxygen into the bottle.

After pressure in the closure decays, oxygen keeps diffusing from the closure into the headspace driven by a concentration difference between the two ends of the closure (Reeves 2009). As oxygen molecules dissolute from the headspace into the wine, a steady state situation in oxygen ingress will be reached after a time period depending on closure characteristics, storage position and temperature. This is the second, linear phase of oxygen ingress. Its slope is called oxygen transmission rate (OTR) and is an indication of the oxygen permeability of the closure. Each month of storage further 0.1 to 0.2 mg oxygen were delivered into the bottle during this phase depending on the type of co-extruded closure. This model is in agreement with the findings of Lopes et al. (2006), about oxygen diffusion through the closure occurring much more intensive during the first month of bottle storage than in the time period after that.

Under screw caps only the linear phase of oxygen ingress was observed as these closures do not have pores. Due to the lower oxygen permeability of their material, OTR, which is the slope of the ingress curve, was in most of the cases lower than this of the cylindrical closures. The oxygen ingress through screw caps used in our study varied from 0.02 to 0.1 mg per month.

Impact of headspace oxygen and closure on wine properties

The impact of headspace and closure on wine post-bottling development in terms of sulfur dioxide (SO_2) losses as well as color, aroma and sensory evolution was evaluated via bottling trials with different HSO and headspace volumes combined with either co-extruded or screw cap closures. Yet DO at bottling was kept the same for all bottles.

In accordance with other studies (Godden et al. 2005, Brajkovich et al. 2005, Kwiatkowski et al. 2007 and Lopes et al. 2009) free SO_2 showed in all treatments a rapid decrease in the first months of storage, followed by a slight but consistent decline till the end of the storage period. The initial decline of free SO_2 in our study was more rapid for wines with high HSO as free SO_2 losses were consistently smaller when lower HSO (and smaller headspace volume) was applied. This initial rapid decline represents the main part of SO_2 loss as approximately 55% of the total free SO_2 decrease under co-extruded bottles and 80% under the screw caps occurred during the first four months in the wines tested. This confirms that the greatest loss of free SO_2 during bottle storage is associated with the oxygen present at bottling, which can be in large part HSO. Since free SO_2 evolution during the first four months was mainly dependant on initial HSO, management of HSO at bottling allows great control on SO_2 decline during bottle storage, and consequently on wine shelf life.

After the first four months only a small further decline of free SO_2 was observed in wines sealed with screw caps. This was due to the very low OTR of this kind of closures. On the contrary, further loss was observed for cylindrical closures, consistent to their permeability to oxygen. At 10 months, the evolution of SO_2 was no longer significantly affected by initial HSO, while closure OTR started to be the main source of differences between the modalities. Initial HSO had a minor impact on the decline of free SO_2, while closure OTR was the main modulator of SO_2 concentration.

From these results it is clear that management of both HSO at bottling and OTR offer the potential to control, to a certain extent, the decline of free SO_2 during bottle storage. However when the headspace contained a large amount of oxygen, even sealing with extremely low OTR closures such as screw cap did not prevent significant loss of SO_2 early in wine life. On the contrary, removal of oxygen by means of inert gas flushing significantly reduced initial SO_2 loss, even for closure with higher OTR. In this study CO_2 inerting of the headspace reduced the loss of free SO_2 up to 17 mg/L in the first 4 months of storage. At this point it should be emphasized that the wine volume in the bottles of this experiment was the half of that we usually got in the practice (375 instead of 750 mL), while the headspace volume and HSO as well as the oxygen ingress through the closure is realistic for 750 mL bottles. Therefore, the time of storage in these small bottles corresponds to twice as much as in big bottles.

Another interesting observation was that, in the first four months, the largest improvement in terms of free SO_2 loss was observed when inerting was applied to screw cap closures with large headspace, which is the typical industry setting for this type of closures. This highlights the importance of management of HSO for screw cap closures and, more in general, in situations where large headspace volumes are used, as they contain large amounts of oxygen.

To evaluate further the impact of HSO on wine color during storage, absorbance at 420 nm (abs420) was estimated. Values were always lower than 0.097, indicating that the degree of oxidation in the wines was relatively low, although several statistically significant differences were observed. Using the data collected at different time points during storage, it was found that HSO only affected color development during the first four months, while from the subsequent time point (10 months), differences in color development were due to OTR. CIELab analysis coupled with calculation of ΔE was carried out to investigate the probability of sensorial relevant differences. The results confirmed that, in general, color variations among the different experimental wines were not likely to be detected by human eye, as ΔE was lower than one. This is in disagreement with the observations of Skouroumonis et al. (2005a) which showed that differences in oxygen exposure during storage resulted in large color differences. Considering that, after 24 months, several wines had already a free SO_2 lower than 10 mg/L, the lack of major color differences observed here is quite interesting, as it has been suggested that below this level there is a high risk of develop advanced color oxidation (Godden et al. 2001). Our data indicate that this value needs to be considered carefully, suggesting that generalizations are not possible. Wine content of phenolic compounds is highly variable, depending on grape variety, region, vintage, and winemaking technology.

Treatments with different headspaces under synthetic closures were analyzed for their aroma fermenting by-products at 24 months post-bottling. Among the 26 compounds measured, only ethyl octanoate, ethyl decanoate and cis-Linalooxide presented considerable differences between the wines. While ethyl octanoate and cis-Linalooxide were present in the wines in concentrations above their thresholds, ethyl decanoate was located below. However, it was also included in this discussion as possible contributor to aroma characteristics of the wines via interactions with other aroma compounds (Laska and Hudson 1991). Ethyl octanoate and ethyl decanoate are related with fruity and grape aromas respectively (Francis and Newton 2005), while cis-Linalooxide is related to sweet and floral attributes (Wang et al. 1994). In general, the concentrations measured in this Riesling wine after 24 months of bottle storage were in line with those reported by other authors for these specific aroma compounds in white wines (Francis and Newton 2005, Knoll et al. 2011).

HSO appeared to influence these aroma compounds positive as wines with higher HSO were characterized by higher concentrations. This trend was more pronounced in ethyl decanoate as treatments with low HSO were significantly lower than treatments with medium HSO, which were significantly lower than treatments with high HSO. However, it was surprising that this ester was not detected in treatments with large headspace volume, indicating that the accumulation pattern of this compound is more complex than just being benefited from higher oxygen exposure. Ethyl octanoate demonstrated a similar trend where higher HSO had a positive effect, whereas large headspace volume a negative. These results indicate that oxygen exposure at bottling in form of high HSO favors these compounds, while large headspace volume not. More research is needed in this field in order to investigate how oxygen at bottling influences the evolution of these aroma compounds post-bottling.

Similar to the above mentioned esters, cis-Linalooxide appeared to be higher in High HSO bottles at 24 months of storage. However, headspace volume did not seem to play a role in the concentration of this compound. Again oxygen exposure at bottling seems to favors cis-Linalooxide.

Furthermore, treatments with different headspaces were analyzed for low volatile sulfur compounds. Among the 10 compounds measured only H_2S and DMS were detected in the wines. H_2S has been described as rotten egg and sewage-like odor (Clarke and Bakker 2004) and DMS as cabbage and sulfur (Francis and Newton 2005). Therefore these compounds are associated with wine´s reductive character. In our study, H_2S concentrations were higher than the aroma threshold which indicates that H_2S could contribute to the aroma characteristics of the wines tested. In contrast, DMS concentrations were below threshold. Nevertheless it could also be contributing to the aroma characteristics of the wines via interactions with other aroma compounds (Laska and Hudson 1991).

Headspace composition at bottling affected the concentration of sulfur compounds, as increasing HSO resulted in lower concentrations of these compounds. All treatments with high HSO had in all cases significantly lower concentrations of H_2S and DMS than treatments with low HSO, while treatments with medium HSO were located between those two. This indicates that oxygen at bottling has the potential to influence wine aroma development during storage. Previous findings have associated low oxygen exposure during storage with higher levels of H_2S (Lopes et al. 2009) and DMS (Vasserot et al. 2001). In our study low oxygen exposure at bottling favored the accumulation of these compounds at 24 months post-bottling in 0.375 L bottles.

Headspace volume also affected final concentration of sulfur compounds. Even if not always significant, H_2S was higher in large headspace volume treatments than in small headspace volume

treatments with the same headspace composition indicating that although higher oxygen exposure at bottling in form of higher HSO eliminates this compound, headspace volume benefits the accumulation of H_2S. This is an indication that the accumulation pattern of H_2S is more complex than just being inhibited by oxygen exposure as HSO affected it negative and headspace volume positive. On the other hand, DMS was always lower in treatments with larger headspace volume than in small headspace volume treatments with the same headspace composition, although not always significant. This indicates that DMS is clearly favored from low oxygen exposure, both in form of HSO and headspace volume.

Wines bottled with different headspace treatments under synthetic closure were analyzed for their sensory properties in the framework of a sensory descriptive analysis at 14 and 24 months of storage. The results imply that different wines **develop** after bottling one single wine with different headspaces. It is known that different grades of oxygen exposure post-bottling due to closures with different oxygen permeability results to wines with different chemical and sensory characteristics (Skouroumounis et al. 2005a, 2005b, Lopes et al. 2006, Godden et al. 2002, Hart and Kleinig 2005, Lopes et al. 2009, O'Brien et al. 2009). However, in our study, different grades of oxygen exposure at bottling due to different headspaces influenced the sensory evolution of the bottled wines even under the same closure.

Among the wines tested, the treatment with the largest headspace volume and highest HSO level got in both tastings the highest scores for *oxidative* and the lowest scores for *citrus* and hedonic *liking* indicating that bottling conditions can influence sensory characteristics of wines already at 14 months post-bottling in 0.375 L bottles. The combination of large headspace volume and high oxygen concentration in the headspaces resulted to excessive oxidative character as well as low *citrus* and *liking* scores. Therefore, the oxidative character of bottled wines is not just a matter of closure, like previous studies of Godden et al. 2002 and Lopes et al. 2009 have shown, but also a matter of bottling and particularly headspace treatment.

The next most similar wine in terms of sensory properties was the treatment with the same HSO level as before but smaller headspace volume. Although at 14 months the two treatments appeared to be statistically the same in terms of *oxidative*, the treatment with the small headspace volume got higher scores for liking which indicates that keeping headspace volume small offers a possibility to protect wines from being negatively perceived. Additionally the treatment with the small headspace volume appeared to be less oxidative at 24 months post-bottling. That means that keeping headspace volume small, even without managing HSO level, offers a possibility to protect wines direct against oxidation, at least on the long term. In other words wines with high HSO und small

headspace appeared somewhat oxidized at 14 months but did not oxidize further at 24 months like wines with high HSO and large headspace did. This is in agreement with Kwiatkowski et al. (2007) who found that large headspace volumes result to more oxidized wines after 24 months of storage in 0.750 L bottles. However, headspace volume has to be considered in relation to wine expansion inside the bottle. Indeed, too small headspace volumes could increase the risk of wine leakage. Therefore inerting a larger headspace could be a safer solution for HSO management than reducing headspace volume.

Finally wines with low HSO but large headspace volume got at the sensory analysis the lowest scores for *oxidativ*, at least at 14 months of storage. This indicates that managing levels of HSO can successfully prevent wine oxidation, even under a large headspace volume. This could also be interesting for bottles sealed with screw caps, since headspace volume under this kind of closures is on average three times greater than under cylindrical closures (Reeves 2009). Furthermore these wines got high ratings for *citrus* and *liking*, indicating that managing headspace composition offers a possibility to avoid negative sensory development of wines post-bottling.

An even longer protection against oxidation up to 24 months post-bottling was provided by adjusting also a smaller headspace volume as wines with low HSO and small headspace were least *oxidative* at 24 months, demonstrating at the same time the highest scores in *citrus* and hedonic *liking*. These results confirm that managing headspace – in volume and composition – offers the possibility to protect wines from excessive oxidation and ensure a positive post-bottling sensory evolution of wines.

Considering the aroma analysis in relation to the sensory analysis we conclude that the analytical differences of the wines in terms of volatile fermenting by-products and sulfur compounds were not great enough to be perceived by the panelists as wines did not differ significantly in the attributes *reductive*, *fruity* or *flowery*. However we cannot exclude the possibility that these analytical differences between the wines played a role on hedonic liking scores. On the other hand, wines differ from each other sensorial in the attributes *citrus*, *oxidative* and hedonic *liking* but these differences were not detected analytically. Apparently more analytical compounds should have been included to the analysis in order to explain this kind of sensorial differences.

Summarizing the aroma and sensory results of the wines bottled with different headspace treatments we can say that wines with high HSO were associated with the attribute *oxidative* and the aroma compound cis-Linalooxide, while wines with low HSO were related to H_2S and DMS as well as *reductive*, *citrus* and CO_2 mouth perception. These results confirm that HSO is an important factor influencing sensory attributes and aroma composition of bottled wine. Within each headspace

treatment, headspace volume also accounted for a significant degree of sensory differentiation across the wines. Under high HSO, a large headspace volume resulted to even more pronounced oxidized character, while small headspace volume was characterized from higher ester concentration. Under low HSO, large headspace volume was more associated to H_2S and DMS, while small headspace volume more to *citrus* aroma.

Impact of dissolved oxygen at bottling and closure on wine properties

The impact of DO at bottling and closure OTR on wine post-bottling development in terms of SO_2 loss and sensory evolution was evaluated during bottling trials with different levels of DO combined with different closures. Yet HSO and headspace volume was kept the same for all bottles.

In agreement with previous observations (Brajkovich et al. 2005, Kwiatkowski et al. 2007, Lopes et al. 2009) free SO_2 decreased during bottle storage, with a rapid decline in the first three months followed by a slower decline the months after in all different treatments. After three months of storage, free SO_2 losses were in most of the cases significantly affected by DO at bottling, with greater loss occurring by higher initial DO. Comparing high and low DO samples with the same closure indicated that, in our study initial DO accounted for up to 8 mg/L of free SO_2 lost. The range of DO at bottling applied in this study (0.5, 2.5 and 5 mg/L) is similar to that reported by other authors (Müller-Späth 1977, Vidal et al. 2001, Valade 2007, Lopes et al. 2009) suggesting that such range could be representative of real-life situations. These results clearly indicate that management of DO at bottling is crucial to SO_2 consumption post-bottling, especially in the short term.

Closure OTR also influenced free SO_2 loss, and this effect became progressively more important with time in the bottle. Comparing co-extruded and screw cap treatments with the same DO level at six or 12 months, we observe that closure can account for up to 10 mg/L free SO_2 loss. In total, screw cap and co-extruded with low OTR showed significantly lower free SO_2 losses at six and 12 months compared to co-extruded with higher OTR, regardless the DO level.

Correlation analyses were conducted to examine the relationship between free SO_2 loss and initial DO. It was found that free SO_2 decline was initially well correlated with DO, but this correlation weakened over time due to the fact that loss of free SO_2 continues after DO is completely consumed. Although our data is consistent with the empirical observation that, at least in the presence of high concentrations of available oxygen (i.e. HSO or DO), a rapid decline of SO_2 is observed (Brajkovich et al. 2005, Kwiatkowski et al. 2007, Lopes et al. 2009), the direct reaction between oxygen and SO_2 is extremely slow under wine conditions (Waterhouse and Laurie 2006). Conversely, SO_2 loss is linked to oxygen through reaction of SO_2 with the products of wine oxidation, in particular hydrogen

peroxide (Danilewicz et al. 2008). Additionally, it has to be considered that DO measurement only reflects the oxygen that is present in the wine at any given time. In a highly reactive environment such as wine, this means that it reflects the occurrence of any excess oxygen that the wine has not yet consumed at that time point. Therefore, once DO reaches a value of zero, consumption of oxygen is still taking place, but cannot be quantified. This explains why a good correlation between SO_2 loss and DO was observed only at the early time points.

In order to overcome this limitation, total consumed oxygen TCO was calculated, as the sum of the oxygen present at bottling (HSO plus DO), plus the oxygen entering the bottle through the closure during storage, minus DO and HSO measured at each time point. TCO was well correlated with free SO_2 loss throughout the whole storage period indicating clearly that, in addition to closure OTR, both DO and HSO at bottling represent a key component of any oxygen management strategy aimed at improving wine shelf-life. Calculation of TCO can therefore provide a valuable tool to predict SO_2 loss during bottle storage, and therefore estimate wine shelf-life.

Further the impact of DO at bottling and closure OTR on sensory properties of the wine was investigated via descriptive sensory analysis. In general, wines sealed with co-extruded closures with high OTR were related to attributes *oxidative* and *phenolic*, while co-extruded closure with lower OTR were mainly related to fruity attributes such as citrus and pear. Finally screw cap closures were partly related to the CO_2 perception in the mouth. Although observed in 375 mL bottles within 15 months, these results confirm that in this bottling trial investigating DO´s and OTR´s role on sensory, closure was the most important factor influencing sensory attributes of bottled wine, as observed in other studies (Godden et al. 2002, Skouroumounis et al. 2005a/b, Hart and Kleinig 2005, Lopes et al. 2006, Kwiatkowski et al. 2007, Lopes et al. 2009, O´Brien et al. 2009). However, within each closure, DO at bottling also accounted for a significant degree of sensory differentiation across the wines, as higher DO values were more closely associated with developed characters (e.g. oxidation). Overall, the trend observed suggest that, in the case of the Riesling wine studied, co-extruded with high OTR resulted in excessive wine development, which was further exacerbated by high DO. This indicates that, in the case of this closure the higher amount of oxygen entering through the closure over fifteen months reduced the influence of DO at bottling, determining an overall dominance of oxidized attributes. Conversely, for the other closures (co-extruded with low OTR and screw caps), the interaction between closures OTR and initial DO was more complex, and allowed overall the development of wines with different aroma characteristics. For example, co-extruded with high DO was related to honey and hay attributes, while co-extruded with medium and low DO were closer to

fruity attributes. Finally screw cap closures with medium and low DO were characterized by higher CO_2 than screw caps with high DO.

Looking in detail the scores for the attribute *oxidative* given to the test wines by the panelists, we observe that wines bottled with high DO showed higher ratings for oxidation, confirming the influence of DO management on the evolution of wine over time. Differences were significant mainly between high and low DO treatments, whereas under the lower OTR of the screw cap closures differences were significant also between high and medium DO treatments. On the contrary, differences between the wines sealed with higher OTR closure (co-extruded) were not significant. These results confirm that sensory differences linked to DO at bottling are more likely to be observed in the presence of lower OTR, while higher OTR could mask them.

4. Summary

In the framework of several bottling trials replicating typical winery conditions, Riesling wines were bottled with different levels of dissolved and headspace oxygen and were sealed with co-extruded or screw cap closures in order to investigate the impact of oxygen at bottling as well as oxygen´s ingress through the closure on wine development. Using the luminescence technology, dissolved and headspace oxygen, as well as closure´s oxygen transfer rate were monitored during bottle storage and SO_2 losses, aroma, color and sensory properties were analyzed throughout the storage period.

Headspace and dissolved oxygen decreased constantly in all treatments due to oxygen dissolution and consumption in the wine and became undetectable in the majority of the cases within one and four months. Decline was slower under cylindrical closures due to additional oxygen ingress from the closure into the bottle. Oxygen ingress through the closure demonstrated two phases: a short but rapid exponential curve during the first weeks followed by a linear phase with smaller slope until the end of storage period – the first representing the oxygen out of the pores of the closure itself, the second the ingress of atmospheric oxygen diffusion through the closure into the bottle, known also as oxygen transmission rate.

Oxygen present in the headspace of bottled wine was found to be the main cause of SO_2 decline during the first months after bottling, where the main SO_2 decline occurs. Headspace oxygen accounted for up to 80% of the total SO_2 loss during storage. However, it did not influence color evolution as the color of the Riesling wine tested remained unchanged throughout the trial. Nevertheless headspace oxygen accounted for a significant degree of sensory differentiation of the wines in terms of *oxidative*. 14 months after bottling wines bottled at low headspace oxygen were perceived as significantly less oxidative than wines with high headspace oxygen even under large headspace volume. This positive effect of headspace management lasted even up to 24 months when small headspace volume was realized. Therefore low concentrations of headspace oxygen via CO_2 flushing combined with small headspace volume offered the best possibility to protect the wine from oxygen. In some cases high headspace oxygen appeared to favor some aroma compounds such as ethyl decanoate, ethyl octanoate and cis-Linalooxide (related to fruity, grape and flowery aroma respectively) and to eliminate some others such as H_2S and DMS (aromas of rotten egg, cabbage and sulfur). Headspace volume had in many cases the opposite effect: large volume favored production of H_2S and reduced DMS and esters. However these effects of headspace management on aroma composition were not perceived by the panelist in the sensory analysis since wines did not differ in terms of fruity, flowery or reductive character.

Under low headspace oxygen dissolved oxygen at bottling appeared to be the main factor influencing SO_2 losses during the first months. The loss of SO_2 was not correlated with the evolution of dissolved oxygen throughout storage, but with the total amount of oxygen consumed by the wine. Dissolved oxygen accounted for significant differences across the wines within the same closure: wines bottled with high dissolved oxygen showed significantly higher ratings for *oxidative*, confirming the influence of dissolved oxygen management on the evolution of wine over time.

In all bottling trials closure was the variable defining the further SO_2 losses after the initial rapid decline during the first months due to HSO and DO at bottling. SO_2 continued to decrease in a slow manner in wines sealed with synthetic closures while it remained almost unchanged under screw cap closures. Therefore both headspace and dissolved oxygen management at bottling as well as closure choice offer the potential to control to a certain extent SO_2 losses. Yet, sealing with closures with low oxygen transmission rate such as screw caps did not prevent significant loss of SO_2 when the headspace oxygen was high. On the contrary, keeping headspace oxygen low significantly reduced initial SO_2 loss, even under closures with higher oxygen transmission rate. Closure choice had also an impact on sensory development of the wines post-bottling: wines with closures with high oxygen transmission rate (synthetic closure type 1) were more oxidative, while those with moderate transmission rate (synthetic closure type 2) were more fruity. Finally those with low transmission rate (screw cap) got higher scores for CO_2 perception and lower for *oxidative*. However, these wines were characterized by increased concentrations of H_2S, a compound described as struck flint and rotten egg.

5. Zusammenfassung

Im Rahmen verschiedener Abfüllungsversuche unter typischen Weingut-Bedingungen wurden mehrere Rieslingweine mit unterschiedlichen Gelöst- und Kopfraumsauerstoffkonzentrationen abgefüllt und mit Kunststoffkorken (Co-extruded) bzw. Schraubverschlüssen versiegelt, um die Auswirkung des Sauerstoffs bei der Abfüllung sowie des Sauerstoffeintrags durch den Verschluss während der Lagerung auf die Weinqualität zu untersuchen. Mithilfe der Lumineszenz-Technologie wurden Gelöst- und Kopfraumsauerstoff sowie Sauerstofftransmission durch den Verschluss während der gesamten Lagerungsperiode gemessen. SO_2, Aroma, Farbe und sensorische Eigenschaften wurden während der Lagerzeit analysiert.

Gelöst- und Kopfraumsauerstoff haben aufgrund des Sauerstoffverbrauchs im Wein während der gesamten Lagerung in allen Varianten abgenommen. In den meisten Fällen wurden sie innerhalb von ein bis vier Monaten unnachweisbar. Bei den Kunststoffkorken war der Rückgang aufgrund des zusätzlichen Sauerstoffeintrags durch den Verschluss langsamer. Der Sauerstoffeintritt durch den Verschluss konnte in zwei Phasen aufgeteilt werden: eine kurze, rasche, exponentielle Kurve in den ersten Wochen und anschließend eine lineare Kurve mit kleinerer Neigung bis zu Ende der Lagerzeit. Die erste Phase repräsentiert das Ausdringen des Sauerstoffs aus den Poren des Verschlusses selbst in den Kopfraum und die zweite den Durchgang von Luftsauerstoff durch den Verschluss, die sogenannte Sauerstofftransmissionsrate.

Der Kopfraumsauerstoff wurde als ein sehr wichtiger Bestandteil des gesamten Sauerstoffs in abgefülltem Wein festgestellt, der die Hauptursache für den Rückgang vom SO_2 in den ersten Monaten nach der Abfüllung - wenn die größte SO_2 Abnahme stattfindet - darstellt. Kopfraumsauerstoff war für bis zu 80% des gesamten SO_2 Verlustes während der Lagerung verantwortlich. Jedoch hat er die Weinfarbe, die während des gesamten Lagerversuchs unverändert blieb nicht beeinflusst. Hohe Kopfraumsauerstoffkonzentration hat Aromakomponente wie Ethyldecanoat, Ethyloctanoat und cis-Linalooxide (fruchtiges, beerenähnliches bzw. blumiges Aroma) begünstigt, während die Produktion von H_2S und DMS (faules Ei, Kohl und Schweflige Aromen) unterdrückt wurden. Das Kopfraumvolum hatte oft den gegenteiligen Effekt: größerer Kopfraum begünstigte H_2S-Produktion und verringert DMS und Esterbildung. Trotz des Einflusses des Kopfraums auf die Aromazusammensetzung konnten die Verkoster diesen Effekt sensorisch nicht wahrnehmen; die verschiedenen Weine wurden in den Eigenschaften *fruchtig*, *blumig* und *reduktiv* ähnlich bewertet. Dennoch beeinflusste der Kopfraum die sensorische Entwicklung der Weine bezüglich des oxidativen Charakters. 14 Monate nach der Abfüllung wurden Weine mit hohem Kopfraumsauerstoff als oxidativer wahrgenommen als Weine mit niedrigem Kopfraumsauerstoff

selbst unter großem Kopfraumvolum. Dieser positive Effekt des Kopfraums dauerte bis zu 24 Monate nach der Abfüllung, wenn zusätzlich ein kleines Kopfraumvolum eingesetzt wurde. Somit erwies sich eine durch CO_2 Spülung minimierte Kopfraumsauerstoffkonzentration kombiniert mit einem kleinen Kopfraumvolum als beste Lösung, um Weine vor einer oxidativen Entwicklung zu schützen.

Bei niedrigem Sauerstoffgehalt im Kopfraum, war der Gelöstsauerstoff bei der Abfüllung der wichtigste Einflussfaktor der SO_2 Verluste während der ersten Monate. Der Verlust von SO_2 korreliert mit der Gesamtmenge an Sauerstoffkonsum im Wein. Die Abnahme von Schwefeldioxid war eng mit der Menge des Sauerstoffverbrauchs während der gesamten Lagerung verbunden. Der Gelöstsauerstoff war auch für signifikante sensorische Unterschiede zwischen Weinen mit dem gleichem Verschluss zuständig: Weine mit hohem Gelöstsauerstoff zeigten signifikant höhere Werte bei der Eigenschaft *oxidativ*. Dies bestätigt die Wichtigkeit des gelösten Sauerstoffs während der Abfüllung bei der Entwicklung des Weines in der Flasche.

In allen Abfüll- und Lagerversuchen war der Verschluss der Faktor, der den weiteren Rückgang der SO_2 - nach der ersten raschen Abnahme durch Gelöscht- und Kopfraumsauerstoff - bestimmte. Während in dieser Phase die SO_2 in den Flaschen mit Schraubverschluss unverändert blieb, nahm diese bei Kunststoffkorken langsam ab. Dies zeigt, dass nicht nur der Kopfraum- und Gelöstsauerstoff bei der Abfüllung, sondern auch die Wahl des Verschlusses eine wichtige Möglichkeit zur Kontrolle der SO_2 Abnahme darstellt. Jedoch konnte das Versiegeln mit Verschlüsse mit niedriger Sauerstoffdurchlässigkeit, wie Schraubverschluss signifikante Verluste von SO_2 direkt nach der Abfüllung bei hohem Kopfraumsauerstoff nicht verhindern. Niedriger Kopfraumsauerstoff dahingegen beugte in den ersten Monaten starke SO_2 Abnahme bedeutsam vor, selbst bei Verschlüssen mit höherer Sauerstoffdurchlässigkeit wie Kunststoffkorken. Die Wahl des Verschlusses hatte auch einen Einfluss auf die sensorische Entwicklung der Weine in der Flasche: Weine mit Kunststoffkorken mit höherer Sauerstoff-Transmissionsrate waren oxidativer, weil Weine unter Kunststoffkorken mit mittlerer Transmissionsrate waren fruchtiger. Weine mit niedriger Transmissionsrate (Schraubverschlüsse) hatten höhere Werte bei CO_2-Gefühl im Mund und niedrigere bei *oxidativ*. Allerdings wurden sie auch durch höhere Konzentrationen von H_2S, eine Verbindung die nach faulen Eiern riecht charakterisiert.

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7. Eidesstattliche Erklärung

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig, ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die ich wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

Geisenheim,

Evdokia Dimkou

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