Effect of oxygen management on white wine composition

by

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Declaration

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Date: March 2020

Summary

Premature oxidation in white wine is a constant problem for winemakers. A number of studies have shown that dissolved oxygen and elevated temperatures have a negative effect on wine composition, but these were often done using extreme conditions such as very high temperatures and excessive oxygen additions. During wine oxidation, compounds associated with positive aromas decrease and those linked to aged and oxidized wines increase in concentration. There are numerous ways to combat oxidation using antioxidants and reductive winemaking techniques. However, a recent study has found wines in South Africa to be bottled at a total packaged oxygen level of between 1.5 and 7.5 mg/L. As these levels could reduce antioxidant capacity, understanding how these levels affect wine ageing is paramount. Furthermore, according to our knowledge, a study of dissolved oxygen concentrations representative of the industry at bottling in conjunction with different storage temperatures has not been done before.

In this study, a Sauvignon blanc and Chenin blanc wine were exposed to no oxygen additions and additions of 3 and 6 mg/L and then aged at 15°C and 25°C for 12 months. These wines were analysed chemically and sensorially after six and twelve months ageing. Temperature and dissolved oxygen concentrations were found to significantly affect antioxidants such as glutathione and sulphur dioxide concentrations. Wine volatiles, such as 3-mercaptohexyl acetate, isoamyl acetate, diethyl succinate, hexanoic acid, octanoic acid and decanoic acid were often influenced by higher storage temperatures. Over time, storage temperature was found to significantly affect the sensory descriptors of the Sauvignon blanc wine more than the Chenin blanc wine.

Furthermore, as winemakers seek to avoid oxidation in wine, removing dissolved oxygen from wine by sparging with inert gasses is a common industry practice. However, little research has been done to investigate the relevant parameters of sparging efficiency and the direct effects of sparging on wine chemical composition. This study sought to build upon limited previous research and, for the first time, investigate the effects of sparging on wine chemical composition. Various parameters of sparging such as temperature, flowrate, gas composition and application of a diffusion stone were investigated and found to affect sparging efficacy. Sparging with both nitrogen and a mixed gas of nitrogen and carbon dioxide significantly affected the concentrations of dissolved carbon dioxide in wine, where the amount of dissolved carbon dioxide lost was dependent on factors such as wine temperature, gas flowrate and gas composition. Sparging wine with inert gasses did not affect the measured white wine aromatic or antioxidant chemical composition.

Opsomming

This thesis is dedicated to my ever supportive family and

Hillary Vos, without whom this would not be possible.

Biographical sketch

James Walls was born in Fresno, California in the United States of America on 1 March 1990. He attended Lincoln Elementary School, Rafer Johnson Middle School, and graduated from Kingsburg High School in 2008. James obtained his B.S. Wine and Viticulture in 2012 from California Polytechnic State University, San Luis Obispo. In 2017, James enrolled for an MScAgric in Oenology at the Department of Viticulture and Oenology, Stellenbosch University.

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Preface

This thesis is presented as a compilation of 4 chapters. Each chapter is introduced separately and is written according to the style of the journal South African journal of Enology and Viticulture.

Chapter 1 **Literature review and project aims**

Chapter 2 **Chapter 2. The effects dissolved oxygen and storage temperature on white wine composition**

Chapter 3 **Chapter 3. The effects of sparging on the dissolved gasses and chemical composition of wine**

Chapter 4 **General discussion and conclusions**

Table of Contents

Chapter 1: Introduction and Literature Review

1.1 Introduction

The role of oxygen (O_2) in wine has been found to be critically important during the winemaking process where dissolved O_2 can have both beneficial and detrimental consequences. The harm or benefit of O_2 is dependent on several criteria such as the stage in the winemaking process, the amount of O_2 added, and the removal of positive or formation of negative aroma compounds (du Toit *et al.,* 2006; Day *et al.*, 2015).

Oxygen additions during fermentation plays a positive role in yeast metabolic functions and can also positively influence red and some white wine ageing in small doses (<22 mg/L/year) (Larue *et al.,* 1980), however, these benefits are highly dependent on the cultivar and the wine style (Larue *et al.,* 1980; Ribéreau-Gayon *et al.,* 2006; Hernández-Orte *et al.,* 2009). However, the dissolution of macro amounts of O_2 (>22 mg/L/year)(Larue *et al.,* 1980) in aromatic white wines during the later stages of the winemaking process can result in premature oxidation (Ugliano, 2013; Morozova *et al.*, 2014; Waterhouse *et al.*, 2016) and an overall decline in wine quality (Singleton *et al.,* 1979; Waterhouse *et al.*, 2016). Some alternative wine styles might rely on O_2 exposure to produce a specific sought after aromatic composition. In these cases, O_2 exposure is intentionally allowed and even induced with care as to not result in objectionable oxidation nuances.

During oxidation, fresh and fruity aromas are significantly reduced, unwanted colouration occurs and oxidative aromas form (Escudero *et al.,* 2002; Ugliano, 2013; Coetzee *et al.*, 2016; Waterhouse *et al.*, 2016). As new chemical compounds form, the aged or oxidative aroma attributes have been described as “honey-like”, “dry fruits”, “farm feed”, “woody-like”, “hay”, “toasted”, “caramel”, “overripe fruit”, “apple”, “oxidised apple”, “acetaldehyde”, “cooked”, “aldehyde” and “liquor” (Thoukis, 1974; Noble *et al.,* 1987; Renouil, 1988; Halliday & Johnson, 1992; Chrisholm *et al.,* 1995; Escudero *et al.,* 2002; Silva Ferreira *et al.,* 2002). These descriptors are considered to contribute negatively to wine aromatic composition. To inhibit the aforementioned aromas formation, winemakers can use both preventative and direct intervention practices to protect their wines from O_2 exposure, thereby safeguarding wine quality.

Most wine production and bottling operations use inert gasses to both prevent O_2 exposure by displacing air (containing O_2) from the surfaces of juice, must, and wine, thereby preventing O_2 exposure and also to remove dissolved O_2 from wine by sparging operations. In the wine industry, carbon dioxide (CO_2), nitrogen (N_2), and argon are used to flush, blanket and sparge wine (Zoecklein *et al.*, 1995; Bird, 2011). Though little research has been conducted into the effects of sparging on wine chemical (including dissolved gases) and sensory composition, industry professionals have speculated that sparging could cause losses of volatiles aromatics (Bird, 2011).

This literature review will focus on two main principles regarding O_2 in wine. The first part will discuss how O_2 enters wine during production, how this dissolution affects the wine composition in terms of the lowering or formation of volatile compounds, and the subsequent effects on the sensorial characteristics of white wine. The second part will explain the principals of Henry's Ideal gas laws and will focus on sparging techniques and the role of N_2 and CO_2 gas in wine production.

1.2 Oxygen pickup during wine processing

Oxidation is one of the main faults found in wine and is a constant concern for winemakers throughout the winemaking process. Without proper prevention strategies in place, O_2 can ingress and dissolve in wine during most winemaking operations (Castellari *et al.,* 2004; Calderón *et al.,* 2014).

Oenological operations can be classified in terms of the potential dissolved O_2 that it can induce, namely, low enrichment and high enrichment operations (Castellari *et al.,* 2004). Studies have identified high enrichment practices to include centrifugation, racking, refrigeration, bottling and continuous tartaric stabilization (Castellari *et al.,* 2004; Calderón *et al.,* 2014). The dissolved O_2 concentrations after various winery processes ranged from <1.0 mg/L to 7.5 mg/L where cold stabilization and refrigeration contributed the largest addition to dissolved O_2. Low enrichment additions are practices such as pumping, heat exchange, electrodialysis and filtration where dissolved O_2 increased up to 1.3 mg/L, filtration being the largest contribution to dissolved O_2 (Calderón *et al.,* 2014).

Additionally, the process of bottling can lead to significant increases in dissolved O_2. After bottling, the O_2 can be present as 1) dissolved O_2 in the wine or 2) as gaseous O_2 present in the headspace. The total packaged oxygen (TPO) is the sum of the dissolved and headspace O_2. A survey conducted on South African bottled white wines showed a large variation of dissolved O_2 concentrations after bottling, ranging from less than 1.0 mg/L to 7.5 mg/L TPO (Van der Merwe, 2013). The final TPO is highly dependent on pre-bottling (dissolved O_2 concentration of the wine while in tank) and bottling practices. After bottling, O_2 can still enter the bottle through the closure, however this O_2 transmission rate varies significantly depending upon the type of closure used (Dimkou *et al.,* 2011). During ageing in tank and barrels, oxidation can also be problematic if wine is stored with ullage containing O_2.

In some cases, intentional O_2 additions can be done to stimulate or enhance certain reactions and activity. A good example is during fermentation where intentional macro O_2 dosage operations such as pump-overs, can quickly increase dissolved O_2 concentrations to around 2-3 mg/L stimulating yeast activity. This O_2 is however quickly consumed by the yeast and will not necessarily be available for oxidation reactions (Schneider, 1998; du Toit *et al.,* 2006; Moenne *et al.,* 2013). The solubility of O_2 in wine is influenced by wine chemical composition and environmental factors such as temperature and pressure (Zoecklein, 1995; Lyons *et al.,* 2015). An increase in ethanol concentration will decrease the potential gas solubility (Liger-Belair *et al.,*

2008), while temperature and the partial pressure of the gas are factors affecting O_2 solubility (Agabaliantz, 1963; Waterhouse & Laurie, 2006). Henry's gas law states that O_2 solubility increases as temperature decreases (Waterhouse & Laurie, 2006; Lyons *et al.,* 2015). Additionally, as the concentration of O_2 in atmosphere increases, O_2 dissolves more rapidly into solutions (Waterhouse & Laurie, 2006). Although increasing temperature lowers the solubility potential of O_2, increasing temperatures exponentially enhances the rate of oxidation reactions in wine mediums (Margalit, 1997; Vivas de Gaulejac *et al.,* 2001; Ribéreau-Gayon *et al.*, 2006).

1.3 Oxidation reactions

In wine, dissolved O_2 is found in an unreactive triplet state, which has minimal potential to react directly with most wine compounds (Waterhouse & Laurie 2006). This reactivity increases in the presence of an oxidation catalyst, which in wine are primarily iron and copper (Cacho *et al.,* 1995; Macris *et al.,* 2000; Danilewicz, 2003). When dissolved in wine, iron donates an electron to dissolved O_2, which inevitable forms the superoxide ion, $^{O}2^{\bullet-}$. Though this superoxide radical exists at wine pH, it is not highly reactivity in wine, and therefore can only react with strong hydrogen-donating species such as phenolics (Wildenradt *et al.,* 1974; Waterhouse & Laurie, 2006). As reactions of superoxide ions with *o*-diphenols occur in wine, it will lead to the formation, *o*-quinones and hydrogen peroxide which are stronger oxidants. Both peroxide and *o*-quinones participate in several chemical reactions affecting the wine chemical composition. By way of a Fenton reaction mechanism, hydrogen peroxide can react with ferrous ions to create hydroxyl radicals, extremely reactive compounds capable of oxidizing most wine components indiscriminately (Waterhouse & Laurie, 2006). Subsequently, ethanol can be oxidized to acetaldehyde, whereas other compounds such as glyoxylic acid are formed from oxidation of tartaric acid or other alcohols (Fenton, 1894; Waterhouse & Laurie, 2006).

1.3.1 Antioxidants

Antioxidants are extremely important contributors to the ageing potential of white wine, where the most common within the wine industry are ascorbic acid, sulphur dioxide (SO_2) and glutathione (GSH). During the phenol oxidation process, these compounds interfere in the Fenton reaction, either by eliminating O_2 from the wine or by combining with the oxidation products. In section 1.3.1.1 and 1.3.1.2, the role of SO_2 and GSH in wine will be briefly discussed.

1.3.1.a Sulphur dioxide

Though the reaction of dissolved O_2 is indirect, a stoichiometric relationship exists between O_2 and sulphite where a ratio of four sulphites to every one O_2 is reacted when both are present in wine (Waterhouse et al., 2016). Sulphur dioxide is an inexpensive but effective additive for the oxidative and microbial preservation of wine and other food products (Doyle & Beuchat, 2007). Though SO_2 naturally occurs in all wines as a by-product of yeast metabolism by way of

fermentation (Rankine & Pocock, 1969), it is typically introduced at several critical stages during conventional winemaking where spoilage or oxidation can occur, such as crushing, settling, post primary and secondary fermentation, transfers, ageing, and bottling (Paul, 1975). That said, overuse of SO_2 is harmful to both the sensorial quality of wine and to consumer health (Kleinhans, 1982), which has led to legal limits.

In wine, SO_2 exists in both free and bound forms, where the sum equals total SO_2. At wine pH (3 to 4), free SO_2 exists in three forms: sulphite (SO_3^{2-}), bisulphite (HSO_3^-) and molecular SO_2. These three forms are existing in an equilibrium dependent upon wine pH, and the presence of bisulphite binding wine constituents and wine temperature (Usseglio-Tomasset, 1992). The most prevalent form of free SO_2 is bisulphite (94-99%) which binds a large array of wine compounds, thus becoming the main constituents of bound SO_2 (Zoecklein *et al.,* 1995; Oliveira *et al.,* 2002). The molecular form of SO_2 is primarily responsible for antimicrobial activity whereby molecular SO_2 pierces the cellular membranes of microorganisms (Beech *et al.,* 1979). Molecular SO2 in only found in small proportions to bisulphite and sulphite due to the pH of wine (Oliveira *et al.,* 2002). Though bisulphite can react directly with dissolved O_2, the concentrations found in wine are insignificant. The direct reaction of bisulphite with O_2 is relatively slow, but bisulphite is a significant antioxidant where o-quinones can go through two reactions in the presence of bisulphite, reduction to o-diphenols or additions resulting in the formation of sulphonic acids, and the reduction of H_2O_2 to H_2O. (Danilewicz, 2007; Arapitsas *et al.*, 2016). Interestingly, sulphonic acid concentrations have been shown to be mediated by dissolved O_2 concentrations at bottling where increased dissolved O_2 concentrations promote the reduction of SO_2 (Arapitsas *et al.*, 2016).

The presence of free SO_2 in wine inhibits the oxidation process and reacts with intermediate oxidation products such as acetaldehyde. (Figure 1.1). The resulting product of the reaction between bisulphite and acetaldehyde is as an odourless and chemically stable sulphite compound known as hydroxysulphonate (Waterhouse & Laurie, 2006). However, more recent research has found that hydroxysulphonate added a ‘sulphur-like’ aroma to a synthetic wine solution (Coetzee *et al.,* 2018).

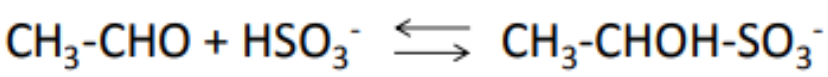

Figure 1.1 Reaction of acetaldehyde with bisulphite.

1.3.1.b Glutathione

In wine, glutathione (GSH), a sulphur-containing tripeptide (L-γ-glutamyl-L-cysteinyl-glycine), acts as an important antioxidant during grape and yeast metabolism (Figure 1.2), and as precursor for thiol formation. Concentration of GSH in must after fermentation is directly influenced by nitrogen uptake by the vine during the growing season (Choné *et al.,* 2006) and GSH starts to accumulate in the berry at the onset of vériason (Adams & Liyanage, 1993). Yeast have been hypothesised to be partly responsible for GSH concentrations found in wines (Lavigne *et al.,*2007), but Fracasetti *et al.,* 2013 found that specific yeast strains did not significantly alter GSH content in wines. However, winemaking procedures have been shown to critically alter GSH concentrations as elevated O_2 exposure led to lower GSH concentrations while higher concentrations are found in reductively treated juices and wines (Du Toit *et al.,* 2007; Maggu *et al.,* 2007; Fracasetti *et al.,* 2013; Coetzee *et al.,* 2016).

During the oxidative processes, the electron-rich nucleophilic mercapto group in glutathione can be substituted by 1,4- Michael substitution into the electrophilic centre of *o*-quinones. The resulting products are known as thioethers, 2-*S*-glutathionyl-caftaric acid, also known as grape reaction product (Figure 1.2) When 2-*S*-glutathionyl-caftaric acid is formed, the *o*-quinone is trapped in a colourless form, preventing further reactions and thereby oxidative browning (Kritzinger *et al.,* 2013a). Glutathione is also sensitive to the oxidant hydrogen peroxide, whereby GSH is oxidised to glutathione disulphide (Anderson, 1998) (Figure 1.2). Cilliers & Singleton, 1990 have argued that disulphide can also form by the reduction of an *o*-quinone back to an *o*-diphenol.

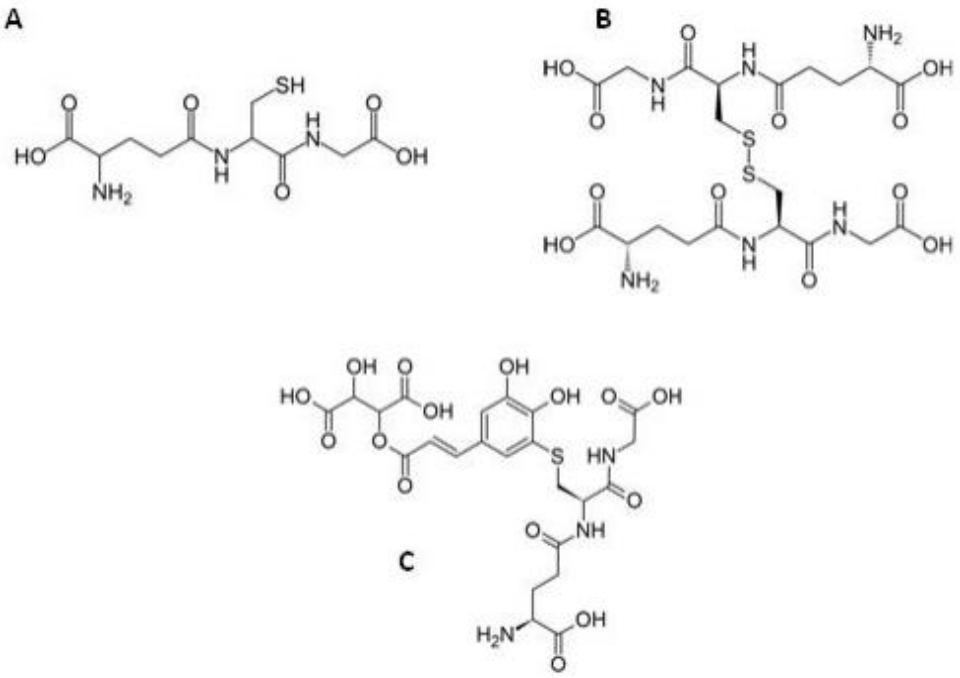

Figure 1.2 Molecular structures of glutathione (A), glutathione disulphide (B) and grape reaction product (C).

1.3.2 Substrates for oxidation: Phenolic compounds

Phenolic compounds are a strong hydrogen donating species, and therefore are excellent oxidation substrates. All phenolic compounds are characterized by the presence of an aromatic ring which contains one or more hydroxyl substituents, including functional derivatives. (Wildenradt & Singleton, 1974). The concentration of phenolic compounds in a wine will be dependent on the grape cultivar, climate, cultivation methods, maturation level at harvest, winemaking practices, and ageing. Both red and white wine can consume considerable amounts of dissolved O_2, though red wine typically has a greater O_2 consumption potential due to greater total phenol content (Rossi & Singleton, 1966). The lower polyphenol content of white wine is typically due different procedures in white wine production as compared to red wine where there is greater phenolic extraction (Rossi & Singleton, 1966).

1.3.3 White wine browning

The presence of oxidation in white wine can be indicated by a prevalence of dark yellow or brown colour. Hydroxycinnamic acids have been shown to contribute to wine browning through coupled oxidation reactions (Simpson, 1982; Fernández-Zurbano *et al.,* 1995). The browning phenomenon in white wine is linked to several key oxidative mechanisms involving phenolic molecules. Phenolic molecules are oxidised to their corresponding *o*-quinones, the *o*-quinones initiate further reactions with phenolic compounds to create dimers (Singleton, 1987). Dimers tend to be more susceptible to oxidation then regular phenolics, thusly accelerating autocatalytic oxidation and phenol polymerisation in wine (Singleton, 1987). The formation of these polymers produces even more severely coloured yellow-brown compounds (Es-Safi *et al.,* 1999; Lopez-Toledano et al, 2004). Research has shown the positive correlation of the total phenolic content of wine with potential of coloration (Simpson, 1982), however, a study have shown the concentrations of hydroxycinnamic acids (a specific class of phenolic compounds) in wines to not correlate strongly with the degree of brown coloration (Fernández-Zurbano *et al.,* 1995).

White wine browning processes accelerate as temperature increases and as pH rises (Ferreira *et al.,* 1997; Escudero *et al.,* 2002; Silva Ferreira *et al.,* 2003; Loscos *et al.,* 2010; Cejudo-Bastante *et al.,* 2013). Iron, copper and O_2 concentration increases have also been linked to increased colouration in white wine (Caputi Jr. & Peterson, 1965; Peterson & Caputi Jr., 1967; Oszmianski *et al.,* 1996). In terms of winemaking techniques influencing the amount of flavan-3-ols, practices such as skin maceration, pressing and/or heat treatment, may consequently impact the browning sensitivity and potential of wine by directly influencing concentrations of flavan-3-ols (du Toit *et al.* 2006). Independent of metal content and winemaking practices, increasing dissolved O_2 concentrations are also known to increase colouration in wine by facilitating oxidation reactions (Ugliano, 2013; Del Caro *et al.,* 2014; Morazova *et al.,* 2014; Coetzee *et al.,* 2016; Waterhouse et al., 2016).

1.4 Effects of oxidation and temperature on white wine volatiles

Dissolved O_2 and elevated storage temperatures (>40°C) have been shown to facilitate oxidation in white wines (Blanchard *et al.* 2004, Nikolantonaki *et al.* 2010; Patrianakou *et al.,* 2013; Ugliano, 2013; Coetzee *et al.,* 2016). As white wine is being oxidized, compounds associated with fruity descriptors such as isoamyl acetate, 2-phenyl acetate, 2-methyl-propyl acetate, and 3-mercaptohexyl acetate have been shown to decrease in intensity (Blanchard *et al.* 2004, Nikolantonaki *et al.* 2010; Patrianakou *et al.,* 2013; Coetzee *et al.,* 2016). Subsequently, the intensity and presence of fruity descriptors such as “peach”, “passion fruit” and “grapefruit” decreased or disappeared entirely as both dissolved O_2 concentrations and storage temperature increases (Presa-Owen & Noble; 1997; Escudero *et al.,* 2002; Cejudo-Bastante *et al.,* 2013; Coetzee *et al.,* 2016).

Compounds associated with oxidative aromas such as various aldehydes, diethyl succinate, ethyl lactate, ethyl hexanoate, octanoic acid and decanoic acid have been shown to increase in the presence of dissolved O_2 and elevated storage temperatures (De la Presa-Owens & Noble, 1997; Escudero *et al.,* 2002; Cejundo-Bastante *et al.,* 2013; Coetzee *et al.,* 2016). This is in part due to Arrhenius activation energy principle whereby every 10°C increase in temperature is known to roughly double the rate of reaction in many compounds (Peleg et al., 2012). Furthermore, sensory attributes associated with oxidation, such as “honey”, “farm feed”, “woody”, “potato bag”, “curry” and “cooked vegetables” (Toukis, 1974; Noble *et al.,* 1987; Renouil, 1988; Halliday & Johnson, 1992; Chrisholm *et al.,* 1995; De la Presa-Owens & Noble, 1997; Escudero *et al.,* 2002; Silva Ferreira *et al.,* 2002; Coetzee *et al.,* 2016), have been found in oxidized wines. The intensity of these descriptors has been shown to increase significantly as dissolved O_2 concentrations and storage temperatures increase (du Toit & Piquet, 2014; Coetzee *et al.,* 2016).

The following sections will address specific aroma compounds that are affected due to oxidation reactions occurring in white wine.

1.4.1 Varietal thiols

Though there are various thiols in food products, a subset of the most important of these are called varietal thiols and are found in Chenin blanc and Sauvignon blanc (Vermeulen *et al.,* 2005; McGorrin, 2011; Coetzee & du Toit, 2012; Weightman, 2014; Wilson, 2017). Varietal thiols are responsible for imparting fruity and tropical organoleptic qualities and have remarkably low sensory thresholds, where organoleptically detectable concentrations are measured in ng/L (Vermeulen *et al.,* 2005). In the past decade, Sauvignon blanc wines have particularly received intensive attention in research circles; however, recently Chenin blanc wines have also been shown to contain high concentrations of varietal thiols (Roland *et al.,* 2011; Coetzee & du Toit, 2012; Coetzee *et al.,* 2013; Weightman, 2014; Aleixandre-Tudo *et al.,* 2015; Wilson, 2017) and are increasingly under investigation.

The main varietal thiols in wine are 4-mercapto-4-methylpentan-2-one (4MMP) (Darriet et al., 1995), which is often described as “box tree”, “passionfruit” and ‘blackcurrant”, 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) (Tominaga et al., 1996; Tominaga et al., 1998), which are linked to attributes described as “passionfruit”, “guava”, and “grapefruit”. New nomenclature for these volatile compounds exists, however, the established nomenclature of 4MMP, 3MH and 3MHA will be utilized as it is more commonly recognized in academic and commercial environments.

Volatile thiols have been detected in juice matrices but in small quantities, however they are detected in significant quantities post alcoholic fermentation. During fermentation, there are two known biogenesis pathways of thiols. The first pathway is where yeast cleave cysteinylated and glutathionylated precursors to release the aromatic thiol, while the second pathway involves the reaction of hydrogen sulphide (or another sulphur contributing compound) directly with *(E)*-2-hexenal mesityl oxide and conjugated carbonyl compounds followed by a reduction phase (Schneider *et al.,* 2006). Not being fully understood, the formation of the volatile thiols is still a mystery as the main precursors have yet to be discovered and, therefore, the synthesis mechanism of varietal thiols requires further investigation. The formation of thiols from the glutathionylated and cysteinylated precursors is still under investigation as only a small percentage (up to 10%) are converted to the aromatic form (Roland *et al.,* 2011).

During ageing, thiols are particularly susceptible to hydrolysis and oxidation. Acid hydrolysis has been found to significantly affect the concentrations 3MHA during the ageing of Sauvignon blanc wines (Herbst *et al.,* 2008; Herbst-Johnstone *et al.,* 2011; Coetzee *et al.,* 2016). Oxidatively, research has found *o*-quinone trapping to be the main mechanism accounting for 3MHA losses in wine being stored under oxidative conditions (Krietman *et al.,* 2013; Coetzee *et al.,* 2016). In a nucleophilic, acid-catalyzed substitution reaction, thiols are known to react with polyphenolic compounds, where the reaction products can degrade quickly due to reactions with phenolic oxidation products, which are primarily o-quinones (Coetzee *et al.,* 2016).

1.4.2 Esters, fatty acids and higher alcohols

Esters, fatty acids and higher alcohols are yeast-derived compounds which are known to contribute towards the aromatic profile of both Sauvignon blanc and Chenin blanc wines. (Schreier *et al.,* 1979; Stashenko *et al.,* 1992; Delfini *et al.,* 2001; Lambrechts *et al.,* 2000; Styger *et al.,* 2011, Louw *et al.,* 2010; Wilson, 2017;). These compounds contribute considerably to overall wine aromatic composition, are produced anabolically or catabolically by yeast during fermentation and are not specific to any cultivar.

1.4.2.a Esters

Esters form by the condensation of an alcohol and an organic acid. Not only in wine, esterification and ester hydrolysis are acid-catalysed into equilibrium reactions (Saerens et al., 2010). Acetate

esters are particularly sensitive to oxidation and elevated storage temperatures where they have been shown to decrease in concentration in several oxidative and aging studies (Herbst-Johnstone et al, 2011; Cejudo-Bastante *et al.*, 2013; Coetzee *et al.,* 2016). The ethyl esters of acetates and straight-chain fatty acids are synthesized during fermentation because of lipid metabolism of yeasts (Díaz-Maroto *et al.,* 2005). Typically, the esters isoamyl acetate, hexyl acetate, 2-phenylethyl acetate, ethyl butyrate and ethyl caprate decrease in concentration during ageing (Chisholm *et al.,* 1995; Patrianakou *et al.,* 2013), while other esters associated with “apple” and “lactic” (Ferreira *et al.*, 2000; Moyano *et al.*, 2002) such as diethyl succinate, ethyl lactate, and ethyl hexanoate have been shown to increase in concentration during the ageing process (Chisholm *et al.,* 1995; Cejudo-Bastante *et al.,* 2013).

1.4.2.b Fatty Acids

Critical aroma contributors, the most abundant fatty acids have been shown to be acetic, hexanoic, octanoic and decanoic acid, where these are shown to contribute towards “fresh” flavours in wine (; Lambrechts & Pretorius, 2000). However, as concentrations of fatty acids increase in wine, unwanted flavours described as “vinegar”, “cheesy”, and “rancid” can develop (Schreier, 1979; Ferreira et al., 2000; Lambrechts & Pretorius, 2000). Hexanoic, octanoic and decanoic acid are medium-chain fatty acids, where these act as intermediates for yeast during the biosynthesis of long-chain fatty acids. As an ethyl ester undertakes hydrolysis, the fatty acid to which the ethyl was bound is released. This process can lead to higher concentrations of fatty acids over time. However, the pattern of these compounds forming during ageing have not always been observed. The concentrations of fatty acids have been shown to be inconsistent during ageing where the formation and degradation of these compounds needs further investigation (Roussis *et al.,* 2005; Câmara *et al.,* 2006; Blake *et al.,* 2009; Lee *et al.,* 2011; Coetzee *et al.,* 2016). It could be that fatty acid formation or degradation is either advanced or inhibited by elevated storage temperatures and dissolved O_2 (Cejudo-Bastante *et al.*, 2013; Coetzee *et al.*, 2016).

1.4.2.c Higher alcohols

Higher alcohols are formed during alcoholic fermentation and are critical precursors for the formation of volatile esters (Soles *et al.,* 1982). Higher alcohols originate from the anabolic synthesis intermediates of sugar metabolism intermediates or are synthesised through the Ehrlich pathway from branched-chain amino acids catabolically (Nykänen, 1986; Boulton *et al.,* 1996; Dickinson *et al.,* 1997; Dickinson *et al.*, 2003). During oxidative ageing, alcohols can form aldehydes thereby lowering the total alcohol concentration in wine (Marais & Pool, 1980).

At higher concentrations, aromas such as “fusal”, “nail polish” and “whiskey” can become pungent too the odour and taste (Nykänen, 1986; Guth, 1997), subsequently masking other aroma contributors. Conversely, it has been shown that when concentrations of higher alcohols

are lower than 300 mg/L in wine, these compounds indirectly contribute to aroma complexity in wine. (Rapp & Mandery, 1986). Though changes to higher alcohol concentrations can be sensorially impactful, numerous studies have observed stable concentrations across wine ageing (Marais, 1978; Roussis *et al.,* 2005; Roussis *et al*., 2007; Blake *et al.,* 2009). Contrarily, the only higher alcohol known to increase in concentration during ageing is hexanolOliveira *et al.,* 2006).

1.4.3 Effects of storage temperature on wine composition

Storage temperature has been shown to significantly affect wine chemical and sensory properties, however, studies did not necessarily report results from conditions which would realistically mimic cellar parameters (De la Presa-Owens and Noble, 1997; Loscos *et al.,* 2010; Robinson *et al.* 2010; Cejundo-Bastante *et al.,* 2013) with methodologies typically including elevated temperatures (>40^{o}C). While beneficial for experimental expediency, raising the temperature to extreme levels could potentially provide catalytic activation energy for compounds which would normally not form in typical cellar conditions (Peleg *et al.,* 2012; Cejundo-Bastante *et al.,* 2013). Further research into the effects of storage temperature on wine composition is therefore warranted.

1.5 Role of sparging wine with inert gas

There are two main dissolved gases present in wine: O_2 and CO_2. The presence of these gasses can have a significant impact on the wine quality and the sensory perception. Nitrogen (N_2) and carbon dioxide (CO_2) are frequently utilised in winemaking to either prevent O_2 dissolution by displacing air in contact with wine or by preventing oxidation by removing dissolved O_2 through sparging operations (Zoecklein *et al.,* 1995; Bird, 2011).

1.5.1 Henry's Ideal gas laws

The dissolution behaviour of gas in wine is based on the principle of Henry's gas law (Lyons *et al.,* 2015). This law was formulated by William Henry in 1803 and states: "At a constant temperature, the amount of a given gas that dissolves in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid." (Agabaliantz, 1963; Liger-Belair, *et al.,* 2012; Lyons *et al.,* 2015). This is expressed as the following equation:

$$c = k_H P_{(gas)}$$

- where "c" is the solubility of a gas at a fixed temperature in a particular solvent
- "$k_{H"}$ is Henry's law constant based on the solubility of a specific gas at a given temperature
- "$P_{(gas)}$" is the partial pressure of a given gas in the vapor phase

Table 1.1 shows Henry's law constant (k_H) of dissolved CO_2 in champagne as a function of temperature (Agabaliantz, 1963). As the temperature of the gas increases, the k_H decreases, resulting in a lower solubility of the particular gas in a particular solution.

The partial pressure ($P_{(gas)}$) is dependent on the nature of the specific gaseous molecule. Understanding and applying the concepts derived from this equation is paramount to researching gas dissolution in wine matrices.

Table 1.1 The Henry's law constant values of champagne for dissolved CO_2 (in g L^{-1} bar^{-1}), as a function of temperature, for a conventional champagne with 12.5% (v/v) of ethanol and 10g L^{-1} of sugars. Compiled from Agabaliantz, 1963.

Temperature °C	Henry's law constant kH (gL^{-1} bar^{-1}) 2.98
1	2.88
2	2.78
3	2.68
4	2.59
5	2.49
6	2.41
7	2.32
8	2.23
9	2.16
10	2.07
11	2
12	1.93
13	1.86
14	1.79
15	1.73
16	1.67
17	1.6
18	1.54
19	1.48
20	1.44
21	1.4
22	1.34
23	1.29
24	1.25
25	1.21

In the wine industry, sparging operations normally utilize inert gases in two methods: static and in-line. Static sparging operations consists of directly applying N_2 into the wine while it is in the storage vessel. In-line sparging is a process which inject inert gas into pipes while the wine is being transferred from one location to another. Thus, the wine is being sparged while moving though pipes.

Sparging wine with fine inert gas bubbles will create a partial pressure difference between the dissolved O_2 and the inert gas (Wilson, 1986; Zoecklein *et al.,* 1995; Liger-Belair *et al.,* 2012; Lyons *et al.,* 2015). Consequently, a partial pressure difference is created between the gasses,

which expels dissolved O_2. Simultaneously, dissolved CO_2 is also expelled from the matrix (when using nitrogen or argon) possibly altering the organoleptic properties of a wine.

1.5.2 Nitrogen as a sparging gas

N_2 gas does not form naturally during winemaking as it is not a by-product of the metabolism of yeast or bacteria. That N_2 has a low solubility at typical cellar temperatures and atmospheric pressure makes it ideal as a sparging gas for the removal of O_2 (thereby preventing oxidation). The low solubility of N_2 means it quickly escapes the wine after sparging, thereby removing dissolved O_2 and preserving the chemical and sensorial properties of the wine (Zoecklein *et al.,* 1995). Though it would seem the above mentioned characteristics make the application of N_2 an ideal tool in reductive winemaking and sparging operations, the effects of sparging on the wine composition still need to be investigated.

1.5.3 Carbon Dioxide as a sparging gas

Carbon dioxide is a natural by-product of alcoholic fermentation and has high solubility in wine at cellar temperatures and atmospheric pressure (Devatine, 2007; Liber-Belier *et al.,* 2012). As CO_2 is heavier than air, it coalesces to the lowest point when introduced to wine storage vessels under normal atmospheric conditions, providing wine with an O_2 scarce protective layer (Baiano, *et al*, 2012). This characteristic makes CO_2 an ideal inert gas to use to fill containers prior to wine movements, thereby preventing air exposure and O_2 dissolution into wine (Zoecklein *et al.,* 1995; Bird, 2011; Cáceres-Mella, A. *et al*, 2013).

In white table wines, dissolved CO_2 concentration is typically between 500 mg/L to 1000 mg/L (Gawel *et al.,* 2018) while it has been described sensorially as ‘prickly’ at 1000 mg/L and ‘spritzy’ at 1800 mg/L (Peynaud, 1983). The higher concentrations of CO_2 found in sparkling wine (2-4 g/L) have been found to increase chemosensory excitation of nociceptors in the oral cavity (Dessirier *et al.,* 2000; Carstens *et al.,* 2002; Chandrashekar *et al.,* 2009; Dunkel *et al.,* 2010) which is described as changing the mouth feel properties to have more ‘bite’ (McMahon *et al.,* 2017).

Dissolved CO_2 and how it interacts with human olfactory systems were first studied in 1980 by Cain and Murphy where it was discovered that dissolved CO_2 could inhibit aromas in carbonated beverages and increase nasal receptor irritation (Cain & Murphy, 1980; Cain, 1981). Yau and McDaniel (1992) later found that in model carbonated solutions, carbonation significantly increased the perception of sourness.

Addition, dissolved CO_2 was found to increase astringency in model cider solutions where increased perceptions of astringency were reported at higher concentrations of dissolved CO_2 (Hewson *et al.,* 2009; Symoneaux *et al.,* 2015). Dissolved CO_2 can form carbonic acid which can lower wine pH (Dessirier *et al.,* 2000; Chandrashekar *et al.,* 2009; Dunkel *et al.,* 2010) and it is known that lowering wine pH increases the organoleptic sensation of astringency (Gawel *et al.,*

2014). Therefore, it could be speculated that by increasing dissolved CO_2 concentrations, the perception of astringency could potentially increase (Gawel *et al.,* 2014).

However, the most current reported research has contradicted this idea, where the perception of astringency in Chardonnay and Viognier wines were significantly reduced by increasing the level of dissolved CO_2 (Smith *et al.,* 2017). However, the authors reported a decrease in the wine pH after the dissolved CO_2 additions (due to the formation of carbonic acid), where the pH in wine treatments was subsequently adjusted to original concentrations prior to sensory evaluation, possibly altering organoleptic properties. As lowering wine pH has been positively correlated with increased perceptions of bitterness and astringency (Gawel *et al.,* 2014), the addition of dissolved CO_2 could indirectly negatively alter the tactile sensations of the wine. The exact nature of how dissolved CO_2 affect organoleptic properties of still white wine is still being investigated (Smith *et al.,* 2017; Gawel *et al.,* 2018).

1.5.4 Wine sparging efficiency

The efficacy of sparging operations seems to be dependent on various factors such as temperature, sparging gas composition, bubble size, flow rate, contact time, wine volume and atmospheric and wine pressure as well as the wine composition (Wilson, 1986). It was found that as wine temperature increases, sparging efficiency improves, but improvements decrease as temperatures rises. The composition of the inert gases being sparged also was found to affect sparging efficacy. The application of diffusion stones with pore sizes ranging from 2 µm to 15 µm were found to increase the rate of CO_2 removal as pore size decreased. Increasing the flow rate of inert gases during sparging increased sparging efficiency, but only until the ratio of gas to wine per minute reached 1:10, after which no additional efficiency gains were observed. How much time inert gases were in contact with wine also effected sparging efficacy, as increased contact time lead to increased efficiency. It was also previously found that atmospheric pressure is inversely related to sparging efficiency where increases in pressure within a given sparging system lowered sparging efficiency (Wilson, 1986). However, it must be stated that these conclusions are only based on the work of Wilson (1986), where very little experimental details were given and performed under commercial conditions, thus requiring confirmation under more controlled experimental conditions.

Additionally, studies found ethanol and residual sugars significantly affect the solubility of dissolved gasses (Joslyn and Supplee, 1949; Agabaliantz, 1963; Liger-Belair, *et al.,* 2012; Lyons *et al.,* 2015). As both ethanol and sugar concentration increases in wine, the solubility of O_2 and CO_2 decreases as this is due to greater osmotic pressure (Joslyn and Supplee, 1949; Agabaliantz, 1963; Lyons *et al.,* 2015).

1.6. Sensory descriptive analysis

Descriptive analysis (DA), provides detailed, qualitative and quantitative information regarding sensory characteristics and it can be used to elucidate even minor differences amongst samples (Lawless & Heymann, 2010). The method is consensus-based and evaluates organoleptic differences between products in relation to the intensities of other products by rating agreed upon descriptors. Throughout product development, DA and similar methods have wide applications, including sensory characterization of products (e.g., treatment effects) (Lawless & Heymann, 2010)

During the initial training, panellists are guided by the panel leader through a series of sessions to identify a succinct list of descriptors, then after the panellists are then trained to determine the intensity of the descriptors across a product set (Lawless & Heymann, 2010). Once the panel has been deemed satisfactorily trained, the panellists are presented with samples in a randomized order, and individually rate the intensity of each descriptor on a scale of 1-100 for each separate product (Lawless & Heymann, 2010). The samples are tasted blindly, and panellists taste each sample from a biological repeat. Up to eight samples are tested in total per analysis session and enforced breaks are taken in between repeats to avoid sensory fatigue. DA has been used before for the sensorial characterisation of a white wine undergoing oxidation (Coetzee *et al.*, 2016). However, a detailed sensorial analyses, using DA, of white wines exposed to different O_2 levels and storage temperatures has not been previously performed.

1.7. Conclusions

The effects of dissolved O_2 and storage temperature on wine quality are critical areas of interest for the wine industry as oxidation and aroma degradation due to elevated temperatures during ageing can lead to the loss of fruity aromas and the development of undesirable oxidative and ageing aromas. By studying the effects of various O_2 concentrations found just after bottling, producers will able to have further insight into the effects thereof on antioxidants, colour development, and the chemical and sensory changes over time.

Evaluating wines which are stored in both ideal and less ideal (realistic) conditions during ageing can provide valuable insight into industry representative wine development. The effects of temperature storage in conjunction with increased concentrations of dissolved O_2 has not been studied before. It is unknown which factor, storage temperature or dissolved O_2 concentrations, will have the most significant impact on the colouration and chemical content as well as the sensorial composition of white wines. Studies done at realistic cellaring temperatures and increased temperatures in combination with varying dissolved O_2 concentrations (mimicking commercial settings), needs to be conducted to also investigate possible interactive and amplifying effects.

Naturally, preventing the dissolution of O_2 in the first place would be considered best practice, however in a situation of elevated dissolved O_2 concentrations in wine, the removal of the O_2 using sparging can be an effective tool to prevent oxidation later on. Having a clearer understanding of the effectivity of different sparging protocols and the possible effects of sparging on wine sensory and chemical composition and the kinetics behind the operation can support producers by providing better tools to protect wine quality while applying remedial treatments effective and economically.

1.8. Research aims

The main aims of this study were:

- To determine the chemical and sensory effects of dissolved O_2 in conjunction with different storage temperatures on white wine composition.
- To determine what environmental and operational factors effect sparging efficacy.
- To determine if the sparging process alters white wine chemical composition.

The objectives of this study were:

- To determine what the effects of O_2 on wine chemical and sensory composition.
- To determine the effects of storage temperature have on wine chemical and sensory composition.
- To determine the combined effects of O_2 and storage temperature on wine chemical and sensory composition.
- To develop methodology to accurately add and remove dissolved O_2 from white wine using inert gases under various functional and environmental conditions.
- To determine if wine chemical composition is affected by sparging under various conditions.

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Chapter 2: The effects of dissolved oxygen and storage temperature on white wine composition

2.1 Introduction

The effects of dissolved O_2 and temperature on white wine chemical and sensory composition has been widely studied before; however, most of the studies used accelerated ageing methodologies (AAM) (Simpson, 1978; De la Presa-Owens and Noble, 1997; Gonzalez *et al.,* 2006; Kallithraka *et al.,* 2009; Loscos *et al.,* 2010; Maury *et al.,* 2010; Cejudo-Bastante *et al.,* 2013) and storage temperatures were higher (>40°C) compared to storage temperatures normally used (15ºC-21ºC) (Robinson *et al.,* 2010; Cejudo-Bastante *et al.,* 2013; Pereira *et al.,* 2014). Using AAM has yielded insightful results, improving the understanding of the effects of ageing on volatile compounds (Loscos *et al.,* 2010; Hopfer *et al.,* 2012; Makhotkina *et al.,* 2012; Cejudo-Bastante *et al.,* 2013; Ugliano *et al.,* 2013), the degradation of flavanols (Wirth, 2010; Arapitsas, 2014; Scrimgeour, 2015), the hydrolysis of esters (Simpson, 1978; Wirth *et al.,* 2010; Hopfer *et al.,* 2012; Makhotkina *et al.,* 2012; Cejudo-Bastante *et al.,* 2013; Scrimgeour *et al.,* 2015) and the effects on the sensory characteristics of wine (De la Presa-Owens and Noble, 1997; Hopfer *et al.,* 2012; Makhotkina *et al.,* 2012; Makhotkina & Kilmartin, 2012; Ugliano, 2013).

The results from AAM is probably not a true reflection of wines aged under realistic or ideal storage conditions. However, the chemical profile of wines put through AAM could be useful in identifying improper handling and storage of wines (Robinson *et al.*, 2010, Cejudo-Bastante *et al.* 2013, Pereira *et al.* 2014). Due to increased storage temperatures in AAM studies, the results from these experiments might not accurately represent the ageing process in a realistic ageing environment. At higher storage temperatures, relevant chemical reactions might differ compared to lower ageing temperatures especially considering the Arrhenius activation energy (Peleg *et al.,* 2012; Scrimgeour *et al.,* 2015) for different chemical reactions being reached.

A recent study investigated the impact of recommended storage temperatures (10-15°C) compared to elevated storage temperatures (25-30°C) on the ageing process of South African Sauvignon blanc and Chenin blanc wines (Mafata *et al.,* 2019). Results showed that storage temperature significantly influenced the sensory profiles of wines. Lower temperatures preserved fruity characteristics while elevated temperatures resulted in the development of ‘biscuit’ and ‘butterscotch’ attributes (Mafata *et al.,* 2019). Further research into the effect of ideal storage temperatures (15°C) compared to elevated storage temperatures (25°C) could provide further information regarding optimal storage conditions for the preservation of wine aroma and quality.

Work investigating the effects of dissolved O_2 during ageing on the chemical and sensory profiles of white wines (Simpson, 1978; Cejudo-Bastante *et al.,* 2013; Fracassetti *et al.,* 2013; Ugliano, 2013; Coetzee *et al.,* 2016) showed that increasing concentration of dissolved O_2 contributed to the loss of aroma compounds associated with fruity characteristics while oxidative aroma compounds increased in concentration (Escudero *et al.,* 2002; Ugliano, 2013; Coetzee *et*

al., 2016). However, the effects of dissolved O_2 concentration at bottling in combination with different ageing temperatures on the chemistry and sensory composition of South Africa wines needs further investigation.

In the current study, the effect of varying dissolved O_2 concentrations at bottling (as reported in literature for South African white wines by Van der Merwe, 2013) in combination with bottle ageing temperatures more representative of industry practices on the chemical and sensory composition of South African Sauvignon blanc and Chenin blanc wines were investigated.

2.2 Materials and methods

2.2.1 Oxygen gas and nitrogen gas

Prior to transferring the wine, all transfer lines, bioreactors and sample bottles were flushed with commercial nitrogen (99.8% pure, Afrox, South Africa) to remove O_2 (<0.3 mg/L oxygen). After filling the bioreactors with wine, the wines were sparged with medical grade oxygen (99.8% pure) to increase the dissolved O_2 from 0.3 mg/L to 3 mg/L and 6 mg/L, respectively.

2.2.2 Bioreactor tanks

Three custom-built stainless-steel tanks (Figure 2.1) were designed to hold 65 L of wine. Each bioreactor was fitted with a temperature probe, a pH probe, a cooling jacket, a diffusion stone connected to a gas inlet, an automated homogenising mixer and an optical oxygen sensor. The tanks were sealed with a rubber gasket fitted to a stainless-steel lid. An automated pressure release valve from Alicat (Duivan, Netherlands) was fitted to each lid to manage internal pressure during sparging operations. The automated homogenising mixer in each bioreactor operated at a rate of 45 rounds per minute (rpm).

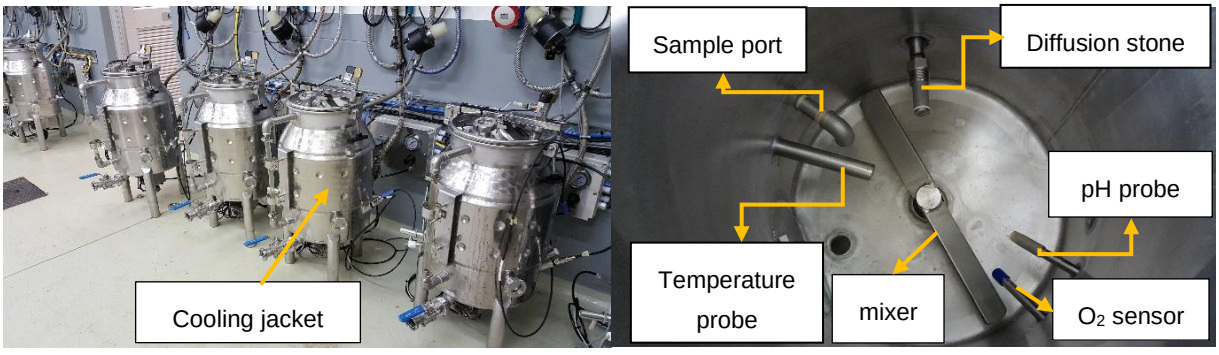

Figure 2.1 Exterior and the interior of bioreactors.

2.2.3. Vinification

Sauvignon blanc and Chenin blanc grapes (vintage 2018) were harvested at 22.3 and 23.1 bawling from the Stellenbosch region and transported to the Department of Viticulture and Oenology experimental cellar at Stellenbosch University (Stellenbosch, South Africa). For each cultivar, 500 kg of grapes were stored in a temperature-controlled room at 4°C until acclimatised.

The grapes were destemmed and crushed, then pressed (up to 1.5 bar) into 300 L stainless steel tanks which were previously flushed with CO_2 to remove O_2. The temperature of the stainless-steel tanks was maintained at 10°C. Forty mg/L of SO_2 and 6 g/hL Lafazym®CL (Laffort, Bordeaux, France) was added to each tank after filling. The juice was settled for 24 hours after which it was racked off the lees. After racking, the juice was inoculated with 30 g/hL *Saccharomyces cerevisiae* VIN 7 (Anchor Technologies, South Africa) yeast and 35 g/hL GoFerm®OMRI (Lallemand, Cape Town, South Africa). Fermentation temperature was maintained at 15°C and the progress was monitored by measuring the specific gravity using a hydrometer. Once fermentation was complete, the wines were racked off the lees and 50 mg/L of SO_2 was added to the wines. The wines were clarified with 75 g/hL bentonite and tartrate stabilized using CELSTAB® (Laffort, Bordeaux, France). After clarification and stabilization, the wines were stored in the 300 L stainless-steel tanks until further treatment.

2.2.4 Oxygen and temperature treatments and sampling

Prior to further processing, the free SO_2 concentration in the Sauvignon blanc and the Chenin blanc wines in the 300 L stainless-steel tanks was increased to 35 mg/L. The wine was then transferred into nine bioreactors previously filled with N_2. Dissolved O_2 was measured before O_2 additions and found to be below 0.3 mg/L in both wines. Measurement confirmed minimal O_2 pickup (<0.3 mg/L) during the transfer (results not shown). The dissolved O_2 concentration was adjusted to 0, 3 or 6 mg/L respectively in triplicate (three bioreactors each) for the different treatments by sparging the wine with pure O_2 gas. This initial process was carried out for the control and O_2 treatments wines. Thirty five litres of wine were thus transferred into the three bioreactors and bottled the same as the control with no O_2 addition. This process was separately repeated for both the 3 and 6 mg/L O_2 treatments in triplicate. Bottling into 750 mL glass bottles commenced by siphoning the wine from the sampling port. No headspace remained in the bottle after filling with wine and the bottles were sealed with Saranex lined screw caps. While filling the bottles from the bioreactors, a constant stream of N_2 gas was applied to the surface of the wine to protect the wine from oxidation.

While bottling, the dissolved O_2 concentration were measured (section 2.2.3.5) in the first, middle, and last bottles of each repeat (total number of bottles per dissolved O_2 repeat: 20 bottles) to ensure no additional O_2 pickup took place. One bottle from each repeat was collected and placed in -4°C overnight for the analyses of free and total SO_2 and colour the next day. Further sampling was done by filling small containers from the initial sampling bottle, bottling (0 months) where after these were stored at -20°C for later analyses of glutathione, varietal thiols, esters, acids and higher alcohols. Prior to sampling and bottling, N_2 gas was used to remove O_2 from the 750 mL bottles and sample containers. No headspace was present in the 750 mL sample bottles after sealing. Sampling and analyses took place again at six and twleve months after bottling. Ten bottles of each dissolved O_2 treatment repeat were stored in either 15°C or 25°C temperature-

controlled rooms. The dissolved O_2 was measured daily for one week after bottling, thereafter weekly for four weeks and then monthly for eleven months.

This process was completed separately for the Sauvignon and Chenin blanc wines.

2.2.5 Chemical analysis

2.2.5.a Free and total sulphur dioxide

Free and total SO_2 were determined by titration (Ripper method) as described in the OIV method: OIV-MA-AS323-04B: R2009 using the Metrohm 862 Compact Tritrosampler (program version 5.862.0024) (Herisau Switzerland).

2.2.5.b Colour analysis

Colour analysis was conducted using a Thermo Fisher Scientific Multiscan Go spectrophotometer (Vantaa, Finland) coupled with a computer equipped with Skanit RE (version 5.0) software. Spectrophotometer measurements were standardized to a 0.2 mL cell. Yellow/brown colour (420 nm) was measured as an indicator of oxidative browning (Singleton, 1976). Samples were measured in triplicate.

2.2.5.c Glutathione

The quantification of reduced glutathione was carried out by ultra-pressure liquid chromatography (UPLC) with a UV detector, as described by Fracassetti *et al.,* 2011. Sample preparation required an ascorbic acid (500 mg/L) and SO_2 (1000 mg/L) addition to 1 mL wine. After, a short centrifugation (10 000 rpm for five minutes) was carried out after which derivatisation was done using p-benzoquinone before analyses on the UPLC.

2.2.5.d Varietal thiols

Two varietal thiols, 3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA), were analysed according to the method published by Coetzee *et al.,* (2018). The method uses a liquid-liquid extraction, followed by ethyl-propiolate derivatization and concentration of the samples before injecting into the gas chromatography-mass spectrometer (GC-MS/MS).

2.2.5.e Major volatiles (ester, acid and alcohol) analysis

Major volatiles consisting of esters, fatty acids and alcohols, were analysed by Gas Chromatography Flame Ionization Detector (GC-FID) using a high-throughput in-house method. The sample preparation consists of the extraction of a 5 mL sample (with 100 µL 0.5 mg/L 4-methyl-2-pentanol as internal standard) with 1 mL diethyl ether (sonicated for 5 minutes). The extract is centrifuged for 3 minutes at 4000 rpm, and the subsequent supernatant is dehydrated with Na_2SO_4 (Merck, 99%) before injecting in duplicate. Details of the method validation are described in Louw, 2007.

2.2.6 Oxygen

Oxygen concentrations in the bioreactors were measured with the PreSens Electro-Optical Module for Oxygen (EOMO) (PreSens GmbH, Regensburg, Germany). The EOMO measurement probe was placed in the bioreactor tanks for the measurement of atmospheric O_2 concentrations before wine transfers. After wine transfers, the EOMO was submerged into the wine for the measurement of dissolved O_2 in mg/L.

Dissolved O_2 in the bottled wine was measured with the NomaSense O2 P300 oxygen meter (Normacorc, Thimister, Germany) coupled with a Pst3 fibreoptic sensor, digital temperature sensor and n2.0.1.1. firmware. The measurement range for the Pst3 oxygen sensors given by the manufacturer was 0-22 mg/L for dissolved O_2 and 0-500 hPa for gaseous and dissolved O_2.

Prior to bottling, O_2 measurements were performed in empty bottles filled with O_2 or CO_2 or ambient air and the presence and/or absence of O_2 was confirmed. The sampling process was also validated by measuring the dissolved O_2 of a selection of samples after the sampling process. The results confirmed the efficiency of the sampling procedure in preventing O_2 dissolution (results not shown).

2.2.7 Descriptive analysis (DA)

Sensory descriptive analysis was conducted after six and twelve months of bottle ageing for both the Sauvignon blanc and Chenin blanc wines using a panel of eight female judges between the ages of 32 and 64 (Lawless & Heymann, 2010). The analysis was conducted at the sensory laboratory at Stellenbosch University's Department of Viticulture and Oenology which is a light- and temperature-controlled environment.

2.2.7.a Training

Panellists attended six two-hour sessions. They were trained using the consensus descriptive analysis method (Lawless & Heymann, 2010). During the first two training sessions, the panel generated terms to describe the aroma for the set of wines (Addendum Table 2A). In training sessions three and four, the panel was presented with standards for the attributes generated for further training and identifying purposes (Addendum Table 2A). During the final training sessions, the panel was trained to reach consensus on the intensity ratings on a scale of 1-100 for each attribute.

2.2.7.b Sensory Analysis

The evaluation of each repeat was performed in triplicate. Sensory analyses were performed in individual booths and panellists were presented with 20 mL wine samples in a randomised order. The wines were evaluated in black International Standards Organisation tasting glasses marked with unique three-digit codes. Panellists were asked to evaluate the samples in the presented

order from left to right and then rate the intensity on a scale of 1-100 for each attribute. Data was captured in Compusense® Five program (Compusense Inc., Guelph, Canada).

2.2.8 Statistical analyses

Statistica (data analysis software system) version 13.5.0.17 from TIBCO Software Inc. (Palo Alto, California, United Statas of America) was used for all statistical analysis. Categorical factors were analysed with one-way analysis of variance (ANOVA) with a significance threshold of $\alpha=0.05$. The Bonferroni post-hoc test was applied for all chemical analysis. A PCA biplot was used to show the relationship between the loadings and scores plot. A mixed modal analysis of variance was used to analyse sensory data from the six and twelve month DA sessions. Random effects in the model were the judge, judge*temperature, judge*O_2 and judge*time. The fixed effects were a full factorial analysis of temperature, O_2 and time. Degrees of freedom was calculated using the Kenward-Rogers method. The Fisher LSD post-hoc test was used in the six and twelve month sensory analysis. Multiple factor analysis was used to evaluate the results of biological repeats which were based on the combination of chemical (Free and total SO2, glutathione, colour, thiols, and major volatiles) and sensory descriptors (Table 2A).

2.3 Results and discussion

2.3.1 Dissolved oxygen concentrations across time.

The dissolved O_2 concentrations fell rapidly in both the 3 mg/L and the 6 mg/L O_2 treatments of both cultivars (Figure 2.2 and 2.3). These findings are similar to previously reported results (Fracassetti *et al.,* 2013; Waterhouse *et al.*, 2016).

Wines stored at higher temperatures experienced a faster decrease in dissolved O_2 compared to similar O_2 treatments stored at lower temperatures. The fact that samples stored at higher temperatures had faster rates of O_2 consumption is supported by the Arrhenius activation energy principle which states that chemical reactions in food products increases by a certain factor (depending on the compounds involved) for every 10°C temperature increase (Peleg *et al.,* 2012; Arapitsas *et al.,* 2014; Scrimgeour *et al.,* 2015). Since dissolved O_2 in the high temperature wines reacts at much higher rate, it follows then (based on Arrhenius activation energy principle) that oxidative and hydrolysis reactions involving other wine compounds would also be occurring at accelerated rates.

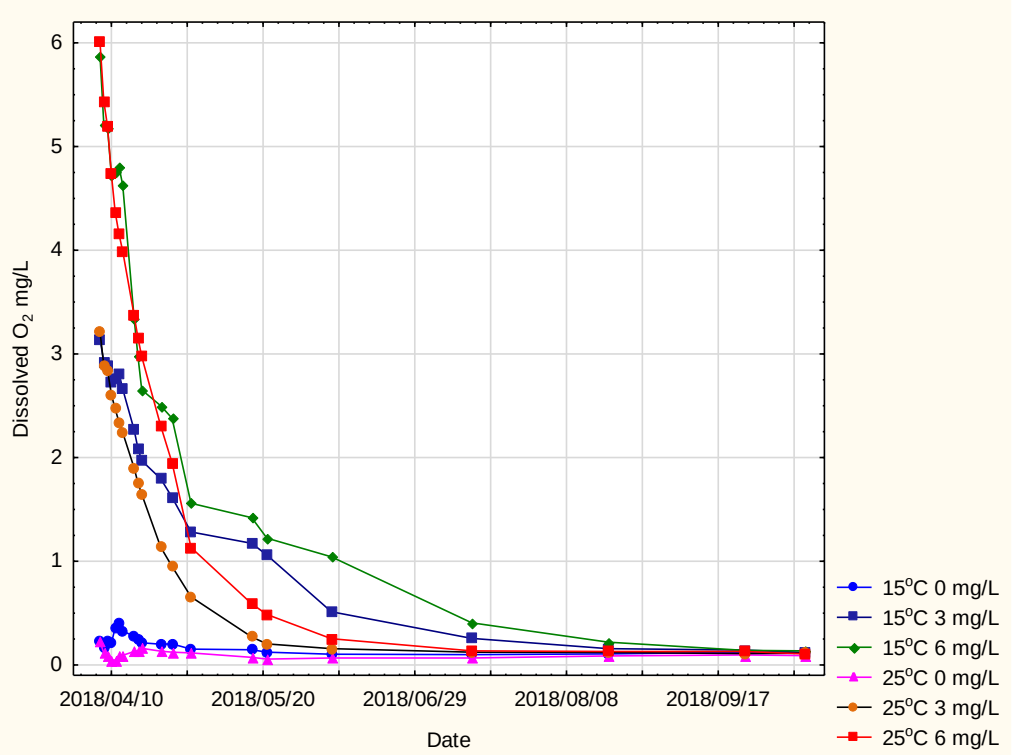

Figure 2.2 Average dissolved O_2 concentrations(mg/L) in the Sauvignon blanc experimental treatments across twelve months. Bottling date is 2018/04/08.

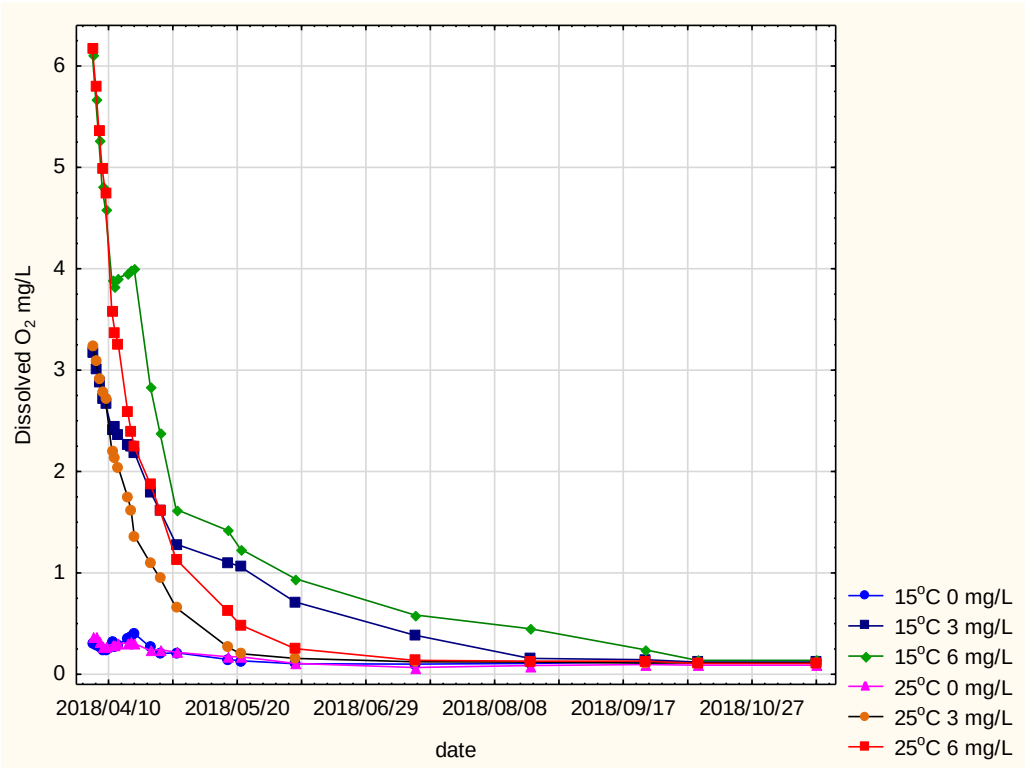

Figure 2.3 Average dissolved O_2 concentrations(mg/L) in the Chenin blanc experimental treatments across twelve months. Bottling date is 2018/04/09.

2.3.2 Initial chemical analyses

Tables 2.1a-2.1c and Tables 2.2a-2.2c contain the results of the chemical analysis for the Sauvignon blanc and Chenin blanc wines at the initial 0 month (a), 6 months (b) and twelve

months (c) sample periods. The concentration of compounds for each cultivar were found within previously reported normal ranges for South African Sauvignon blanc and Chenin blanc wines (Louw *et al.,* 2010; Coetzee & du Toit, 2012; Aleixandre-Tudo *et al.,* 2015; Coetzee *et al.,* 2016; Wilson, 2017).

Significant differences were found only for a few compounds for the 0 month analyses (Tables 2.2a and 2.3a). Significant differences between SO_2 concentration (Chenin blanc) and certain fatty acids (Sauvignon blanc) were seen between the O_2 treatments concentrations for the Chenin blanc wines only, but these were still relatively small .The results obtained after six months and twelve months' storage are discussed in sections 2.3.3 – 2.3.8.

Table 2.1a The initial chemical analysis of the Sauvignon blanc wine (0 months). Letters 'a' and 'b' indicate significant differences between samples. Lines without letters indicates no significant difference between treatments.

Initial Analysis		15°C_0 mg/L	15°C_3 mg/L	15°C_6 mg/L	25°C_0 mg/L	25°C_3 mg/L	25°C_6 mg/L
Antioxidants	unit						
Free sulphur dioxide	mg/L	28.00 ± 0.33	28.00 ± 0.33	28.00 ± 0.33	28.00 ± 0.33	28.00 ± 0.33	28.00 ± 0.33
Total sulphur dioxide	mg/L	102.00 ± 0.88	101.00 ± 0.33	97.00 ± 1.20	102.00 ± 0.88	101.00 ± 0.33	97.00 ± 1.20
Glutathione	mg/L	20.44 ± 0.46	20.57 ± 0.38	19.90 ± 0.52	20.44 ± 0.46	20.57 ± 0.38	19.90 ± 0.52
Spectroscopy							
Brown/yellow colour	AU	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Thiols							
3-mercaptohexyl acetate	ng/L	89.00 ± 0.27	88.00 ± 0.83	84.00 ± 12.13	89.00 ± 0.27	88.00 ± 0.83	84.00 ± 12.13
3-mercaptohexan-1-ol	ng/L	207.00 ± 16.12	197.00 ± 5.03	207.00 ± 8.21	207.00 ± 16.12	197.00 ± 5.03	207.00 ± 8.21
Esters							
Isoamyl Acetate	mg/L	5.26 ± 0.04	5.28 ± 0.12	6.12 ± 0.41	5.26 ± 0.04	5.28 ± 0.12	6.12 ± 0.41
2-Phenyl acetate	mg/L	0.44 ± 0.00b	0.51 ± 0.08a	0.51 ± 0.04a	0.44 ± 0.00b	0.51 ± 0.08a	0.51 ± 0.04a
Ethyl Acetate	mg/L	49.22 ± 1.18	45.88 ± 0.97	48.49 ± 0.75	49.22 ± 1.18	45.88 ± 0.97	48.49 ± 0.75
Ethyl Butyrate	mg/L	0.44 ± 0.00	0.43 ± 0.01	0.41 ± 0.04	0.44 ± 0.00	0.43 ± 0.01	0.41 ± 0.04
Ethyl Caprylate	mg/L	0.69 ± 0.01b	0.74 ± 0.05ab	0.95 ± 0.08a	0.69 ± 0.01b	0.74 ± 0.05ab	0.95 ± 0.08a
Ethyl Lactate	mg/L	0.95 ± 0.01a	0.87 ± 0.02b	0.74 ± 0.07b	0.95 ± 0.01a	0.87 ± 0.02b	0.74 ± 0.07b
Ethyl Hexanoate	mg/L	1.31 ± 0.01	1.32 ± 0.05	1.37 ± 0.00	1.31 ± 0.01	1.32 ± 0.05	1.37 ± 0.00
Ethyl-2-Methyl-Propanoat	mg/L	1.59 ± 0.00	1.59 ± 0.00	1.59 ± 0.00	1.59 ± 0.00	1.59 ± 0.00	1.59 ± 0.00
Diethyl Succinate	mg/L	0.09 ± 0.00	0.09 ± 0.00	0.08 ± 0.01	0.09 ± 0.00	0.09 ± 0.00	0.08 ± 0.01
Acids							
Acetic Acid	mg/L	517.17 ± 3.93	475.72 ± 6.84	497.04 ± 17.75	517.17 ± 3.93	475.72 ± 6.84	497.04 ± 17.75
Propionic Acid	mg/L	1.74 ± 0.10	1.63 ± 0.04	2.18 ± 0.31	1.74 ± 0.10	1.63 ± 0.04	2.18 ± 0.31
Isobutyric Acid	mg/L	2.39 ± 0.03b	2.30 ± 0.02b	2.72 ± 0.08a	2.39 ± 0.03b	2.30 ± 0.02b	2.72 ± 0.08a
Butyric Acid	mg/L	1.10 ± 0.02	1.05 ± 0.01	1.10 ± 0.07	1.10 ± 0.02	1.05 ± 0.01	1.10 ± 0.07
Isovaleric Acid	mg/L	1.77 ± 0.03	1.74 ± 0.04	1.88 ± 0.00	1.77 ± 0.03	1.74 ± 0.04	1.88 ± 0.00
Hexanoic Acid	mg/L	3.55 ± 0.06b	3.54 ± 0.14b	4.06 ± 0.03a	3.55 ± 0.06b	3.54 ± 0.14b	4.06 ± 0.03a
Octanoic Acid	mg/L	4.04 ± 0.08b	4.07 ± 0.23b	4.98 ± 0.17a	4.04 ± 0.08b	4.07 ± 0.23b	4.98 ± 0.17a
Decanoic Acid	mg/L	1.38 ± 0.02	1.39 ± 0.07	1.61 ± 0.01	1.38 ± 0.02	1.39 ± 0.07	1.61 ± 0.01
Alcohols							
Methanol	mg/L	51.09 ± 1.59	46.67 ± 1.34	46.73 ± 0.72	51.09 ± 1.59	46.67 ± 1.34	46.73 ± 0.72
Propanol	mg/L	29.13 ± 0.31	26.95 ± 0.46	24.98 ± 0.69	29.13 ± 0.31	26.95 ± 0.46	24.98 ± 0.69
Pentanol	mg/L	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
Butanol	mg/L	0.68 ± 0.01	0.66 ± 0.01	0.78 ± 0.08	0.68 ± 0.01	0.66 ± 0.01	0.78 ± 0.08
Isobutanol	mg/L	48.88 ± 0.77	46.16 ± 0.43	44.35 ± 1.83	48.88 ± 0.77	46.16 ± 0.43	44.35 ± 1.83
Isoamyl Alcohol	mg/L	305.01 ± 4.82	295.81 ± 2.60	293.28 ± 10.04	305.01 ± 4.82	295.81 ± 2.60	293.28 ± 10.04
Phenyl ethanol	mg/L	37.03 ± 0.45	36.05 ± 0.70	48.54 ± 3.88	37.03 ± 0.45	36.05 ± 0.70	48.54 ± 3.88

Table 2.1b The six month chemical analysis of the Sauvignon blanc wine. Letters ‘a’, ‘b’ ‘c’ and ‘d’ indicate significant differences between samples . Lines without letters indicates no significant difference between treatments.

Six month Analysis		15°C_0 mg/L	15°C_3 mg/L	15°C_6 mg/L	25°C_0 mg/L	25°C_3 mg/L	25°C_6 mg/L
Antioxidants							
Free sulphur dioxide	mg/L	27.00 ± 0.33a	23.00 ± 0.33b	20.00 ± 0.33c	24.00 ± 0.33b	21.00 ± 0.58c	18.00 ± 0.33d
Total sulphur dioxide	mg/L	89.00 ± 1.20a	83.00 ± 0.88bc	75.00 ± 0.88d	86.00 ± 1.20ab	79.00 ± 1.00c	74.00 ± 0.58d
Glutathione	mg/L	10.63 ± 0.69a	8.16 ± 1.49ab	6.73 1.11bc	3.36 ± 0.26d	4.56 ± 0.81cd	2.38 ± 0.80d
Spectroscopy							
Brown/yellow colour	AU	0.07 ± 0.00b	0.07 ± 0.00a	0.07 ± 0.00ab	0.07 ± 0.00a	0.07 ± 0.00a	0.07 ± 0.00a
Thiols							
3-mercaptohexyl acetate	ng/L	48.00 ± 2.70	50.00 ± 0.75	50.00 ± 1.22	44.00 ± 0.45	43.00 ± 2.59	46.00 ± 1.05
3-mercaptohexan-1-ol	ng/L	450.00 ± 42.61b	502.00 ± 11.30ab	497.00 ± 36.60ab	529.00 ± 16.42ab	613.00 ± 26.78a	549.00 ± 30.92ab
Esters							
Isoamyl Acetate	mg/L	4.07 ± 0.02	4.07 ± 0.02	4.07 ± 0.03	3.96 ± 0.33	3.65 ± 0.01	3.72 ± 0.03
2-Phenyl acetate	mg/L	0.24 ± 0.05	0.28 ± 0.01	0.28 ± 0.01	0.23 ± 0.02	0.22 ± 0.00	0.23 ± 0.01
Ethyl Acetate	mg/L	57.11 ± 0.53b	56.50 ± 0.90b	54.93 ± 1.87b	65.40 ± 1.13a	63.98 ± 1.27a	64.33 ± 1.11a
Ethyl Butyrate	mg/L	0.29 ± 0.13	0.42 ± 0.00	0.42 ± 0.01	0.47 ± 0.03	0.44 ± 0.00	0.46 ± 0.01
Ethyl Caprylate	mg/L	0.55 ± 0.01	0.54 ± 0.03	0.54 ± 0.01	0.55 ± 0.02	0.56 ± 0.02	0.62 ± 0.02
Ethyl Lactate	mg/L	16.05 ± 0.049b	16.59 ± 0.37b	16.71 ± 0.56b	24.04 ± 0.69a	24.04 ± 0.40a	24.38 ± 0.21a
Ethyl Hexanoate	mg/L	1.17 ± 0.01	1.17 ± 0.01	1.19 ± 0.02	1.26 ± 0.07	1.22 ± 0.01	1.28 ± 0.03
Ethyl-2-Methyl-Propanoat	mg/L	1.70 ± 0.01	1.69 ± 0.00	1.69 ± 0.00	1.96 ± 0.16	1.79 ± 0.01	1.81 ± 0.01
Diethyl Succinate	mg/L	1.33 ± 0.03b	1.34 ± 0.05b	1.38 ± 0.00b	4.14 ± 0.11a	4.11 ± 0.10a	4.39 ± 0.16a
Acids							
Acetic Acid	mg/L	422.71 ± 4.91	449.22 ± 2.16	411.71 ± 25.20	463.38 ± 14.68	461.05 ± 7.31	462.58 ± 8.26
Propionic Acid	mg/L	1.49 ± 0.03ab	1.44 ± 0.01b	1.42 ± 0.04b	1.62 ± 0.04a	1.67 ± 0.06a	1.56 ± 0.02a
Isobutyric Acid	mg/L	1.92 ± 0.03ab	1.97 ± 0.05b	1.89 ± 0.03b	2.14 ± 0.03ab	2.11 ± 0.04a	2.17 ± 0.02ab
Butyric Acid	mg/L	0.94 ± 0.02ab	0.95 ± 0.02b	0.92 ± 0.02b	1.07 ± 0.02a	1.07 ± 0.01a	1.07 ± 0.00a
Isovaleric Acid	mg/L	1.48 ± 0.03b	1.48 ± 0.05b	1.47 ± 0.02b	1.61 ± 0.04a	1.60 ± 0.03a	1.68 ± 0.04a
Hexanoic Acid	mg/L	3.09 ± 0.06c	3.11 ± 0.14c	3.13 ± 0.10c	3.39 ± 0.10b	3.37 ± 0.08b	3.68 ± 0.19a
Octanoic Acid	mg/L	3.65 ± 0.07c	3.69 ± 0.14c	3.62 ± 0.15c	4.00 ± 0.14a	3.91 ± 0.09ab	4.37 ± 0.26a
Decanoic Acid	mg/L	1.40 ± 0.04c	1.47 ± 0.05bc	1.28 ± 0.04c	1.65 ± 0.0a	1.56 ± 0.0ab	1.78 ± 0.07a
Alcohols							
Methanol	mg/L	39.69 ± 1.01	44.55 ± 1.79	39.88 ± 0.87	45.01 ± 1.68	43.80 ± 0.51	46.23 ± 2.22
Propanol	mg/L	24.91 ± 0.14ab	26.10 ± 0.31ab	24.29 ± 1.20b	27.72 ± 0.56a	27.61 ± 0.31a	27.59 ± 0.51a
Pentanol	mg/L	0.07 ± 0.00bc	0.07 ± 0.00bc	0.07 ± 0.00c	0.07 ± 0.00ab	0.07 ± 0.00ab	0.07 ± 0.00a
Butanol	mg/L	0.60 ± 0.00b	0.62 ± 0.00ab	0.59 ± 0.02b	0.67 ± 0.01a	0.67 ± 0.01a	0.67 ± 0.00a
Isobutanol	mg/L	41.94 ± 0.39c	42.63 ± 0.26bc	40.76 ± 1.55c	46.67 ± 0.83a	46.44 ± 0.56ab	46.34 ± 0.16ab
Isoamyl Alcohol	mg/L	260.13 ± 4.19b	261.24 ± 4.77b	256.89 ± 2.21b	289.60 ± 6.02a	289.16 ± 2.5a	299.52 ± 3.36a
Phenyl ethanol	mg/L	35.09 ± 0.57b	37.15 ± 1.22b	34.32 ± 0.24b	38.95 ± 0.82a	38.98 ± 0.36a	39.22 ± 0.61a

Table 2.1c The twelve month chemical analysis of the Sauvignon blanc wine. Letters 'a', 'b' 'c' and 'd' indicate significant differences between samples. Lines without letters indicates no significant difference between treatments.

Twelve month Analysis		15℃_0mg/L	15℃_3mg/L	15℃_6 mg/L	25℃_0 mg/L	25℃_3 mg/L	25℃_6 mg/L
Antioxidants							
Free sulphur dioxide	mg/L	24.00 ± 0.33a	21.00 ± 0.33ab	15.00 ± 0.57cd	22.00 ± 0.58	17.00 ± 0.57c	14.00 ± 0.33d
Total sulphur dioxide	mg/L	84.00 ± 1.15a	76.00 ± 1.20b	70.00 ± 0.67c	76.00 ± 1.20	68.00 ± 1.00c	61.00 ± 0.67d
Glutathione	mg/L	0.58 ± 0.02a	0.58 ± 0.19a	0.39 ± 0.05a	0.09 ± 0.00b	0.09 ± 0.01b	0.08 ± 0.01b
Spectroscopy							
Brown/yellow colour	AU	0.07 ± 0.00b	0.07 ± 0.00b	0.07 ± 0.00b	0.07 ± 0.00a	0.07 ± 0.00a	0.08 ± 0.01a
Thiols							
3-mercaptohexyl acetate	ng/L	29.00 ± 1.38ab	30.00 ± 2.97a	24.00 ± 0.83ab	30.00 ± 0.10a	22.00 ± 1.09b	24.00 ± 0.32ab
3-mercaptohexan-1-ol	ng/L	459.00 ± 13.29ab	478.00 ± 28.62ab	413.00 ± 11.70b	497.00 ± 14.72ab	550.00 ± 22.11a	512.00 ± 18.20a
Esters							
Isoamyl Acetate	mg/L	4.10 ± 0.01b	4.25 ± 0.07ab	4.33 ± 0.01a	3.79 ± 0.01c	3.86 ± 0.01c	3.90 ± 0.00c
2-Phenyl acetate	mg/L	0.27 ± 0.01ab	0.31 ± 0.04a	0.35 ± 0.00a	0.17 ± 0.00c	0.18 ± 0.00c	0.20 ± 0.00c
Ethyl Acetate	mg/L	53.40 ± 1.16	60.00 ± 5.82	57.58 ± 2.14	58.88 ± 6.35	64.29 ± 12.94	67.11 ± 10.63
Ethyl Butyrate	mg/L	0.43 ± 0.01c	0.45 ± 0.01c	0.48 ± 0.00b	0.46 ± 0.00c	0.48 ± 0.00bc	0.51 ± 0.012a
Ethyl Caprylate	mg/L	0.07 ± 0.01b	0.08 ± 0.01a	0.09 ± 0.00a	0.07 ± 0.00b	0.07 ± 0.00ab	0.08 ± 0.00b
Ethyl Lactate	mg/L	10.52 ± 0.02b	10.45 ± 1.53b	9.58 ± 0.50b	15.10 ± 0.17a	16.86 ± 0.32a	16.54 ± 0.69a
Ethyl Hexanoate	mg/L	0.96 ± 0.01c	0.99 ± 0.02c	1.02 ± 0.00b	1.01 ± 0.02bc	1.06 ± 0.00ab	1.10 ± 0.01a
Ethyl-2-Methyl-Propanoat	mg/L	1.80 ± 0.00c	1.82 ± 0.01c	1.84 ± 0.00c	1.92 ± 0.00b	1.98 ± 0.01ab	2.00 ± 0.03a
Diethyl Succinate	mg/L	1.74 ± 0.00b	1.90 ± 0.10b	2.03 ± 0.02b	5.23 ± 0.23a	5.82 ± 0.07a	6.59 ± 0.25a
Acids							
Acetic Acid	mg/L	441.15 ± 12.07	458.98 ± 38.84	414.38 ± 7.86	418.72 ± 6.11	406.33 ± 58.61	449.44 ± 14.99
Propionic Acid	mg/L	1.48 ± 0.06b	1.76 ± 0.19ab	1.62 ± 0.10b	1.86 ± 0.04ab	1.94 ± 0.16a	2.03 ± 0.06a
Isobutyric Acid	mg/L	1.92 ± 0.00	1.99 ± 0.11	2.04 ± 0.04	2.01 ± 0.05	2.05 ± 0.12	2.25 ± 0.06
Butyric Acid	mg/L	1.07 ± 0.00a	0.79 ± 0.03b	0.79 ± 0.00b	0.79 ± 0.02b	0.87 ± 0.04a	0.87 ± 0.03a
Isovaleric Acid	mg/L	0.99 ± 0.01c	1.07 ± 0.06bc	1.15 ± 0.02b	1.13 ± 0.06b	1.24 ± 0.012ab	1.38 ± 0.04a
Hexanoic Acid	mg/L	2.07 ± 0.01c	2.35 ± 0.17bc	2.57 ± 0.03b	2.62 ± 0.13b	2.96 ± 0.04ab	3.34 ± 0.09a
Octanoic Acid	mg/L	2.73 ± 0.03c	3.19 ± 0.36bc	3.69 ± 0.05b	3.93 ± 0.19b	4.45 ± 0.07ab	5.03 ± 0.08a
Decanoic Acid	mg/L	0.92 ± 0.03c	0.97 ± 0.14c	1.17 ± 0.05bc	1.41 ± 0.06b	1.57 ± 0.00ab	1.59 ± 0.07a
Alcohols							
Methanol	mg/L	47.67 ± 2.21	53.71 ± 5.57	48.13 ± 0.90	45.99 ± 1.00	50.52 ± 7.10	53.00 ± 6.03
Propanol	mg/L	21.89 ± 0.74	23.42 ± 3.05	21.02 ± 0.69	20.40 ± 1.75	19.96 ± 3.14	21.25 ± 2.12
Pentanol	mg/L	0.28 ± 0.00	0.28 ± 0.01	0.29 ± 0.00	0.28 ± 0.00	0.28 ± 0.01	0.29 ± 0.01
Butanol	mg/L	0.75 ± 0.01	0.80 ± 0.07	0.75 ± 0.03	0.75 ± 0.02	0.75 ± 0.11	0.80 ± 0.05
Isobutanol	mg/L	34.70 ± 1.41	35.65 ± 2.81	33.99 ± 0.93	33.45 ± 0.85	32.98 ± 4.43	35.42 ± 2.09
Isoamyl Alcohol	mg/L	188.09 ± 0.27b	201.43 ± 8.08b	205.52 ± 3.4ab	208.87 ± 5.92ab	216.03 ± 9.77ab	237.83 ± 5.38a
Phenyl ethanol	mg/L	34.81 ± 0.08	35.57 ± 1.55	32.44 ± 0.40	33.84 ± 1.38	32.31 ± 1.58	36.28 ± 1.35

Table 2.2a The initial chemical analysis of the Chenin blanc wine (0 months). Letters 'a' and 'b' indicate significant differences between samples. Lines without letters indicates no significant difference between treatments.

Initial analysis		15°C_0mg/L	15°C_3mg/L	15°C_6mg/L	25°C_0mg/L	25°C_3mg/L	25°C_6mg/L
Antioxidants	unit						
Free sulpher dioxide	mg/L	28.00 ± 0.00a	27.70 ± 0.33ab	27.00 ± 0.00b	28.00 ± 0.00a	27.70 ± 0.33ab	27.00 ± 0.00b
Total sulphur dioxide	mg/L	95.30 ± 1.45a	92.70 ± 1.45ab	89.70 ± 0.88b	95.30 ± 1.45a	92.70 ± 1.45ab	89.70 ± 0.88b
Glutathione	mg/L	16.60 ± 0.54	16.40 ± 0.31	16.00 ± 0.40	16.60 ± 0.54	16.40 ± 0.31	16.00 ± 0.40
Spectroscopy							
Brown/yellow colour	AU	0.09 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.08 ± 0.00
Thiols							
3-mercaptohexyl acetate (3MHA)	ng/L	120.40 ± 1.10	119.30 ± 4.23	125.60 ± 2.19	120.40 ± 1.10	119.30 ± 4.23	125.60 ± 2.19
3-mercaptohexan-1-ol (3MH)	ng/L	199.40 ± 16.35	182.80 ± 5.94	202.20 ± 11.28	199.40 ± 16.35	182.80 ± 5.94	202.20 ± 11.28
Esters							
Isoamyl Acetate	mg/L	4.08 ± 0.02	4.11 ± 0.02	4.13 ± 0.04	4.08 ± 0.02	4.11 ± 0.02	4.13 ± 0.04
Ethyl Acetate	mg/L	66.26 ± 0.81	66.16 ± 5.15	60.74 ± 1.35	66.26 ± 0.81	66.16 ± 5.15	60.74 ± 1.35
Ethyl-2-Methyl-Propanoate	mg/L	1.78 ± 0.01	1.78 ± 0.00	1.78 ± 0.01	1.78 ± 0.01	1.78 ± 0.00	1.78 ± 0.01
Hexyl Acetate	mg/L	0.48 ± 0.00	0.49 ± 0.00	0.47 ± 0.02	0.48 ± 0.00	0.49 ± 0.00	0.47 ± 0.02
Ethyl Hexanoate	mg/L	1.14 ± 0.00	1.16 ± 0.01	1.19 ± 0.02	1.14 ± 0.00	1.16 ± 0.01	1.19 ± 0.02
Ethyl Butyrate	mg/L	0.42 ± 0.00	0.42 ± 0.00	0.43 ± 0.00	0.42 ± 0.00	0.42 ± 0.00	0.43 ± 0.00
Ethyl Lactate	mg/L	20.96 ± 0.44	20.79 ± 1.00	19.33 ± 6.30	19.96 ± 0.44	20.79 ± 1.00	19.33 ± 6.30
Ethyl Caprylate	mg/L	0.55 ± 0.01	0.58 ± 0.02	0.62 ± 0.03	0.55 ± 0.01	0.58 ± 0.02	0.62 ± 0.03
Diethyl Succinate	mg/L	2.58 ± 0.03	2.83 ± 0.04	2.88 ± 0.11	2.58 ± 0.03	2.83 ± 0.04	2.88 ± 0.11
Acids							
Acetic Acid	mg/L	448.72 ± 11.98	464.24 ± 21.16	442.73 ± 10.18	448.72 ± 11.98	464.24 ± 21.16	442.73 ± 10.18
Propionic Acid	mg/L	1.39 ± 0.01b	1.70 ± 0.05a	1.61 ± 0.06ab	1.39 ± 0.01b	1.70 ± 0.05a	1.61 ± 0.06ab
Isobutyric Acid	mg/L	2.35 ± 0.01	2.46 ± 0.06	2.42 ± 0.02	2.35 ± 0.01	2.46 ± 0.06	2.42 ± 0.02
Butyric Acid	mg/L	0.89 ± 0.00	0.94 ± 0.02	0.91 ± 0.01	0.89 ± 0.00	0.94 ± 0.02	0.91 ± 0.01
Isovaleric Acid	mg/L	1.50 ± 0.02	1.56 ± 0.02	1.56 ± 0.04	1.50 ± 0.02	1.56 ± 0.02	1.56 ± 0.04
Hexanoic Acid	mg/L	3.00 ± 0.03	3.18 ± 0.06	3.25 ± 0.16	3.00 ± 0.03	3.18 ± 0.06	3.25 ± 0.16
Octanoic Acid	mg/L	4.02 ± 0.04	4.25 ± 0.13	4.44 ± 0.27a	4.02 ± 0.04	4.25 ± 0.13	4.44 ± 0.27
Decanoic Acid	mg/L	1.58 ± 0.02	1.69 ± 0.07	1.72 ± 0.05	1.58 ± 0.02	1.69 ± 0.07	1.72 ± 0.05
Alcohols							
Acetoin	mg/L	4.47 ± 0.24	4.54 ± 0.09	4.16 ± 0.23	4.47 ± 0.24	4.54 ± 0.09	4.16 ± 0.23
Methanol	mg/L	46.08 ± 1.86	4.51 ± 4.56	43.37 ± 1.10	46.08 ± 1.86	4.51 ± 4.56	43.37 ± 1.10
Butanol	mg/L	0.92 ± 0.01	0.95 ± 0.04	0.92 ± 0.01	0.92 ± 0.01	0.95 ± 0.04	0.92 ± 0.01
Isoamyl Alcohol	mg/L	243.14 ± 2.23	255.01 ± 5.81	249.67 ± 0.76	243.14 ± 2.23	255.01 ± 5.81	249.67 ± 0.76
Isobutanol	mg/L	3.97 ± 0.01	4.12 ± 0.02	38.98 ± 0.04	3.97 ± 0.01	4.12 ± 0.02	38.98 ± 0.04
Hexanol	mg/L	1.31 ± 0.51	1.36 ± 1.53	1.38 ± 1.15	1.31 ± 0.51	1.36 ± 1.53	1.38 ± 1.15
Propanol	mg/L	25.07 ± 0.00	2.48 ± 0.00	23.30 ± 0.00	25.07 ± 0.00	2.48 ± 0.00	23.30 ± 0.00
Pentanol	mg/L	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.09 ± 1.72	0.09 ± 0.00

Table 2.2b The six month chemical analysis of the Chenin blanc wine. Letters 'a', 'b' 'c' and 'd' indicate significant differences between samples. Lines without letters indicates no significant difference between treatments.

Six month analysis		15°C_0mg/L	15°C_3mg/L	15°C_6mg/L	25°C_0 mg/L	25°C_3 mg/L	25°C_6 mg/L
Antioxidants	unit						
Free sulpher dioxide	mg/L	25.70 ± 0.33a	22.70 ± 0.33b	20.30 ± 0.33c	23.30 ± 0.33	20.00 ± 0.58c	18.00 ± 0.00C
Total sulphur dioxide	mg/L	94.30 ± 1.20a	86.00 ± 1.15b	79.00 ± 1.52c	86.00 ± 1.53	73.30 ± 0.88ab	67.00 ± 1.52c
Glutathione	mg/L	8.10 ± 0.08a	8.10 ± 0.51a	8.40 ± 0.26a	3.70 ± 0.35b	3.00 ± 0.38ab	2.30 ± 0.62b
Spectroscopy							
Brown/yellow colour	AU	0.11 ± 0.00b	0.11 ± 0.00b	0.11 ± 0.00b	0.12 ± 0.00a	0.12 ± 0.00a	0.12 ± 0.00a
Thiols							
3-mercaptohexyl acetate (3MHA)	ng/L	72.80 ± 13.07b	85.30 ± 3.28a	73.30 ± 2.42ab	48.00 ± 1.47bc	47.30 ± 1.51c	43.30 ± 3.18c
3-mercaptohexan-1-ol (3MH)	ng/L	639.00 ± 66.27b	689.90 ± 45.70a	693.70 ± 79.88a	715.90 ± 39.5a	704.60 ± 37.06a	750.60 ± 49.38a
Esters							
Isoamyl Acetate	mg/L	3.84 ± 0.017a	3.86 ± 0.02a	3.88 ± 0.04a	3.76 ± 0.04a	3.69 ± 0.13ab	3.78 ± 0.01a
Ethyl Acetate	mg/L	6.23 ± 0.81	6.22 ± 5.15	5.71 ± 1.35	6.31 ± 5.60	6.37 ± 4.52	6.22 ± 0.84
Ethyl-2-Methyl-Propanoate	mg/L	1.67 ± 0.01	1.67 ± 0.00	1.67 ± 0.01	1.66 ± 0.01	1.66 ± 0.02	1.66 ± 0.00
Hexyl Acetate	mg/L	0.46 ± 0.00	0.46 ± 0.00	0.45 ± 0.02	0.45 ± 0.00	0.45 ± 0.01	0.44 ± 0.01
Ethyl Hexanoate	mg/L	1.07 ± 0.00a	1.09 ± 0.01a	1.12 ± 0.02a	1.03 ± 0.03b	1.10 ± 0.03a	1.05 ± 0.00ab
Ethyl Butyrate	mg/L	0.39 ± 0.00	0.40 ± 0.00	0.40 ± 0.00	0.38 ± 0.01	0.40 ± 0.01	0.39 ± 0.00
Ethyl Lactate	mg/L	18.44 ± 0.44	19.54 ± 1.00	18.53 ± 1.06	19.69 ± 3.05	20.61 ± 1.20	18.79 ± 0.07
Ethyl Caprylate	mg/L	0.52 ± 0.01ab	0.55 ± 0.02a	0.58 ± 0.03a	0.45 ± 0.02b	0.52 ± 0.01ab	0.48 ± 0.01b
Diethyl Succinate	mg/L	2.42 ± 0.03c	2.66 ± 0.00b	2.70 ± 0.11b	2.84 ± 0.02b	3.40 ± 0.51ab	4.49 ± 0.03a
Acids							
Acetic Acid	mg/L	421.79 ± 11.98	436.38 ± 21.16	416.17 ± 10.18	462.67 ± 73.23	443.47 ± 2.95	426.70 ± 4.76
Propionic Acid	mg/L	1.31 ± 0.10	1.60 ± 0.05	1.58 ± 0.06	1.40 ± 0.14	1.41 ± 0.17	1.22 ± 0.02
Isobutyric Acid	mg/L	2.21 ± 0.01	2.32 ± 0.06	2.28 ± 0.02	2.31 ± 0.16	2.27 ± 0.13	2.23 ± 0.01
Butyric Acid	mg/L	0.83 ± 0.04b	0.88 ± 0.02ab	0.85 ± 0.01ab	0.89 ± 0.08ab	0.93 ± 0.05a	0.86 ± 0.0ab
Isovaleric Acid	mg/L	1.41 ± 0.02	1.47 ± 0.02	1.46 ± 0.04	1.41 ± 0.05	1.46 ± 0.05	1.39 ± 0.00
Hexanoic Acid	mg/L	2.18 ± 0.38b	2.01 ± 0.58bc	2.35 ± 1.12b	2.41 ± 1.80b	2.44 ± 0.10ab	2.80 ± 0.40a
Octanoic Acid	mg/L	3.78 ± 0.04ab	3.99 ± 0.13ab	4.17 ± 0.26a	3.57 ± 0.20b	3.73 ± 0.05ab	3.69 ± 0.07b
Decanoic Acid	mg/L	1.49 ± 0.03ab	1.59 ± 0.07a	1.65 ± 0.09a	1.40 ± 0.03ab	1.46 ± 0.06ab	1.38 ± 0.06b
Alcohols							
Acetoin	mg/L	4.20 ± 0.24	4.27 ± 0.09	3.91 ± 0.23	4.38 ± 0.61	4.27 ± 0.21	3.84 ± 0.05
Methanol	mg/L	43.32 ± 1.86	42.40 ± 4.56	40.77 ± 1.10	47.41 ± 7.29	41.94 ± 1.81	43.49 ± 1.04
Butanol	mg/L	0.87 ± 0.01	0.90 ± 0.04	0.86 ± 0.01	0.85 ± 0.10	0.79 ± 0.07	0.84 ± 0.01
Isoamyl Alcohol	mg/L	228.55 ± 2.23	239.71 ± 5.81	234.69 ± 0.76	226.37 ± 11.35	244.35 ± 17.05	223.49 ± 1.24
Isobutanol	mg/L	37.35 ± 0.01	38.77 ± 0.02	36.64 ± 0.04	37.89 ± 0.01	39.14 ± 0.17	36.09 ± 0.01
Hexanol	mg/L	1.23 ± 0.51	1.28 ± 1.53	1.30 ± 1.15	1.18 ± 3.41	1.39 ± 1.44	1.20 ± 0.30
Propanol	mg/L	2.36 ± 0.00	2.33 ± 0.00	21.90 ± 0.00	24.11 ± 0.01	23.78 ± 0.00	22.25 ± 0.00
Pentanol	mg/L	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00

Table 2.2c The twelve month chemical analysis of the Chenin blanc wine. Letters 'a', 'b' 'c' and 'd' indicate significant differences between samples. Lines without letters indicates no significant difference between treatment.

Twelve month analysis		15°C_0 mg/L	15°C_3 mg/L	15°C_6 mg/L	25°C_0 mg/L	25°C_3 mg/L	25°C_6 mg/L
Antioxidants	unit						
Free sulpher dioxide	mg/L	24.30 ± 0.33a	4.00 ± 0.33b	17.00 ± 0.57c	20.70 ± 0.66a	16.70 ± 0.33c	13.70 ± 0.33c
Total sulphur dioxide	mg/L	88.30 ± 0.33a	81.00 ± 1.50b	71.00 ± 0.58c	78.70 ± 0.90a	69.00 ± 0.57c	62.00 ± 1.15c
Glutathione	mg/L	1.50 ± 0.02a	1.50 ± 0.10a	1.50 ± 0.08a	0.40 ± 0.01b	0.50 ± 0.12b	0.10 ± 0.01b
Spectroscopy							
Brown/yellow colour	AU	0.13 ± 0.00b	0.13 ± 0.00b	0.14 ± 0.00b	0.16 ± 0.00a	0.16 ± 0.00a	0.16 ± 0.00a
Thiols							
3-mercaptohexyl acetate (3MHA)	ng/L	31.20 ± 3.66ab	36.00 ± 2.13a	40.40 ± 3.64a	32.90 ± 1.40ab	32.20 ± 1.51ab	28.10 ± 3.18b
3-mercaptohexan-1-ol (3MH)	ng/L	565.80 ± 29.00	656.60 ± 40.50	607.40 ± 66.43	649.40 ± 39.05	637.00 ± 30.64	677.30 ± 44.02
Esters							
Isoamyl Acetate	mg/L	3.83 ± 0.14a	3.91 ± 0.15a	3.85 ± 0.04a	3.65 ± 0.05ab	3.59 ± 0.01b	3.61 ± 0.00b
Ethyl Acetate	mg/L	67.48 ± 1.17	56.20 ± 3.02	56.60 ± 4.04	61.73 ± 8.44	66.15 ± 1.40	62.58 ± 5.01
Ethyl-2-Methyl-Propanoate	mg/L	1.85 ± 0.03b	1.79 ± 0.01c	1.83 ± 0.01b	1.88 ± 0.04ab	1.91 ± 0.02a	1.93 ± 0.01a
Hexyl Acetate	mg/L	0.46 ± 0.01	0.47 ± 0.00	0.47 ± 0.00	0.47 ± 0.00	0.49 ± 0.00	0.49 ± 0.00
Ethyl Hexanoate	mg/L	0.99 ± 0.02b	0.97 ± 0.00b	1.02 ± 0.03a	1.01 ± 0.02ab	1.01 ± 0.02ab	1.06 ± 0.00a
Ethyl Butyrate	mg/L	0.39 ± 0.01	0.37 ± 0.01	0.38 ± 0.01	0.38 ± 0.02	0.38 ± 0.01	0.39 ± 0.00
Ethyl Lactate	mg/L	13.86 ± 1.10ab	12.81 ± 0.48b	11.56 ± 0.10b	13.65 ± 2.24ab	16.67 ± 0.85a	12.55 ± 0.87b
Ethyl Caprylate	mg/L	0.07 ± 0.06	0.07 ± 0.05	0.07 ± 0.00	0.06 ± 0.02	0.06 ± 0.00	0.07 ± 0.00
Diethyl Succinate	mg/L	2.59 ± 0.010cd	2.00 ± 0.11d	2.68 ± 0.18cd	3.85 ± 0.74b	4.19 ± 0.40ab	4.94 ± 0.12a
Acids							
Acetic Acid	mg/L	438.90 ± 53.83	427.70 ± 16.75	379.79 ± 15.27	366.60 ± 24.23ab	406.18 ± 9.59a	336.62 ± 12.80b
Propionic Acid	mg/L	1.61 ± 0.06b	1.43 ± 0.07b	1.56 ± 0.09b	1.77 ± 0.12ab	1.95 ± 0.11ab	2.47 ± 0.28a
Isobutyric Acid	mg/L	1.71 ± 0.14	1.66 ± 0.18	1.74 ± 0.11	1.61 ± 0.10	1.61 ± 0.07	1.70 ± 0.03
Butyric Acid	mg/L	0.81 ± 0.02ab	0.73 ± 0.00b	0.81 ± 0.02ab	0.84 ± 0.06ab	0.86 ± 0.03a	0.89 ± 0.00a
Isovaleric Acid	mg/L	1.03 ± 0.08b	0.96 ± 0.05c	1.08 ± 0.056ab	1.10 ± 0.07ab	1.09 ± 0.06ab	1.21 ± 0.02a
Hexanoic Acid	mg/L	1.82 ± 0.24b	1.97 ± 0.09b	2.12 ± 0.21ab	2.19 ± 0.17ab	2.22 ± 0.17a	2.57 ± 0.06a
Octanoic Acid	mg/L	2.93 ± 0.33bc	2.76 ± 0.28c	3.08 ± 0.31b	3.23 ± 0.21ab	3.31 ± 0.26a	3.82 ± 0.10a
Decanoic Acid	mg/L	1.22 ± 0.25ab	1.08 ± 0.05b	1.17 ± 0.07b	1.39 ± 0.10ab	1.39 ± 0.08ab	1.54 ± 0.03a
Alcohols							
Acetoin	mg/L	4.19 ± 0.92	4.35 ± 0.12	4.62 ± 1.04	4.19 ± 1.58	4.35 ± 1.29	4.62 ± 0.81
Methanol	mg/L	51.99 ± 4.33	52.10 ± 0.87	42.12 ± 1.83	42.79 ± 2.23	50.44 ± 1.06	37.48 ± 1.27
Butanol	mg/L	0.61 ± 0.13	0.45 ± 0.02	0.46 ± 0.02	0.48 ± 1.43	0.52 ± 0.00	0.45 ± 0.03
Isoamyl Alcohol	mg/L	208.58 ± 9.82	186.89 ± 2.20	208.98 ± 5.11	217.76 ± 15.28b	227.26 ± 7.32	232.04 ± 4.15
Isobutanol	mg/L	38.28 ± 1.15	32.65 ± 1.04	34.36 ± 1.14	34.94 ± 1.21	37.58 ± 0.12	33.19 ± 0.00
Hexanol	mg/L	1.28 ± 0.01b	1.19 ± 0.09b	1.38 ± 0.08ab	1.51 ± 0.06a	1.38 ± 0.05ab	1.68 ± 0.02a
Propanol	mg/L	25.22 ± 0.01	22.79 ± 0.01	21.37 ± 0.00	20.95 ± 0.00	22.90 ± 0.01	18.53 ± 0.00
Pentanol	mg/L	0.27 ± 0.00	0.27 ± 0.00	0.27 ± 0.00	0.26 ± 0.00	0.27 ± 0.00	0.26 ± 0.00

2.3.3 Free and total sulphur dioxide analysis

The control treatment (0 mg/L O_2) had the highest concentration of free and total SO_2, while the 6 mg/L O_2 treatment resulted in the lowest concentration of SO_2 at both storage temperatures after six and twelve months (Figures 2.4, Figure 2.5, Figure 2.6 and Figure 2.7; Tables 2.1b-2.1c and Tables 2.2b-2.2c).

For the Chenin blanc wines, both the six and twelve months storage the wine stored at 25°C had lower free and total SO_2 concentrations compared to the 15°C treatments (Addendum 2A); however, this result was only significant for the Chenin blanc wine. This indicates that both temperature (in some cases) and dissolved O_2 had significant effects on free SO_2 concentration, but dissolved O_2 had a greater effect in reducing the initial free and total SO_2 concentrations in

both wines (Figures 2.4, Figure 2.5, Figure 2.6 and Figure 2.7). This is in line with previous results where both elevated storage temperatures and dissolved O_2 concentrations lowered the free and total SO_2 content in wine (Blake *et al.,* 2010; Fracassetti *et al.,* 2013; Morozova *et al.,* 2014; Comuzzo *et al.,* 2015; Arapitsas *et al.,* 2014; Arapitsas *et al.*, 2016; Coetzee *et al.,* 2016; Benucci, 2019). It is known that as concentrations of dissolved O_2 increase, the concentrations of peroxide and *o*-quinones (through the Fenton reaction) will also increase. These compounds primarily react with bisulphite, therefore lowering the free SO_2 present in wine (Fenton, 1984; du Toit *et al.,* 2006; Danilewicz, 2007; Arapitsas *et al.,* 2016).

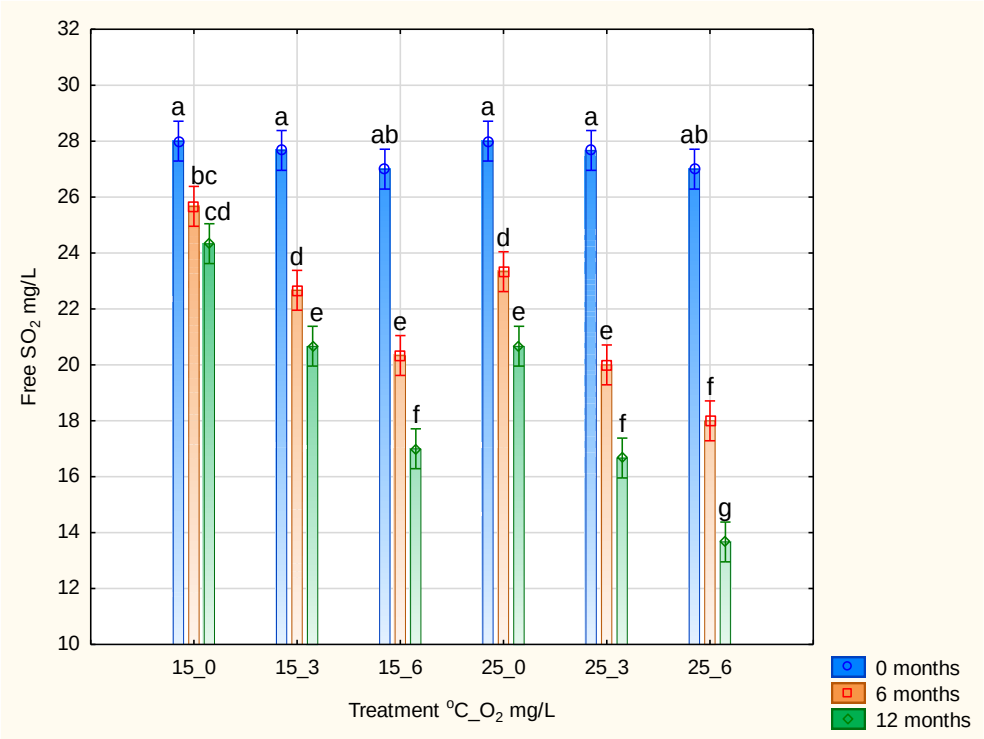

Figure 2.4. Free SO_2 concentrations of the Sauvignon blanc wine comparing the effects of O_2 and temperature across time.

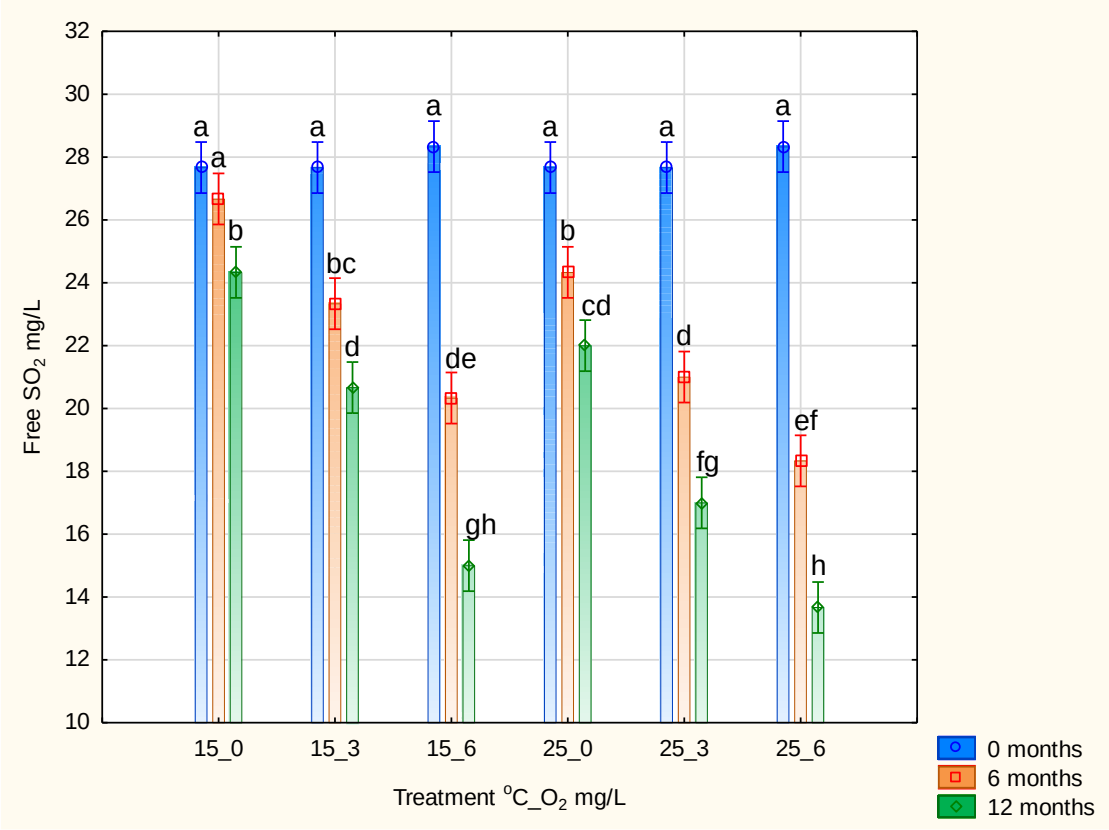

Figure 2.5 Free SO_2 concentrations of the Chenin blanc wine comparing the effects of O_2 and temperature across time.

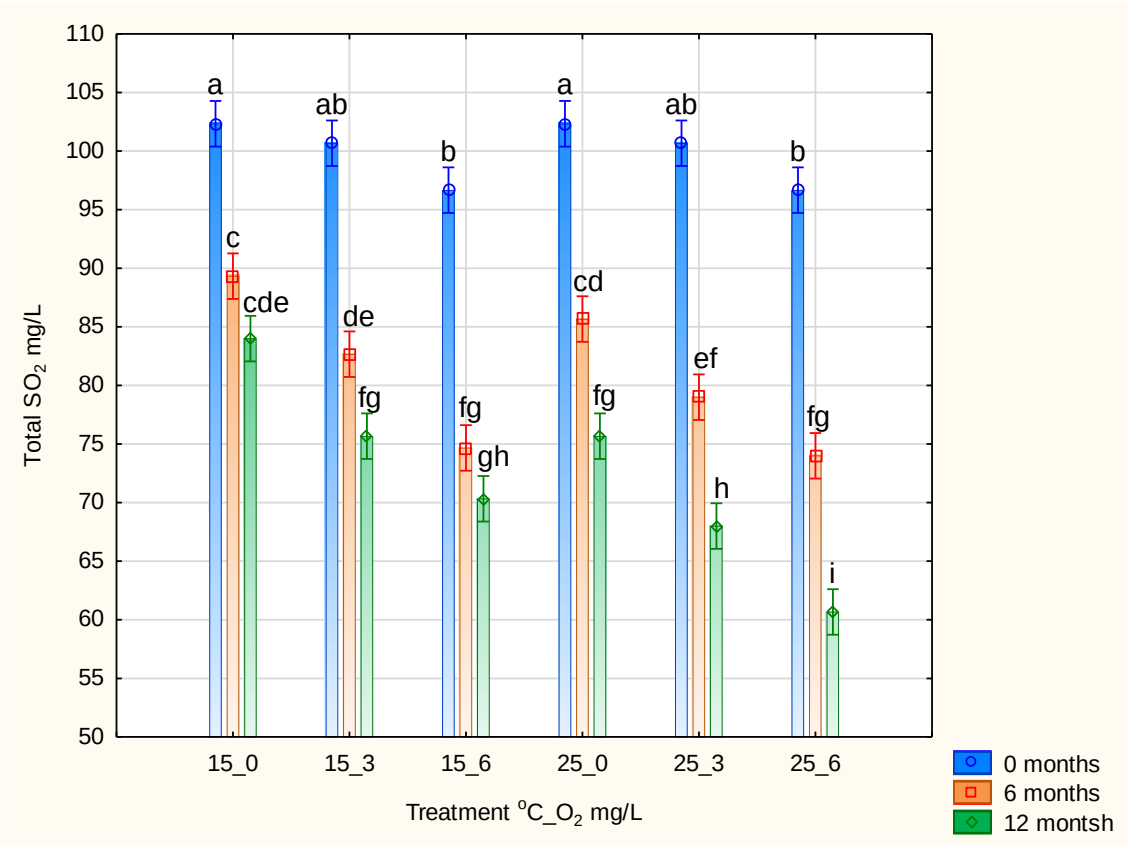

Figure 2.6 Total SO_2 concentrations of the Sauvignon blanc wine comparing the effects of O_2 and temperature across time.

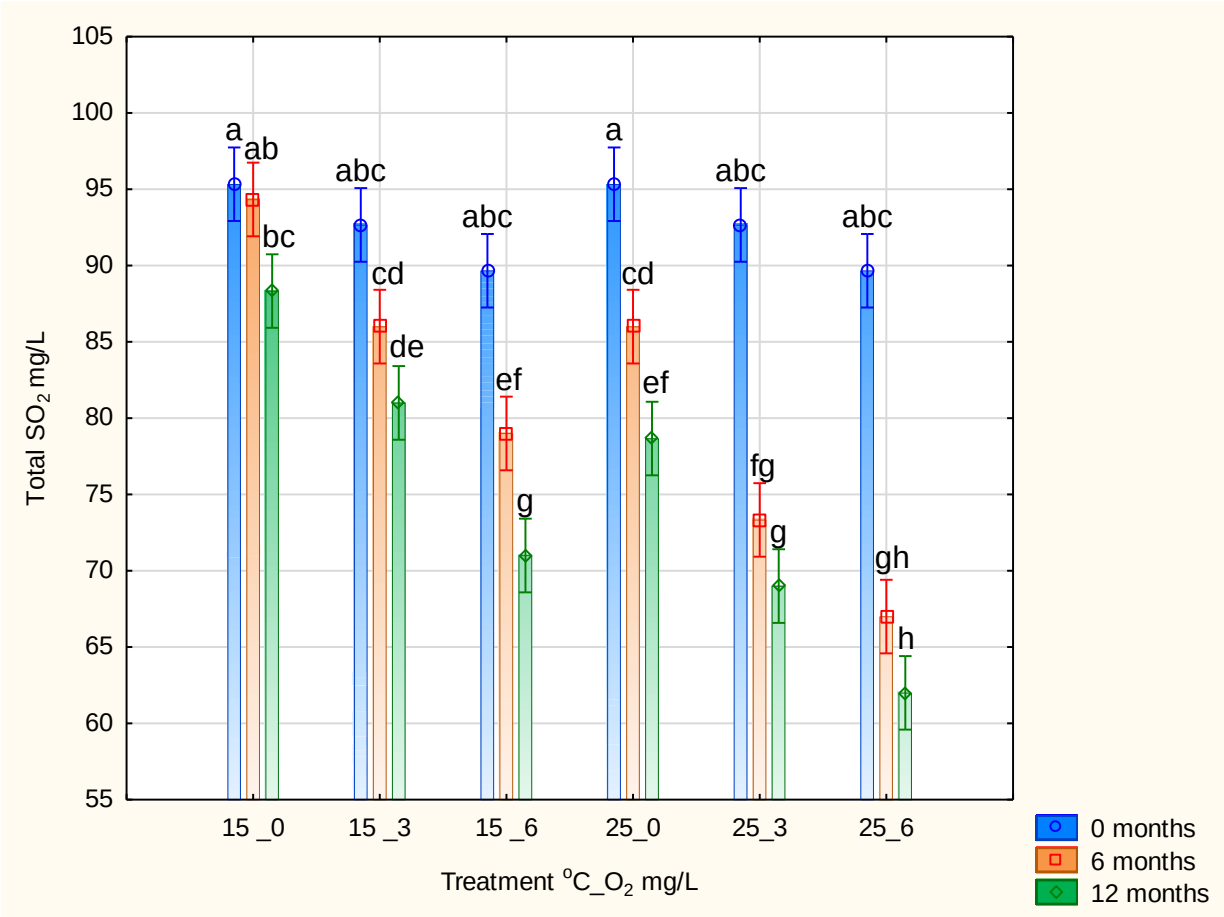

Figure 2.7 Total SO_2 concentrations of the Chenin blanc wine comparing the effects of O_2 and temperature after across time.

2.3.4 Colour analysis

Colour differences observed between O_2 treatments (six and twelve months) for both wines were not significant (Table 2.1b-2.1c and Table 2.2b-2.2c). Differences between storage temperatures were significant where wines stored at higher storage temperatures had increased yellow/brown colour intensity (Figure 2.7 and 2.8) compared to wines stored at lower temperatures. This is supported by previous AAM studies where increasing storage temperatures led to increased concentrations of yellow/brown colour intensity measured at 420 nm (Singleton, 1976; Recamales *et al.*, 2006; Killithraka *et al.,* 2009; Loscos *et al.,* 2010; Cejudo-Bastante *et al.,* 2013; Mafata *et al.,* 2019). However, when observing and comparing all three measurement points, time itself was the largest contributor to colour development as colour absorbance was greater between time points than all other factors (dissolved O_2 and temperature) (Addendum Figures 2B and Figure 2C).

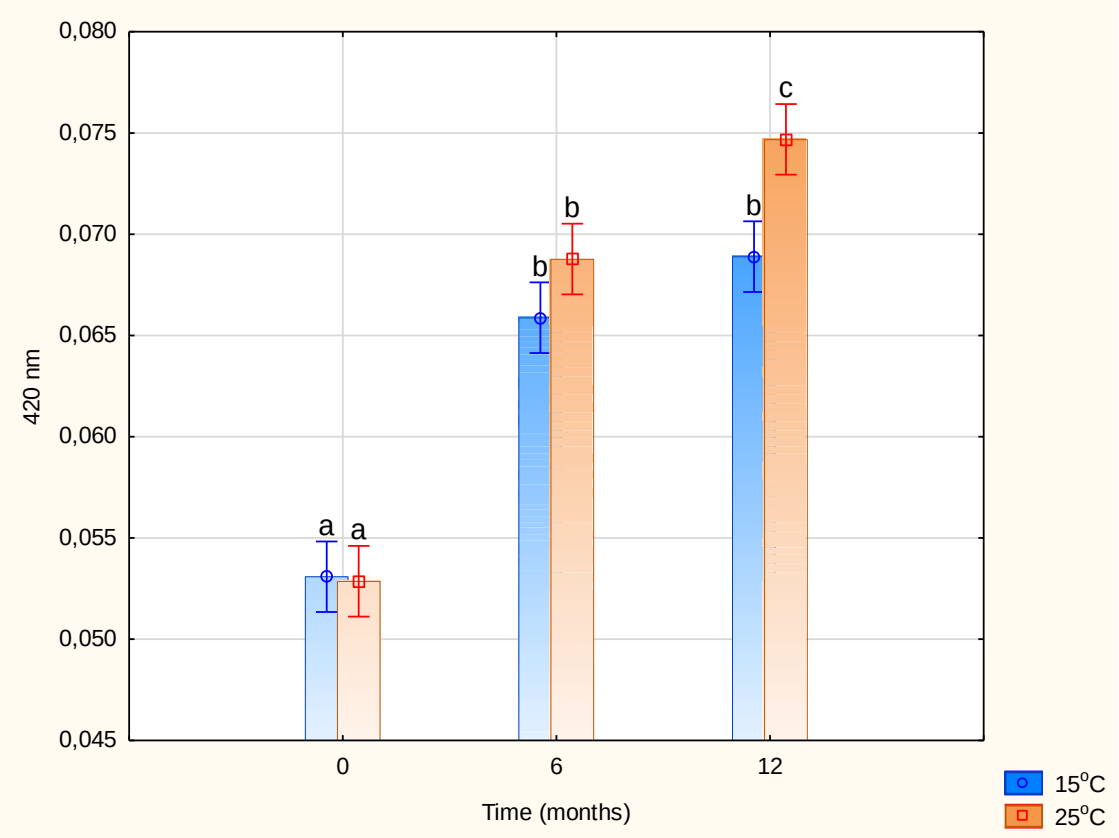

Figure 2.7 Yellow/brown colour absorbance measurements of Sauvignon blanc temperature treatments across time. All dissolved O_2 treatments were combined.

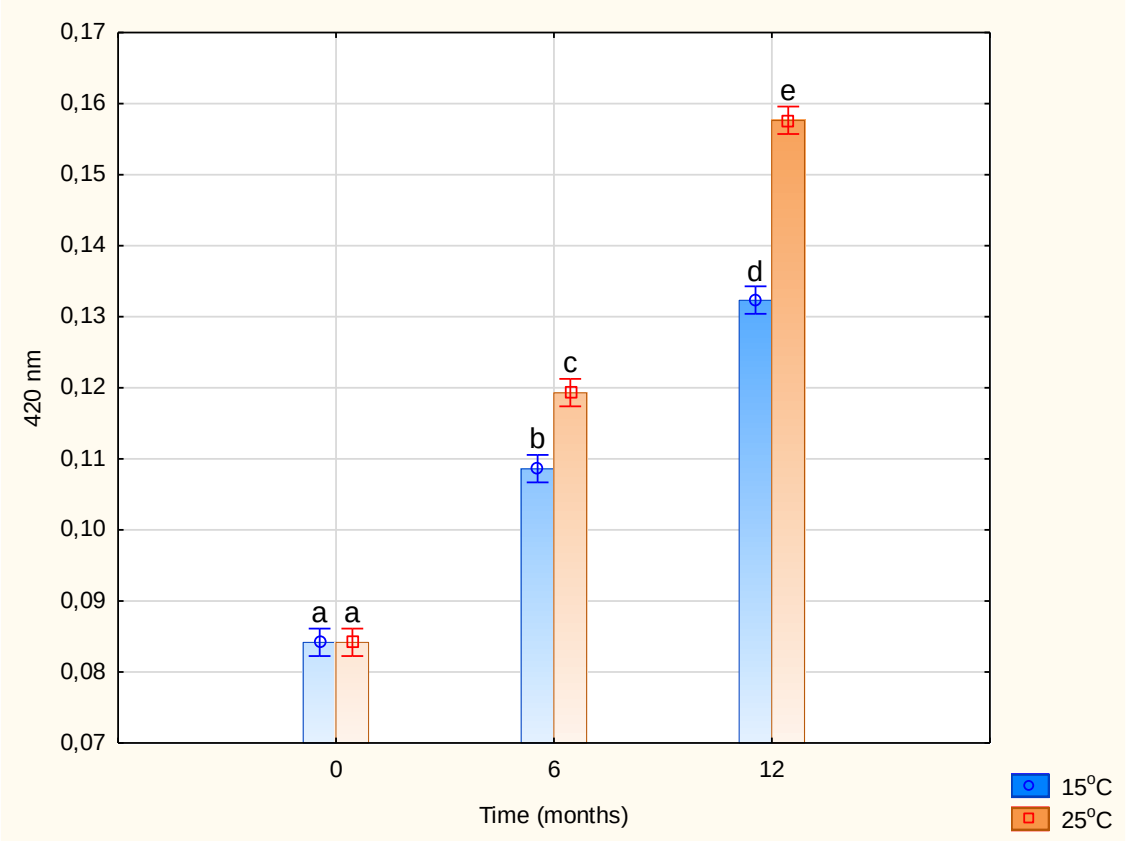

Figure 2.8 Yellow/ brown colour absorbance values of Chenin blanc temperature treatments across time. All dissolved O_2 treatments were combined.

2.3.5 Glutathione analysis

During oxidation, glutathione is changed to oxidised glutathione, with Grape Reaction Product and glutathionyl-caffeic acid also being formed (Fracassetti *et al.,* 2013; Coetzee *et al.,* 2016). During the experiment, the glutathione concentrations in the Sauvignon blanc wines (at six months) were found to be significantly different between both dissolved O_2 and temperature treatments (Figure 2.9, Table 2.1b). At twelve months the only significant differences found were between storage temperature treatments, with time significantly lowering glutathione concentrations (Addendum Figure 2D). Coetzee *et al.*, 2016 also found dissolved O_2 concentration and time to significantly reduce glutathione concentrations in a Sauvignon blanc wine at normal storage temperatures (15°C) (Coetzee *et al.,* 2016). In the Chenin blanc wines, storage temperature and time predominantly influenced glutathione concentrations (Table 2.1b-2.1c, 2.2b-2.2c, Addendum Figure 2F).

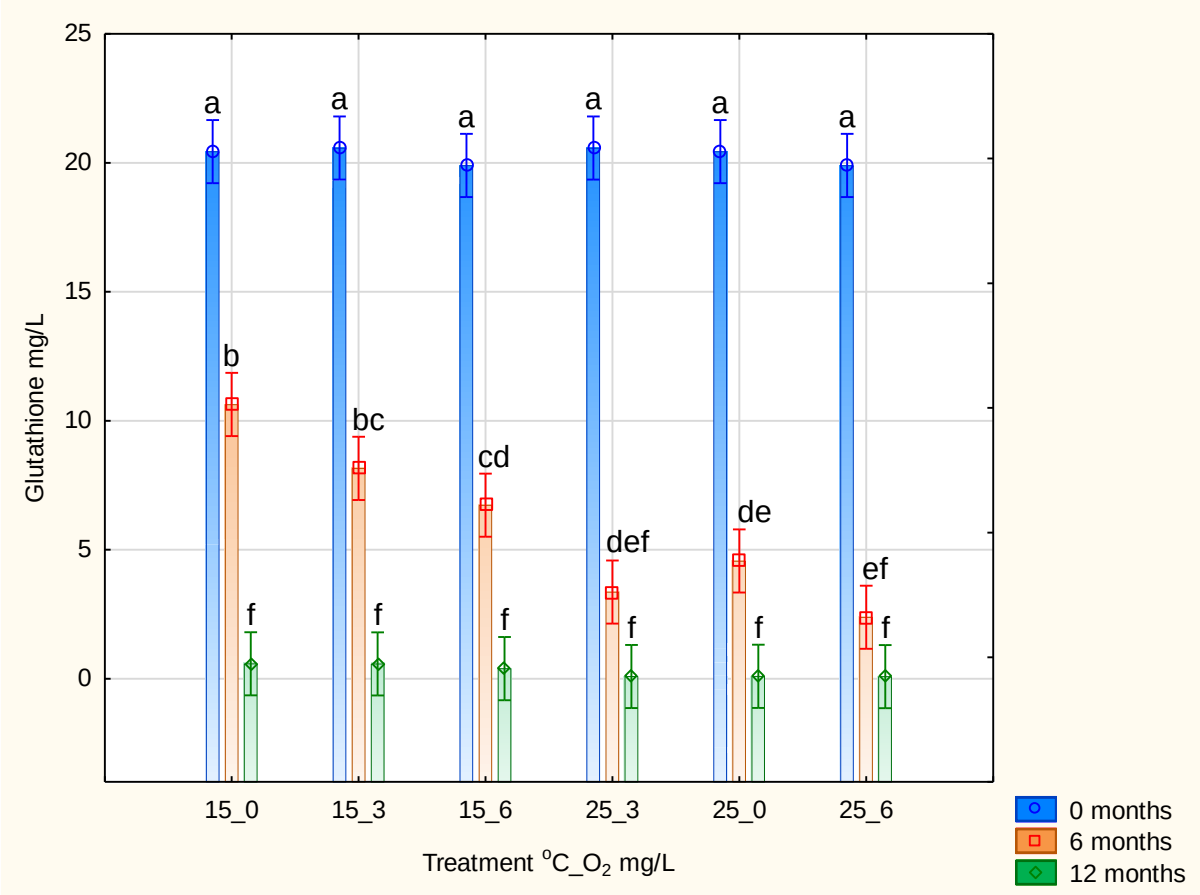

Figure 2.9 Glutathione concentrations in the Sauvignon blanc dissolved O_2 and storage temperature treatments across time.

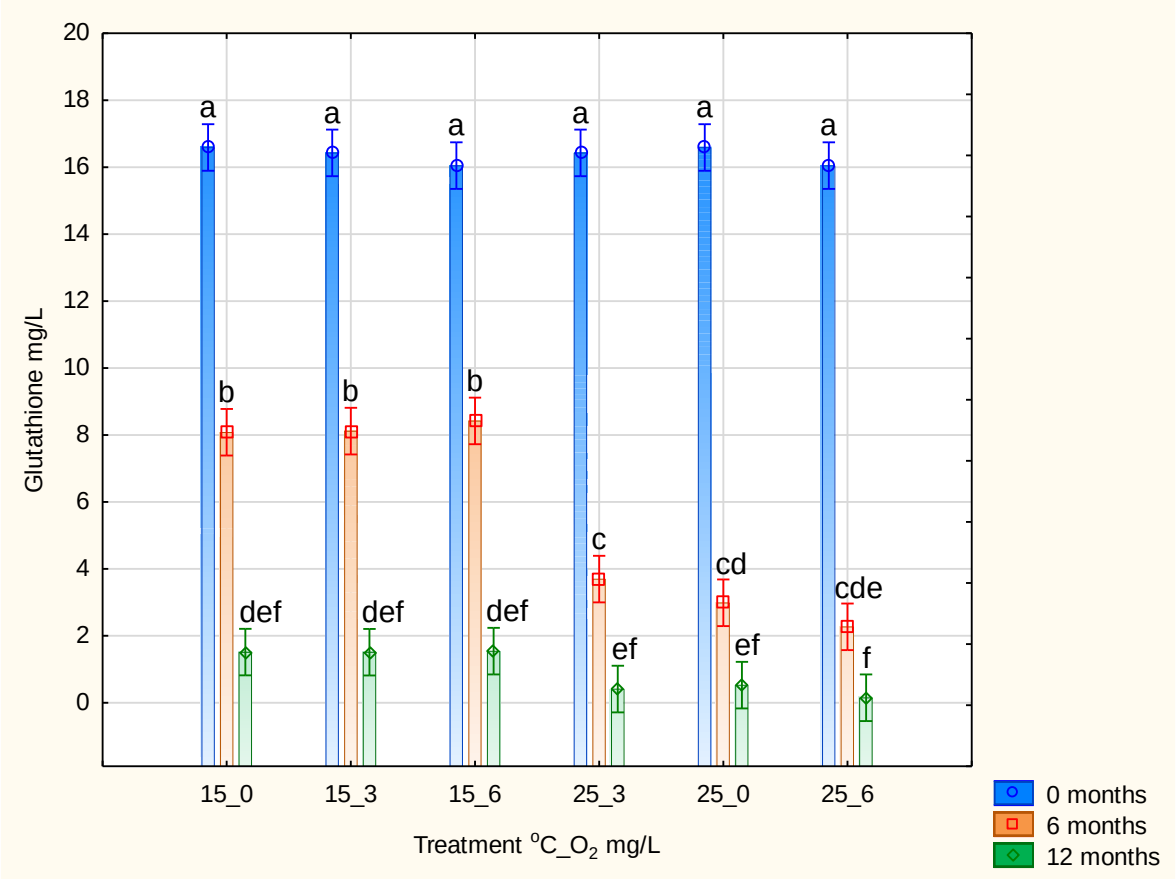

Fig 2.10 Glutathione concentrations in the Chenin blanc storage temperature treatments across time.

2.3.6 Varietal thiols

The initial 3MHA concentrations for the Sauvignon blanc were between 84 and 89 ng/L and between 119 and 125 ng/L for the Chenin blanc. The initial 3MH concentrations for the Sauvignon blanc were between 182 and 202 ng/L and between 197-207 ng/L for the Chenin blanc (Table 2.1b-2.1c, Table 2.2b-2.2c).

3MHA concentrations found in Sauvignon blanc and Chenin blanc wines mostly did not show significant differences between O_2 treatments after six and twelve months (Table 2.1b-2.1c, Table 2.2b-2.2c, Figures 2.11, 2.12). However, both cultivars did show significant differences in 3MHA concentration between storage temperatures at six months (Addendum Figures 2F and 2G). 3MHA is associated with tropical aromas (Tominaga *et al.*, 1996; Addendum 2B), and as concentrations lower, losses of fruity aroma could occur.

Previous works have found that 3MHA is sensitive to oxidation (Blanchard *et al.* 2004, Nikolantonaki *et al.* 2010). However, at the six-month sampling period for the Chenin blanc wine only, the 3MHA concentrations were found to be at significantly lower concentrations in wines stored at higher temperatures. While differences between the O_2 treatments were insignificant, indicating that the presence of O_2 did not have a major role in the decrease in 3MHA concentration for the conditions of this study, but storage temperature did. This result is supported by previous studies where elevated temperatures lead to lower 3MHA concentrations, due to hydrolyses of 3MHA to 3MH (Makhotkina *et al.,* 2012; Bruwer, 2018; Mafata *et al*., 2019).

That dissolved O_2 treatments did not significantly affect 3MHA concentrations is in part contrary to previous research which found thiols concentrations to decrease with increased concentrations of dissolved O_2 (Krietman *et al.,* 2013; Coetzee *et al.,* 2016). However, the dissolved O_2 concentrations administered in the current study are more reflective of bottling procedures (Van der Merwe, 2013). This means that greater amounts of dissolved O_2 could have more significant effects, but the levels found after bottling do significantly effect thiol composition.

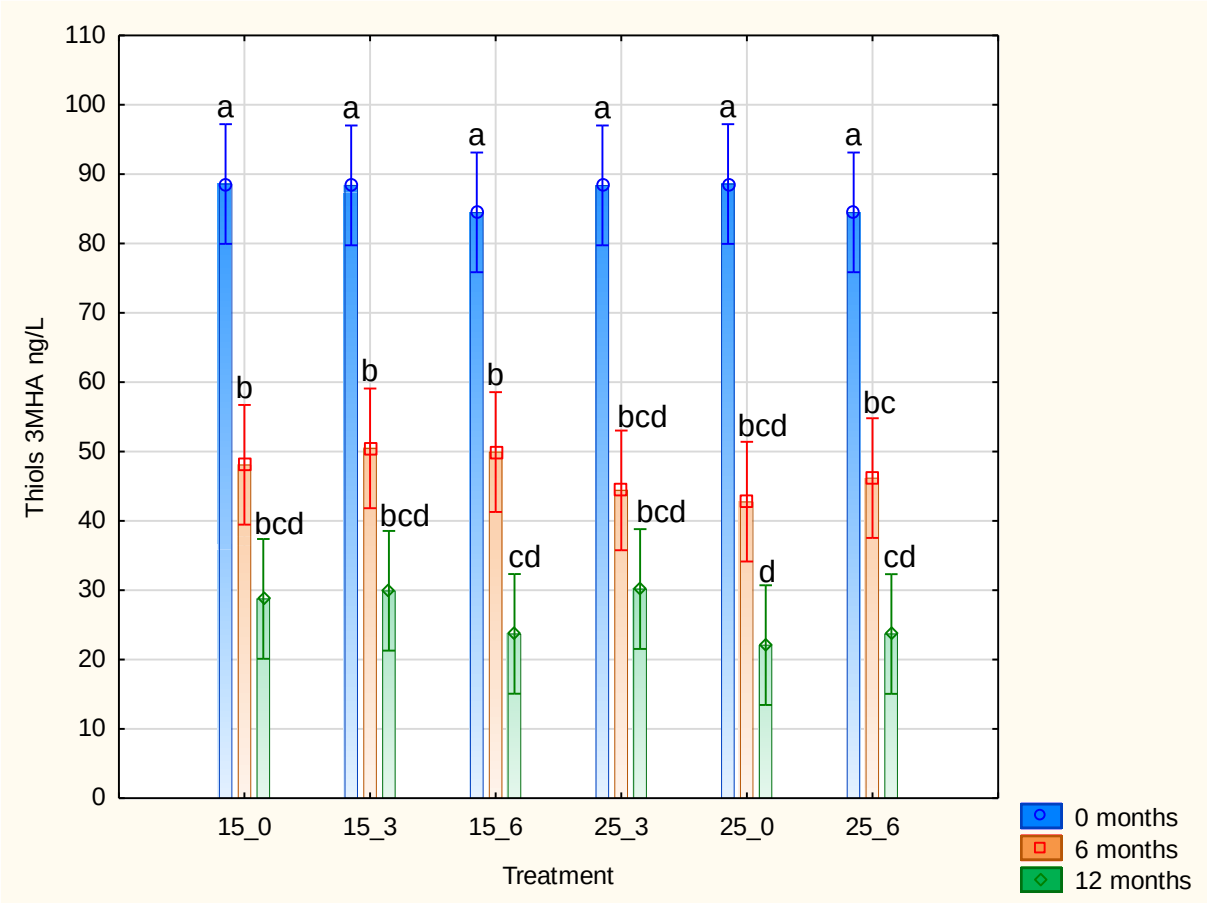

Figure 2.11 3MHA concentrations in the Sauvignon blanc wine for different storage temperatures and O_2 treatments across time.

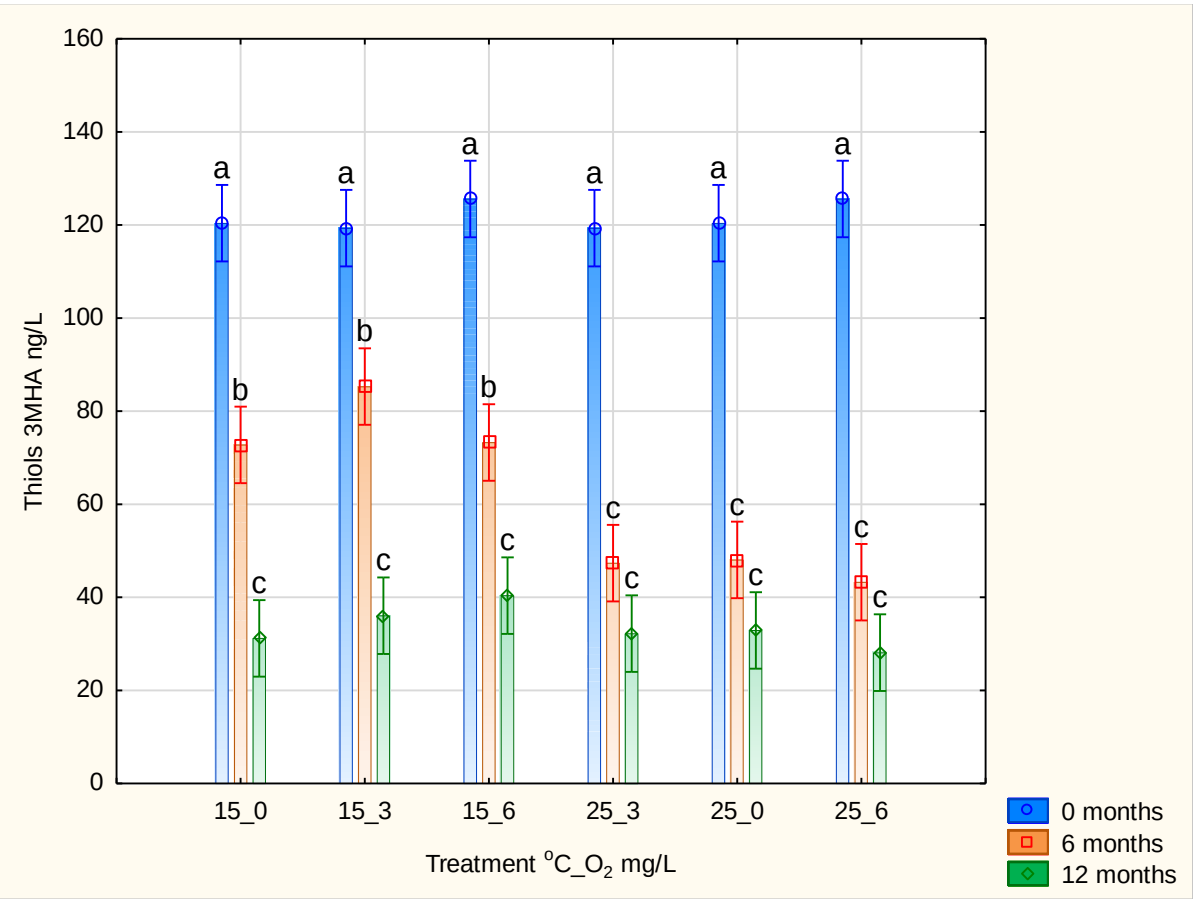

Figure 2.12 3MHA concentrations in the Chenin blanc wine for different storage temperatures and O_2 treatments across time.

3MH concentrations in the Sauvignon blanc and Chenin blanc wines were mostly not significantly different between the dissolved O_2 treatments (Figures 2.13 and 2.14). For the Sauvignon blanc wine where no O_2 addition took place, 3MH concentrations were higher after six months when stored at 25°C compared to 15°C (Figure 2.13). After twelve months, there was no significant difference. Combined data from the O_2 treatments showed significant increases in 3MH concentration between storage temperatures at both six and twelve months, with wines stored at 25°C having higher concentrations of 3MH compared to wines stored at 15°C (Addendum 2H and 2I).

3MH concentrations in wine have been known to increase over time, partially due to the hydrolysis of 3MHA (Herbst-Johnstone *et al.,* 2011). Furthermore, elevated storage temperatures could accelerate 3MHA hydrolysis (Peleg *et al.,* 2012) resulting in higher concentrations of 3MH. Makhotkina *et al.* (2012) reported that wines aged at elevated temperatures resulted in stable or increased levels of 3MH found at higher storage temperatures from six to twelve months, despite the potential for 3MH to oxidise.

However, in these wines the increase in 3MH concentrations from the initial to six-month sample period cannot be solely accounted for by 3MHA hydrolysis as the amount of 3MHA loss does not stoichiometrically account for the of 3MH gained. This finding is similar to results reported by Makhotkina *et al.* (2012) and Mafata *et al.*,(2019) where observed increases to 3MH

in elevated storage temperature experiments involving white wine. The increase in 3MH concentrations during ageing beyond what can be gained from 3MHA hydrolysis is opportunity for further study into sources of 3MH. However, as 3MHA is a stronger odorant then 3MH (Tominaga *et al.*, 1996; Tominaga *et al.*, 1998) small increases in 3MH concentration might not significantly affect wine aroma nearly so much as 3MHA loss.

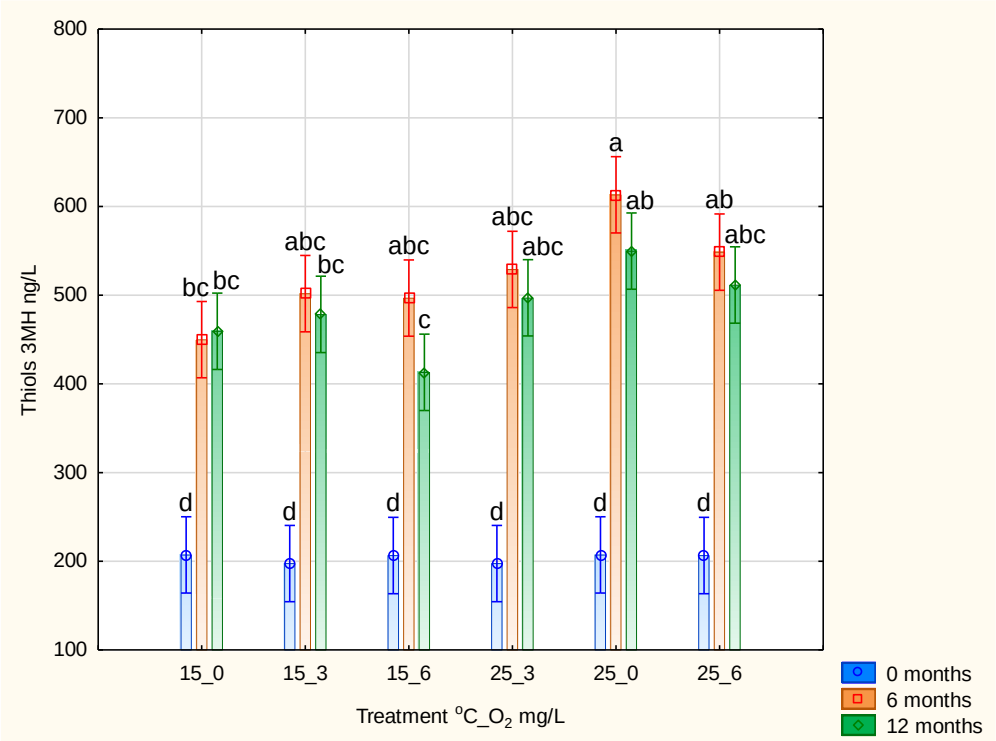

Figure 2.13 3MH concentrations in the Sauvignon blanc wine for different storage temperatures and O_2 treatments across time.

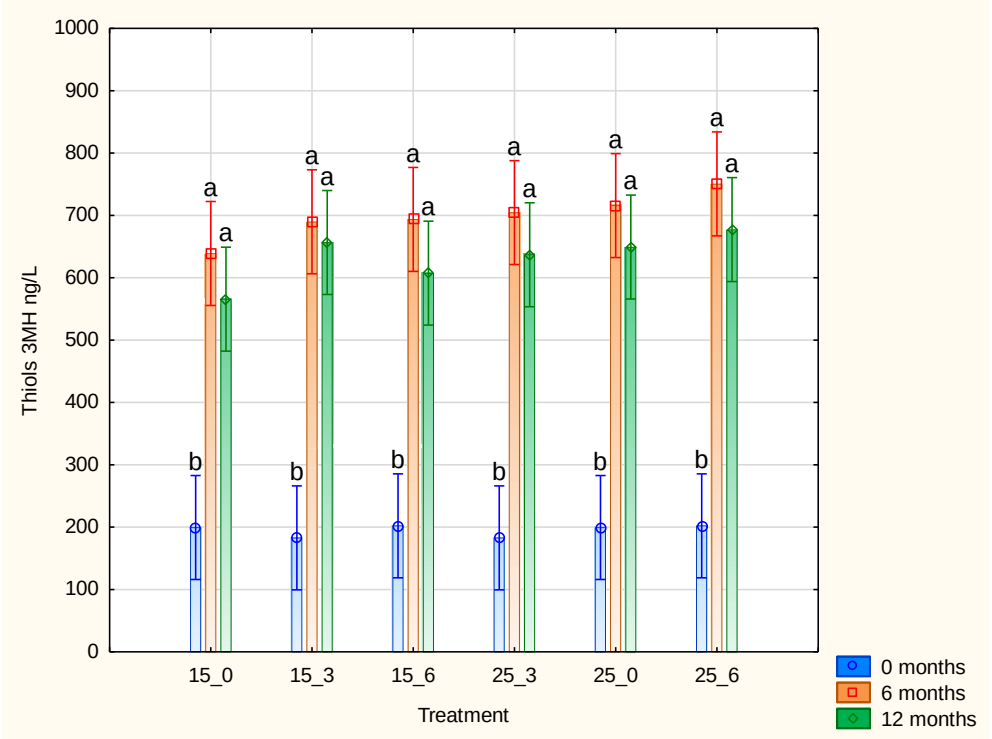

Figure 2.14 3MH concentrations in the Chenin blanc wine for different storage temperatures and O_2 treatments at across time.

2.3.7 Esters, fatty acids, and higher alcohols

The major volatiles analysis provided insightful results regarding the development and degradation of esters, fatty acids, and alcohols over the course of twelve months storage. The ester isoamyl acetate, which is associated with pleasant fruity aromas (Benkwitz *et al.*, 2012; Addendum Table 2B), decreased in concentration when stored at higher temperatures for both wines, especially after twelve months (Table 2.1b-2.1c and Table 2.2b-2.2c). Correspondingly, the concentration of isoamyl alcohol, which is described as “whisky”, “malt” and “burnt” (Guth, 1997; Addendum Table 2B), also increased at higher storage temperatures. The decline of acetate esters during storage has been reported in literature previously (Marais & Pool, 1980; Ramey & Ough, 1980; Ferreira *et al.,* 1997; Pérez-Coello *et al.*, 2003; Makhotkina & Kilmartin, 2012; Patrianakou & Roussis, 2013; Coetzee *et al.,* 2016).

Diethyl succinate concentrations were significantly higher in in both the Chenin blanc and Sauvignon blanc stored at the higher temperature, especially by twelve months (Table 2.1b-2.1c and Table 2.2b-2.2c). In the Sauvignon blanc wine, ethyl lactate concentrations were also found at significantly higher quantities as storage temperature increased. Diethyl succinate and ethyl lactate are typically associated with malolactic fermentation (Louw *et al.,* 2010) and contribute odours such as “Melon”, “Lactic” and “fruity” (Addendum Table 2B). As these wines did not go through malolactic fermentation, the appearance of these compounds could also result from the transformation of lactic and succinic acids to form ethyl lactate and diethyl succinate during fermentation and maturation (De Villiers *et al.,* 2003). The increase of these compounds during ageing have been reported in literature previously (Rapp, 1988; Ferreira *et al.,* 1997; Pérez-Coello *et al.,* 2003; Hernanz *et al.,* 2009; Coetzee *et al.,* 2016).

In most cases compounds such as propionic acid, hexanoic acid, octanoic acid and decanoic acid, which are associated with ‘rancid’ aromas in aged wines (Ferreira *et al.*, 2000; Addendum Table 2B), were sometimes found to be significantly higher in the 25°C samples for both varieties (Table 2.1c,and Table 2.2c). This is supported by previous studies (Marais & Pool, 1980; Ferreira *et al.,* 1997; Câmara *et al.,* 2006; Blake *et al.,* 2010; Lee *et al.,* 2011) where higher concentrations of these compounds were found after ageing at elevated temperatures. However, there are mixed results in current research where increased concentrations were not reported in an oxidation study done at 15°C (Coetzee *et al.,* 2016). It could be that the formation of these acids is more closely associated with ageing compared to O_2 exposure, especially as storage temperatures increase. As increases in these compounds have been reported to have negative aromas (Addendum Table 2B), preventing their formation is key to preserving fresh and fruity aromas, especially for young white wines.

2.3.8 Descriptive analysis

2.3.8.a Sauvignon blanc

Significantly different descriptors in the Sauvignon blanc six month descriptive analyse were ‘fresh green’, ‘green apple’ ‘dust/tea’, ‘baked apple’ and ‘cooked veg’. The most intense descriptors being ‘fresh green’ and ‘baked apple’, and the least intense being ‘cooked veg’ At the twelve month descriptive analysis, ‘passionfruit’, ‘grapefruit’, ‘dried fruit’, ‘apple’, and ‘cooked veg’ were significant. The descriptive analysis yielded differences between the treatments for both Sauvignon blanc and Chenin blanc wines, but the Sauvignon blanc wines tended to have more significant results and stronger correlations between temperature treatments. Oxygen treatments did not produce strong significant differences between samples for most descriptors (Table 2.3 and Table 2.4).

Table 2.3 Descriptor intensities from the six and twelve month sensory analysis for the Sauvignon blanc wines. Letters ‘a’, ‘b’ and ‘c’ indicate the degree of significant difference.

6 month descriptive analysis	15°C_0 mg/L	15°C_3 mg/L	15°C_6 mg/L	25°C_0 mg/L	25°C_3 mg/L	25°C_6 mg/L
Descriptor						
Passionfruit	11 ± 2a	10 ± 2a	12 ± 2a	8 ± 2a	6 ± 2a	7 ± 2a
Guava	13 ± 2a	14 ± 2a	10 ± 1a	9 ± 1a	13 ± 2a	6 ± 2a
Grapefruit	36 ± 2a	37 ± 2a	31 ± 2a	29 ± 2a	29 ± 2a	26 ± 2a
Pineapple	31 ± 3a	34 ± 3a	35 ± 3a	29 ± 3a	29 ± 2a	31 ± 3a
Fresh green	26 ± 3a	25 ± ab	23 ± ab	20 ± 3ab	20 ± 3ab	14 ± b
Green apple	14 ± 3a	15 ± 4a	16 ± 3a	8 ± 3ab	7 ± 2ab	9 ± 2b
Dust/tea	12 ± 2b	13 ± 2b	10 ± 2b	18 ± 2a	19 ± 2a	14 ± 2ab
Dried fruit	30 ± 3a	29 ± 3a	33 ± 2a	29 ± 3a	30 ± 2a	32 ± 3a
Baked apple	13 ± 3b	12 ± 3b	13 ± 3b	21 ± 3ab	22 ± 3ab	27 ± 2a
Cooked veg	5 ± ab	6 ± ab	1 ± b	4 ± ab	10 ± a	4 ± ab

12 month descriptive analysis	15°C_0 mg/L	15°C_3 mg/L	15°C_6 mg/L	25°C_0 mg/L	25°C_3 mg/L	25°C_6 mg/L
Descriptor						
Passionfruit	32 ± 4a	28 ± 4ab	29 ± 3ab	16 ± 3bc	19 ± 3abc	15 ± 4c
Guava	10 ± 2a	10 ± 2a	7 ± 2a	8 ± 3a	10 ± 2a	6 ± 2a
Grapefruit	37 ± 2a	37 ± 2a	36 ± 2a	24 ± 3b	27 ± 3ab	21 ± 3b
Pineapple	39 ± 3a	38 ± 3a	38 ± 3a	31 ± 3a	28 ± 4a	29 ± 4a
Fresh green	23 ± 3a	22 ± 3a	22 ± 3a	12 ± 2a	19 ± 3a	13 ± 3a
Green apple	28 ± 4a	25 ± 4a	27 ± 4a	14 ± 4a	19 ± 4a	16 ± 4a
Dust/tea	11 ± 3a	12 ± 3a	15 ± 3a	14 ± 2a	13 ± 3a	8 ± 2a
Dried fruit	28 ± 3b	30 ± 4b	31 ± 3ab	34 ± 3ab	34 ± 3a	38 ± 3a
Baked apple	21 ± 3c	23 ± 4c	22 ± 3c	36 ± 4a	26 ± 3ab	41 ± 3a
Cooked veg	4 ± 1ab	3 ± 1b	5 ± 2ab	7 ± 1ab	11 ± 1a	6 ± 2ab

However, at six months in the Sauvignon blanc wine, the ‘fresh green’ descriptor was significantly less intense in the 25°C treatment that was bottled with 6 mg/L O_2 than the 15°C with no O_2 added at bottling. Dust/tea and baked apple was in some cases also higher in the Sauvignon blanc wine stored at the higher temperature at this time. After twelve months in some cases passion fruit and grapefruit were also significantly lower in the Sauvignon blanc wine stored at 25°C. Baked apple and cooked veg, were also in some cases significantly higher in the Sauvignon blanc wines stored at the higher temperature, although in the case of cooked veg the differences were relatively small.

Though the 3MHA concentrations in the different storage temperature treatments were similar, the sensory perception of ‘passion fruit’ was often significantly lower in the high storage temperature wines after twelve months. This might be in part due to the formation of higher alcohols and fatty acids associated with off aromas, such as diethyl succinate and octanoic acid. As those compounds’ concentration increased with higher temperatures, their sensorial contribution is also likely to increase, as well as some oxidation or overaged related compounds being formed that may lower the intensity of or mask the aromas associated with varietal thiols (Coetzee *et al.,* 2015; Coetzee *et al.,* 2016).

Figure 2.15 shows a PCA biplot with loadings and scores of the descriptive analysis samples from the six and twelve month sensory results. PC1 at 72% (effect of storage temperature) explained most of the variance, with PC2 (time), explaining 17% of the variance. The wines stored at 15°C tended to correlate more with the ‘grapefruit’, ‘pineapple’, ‘guava’, ‘green apple’, ‘fresh green’ and ‘passion fruit’ descriptors. The wines stored at 25°C were correlated better with ‘baked apple’, ‘cooked veg’, ‘dust/tea’ and ‘dried fruit’. As the wine aged, wines stored at lower temperatures become more correlated to ‘fresh green’, ‘guava’ and ‘green apple’.

As the 25°C storage samples aged, these became more strongly correlated to ‘baked apple’ which is supported by the fact that positive fruity esters decreased, and compounds associated with aromas related to aged aromas probably increased. Similar results were found by Du Toit and Piquet (2014) who also found a decrease in fruity descriptors and an increase in negative associated descriptors at higher storage temperatures in South African Sauvignon blanc wines. The oxygen treatments clustered loosely on the PCA, giving a further indication of its lower contribution to the wines’ sensorial differences, which is supported by the major volatile analyses where significant differences were mainly observed between storage temperatures.

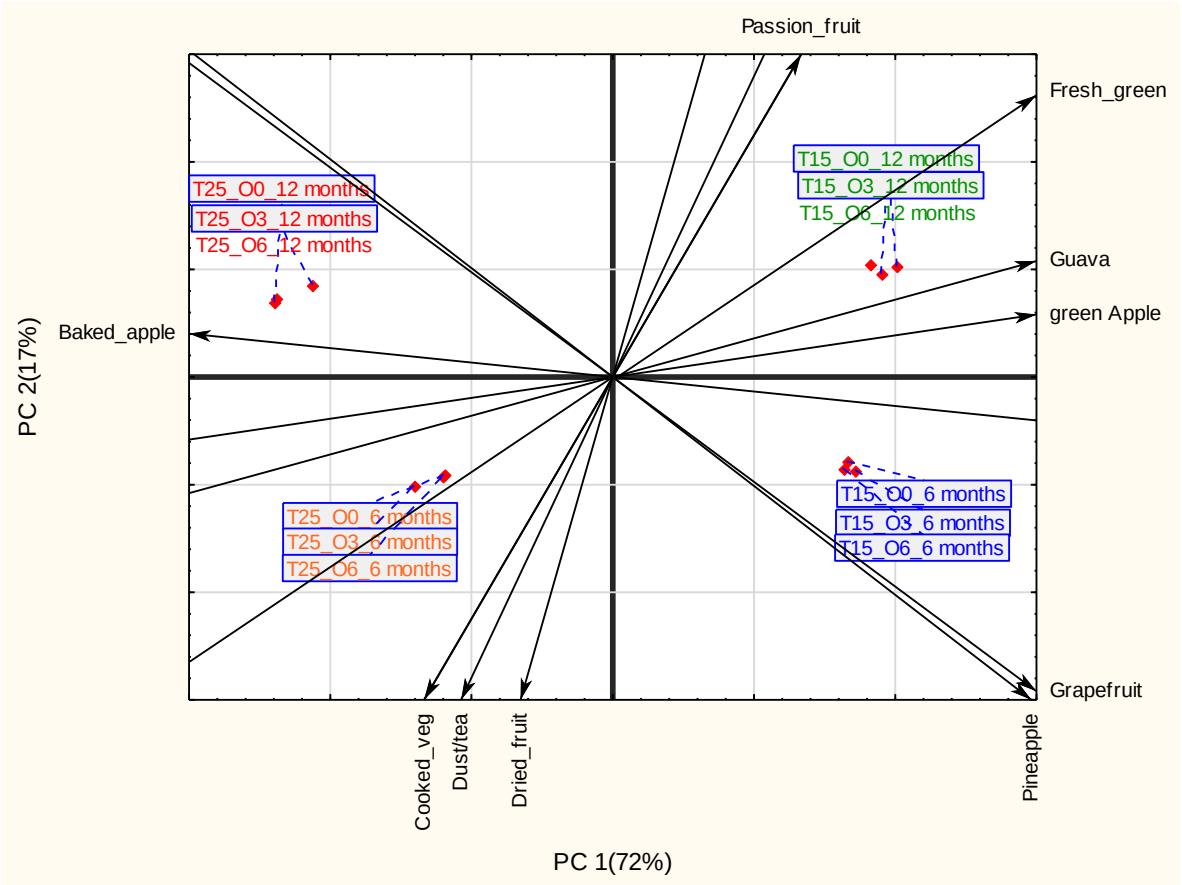

Figure 2.15 PCA biplot of the Sauvignon blanc wines' sensory results after six and twelve month. 'T' indicates temperature and 'O' indicates oxygen level at bottling.

2.3.8.b Chenin blanc

Significantly different descriptors in the Chenin blanc six month descriptive analyse were 'fresh green', 'green apple' 'dust/tea', 'baked apple' and 'cooked veg'. At the twelve month descriptive analysis, no descriptors were significantly different.

Table 2.4 Descriptor intensities from the six and twelve month sensory analysis for the Chenin blanc wines. Letters 'a', 'b' and 'c' indicate significant difference.

6 month descriptive analysis	15°C_0 mg/L	15°C_3 mg/L	15°C_6 mg/L	25°C_0 mg/L	25°C_3 mg/L	25°C_6 mg/L
Descriptor						
Guava	22 ± 2a	21 ± 2a	25 ± 2a	24 ± 2a	7 ± 2b	5 ± 2b
Grapefruit	37 ± 2a	35 ± 2a	36 ± 1a	34 ± 1a	37 ± 2a	31 ± 2a
Pineapple	30 ± 2a	32 ± 2a	32 ± 2a	30 ± 2a	39 ± 2a	33 ± 3a
Fresh green	26 ± 3a	27 ± 3a	24 ± 2a	23 ± 2a	27 ± 2a	21 ± 3a
Green apple	16 ± 2a	16 ± 2a	17 ± 2a	23 ± 1a	24 ± 4a	21 ± 4a
Hay/tea	20 ± 2a	20 ± 2a	19 ± 2a	20 ± 2a	7 ± 2b	11 ± 3b
Dried/stewed_fruit	33 ± 1a	33 ± 1a	35 ± 1a	34 ± 1a	34 ± 3a	32 ± 2a
Baked apple	33 ± 1a	33 ± 1a	35 ± 1a	34 ± 1a	34 ± 3a	32 ± 2a
Caramel	14 ± 1a	16 ± 2a	14 ± 1a	10 ± 1a	9 ± 1a	11 ± 3a
Honey	15 ± 2ab	21 ± 2a	18 ± 2a	13 ± 2b	16 ± 3ab	12 ± 2b
Cooked veg	3 ± 1ab	2 ± 1b	3 ± 1ab	2 ± 1b	4 ± 1ab	9 ± 1a

12 month descriptive analysis	15°C_0 mg/L	15°C_3 mg/L	15°C_6 mg/L	25°C_0 mg/L	25°C_3 mg/L	25°C_6 mg/L
Descriptor						
Guava	10 ± 2a	10 ± 2a	7 ± 2a	12 ± 2a	12 ± 2a	12 ± 3a
Grapefruit	36 ± 2a	35 ± 2a	33 ± 3a	31 ± 2a	31 ± 3a	31 ± 3a
Pineapple	35 ± 2a	36 ± 2a	33 ± 3a	38 ± 3a	34 ± 3a	34 ± 2a
Fresh green	27 ± 2a	29 ± 3a	24 ± 2a	22 ± 2a	22 ± 2a	20 ± 3a
Green apple	22 ± 3a	19 ± 4a	17 ± 3a	16 ± 2a	17 ± 4a	16 ± 3a
Hay/tea	14 ± 2a	12 ± 2a	11 ± 2a	13 ± 2a	11 ± 2a	9 ± 2a
Dried/stewed_fruit	32 ± 3a	29 ± 3a	35 ± 2a	34 ± 2a	34 ± 3a	33 ± 2a
Baked apple	30 ± 3a	28 ± 3a	35 ± 2a	33 ± 2a	33 ± 3a	31 ± 2a
Caramel	8 ± 2a	12 ± 2a	15 ± 3a	13 ± 3a	13 ± 3a	11 ± 2a
Honey	11 ± 2a	15 ± 2a	18 ± 2a	12 ± 2a	17 ± 2a	11 ± 1a
Cooked veg	8 ± 2a	6 ± 2a	4 ± 1a	5 ± 2a	6 ± 2a	3 ± 1a

The Chenin blanc descriptive analysis did not show many significant differences in intensities most descriptors in both six and twelve month analyses. At six months, no differences in the 15°C temperature is seen, but once O_2 was introduced in 25°C storage samples at bottling, the perception of 'guava' significantly decreases at this stage. This is supported by the varietal thiol data where the 3MHA concentration were significantly higher in the wines stored at 15°C at this stage. By twelve months, the 3MHA concentration were not significantly different from each other and this is also reflected in the guava descriptor (Table 2.4) where no significant differences were observed.

In Figure 2.16, the PCA biplot shows the results from the six and twelve month descriptive analysis for the Chenin blanc wine. PC1 explained 56% of the variance, with the 15°C storage/6 month samples separating from the other samples. The 25°C _6 mg/L O_2 treatment, which correlated with 'dried/stewed fruit' and inversely correlated to 'guava' and 'hay/tea'. Interestingly,

though the 15°C samples were correlated to higher intensities of guava, this was is not reflected in the varietal thiol data and might be due some enhancing effects of esters on varietal thiol derived descriptors, as described by King et al. 2011. The 0 and 3 mg/L O_2 treatments stored at 15°C after twelve months correlated better with the 25°C six months storage treatments that received O_2 at bottling. The 15°C_O6_T12 treatments did not correlate well to the other 15°C storage treatments as it correlated more with the dried fruit descriptor. Overall, the Chenin blanc results show fewer significant differences in descriptor intensities when compared to the Sauvignon blanc wines. However, in both cultivars from the 6 month analysis the 15°C stored wines correlated closely to tropical descriptors; and in both cultivars from the 12 month analysis the wines stored at 25°C correlated towards oxidative descriptors.

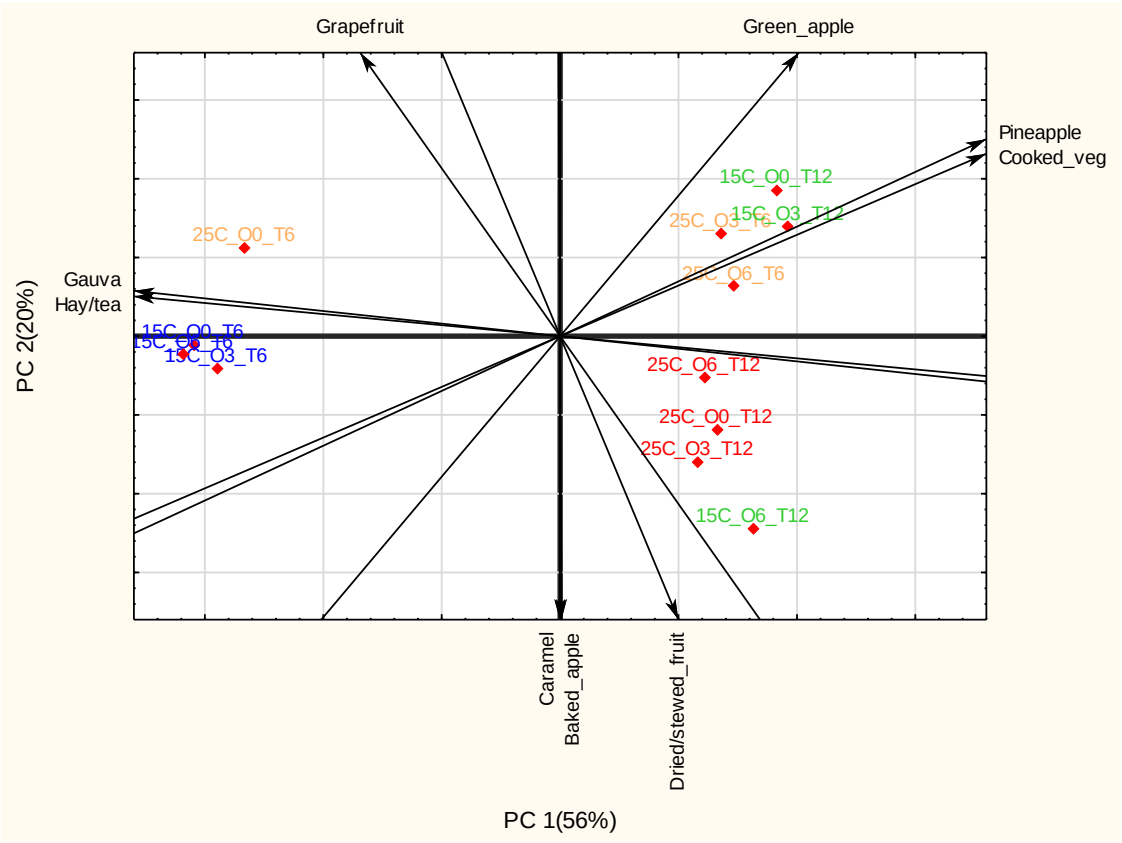

Figure 2.16 PCA biplot of the Chenin blanc wines' sensory results after six and twelve months. 'T' indicates storage temperature and 'O' indicates dissolved oxygen level at bottling.

2.4. Multiple factor analysis

2.4.1 Sauvignon blanc

An individual sample multiple factor analysis was used separately for the six (Figure 2.17) and twelve month (Figure 2.18) chemical and sensory data as the combined six and twelve month data sets did not yield clear patterns (results not shown). The twelve month Sauvignon blanc multiple factorial analysis samples (Figure 2.18) were strongly grouped by storage temperature similar to the six month (Figure 2.17). However, the samples were not clearly organized by O_2 treatments as was the case in the six month multiple factor analysis. This seems to imply that the

effects of O_2 were in fact more significant at six months and became less impactful over time. In this study, temperature had a strong effect on the Sauvignon blanc wine chemistry, which suggests it could have significant effects on sensory characteristics.

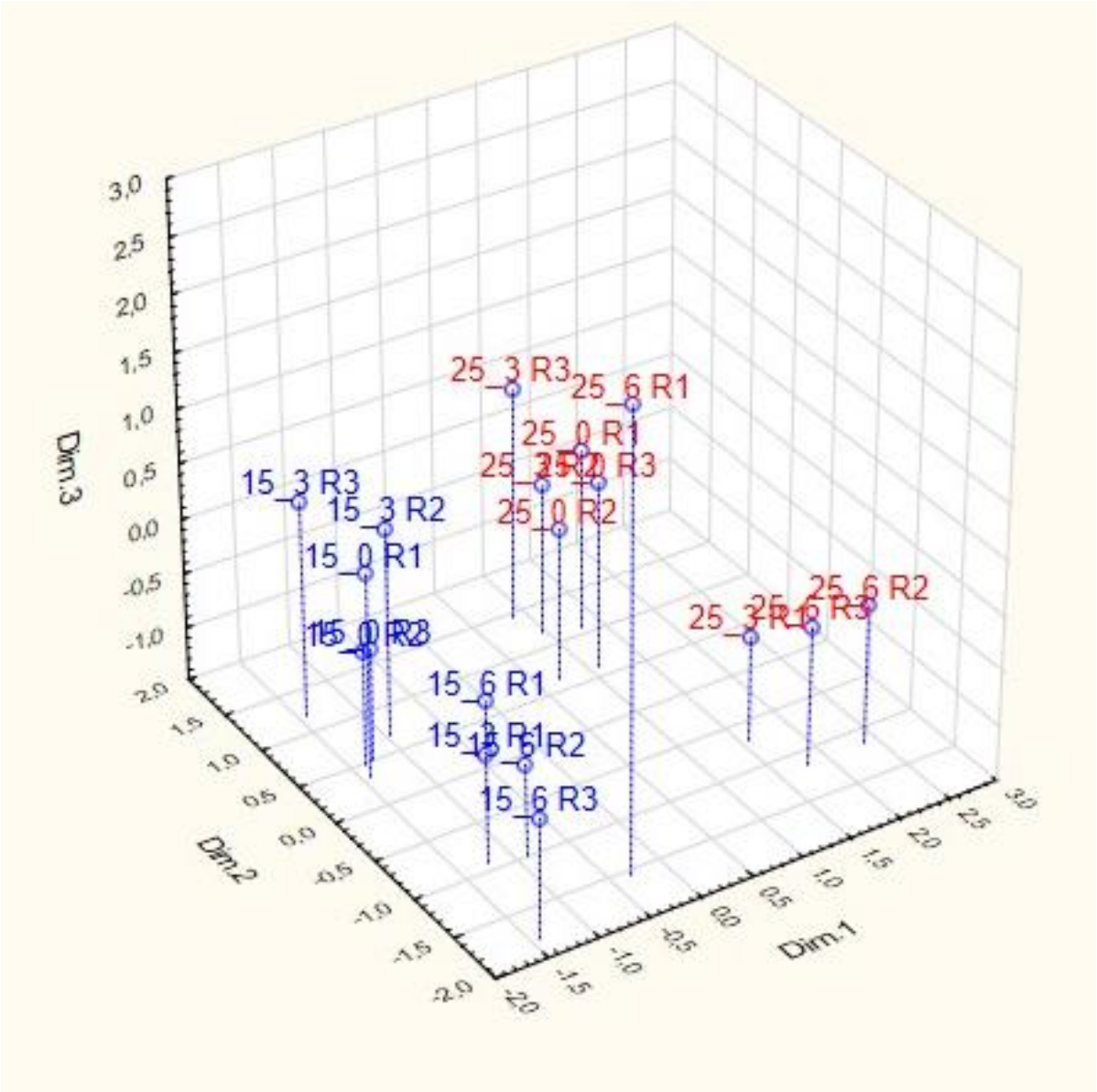

Figure 2.17 Individual sample multiple factor analysis of the Sauvignon blanc wine six month chemical and sensory analysis: 3D individual sample scatterplot. Blue samples designate 15°C storage and red samples designates 25°C storage. R1, R2 and R3 indicate the biological repeat. Samples are correlated along dimension 1 and dimension 2 where dimension 1 sample groups are separated by storage temperatures and dimension 2 more so by dissolved O_2 concentration.

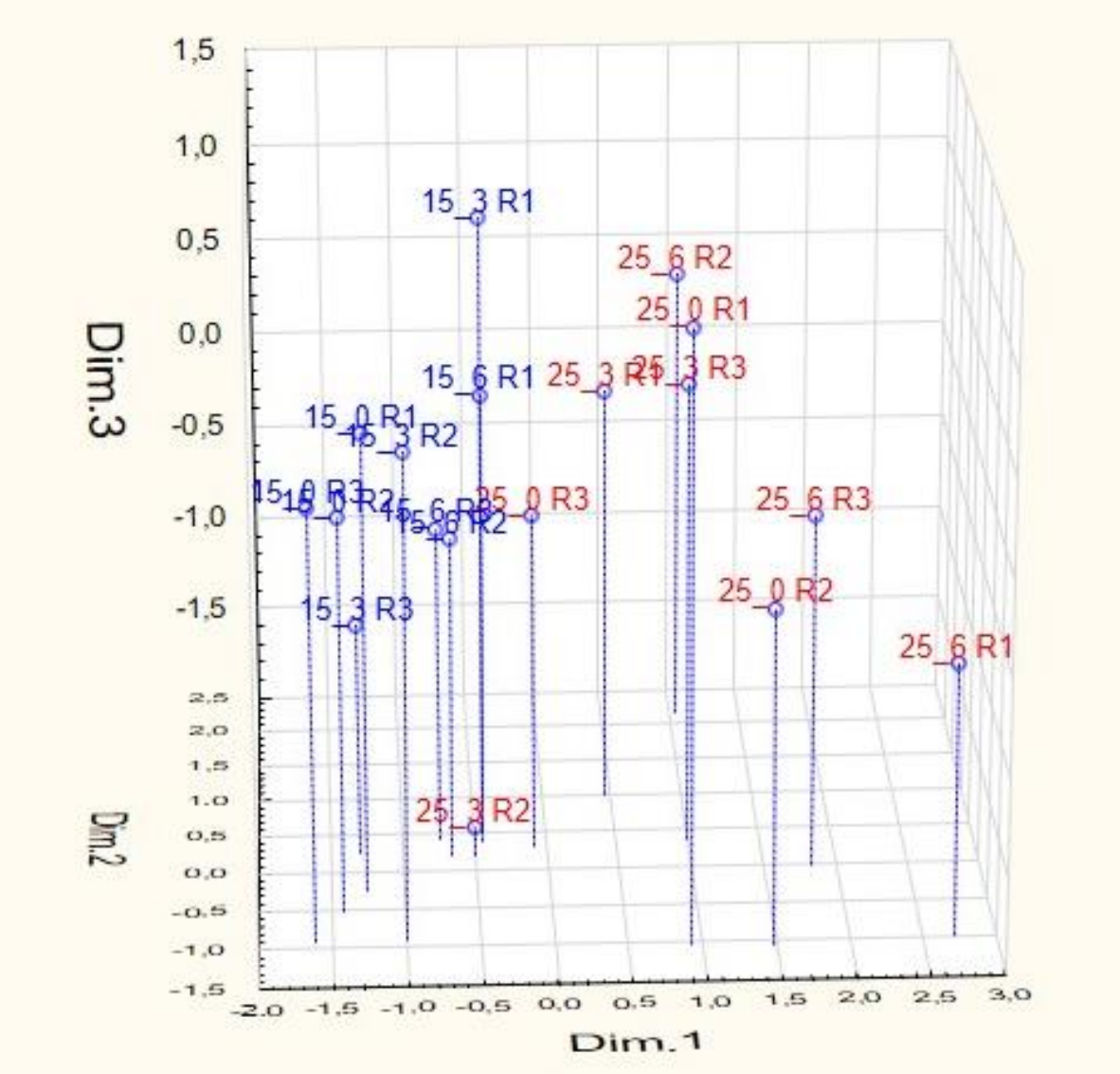

Figure 2.18 Individual sample multiple factor analysis of the Sauvignon blanc wine twelve month chemical and sensory analysis: 3D individual sample scatterplot. Blue samples designate 15°C storage and red samples designates 25°C storage. R1, R2 and R3 indicate the biological repeat. Samples are correlated along dimension 1 by storage temperature.

2.4.2 Chenin blanc

An individual sample multiple factor analysis (ISMFA) was used separately for the six and twelve month chemical and sensory data as the combined six and twelve month data sets did not yield clear patterns (results not shown).

Compared to the six month multiple factor analysis (Figure 2.19), the twelve month analysis (Figure 2.20) did not show groupings as clearly to either temperature treatments or dissolved O_2 treatments. That said, the wines did seem to correlate to storage temperature to some degree. This would make sense as there were fewer significant differences in the twelve month sensory analysis, but still certain significant differences in the chemical analysis where attributes such as free and total sulphur dioxide, yellow/brown colour, glutathione, diethyl succinate, hexanoic acid, octanoic acid and decanoic acid were still significantly different between temperature treatments, which could explain why the wines still correlated to storage temperature treatments.

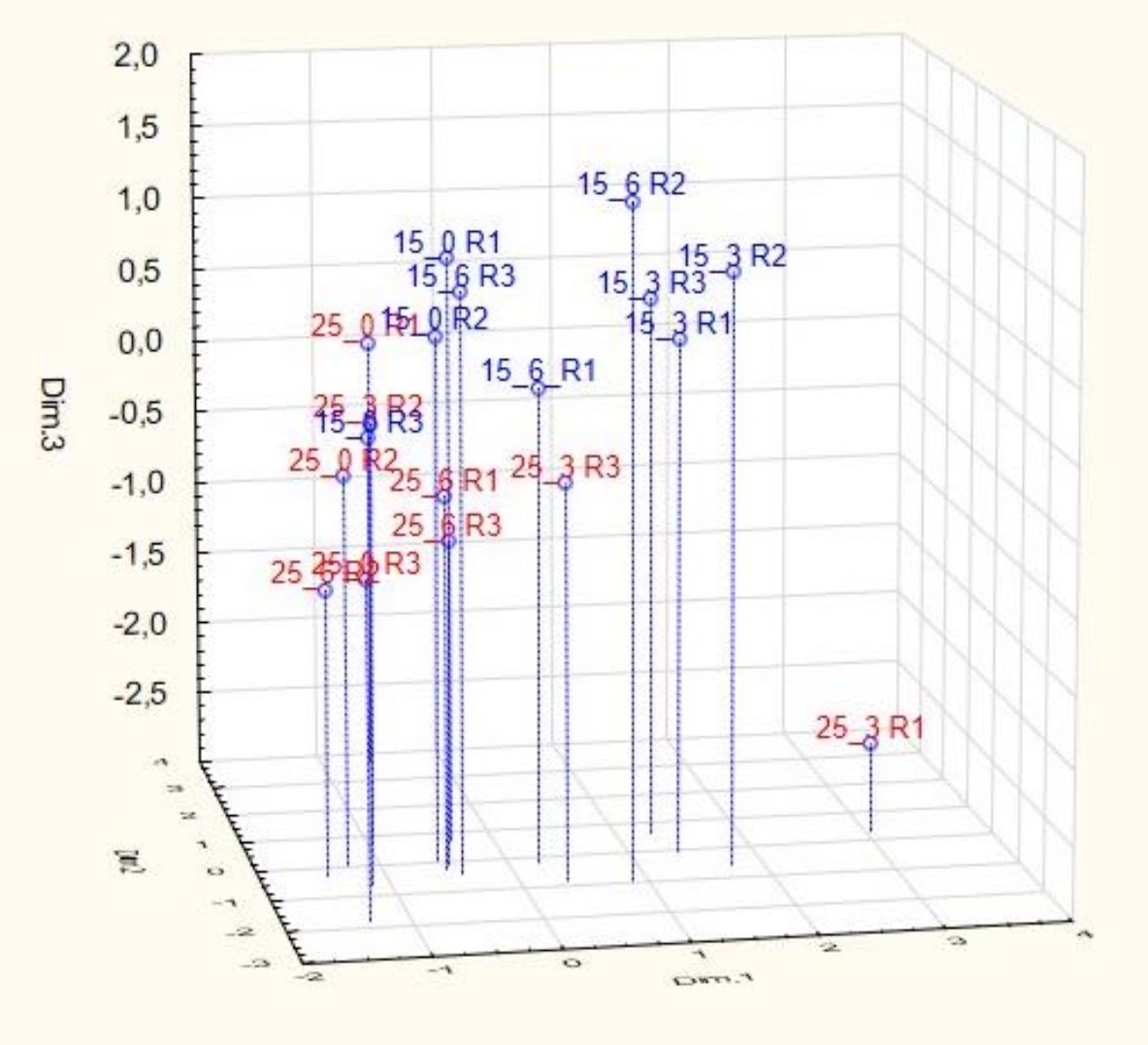

Figure 2.19 Individual sample multiple factor analysis of Chenin blanc 6 month chemical and sensory data. The 15°C storage samples are marked blue and the 25°C samples are marked red. R1, R2 and R3 indicate the biological repeat. Samples seem to be strongly correlated along dimensions 1 and 2 where both dimensions separate, to an extent, the samples by storage temperature.

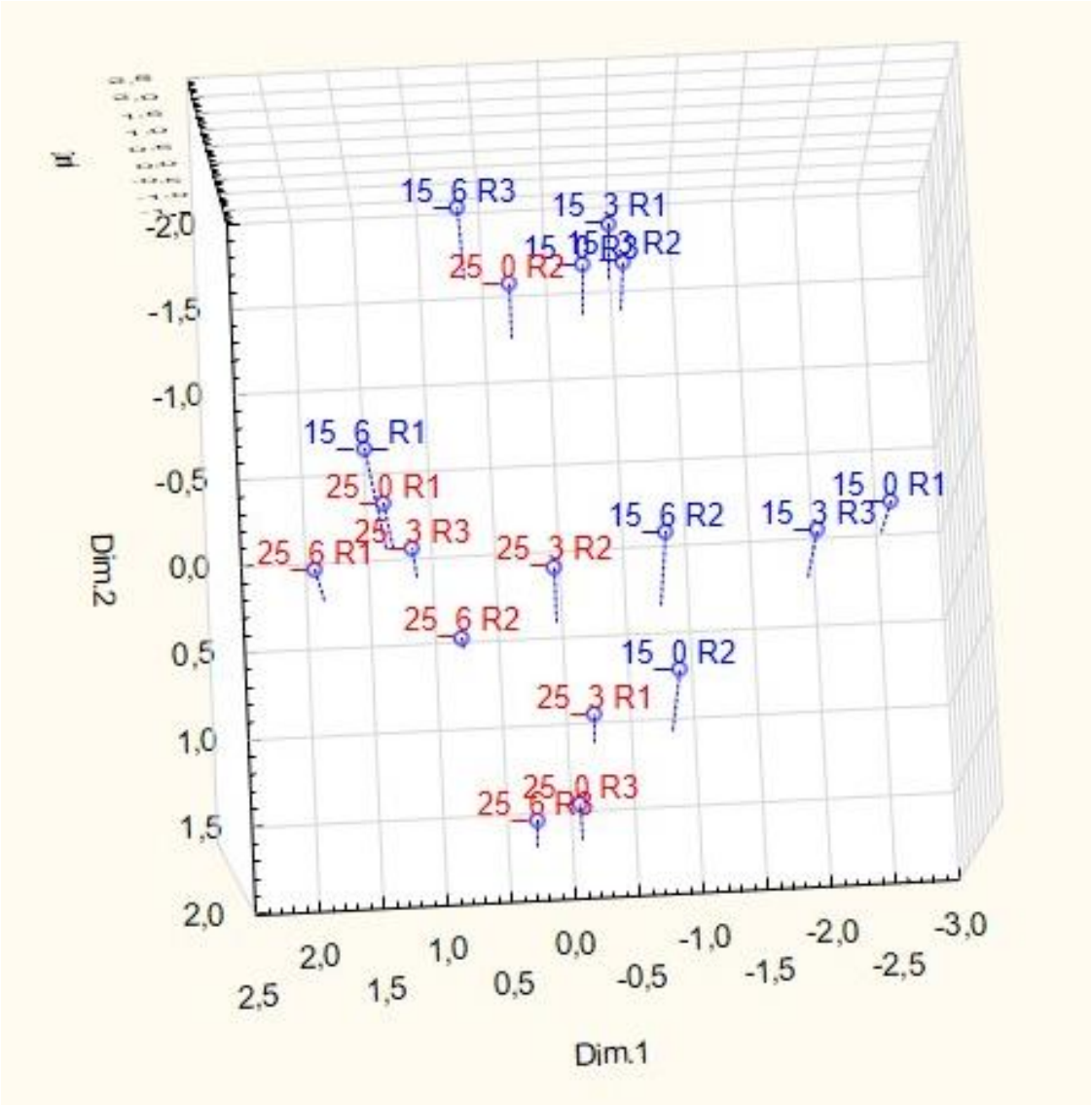

Figure 2.20 Individual sample multifactor analysis of Chenin blanc twelve month chemical and sensory data. The 15°C storage samples are marked blue and the 25°C samples are marked red. R1, R2 and R3 indicate the biological repeat. Samples correlate to storage temperature along dimension 1 and dimension 2.

2.5 Conclusion

Based on chemical and sensory evidence from both experimental cultivars, and especially the Sauvignon blanc samples, time and storage temperature had the largest effects on wine evolution with dissolved O_2 at bottling to a lesser extent. Many chemical analyses such as glutathione, brown colour and varietal thiols concentration were more affected more by time as the experiment progressed. Glutathione concentrations were significantly affected by temperature and dissolved O_2 in the Sauvignon blanc six month analysis but was then only seemingly affected by storage temperature at the twelve month analysis.

Many major volatiles such as acetate esters, fatty acids, and higher alcohols were often affected by storage temperature. Isoamyl acetate concentration was found to be in higher concentrations at 15°C storage and ethyl lactate, diethyl succinate, hexanoic acid, octanoic acid, decanoic acid were sometimes found in greater concentrations at 25°C storage temperatures.

The dissolved O_2 concentration found in these wines combined with elevated storage temperatures significantly lowered the antioxidants free and total sulphur and glutathione in both cultivars. Lowering free and total SO_2 and glutathione in white wine could have detrimental effects to white wine ageing potential as the O_2 consumption capacity is severely reduced early in a wine's life. In the twelve month analysis of this study, higher dissolved O_2 treatments (which lead to lower free and total SO_2 and glutathione concentrations) did not drastically alter sensory descriptor intensities in the Sauvignon blanc wines stored at 15°C, but did seem to influence the increased intensity of oxidative descriptors in wines stored at 25°C.

Winemakers should seek to improve bottling procedures to retain SO_2 and glutathione concentrations in white wines as oxidation characters are still viewed negatively by consumers. More importantly, winemakers should strive to protect wine from elevated temperatures during storage and bottle ageing. Despite lower antioxidant capacity, the O_2 treated wines stored at 15°C were, especially in the Sauvignon blanc wines, more similar to the control wine than the control wines stored at 25°C. Though fewer significant differences were found in the Chenin blanc wines, key differences in chemistry and sensory results were found at 6 months and had similar results to the Sauvignon blanc wines. These results seems to indicate that temperature can have a stronger effect on wine chemical and sensory attributes than dissolved O_2 concentrations at bottling.

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2.7 Addendum (Chapter 2)

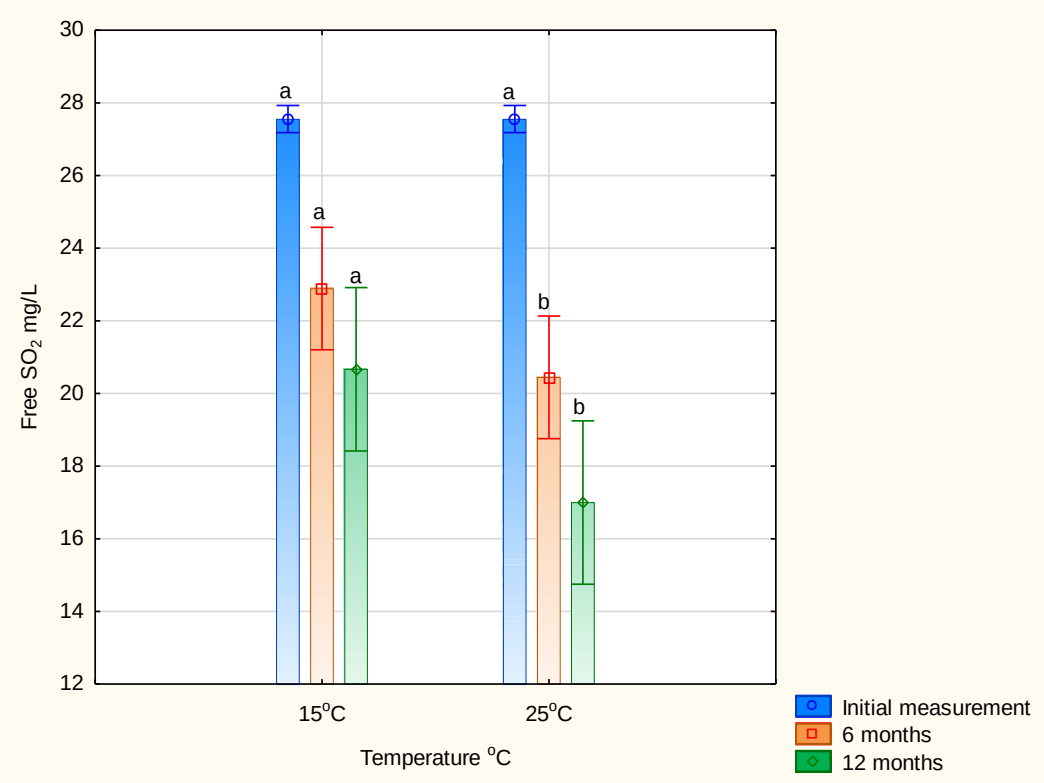

Figure 2A Free SO_2 concentrations of the Chenin blanc wine comparing the effects of storage temperature across time. All dissolved O_2 treatments were combined to corresponding storage treatments for the purpose of demonstrating the significance of the different temperatures in each sample period.

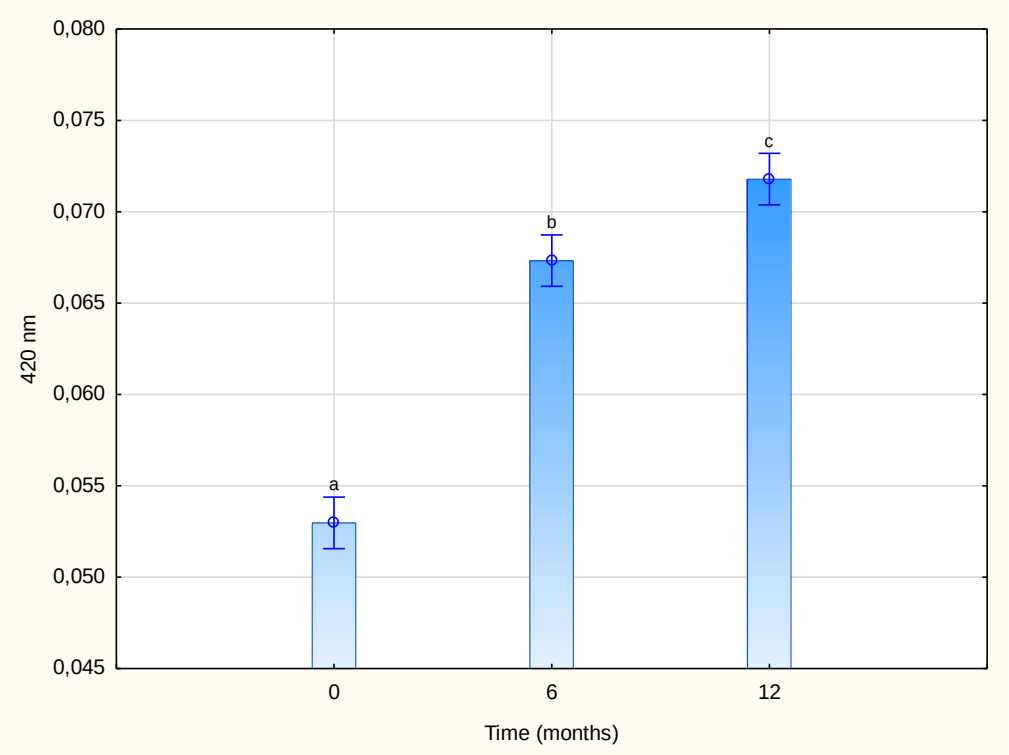

Figure 2B Measurement of yellow/brown colour absorbance at 420 nm in the Sauvignon blanc wine over time. All dissolved O_2 treatments and storage temperatures were combined.

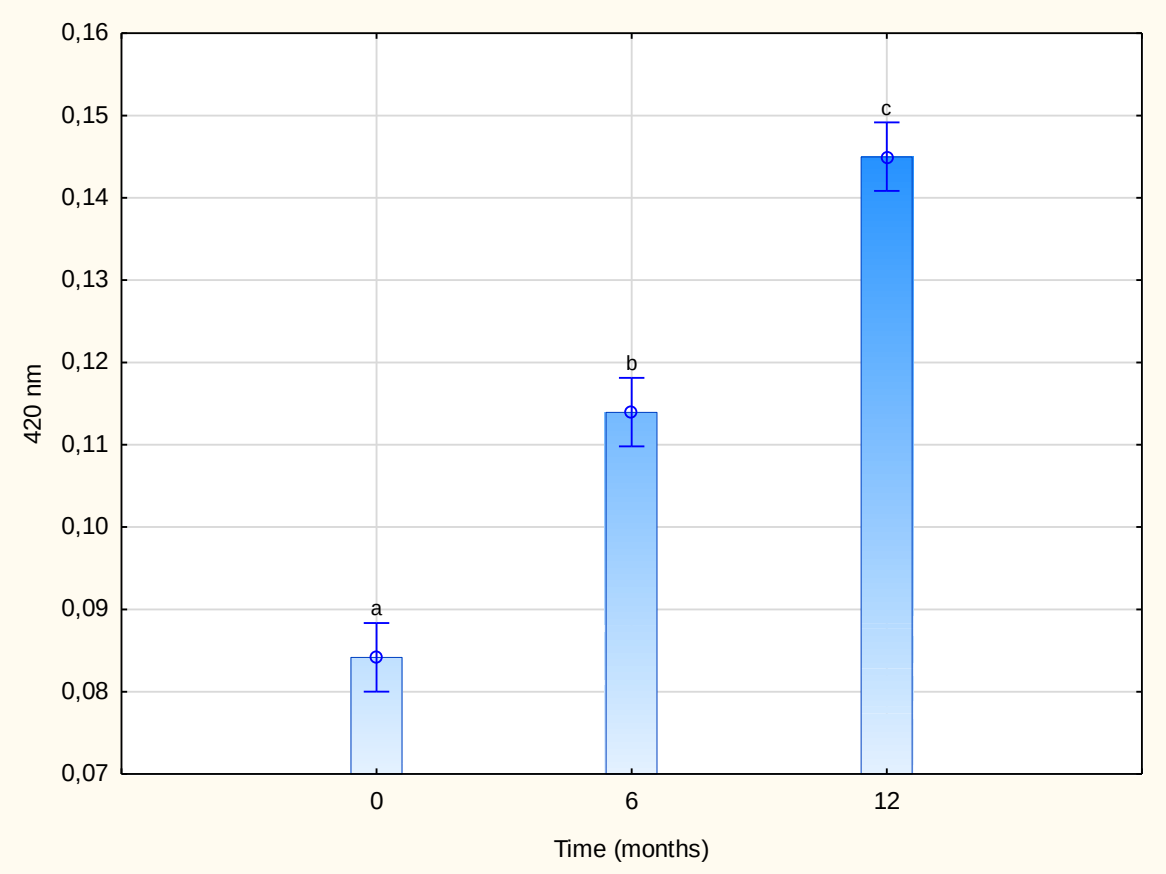

Figure 2C Measurement of yellow/brown colour in the Chenin blanc wine over time. All dissolved O_2 treatments and storage temperatures were combined.

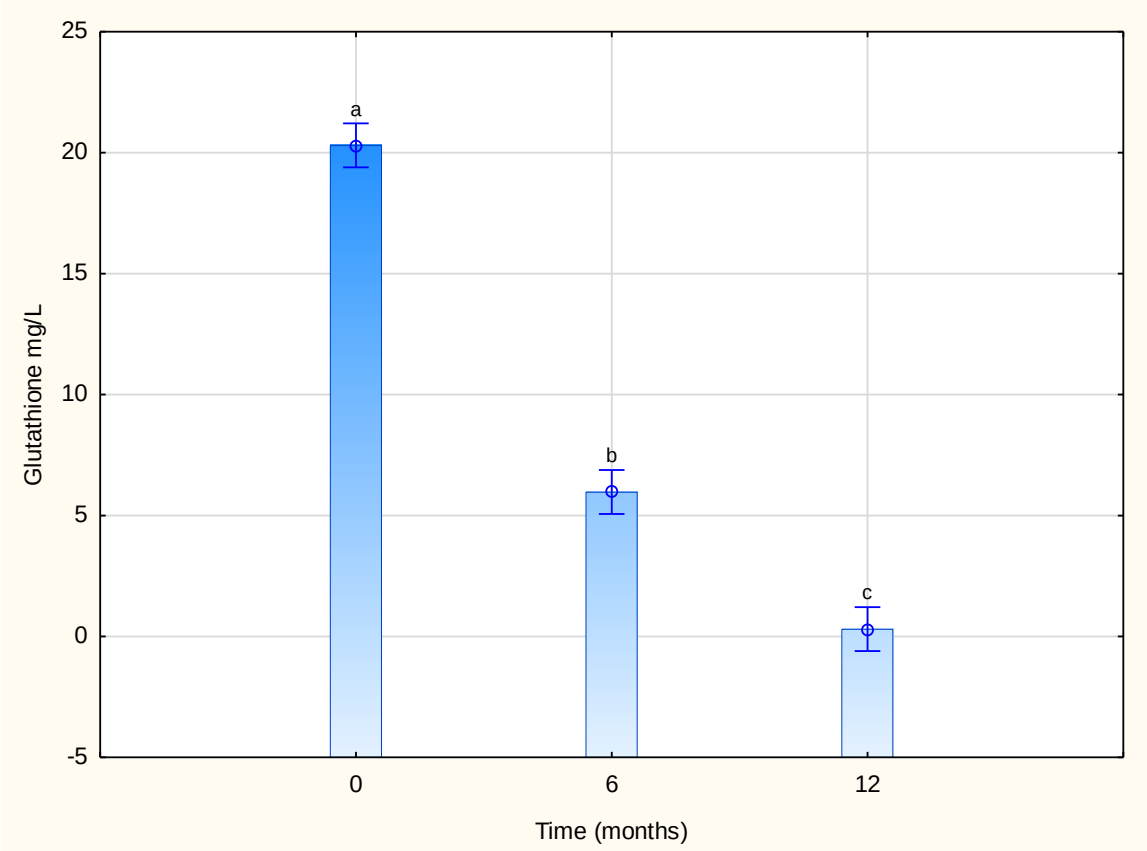

Figure 2D Glutathione concentrations in Sauvignon blanc across time. All dissolved O_2 treatments and storage temperatures were combined.

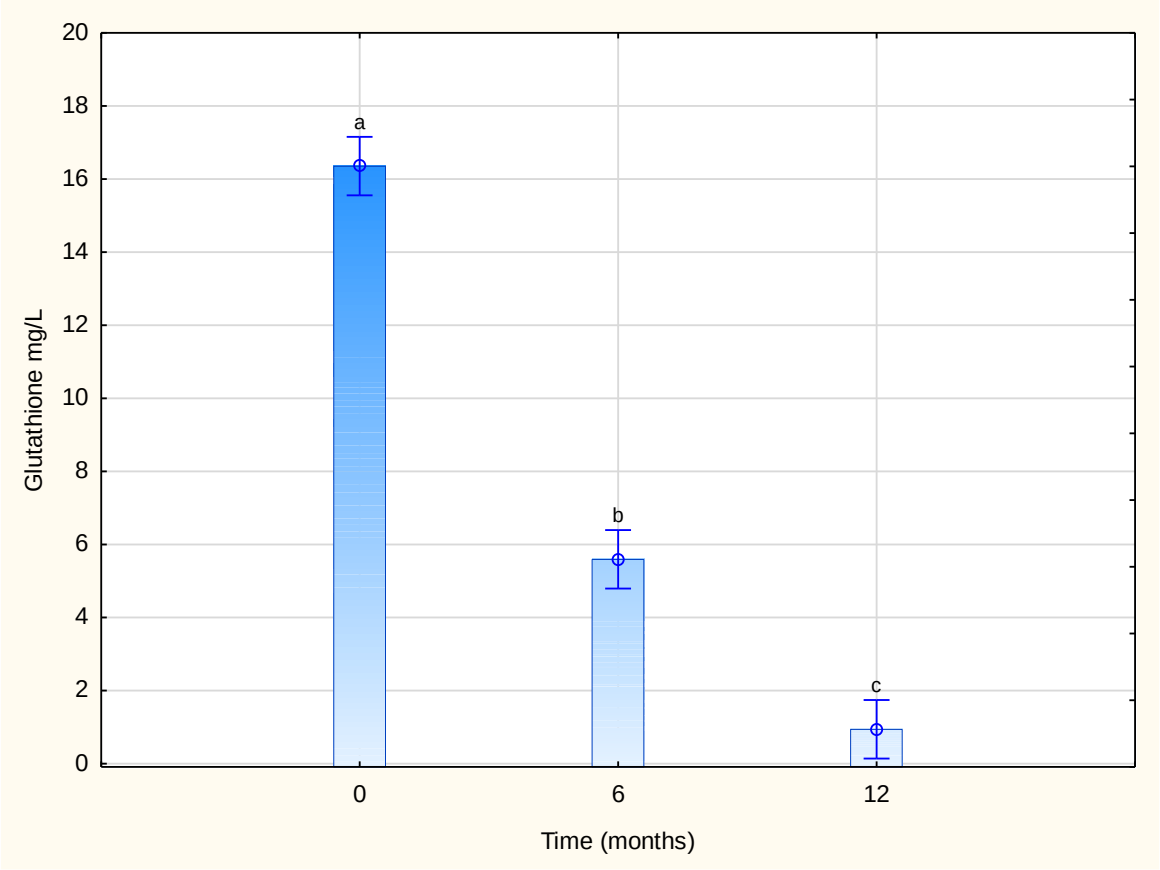

Figure2E Glutathione concentrations in Chenin blanc wine across time. All dissolved O_2 treatments and storage temperatures were combined.

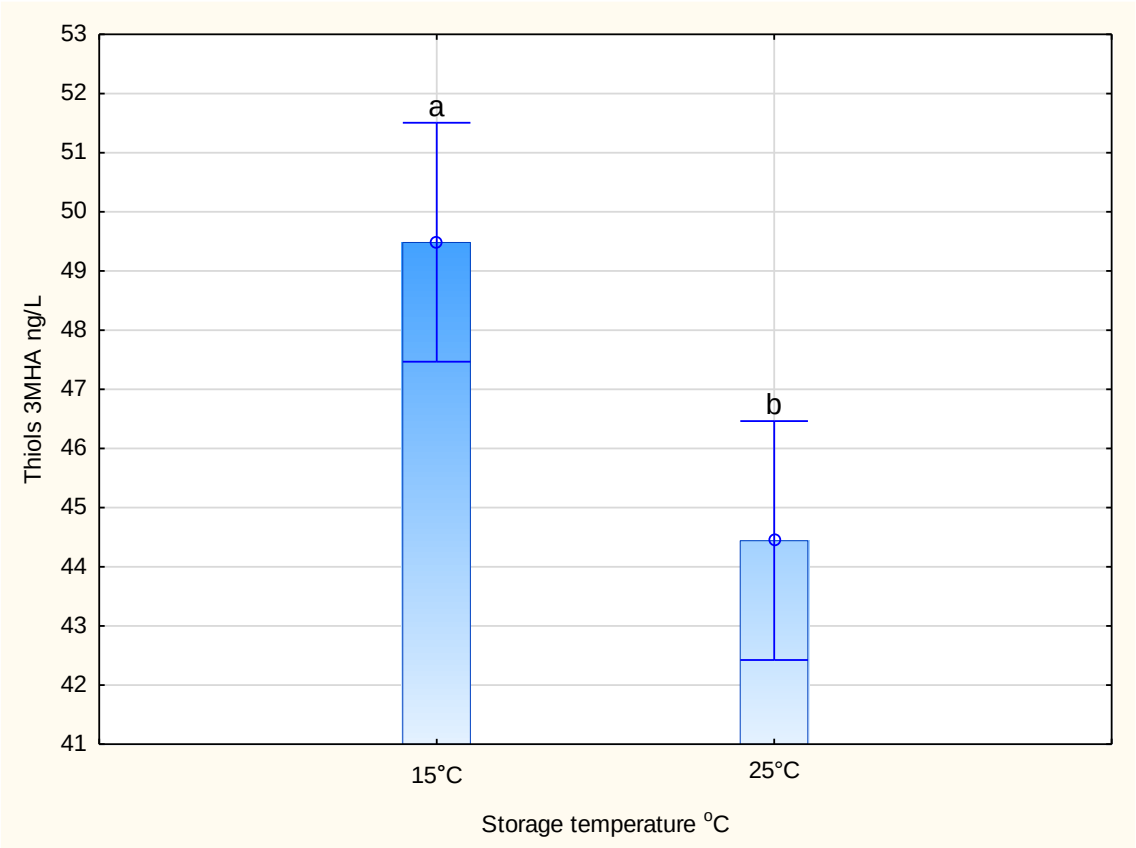

Figure 2F 3MHA concentrations in Sauvignon blanc wine at different storage temperatures at six months. All dissolved O_2 treatments are combined.

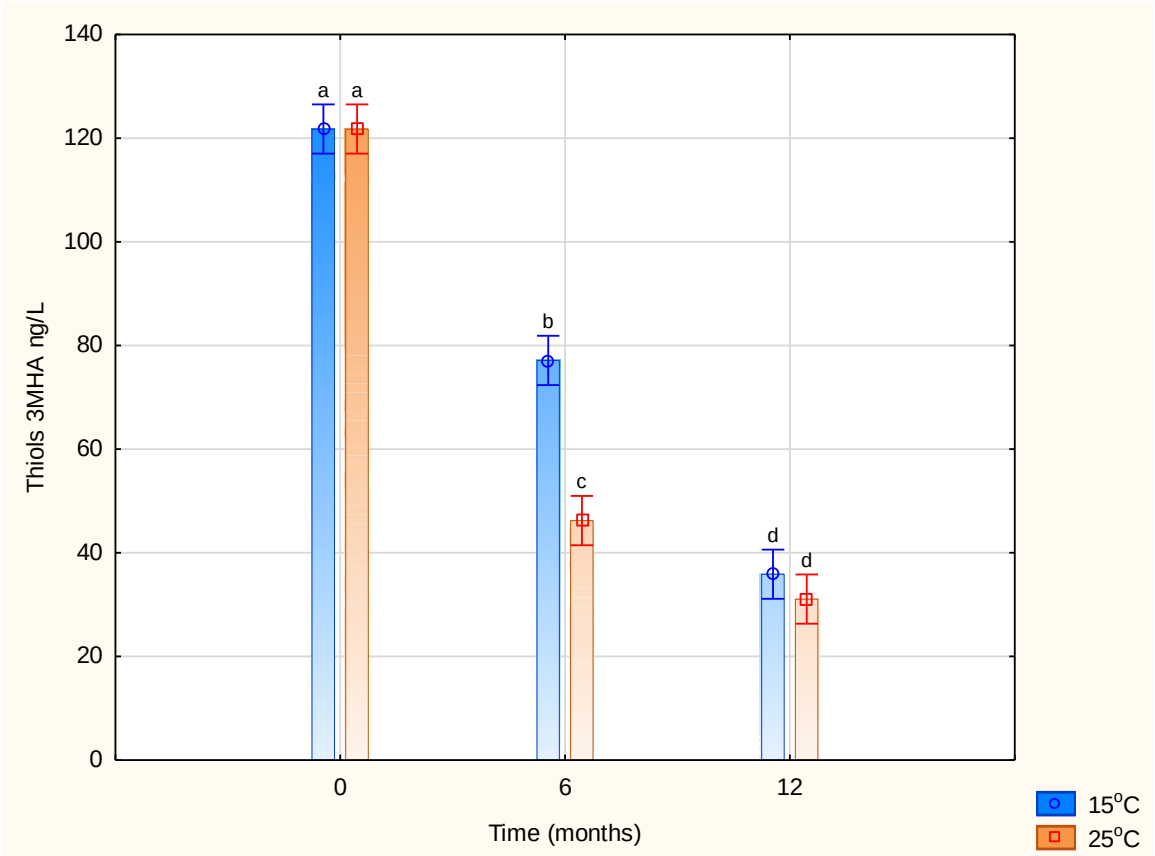

Figure 2G 3MHA concentrations in Sauvignon blanc wine at different storage temperatures across time. All dissolved O_2 treatments are combined.

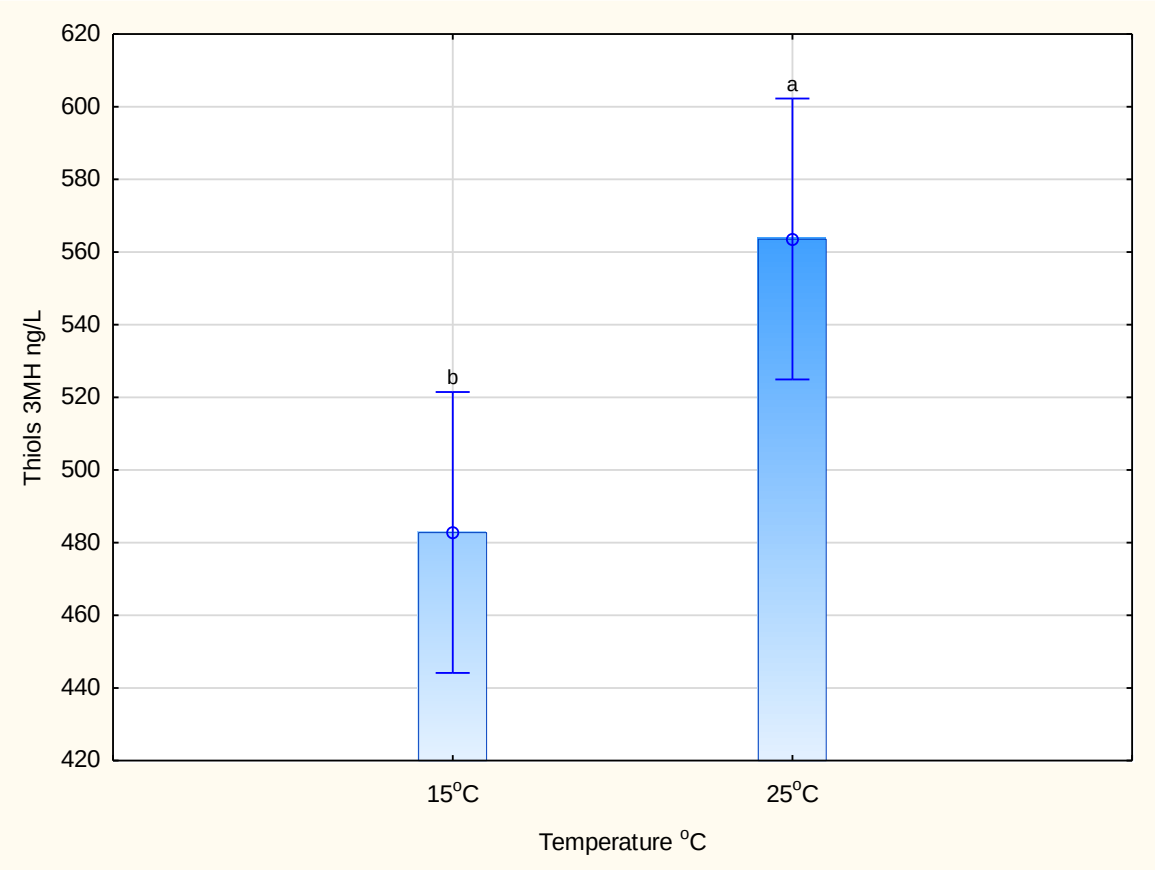

Figure 2H Effects of storage temperature on 3MH concentrations in Sauvignon blanc wines at six months. All dissolved O_2 treatments were combined.

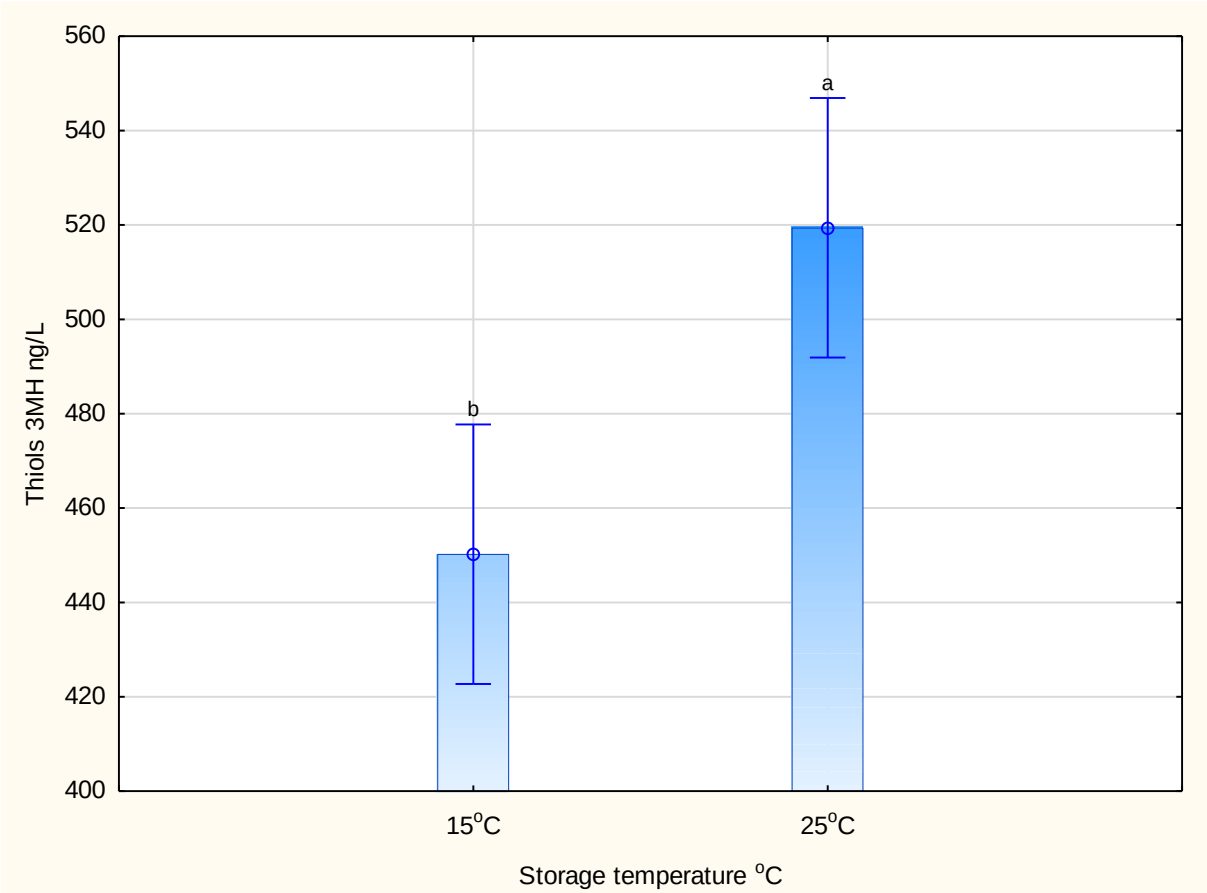

Figure 2I Effects of storage temperature on 3MH concentrations in Sauvignon blanc wines at twelve months. All dissolved O_2 treatments were combined.

Table 2A Final lists of aroma attributes and reference standards used for descriptive analysis of the Sauvignon blanc and Chenin blanc wines.

Descriptors	Standard composition	Sauvignon blanc
Passion_fruit	20 mL fresh passion fruit pulp	
Guava	¼ freshly slice guava	
Grapefruit	¼ freshly slice grapefruit	
Pineapple	¼ freshly slice pineapple	
Fresh_green	5 g freshly chopped grass	
green Apple	¼ slice grannysmith apple	
Dust/tea	1.5 g black tea "Five Roses®"	
Dried_fruit	1 piece apple, apricot, peach, prune, pear chopped (Safari)	
Baked_apple	¼ freshly baked Golden Delicious® apples	
Cooked_veg	5 mL canned green bean brine "KOO" + 5 mL canned asparagus brine "Food Lover's Signature"	

Descriptors	Standard composition	Chenin blanc
Gauva	¼ freshly slice guava	
Grapefruit	¼ freshly slice grapefruit	
Pineapple	¼ freshly slice pineapple	
Fresh_green	5 g freshly chopped grass	
Green_apple	¼ freshly slice grannysmith apple	
Hay/tea	1.5 g black tea "Five Roses®" and 3 grams dried grass	
Dried/stewed_fruit	1 piece apple, apricot, peach, prune, pear chopped (Safari)	
Baked_apple	¼ freshly baked Golden Delicious® apples	
Caramel	5 g caramel Cadbury®	
Honey	1 tsp. in 10 mL water Woolworths®	
Cooked_veg	5 mL canned green bean brine "KOO" + 5 mL canned asparagus brine "Food Lover's Signature"	

Table 2B List of the aromatic compounds found in white wine, aroma perception thresholds and attributes used to describe the various odours. Table used with permission from Coetzee, 2014.

Compound	Perception threshold	Threshold determined in	Descriptors	Reference
Volatile Thiols				
4-Mercapto-4-methylpentan-2-one (4MMP)	0.8 ng/L	12% ethanol, pH 3.5	Passion fruit, broom, black current	Darriet *et al.*, 1995
3-Mercaptohexylacetate (3MHA)	4.2 ng/L	12% ethanol, pH 3.5	Passion fruit, grapefruit, gooseberry, guava	Tominaga *et al.*, 1996
3-Mercaptohexanol (3MH)	60 ng/L	12% ethanol, pH 3.5	Passion fruit, grapefruit, gooseberry, guava	Tominaga *et al.*, 1998a
Esters				
Isoamyl acetate	0.05 mg/L	12.5% ethanol, pH3.2	Banana, fruity, sweet	Benkwitz *et al.*, 2012b
Hexyl acetate	0.4 mg/L	12.5% ethanol, pH3.2	Apple, cherry, pear, flower	Benkwitz *et al.*, 2012b
2-Phenylethyl acetate	0.25 mg/L	10% ethanol	Rose, honey, tobacco, flower	Guth, 1997
Ethyl acetate	12.3 mg/L	10% ethanol, pH 3.2	Pineapple, fruity, varnish, solvent	Escudero *et al.*, 2004
Ethyl butyrate	0.02 mg/L	10% ethanol	Acidic, fruity, apple	Guth, 1997
Ethyl lactate	146 mg/L	14% ethanol, pH3.5	Lactic, buttery, fruity	Moyano *et al.*, 2002
Ethyl hexanoate	0.014 mg/L	11% ethanol, pH3.4	Green apple peel, fruit, banana, brandy	Ferreira *et al.*, 2000
Ethyl octanoate	0.005 mg/L	11% ethanol, pH3.4	Sweet, ripe banana, pear, soapy	Ferreira *et al.*, 2000
Ethyl decanoate	0.2 mg/L	11% ethanol, pH3.4	Fruity, floral, grape, soapy, brandy	Ferreira *et al.*, 2000
Diethyl succinate	1.2 mg/L	10% ethanol, pH 3.5	Fruity, melon	Peinado *et al.*, 2004
Acids				
Acetic acid	300 mg/L	10% ethanol, pH 3.2	Vinegar	Escudero *et al.*, 2004
Propionic acid	8.1 mg/L	11% ethanol, pH3.4	Rancid, pungent, soy	Ferreira *et al.*, 2000
Butyric acid	0.173 mg/L	11% ethanol, pH3.4	Rancid, cheese, sweat	Ferreira *et al.*, 2000
Isobutyric acid	2.3 mg/L	11% ethanol, pH3.4	Acidic	Ferreira *et al.*, 2000
Isovaleric acid	0.033 mg/L	11% ethanol, pH3.4	Blue cheese	Ferreira *et al.*, 2000
Hexanoic acid	0.42 mg/L	11% ethanol, pH3.4	Sweat, cheesy, fatty	Ferreira *et al.*, 2000
Octanoic acid	0.50 mg/L	11% ethanol, pH3.4	Sweaty, rancid, harsh, fatty	Ferreira *et al.*, 2000
Decanoic acid	1.00 mg/L	11% ethanol, pH3.4	Rancid, fatty	Ferreira *et al.*, 2000
Alcohols				
Propanol	306 mg/L	10% ethanol, pH 3.5	Alcohol, ripe fruit	Peinado *et al.*, 2004
Butanol	150 mg/L	10% ethanol, pH 3.5	Fusel odour, medicinal	Peinado *et al.*, 2004
Isobutanol	40 mg/L	10% ethanol	Fusel, alcohol, nail polish	Guth, 1997
Isoamyl alcohol	30 mg/L	10% ethanol	Whiskey, malt, burnt	Guth, 1997
Hexanol	8 mg/L	10% ethanol	Grassy, green, resin, flower, woody	Guth, 1997
Phenylethanol	14 mg/L	10% ethanol, pH3.5	Honey, spice, rose, lilac	Peinado *et al.*, 2004
Methionol	1 mg/L	10% ethanol, pH3.5	Cauliflower, cooked cabbage, sweet, potato	Peinado *et al.*, 2004

Chapter 3: The effects of sparging on the dissolved gasses and chemical composition of wine

3.1. Introduction

The previous chapter demonstrated how dissolved oxygen (O_2) concentrations found in South African white wines can significantly affect sulphur dioxide concentrations and to lesser extent, contribute towards oxidized aromas. As these effects can be undesirable, winemakers can seek to remove dissolved O_2 from white wines using inert gas sparging techniques.

Though sparging operations are common in the wine industry, factors affecting sparging have been scarcely investigated. Practical work examining variables that can potentially alter sparging efficacy in wine have been investigated to a limited extent (Wilson, 1986). Though pioneering, the methodology from this work has become dated and does not provide crucial experimental parameters, thereby failing to provide critical knowledge for industry professionals. Accordingly, several books and guidelines have been produced by research institutions and industry professionals broaching the topic of sparging, but without providing in-depth details regarding effects of sparging on wine chemical composition (Bird, 2011). That said, the nebulous consensus is that sparging with nitrogen (N_2) gas can remove dissolved O_2 along with other dissolved gases, particularly carbon dioxide (CO_2) (Wilson, 1986; Zoecklein *et al.,* 1995; Bird, 2011).

A study published in The Australian Grapegrower & Winemaker in 1986, was instrumental as a resource to communicate current research on sparging in wine at the time (Wilson, 1986). However, the study failed to report the exact gas flow rates used. The current research results discussed in this chapter, ensured to include the exact flowrates (mL gas/L of wine/minute), therefore allowing for precise interpretation of results as well as reproducibility of the experiments. Unlike the study of Wilson (1986), where wine was being transferred whilst being sparged, this study used static wine held in tanks. The current study also included replicates for calculating significant differences between the treatments.

Furthermore, research into sparging has, thus far, focused on understanding the influence of various parameters on the efficacy of removing dissolved gases during sparging. The potential effects of sparging on the concentration of volatile aromatic compounds remain unknown, however, some speculate that sparging processes can inadvertently remove aroma compounds (Bird, 2011). “The danger with sparging, as with so many other wine treatments, is that it can easily be over-used…It will remove anything volatile and flavour components are by their very nature volatile…” (Bird, 2011).

According to Henry’s Ideal gas laws, an inert gas passing through a liquid would create a partial pressure difference between volatile compounds and the inert gas (Bird, 2011; Lyons *et al.,* 2015). The difference in partial pressures could cause the volatile compounds to equilibrate,

transferring aromatic compounds from the liquid medium into the air space. Therefore, it is possible for volatile compounds to be removed by sparging from wine, however, the significance of potential losses needs to be qualified and quantified. There is thus no published research, that we know of, investigating the direct effects of inert gas sparging on the concentration of aromatic compounds in wine. Given the widespread application of sparging in the wine industry, this warrants further investigation.

In the current study, exploratory experiments were conducted by sparging a constant, specified volume of Chenin blanc and Sauvignon blanc wine with N_2 and a N_2/CO_2 mixed gas. Variables such as the flow rate, gas composition, duration of sparging, number of sparging treatments, wine temperature and the utilization of a diffusion stone, were evaluated to determine the efficacy of removing dissolved gasses as well as the effect on the wine chemical composition.

3.2 Materials and Methods

3.2.1 Wine Samples

A Chenin blanc wine (vintage 2018) was obtained from Brandvlei Cellar (Breede River Valley, South Africa), a Sauvignon blanc wine (vintage 2019) was collected from Kleine Zalze Wine Estate (Stellenbosch, South Africa). The grapes were harvested by hand when considered ripe for commercial harvesting. These wines were made according to the respective wineries' standard practices. Both wines had been stabilized at the respective wineries and were ready for bottling. The v/v% alcohol, total acidity, pH and residual sugar information for these wines obtained using the WineScan FT 120 instrument (FOSS Analytical, Denmark) (Nieuwoudt, *et al.* 2006).

The wines were collected in 20 L kegs filled with N_2 and stored at -4°C. The wines (separate trials) were then transferred into a 1000 L stainless-steel tank, also previously filled with N_2 gas, prior to distribution into the bioreactors (see section 3.2.3). A 15 % SO_2 solution was added to the wines before experimentation to increase free SO_2 to 30 mg/L (refer to section 3.2.5.2).

3.2.2 Gases and diffusion stone

All gases used in the study were obtained from Afrox, South Africa. Prior to transferring the wine, all transfer lines, bioreactors and sample bottles were flushed with commercial N_2 (99.8% pure) to remove the O_2 (<0.3 mg/L atmospheric O_2). After filling the bioreactor, the wines were sparged with medical grade oxygen (99.8% pure) (where applicable) to increase the dissolved O_2 to 3 mg/L. For the sparging of the wine, N_2 and a mixed gas (Aligal 13) consisting of 70% N_2 and 30% CO_2 (99.8% pure), was used. A stainless steel diffusion stone with 15 µm pore size obtained from Wine Machinery (Stellenbosch, South Africa) was used to sparge the wine with

the gas. The gas flow-rate was monitored using a M-Gas Mass Flow Meter from Alicat (Duiven, Netherlands).

3.2.3 Bioreactor tanks

Four custom-built stainless-steel tanks (designated as bioreactors, Figure 3.1) were designed to hold 65 L of wine. Each bioreactor was fitted with a temperature probe, a cooling jacket, a diffusion stone connected to a gas inlet, an automated homogenising mixer and optical oxygen sensors. The tanks were sealed with a rubber gasket fitted to a stainless-steel lid. An automated pressure release valve from Alicat (Duivan Netherlands) was fitted to each lid to manage internal pressure during sparging operations. The automated homogenising mixer in each bioreactor operated at a rate of 45 rpm.

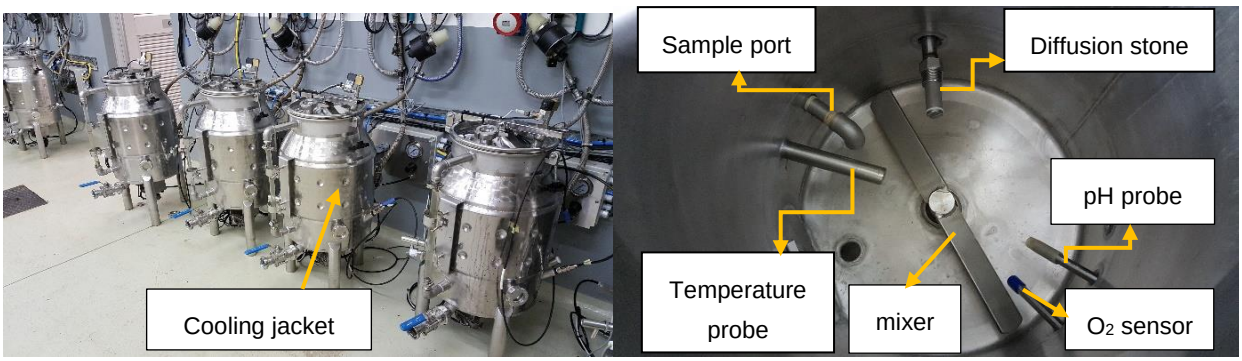

Figure 3.1 Exterior and the interior of bioreactors.

3.2.4 Sampling procedure

Sampling took place after the initial wine transfer into the bioreactor (before any sparging treatment), after O_2 additions and after sparging with N_2 or a mixed gas. Prior to sampling, 750 mL sample bottles were filled with nitrogen gas to remove O_2 from the bottle. Samples were drawn from a sampling port on the bioreactor with a plastic pipe that allows for the gentle flow of the wine into the bottles. No headspace was present in the 750 mL sample bottles after sampling and the bottles sealed with screw caps. The bottles were stored at -4°C for 1 day after which they were transferred into smaller sample containers, also previously filled with N_2 gas. A 100 mg/L SO_2 was added to samples for varietal thiol analyses (as well as major volatile analyses in the case of the Sauvignon blanc) and 100 mg/L SO_2 and 500 mg/L ascorbic acid were added to samples for glutathione analysis then stored at -20°C for future analysis. Free and total SO_2, colour, and dissolved CO_2 were measured on the same day as sample separation.

3.2.5 Chemical analysis

3.2.5.a Free and total sulphur dioxide analysis

Free and total SO_2 were determined by titration (Ripper method) as described in the OIV method: OIV-MA-AS323-04B: R2009 using a Metrohm 862 Compact Tritrosampler (program version 5.862.0024) (Herisau Switzerland).

3.2.5.b Colour analysis

Colour analysis of wine was conducted using a Thermo Fisher Scientific Multiscan Go spectrophotometer (Vantaa, Finland) coupled with a computer equipped with Skanit RE (version 5.0) software. Spectrophotometer measurements were standardized to a 0.2 mL cell. Yellow/brown colour (420 nm) was measured as an indicator of oxidative browning (Singleton, 1976). Samples were measured in triplicate.

3.2.5.c Glutathione analysis

The quantification of reduced glutathione was carried out by ultra-pressure liquid chromatography (UPLC) with a UV detector, as described by Fracassetti *et al.,* 2011. Sample preparation required an ascorbic acid (500ppm) and SO_2 (1000ppm) addition to a 1 mL wine sample. After, a short centrifugation (10,000 rpm for five minutes) was carried out after which derivatisation was done using p-benzoquinone before analysis.

3.2.5.d Varietal thiols analysis

Three varietal thiols, 3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA), and 4-mercapto-4-methylpentan-2-one (4MMP) were analysed according to the method published by Coetzee *et al.,* (2018). The method used a liquid-liquid extraction, followed by propiolate derivatization and concentration of the samples before injecting into the gas chromatography-mass spectrometer (GC-MS/MS).

3.2.5.e Major volatiles (ester, acid and alcohol) analysis

Major volatiles consisting of esters, fatty acids and alcohols, were analysed by Gas Chromatography Flame Ionization Detector (GC-FID) using a high-throughput in-house method. The sample preparation consists of the extraction of a 5 mL sample (with 100 µL 0.5 mg/L 4-methyl-2-pentanol as internal standard) with 1 mL diethyl ether (sonicated for 5 minutes). The extract is centrifuged for 3 minutes at 4000 rpm, and the subsequent supernatant is dehydrated with Na_2SO_4 (Merck, 99%) before injecting in duplicate. Details of the method validation are described in Louw, 2007. This analysis was only performed on the Sauvignon blanc wines due to limited availability of analysis.

3.2.5.f Oxygen

Oxygen in the bioreactors was measured with the PreSens Electro-Optical Module for Oxygen (EOMO) (PreSens GmbH, Regensburg, Germany). The EOMO measurement probe was placed

in four bioreactor tanks for the measurement of atmospheric O_2 before wine transfers. After wine transfers, the EOMO was submerged into the wine for the measurement of dissolved O_2 in mg/L. Dissolved O_2 in bottled wine was measured with the NomaSense O2 P300 oxygen meter (Normacorc, Thimister, Germany) coupled with a Pst3 fibreoptic sensor, digital temperature sensor and n2.0.1.1. firmware. The measurement range for the Pst3 oxygen sensor was given by the manufacturer to be 0-22 mg/L for dissolved O_2 and 0-500 hPa for gaseous and dissolved O_2.Oxygen measurements were performed in empty bottles filled with O_2 or CO_2 or ambient air prior to bottling and sampling, where the presence and absence of O_2 was confirmed. The sampling process was also validated by measuring the dissolved O_2 of a selection of samples after the sampling process. The results confirmed the efficiency of the sampling procedure in preventing O_2 dissolution (results not shown).

3.2.5.g Dissolved carbon dioxide

Dissolved CO_2 was monitored using a Carbodoseur (Dujardin-Salleron laboratories, Noizay, France). Wine (100 mL) is sampled from the bioreactor into a graduated cylinder and sealed with a cap. A narrow tube extends through the cap to near the base of the cylinder. The cylinder is shaken to agitate dissolved CO_2 while keeping the cap and narrow tube closed. Immediately after sample agitation, the cylinder is placed vertically, and the tube is uncovered. The agitated dissolved CO_2 gas entering vapour phase increases the internal pressure in the cylinder until wine is expelled through the tube, ceasing when internal pressure equalises with atmospheric pressure. This process is repeated until no wine is expelled from the cylinder. The remaining wine volume and temperature are measured to calculate the level of dissolved CO_2 in mg/L on a correlational table (appendix) as described in Vidal, 2011.

3.2.5.h Statistical analysis

Categorical factors were analysed with one-way analysis of variance (ANOVA) and the significance threshold was set at $\alpha=0.05$. The most conservative post-hoc test, Bonferroni, was utilized for all chemical analysis. A full parameter logistic graph curve was calculated for each sparging treatment. One of the parameters calculated was the slope parameter which was used in part to represent the rate of dissolved O_2 removal. Statistica (data analysis software system) version 13.5.0.17 from TIBCO Software Inc. (Palo Alto, California) was used for all statistical analysis.

3.3 Experimental details of sparging experiments

3.3.1 Testing the effect of wine temperature and gas flow rate during sparging

Four bioreactors (duplicate treatment) were filled with 40 L of Chenin blanc wine and kept at 18°C. Oxygen was then sparged into the wine until 3 mg/L of dissolved O_2 was achieved.

Immediately after reaching the required dissolved O_2, the wine was sparged with N_2 using a 15 µm diffusion stone. Two flow rates of N_2 were tested; 120 mL N_2/L of wine/minute and 280 mL N_2/L of wine/minute. N_2 gas sparging ceased once the dissolved O_2 level reached < 0.3 mg/L. The dissolved O_2 was measured and automatically recorded by the O_2 meter every 3 seconds during the sparging process. Sample collection protocol is specified in section 3.2.4. The entire process was repeated at 10°C.

3.3.2 Testing the effects of mixed gasses during sparging

Two bioreactors (duplicate treatment) were filled with 40 L of Chenin blanc wine and kept at 18°C. The wine was then sparged with O_2 until 3 mg/L of dissolved O_2 was achieved. A mixed gas of 70% N_2 and 30% CO_2 was sparged into the wine using a 15 µm diffusion stone at a rate of 120 mL gas/L of wine/minute until the dissolved O_2 level dropped to below 0.3 mg/L. This experimental process was subsequently repeated at 10°C. Sample collection protocol is specified in section 3.2.4.

3.3.3 Testing the effect of a diffusion stone during sparging

Two bioreactors (duplicate treatment) were filled with 40 L of Chenin blanc wine and kept at a temperature of 18°C. The wine was then sparged with O_2 until 3 mg/L of dissolved O_2 was achieved. In the control treatment, N_2 was sparged into the wine using a 15 µm diffusion stone at a rate of 120 mL gas/L of wine/minute until the dissolved O_2 level dropped below 0.3 mg/L. This experimental process was then repeated without the use of the 15 µm diffusion stone with gas freely flowing from the open-ended pipe (also at a flow rate of 120 mL N_2/L of wine/minute). The sparging duration was equal to the that of the control treatment with the sparging stone. The sample collection protocol was identical to the protocol specified in section 3.2.4.

3.3.4 Testing the effect of repetitive sparging

Three bioreactors (triplicate treatment) were filled with 40 L of Chenin blanc wine and kept at 18°C. The dissolved O_2 in the wine was raised to 3 mg/L. N_2 gas was sparged into the bioreactors at a rate of 120 mL N_2/L of wine/minute with a 15 µm diffusion stone until the dissolved O_2 level reached 0.3 mg/L. This process was repeated four times in total alternating the sparging of O_2 and N_2. The sampling protocol was identical to the protocol specified in section 3.2.4, where samples were collected before and after each O_2 and N_2 sparging treatment.

3.3.5 Testing the effect of extended sparging times

Three bioreactors (triplicate treatment) were filled with 40 L of Sauvignon blanc wine and kept at 18°C. In this treatment, no O_2 was added prior to the sparging of the inert gas. N_2 gas was sparged into the bioreactors with a 15 µm diffusion stone at a rate of 120 mL gas/L of wine/minute for 68 minutes. After the initial eight minutes of sparging, a sample was taken for

analyses after which sparging continued for an additional 60 minutes. The sample collection protocol was identical to the protocol specified in section 3.3.4.

3.4 Results and discussion

3.4.1.a Analyses prior to treatment

Both the Chenin blanc and Sauvignon blanc were chemically analysed before experimentation, the results are listed in the Addendum as Table 3A. Only the Sauvignon blanc was analysed for esters, fatty acids, and higher alcohols due to availability of the analysis only occurring during the time frame of that experiment. The initial dissolved CO_2 concentrations found in the Chenin blanc base wine before each experiment did lower slightly over the time of the experiments, but in the worst case, was only 50 mg/L less than the first analysis.

3.4.1.b Sparging flow rate and wine temperature

Figure 3.2 shows the average dissolved O_2 concentration over time when sparged at different flow rates at temperatures of 10°C and 18°C, respectively. Wilson (1986) tested the efficacy using a flow rate of 100 mL N_2/L wine/minute and reported no improvement when using higher flow rates. For the current study, a flow rate of 120 mL N_2/L of wine/minute was chosen in order to maintain a flow rate above 100 mL N_2/L of wine/minute (flow rates periodically fluctuated during sparging). The flow rate of 280 mL N_2/L of wine/minute of wine was selected for this was the highest flow rate the sparging system could maintain.

There was no significant difference in the rate of O_2 removal (the regression slopes of the graph) between the two flow rates tested. This was seen at both 10°C and 18°C. This result is in part supported by the findings of Wilson (1986) where sparging efficiency ceased to improve above certain flowrates. This could be due to a saturation of the inert gas in the wine, where after a certain ratio of gas to liquid is reached the surface area of the gas per litre of wine diminishes (Lyons *et al.,* 2015). Additionally, the static sparging experimental system herein is probably more efficient than in commercial settings due to the automated homogenising mixer, small volumes and high flowrate compared to industry practices. As there was no improvement to sparging efficacy after the flow rates were more than doubled, additional studies should thus be conducted at lower these and lower than measured flow rates to evaluate the optimal flow rate for efficient O_2 removal (saving time and resources).

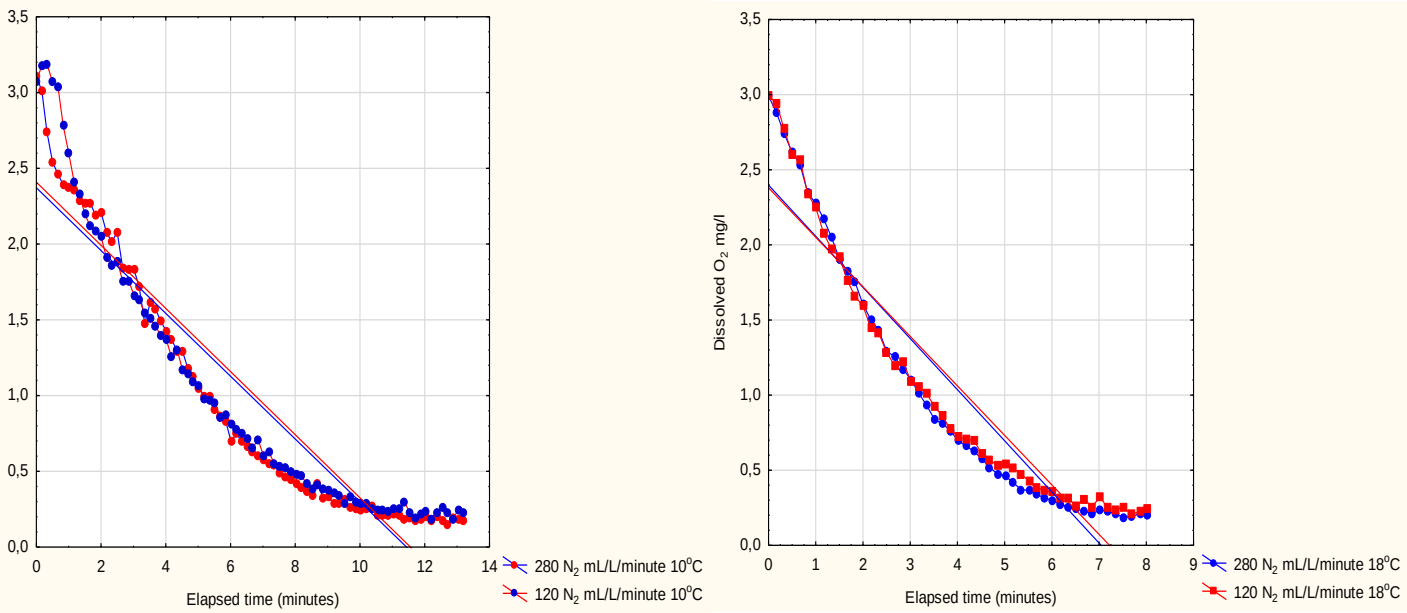

Figure 3.2 The average level of dissolved O_2 at two different flow rates of N_2 sparging at 10°C (left) & 18°C (right) over time. The straight lines represent the slope of the regression curve for each flow rate.

Significant differences in sparging efficacy were found between the different temperatures tested. The slope of the regression curve of dissolved O_2 removal at 18°C was significantly higher (0.59) compared to when the wine was at a temperature of 10°C (0.42) (Figure 3.3). This is supported by the results reported by Wilson (1986) which showed an increase in sparging efficacy as the wine temperature increased from 0°C to 30°C.

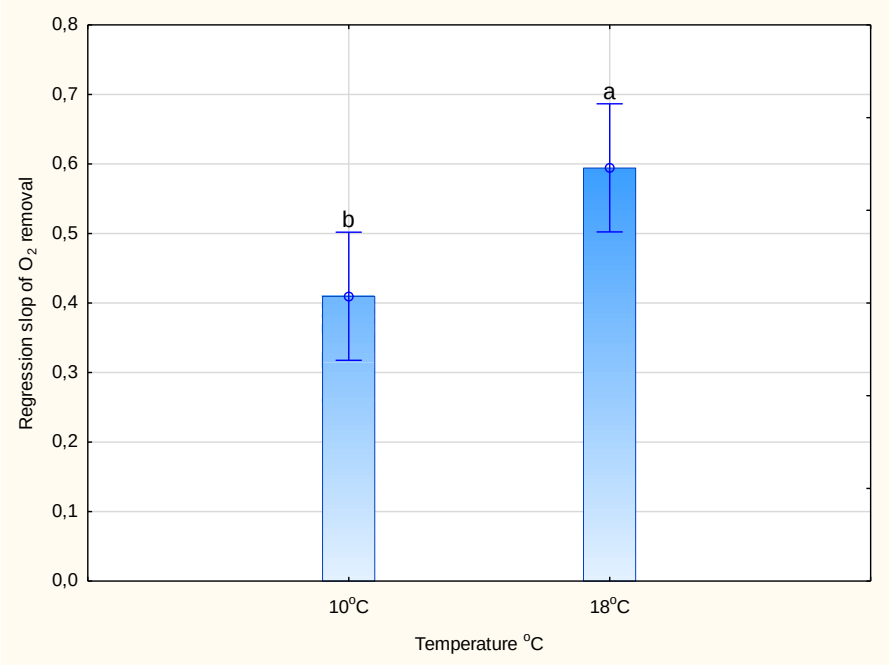

Figure 3.3 Comparing the slopes of dissolved O_2 removal for sparging at 10°C and 18°C. All flow rates are included in the analysis.

These results are in accordance with Henry's Ideal gas laws where the solubility ofgases in solution decreases as temperature increases (Agabaliantz, 1963; Lyons *et al.,* 2015). For the purpose of sparging, this means that as the temperature increases, the difference in the partial pressure value required for the expulsion of dissolved O_2, decreases, resulting in faster removal of dissolved O_2. The practical implication of these results is that when flow rate is constant, more time and therefore more sparging gas is required to remove the same amount of dissolved O_2 when wine is at 10°C compared to 18°C (Table 3.1).

Table 3.1 The average time and N_2 volume needed to remove 80% and 90% (ending at 0.3 mg/L) dissolved O_2 from 40 L wine at 10°C and 18°C (under the specific experimental conditions).

80% of dissolved O_2 removal			90% of dissolved O_2 removal		
Temperature	Avg. Time (minutes)	Volume N_2 (L)	Temperature	Avg. Time (minutes)	Volume N_2 (L)
10°C	6.94	27.7 L	10°C	9.36	37.4 L
18°C	4.60	18.4 L	18°C	7.27	29.1 L

Sparging the wine with inert gasses will not only affect the dissolved O_2 concentration, but it could also alter the concentration of other gases present in the wine. The effects of the treatments on the removal of dissolved CO_2 are shown in Figure 3.4. The average initial CO_2 concentration in the wine was 1067 mg/L for 18°C and 1045 mg/L for 10°C, which was not significantly different. The concentration of dissolved CO_2 did not decrease significantly after the addition of O_2 (Figure 3.4), however the CO_2 concentration did decrease drastically when the wine was sparged with N_2. Unlike the rate of O_2 removal (which stayed constant between the two flow rates tested for the same temperature) (Figure 3.3), sparging the wine at a higher N_2 flow rate resulted in a greater loss of dissolved CO_2 compared to the lower N_2 flow rate (for both the temperatures tested) (Figure 3.4).

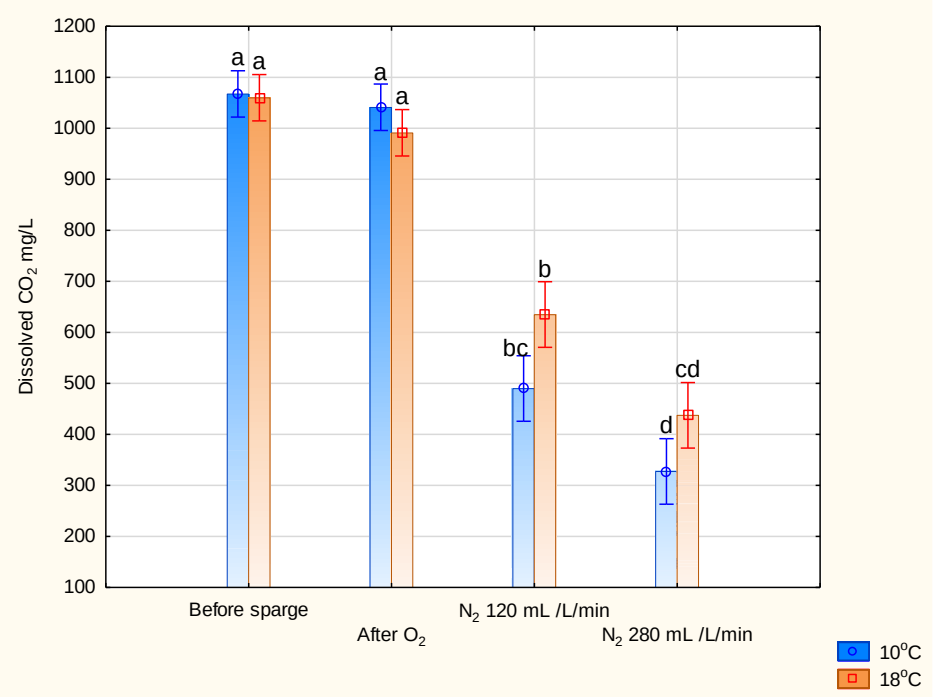

Figure 3.4 Comparing the dissolved CO_2 in solution before sparging, after O_2 addition, after sparging 120 mL N_2 gas/L wine/min and after sparging 280 mL N_2 gas/L wine/min.

The temperature of the wine had an indirect effect on the dissolved CO_2 concentration. Due to the lower O_2 removal rate at 10°C, more time and N_2 gas was needed to remove the O_2 at 10°C compared to 18°C (Table 3.2). This means that the sparging ceased much earlier when the wine was at 18°C while the sparging continued for an additional four minutes at 10°C due to O_2 still being removed (at a slower rate). The additional sparging time led to further decreases in CO_2 at 10°C (Figure 3.4). However, no significant differences were found for free and total SO_2 levels, colour, glutathione or varietal thiols concentrations between the different treatments (results not shown). The total amounts of inert gas used for this and the following experiments can be seen in Table 3.2.

Table 3.2 Total volume of N_2 (L)/L of wine used in each experiment to lower the O_2 levels to below 0.5 mg/L. *Total volume of N_2 sparged without a diffusion stone was extrapolated from O_2 removal rate.

Experiment	Flow rate (N_2 mL/L of wine/minute)	Temperature	Total N_2 gas or mixed gas (L)/L of wine
Temperature and flowrate	120	10°C	1.44
	120	18°C	0.87
	280	10°C	3.41
	280	18°C	2.24
Mixed gas	120	10°C	1.51
	120	18°C	1.26
Diffusion stone	120 (with stone)	18°C	0.81
	120 (no stone)*	18°C	6.89*
Repeated sparging	120 (28 minutes)	18°C	3.36
Extended sparging	120 (68 minutes)	18°C	8.16

3.4.1.c Mixed gas sparging

Consistent with section 3.4.1.b, sparging the wine at a higher temperature resulted in a significantly steeper regression slope (0.47) compared to sparging at a lower temperature (0.38) (Figure 3.5). Comparing the efficacy of mixed gas vs N_2-only sparging, it was evident that the regression slope of O_2 removal was significantly lower when using mixed gas (0.47) compared to N_2 at 18°C (0.59). This result is supported by similar findings from Wilson (1986) where N_2 sparging was found to be more expedient than sparging with CO_2. At 10°C, the slope of O_2 removal using mixed gas was slightly lower (0.38) compared to N_2 sparging at the same temperature (0.42), however, this difference was not significant.

Sparging the wine with the mixed gas had varying effects on the CO_2 concentration, depending on the temperature of the wine. When sparging the wine at 18°C, the CO_2 concentration did not change significantly (Figure 3.6). When sparging the wine at 10°C, a significant increase in the dissolved CO_2 level was seen.

Using a mixed gas was less efficient in terms of removing dissolved O_2 at higher temperatures compared to sparging with N_2 only: however, there was no loss of dissolved CO_2 when using the mixed gas (Figure 3.6). What this implies for winemakers is that it is possible to remove dissolved O_2 while maintaining or increasing dissolved CO_2 in wine. Sparging with a mixed gas, therefore, can reduce production time where dissolved CO_2 will not have to be replenished after the process, thereby increasing efficiency in terms of time. Furthermore, it is possible to increase dissolved CO_2 when sparging with a mixed gas, but that result is highly dependent on temperature when other factors such as alcohol v/v% remains constant.

No significant differences were found for free and total SO_2 levels, colour, glutathione concentrations and varietal thiol concentrations between the different treatments (results not shown). Aside from dissolved CO_2, this suggests that sparging with a mixed gas should not affect white wine's chemical composition drastically when using the described wine volumes, flow rates and temperatures.

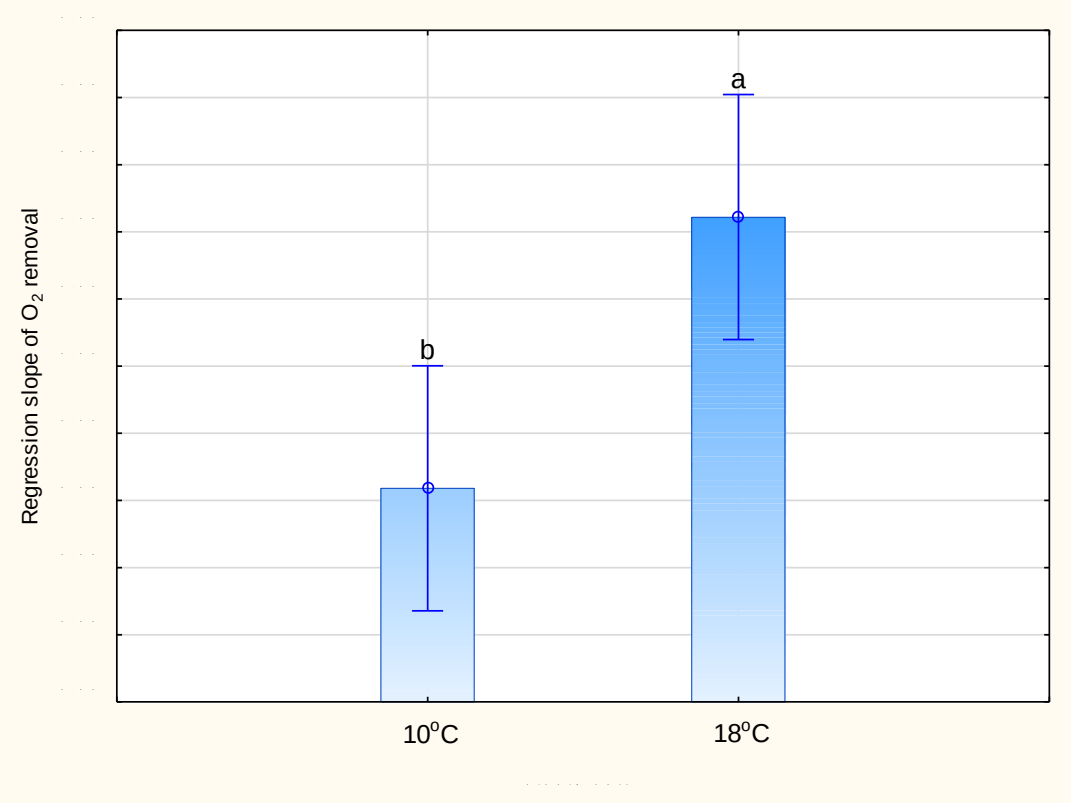

Figure 3.5 Comparing the slopes (rate of decrease) of dissolved O_2 removal in a Chenin blanc wine at 10°C and 18°C using a mixed gas. All flow rates are included in analysis.

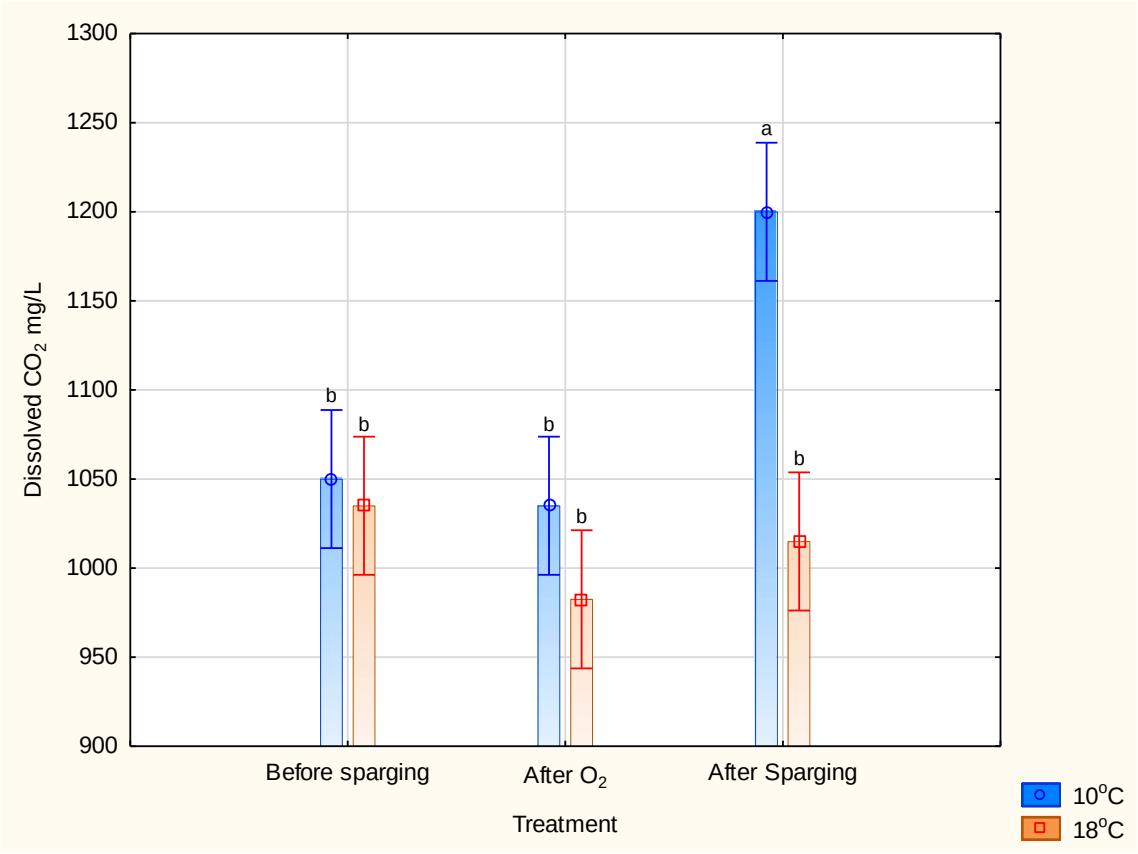

Figure 3.6 Comparing the dissolved CO_2 in solution at different temperature treatments when sparging with a mixed gas.

3.4.1.d Testing the effect of a diffusion stone during sparging

The use of a 15 µm diffusion stone dramatically increased sparging efficacy. The slope of the O_2 removal when using a diffusion stone was significantly greater (0.62) compared to sparging without a diffusion stone (0.024) (Figure 3.7). The regression slopes of O_2 removal were calculated using data from the same time window (sparging ceased for both treatments when the O_2 concentration for the treatment with the diffusion stone reached below 0.3 mg/L) and the same sparging flow rate was used.

The dissolved CO_2 concentrations decreased significantly with and without the diffusion stone, however the loss of CO_2 was greater when a diffusion stone was used (Figure 3.8). Presumably, the smaller bubble size produced by the diffusion stone was more effective at removing dissolved CO_2. Again, no significant differences were found for free and total SO_2 levels, colour, glutathione concentrations and varietal thiol concentrations between the different treatments (results not shown). As many wine production operations currently use open pipes instead of diffusion stones in static sparging operations, (personal communication with several South African bottling operation managers) there is great potential to increase sparging efficacy by using a 15 µm diffusion stone. By using diffusion stones with the smallest applicable pore size, a greater surface are of the inert gases is utilized, meaning that sparging will be more efficient in both time and resources, which is also reflected in the volumes of N_2 gas used in this experiment (Table 3.2).

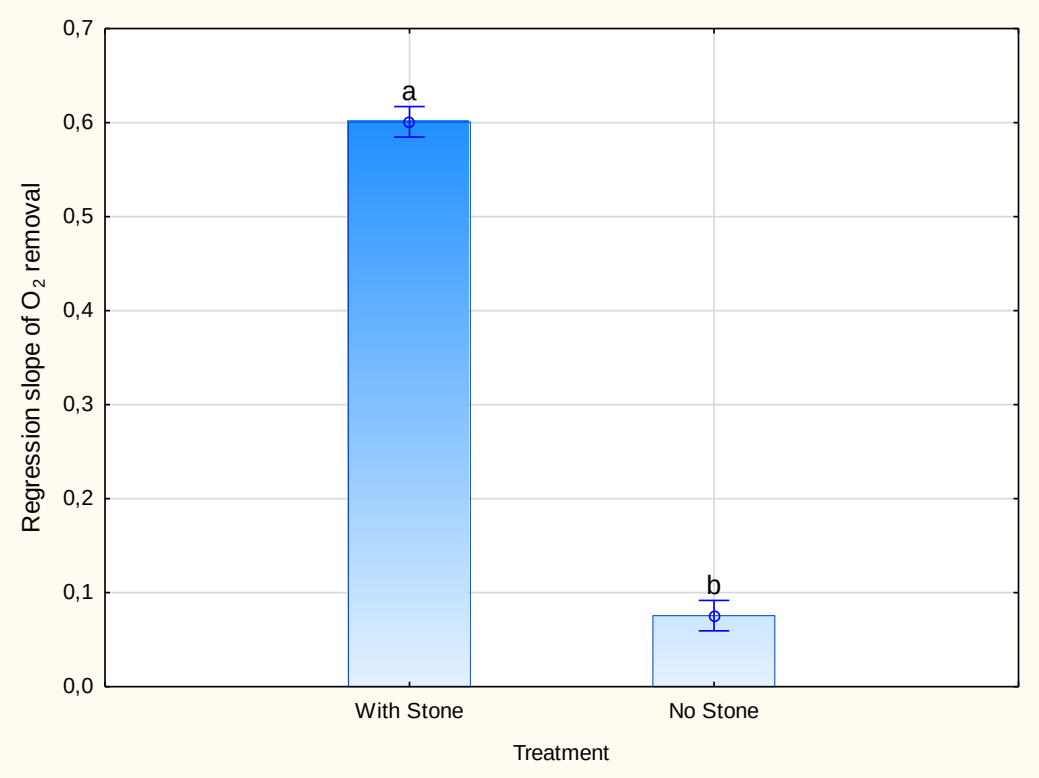

Figure 3.7 Comparing the regression slopes of dissolved O_2 removal comparing sparging while using a diffusion stone compared to sparging while not using a diffusion stone.

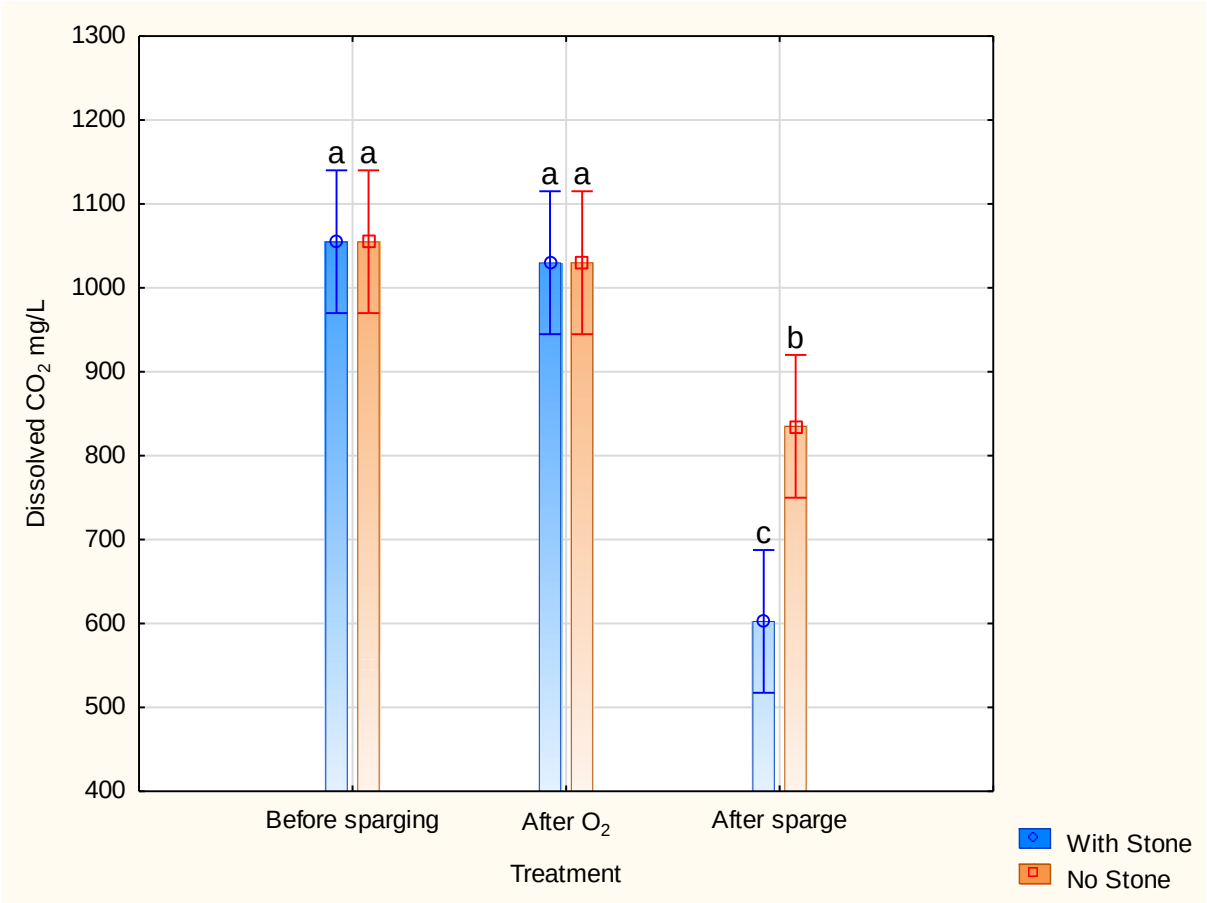

Figure 3.8 Comparing the dissolved CO_2 in solution before and after using, and not using, a diffusion stone.

3.4.1.e Repeated sparging

Repetitive sparging did not significantly alter the chemical composition of the wine for all the compounds analysed (results not shown), except for dissolved CO_2 concentrations that decreased significantly after each successive N_2 sparging treatment (Figure 9). No differences were thus found for free and total SO_2 levels, colour, glutathione concentrations and varietal thiol concentrations between the different treatments (results not shown).

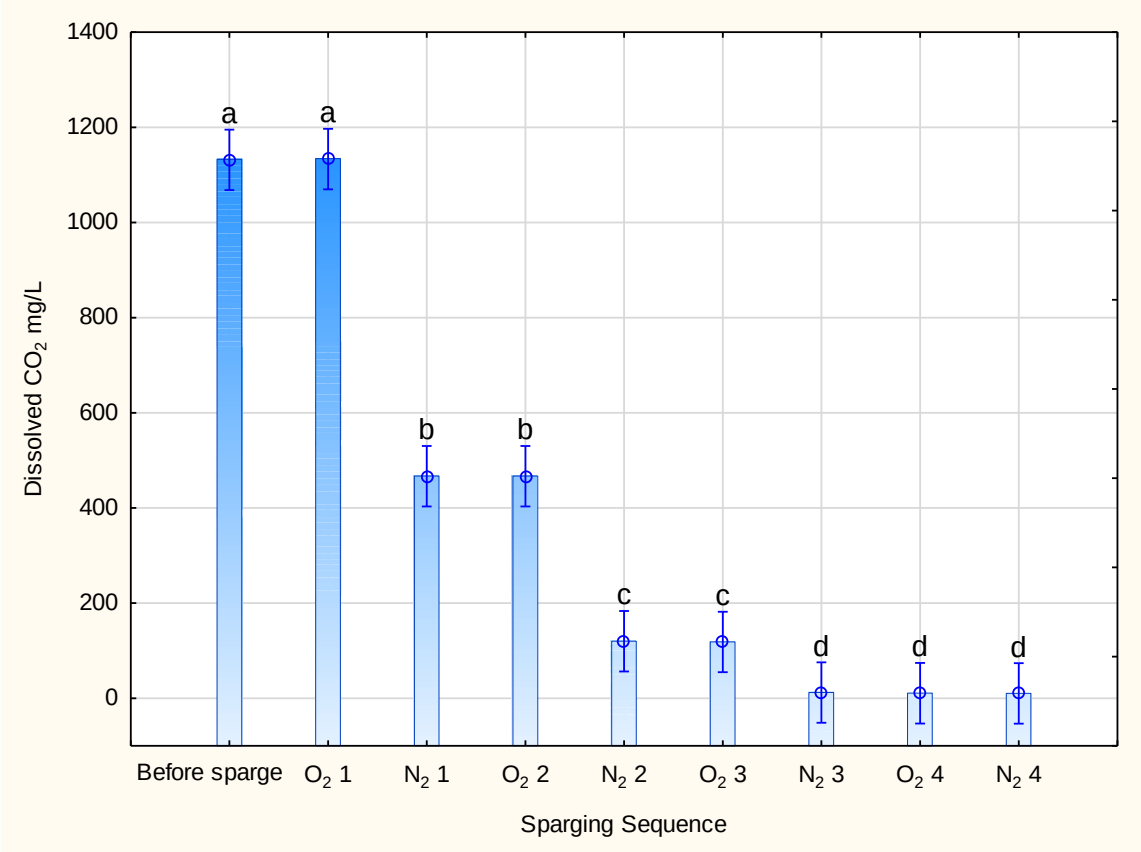

Figure 3.9 The average dissolved CO_2 concentrations after each gas treatment in the repeated sparging experiment. The numbers next to the O_2 and N_2 symbols indicate the sparging repetition for O_2 and N_2.

3.4.1.f Extended sparging

A dry Sauvignon blanc wine from 2018 was sparged with N_2 for a total of 68 minutes (a sample was taken after 8 and 68 minutes) at 18°C to investigate if the sparging process would affect the wine composition. In most previous experiments conducted under the same parameters, dissolved O_2 levels were below 0.5 mg/L after 8 minutes. Results from before and after normal and extended sparging were compared to assess the effects of these treatments on the chemical composition of the wine.

Experiments in the previous sections investigated the effects of adding O_2 and then removing it with inert gas sparging. However, this experiment only sought to investigate the direct effects of sparging with inert gas (omitting any possible oxygen-induced effects). Secondly, in the previous sections the duration of sparging had been limited to the time needed to remove specific amounts of O_2. To further investigate the effects of inert gas sparging on white wine chemical composition, a wine was sparged for an extended amount of time.

This experiment used the largest volume of N_2 (8.16 L of N_2/L of wine) that was sparged in total. The second largest volume of N_2 sparged was used in the repeated sparging experiment (3.40 L N_2/L of wine)(Table 1A). As previously found in sections 3.4.1.b through 3.4.1.e, the only compound tested showing significant difference in concentration before and after sparging was the level of dissolved CO_2 (Figure 3.10). After the first eight minutes of sparging, 40% of the dissolved CO_2 was removed. This result is consistent with results found in

sections 3.4.1.2, and 3.4.1.4 and 3.4.1.5. After an additional 60 minutes of sparging, the dissolved CO_2 was undetectable (Figure 3.10).

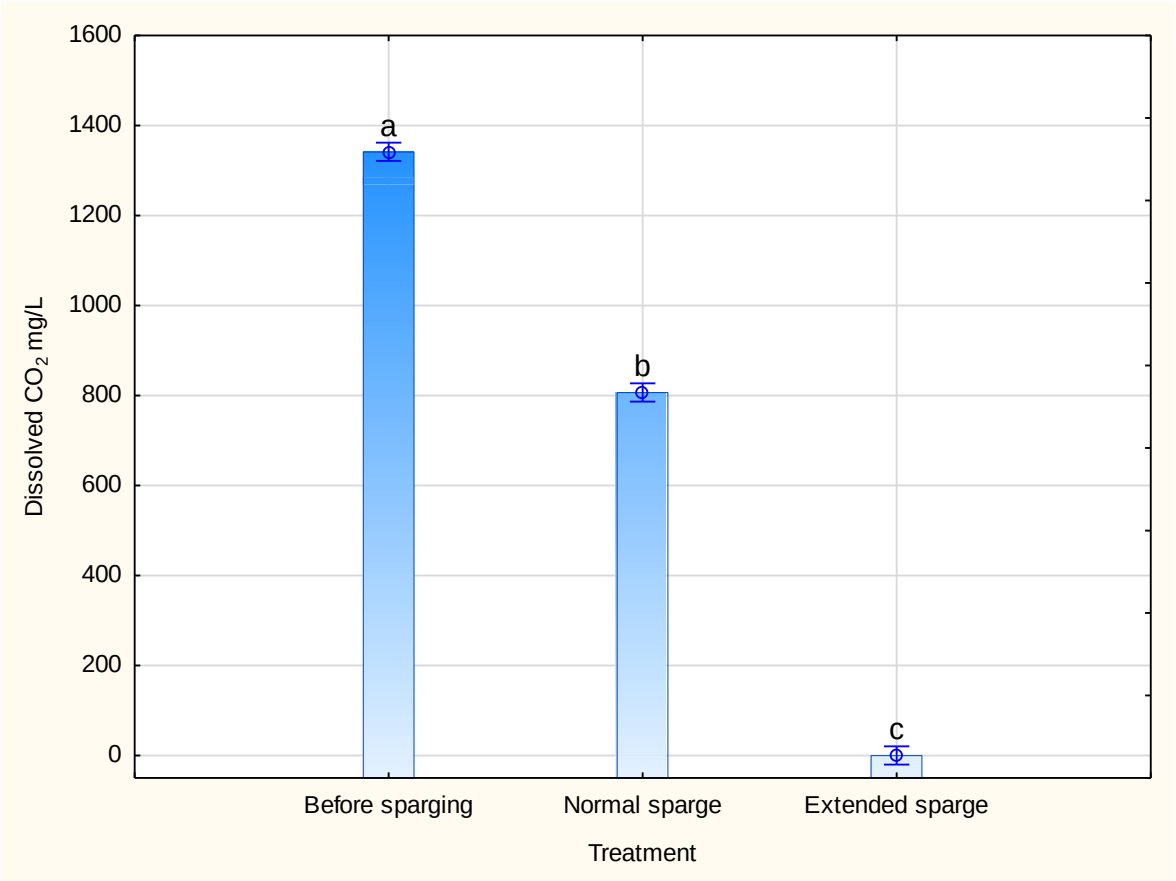

Figure 3.10 The average dissolved CO_2 after each sparging treatment.

Again, no significant differences were found for concentrations of free and total SO_2, colour, glutathione, varietal thiols as well as for found for concentrations of esters, fatty acids, and higher alcohols (results not shown). These results further indicate that inert gas sparging does not significantly affect the aromatic compounds measured in this study. In terms of industry implications, when done correctly, it seems that winemakers should not be overly concerned with altering the chemical composition of wine when sparging, other than dissolved CO_2 and possible organoleptic effects therein.

3.4.2 Carbon dioxide in still wine

The effects of inert gas sparging on dissolved CO_2 concentrations in these wines was the most significant result found in sections 3.4.1.2 through 3.4.1.6. When sparging with N_2 only, it was found that significantly more dissolved CO_2 was lost at greater sparging flow rates and at lower temperatures. At lower temperatures, more time is spent sparging wine to remove similar amounts of dissolved O_2, and this additional time sparging removes significantly more dissolved CO_2 despite the solubility of CO_2 increasing as liquid temperature decreases (Agabaliantz, 1963). Further research could examine the losses of dissolved CO_2 at lower flow rates to see how CO_2 removal efficiency is related to dissolved O_2 removal efficiency.

Concentrations of dissolved CO_2 in still wines normally range from 500-1000 mg/L (Gawel *et al.,* 2018). Increasing dissolved CO_2 concentrations in wine within this range has recently been found in some cases to be beneficial to white wine where it can increase perceived freshness and/or fruitiness (Smith *et al.,* 2018). There are several issues with the previously cited study, however. The changes to pH caused by increasing dissolved CO_2 were corrected to the original pH levels before sensory evaluation; this correctional pH adjustment could possibly result in lower sensory perception of astringency and bitterness, perhaps influencing the result of increased perceptions of freshness and/or fruitiness (Smith *et al.,* 2017; Gawel *et al.,* 2018). As dissolved CO_2 is sensorially undetectable below 500 mg/L (Peynaud, 1983; Zoecklein, *et al.* 1995), this studies results indicate that the organoleptic properties associated with dissolved CO_2 could induce a potentially significant sensorial change. Further research is needed to determine what degree of dissolved CO_2 needs to occur in still wine for organoleptic differences to be observed. Winemakers should, therefore, be cautious when sparging to ensure sparging procedures and parameters are in line with desired sensorial goals of any wine, especially in terms of the effects dissolved CO_2 has on wine.

3.5 Conclusion

Factors such as temperature, diffusion stone application, and gas composition were found to significantly affect sparging efficacy. As seen in previous studies, sparging efficacy increased as the temperature of the wine increased and with the application of a 15 µm diffusion stone. Using a mixed gas of N_2 and CO_2 is slightly less efficient in removing O_2 at higher temperatures, however, by using mixed gas, the CO_2 concentration of the wine can be maintained and even increased if desired. A mixed gas of N_2 and CO_2 can be more expensive compared to pure N_2 or CO_2. However, a simple manifold can be utilised for mixing less expensive pure gases in-line.

It is clear that differing sparging parameters have significant impact on sparging efficacy (Figure 3.11). Winemakers should understand the range of variables which alter sparging efficacy when deciding to sparge wine in order to maximize efficiency. It was previously speculated that inert gas sparging can potentially remove aromatic compounds from wine, however, the current study did not deliver evidence to support this hypothesis. The only chemical compound which was consistently affected by N_2 and mixed gas sparging was the dissolved CO_2 concentration. The fact that sparging did not alter the aromatic composition of the wine is a significant result for the wine industry. However, further investigations using wines produced from more varieties and that possess a wider range of aromatic concentrations needs to be conducted.

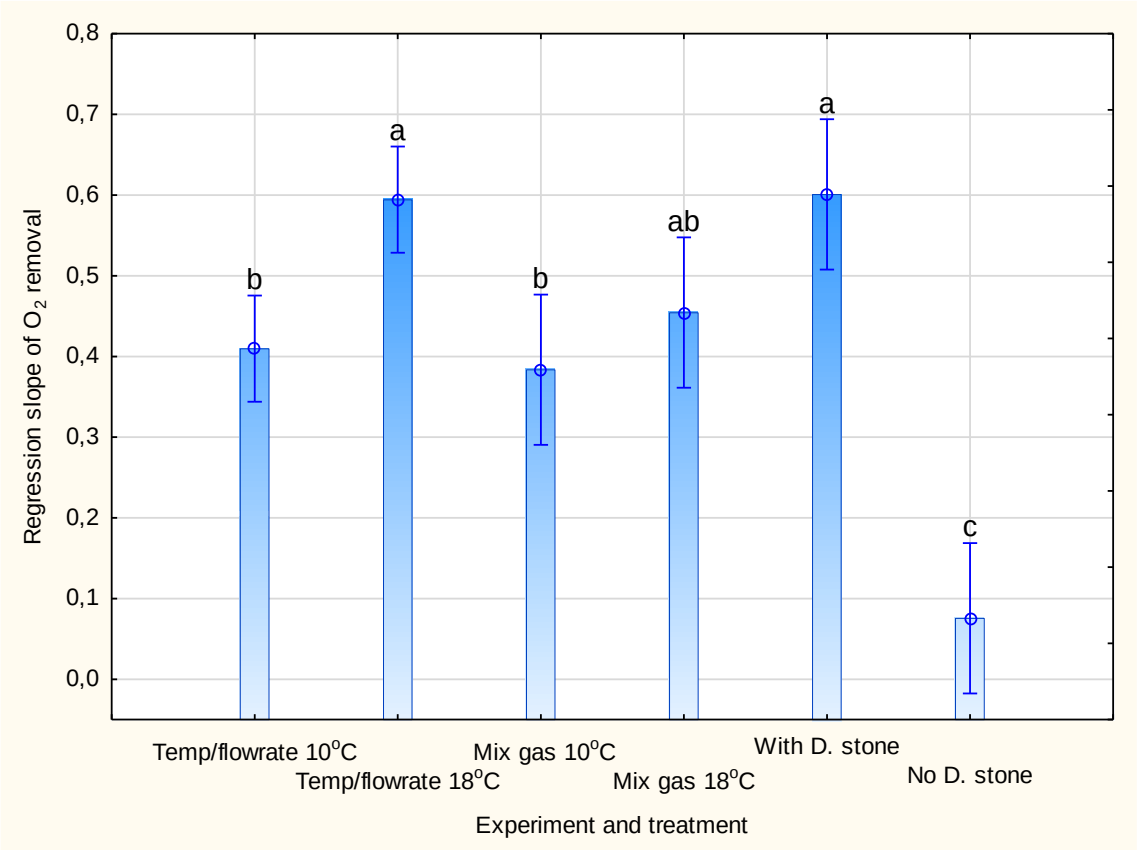

Figure 3.11 Comparing the regression slopes of O_2 removal between the temperature and flow rate experiment, mixed gas experiment and the diffusion stone experiment.

Though the varietal thiol concentrations found in the wines used in this study were in the typical range of South African Chenin blanc and Sauvignon blanc wines, these were toward the lower to medium ranges when compared to other South African white wines (Coetzee & du Toit, 2012; Aleixandre-Tudo *et al.,* 2015; Wilson *et al.,* 2017). The effect of sparging on a high varietal thiol-containing wines could also be evaluated to see if similar results are found. However, small decreases in certain volatile compounds, such as varietal thiols in wine, might not always translate into large sensorial differences.

However, the sensorial effects of CO_2 in wine should not be underestimated and should be considered when sparging. Only when sparging with a mixed gas of N_2 and CO_2 did the dissolved CO_2 concentrations remain unchanged after sparging compared to before sparging (at 18°C). Hence, sparging with inert gasses can be an effective tool to remove dissolved O_2, while the effects on and of CO_2 can be manipulated by adjusting the wine temperature during sparging or using a gas mixture.

The sparging treatments in this study also did not directly affect the aromatic compounds measured, however, changes to wine dissolved CO_2 concentrations could indirectly influence the sensory perception of wine aroma compounds. As dissolved CO_2 removal in sparging operations is a common occurrence, understanding the potential organoleptic effects on wine is paramount for winemakers. The exact sensory effects of varying concentrations of dissolved

CO_2 in wine are unclear, and the possible sensory effects of sparging (either to remove dissolved O_2 and by extension, CO_2, or to replenish CO_2) should be further investigated.

3.6 References

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3.7 Addendum

Table 3A Initial analysis for the Chenin blanc and Sauvignon blanc before experimentation.

Attribute	unit	Chenin blanc	Sauvignon blanc
Vintage	year	2017	2018
Alcohol	%v/v	13.4	13.0
Residual sugar	g/L	4.1	3.4
Total acidity	g/L	5.4	7.0
pH		3.61	3.36
		Control	**Control**
Dissolved gas			
Carbon dioxide	mg/L	1090 ±24.8	1341 ±8.3
Antioxidents			
Free sulphur dioxide	mg/L	33 ±0.71	35 ±0.33
Total sulphur dioxide	mg/L	99 ±0.73	102 ±0.98
Glutathione	mg/L	1.958 ±0,016	0.947 ±0.02
Colour			
Yellow/ Brown at 420 nm	AU	0.1104 ±0,002	0.0947 ±0.003
Thiols			
4-mercapto-4-methylpentan-2-one (4MMP)	ng/L	17.630 ±2.29	10.757 ±0.54
3-mercaptohexylacetate (3MHA)	ng/L	40.135 ±2.912	29.475 ±1.15
3-mercaptohexanol (3MH)	ng/L	681.892 ±34.11	144.968 ±4.31
Esters			
2-Methyl Propyl Acetate	mg/L	NA	1,57 ±0.03
Diethyl Succinate	mg/L	NA	1,23 ±0.08
Ethyl Acetate	mg/L	NA	79,62 ±3.81
Ethyl Butyrate	mg/L	NA	0,69 ±0.02
Ethyl Caprate	mg/L	NA	0,13 ±0.06
Ethyl Hexanoate	mg/L	NA	1,35 ±0.02
Ethyl Lactate	mg/L	NA	38,75 ±1.72
Ethyl Phenethylacetate	mg/L	NA	0,60 ±0.01
Hexyl Acetate	mg/L	NA	0,69 ±0.02
Isoamyl Acetate	mg/L	NA	6,19 ±0.20
Phenylacetate	mg/L	NA	0,25 ±0.02
Acids			
Acetic Acid	mg/L	NA	652,14 ±28.83
Butyric Acid	mg/L	NA	1,29 ±0.07
Decanoic Acid	mg/L	NA	2,37 ±0.20
Hexanoic Acid	mg/L	NA	3,82 ±0.19
Isobutyric Acid	mg/L	NA	0,65 ±0.04
Isovaleric Acid	mg/L	NA	0,32 ±0.02
Octanoic Acid	mg/L	NA	4,66 ±0.21
Propionic Acid	mg/L	NA	1,01 ±0.05
Valeric Acid	mg/L	NA	0,04 ±0.003
Alcohols			
Acetoin	mg/L	NA	20,57 ±1.02
Butanol	mg/L	NA	0,86 ±0.04
Ethoxy-1-Propanol	mg/L	NA	0,27 ±0.02
Hexanol	mg/L	NA	1,75 ±0.06
Isobutanol	mg/L	NA	29,51 ±1.16
Isoamyl Alcohol	mg/L	NA	131,13 ±3.39
Methanol	mg/L	NA	84,49 ±4,70
Pentanol	mg/L	NA	0,28 ±0.004
Phenylethanol	mg/L	NA	12,82 ±0.64
Propanol	mg/L	NA	33,44 ±1.46

Chapter 4: General discussion and conclusions

4.1 General discussion and conclusions

The effects of elevated storage temperature and dissolved O_2 on white wine chemical and sensory composition has been studied separately before, but these factors have not been studied in conjunction under conditions simulating those that occurs commercially .

Results from the dissolved O_2 and storage temperature experiments (Chapter 2) showed that the storage temperature, time, and to a lesser extent, the levels of dissolved O_2 at bottling, significantly affect the wine composition. Antioxidants, such as sulphur dioxide and glutathione, significantly decreased due to increasing O_2 levels as well as from increased storage temperature. Brown colour increased when stored at higher temperatures compared to lower temperatures, but also increased significantly from ageing. Varietal thiols concentrations were not found to be significantly altered by the dissolved O_2 concentrations in this study; however, 3MHA naturally hydrolysed in some cases at a faster rate when stored at higher temperatures. In these wines, the 3MH content was sometimes higher when stored at higher temperatures probably due to the conversion of 3MHA to 3MH. Interestingly, a large increase in 3MH was also observed in the first six months of ageing. Major volatiles associated with fruity aromas such as isoamyl acetate and ethyl caprylate decreased during overall storage and were found in lower quantities when stored at higher temperatures compared to lower temperatures. The esters ethyl lactate and diethyl succinate were found in greater quantities when stored at higher temperatures and, in some cases, increased dissolved O_2, but this was only seen when the wines were stored at higher temperatures. Propionic acid, butyric acid, octanoic acid and decanoic acid were found in greater quantities when stored at higher temperatures. From the sensory analysis, the Sauvignon blanc wines were found to have higher intensities of fruity descriptors such as ‘passion fruit’ and ‘grapefruit’ at lower storage temperatures after 12 months and higher intensities of oxidized descriptors such as ‘baked apple’ at higher storage temperatures. The Chenin blanc wines did not exhibit any significant differences in the twelve month descriptive analysis, but at six months guava was found in higher intensities at lower temperatures and in high storage temperature wine with no O_2 additions.

These results indicate that dissolved O_2 concentrations found at bottling may significantly impact antioxidants of white wines; however, storage temperature seems to be more important regarding the sensorial development of South African white wine in bottled wine. That being said, the combination of high O_2 at bottling and high bottle storage temperatures during bottle ageing is probably the most detrimental conditions delicate white wines can face.

Dissolved O_2 might have an amplifying effect as some descriptors, such as ‘baked apple’, found in the Sauvignon blanc wines increased even more when both factors (higher storage temperature and elevated dissolved O_2), were present. The Sauvignon blanc wines seem to be

more affected by temperature treatments when compared to the Chenin blanc wines. This could be due to Sauvignon blanc containing more oxidation sensitive chemical species on average compared to Chenin blanc, but requires further investigation.

It is important to note that dissolved O_2 additions in this experiment were representative of concentrations found during bottling in South Africa. In commercial wineries however, O_2 pick can take place before bottling which might limit the oxidative capacity of wine during bottling. This could increase the influence of the O_2 at bottling to a larger extent than what was found and should be included in future studies. Future studies can also include more untargeted volatile analyses, as well as include compounds such as aldehydes, knowns to form due to oxidation.

As dissolved O_2 concentrations at bottling can significantly affect the concentrations of antioxidants in white wines, understanding how to remove dissolved O_2 from wine is paramount to winemakers. In chapter 3, the effects of sparging white wine with various inert gases showed that environmental and procedural factors influenced the efficiency of sparging and inevitably the concentration of dissolved gases in wine. The temperature of the wine during sparging significantly influenced the rate of dissolved O_2 removal where wines sparged at lower temperatures had slower removal rates due to the higher solubility of O_2 at low temperatures. The rate of O_2 removal was not increased with increased flow rate of the sparged gas (from 120 mL gas/L wine/min to 280 mL gas/L wine/min). This indicates that there is a limit to sparging efficiency in terms of inert gas flow rate. Despite the rate of O_2 removal not increasing, greater concentrations of dissolved CO_2 were lost at greater gas flow rates and at lower temperatures.

Compared to sparging with pure N_2, sparging with a mixed gas of N_2 and CO_2 was found to be slightly less efficient in removing dissolved O_2, however, no loss of dissolved CO_2 was found when sparging with the mixed gas at higher temperatures, while an increase in dissolved CO_2 was observed when sparging with mixed gas at lower temperatures. Even though sparging with the mixed gas is less efficient in removing dissolved O_2, the fact that there are no CO_2 losses can be a significant advantage due to the fact that nitrogen sparging might necessitate the replenishment of the removed CO_2 with an additional sparging process (with CO_2 gas). To maximize sparging efficiency, winemakers should utilize a diffusion stone as it significantly improves sparging efficacy.

After repeatedly and continuously sparging a Chenin blanc wine with O_2 and N_2 and sparging a Sauvignon blanc wine extensively with N_2, no significant differences in free and total sulphur dioxide, glutathione, colour, varietal thiols, esters, fatty acids, and alcohols concentrations were found. These results suggest that, other than dissolved CO_2 concentration, inert gas sparging does not significantly affect the chemical composition of wines. As the cultivars in chapter 2 responded significantly different to similar treatments, future studies on this topic should investigate more wine volatiles, wine styles and other cultivars to examine how universal the reported results are.

Dissolved CO_2 was consistently affected by sparging operations and is still not a fully understood phenomena in terms of potential sensorial effects. Additional research is needed to determine to what degree of change in dissolved CO_2 due to sparging needs to occur in still wine for organoleptic differences to be observed. This study did not include a sensory analysis of the sparged wines, particularly of the wines which went through prolonged sparging treatments where all measurable dissolved CO_2 was removed. Future studies investigating the effects of sparging on wine should incorporate sensory analysis of aroma and mouth feel to determine if any significant organoleptic differences emerge. Since only a limited number of wine volatiles were measured in this study, a sensory analysis could have provided evidence whether inert gas sparging affected aroma or flavour descriptors associated with those compounds.

A greater understanding of how dissolved O_2, storage temperature, and the mechanics of sparging can help the industry to protect and improve wine quality, integrity, and operational efficiency. The knowledge that dissolved O_2 and storage temperature can affect the chemistry and sensory profile of a wine will encourage winemakers to evaluate bottling and storage practices to achieve desired outcomes. Previous research has provided clear insights into the effects of dissolved O_2 and elevated storage temperatures in white wines (Cejudo-Bastante *et al.,* 2013; Fracassetti *et al.,* 2013; Ugliano, 2013; Pereira *et al.,* 2014; Coetzee *et al.,* 2016). Until now, very little research on sparging efficacy has been published (Wilson, 1986) and, to our knowledge, no research regarding the effect of sparging on the chemical composition of a wine exists. The findings of this study can help winemakers to create optimal conditions for sparging operations. Further, more knowledge on how sparging affects wine chemistry will give winemakers confidence when making decisions to use inert gases in winemaking.

4.2 References

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