# Effect of foliar Nitrogen and Sulphur spraying on white wine composition (*Vitis vinifera* L. cv. Chenin Blanc and Sauvignon Blanc)

by

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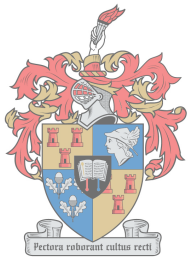


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# Declaration

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# Summary

Chenin Blanc and Sauvignon Blanc, as the most planted wine cultivars in South Africa, are of great interest to researchers worldwide, due to its increased high wine quality. Wine quality is interlinked with wine aroma. Vine nitrogen fertilization influence the vine physiology and composition of the grapes, and enhanced aroma expression. By addressing Yeast Assimilable Nitrogen (YAN) deficiency with foliar fertilization, during the ripening season, to low nitrogen containing vines, the aroma potential of the wines can be potentially influenced.

The main aim of this research study was to assess the influence of different foliar fertilization treatments on Chenin Blanc and Sauvignon Blanc vines planted in various locations in South Africa. For each season, two vineyards with a history of producing low nitrogen content grapes were used, one vineyard per cultivar. The vineyards received sulphur and nitrogen foliar treatments twice before véraison. During winemaking, the juices and wines underwent analysis for non-volatile and volatile content. The wines underwent maturation for three and nine months, and then sensorially and chemically analysed.

In Chapter 2 the various wine compounds and classes of compounds present in Chenin Blanc and Sauvignon Blanc wines were analysed. The specific characteristics, aroma composition and its implications on the sensory perception of the cultivars were reviewed. The influence and contribution of different fertilization practices on the chemical compounds and resulting wine's aromatic expression were investigated.

The first part of the research study investigated the effect of foliar fertilization on the non-volatile content in the juices and wines. In Chapter 3, the nitrogen containing foliar fertilization applications increased the YAN levels. This increase is relevant not only for yeast metabolism, but also for the aromatic potential of a wine, as certain amino acids being precursors of aroma compounds. Glutathione were also influenced by the treatments for both years and both cultivars, but the trends were not as evident as with YAN.

The second part of the study assessed the effect of various fertilization treatments on the volatile content of the juices and aged wines. Sensory analysis and chemical analysis were used to assess the wines after three and nine months of bottle maturation. Chapter 4 highlighted that sulphur containing foliar treatments influenced the volatile content of major volatiles and volatile thiols. The overall volatile content of the wines was very similar but identified a clear vintage and age effect during maturation. Sensory analysis classified the Chenin Blanc wine with 'tropical' and 'fruity' aromas, while Sauvignon Blanc wines had prominent 'tropical', 'passion fruit', and 'grapefruit' aromas. During bottle maturation, some notes and aroma characters were maintained but their frequency of citations changed.

The results of this research study contributed to the knowledgebase on South African Chenin Blanc and Sauvignon Blanc wines, but also concluded that foliar fertilization can influence the non-volatile and volatile content of wines. South African winemakers and the industry can use this information to make decisions at the viticulture and winemaking level to produce wines with more desirable sensory attributes.

# Opsomming

Chenin Blanc en Sauvignon Blanc is die mees aangeplante witwyn-kultivars in Suid-Afrika en is van groot belang vir navorsers wêreldwyd weens die hoë wynkwaliteit. Wynkwaliteit is gekoppel aan wyn aroma. Stikstof bemesting tot die wingerdstok kan die wingerd fisiologie en samestelling van die druiwe beïnvloed, asook die aroma uitdrukking verbeter. Deur die gisbare stikstof (YAN) tekort aan te vul in lae stikstofbevattende wingerde met blaarvoeding gedurende die rypwording seisoen, kan die aroma van die wyne potensieël beïnvloed word.

Die hoofdoel van hierdie navorsing studie was om die invloed van verskillende blaarvoeding behandelinge op Chenin Blanc- en Sauvignon Blanc wingerdstokke op verskillende plekke in Suid-Afrika te beoordeel. Vir elke seisoen is twee wingerde met 'n geskiedenis van lae druif stikstofinhoud gebruik, een wingerd per kultivar. Die wingerd het twee keer voor deurslaan swael- en stikstof-blaartoevoegings ontvang. Tydens die wynmaakproses is die sappe en wyne geanaliseer vir nie-vlugtige en vlugtige inhoud en het onderskiedelik vir drie en nege maande veroudering ondergaan en is daarna sensories en chemies ontleed.

In Hoofstuk 2 is verskillende wynverbindings en klasse van verbindings wat teenwoordig is in Chenin Blanc- en Sauvignon Blanc wyne geanaliseer. Die spesifieke eienskappe, aroma-samestelling en uitwerking daarvan op die sensoriese persepsie van die kultivars is geevalueer. Die invloed en bydrae van verskillende blaarvoeding behandelinge op die chemiese verbindings en gevolglike aromatiese uitdrukking van die wyne is ondersoek.

Die eerste deel van die navorsing het ondersoek ingestel op die effek van blaarvoeding op die nie-vlugtige inhoud in die sappe en wyne. In Hoofstuk 3 het die stikstofbevattende blaarvoeding behandelinge die YAN-vlakke verhoog. Hierdie toename is nie net relevant vir gismetabolisme nie, maar ook vir die aromatiese potensiaal van 'n wyn, aangesien sekere aminosure voorlopers van aromaverbindings is. Glutatione was ook beïnvloed deur die behandelings vir beide jare en albei kultivars, maar die neigings was nie so duidelik soos met YAN nie.

Die tweede deel van die studie het die effek van verskillende blaarvoeding behandelinge geevalueer op die vlugtige inhoud van die sappe en verouderde wyne. Sensoriese - en chemiese analise is gebruik om die wyne na drie en nege maande se bottelveroudering te beoordeel. Hoofstuk 4 het uitgewys dat swael-blaartoevoegings die vlugtige inhoud van esters, hoër alkohole, vetsure en positiewe vlugtige tiole beïnvloed het. Die algehele vlugtige inhoud van die wyne was baie soortgelyk, maar het 'n duidelike oesjaar en verouderingseffek tydens veroudering getoon. Sensoriese analise het die Chenin Blanc wyn met 'tropiese' en 'vrugtige'-aromas geklassifiseer, terwyl Sauvignon Blanc wyne prominente' tropiese', ‘grenadella' en ‘pomelo' aromas gehad het. Tydens bottelveroudering is die teenwoordigheid van sommige aromatiese karakters behou, maar die hoeveelheid keer wat dit voorkom het verander.

Resultate van hierdie navorsing studie het bygedra tot die kennisbasis oor die Suid-Afrikaanse Chenin Blanc- en Sauvignon Blanc wyne, maar het ook tot die gevolgtrekking gekom dat blaarvoeding behandelinge die nie-vlugtige en vlugtige inhoud van wyne kan beïnvloed. Suid-Afrikaanse wynmakers en die bedryf kan hierdie inligting gebruik om besluite te neem op wingerdbou en wynmaak om wyne met meer wenslike sensoriese eienskappe te produseer.

This thesis is dedicated to my close friends, family, and to the mystification of that which is wine.

*“I enjoy making wine, because this sublime nectar is quite simply incapable of lying. Picked too early, picked too late, it matters not - the wine will always whisper into your mouth with complete, unabashed honesty every time you take a sip.”*

*Henry Skinner, A Good Year*

# Biographical sketch

Aléta Bruwer was born on 27 May 1991 in Ashton, South-Africa on a vineyard farm. She went to Ashton Primary School and matriculated at Prospect Home School in 2009. In 2011 she enrolled for a BSc Agric degree, majoring in Viticulture and Oenology at Stellenbosch University.

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# Preface

This thesis is presented as a compilation of 5 chapters.

**Chapter 1** **General introduction and research aims**

**Chapter 2** **Literature review**

Nitrogen and Sulphur foliar fertilization: Contribution to non-volatile and volatile compounds of Chenin Blanc and Sauvignon Blanc juices and wines.

**Chapter 3** **Research results**

Effect of foliar Nitrogen and Sulphur fertilization applications on non-volatile content of Chenin Blanc and Sauvignon Blanc juices and wines.

**Chapter 4** **Research results**

Effect of foliar Nitrogen and Sulphur fertilization applications on Chenin Blanc and Sauvignon Blanc juices and wines: Volatile chemistry and sensory expression.

**Chapter 5** **General discussions and conclusions**

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# List of Abbreviations

| | | |
|---|---|---|
| **Amino Acids** | ASP | Aspartic acid |
| | GLU | Glutamic acid |
| | ASN | Asparagine |
| | SER | Serine |
| | ARG | Arginine |
| | ALA | Alanine |
| | GLN | Glutamine |
| | PRO | Proline |
| | VAL | Valine |
| | LEU | Leucine |
| | PHE | Phenylalanine |
| | ILE | Isoleucine |
| | MET | Methionine |
| | CYS | Cysteine |
| | CYS-CYS | Cystine |
| | SRS | Sulfate reduction sequence |
| | GABA | Aminobutyric acid |
| | TYR | Tyrosine |
| | LYS | Lysine |
| | THR | Threonine |
| | GLY | Glycine |
| | TRP | Tryptophan |
| | HIS | Histidine |
| | ORN | Ornithine |
| | HYP | Hydroproline |
| **Glutathione** | GSH | Glutathione |
| | GSH-O | Oxidised GSH form |
| | GSH-R | Reduced GSH form |
| | GRP2 | 2,5-di-sulphur-glutathionylcaftaric acid |
| | GRP1 | 2-S-glutathionyl-caftaric acid or Grape Reaction Product |
| **Methoxypyrazine** | IBMP | 2-isobutyl-3-methoxypyrazine |
| | SBMP | 2-*sec*-butyl-3-methoxypyrazine |
| | IPMP | 2-isopropyl-3-methoxypyrazine |
| **Chemical analysis methods** | LC-MS/MS | Liquid chromatography-tandem mass spectrometry |
| | GC-FID | Gas chromatography-flame ionisation detection |
| | MS | Mass spectrometry |
| | NOPA | Nitrogen by o-phthaldialdehyde assay |
| | HS-SPME | Gas chromatography coupled with head space solid-phase micro-extraction |
| | FT-MIR | Fourier transform mid-infrared spectroscopy |

| | | |
|---|---|---|
| | UPLC-MS/MS | Ultra performance liquid chromatography-tandem mass spectrometric |
| | OPA-IDA-FMOC | *O*-phthaldialdehyde, iodoacetic acid, and 9-fluorenyl-methylchloroformate |
| | HPLC-FLD | High pressure liquid chromatography–fluorescence detection |
| | SPE | Solid phase extraction |
| **Volatile thiols** | 3MHA | 3-mercapto-hexyl acetate |
| | 3MH | 3-mercaptohexan-1-ol |
| | CYS-3MH | S-3-(hexan-1-ol)-cysteine |
| | CYS-4MMP | S-3-(4-mercapto-4-methylpentan-2-one)-cysteine |
| | GSH-3MH | S-3-(hexan-1-ol)-glutathione |
| | PGSH-3MH | Glutathionylated precursor of 3MH |
| | PCYS-3MH | Cysteinylated precursor of 3MH |
| | 4MMP | 4-mercapto-4-methyl-pentan-2-one |
| **Wine styles** | RRUW | Rich and ripe–unwooded |
| | FF | Fresh and fruity |
| | RRW | Rich and ripe–wooded |
| **Statistical analysis** | CA | Correspondence analysis |
| | MDS | Multi-dimensional scaling |
| | ANOVA | Analysis of variance |
| | LSD | Least significance difference |
| | PCA | Principal Component Analysis |
| | AHC | Agglomerated hierarchical cluster |
| **Other** | YAN | Yeast assimilable nitrogen |
| | $Na_2SO_4$ | Sodium sulphate |
| | $CO_2$ | Carbon dioxide |
| | TA | Total acidity |
| | ˚B | Balling |
| | $NH_3$ | Ammonia nitrogen / ammonium |
| | DAP | Di-ammonium phosphate |
| | $H_2S$ | Hydrogen sulfide |
| | VSP | Vertical shoot positioning |
| | FAN | Free amino nitrogen |
| | $SO_2$ | Sulphur dioxide |

# Chapter 1

# General introduction and research aims

# Chapter 1. General introduction and research aims

## 1.1 Introduction

Wine is regarded as one of the oldest beverages in the world (Amerine *et al*., 1982) and was made by Jan van Riebeeck for the first time in South Africa in 1669 (Fischer, 2007). Even though South Africa has been producing wines for almost 300 years, it is still classified as a New World country because of the specific wine styles produced (Fischer, 2007). Viticultural practices and oenological techniques have changed over the years to improve the quality and aroma of wines. In today's wine industry, winemakers have to study and consider the preferences of consumers and quality wine production demands (Pretorius & Bauer, 2002). Vineyard practices and their influence on the table wine cultivars have been studied extensively the past decade, mainly focusing on the chemical compounds and aroma expression of the wines (Dufourcq *et al*., 2007; Lacroux *et al*., 2008; Jreij *et al*., 2009).

Vine nutrition plays a crucial role in vine development, canopy growth, and composition of the grape berry (Bell & Henschke, 2005; Choné *et al*., 2006). Traditionally, vineyard nutrition was carried out by adding fertilizers to the soil to be absorbed by the roots of the plant. Due to climate change and more frequent summer droughts, soil fertilization is no longer the best solution to increase the nitrogen levels of the must and vines (Fischer, 2007; Laget *et al*., 2008; Keller, 2010). Foliar spray fertilization is a widely-used technique on various crops and can lead to a quick nutrient uptake through the leaves and is cost effective (Christensen, 2005; Jreij *et al.*, 2009; Lasa *et al*., 2012). Nitrogen foliar fertilization is only effective in plants approaching nitrogen deficiency, and deficiencies can be overcome temporarily (Delas, 2000).

In recent years, winemakers have become increasingly interested in the bouquet and aroma expression of wines (Robinson *et al*., 2014). Aroma components play an important role and contribute directly to the quality of wine (Marais, 1994). Vine nutrition deficiency and low Yeast Assimilable Nitrogen (YAN) can negatively influence the aroma profile due to sluggish or stuck alcoholic fermentation (Monteiro & Bisson, 1991). A common practice among winemakers is to increase the nutrient or YAN levels of must by adding diammonium phosphate (DAP) or complex nutrients (Lorenzini & Vuichard, 2012). Few researches reported Sauvignon Blanc vines having received urea foliar fertilization before or during véraison, resulting in increased concentration levels of YAN and amino acids in the grape must and wine (Dufourcq *et al.*, 2007; Lacroux *et al.*, 2008; Verdenal *et al*., 2015). The formation of aromatic expression is influenced by the amino acid composition of grape juice and nitrogen foliar fertilization can influence the levels of these compounds (Fischer, 2007).

Understanding the role of aromatic compounds in wines and how the precursors and flavour components develop during the winemaking process is important to wine producers (Stashenko *et al.*, 1992). Aroma compounds present in wine that are mainly responsible for the characteristic aromas are methoxypyrazines, volatile thiols, esters, higher alcohols, and fatty acids (Fischer, 2007). For example, volatile aroma thiols, ethyl and acetate esters, and higher alcohols contribute to the 'fruity' and 'tropical' aromas of Sauvignon Blanc wines (Marais, 1983; Darriet *et al*., 1995; Tominaga *et al*., 1996; Tominaga *et al*., 1998; Antalick *et al*., 2014). Meanwhile, methoxypyrazines contribute to the 'fresh' and 'green' aroma style (Marais, 1994). The wine quality together with grape yield and juice can be affected by foliar fertilization (Lasa *et al.*, 2012).

In South Africa, Chenin Blanc (17965 ha) and Sauvignon Blanc (9263 ha) are two of the most planted white wine cultivars (SAWIS, 2016). Traditionally, Chenin Blanc grapes were of low interest and were used to make low priced wine, brandy, and other spirits (Coetzee & Du Toit, 2012). By means of renewed interest by researchers and industry, Chenin Blanc wines have increased in quality and aroma styles in the past few years. Currently three dry Chenin Blanc wine styles are recognised such as 'fresh and fruity', 'rich and ripe wooded', and 'rich and ripe unwooded' (CBA, 2016). Research by Lawrence (2012) on Chenin Blanc investigated analytical methods and aroma compounds such as esters, monoterpenes, higher alcohols, and fatty acids. Only recently volatile thiols levels in Chenin Blanc have been reported (Wilson, 2017).

Sauvignon Blanc wines can be classified into two styles, explicitly 'fruity' or 'tropical' and 'fresh' or 'green'. Many studies have been performed on Sauvignon Blanc with the key focus on aroma compounds that influence aroma expression. Recent studies with nitrogen and sulphur foliar fertilization applications resulted in Sauvignon Blanc juice and wine having higher volatile thiols levels and improved aromatic potential (Dufourcq *et al.*, 2007a; Lacroux *et al.*, 2008; Jreij *et al.*, 2009). Increased glutathione (GSH) levels are obtained where soil nitrogen as well as foliar nitrogen and sulphur foliar applications were done (Lacroux *et al.*, 2008). Due to its antioxidant properties, GSH plays an important role in Sauvignon Blanc wines by protecting the aroma compounds such as volatile thiols (Dubourdieu & Lavigne, 2004).

The positive results of foliar fertilization studies in vineyards have gained the attention of South Africa's wine industry. Wine producers want to implement methods to influence the aroma of the wines positively and to increase the complexity as well. South Africa is a developing country and new international markets and trends are influencing the choices wine producers are making regarding oenological or viticultural practices.

## 1.2 Problem statement and research questions

Aroma plays a key role in wine and a current goal of winemakers is to improve the aroma expression of wines positively and to increase the complexity of the wines (Loubser, 2008). Various factors such as canopy management and nutrition can influence the quality and aroma expression of wine (Choné *et al.*, 2006; Lacroux *et al.*, 2008). Grapes with low YAN can lead to low yeast populations, poor fermentation vigour, and increased risk of sluggish or stuck alcoholic fermentations (Monteiro & Bisson, 1991). Nutrition levels can be adjusted or supplied to the vines through soil fertilization or by applying foliar fertilization sprays, meanwhile DAP or complex nutrients can be added to grape musts (Lorenzini & Vuichard, 2012). Previously research proved that nitrogen foliar fertilization result in increased levels of amino acids in the must (Lacroux *et al.*, 2008). Therefore nitrogen with or without sulphur foliar nutrition can enhance the aroma expression in Sauvignon Blanc wines (Choné *et al*., 2006; Lacroux *et al*., 2008).

Research proved that aromatic Sauvignon Blanc wines were obtained with nitrogen and sulphur foliar fertilization (Dufourcq *et al.*, 2007a), and by performing vine treatments, the aroma of Sauvignon Blanc wine can be manipulated and improved. Chenin Blanc is one of South Africa's most important and planted white cultivars in the industry (SAWIS, 2016), and no foliar fertilization trials have included this cultivar to date. Since few research studies regarding foliar fertilization trials have been published to date and most trials have been conducted under European conditions on various white and red wine cultivars (Dufourcq *et al.*, 2007b; Lacroux *et al.*, 2008; Jreij *et al.*, 2009; Lasa *et al.*, 2012; Verdenal *et al.*, 2015, 2016; Garde-Cerdán *et al.*, 2016; Geffroy *et al.*, 2016b), it is important to perform such foliar fertilization trials in South Africa (Fischer, 2007).

Such research trials are therefore required to compare the outcomes to previous trials (Sauvignon Blanc), to evaluate in local context (Chenin Blanc and Sauvignon Blanc) and to contribute to the knowledge regarding Chenin Blanc as a whole.

A specific combination of odour-active aroma compounds gives the aromatic character of Sauvignon Blanc, namely methoxypyrazines are found in the grape, while major volatiles and thiols are released during alcoholic fermentation (Fischer, 2007). Most foliar nutrition research has focused widely on those compounds (Dufourcq *et al*., 2007; Lacroux *et al*., 2008; Geffroy *et al*., 2016).

Overall, there is a lack of studies done with Chenin Blanc regarding the volatile and non-volatile compounds and the influence thereof on the aroma expression. Chenin Blanc have increased in quality over the past few years and have the potential to produce world recognized wines. Renewed interest and current research studies will broaden the knowledge regarding Chenin Blanc and guide South African winemakers to produce the best Chenin Blanc possible in the future.

## 1.3 Research aims and objectives

The aim of this research project was to study the effect of nitrogen and sulphur foliar fertilization treatments on the chemical composition of the juice and wine of *Vitis vinifera* L. cultivars Chenin Blanc and Sauvignon Blanc. Furthermore, the effect on the aroma composition of the wines was also evaluated sensorially. The main objectives of this study were as follow:

- To evaluate the effect of foliar fertilization applications on non-volatile content (amino acids, GSH, and YAN) at various stages of winemaking;
- To evaluate the effect of foliar fertilization applications on the volatile composition (major volatiles, methoxypyrazines, and volatile thiols);
- To determine the effect of foliar fertilization applications on the sensory properties.

The secondary objectives of this study were as follows:

- To determine the effect of wine maturation on the sensory properties and chemical composition of the wines matured for three and nine months

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# Chapter 2

# Literature Review

## Nitrogen and Sulphur foliar fertilization: Contribution to non-volatile and volatile compounds of Chenin Blanc and Sauvignon Blanc juices and wines

# Chapter 2. Nitrogen and Sulphur foliar fertilization: Contribution to non-volatile and volatile compounds of Chenin Blanc and Sauvignon Blanc juices and wines

## 2.1 Introduction

A wine's aroma, taste, and flavour can contribute to the wine's overall quality and determine if winemakers and consumers find it appealing (Marais, 1994). The aroma of a wine is a result of various interactions between different chemical compounds found in the wine. These compounds are generated at different stages and through various processes; some compounds originate from the grape, while others are generated during fermentation or wine aging. The composition of a grape berry depends on various factors such as grape variety, environmental, viticultural practices and terroir. Monoterpenes and methoxypyrazines are grape-derived, while volatile thiols, esters, higher alcohols, and fatty acids are released by yeast from their precursors during alcoholic fermentation (Fischer, 2007).

Nutrient requirements of a grapevine depend on its age, cultivar variety, yield, soil type, and properties (Holzapfel & Treeby, 2007). Vine nutrition deficiency often occurs due to various reasons, and negatively affects the aroma profile of a wine due to sluggish or stuck fermentation (Monteiro & Bisson, 1991). Nutrition deficiencies can be corrected and rectified by carrying out fertilization applications. Foliar nitrogen and sulphur fertilization applications can positively influence the levels of nitrogen and sulphur compounds in the must, success rate of alcoholic fermentation, and the resulting wine's composition and aroma (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Lasa *et al.*, 2012; Hannam *et al.*, 2014; Geffroy *et al.*, 2016a; Dienes-Nagy *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2017b; Helwi *et al.*, 2017; Kelly *et al.*, 2017). Results obtained through aroma and chemistry research in foliar fertilization studies can contribute to the knowledge base of aromas and chemical compounds of specific cultivar varieties.

Sauvignon Blanc and Chenin Blanc are two of the most widely planted white wine cultivars in South Africa (SAWIS, 2016), and currently Chenin Blanc is of great interest to researchers and the wine industry. The typical aroma of a Sauvignon Blanc wine can be described as being 'green' or 'tropical', depending on the wine style, and these characteristics express before and during alcoholic fermentation from various volatile compounds such as major volatiles, methoxy-pyrazines, and volatile thiols (Lacey *et al.*, 1991; Marais, 1994; Dubourdieu *et al.*, 2006). Chenin Blanc wines can be described, again depending on the winemaking style, as being 'fresh and fruity', 'rich and ripe unwooded', or 'rich and ripe wooded', and carbonyls, esters, higher alcohols, monoterpenes, volatile thiols, and wood-derived compounds can be linked to these aromas (Bester, 2011; Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012; Botha, 2015; Wilson, 2017).

Various research and studies have focused on the sensory and chemical compounds of Chenin Blanc and Sauvignon Blanc. A better understanding of the levels and perceptions of this class of aroma compounds in Sauvignon Blanc and Chenin Blanc wines can contribute to the chemical and sensory profiling of these varieties. This knowledge could ultimately aid researchers and winemakers to understand these compounds, produce a specific wine style, and produce wines with more complexity and higher quality.

The first part of this literature review will focus on various wine compounds and classes of compounds present in Chenin Blanc and Sauvignon Blanc wines. The second part will present cultivar characteristics with an accent on the aroma composition and its implications on the sensory perception of these cultivars. The literature review then proceeds to investigate the influence and contribution of different fertilization practices on the chemical compounds of the grapes, juice, and wine, but also on the resulting wine's aromatic expression.

## 2.2 Classes of chemical compounds present in the grape, must, juice, and wine

Generally, chemical compounds can be grouped according to their properties or the role which they play. In wine non-volatile compounds influence the taste and flavour of wines, while volatile compounds play an important role in influencing the aromatic expression. This part of the literature will include information on the presence, (bio)synthesis, and evolution throughout winemaking, chemical properties, and their implications for the various classes of compounds. The methods of analysis and reported values in the literature will also be presented.

### 2.2.1 Non-volatile compounds

Various non-volatile compounds are constituents of grapes, juices, and wines. These compounds are not aromatic, but some can be considered precursors to aromatic compounds while others can influence the perception of aroma compounds through interactions. Compounds reviewed in this section will include normal oenological parameters, ammonia and amino acids, and glutathione (GSH). Standard oenological parameters including pH, total acidity (TA), sugar content (Balling), and ethanol content are commonly measured during the winemaking process. Knowing the nitrogen levels of the must is crucial for successful alcoholic fermentation, and therefore the Yeast Assimilable Nitrogen (YAN) levels are measured. GSH includes the reduced and oxidised forms and can be considered an indicator of the level of oxidation and one of its functions is to protect volatile thiols from oxidation.

#### 2.2.1.1 Standard oenological parameters

***pH, total acidity, Balling, and Ethanol %***

In South Africa, Balling (˚B) was used extensively to determine the optimum maturity of grapes. The determination of harvest date in the past did not include other parameters such as pH and TA and nowadays relying solely on sugar level is considered not a very accurate index to follow (Du Plessis & Van Rooyen, 1982). Optimal grape ripeness can be determined by including the levels of ˚B, pH, TA, and colour of the grape skin and seeds (Deloire, 2012). Van Schalkwyk and Archer (2000) reported various optimal grape ripeness ranges for different classes of South African wines. The sugar content for white wine grapes at harvest ranges from 19.5-23˚B, while grapes for sparkling wines are harvested at lower levels (18-20˚B), red wines fall within the same range or can be slightly higher nowadays as white wine, and sweet and dessert wine grapes are harvested at higher sugar levels (22-26˚B) (Van Schalkwyk & Archer, 2000). The pH ranges for white, red, and sweet wine grapes are similar (3.2-3.4), while sparkling wines have lower levels (2.8-3.2), and dessert wines higher (3.3-3.7) (Van Schalkwyk & Archer, 2000). The TA levels of red (6.5-7.5 g/L), sweet (6.5-8 g/L), and dessert wine grapes (6.5-8 g/L) are lower compared to sparkling wines (7-9 g/L) and white wines which range from 7-8 g/L (Van Schalkwyk & Archer, 2000).

During winemaking, methods such as skin contact, maceration, and pressing can influence the pH and TA levels in must and juice. Free-run juice results in higher sugar and lower acid levels compared to the pressed juice (Amerine *et al.*, 1982). The method of adding tartaric acid to wines to lower the pH levels is effective and should be performed before the start of alcoholic fermentation (Pambianchi, 2001). During alcoholic fermentation the yeast converts the sugar to alcohol and carbon dioxide ($CO_2$). If the acidity is below 3 g/L the alcoholic fermentation is somewhat reduced; on the other hand, low pH levels help inhibiting the growth of undesirable bacteria (Amerine *et al.*, 1982). During alcoholic fermentation the alcohol content increases and the solubility of the acids decreases; therefore TA levels decrease and cause the pH to increase (Pambianchi, 2001; Robinson & Harding, 2014). White wine cold and protein stabilisation is done generally at -4˚C for a minimum of two weeks. During this procedure tartaric acid precipitates as potassium bitartrate salt and the resulting wine's TA decreases and pH increases further (Pambianchi, 2001; Robinson & Harding, 2014).

The initial sugar level of the harvested grapes and resulting wine's alcohol levels are correlated. According to South African legislation, alcohol levels of natural wines should range between 4.5-16.5% (WOSA, 2017). The residual sugar levels differ for different classes of wines: sparkling wines (<3 to >50 g/L), dry still wines (<5 g/L), semi-dry (>5 to ≤12 g/L), semi-sweet (>12 to <30 g/L), late harvest (≥20 g/L), natural sweet (>20 g/L), and noble late harvest wines (>50 g/L) (WOSA, 2017). During wine maturation in barrels wine diffuses through the small oak pores, and alcohol diffuse slower than water. Thus, in a dry cellar the ethanol strength can increase, while in a humid cellar, the ethanol strength will decrease. It is therefore important to top up the barrels regularly during maturation (Robinson & Harding, 2014).

#### 2.2.1.2 Yeast assimilable nitrogen

Grapes contain various nitrogen compounds which are grouped into two forms: mineral ($NH_4^+$, $NO_3^-$, and $NO_2^-$) and organic (free amino acids, nucleic acids, proteins, ethyl carbamate, and urea) (Conde *et al.*, 2007). Grape juice and must contain various nutrients and it is of importance to know which nitrogen compounds are abundant and required by the yeast for metabolism (Henschke & Jiranek, 1993). The major sources of nitrogen utilised by yeast are known as YAN and the constituents thereof are free amino nitrogen (FAN) and ammonia nitrogen. During grape ripening, Bell (1994) reported a gradual increase of total nitrogen and amino acid nitrogen levels for Cabernet Sauvignon grapes, while ammonium levels decreased. Similar increases and decreases during ripening have been reported by various researchers (Kliewer, 1968; Löhnertz & Schaller, 1992; Hilbert *et al.*, 2003). Henschke & Jiranek (1993) reported that grape juice nitrogen content ranges from 60-2400 mg N/L. The yeast assimilable amino acid nitrogen are distributed in different parts in the berry: 10-15% in the seed, 19-29% in the skin, and 61-65% in the pulp (Stines *et al.*, 2000). Assimilable nitrogen content of must provide a good estimation of the vine nitrogen status (van Leeuwen *et al.*, 2000).

A current ongoing study at Stellenbosch University, focuses on determining the YAN levels of different cultivars situated in different wine regions and districts in South Africa (Bieszczad & Buica, 2016; Buica & Bieszczad, 2016). YAN levels for Chenin Blanc were 82-288 mg N/L in 2016 and 77-250 mg N/L in 2017, while for Sauvignon Blanc they were 97-357 mg N/L in 2016 and 78-438 mg N/L in 2017 (Bieszczad & Buica, 2016; Buica & Bieszczad, 2016). During alcoholic fermentation yeast uses free alpha amino acids and ammonium ions for growth, metabolism, and to ferment grape juice and must (Henschke & Jiranek, 1993; Jiranek & Langridge, 1995).

YAN measurements should ideally be performed directly on must or juice just before alcoholic fermentation to get the most representative results (Bell & Henschke, 2005). Juice samples can underestimate the total berry YAN, because the majority of amino acids are contained in the skins of the grape (Stines *et al.*, 2000). Grapes from vineyards with a history of low YAN level, could be analysed for YAN before harvest to get an indication of the levels. In such cases, nitrogen supplementation with di-ammonium phosphate (DAP) at the start of alcoholic fermentation is suggested. Research studies recommend the minimum YAN level required by the yeast before alcoholic fermentation to be between 140-150 mg N/L (Henschke & Jiranek, 1993; Spayd *et al.*, 1993; Bell & Henschke, 2005). Lower YAN levels increase the risk of having slow, lagging, or stuck alcoholic fermentations and also the risk of producing hydrogen sulphide (Henschke & Jiranek, 1991). Even though 140-150 mg N/L is seen as the critical value for YAN, practically nitrogen levels should be increased to at least 200 mg N/L for a successful fermentation (Leonardelli, 2013; Petrovic & Buica, 2018). It was suggested that the nitrogen requirement of yeast differs according to sugar content of the juice: <21˚B (200-250 mg N/L), 21-23˚B (250-300 mg N/L), 23-25˚B (300-350 mg N/L), and >25˚B (350-400 mg N/L) (Wilton, 2015).

Current methods used can quantify primary amino acids and ammonia nitrogen, and include enzymatic assay kits, the Formol titration (Shively & Henick-Kling, 2001; Bell & Henschke, 2005; AWRI, 2017), and high-performance liquid chromatography (HPLC) for individual constituents of FAN, namely amino acids (Wilton, 2015; Waters, 2017).

### *Ammonia nitrogen*

Ammonia nitrogen ($NH_3$ or $NH_4^+$ for the ionic form, ammonium) is an important component of YAN. As mentioned above, during grape ripening, the ammonia nitrogen concentration declines over time (Bell, 1994). Various researchers have reported the percentage of ammonia nitrogen of the total YAN content found in berries and juices from different cultivars (Bell, 1994; Spayd *et al.*, 1994; Conradie, 2001; Ribéreau-Gayon *et al.*, 2006). Of the total YAN, ammonia nitrogen levels varied from 32-80% in berries and from 9-40% in juice (Huang & Ough, 1989). Ammonia nitrogen is readily assimilated by the yeast and is the most preferred nitrogen source during alcoholic fermentation (Monteiro & Bisson, 1991; Henschke & Jiranek, 1993). At the end of alcoholic fermentation, the ammonia nitrogen levels are usually depleted and it is therefore important to know the levels in the grape must before alcoholic fermentation (Ribéreau-Gayon *et al.*, 2006).

The major source of ammonia nitrogen is the berry itself, but additions of DAP to deficient musts can influence the levels thereof and also the total YAN concentration (Henschke & Jiranek, 1991, 1993; Monteiro & Bisson, 1991). Henschke & Jiranek (1993) reported a range of 5-325 mg N/L ammonia nitrogen in grapes. A recent ongoing study on South African wines have shown that ammonia nitrogen levels ranged from 16-86 mg N/L for Chenin Blanc and 27-104 mg N/L for Sauvignon Blanc (Bieszczad & Buica, 2016; Buica & Bieszczad, 2016).

### *Free amino nitrogen*

FAN includes free or primary amino acids, while secondary amino acids do not fall under this group. All amino acids contain the carboxyl (-COOH) and amino (-$NH_2$ or -NH-) functional groups (Ribéreau-Gayon *et al.*, 2006). From a structural point of view, the difference between primary and secondary amino acids is due to the level of substitution of the N in the amino group; in this case, -$NH_2$ is for primary and -NH- for secondary amino structures (Ribéreau-Gayon *et al.*, 2006). The implications of the structural differences can be observed in the method for the determination of these compounds. Various methods are available to analyse the amino acids (Section 2.2.1.2)

and the reagent most commonly used, nitrogen by *o*-phthaldialdehyde assay (NOPA), does not react with secondary amino acids. For quantification of individual compounds, some researchers focus only on the most important amino acids and do not include all the amino acids in their research due to the availability of methods for the quantification of these compounds (Wang *et al.*, 2016).

Amino acids are the most prevalent form of total nitrogen in grape juice and wine. Amino nitrogen distribution present in Riesling and Cabernet Sauvignon berries are 10-15% in the seeds, 19-29% in the skin, and 61-65% in the pulp (Stines *et al.*, 2000). The total FAN levels vary in grapes or grape juice depending on the year and amino acids usually represent 30-40% of the total nitrogen in ripe grapes (Ribéreau-Gayon *et al.*, 2006) and 51-92% of juice YAN at harvest (Bell, 1994; Spayd *et al.*, 1994; Conradie, 2001). An ongoing study on South African wines have shown that FAN levels ranged from 64-221 mg N/L for Chenin Blanc and Sauvignon Blanc levels were 60-267 mg N/L (Bieszczad & Buica, 2016; Buica & Bieszczad, 2016).

Several factors can influence the amino acid composition and concentration levels in grapes, including the grape cultivar, rootstock, site, seasonal conditions, and viticultural management (Kliewer, 1968; Etievant *et al.*, 1988). Bell and Henschke (2005) compiled a list of all the amino acids found in whole grapes and/or juice at harvest (Table 2.1). During the growth phase of alcoholic fermentation, yeast metabolises grape amino acids, while some others are produced by enzymatic degradation of proteins and others excreted by live yeasts at the end of fermentation (Lehtonen, 1996). Under anaerobic conditions amino acids are not metabolised by the yeast during alcoholic fermentation (Duteutre *et al.*, 1971; Ingledew *et al.*, 1987; Long *et al.*, 2012).

**Table 2.1** Concentration of amino acids found in the whole grape and/or juice at harvest (Bell & Henschke, 2005).

| Amino Acid | Concentration range (mg/L) | Amino Acid | Concentration range (mg/L) |
|---|---|---|---|
| Alanine | 10 - 227 | Lysine | 2 - 160 |
| Arginine | 20 - 2322 | Methionine | 1 - 33 |
| Asparagine | 1 - 171 | Ornithine | 0.1 - 27.2 |
| Aspartic acid | 10 - 138 | Phenylalanine | 2.8 - 138 |
| Cysteine | 1 - 8.2 | Proline | 9 - 2257 |
| Glutamine | 9 - 4499 | Serine | 13 - 330 |
| Glutamic acid | 27 - 454 | Threonine | 9 - 284 |
| Glycine | 1 - 20 | Tryptophan | 0.2 - 11 |
| Histidine | 5 - 197 | Tyrosine | 2 - 33 |
| Isoleucine | 1 - 117 | Valine | 7 - 116 |

Nitrogen compounds, including amino acids, contribute to the formation of compounds like esters, higher alcohols, hydrogen sulfide ($H_2S$), monoterpenes, and volatile thiols during the winemaking process (Henschke & Jiranek, 1993; Bell & Henschke, 2005). The formation of aroma compounds by the yeast during alcoholic fermentation is schematically presented in Figure 2.1 (Bell & Henschke, 2005). Various volatile compounds are formed from amino acids during alcoholic fermentation, therefore amino acids can be considered precursors of certain aroma compounds (Henschke & Jiranek, 1993; Bell & Henschke, 2005).

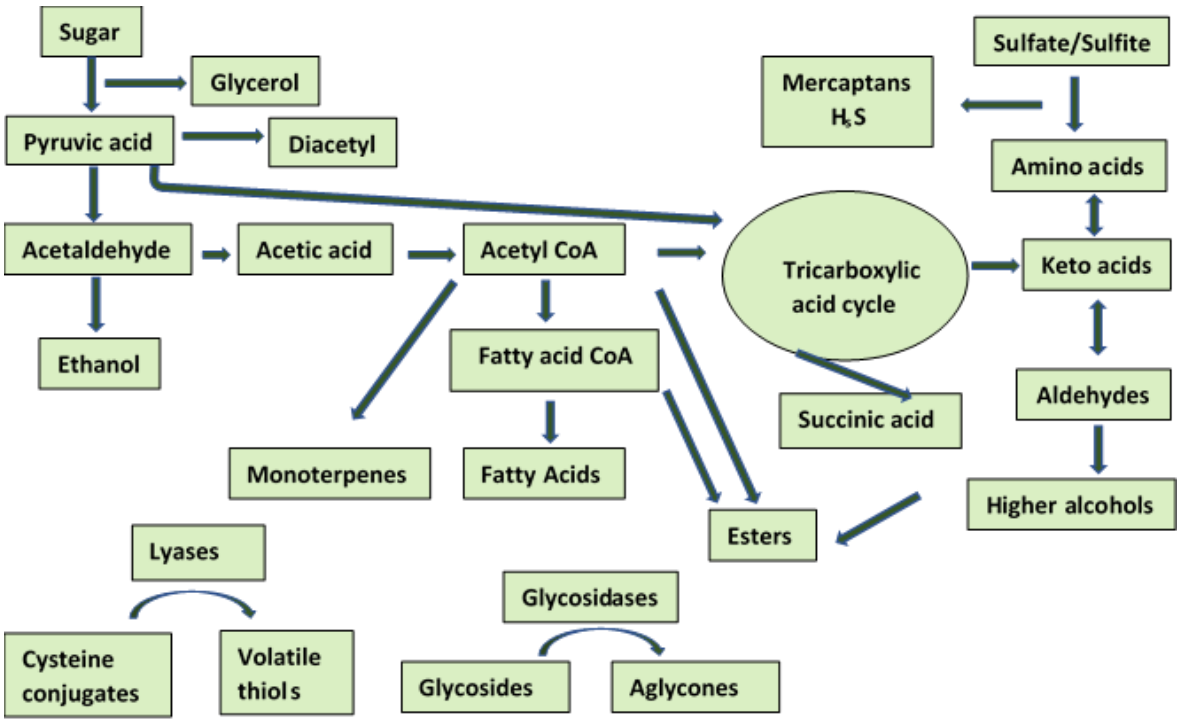


**Figure 2.1** A brief summary of the principal flavour metabolism by yeast in wine (Bell & Henschke, 2005).

Amino acids can be divided into different groups based on their role or structure: yeast-preferred, branched, sulphur-containing, and other amino acids (Godard *et al.*, 2007; Ljungdahl & Daignan-Fornier, 2012). Yeast preferred amino acids (Aspartic acid (ASP), Glutamic acid (GLU), Asparagine (ASN), Serine (SER), Arginine (ARG), Alanine (ALA), and Glutamine (GLN)) are considered the most important, because yeast metabolises them first (Monteiro & Bisson, 1991; Godard *et al.*, 2007). ARG and Proline (PRO) are usually the most abundant amino acids in grapes and ARG is preferred by the yeast as a nitrogen source (Garde-Cerda & Ancin-Azpilicueta, 2008; Holzapfel *et al.*, 2015). Addition of DAP to juice inhibits the ARG utilisation, and only after ammonia nitrogen has been metabolised by the yeast, ARG will be used. Keto acids, such as pyruvic and α-ketoglutaric acid, bind to sulphur dioxide ($SO_2$) and react to phenols during winemaking and are formed during the Ehrlich pathway by amino acids ALA and GLU (Ough *et al.*, 1990).

Branched amino acids (Valine (VAL), Leucine (LEU), Phenylalanine (PHE), and Isoleucine (ILE)) are precursors of volatile esters (Antalick et al., 2014). Higher alcohols are formed during the Ehrlich pathway from these amino acids, but a greater proportion is synthesised from sugars (Bell & Henschke, 2005). The relationship between higher alcohols and amino acid assimilation during the fermentation cycle is not clear (Bell & Henschke, 2005). The acids along with these alcohols can form esters such as isoamyl acetate and phenylethyl acetate. These amino acids are accumulated in the early stages of alcoholic fermentation and do not support and contribute to the high growth rates during fermentation. The majority of esters are enzymatically synthesised by the yeast from alcohols and medium and long fatty acids through esterification reactions (Lambrechts & Pretorius, 2000).

Sulphur-containing amino acids are Methionine (MET), Cysteine (CYS), and Cystine (Cys-Cys). They are involved in yeast metabolism under certain conditions and can result in $H_2S$ production (Henschke & Jiranek, 1991; Giudici & Kunkee, 1994). When MET becomes depleted in the early stages of alcoholic fermentation, the Sulfate Reduction Sequence (SRS) pathway is activated to reduce sulfate to $H_2S$ and release a surplus thereof alongside mercaptans from the cell (Bell &

Henschke, 2005). DAP additions before or during alcoholic fermentation can inhibit the production of $H_2S$ (Bell & Henschke, 2005). GSH is formed during alcoholic fermentation from Glutamate, GLY, and CYS (Castellarin *et al.*, 2012). Research done by Elskens *et al.* (1991) and Hallinan *et al.* (1999) suggest that GSH can be degraded to CYS and finally $H_2S$ in nitrogen deficient conditions. Volatile thiols are found in small amounts in juice must and their precursors such as non-volatile, non-glycosylated, odourless S-CYS conjugates have been identified (Tominaga *et al.*, 1998b, 1998a, 1998c). During alcoholic fermentation the yeast degrade the S-CYS thiol precursors to release the volatile thiols (Tominaga *et al.*, 1998b, 1998a, 1998c; Murat *et al.*, 2001a).

All other amino acids (Aminobutyric acid (GABA), Lysine (LYS), Threonine (THR), Glycine (GLY), Tyrosine (TYR), Tryptophan (TRP), Histidine (HIS) and Ornithine (ORN) – Hydroproline (HYP) and PRO are secondary amino acids and are not included in FAN value)) are used by the yeast only in the case that other nitrogen sources are depleted first (Duteutre *et al.*, 1971). PRO is among the two dominant amino acids that makes up the bulk of the total amino acids (Kliewer, 1968). PRO cannot be assimilated by yeast in the absence of oxygen, therefore, at the end of alcoholic fermentation PRO levels have not changed or decreased and high levels are still found in the resulting wine (Ribéreau-Gayon *et al.*, 2006).

### 2.2.1.3 Glutathione

GSH was first discovered in 1989 by Cheynier *et al.* (1989) in red and white French grapes. It is a sulphur-containing tri-peptide (γ-glutamyl-L-cysteinyl-GLY) and occurs as a natural antioxidant in grapes and must (Anderson, 1998). GSH is formed from amino acids GLU, GLY, and CYS and contains a nucleophilic -SH centre (Anderson, 1998; Castellarin *et al.*, 2012). GSH is synthesised enzymatically in the grape berry and the reduced GSH (GSH-R or simply GSH, Figure 2.2) form is the most abundant thiol-containing compound present at harvest. Šuklje *et al.* (2012) reported that of the total GSH content in grapes more than 90% of it is present in its reduced form.

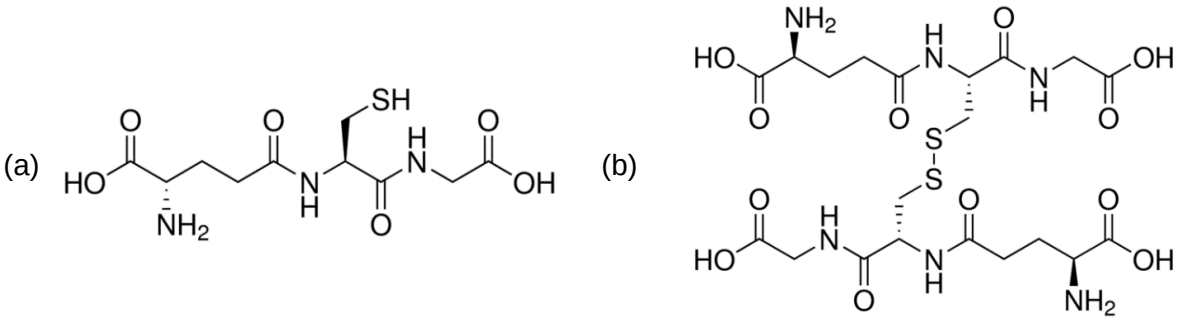


**Figure 2.2** L-Glutathione reduced form (GSH-R) (a), and Glutathione oxidised form (GSH-O) (b).

During crushing, GSH needs to be present in high levels in the must to protect it fully (Singleton *et al.*, 1985). Du Toit *et al.* (2007) and Kritzinger (2012) reported that GSH can be used as a marker of oxidation in winemaking, due to its sensitivity to oxidation. GSH reacts with polyphenol *o*-quinones in juice and wine and consequently limit the effect of oxidation (Cheynier *et al.*, 1989; Makhotkina *et al.*, 2014). Being reactive to quinones, GSH plays an important role in juice by protecting various volatile thiols from oxidation (Choné *et al.*, 2006) and also stopping the formation of unstable aromas in wine (Papadopoulou & Roussis, 2001).

GSH-R's electrophilic -SH group is reactive leading to the formation of 2-S-glutathionyl-caftaric acid or Grape Reaction Product (GRP) (Singleton *et al.*, 1985). The *o*-quinone of caffeic acid reacts most readily with GSH-R, and phenols can form derivatives of GSH. The GRP is colourless and traps the *o*-quinone and prevents the must from browning and other reactions from taking place (Kritzinger *et al.*, 2013). The GRP can be further oxidised by laccase and yields a *o*-quinone, which can brown the polymers and also produce 2,5-di-sulphur-glutathionylcaftaric acid by the addition of another GSH (Singleton *et al.*, 1985; Cheynier *et al.*, 1989). GSH can also react with oxygen and undergo oxidation resulting into the oxidised GSH form (GSH-O or GSSG) (Figure 2.2).

GSH concentration increases during grape ripening and the highest levels occur at the start of véraison (Adams & Liyanage, 1993). Due to enzymatic and redox reactions, GSH levels decrease rapidly during crushing (Adams & Liyanage, 1993). Free run juices during pressing are reported to have higher GSH concentrations compared to other higher press fractions (Patel *et al.* 2010). Levels of GSH-R during alcoholic fermentation usually decrease and this can be due to the metabolism of the yeast (Du Toit *et al.*, 2007). During wine ageing, GSH (unspecified GSH-R or GSH-O) concentrations decrease (Lavigne *et al.*, 2007; Ugliano *et al.*, 2011). South African GSH-R concentrations present ranges from 1-71 mg/L in juice and up to 35 mg/L in wine (Du Toit *et al.*, 2007; Janeš *et al.*, 2010; Fracassetti *et al.*, 2011). Reported GHS-O levels found in South African Sauvignon Blanc juices ranged from 0.46-2.93 mg/L (Du Toit *et al.*, 2007).

A method currently used in South Africa for GSH determination in grape juice and wine is the liquid chromatography-tandem mass spectrometry (LC-MS/MS) and can measure both reduced and oxidised forms (Du Toit *et al.*, 2007).

### 2.2.2 Volatile compounds

During alcoholic fermentation, the metabolism of sugar by the yeast, leads to the formation of volatile compounds such as esters, fatty acids, and higher alcohols (Francis & Newton, 2005). These compounds arise as primary metabolites of yeast and sugar and the metabolism of amino acids (Henschke & Jiranek, 1993; Swiegers *et al.*, 2005). The nitrogen status of the must also contribute to the formation of these compounds (Henschke & Jiranek, 1993), but too high nitrogen content can reduce the production thereof. The odour thresholds for major volatiles are measured in mg/L, while methoxypyrazines and volatile thiols are much lower and measured in ng/L in juice and wine. The volatile compounds reviewed in this section will include major volatile compounds such as esters, fatty acids, higher alcohols, methoxypyrazines, and volatile thiols which correlates with the aromatic expression of the wine.

#### 2.2.2.1 Major volatile compounds

##### *Esters*

Esters are an important group of volatile compounds and contribute to the pleasant 'fruity' and 'floral' aromas in wines (Swiegers *et al.*, 2005). Esters can be grouped into two groups, namely acetate esters and ethyl esters. The most significant esters are ethyl ethanoate (ethyl acetate), 3-methylbutyl acetate (isoamyl acetate), 2-methylpropyl ethanoate (isobutyl acetate), ethyl hexanoate (ethyl caproate), and 2-phenylethyl acetate (phenethyl acetate) (Thurston *et al.*, 1981). Ethyl esters contribute 'apple' aromas, while acetate esters are associated with 'fruity' aromas (Saerens *et al.*, 2008). Yeast plays a crucial role in the formation of esters. Esters are produced

by the metabolism of yeast through lipid and acetyl-CoA metabolism (Figure 2.1), through the action of alcohol acetyl transferase, an alcohol and a coenzyme-A-activated acid condensate (Lambrechts & Pretorius, 2000).

Various factors, such as yeast strain, clarification, and temperature during the winemaking processes can influence the levels of esters present in wines. During ageing, ester concentrations can decrease due to hydrolysis or oxidation and the wines can result in having a loss of 'fruity' aromas (Marais, 1978). During bottle maturation of white wine, loss of 'fruitiness' can be linked to the loss of acetate esters and they tend to diminish more rapidly than ethyl esters (Ramey & Ough, 1980). Ethyl acetate concentrations are much higher compared to other esters found in wine. The sensory detection thresholds of esters in wine range from 0.08-60 mg/L for the various compounds (Von Mollendorff, 2013). Ethyl acetate range from 30-234 mg/L (Van Wyngaard, 2013) in South African Sauvignon Blanc wines, and from 48-210 mg/L in Chenin Blanc wines (Lawrence, 2012).

Major volatiles, which include esters, fatty acids, and higher alcohols, are determined using various methods. Most are based on gas chromatography, coupled with flame ionisation detection (GC-FID) or mass spectrometry (MS) detection (Louw *et al.*, 2009).

***Fatty acids***

Fatty acids contribute to the wine aroma and have an important impact on wine quality (Bell & Henschke, 2005). The most abundant fatty acids in wine include acetic, decanoic, hexanoic, octanoic, and decanoic acid. Volatile fatty acid composition range between 500-1000 mg/L, and acetic acid ranging between 0.2-2 g/L accounts for more than 90% of the fatty acids. Generally, red wines have higher concentrations of these acids than white wine (Lambrechts & Pretorius, 2000). Low concentrations can positively contribute to the complexity and aroma of wines (Coetzee, 2011), while unwanted flavours like 'cheesy', 'vinegar', and 'rancid' are due to too high concentrations of fatty acids (Lambrechts & Pretorius, 2000). Bacterial spoilage can be linked to elevated levels of acetic acid (Ribéreau-Gayon *et al.*, 2006).

During the early stages of alcoholic fermentation and biosynthesis of long chain fatty acids, medium chain fatty acids like hexanoic, octanoic, and decanoic acid are produced as intermediates. Acetic acid is formed as a metabolic intermediate in the synthesis of acetyl-CoA from pyruvic acid or is formed directly from acetaldehyde by aldehyde dehydrogenases (Bell & Henschke, 2005). Long chain unsaturated fatty acids, such as oleic and linoleic acid, can enhance alcoholic fermentation, but are not yeast-derived products and originate from the waxy cuticle of grape skins (Lambrechts & Pretorius, 2000). Many factors that can influence the fatty acid levels include yeast strain, sugar concentration, inoculation rate, juice clarification, fermentation temperature, nitrogen, oxygen exposure, and $SO_2$ additions (Henschke & Jiranek, 1993; Garde-Cerdán *et al.*, 2009; Coetzee, 2011). The nitrogen concentration levels of the must play a crucial role in the volatile acidity in wine.

Fatty acid sensory detection thresholds in wine or spirits range from 0.7-1000 mg/L for the various compounds (Von Mollendorff, 2013). Contradictory results, related to increases, decreases, or stability of certain fatty acids have been reported during wine aging (Roussis *et al.*, 2005; Blake *et al.*, 2009; Lee & Steenwerth, 2011).

***Higher alcohols***

Higher alcohols, also known as fusel alcohols, are secondary yeast metabolites, and can positively or negatively influence the aroma of the wine (Bell & Henschke, 2005). Higher alcohols have more than two carbon atoms in their structure and can be grouped into two categories: aliphatic and aromatic alcohols (Lambrechts & Pretorius, 2000). Aliphatic alcohols include pentan-1-ol (amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol), 2-methylpropan-1-ol (isobutanol), and propan-1-ol (propanol), while aromatic alcohols include 2-phenylethanol (phenylethyl alcohol) and 4-(2-Hydroxyethyl) phenol (tyrosol).

Higher alcohols are formed in two ways during alcoholic fermentation. Firstly, by being synthesised anabolically from intermediates of the sugar metabolism (e.g. glucose), and secondly synthesised catabolically from branched-amino acids, such as LEU, ILE, THR, and VAL (Boulton *et al.*, 1996; Dickinson *et al.*, 2003). Alcohols are formed via the Ehrlich pathway, and the branched amino acids are deaminated, α-keto-acids are decarboxylated, and reduced to the corresponding alcohol (Bell *et al.*, 1979) (Figure 2.1).

Must containing high amino acids will produce higher levels of higher alcohols (Swiegers *et al.*, 2005). The sensory detection thresholds of various higher alcohols in spirits, beer, or wine range from 4-800 mg/L for the various compounds (Von Mollendorff, 2013). Concentrations below 300 mg/L add positively to the complexity of wines, whereas more than 400 mg/L can have a detrimental effect and display unpleasant ‘fusel’ and ‘solvent-like’ aromas, with the exception of 2-phenyl ethanol (‘rose’ and ‘floral’ aromas) (Lambrechts & Pretorius, 2000). Isoamyl alcohol, is usually found in wines with the highest levels ranging from 45-490 mg/L at the end of alcoholic fermentation (Lambrechts & Pretorius, 2000). During aging, alcohols can be oxidised to form aldehyde, causing the concentration levels to decrease (Marais & Pool, 1980).

### 2.2.2.2 Methoxypyrazines

Methoxypyrazines are nitrogen-containing compounds that are derived from grapes and are situated in the skin and exocarp of grape berries (Marais, 1994). Methoxypyrazines are formed by the catabolism of secondary amino acids such as VAL, GLY, and MET present in the grape (Cheng *et al.*, 1991). In Sauvignon Blanc, three methoxypyrazine compounds were identified, namely 2-isobutyl-3-methoxypyrazine (IBMP), 2-*sec*-butyl-3-methoxypyrazine (SBMP), and 2-isopropyl-3-methoxypyrazine (IPMP) (Lacey *et al.*, 1991; Marais, 1998) (Figure 2.3).

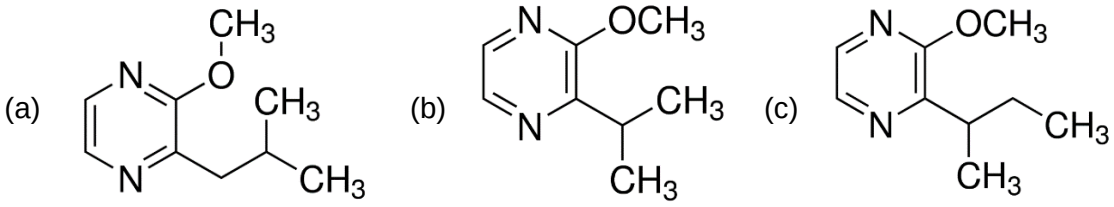


**Figure 2.3** 3-Isobutyl-2-methoxypyrazine (IBMP) (a), 3-isopropyl-2-methoxypyrazine (IPMP) (b), and 3-sec-butyl-2-methoxypyrazine (SBMP) (c).

The accumulation of methoxypyrazines in the berries can be influenced by various factors, such as environmental parameters, clone, canopy management, soil, and terroir (Swiegers *et al*., 2006). During berry ripening, methoxypyrazine levels increase and the highest levels are obtained

at véraison, but during véraison the levels start to decrease (Lacey *et al.*, 1991; Swiegers *et al.*, 2006). Lacey *et al.* (1988) reported that higher climatic temperatures will lead to lower methoxypyrazine concentrations due to their degradation during ripening and sensitivity to sunlight, while in cooler climates higher concentrations are obtained. Various research studies links vines with excessive growth to produce grapes with higher methoxypyrazine concentrations before harvest, irrespective of cluster light exposure. The majority (95.5%) of methoxypyrazines are located in the skin of grapes at harvest time (Roujou de Boubee *et al.*, 2000). The highest levels of IBMP are found in the free run juice and also increased levels are found after skin contact (Marais, 1998; Marais *et al.*, 1999; Roujou de Boubee *et al.*, 2000). Methoxypyrazine concentrations can decrease due to clarification processes while during alcoholic fermentation the levels are reported to be stable (Sala *et al.*, 2004; Kotserides *et al.*, 2008). It can be concluded that viticultural practices can influence the methoxypyrazine concentrations much more than winemaking practices (Roujou de Boubee *et al.*, 2000).

Methoxypyrazine sensory detection thresholds in water are very low: 2 ng/L (IBMP), 1 ng/L (SBMP), and 2 ng/L (IPMP) (Lacey *et al.*, 1991; Alberts *et al.*, 2009). Methoxypyrazines are known to contribute to the 'green' aromas present in Sauvignon Blanc wines. IBMP contribute to the 'herbaceous' and 'green pepper' aromas, while SBMP contributes to the 'asparagus' and 'green beans', and IPMP is associated with 'pea' and 'bell pepper' aromas (Ebeler & Thorngate, 2009). Wines with high methoxypyrazine levels can be perceived negatively if the aroma notes are not in balance with other compounds. IBMP is the methoxypyrazine that is found with the highest concentration in Sauvignon Blanc musts and wines, while IPMP and SBMP are found at much lower concentrations (Lacey *et al.*, 1991). Van Wyngaard (2013) performed a survey on South African Sauvignon Blanc wines and levels of IBMP ranged from 0.4-44 ng/L.

Methods used to analyse methoxypyrazines, such as IBMP and IPMP, are commonly based on gas chromatography coupled with head space solid-phase micro-extraction (HS-SPME) (Coetzee, 2014).

#### 2.2.2.3 Volatile thiols

Volatile thiols are sulphur-containing compounds and are present in wine at very low concentrations and the aromas they produce are powerful. Volatile thiols are generally known to contribute to the positive 'tropical' aroma characteristics such as 'citrus', 'gooseberry', 'grapefruit', and 'passion fruit' of Sauvignon Blanc wines (Tominaga *et al.*, 1998c, 2000). These compounds are sulphur-containing substances with additional functional groups such as alcohol, ester, or ketone. Darriet *et al.* (1995) identified the first volatile thiol, 4-mercapto-4-methyl-pentan-2-one (4MMP), in 1995 in Sauvignon Blanc wines. Since then, various researchers focused on these compounds and Tominaga *et al.* (1996, 1998a) identified four other volatile thiols in Sauvignon Blanc. In Figure 2.4, the three major volatile thiol compounds contributing to the aroma expression of Sauvignon wines are 4MMP, 3-mercapto-hexyl acetate (3MHA), and 3-mercaptohexan-1-ol (3MH) (Darriet *et al.*, 1995; Tominaga *et al.*, 1998c). 3MH and 3MHA were only recently identified in South African Chenin Blanc wines (Wilson, 2017).

The odour threshold of these volatile thiols in model wine solution are 0.8 ng/L for 4MMP, 60 ng/L for 3MH, and 4 ng/L for 3MHA (Tominaga *et al*., 1998b). Aromas associated with these compounds are 'box tree', 'guava', and 'blackcurrant' for 4MMP, 'grapefruit' and 'passion fruit' for 3MH, and 'box tree' and 'passion fruit' for 3MHA (Darriet *et al*., 1995; Tominaga *et al*., 1998b, 2000; Swiegers *et al*., 2006; Roland *et al*., 2011).

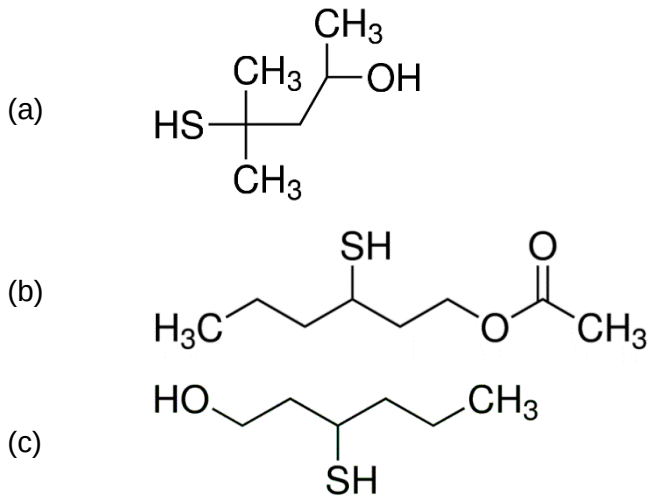


**Figure 2.4** 4-mercapto-4-methyl-pentan-2-one (4MMP) (a), 3-mercaptohexyl acetate (3MHA) (b), and 3-mercapto-hexan-1-ol (3MH) (c).

In Figure 2.5, the three identified pathways that can lead to the formation of 4MMP and 3MH are presented (Roland *et al.*, 2011). One pathway leading to the formation of 3MH includes the trans-2-hexenal and trans-2-hexenol alongside $H_2S$, which acts as a sulphur donor. 4MMP and 3MH share the other two pathways and include cysteinylated and glutathionylated precursors. 4MMP and 3MH are synthesised in the berry and their precursors are in a cysteinylated bound form.

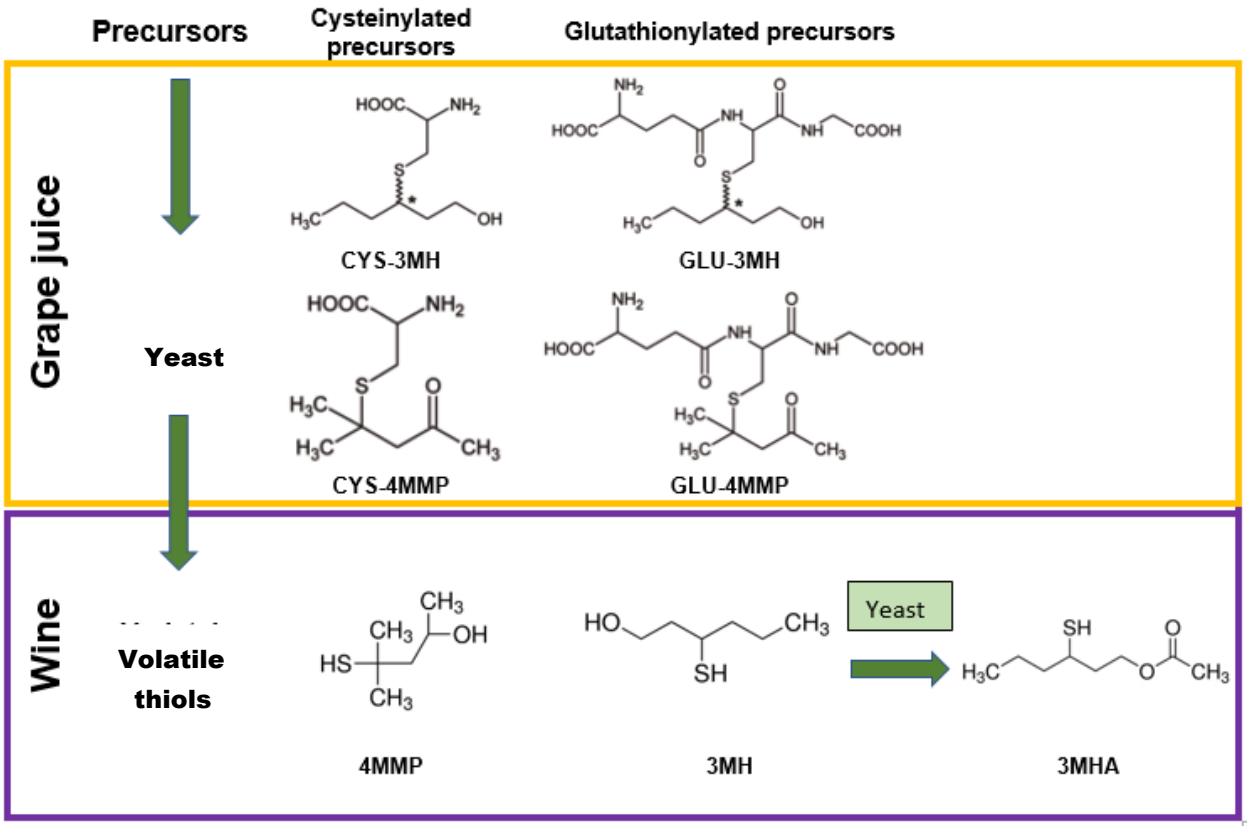


**Figure 2.5** Biogenesis pathways for volatile thiols during alcoholic fermentation (Adapted from Roland *et al.*, 2010a).

Tominaga *et al.* (1998c) identified the precursors of volatile thiols as odourless, non-volatile, non-glycosylated sulphur-CYS conjugates. These conjugates are cleaved by the yeast, via its beta-lyase activity, during alcoholic fermentation and the non-volatile thiols S-3-(hexan-1-ol)-cysteine (CYS-3MH) and S-3-(4-mercapto-4-methylpentan-2-one)-cysteine (CYS-4MMP) becomes an

active compound (Roland *et al.*, 2010b). The last pathway includes the glutathionylated precursor such as S-3-(hexan-1-ol)-glutathione (GSH-3MH) which are released during alcoholic fermentation. 3MH precursors are mainly located in the skins, while 4MMP are mostly found in the skin and pulp (Roland *et al.*, 2011).

Only trace amounts of 4MMP and 3MH are found in grapes and musts (Dubourdieu *et al.*, 2006). During alcoholic fermentation these volatile thiols are released from their odourless non-volatile precursors. 3MHA is produced from the acetylation of 3MH during alcoholic fermentation by the yeast ester and forms alcohol acetyltransferase (Roland *et al.*, 2011). Various factors such as terroir and winemaking processes prior to alcoholic fermentation can influence the levels of precursors present in the grapes (Murat *et al.*, 2001b; Dubourdieu *et al.*, 2006). Volatile thiols are susceptible to oxidation during aging and a decrease in levels can be expected (Coetzee, 2014). During wine ageing, 3MHA is usually converted to 3MH via acid hydrolysis (Nikolantonaki *et al.*, 2010; Herbst-Johnstone *et al.*, 2011) or by the breakdown of 3MH disulphide present in the wines (Capone *et al.*, 2010; Sarrazin *et al.*, 2010).

Various methods have been used to quantify volatile thiols such as 3MH and 3MHA. Most are based on the determination of thiols by MS detection (Tominaga *et al.*, 1998a; Herbst-Johnstone *et al.*, 2013; Capone *et al.*, 2015; Piano *et al.*, 2015).

## 2.3 Cultivar characteristics of Chenin Blanc and Sauvignon Blanc wines based on the aroma compound classes: Linking the chemistry composition to the sensory perception

Wine aromas are the result of various volatile aroma compounds and their interactions with other compounds (volatile and non-volatile) present in wines. The origin of various aromas contributing to the overall aroma profile of wine are divided into four categories: primary grape aroma, secondary grape aroma, fermentation bouquet, and maturation bouquet (Rapp, 1998). Primary aromas are present in the grapes and are a result of a specific cultivar and terroir. Methoxypyrazines and some monoterpenes fall under this group. The secondary grape aromas are formed during the processing of the grapes and by chemical, enzymatic, and thermal reactions in the must. This specific group includes other monoterpenes. The third category, fermentation bouquet aromas, are aroma compounds formed during alcoholic fermentation and malolactic fermentation. Various factors such as yeast strain, temperature of fermentation, lees contact, and malolactic fermentation can contribute and influence these compounds. This category is responsible for major volatile compound composition of the wine and compounds such as esters, fatty acids, higher alcohols, and volatile thiols are included (Rapp, 1998; Ferreira *et al.*, 2000). The final category, maturation bouquet aromas, are influenced by chemical reactions during the maturation of wine in barrels and bottles. Chemical changes can occur during ageing between esters, fatty acids, higher alcohols, and volatile thiols. Understanding and linking the chemical composition to the winemaking methods, can aid winemakers in producing quality wines of specific styles (Marais, 2006).

Being two of the most planted white wine cultivars in South Africa, Chenin Blanc and Sauvignon Blanc are of great interest to researchers currently (SAWIS, 2016). Various studies focused mainly on yeast metabolism and winemaking techniques and investigated the aroma compounds of specific cultivars and their contributing chemical compounds in wines (Swiegers *et al.*, 2005; Malherbe *et al.*, 2013; Van Wyngaard, 2013; Von Mollendorff, 2013; Botha, 2015).

### 2.3.1 Chenin Blanc

Chenin Blanc is considered as being a neutral grape variety and was used in South Africa in the past mainly for distillation and production of brandy, other spirits, and low quality wines (Clarke, 2007). Wines were exported in bulk to other countries or frequently blended with other varieties (Viognier, Sauvignon Blanc, and Semillon), as opposed to being bottled as a varietal wine (SAWIS, 2016). Currently, it is the most planted white cultivar in South Africa and the focus has shifted towards producing high quality wines with amazing aromas (Loubser, 2008). Due to various winemaking processes and methods, different Chenin Blanc wine styles are being made (CBA, 2016). According to the Chenin Blanc Association of South Africa different Chenin Blanc styles have been identified: 'fresh and fruity' (FF), 'rich and ripe-unwooded' (RRUW), and 'rich and ripe-wooded' (RRW) (CBA, 2016).

Various researchers have shown that sensory panelists have difficulties distinguishing between the three styles, and the wines are often rather grouped into two groups: FF/RRUW and RRW or FF and RRW/RRUW (Bester, 2011; Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012; Botha, 2015; Wilson, 2017). Generally, the aromatic descriptors for Chenin Blanc wines are varied, and include: 'buttery', 'caramel', 'citrus', 'earthy', 'floral', 'fresh fruit', 'fruity', 'guava', 'grapefruit', 'honey', 'lemon', 'marmalade', 'nutty', 'oak', 'pineapple', 'peach', 'rich fruit', 'ripe fruits', 'spicy', 'sweet', 'toasted bread', 'tropical', 'vegetative', 'vanilla', and 'wood' descriptors (Bester, 2011; Hanekom, 2012; Van Antwerpen, 2012; Botha, 2015; Wilson, 2017). The three styles mentioned overlap in their descriptions, with FF wines driven more by 'freshness' and 'fruit-associated' aroma attributes, while, at the other end of the spectrum, RRW wines are described more with 'oak-associated' attributes.

FF wines are associated with volatile compounds such as esters and volatile thiols. Acetate and ethyl esters consist of ethyl butyrate, ethyl hexanoate, 2-phenylethyl acetate, isoamyl acetate, and hexyl acetate (Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012; Wilson, 2017). These fermentation-derived compounds can be influenced by the type of yeast strain and can be altered by fermentation temperature, nitrogen content, and oxygen exposure (Garde-Cerda & Ancin-Azpilicueta, 2008; Coetzee, 2011). Acetate esters are associated with aromas like 'banana', 'honey', 'pear', and 'rose', while ethyl esters are linked to 'apple', 'banana', 'floral', 'fruity', 'pear', and 'pineapple' (Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012). The FF group is also associated with volatile thiols, 3MHA and 3MH (Lawrence, 2012; Weightman, 2014; Buica *et al.*, 2016; Wilson, 2017). 3MHA is formed by the esterification of 3MH and is associated with 'passion fruit' and 'grapefruit' aromas, while 3MH is associated with 'passion fruit', 'grapefruit', 'fresh', and 'herbaceous' aromas (Wilson, 2017).

RRUW wines are associated with volatile compounds such as esters, monoterpenes, and volatile thiols and include compounds such as ethyl butyrate, ethyl hexanoate, geraniol, limonene, β-ionone, 3MH, and 3MHA (Bester, 2011; Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012; Botha, 2015; Wilson, 2017). The fermentation-derived esters are linked to aromas such as 'apple', 'banana', 'violets', while monoterpenes are associated with 'floral', 'rose', 'citrus', and 'geranium' aromas (Lambrechts & Pretorius, 2000; Swiegers *et al.*, 2005; Coetzee & Du Toit, 2012; Buica *et al.*, 2017; Wilson, 2017). Volatile thiols such as 3MH and 3MHA contribute to 'passion fruit', 'grapefruit', and 'fresh and herbaceous' aromas (Lambrechts & Pretorius, 2000; Swiegers *et al.*, 2005; Wilson, 2017).

RRW wines are associated with esters, carbonyl, and wood-derived compounds such as ethyl lactate, furfural, diacetyl, diethyl succinate, acetoin, and cis- and trans-whiskey lactones (Bester, 2011; Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012; Botha, 2015; Wilson, 2017). Aromas linked to these specific compounds are 'buttery', 'toasty', 'vanilla', 'nutty', 'spicy', 'oaky/ wooded', 'toasted bread', and 'creamy' aromas (Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012). The wood-derived compounds are mainly extracted from the oak wood into the wine during alcoholic or malolactic fermentation and/or maturation. During wine maturation, volatile thiols are unstable and influenced by oxygen, and wood derived compounds can suppress and overpower them (Wilson, 2017).

### 2.3.2 Sauvignon Blanc

Sauvignon Blanc is one of the most famous white cultivars in the world and has characteristic 'asparagus', 'green pepper', 'grassy', 'herbaceous', and 'vegetative' aromas which come from a volatile compound group, methoxypyrazines (Marais, 1994). Currently, Sauvignon Blanc is the third most planted white cultivar in South Africa and plays an important role locally and internationally (SAWIS, 2016). The taste of Sauvignon Blanc wines is described as fresh, crisp, and acidic on the palate. Most Sauvignon Blanc wines are made by fermenting in stainless steel tanks and barrel fermentation with aged lees are rarely performed during winemaking. Typical aromas of South African Sauvignon Blanc wines include 'asparagus', 'capsicum', 'gooseberry', 'grapefruit', 'grassy', 'green pepper', 'herbaceous', passion fruit', 'tomato leaf', and 'vegetative' (Swiegers *et al.*, 2006). Sauvignon Blanc wines can be grouped into two style groups: 'fresh and crisp green style' and 'rich and tropical/fruity style' (Coetzee & Du Toit, 2012)'.

The 'green style' is described by aroma attributes such as 'asparagus', 'capsicum', 'grassy', 'green pepper', 'herbaceous', 'tomato leaf', and 'vegetative'. The grape-derived volatile compounds, methoxypyrazines, are mainly responsible for these 'green' aromas (Marais, 1994). IBMP is associated with 'bell pepper', 'green bean', and 'herbaceous' aromas, while IPMP is associated with 'pea', 'asparagus', and 'vegetal' aromas (Lacey *et al.*, 1988, 1991; Marais, 1994; Swiegers *et al.*, 2006). Yeast cannot modify the methoxypyrazine levels, and their final levels in wine are dependent on viticultural practices. When present above certain levels, thiols can also impart a green associated aroma such as 'tomato leaf' (Tominaga *et al.*, 1998a). In addition, fermentation-derived compounds can also add to the complexity of wines if they are at optimal levels.

The 'tropical/fruity style' includes aromas such as 'gooseberry', 'grapefruit', 'pineapple', and 'passion fruit' (Treurnicht, 2011). Various volatile compounds like esters, higher alcohols, mono-terpenes, and volatile thiols are associated with aromas of this style. Volatile thiols contribute to the 'passion fruit', 'grapefruit', 'box tree', and 'cat urine' attributes depending on their levels (Darriet *et al.*, 1995; Tominaga *et al.*, 1996, 1998a; Dubourdieu *et al.*, 2006). Monoterpenes generally contribute to the 'floral aromas' in Sauvignon Blanc wines. Esters contribute to the 'fresh' and 'fruity' flavours of Sauvignon Blanc. Acetate esters are usually present at higher levels than ethyl esters. Ethyl acetate is the most abundant ester and contributes to 'fruity', 'nail polish', and 'varnish' aromas, while isoamyl acetate produces 'pear' and 'banana' aromas. A higher alcohol, isoamyl alcohol, is found at the highest levels found in wines and 'marzipan' aromas are produced.

Sauvignon Blanc is sensitive to oxidation, and when winemakers prevent the wine to come into contact with oxygen, reductive aromas such as 'rubbery', 'egg', 'flinty', or 'cabbage' can be produced from sulphur-containing compounds such as $H_2S$, methanethiol, and dimethylsulfide (Swiegers *et al.*, 2005; Swiegers & Pretorius, 2007).

Sauvignon Blanc wines from cooler regions have more distinctive 'green and vegetative' characteristics, and warmer regions are associated with a more 'tropical and fruity' aroma (Marais, 1994, 1998). It is known that a 'green' Sauvignon Blanc wine can be created in the vineyard, while a 'tropical' wine can be manipulated by yeast during the winemaking process (Von Mollendorff, 2013). It is important to understand the importance of yeast strain choice and the influence it has on various volatile compounds. Adjusting and correcting nitrogen deficient musts before the onset of alcoholic fermentation, will prevent the production of off-flavours such as 'egg', 'cabbage', and other negative associated aromas.

## 2.4 Fertilization effects: From the vineyard to the finished wine

Grapevine has the potential to grow successfully and produce quality grapes grown in favourable environmental conditions. The nutrition levels of a grapevine can influence the grape's composition and eventually the wine's composition and quality (Bell & Henschke, 2005). A grapevine's nutrition can be affected by various factors such as the canopy shading (Perez-Harvey & Witting, 2001), canopy temperature (Ewart & Kliewer, 1977), cultivar (Christensen, 1984; Huang & Ough, 1989), rootstock (Christensen, 1984; Huang & Ough, 1989), season (Bell & Robson, 1999), site (Huang & Ough, 1989), soil management (Bell *et al.*, 1979), training system (Kliewer *et al.*, 1991), and fertilization timing, rate, and form of application (Bell *et al.*, 1979; Peacock *et al.*, 1991; Spayd *et al.*, 1994; Christensen & Peacock, 2000; Conradie, 2001; Dufourcq *et al.*, 2007; Lacroux *et al.*, 2008; Jreij *et al.*, 2009).

The nutrient requirements of a grapevine depend on its age, cultivar, yield, soil type, and soil properties (Holzapfel & Treeby, 2007). Nutrition deficiencies often occur due to various reasons and can be rectified by carrying out fertilization applications. In South Africa, farmers must adhere to government laws set out in Fertilisers, Farm Feeds, Agricultural Remedies And Stock Remedies Act, 1947 (Act No. 36 of 1947) regarding application and requirements of fertilizers to plants (FERTASA, 2018; Macaskill, 2018). Different types of fertilizers are available in the industry, such as: solid and liquid plant nutrient fertilizers, chemically compounded solid fertilizer, chemically compounded liquid fertilizer, micro- and macro-element fertilizers, compost, animal and bird manure, carcass, hoof, bone, and horn meal, organic and enriched organic fertilizer mixtures, and liming materials (FERTASA, 2018). These fertilizers are available in different forms such as crystal, micro granule, macro granule, powder, suspension, and solution (FERTASA, 2018). Independent laboratory analysis of soil samples and plant sap, leaf, and shoot samples can be used to see if a plant is nutrient deficient and can guide a farmer by indicating which nutrients are required and prevent unnecessary fertilizer applications (Macaskill, 2018). Under or over fertilizing can influence the growth, quality, or yield of the plant, while over fertilizing can lead to the pollution of soil and water sources.

In South Africa, soil fertilization has been applied traditionally to the root zones of grapevines showing nutrition deficiencies of elements such as nitrogen, potassium, phosphorus, zinc, and boron, which are essential for plant growth (Christensen & Peacock, 2000; Christensen & Smart, 2005). Other elements such as copper, calcium, carbon, manganese, magnesium, molybdenum, and sulphur are also required by plants. Macro elements such as nitrogen, phosphate, and potassium are most commonly added to the soil for fertilization because the plants take up relatively large amounts of these elements from the soil (Macaskill, 2018).

Nitrogen supplementation is the most common performed soil fertilization, as it is a primary constituent and is required by the grapevine for growth and reproduction. How much nitrogen to

apply depends on the production and quality of the crops, but also depend on numerous research trials and years of grower experience (Christensen & Peacock, 2000). Drip irrigation is the most common way of nitrogen applications, and liquid materials such as aqua ammonia, urea, and ammonium nitrate solution usage have increased, while anhydrous ammonia use has declined due to high cost (Christensen & Peacock, 2000). Application rates of drip irrigation depend on vineyard conditions and are usually applied in spring. In South Africa, the minimum plant nitrogen requirement for a plant is 450 g N/kg urea, 200 g N/kg ammonium sulphate, or 150 g N/kg aqua ammonia (FERTASA, 2018). Research studies have reported foliar rates to vines: 11-28 kg/ha N applied to medium vigour vines and 33-44 kg N/ha to below average vines (Christensen & Peacock, 2000). Organic sources of nitrogen include farm manure, grape pomace, and compost, while cover crops can also be used to add nitrogen to the soil (Christensen & Peacock, 2000).

In the past few years, foliar fertilization has been widely used where deficiencies or imbalances cannot be rectified by soil fertilization applications. Foliar fertilization is also applied as a method to improve a crop's quality and yield (Christensen & Peacock, 2000). Foliar fertilization applications show advantages such as low cost, lack of soil fixation, independent of root uptake, use small quantities of fertilization, increase quality and yield of crops, and have quick plant uptake, response, and assimilation (Oosterhuis, 2009; Lasa *et al.*, 2012). Foliar applications are preferred over soil applications when the topsoil is dry, the soil has low available nutrients, or with decreased root activity. Foliar applications are applied mostly in the case when small fertilization corrections are to be made.

The limitations of foliar fertilization applications include leaf burn, leaf necrosis, low penetration rate, solubility problems, washing off by rain, limited amounts can be applied at a time, and correct weather conditions (Watson *et al.*, 2000). Many foliar fertilizers are soluble in water and can be applied directly to the leaves of a grapevine. Foliar fertilization chemicals can traverse from the leaf to the stomata via two pathways, namely an aqueous pathway and a lipoidal route (Oosterhuis, 2009). The uptake of the nutrients depends on the element's inorganic form, combined in an organic form, ionic concentration, or on the environmental conditions which influence the time the nutrients remains in solution on the leaf (Oosterhuis, 2009). Water deficit increases the wax of the cuticle and reduces the absorption of the foliar-applied nutrient (Oosterhuis, 2009), and foliar fertilization should therefore not be applied to grapevines undergoing a drought.

Nitrogen foliar fertilization can be applied using urea (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Jreij *et al.*, 2009; Wolf, 2012; Garde-Cerdán *et al.*, 2015; Juhasz, 2015; Verdenal *et al.*, 2015a; Garde-Cerdán *et al.*, 2016; Geffroy *et al.*, 2016a; Hannam *et al.*, 2016; Verdenal *et al.*, 2016; Dienes-Nagy *et al.*, 2017) or amino acids (Garde-Cerdán *et al.*, 2015). Application levels for these studies ranged from 2-36 kg N/ ha. Sulphur foliar applications (5 and 10 kg S/ha) used elemental soluble sulphur (Dufourcq *et al.*, 2009; Juhasz, 2015; Geffroy *et al.*, 2016a) or micronised sulphur (Lacroux *et al.*, 2008).

Elemental sulphur is a slow release substrate and is widely and frequently applied in the industry at various stages during the growing season of vines. Sulphur has a direct fungicidal effect and fungistatic activity on plants, by increasing the plant's resistance to powdery mildew (*Uncinula* spp.), downy mildew, and botrytis (*Botrytis cinerea*) (Omnia, 2015). Sulphur can also be sprayed to control mites, which can have a detrimental effect on the growth of buds and leaves during the growing season. In South Africa, the minimum sulphur content or requirement for a plant is 900 g S/kg elemental sulphur per year (FERTASA, 2018). A 30 day withholding period of sulphur

applications are required with 10 day intervals between applications, otherwise residual sulphur can influence the chemical composition of the grapes and can have negative associated aromas from compounds such as $H_2S$. Studies with foliar applications have not focused on applying sulphur separately to vines (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009). A synergy between nitrogen and sulphur result in interactions between the pathways (Téa *et al.*, 2003; Lacroux *et al.*, 2008). The timing of foliar sprays is critical, and nitrogen and sulphur can be applied at different stages, such as during véraison (Dufourcq *et al.*, 2009; Jreij *et al.*, 2009; Garde-Cerdán *et al.*, 2015; Juhasz, 2015; Verdenal *et al.*, 2015a; Garde-Cerdán *et al.*, 2016; Geffroy *et al.*, 2016a; Hannam *et al.*, 2016; Verdenal *et al.*, 2016; Dienes-Nagy *et al.*, 2017) or at flowering stage (Wolf, 2012; Verdenal *et al.*, 2015a).

Foliar fertilization studies with nitrogen and sulphur applications cover a wide range of red and white cultivars and were performed in various places such as Canada, Chile, France, Spain, South Africa, Switzerland, and Virginia. White cultivars included Chardonnay (Dienes-Nagy *et al.*, 2017), Chasselas (Verdenal *et al.*, 2015a, 2015b, 2016; Dienes-Nagy *et al.*, 2017; Koestel *et al.*, 2017), Colombard (Dufourcq *et al.*, 2005, 2009, Geffroy *et al.*, 2016a, 2016b), Gewürztraminer (Dienes-Nagy *et al.*, 2017), Gros Manseng (Dufourcq *et al.*, 2007, 2009, Geffroy *et al.*, 2016a, 2016b), Melon (Dufourcq *et al.*; Geffroy *et al.*, 2016a, 2016b), Négrette (Dufourcq *et al.*, 2005, 2009, Geffroy *et al.*, 2016a, 2016b), Sauvignon Blanc (Dufourcq *et al.*; Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Jreij *et al.*, 2009; Geffroy *et al.*, 2010; Lasa *et al.*, 2012; Wolf, 2012; Helwi *et al.*, 2014; Juhasz, 2015; Geffroy *et al.*, 2016a, 2016b; Dienes-Nagy *et al.*, 2017; Helwi *et al.*, 2017), and Viognier (Hannam *et al.*, 2014).

Red cultivars such as Cabernet Sauvignon (Hannam *et al.*, 2014; Gutiérrez-Gamboa *et al.*, 2017a), Carignan (Geffroy *et al.*, 2012, 2016a, 2016b), Duras (Dufourcq *et al.*, 2005), Fer Servadou (Geffroy *et al.*, 2012, 2016a, 2016b), Malbec (Dufourcq *et al.*, 2005), Merlot (Lasa *et al.*, 2012; Wolf, 2012; Hannam *et al.*, 2014, 2016), Petit Manseng (Wolf, 2012; Kelly, 2013; Kelly *et al.*, 2017), Pinot Gris (Hannam *et al.*, 2014, 2016), Pinot Noir (Hannam *et al.*, 2014), and Tempranillo (Garde-Cerdán *et al.*, 2015) were included. Only soil nitrogen fertilization studies have been performed on Chenin Blanc (Conradie, 1981; Conradie & Saayman, 1989). Foliar nitrogen fertilization applications do not only influence the concentrations and composition of nitrogen present in the grape berry, but also indirectly influence the nitrogen levels in the must, alcoholic fermentation, and the resulting wine's composition and aroma (Petering *et al.*, 1991; Bell & Henschke, 2005).

It was shown in the previous section (Section 2.3) that Sauvignon Blanc and Chenin Blanc wines can be linked to various nitrogen and sulphur compounds such as methoxypyrazines, volatile thiols, esters, higher alcohols, monoterpenes (Marais, 1983, 1994; Lacey *et al.*, 1991; Tominaga *et al.*, 1998b; Dubourdieu *et al.*, 2006; Swiegers *et al.*, 2006) and volatile thiols, esters, higher alcohols, monoterpenes, carbonyl, and wood derived compounds (Bester, 2011; Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012; Botha, 2015; Wilson, 2017). Since various fertilization studies have found increased levels of nitrogen or sulphur derived compounds by using foliar applications (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; 2009; Mundy *et al.*, 2009; Lasa *et al.*, 2012; Hannam *et al.*, 2014; Geffroy *et al.*, 2016a; Dienes-Nagy *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2017b; Helwi *et al.*, 2017; Kelly *et al.*, 2017), it is expected that sulphur and nitrogen foliar fertilization should have an effect on the chemical composition of Sauvignon Blanc and Chenin Blanc grapes, must, and wine.

### 2.4.1 Vineyard

A grapevine's response to fertilization applications depends on various factors such as the genetic makeup of the cultivar, canopy management, and terroir. The chemical analysis of vine leaves, petioles, and soil can indicate the nitrogen content of a grapevine and can affect how the vine reacts to an application of foliar nitrogen (Bell *et al.*, 1979; Kliewer *et al.*, 1991; Spayd *et al.*, 1994; Bell & Robson, 1999; Conradie, 2001; Lacroux *et al.*, 2008; Kelly, 2013; Hannam *et al.*, 2014; Kelly *et al.*, 2017). The nitrogen requirement for every wine cultivar is different, for instance the shoots, leaves, and fruit of Chenin blanc accumulated up to 27 kg N/ha after soil application, and up to 60% of nitrogen reserves originates from nitrogen absorbed during post-harvest (Conradie, 1986). Nitrogen deficient grapevines (low YAN levels) that received nitrogen resulted in an increased vine nitrogen status which leads to nitrogen metabolism and carbohydrate accumulation (Bell & Henschke, 2005).

Increased levels of nitrogen in the roots, canes, and trunk increase the grapevine's ability to store nitrogen and carbohydrates (Kliewer *et al.*, 1991; Bell & Robson, 1999). Therefore, with an increased growth of the roots, the uptake of nutrients, water, and nitrogen are increased (Kliewer & Cook, 1971). By applying small additions of nitrogen to a vineyard with adequate nitrogen levels, no increases in the growth and yield will happen (Kliewer *et al.*, 1991). High vine nitrogen status may interrupt the grapevines' natural balance, become excessively vegetative, and maintain vegetative growth at the expense of the yield (Kliewer & Cook, 1971). It is very important that the source and sink ratio are balanced in vines, otherwise competition between the sinks for carbohydrates can occur, and grapes can have reduced composition and quality (Ewart & Kliewer, 1977). When a canopy changes due to increased growth the microclimate including factors such as light, temperature, radiation, wind, and humidity in the canopy are altered.

Only a few foliar fertilization studies focused on studying the effect of the applications on the grapevines. Most research studies on fertilization made use of urea, an inorganic form of nitrogen, because of the easy control of application. Factors such as the canopy nitrogen status, interior leaves, clusters, leaf layers, leaf area, pruning mass, bunch weight, yield, and bunches per vine in the vineyard during ripening were studied. Kelly *et al.* (2017) reported that the petioles had relatively low nitrogen levels prior to fertilization and found no differences in the number of interior leaves or clusters among fertilized treatments (15 kg/ha urea and 15 kg/ha urea with 5 kg/ha micronised sulphur (Microthiol Disperss)-applied twice before véraison)), but the control had deficient YAN levels and had a greater percentage of leaf layers compared to the fertilization applications.

Another study, where 15 kg/ha urea were applied thrice to Petit Manseng, showed no impact on the primary or secondary leaf area, however the pruning mass per vine increased significantly compared to the control (Helwi *et al.*, 2014). An alternative study with foliar urea applications at 1% and 2% w/v rates applied on Merlot two weeks before véraison, at véraison, and 2 weeks after véraison, resulted in no difference in the grapevine nitrogen status, but in the second year of the experiment, an increase in canopy density occurred (Hannam *et al.*, 2014). Lacroux *et al.* (2008) reported the plots were nitrogen deficient (low initial N-tester values) before performing nitrogen and nitrogen with sulphur foliar applications twice before véraison on Sauvignon Blanc vines. Higher levels compared to the initial nitrogen levels were observed with both foliar applications with the N-tester (leaf blade nitrogen content) analysis and an 60% increase with YAN levels (Lacroux *et al.*, 2008).

The total yield increases upon the application of nitrogen to low nitrogen containing vines (Bell *et al.*, 1979; Kliewer *et al.*, 1991; Spayd *et al.*, 1994; Bell & Robson, 1999; Conradie, 2001; Hannam *et al.*, 2014), while other studies found no increases in the yield (Lacroux *et al.*, 2008; Kelly, 2013; Kelly *et al.*, 2017). This can be due to the initial nitrogen levels present in the vines prior to foliar fertilization applications. Lacroux *et al.* (2008) also found no significant differences in bunch weight, yield, bunches per vine, and pruning weight and this can be due to the late application of nitrogen in the season. The study using 2% w/v (28-36 kg N/ha/year) urea application found increased yield of Pinot Gris, increased number of clusters/vine for Merlot (plot 2) and Viognier, while the following season, another Merlot plot and Pinot Gris vineyards had increased number of clusters/vine (Hannam *et al.*, 2014) Variable yields obtained can be due to the optimal or suboptimal timing of applications, fertilizer materials, dosage, and climatic and environmental conditions (Oosterhuis, 2009).

### 2.4.2 Grapes

During the ripening period of the grape berry, the composition, colour, flavour, size, texture, and susceptibility to pathogens change (Conde *et al.*, 2007). Tartaric acid concentrations increase during the first stages of ripening and usually remain constant after véraison. Compounds such as amino acids, micro-nutrients, and volatile aroma compounds accumulate during the first phase of berry growth, affecting the composition of the grape berry, and eventually the composition of the resulting wine (Conde *et al.*, 2007). During véraison, the grape berry undergoes dramatic changes and transforms from small, acidic and hard berries with very little sugar to softer, sweeter, larger, less acidic, and strongly flavoured and coloured. Aromatic compounds such as methoxypyrazines, which are produced during the first stage, decline during the ripening of the grape berries (Conde *et al.*, 2007). This can be due to the increase of sunlight and temperature levels in the cluster and by applying leaf removal practices (Šuklje *et al.*, 2016).

During ripening, the grape berries rely on carbohydrates produced from photosynthesis for their development and growth. Most of the accumulation of fructose and glucose occurs after véraison. The physiological ripeness of grape berries is reached when the grapes achieve sufficient sugar levels without losing too much acidity. The phenolic and aromatic content of the berries should also be taken into consideration (Conde *et al.*, 2007). For a winemaker, the optimal grape maturity is essential for successful alcoholic fermentation and producing quality wines. Generally, canopies with low light produced grapes with reduced concentrations of soluble solids, total phenols, and anthocyanins, while levels of pH, TA, methoxypyrazines, and potassium in grapes increased (Kliewer, 1968; Ewart & Kliewer, 1977; Šuklje *et al.*, 2016).

Foliar fertilization research studies analysed ˚B, TA, pH, berry composition, total nitrogen content, berry volume, YAN, amino acids, ammonium, IBMP, and susceptibility to pathogens in the grapes during grape berry ripening and at harvest (Lacroux *et al.*, 2008; Jreij *et al.*, 2009; Mundy *et al.*, 2009; Hannam *et al.*, 2014; Dienes-Nagy *et al.*, 2017; Helwi *et al.*, 2017; Kelly *et al.*, 2017). No foliar fertilization studies focused on all the methoxypyrazine compounds, except on IBMP, and on the separate amino acid composition during ripening or at harvest.

Both 1% (14-18 kg N/ha/year) and 2% w/v (28-36 kg N/ha/year) foliar urea applications increased the soluble solids at harvest for Merlot and Pinot Gris in 2010, but the 2% urea treatment resulted in a reduction in soluble solids in Merlot in the two following seasons, 2011 and 2012 (Hannam *et al.*, 2014). The 2% urea treatment caused increases in the pH levels in Merlot and Pinot Gris in 2010 and only in Pinot Gris in 2012. For all three vintages, TA levels were reduced in the grape

juice in Pinot Gris with the 2% urea foliar application (Hannam *et al.*, 2014). Similarly, Kelly *et al.* (2017) also found increased pH levels with foliar urea (15 kg N/ha–twice before véraison) and urea with micronised sulphur (15 kg N/ha with 5 kg S/ha–twice before véraison) treatments in 2011 compared to the control, while no difference were observed with the ˚B and TA levels (Kelly *et al.*, 2017). Lacroux *et al.* (2008) found no differences in the grape composition at the harvest date for oenological parameters for Sauvignon Blanc.

During ripening, the total nitrogen content of the grapes increases, but the levels can plateau after an initial increase, and in some cases even decline to the end of ripening (Kliewer & Cook, 1971; Bell, 1994). Jreij *et al.* (2009) showed that a soil application of 60 kg N/ha ammonium nitrate (1 week after bud break) with a 5 kg N/ha foliar application (véraison) treatment resulted in an 11% increase in berry volume compared to the control and to the 30/2.6 kg N/ha treatment. The 60/5 kg N/ha treatment also showed 11% increase in total berry nitrogen at the maturity stage compared to the control and other treatment (Jreij *et al.*, 2009).

Hannam *et al.* (2014) reported that foliar applications of 2% urea solution (28-36 kg N/ha/year) increased the YAN levels of grape juice at harvest in almost all the vineyards in all three vintages of the study. These YAN levels were more than double compared to the control (Hannam *et al.*, 2014). YAN levels were increased by 60% with foliar nitrogen (10 kg N/ha urea–two applications prior véraison) and foliar nitrogen (10 kg N/ha urea–twice before véraison) with sulphur (5 kg S/ha micronised sulphur–twice before véraison) applications compared to the control (Lacroux *et al.*, 2008). Dienes-Nagy *et al.* (2017) reported that YAN levels increased proportionally with foliar urea applications compared to the control over three vintages. This shows that the vines are able to redistribute the nitrogen which are absorbed from the leaves to the grape berry.

From véraison to harvest the total amino acid concentration increases (Kliewer, 1968; Bell, 1994), while in other cases the concentration stabilised and declined after véraison (Kliewer, 1968). During grape berry ripening, the ammonium concentration of grape berries decline (Bell, 1994). Foliar urea applied to white cultivars, resulted in an increase of primary and secondary amino acids, but a decrease in ammonium nitrogen levels was observed during the grape berry ripening compared to the control (Dienes-Nagy *et al.*, 2017). Bell (1994) reported that PRO accumulation increases during grape berry ripening, while some reported PRO levels stabilised and slowly decline until harvest (Bell & Henschke, 2005). PRO generally accumulate during physiological conditions due to abiotic stresses such as salt stress, cold stress, water stress, and heat stress (Kavi Kishor & Sreenivasulu, 2014). ARG increase from véraison and reached a maximum and start to decline during ripening (Kliewer, 1968; Bell, 1994), while in other studies ARG continued to increase until harvest.

The susceptibility of grapes to the fungal pathogen, *Botrytis cinerea*, can be enhanced by high content of foliar urea applications, but Lacroux *et al.* (2008) found contradictory results and showed no increase in the incidence of *Botrytis cinerea*. A strong positive correlation between sugar concentration and susceptibility to *Botrytis cinerea* in intact grape berries of Sauvignon Blanc was concluded, and wounding of unripe berries with low sugar can also increase the susceptibility to the fungal pathogen (Mundy *et al.*, 2009).

### 2.4.3 Grape must to wine

After grapes are harvested, some grape compounds can be subject to degradation due to biological, chemical, or physical processes. These processes can influence the composition and the quality of grapes and also the resulting wine. The nitrogen composition of grape berries at harvest, including the total nitrogen content, primary and secondary amino acids, ammonium, and thiol precursors, can positively be influenced by nitrogen fertilization (Conradie, 2001; Choné *et al.*, 2006). Mechanical machine harvesting can activate a variety of processes such as oxidation, releasing the precursors of volatile thiols, or microbial metabolism. Harvested white grapes usually undergo crushing and destemming and $SO_2$ is added to prevent oxidation. Thereafter the grape must can undergo a period of skin contact and is pressed afterwards. During alcoholic fermentation, the grape must is transformed into wine by the activity of the yeast. In other words, the high sugar concentration of glucose and fructose is converted by various metabolic steps and $CO_2$ and ethanol are produced. The yeast growth depends on the available nutrients, such as nitrogen which were provided from the grapes (Henschke & Jiranek, 1993). When the must is deficient in nitrogen content (<140 mg N/L, low YAN), the winemaker can adjust the nitrogen levels by adding a source of nitrogen, usually DAP. During alcoholic fermentation, a large amount of aroma compounds such as volatile thiols and major volatiles are released from their precursors (Lambrechts & Pretorius, 2000; Swiegers *et al.*, 2005) and contribute to the 'fermentation bouquet' (Bell & Henschke, 2005). The maturation of wine in bottle or in an oak barrel can influence the chemical compound makeup and aromatic expression (Botha, 2015).

Foliar and soil fertilization research studies focused on measuring ˚B, alcohol, CYS-3MH, FAN, GSH, GSH-3MH, malic acid, pH, TA, tartaric acid, YAN, amino acids, esters, methoxypyrazines, higher alcohols, and volatile thiols in the must, juice, fermentation juice, and/or resulting wines (Conradie, 2001; Choné *et al.*, 2006; Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Lasa *et al.*, 2012; Helwi *et al.*, 2014; Juhasz, 2015; Geffroy *et al.*, 2016a; Dienes-Nagy *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2017b) .

Oenological parameters such as ˚B, alcohol, pH, TA, tartaric acid, and malic acid were measured and no differences were found in the composition of the grape juice and must between the treatments (Lacroux *et al.*, 2008). Lacroux *et al.* (2008) reported that with Sauvignon Blanc, the soil ammonium nitrate application (30 kg N/ha) resulted in higher alcohol levels in the wines compared to the control and foliar fertilization applications. Foliar applications did not significantly affect the grape juice's oenological parameters with Tempranillo and Monastrell (Garde-Cerdán *et al.*, 2015), but late foliar applications of urea (15 days after véraison) increased the acidity of the resulting wine of Sauvignon Blanc and Merlot (Lasa *et al.*, 2012).

GSH in musts and wine plays a crucial role by protecting varietal volatile thiols from oxidation, and therefore protects the aroma production of Sauvignon Blanc wines (Lavigne *et al.*, 2007). GSH levels in wines, were higher for foliar urea (10 kg N/ha-twice before véraison) and foliar urea (10 kg N/ha-twice before véraison) with sulphur (5 kg S/ha–twice before véraison) applications compared to the control (nitrogen deficient) and soil fertilization (Lacroux *et al.*, 2008). The urea with sulphur application did not increase the GSH levels compared to the urea treatment (Lacroux *et al.*, 2008). Foliar urea and foliar urea with sulphur applied at véraison and two weeks later, did not show significant effects on the GSH levels in the musts compared to the control (Gutiérrez-Gamboa *et al.*, 2017a). Soil nitrogen fertilization of 60 kg N/ha at berry set to Sauvignon Blanc showed an 670% increase of GSH compared to the control (Choné *et al.*, 2006).

Helwi *et al.* (2014) applied soil fertilisation (50 kg N/ha-two applications), soil (100 kg N/ha-two applications), and foliar fertilisation of urea (15 kg N/ha–three applications) to Sauvignon Blanc in two different locations. The glutathionylated precursor of 3MH (PGSH-3MH) increased during ripening for all the treatments, and with soil and foliar treatments the levels were higher compared to the control. The results were highly correlated to the nitrogen status of the grapes. The cysteinylated precursor of 3MH (PCYS-3MH) was stable in one of the Sauvignon Blanc plots' berries during berry development, but increased with nitrogen foliar application at mid-maturity and decreased thereafter during maturity for all the treatments. Conversely, with the other Sauvignon Blanc plot, the PCYS-3MH concentration increased during ripening and was higher in the soil and foliar nitrogen applications compared to the control (Helwi *et al.*, 2014).

The nitrogen content of the must were increased by 50% and 100% respectively with 10 kg N/ha and 20 kg N/ha foliar applications to white cultivars (Sauvignon Blanc) (Geffroy *et al.*, 2016a). Dufourcq *et al.* (2009) reported significant increases of berry YAN with urea foliar fertilization at véraison. Nitrogen soil applications resulted with a 10% decrease in amino acids in relation to YAN occurred (Spayd *et al.*, 1994). Foliar urea treatments on white cultivars, such as Chasselas, Sauvignon Blanc, and Chardonnay, showed a correlation between YAN and PRO levels in the wines (Dienes-Nagy *et al.*, 2017). The amino acid content of a vine can be influenced and is dependent on various factors such as the cultivar and rootstock genetics, climatic conditions, terroir, nitrogen content, soil, and canopy management practices (Conradie, 2001; Bell & Henschke, 2005; Lee & Schreiner, 2010). Increases of 44% and 75% of ARG and ALA respectively were obtained with urea with sulphur applications at véraison and two weeks later (Gutiérrez-Gamboa *et al.*, 2017a). This study also showed that by comparing applications of foliar urea to foliar urea with sulphur, the latter application resulted in having improved amino acid content (Gutiérrez-Gamboa *et al.*, 2017a). Lasa *et al.* (2012) showed that with foliar applications of urea the accumulation and synthesis of amino acids, such as ARG, GLN, THR, and ALA were significantly increased. Therefore better alcoholic fermentation kinetics and higher production of ethyl esters which are essential for quality wines are produced (Lasa *et al.*, 2012).

Methoxypyrazine levels in the must can determine the levels in the resulting wine. Helwi *et al.* (2017) reported that nitrogen soil fertilization did not significantly affect the IBMP levels in the Sauvignon Blanc berries, wine, or must compared to the control (deficient YAN). IBMP levels were slightly higher in the resulting wine than the must (3-5 ng/L *vs* 0.5-1 ng/L) (Helwi *et al.*, 2017). Another study reported that a high level of irrigation with additional nitrogen (60 kg/ha at fruit set) promoted canopy growth with higher levels of IBMP during fruit maturation in Merlot vines (Mendez-Costabel *et al*., 2014). Only one foliar fertilization study focused on the impact of foliar nitrogen fertilization on the methoxypyrazine content (IBMP and IPMP levels), and found no significant differences (Juhasz, 2015). Researchers speculate the possibility that foliar fertilization with urea can increase these levels in the grapes and consequently in the resulting wine.

Various wines produced during nitrogen fertilization studies resulted in lower concentrations of higher alcohols. Higher alcohols are directly related to amino acid metabolism and are influenced directly by the YAN levels. Higher alcohols reach a peak between 200-300 mg N/L YAN, and degrade if the YAN levels increase (Ugliano *et al.*, 2007). Increased levels of esters are mostly found with nitrogen fertilization applications (Lasa *et al.*, 2012). Wines were described as being more 'fruitier' if compared to the control. Fatty acids, ethyl esters, and acetates contribute to the fruity aroma of white wines, and their levels increase with DAP additions and higher YAN levels in the must (Ugliano *et al.*, 2007).

In low (<140 mg N/L) and moderate nitrogen (140-300mg N/L) (Gardner, 2014; Petrovic & Buica, 2018) containing vineyards where nitrogen fertilization applications were applied, an increase in YAN, 3MH, 3MHA, and 4MMP levels occurred and were much higher compared to the control (Peyrot Des Gachons *et al.*, 2005; Choné *et al.*, 2006). 4MMP was at higher levels for the foliar urea application (10 kg N/ha-twice before véraison) compared to the control for Sauvignon Blanc, while 3MHA and 3MH levels were not higher (Lacroux *et al.*, 2008). The foliar urea (10 kg N/ha - twice before véraison) with sulphur (5 kg S/ha–twice before véraison) application resulted in higher concentrations for 3MH, 3MHA, and 4MMP compared to the control (Lacroux *et al.*, 2008). Geffroy *et al.* (2016a) also reported that 10 and 20 kg N/ha foliar applications with 5 and 10 kg S/ha, respectively, resulted in musts with three to four–fold gain in varietal thiols in the case of white cultivars, including Sauvignon Blanc. Dufourcq *et al.* (2009) reported significant increases of berry YAN with urea foliar fertilization at véraison, and these musts correlated with a higher 3MH and 3MHA concentrations in the resulting wine. Helwi *et al.* (2014) applied soil fertilisation (50 and 100 kg N/ha - two applications) and foliar fertilisation of urea (15 kg/ha–three applications) to Sauvignon Blanc and reported that all the wines produced from vines with higher nitrogen status contained more 3MH.

Wines produced from grapevines that received applications of soil ammonium nitrate (30 kg/ha), foliar urea (10 kg N/ha-twice before véraison) and foliar urea (10 kg N/ha-twice before véraison) with sulphur (5 kg S/ha–twice before véraison) applications underwent sensory evaluation (Lacroux *et al., 2008,* Geffroy *et al.*, 2016a). The foliar urea with sulphur application wines resulted in significantly higher aroma intensity, while foliar urea application wines had decreased intensities (Lacroux *et al.*, 2008). Geffroy *et al.* (2016a) also reported that the 10 and 20 kg/ha N and 5 and 10 kg/ha S foliar applications resulted in wines with more intense and increased notes of ‘grapefruit’ and ‘tropical fruit’, while no undesirable sulphur-related notes were perceived.

## 2.5 Conclusions

A wine’s flavour and aroma expression are influenced by various reactions and interactions of chemical compounds (Fischer, 2007). These non-volatile and volatile compounds can be derived from the grape, produced from the yeast metabolism during alcoholic or malolactic fermentation, or produced during barrel or bottle ageing (Fischer, 2007). Sauvignon Blanc and Chenin Blanc are both very important white wine varieties in South Africa, and these wines can have very different aroma styles from each other.

Various foliar fertilization studies show that nitrogen and nitrogen with sulphur applications can positively affect various volatile and non-volatile compounds in grapes, musts, and resulting wines. Increased levels of nitrogen and sulphur compounds such as YAN, FAN, amino acids, higher alcohols, and volatile thiols occurred (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Jreij *et al.*, 2009; Mundy *et al.*, 2009; Lasa *et al.*, 2012; Hannam *et al.*, 2014; Geffroy *et al.*, 2016a; Dienes-Nagy *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2017b; Helwi *et al.*, 2017; Kelly *et al.*, 2017). By analysing the oenological parameters in the grapes, juice, and wine, the harvest date can be decided on, but also see a correlation between the ˚B and ethanol % of the wines.

Foliar fertilization applications of nitrogen and nitrogen with sulphur applied at véraison have positively impacted the berry chemical content, yeast growth, and metabolism, and produce more aromatic wines. By doing nitrogen and sulphur foliar applications at véraison, sulphur and nitrogen containing compound concentrations can be increased. Methoxypyrazines and major volatiles are

important volatile compounds and have not been analysed in foliar fertilization research studies. Only a few research studies determined the vine nitrogen status before and after foliar applications (Lacroux *et al.*, 2008; Jreij *et al.*, 2009; Helwi *et al.*, 2014). Only two studies have done sensory analysis of the finished wines, but do not mention the age of the wines (Lacroux *et al.*, 2008; Geffroy *et al.*, 2016a). Another void in foliar fertilization research studies, is that chemical and sensory evolution of wines during bottle maturation have not been considered. Therefore, the study of the evolution of volatile chemical compounds, such as major volatiles and volatile thiols, during ageing is needed to better understand the relationships between the mechanisms and chemical compounds. The information gained by performing sensory analysis during different stages of bottle maturation, can add to the knowledgebase of the aroma of these wines.

Various foliar fertilization studies have been performed on Sauvignon Blanc in different locations and vary in application products, rates, and times. Only one study has been performed in South Africa and in a warm climate zone (Juhasz, 2015). Only two soil fertilization studies have been done on Chenin Blanc (Conradie, 1981; Conradie & Saayman, 1989). Therefore, it will be of interest to perform nitrogen and nitrogen with sulphur foliar applications at véraison on Chenin Blanc and Sauvignon Blanc in warm and cold climatic regions (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009). Most foliar research studies have mentioned and have proven that they have found positive correlations were fertilization applications were performed prior to and at véraison, due to the vine's nutrient uptake patterns and requirements.

Many research studies have been done on the biochemical and chemical origins of non-volatile and aroma compounds and the effects of the environment, viticultural, and winemaking practices on the concentrations on these compounds. By performing chemical and sensory analysis to understand the influence of the effect nitrogen and nitrogen with sulphur foliar fertilization on Chenin Blanc and Sauvignon Blanc, South African winemakers and the industry can use this information to make decisions at the viticulture and winemaking level to produce wines with more desirable sensory attributes (Francis & Newton, 2005).

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# Chapter 3

# Research Results

## Effect of foliar Nitrogen and Sulphur fertilization applications on non-volatile content of Chenin Blanc and Sauvignon Blanc juices and wines

# Chapter 3 Effect of foliar Nitrogen and Sulphur fertilization applications on non-volatile content of Chenin Blanc and Sauvignon Blanc juices and wines

## 3.1 Introduction

The soil is the main source of nutrients for the vine, but recently foliar fertilization applications were used to combat certain deficiencies occurring in vines for a quicker and more efficient uptake (Mengel, 2002; Oosterhuis, 2009; Lasa *et al.*, 2012). Various studies have looked into applying nitrogen fertilization at various doses, soil versus foliar fertilization applications together and separately, and the timing of the applications (Lacroux *et al.*, 2008; Jreij *et al.*, 2009; Lasa *et al.*, 2012; Geffroy *et al.*, 2016; Garde-Cerdán *et al.*, 2017; Helwi *et al.*, 2017). Nitrogen soil and foliar applications and sulphur foliar applications on Sauvignon Blanc vines have been reported to have influenced oenological parameters and Yeast Assimilable Nitrogen (YAN) levels of the musts, juices, and wines (Lacroux *et al.*, 2008; Jreij *et al.*, 2009; Lasa *et al.*, 2012; Helwi *et al.*, 2014, 2017). These treatments also resulted in increasing the levels of precursors of aroma compounds (glutathione- and cysteine-conjugated compounds and amino acids) present in the musts, juices, and wines (Garde-Cerdán *et al.*, 2017). Dufourcq *et al.* (2007) reported that urea and urea with sulphur foliar applications to vines having a history of producing low YAN grapes resulted in wines that were more aromatic sensorially and chemically.

Vine nitrogen fertilization has been shown to have a major impact on the vine physiology and grape composition (Bell & Henschke, 2005; Lacroux *et al.*, 2008). Nowadays, the optimal ripeness of grapes are determined by including parameters such as pH, total acidity (TA), sugar content (Balling-˚B), and the colour of grape skin and seeds (Deloire, 2012). Optimal ripeness plays a crucial role by determining the harvest date and various methods prior to and after harvest can influence these levels in the grapes, juice, or wines. The nitrogen content of grapes known as YAN are utilised by yeast during alcoholic fermentation. Even though 140 mg N/L is seen as the critical value for YAN, nitrogen levels should be increased for successful fermentation (Leonardelli, 2013; Petrovic & Buica, 2018). Glutathione (GSH) is a natural antioxidant in grapes and must (Anderson, 1998), and plays an important role in juice by protecting various volatile thiols from oxidation (Choné *et al.*, 2006) and also stopping the formation of unstable aromas in wine (Papadopoulou & Roussis, 2001).

With South Africa's unique climate foliar fertilization studies can be performed to provide new and additional information for researchers and producers in the industry. Due to drought and cost increases fertilization applications to the soil or with irrigation are not the best option for small deficiencies. Foliar fertilization have been used more often and are proven to be more effective for quick uptake (Lacroux *et al.*, 2008; Oosterhuis, 2009). In South Africa, Sauvignon Blanc and Chenin Blanc are two of the most widely planted white cultivars (SAWIS, 2016). Commonly Sauvignon Blanc has been included in soil and foliar fertilization studies, while only two soil fertilization studies focused solely on Chenin Blanc (Conradie, 1981; Conradie & Saayman, 1989). Currently Chenin Blanc and Sauvignon Blanc are of great interest to researchers and various studies have investigated the contribution of chemical compounds to the aromatic expression of the wines (Swiegers *et al.*, 2005; Malherbe *et al.*, 2013; Van Wyngaard, 2013; Von Mollendorff, 2013; Weightman, 2014; Botha, 2015; Wilson, 2017).

Consequently, in this study, different Chenin Blanc and Sauvignon Blanc vineyards with low YAN levels received foliar fertilization applications during véraison. The aim of this study was to assess the effect of different fertilization treatments on the non-volatile content of the grapes, juices, and/or aged wines. This chapter will focus mainly on glutathione, oenological parameters, and YAN. The levels of each parameter or compound will be investigated to see how they were influenced from season to season, but also the evolution of GSH during bottle maturation. Results obtained from foliar fertilization studies can contribute to a better understanding of the impact of these practices on various non-volatile compounds and the data obtained was used to explore trends and correlations found between the various treatments applied to the cultivars. This knowledge could ultimately aid researchers and winemakers to understand the foliar influence on these compounds, produce a specific wine style, and produce better quality wines.

## 3.2 Materials and methods

### 3.2.1 Experiment layout

Three different vineyards were used during the (2014/2015 and 2015/2016) seasons for this research project. Vineyard plots used for Chenin blanc are Farm A in 2014/2015 and Farm B in 2015/2016, while Farm C was used for Sauvignon Blanc trials for both seasons, 2014/2015 and 2015/2016.

#### 3.2.1.1 Farm A

The Chenin Blanc vineyard is situated in the Stellenbosch district situated in the Coastal wine region near Somerset West, South Africa (Figure 3.1, 34°2'15.63"S, 18°45'13.28"E). The planting year and clones of the scion and rootstock were not made known. This vineyard has a history of producing grapes with natural low YAN levels. The vineyard received soil fertilization during soil preparation for vineyard planting, but no soil fertilization such as urea has been applied the past few years with irrigation. No foliar sulphur has been sprayed to prevent pathogens such as mites and diseases including powdery mildew and downy mildew. The vines receive drip irrigation during the growing season and were trained in a five-wire vertical shoot positioning system (VSP) with movable canopy wires. The 0.25 ha vineyard block has 749 vines and spacing is 2.5 m x 1.2 m. The treatments (Section 3.2.1.4) were applied to the vines in accordance to the experiment design in Table 3.1. This plot was used only once for the 2014/2015 season.


**Figure 3.1** Farm A with the Chenin Blanc vineyard in Somerset West.

**Table 3.1** Farm A - Chenin Blanc vineyard with foliar applications experiment layout.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Row 1** | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines |
| **Row 2** | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines |
| **Row 3** | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines |
| **Row 4** | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines |

### 3.2.1.2 Farm B

The Chenin Blanc vineyard is situated on Farm B in the Paarl and Wellington district in the Coastal region, South Africa (Figure 3.2, 33°41'59.0"S, 18°53'53.9"E). The vineyard was planted in 2004 with 2.5 m x 1.2 m spacing and has a history of producing low YAN grapes. Various clones of the scion were planted but were unknown, while several different Richter (*Vitis Berlandieri* and *Vitis rupestris)* rootstocks were used, such as Richter 99 and Richter 110. This plot is considered as a dry-land vineyard and do not receive any irrigation during the growing season.


**Figure 3.2** Farm B with the Chenin Blanc vineyard in Paarl.

**Table 3.2** Farm B - Chenin Blanc vineyard with foliar applications experiment layout.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Row 9** | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines |
| **Row 8** | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines |
| **Row 7** | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines |
| **Row 6** | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines |

The vineyard received soil fertilization during soil preparation for vineyard planting, but no soil fertilization such as urea has been applied the past few years. No foliar sulphur has been sprayed to prevent pathogens such as mites and powdery mildew and downy mildew diseases if necessary. The vines are trained in a five-wire VSP with movable canopy wires. Foliar treatments were applied to vines of four adjacent rows that grows homogenously (Figure 3.2), in accordance to the experiment design (Table 3.2). This plot was used only for the 2015/2016 season.

### 3.2.1.3 Farm C

This Sauvignon Blanc vineyard is situated on Farm C in the Elgin district in the Cape South coast region, South Africa (Figure 3.3, 34°10'38.20"S, 19°2'13.66"E). This vineyard was planted in 2006 with 2.5 m x 1.2 m spacing and the soil consists mostly of Koffieklip with an underlying 40-50 cm clay layer. The Sauvignon Blanc 376 (ENTAV-INRA, France) clone is grafted on 101-14 Mgt rootstock and receives drip irrigation. The vineyard has a history of producing low YAN grapes and no soil fertilization has been applied previously.


**Figure 3.3** Farm C with the Sauvignon Blanc vineyard in Elgin.

**Table 3.3** Farm C - Sauvignon Blanc vineyard with foliar experiment layout.

| | | | | | |
|---|---|---|---|---|---|
| **Row 1** | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines |
| **Row 2** | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines |
| **Row 3** | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines |
| **Row 4** | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines |
| **Row 5** | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines |
| **Row 6** | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines |
| **Row 7** | **Buffer** 3 vines | **Sulphur** 12 vines | **Buffer** 3 vines | **Control** 12 vines | **Buffer** 3 vines |
| **Row 8** | **Buffer** 3 vines | **Nitrogen** 12 vines | **Buffer** 3 vines | **Nitrogen+ Sulphur** 12 vines | **Buffer** 3 vines |

The vineyard received soil fertilization during soil preparation for vineyard planting, but no soil fertilization such as urea has been applied the past few years. No foliar sulphur has been sprayed to prevent powdery mildew, downy mildew and mites only if necessary. The vines are trained in a five-wire VSP with movable canopy wires and usually produces 6-8 tonnes/ha per harvest. The treatments were applied to the vines of eight adjacent rows, which grow homogenously, in accordance to the experiment design in Table 3.3. This plot was used for the 2014/2015 and 2015/2016 season.

#### 3.2.1.4 Foliar fertilization treatments

The research study consists of four treatments with four repeats applied to the foliage of the vines: Control (no foliar fertilization applied), Nitrogen (10 kg/ha N as Low-Biuret (LB) Urea (NUTRICO SA, Gauteng Province, South Africa)), Sulphur (5 kg/ha S as Microthiol Special (OTAL South Africa, Saxonwold, South Africa)), and Nitrogen with Sulphur (N+S-10 kg/ha N as Urea LB and 5 kg/ha S as Microthiol). Microthiol Special is a water dispersible granule containing 80% w/w sulphur and Urea LB is a water-soluble fertilizer with low biuret urea (45% urea nitrogen and 5% biuret). This was the same method used as reported by Lacroux *et al.* (2008). The vine and row spacing of the vineyard was taken into consideration to calculate the dosage of the different foliar applications. The experiment layout was arranged in a checkered order and the foliar treatments were applied to twelve selected vines per treatment, with three vines between each treatment serving as a buffer zone. The buffer zone prevented treatments from overlapping.

The foliar treatments were applied three weeks (<50% véraison) and 1 week (>80% véraison) before full véraison with a Pressure sprayer with a 6,5 L volume (Shixlasx-68). Only the canopy was sprayed on both sides and the nozzle was set to a fine misty spray (no big droplets). The sprays were performed on wind still days and early in the morning to prevent the application products to be blown away and prevent sulphur burn. The nitrogen and sulphur products used are available in the industry from various chemical companies that supply various products to producers and farmers. The layout of the treatments for the Chenin Blanc vineyards can be seen in Table 3.1 and Table 3.2, while Table 3.3 shows the Sauvignon Blanc vineyard. For ease of reading, the foliar treatments are coded with the following codes throughout the rest of the thesis: C–Control, N–Nitrogen, S–Sulphur, and N+S–Nitrogen and Sulphur.

### 3.2.2 Vinification and sampling

#### 3.2.2.1 Small scale vinification

The Chenin Blanc and Sauvignon Blanc grapes were harvested at the same ripeness levels as required by winemakers in the South African industry (Deloire, 2012). For each replicate of each treatment, ± 40 kg grapes were hand harvested separately early in the morning from 12 vines of the specific treatment (4 treatments x 4 vineyard repeats). The harvested grapes were stored overnight in the 4˚C cold room at the experimental cellar at the Department of Viticulture and Oenology, Stellenbosch University. A standard vinification method as used by the experimental cellar was used to perform the small scale vinification. For each cultivar and each season, 16 wines were made, corresponding to the vineyard repeats.

After the grapes were destemmed and crushed, 40 mg/L sulphur dioxide ($SO_2$) and 30 g/ton pectolytic enzyme (Lafazym Extract Enzyme, Laffort, South Africa) were added to the grape must. The must was left overnight for skin contact at the 4˚C cold room, pressed with a vertical hydro-

press to 1 bar, and the pressed juice was placed into 20 L plastic canisters. 4 mL/hL of an enzyme (Rapidase CLEAR enzyme) was added to obtain better juice sedimentation overnight for settling in the 4°C. The pressed juice was racked via syphoning into 20 L stainless-steel fermenters and left to reach room temperature. Upon reaching 20˚C, the juice was inoculated with a dried yeast strain, QA23 (Lallemand®, South Africa), at 30 g/hL according to the manufacturer's advice and placed in a 15˚C temperature-controlled room. To exclude YAN as a limiting factor for fermentation and for variability between treatments, d-ammonium phosphate (DAP) was added to increase all the YAN levels to 250 mg/L N in the must. The alcoholic fermentation was monitored twice daily by measuring the weight loss of the canisters due to carbon dioxide ($CO_2$) release. During alcoholic fermentation the canisters were topped up with $CO_2$ (Afrox, South Africa) every two days to maintain an anaerobic environment. Alcoholic fermentation was considered finished when the weight loss stopped and residual sugar levels (glucose + fructose) were below 5 g/L.

The settled wines were racked with $CO_2$ off the lees by syphoning into clean 20 L stainless-steel canisters. 60 mg/L $SO_2$ and 50 g/hL of bentonite for protein stabilisation were added to the racked wine. The wines were then placed into the -4˚C fridge to undergo cold stabilisation for two weeks. The canisters were topped up with $CO_2$ every three days. After two weeks, the wine was racked off the lees and the free $SO_2$ levels were adjusted to 40 mg/L. Two hours prior to bottling, green glass bottles (Consol glass, South Africa) were cleaned and sterilized with an iodine solution and left to drip dry upside down. The wine was left to reach room temperature (18-20˚C) and was bottled unfiltered. The wines were syphoned into 750 mL green glass bottles and sealed under $CO_2$ with SAVin (Guala Closures South Africa (Pty) Ltd, Paarden Eiland, South Africa) aluminium screw caps. The bottled wines were stored at a constant temperature in the 15˚C temperature-controlled room for three and nine months until wine sensory and chemical evaluation was performed. The same winemaking procedure was followed for both 2015 and 2016 vintages.

#### 3.2.2.2 Juice and wine sampling stages and storage

During the small scale vinification process, berry, juice, must, and wine samples were taken at various stages from each repeat (Figure 3.4). After harvesting, 100 berries were selected at random from 20 bunches (5 berries per bunch) from each treatment and repeat (16 in total) and stored in the -20°C fridge for amino acid analysis. In duplicate, 20 berries from 10 random bunches (2 berries per bunch) from each treatment were taken and 200 mg/L $SO_2$ was added and stored in the -20°C fridge; only one replicate's berries were crushed while the other replicate was kept intact.

After crushing and destemming, juice samples were taken to analyse the standard oenological parameters (°B, pH, and TA). Racked juice samples were taken to analyse free $SO_2$ and YAN levels. For GSH, 10 mL juice sample with 200 mg/L $SO_2$ added to prevent oxidation was placed in the -20°C fridge. At the end of alcoholic fermentation, the residual sugar (glucose and fructose) levels were analysed to confirm that the alcoholic fermentation was complete and the ethanol % was also measured. After protein- and cold stabilisation, only samples for GSH analysis were taken. Just before bottling the $SO_2$ levels were adjusted to 40 mg/L free if required. The three- and nine-month aged wines were submitted to standard parameter analysis as well as ethanol %. All the analyses were performed immediately, except for the YAN samples that were placed in the 4°C cold room and the GSH and amino acid samples in the -20°C freezer.

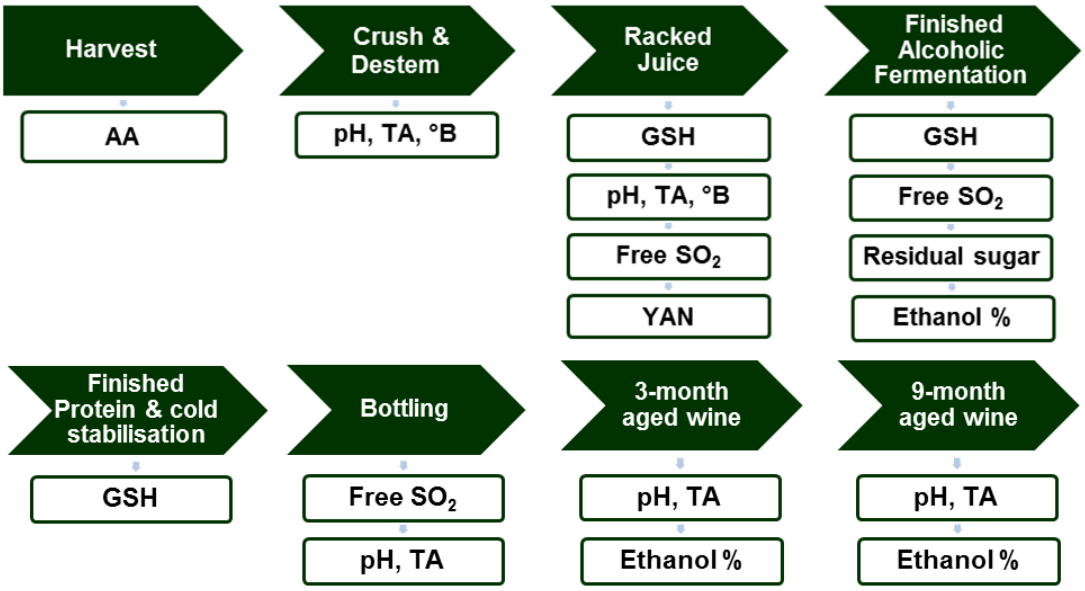


**Figure 3.4** Summary of the sampling and analyses performed during the winemaking process.

### 3.2.3 Chemical analysis

#### 3.2.3.1 Standard oenological parameters

A potentiometric titrator from Metrohm, type 702 SM Titrino (Metrohm Ltd., Switzerland) was used to analyse the pH, TA, and free $SO_2$ of the juice and wine samples. The ˚B was measured with the use of a hydrometer by measuring the relative density of the sugar content of the juice and wine. The ethanol % was determined by using the Fourier transform mid-infrared spectroscopy (FT-MIR) (WineScan FT 120, Foss Analytical, Denmark). The pH and TA of the aged wines were analysed by the FT-MIR after the completion of the sensory analysis sessions.

#### 3.2.3.2 Yeast assimilable nitrogen and amino acids

YAN (alpha-amino nitrogen or free amino nitrogen (FAN) + ammonium ($NH_4^+$)) analyses were outsourced and done by VinLAB, which is an ISO17025 and B-BBEE accredited and independent laboratory in Stellenbosch, South Africa. A colorimetric procedure was followed for the FAN analysis and an enzymatic procedure for $NH_4^+$ analysis. The amino acids were analysed by *o*-phthaldialdehyde, iodoacetic acid, and 9-fluorenylmethylchloroformate (OPA-IDA-FMOC) derivatization and high pressure liquid chromatography–fluorescence detection (HPLC-FLD) according to the method of (Šuklje *et al.*, 2016).

#### 3.2.3.3 Glutathione

GSH concentrations were analysed by ultra-performance liquid chromatography-tandem mass spectrometric (UPLC-MS/MS) analysis according to the method published by Fracassetti *et al,.* (2011). The sample was transferred into 2 mL microcentrifuge tubes and thereafter a 800 µL dilution solvent of HPLC graded Milli-Q-Water with 1000 mg/L $SO_2$ and 500 mg/L ascorbic acid was added to dilute the sample five times. The diluted sample was then centrifuged (Centrifuge 5415 D, Eppendorf, Hamburg, Germany) at 10000 r.p.m. for five minutes and a 950 µL sample was transferred to an amber coloured vial and capped. It was thereafter injected into the MS.

### 3.2.4 Statistical data analysis

For the statistical analysis of the chemical data, Statistica® software version 13 (Dell Inc., Tulsa, USA) was used. Differences between the treatments were tested for significance by applying the analysis of variance (ANOVA). Separate statistical tests were performed for each cultivar and vintage. Even though the same vineyard was used for Sauvignon Blanc for both years, it was decided to do separate tests as with Chenin Blanc. For oenological parameters (pH, TA, ˚B, ethanol %) and YAN (FAN, amino acids, and $NH_4^+$) the treatment effect was tested, while for GSH the treatment*time interaction was tested. For the treatment*time interaction, a mixed repeated ANOVA model, Variance Estimation, Precision and Comparison (VEPAC), was performed. For the treatment effect the least squares means (LS Means) were calculated form the linear model, ANOVA. Fisher's least significance difference (LSD) tests were used for post-hoc analysis and a *p* value threshold of 0.05 (different letters account for significance level at $p<0.05$) was used for the determination of statistical significance. If a sample was considered an outlier in the ANOVA's linear regression, the data was excluded from the LS Means and VEPAC computed graphs.

## 3.3 Results and discussions

### 3.3.1 Standard oenological parameters

The influence of treatment, cultivar, and vintage was evaluated on pH, TA, ˚B, and ethanol % to assess if there was an effect on the grapes and resulting wines. The homogeneity within and between treatments were evaluated. Appendix A show full chemical analysis of these parameters.

#### 3.3.1.1 pH and TA

Although the pH and TA levels were analysed at the critical winemaking stages, the initial levels in the grapes are of importance to determine the effect of the treatments. All the pH and TA values of can be seen in Appendix A-Table A.1 and A.2. The racked juice sampling stage showed grape homogeneity and treatment effect on the grapes (Figure 3.5 and 3.6). Significant higher pH levels were obtained for Chenin Blanc with the N treatment (2015 and 2016) and N+S treatment (2016).

The pH and TA results showed that even though in some cases there were significant differences observed, in real winemaking terms the differences were not large. The pH levels in South Africa for dry white wines generally range between 3.2-3.4 (Van Schalkwyk & Archer, 2000). No acid additions were performed during the winemaking process. For example, for both vintages the Chenin Blanc grapes had a higher pH than the Sauvignon Blanc grapes (Figure 3.5 and 3.6). The high pH levels for Chenin Blanc in 2016 may indicate that the grapes were overripe when harvested, but the ˚B levels ranged from 20.3-23.2˚B. In 2016, the grapes were harvested after a sudden heat wave hit the Paarl area and could have caused the increase of pH levels. The lower pH levels of the Sauvignon Blanc and Chenin Blanc (2015) grapes can possibly be explained by the fact that the vineyard is located in a cool climate region (Barnuud *et al.*, 2014).

pH and TA were also analysed at other winemaking stages: after alcoholic fermentation and bottle maturation. The other stages were at 3 and 9 months of maturation after bottling, which correspond to the sensory evaluation (Section 4.2). There were significant differences found between the treatments, but they were not relevant because the differences were small.

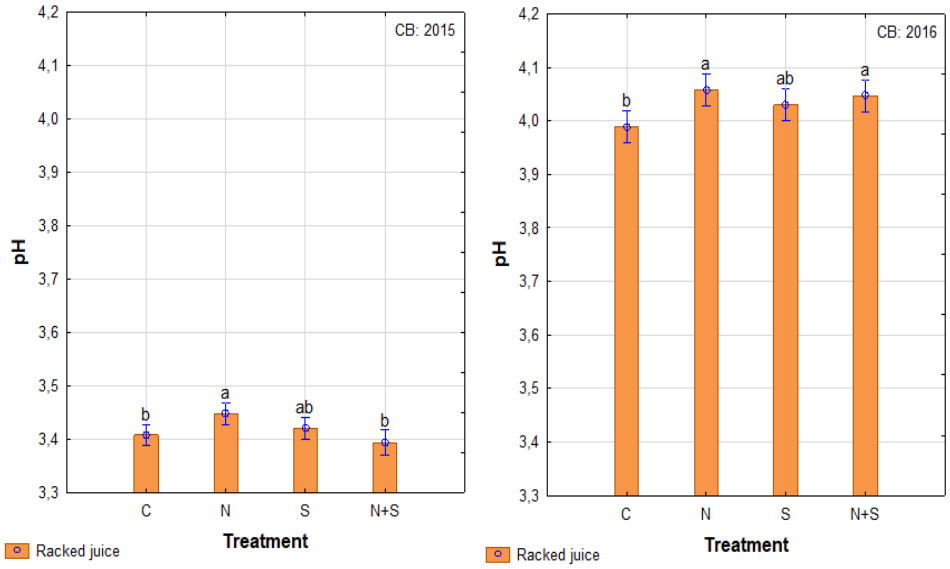


**Figure 3.5** LS means plot illustrating the treatment effect on pH levels of Chenin Blanc racked juices in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

After alcoholic fermentation, the Chenin Blanc wines had a higher pH than the Sauvignon Blanc wines. The pH levels were 3.53 *vs.* 3.05 in 2015 and 3.95 *vs.* 3.06 in 2016 for Sauvignon Blanc and Chenin Blanc respectively (Figure 3.5 and 3.6 and Appendix A-Table A.1). In 2015, the pH decreased slightly during bottle maturation of 3 to 9 months (Chenin Blanc: 3.53 to 3.49 and Sauvignon Blanc: 3.09 to 3.03), while in 2016 no differences between these two sampling stages were found. Only the N treatment showed significant higher pH levels compared to the C for 3- and 9-month aged Chenin Blanc wines in 2015 and 9-month aged wines in 2016. Most of the wines had significant decreased pH levels for the treatments from the racked juice to aged wines.

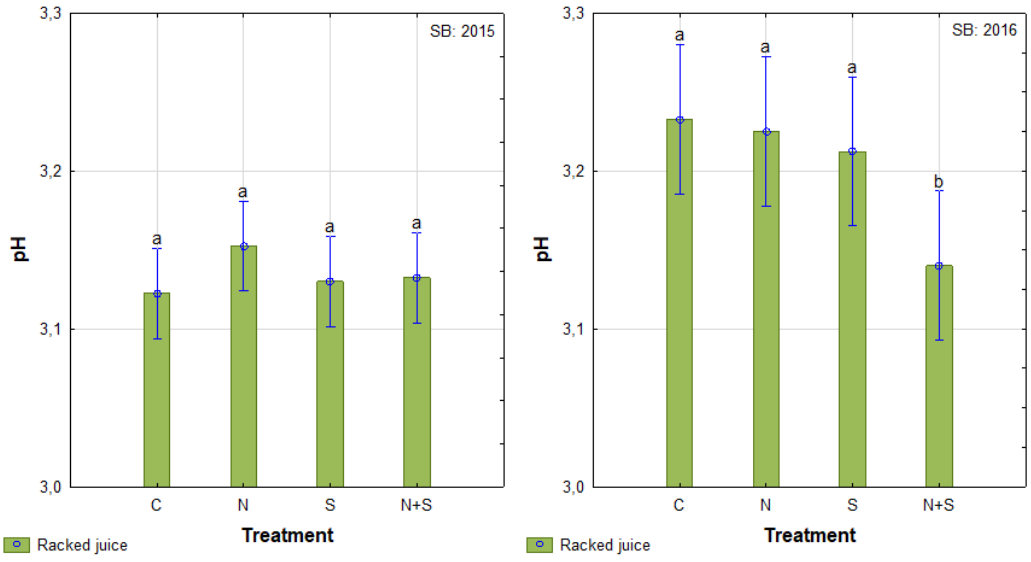


**Figure 3.6** LS means plot illustrating the treatment effect on pH levels of Sauvignon Blanc racked juices in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

TA and pH are in correlation with each other and this 'seesaw' relationship can be seen in all the graphs (Appendix A-Table A.1 and A.2). For both cultivars in 2015, the N+S treatments showed significant higher levels compared to the C with the racked juices (Figure 3.7 and 3.8). Although the sulphur treatments (S and N+S) were higher compared to the C, it was not significant. The low TA levels of Chenin Blanc in 2016 can be due to the grapes being overripe with harvest.

General TA levels in dry white South African wines range from 7 to 8 g/L. TA levels decreased from after alcoholic fermentation to 3 month aged wines as expected due to the cold stabilization step where the tartaric acid precipitates as potassium bitartrate salt (Ribéreau-Gayon *et al.*, 2006). For Sauvignon Blanc the average TA levels decreased during vinification from 8.72 to 6.34 g/L in 2015 and 8.35 to 6.54 g/L in 2016, while in Chenin Blanc TA levels decreased from 8.16 to 5.58 g/L in 2015 and from 5.18 to 4.15 g/L in 2016 (Figure 3.7 and 3.8 and Appendix A-Table A.2). There were some significant differences between the treatments, but they were not relevant because the differences were small (Appendix A-Table A.2). During bottle maturation the TA levels increased for Chenin Blanc for both vintages, while for Sauvignon Blanc most of the treatments showed slight decreases in TA levels.

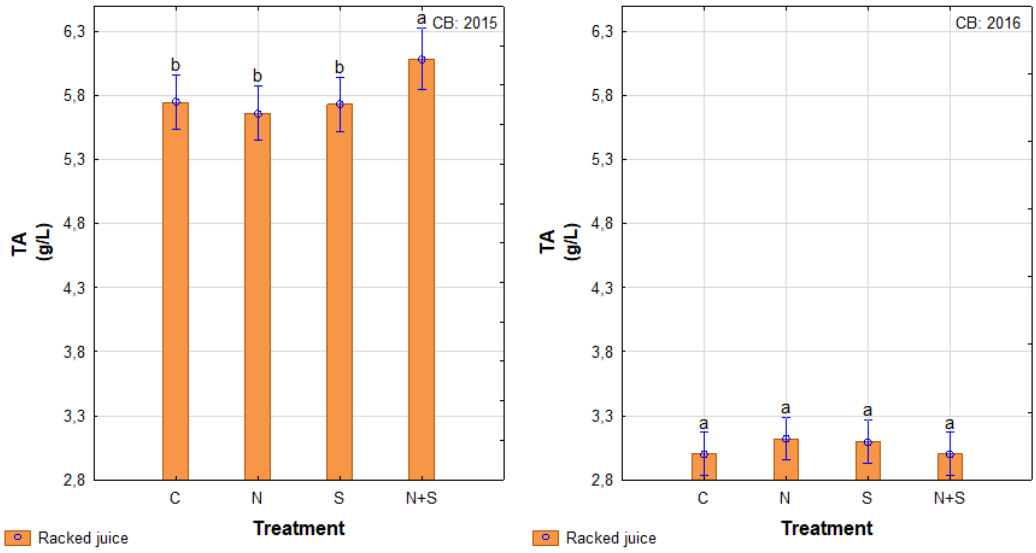


**Figure 3.7** LS means plot illustrating the treatment effect on TA levels of Chenin Blanc racked juices in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

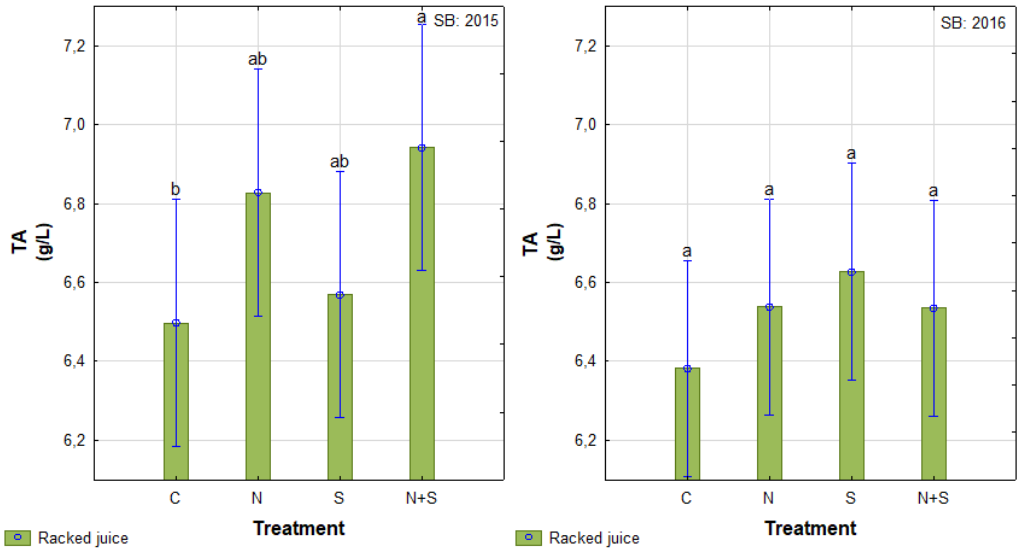


**Figure 3.8** LS means plot illustrating the treatment effect on TA levels of Sauvignon Blanc racked juices in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

These results contradict or agree with findings reported by literature studies. Geffroy *et al.* (2016a) found a significant increase for pH, but not for TA in the case of N foliar application for the red

cultivars, while Hannam *et al.* (2016) observed significant decreased levels with foliar urea application compared to the control in the TA levels and not in the pH for Merlot and Pinot Gris. Various researchers, Juhasz (2015) and Lacroux *et al.* (2008), found no significant differences between the treatments with the pH and TA levels of the grapes. These differences reported in the literature are small in real terms and can be ascribed possibly to variations in measurement.

### 3.3.1.2 Balling and ethanol %

The homogeneity of the grapes from different treatments indicated by the sugar levels at harvest should reflect in the homogeneity of the wines shown by the lack of difference in ethanol content at tasting. For both cultivars and vintages, no significant differences were observed between the treatments for both the sugar content in the grapes (Figure 3.9 and 3.10) and ethanol content (Figure 3.11 and 3.12) in the wines.

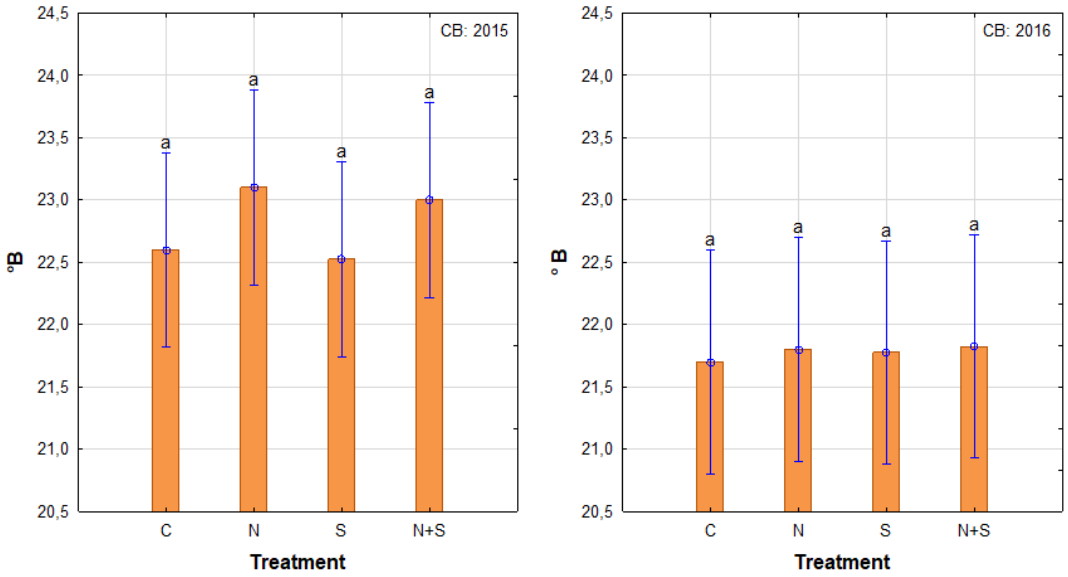


**Figure 3.9** LS means plot illustrating the treatment effect on Balling levels of Chenin Blanc juices in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

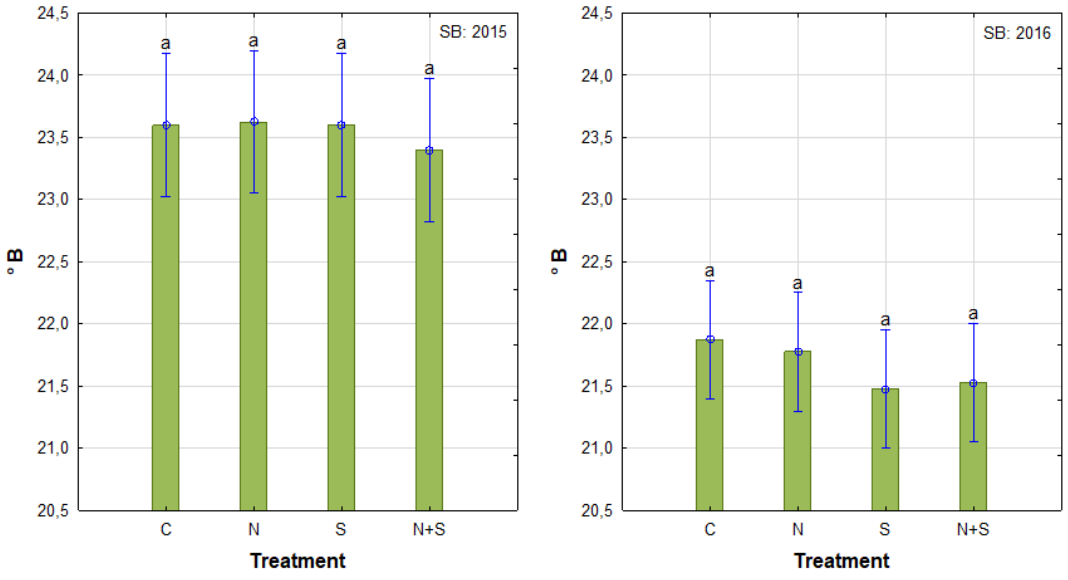


**Figure 3.10** LS means plot illustrating the treatment effect on Balling levels of Sauvignon Blanc juices in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

The harvest in 2015 were riper compared to 2016, Sauvignon Blanc grapes ranged from 22.5-24.4˚B and the Chenin Blanc grapes from 21.7-24.2˚B. In 2016 both cultivars were harvested at almost the same ripening level ranging from 20.3-23.2˚B for Chenin Blanc and 20.5-21.88˚B for Sauvignon Blanc (Figure 3.9 and 3.10). In South Africa the sugar levels for dry white wines range between 19.5-23˚B, but both cultivars were riper in 2015 compared to 2016. Just before the planned harvest date, a heat wave befell over the weekend and caused higher sugar levels.

Consequently, after alcoholic fermentation for all the cultivars, the ethanol levels for Sauvignon Blanc wines (13.55-14.79%) in 2015 were higher than for the Chenin Blanc wines (12.95-14.16%). Although in 2016, ethanol levels of the wines were at similar levels for both cultivars, Chenin Blanc levels were 12.95-14.16% and Sauvignon Blanc levels ranged from 12.29-14.04% (Figure 3.11 and 3.12). 2016 grapes were harvested at lower sugar levels than 2015 grapes, this can be due to the cooler and rainy conditions during the ripening season (Barnuud *et al.*, 2014). This reflected in the levels of ethanol % which were lower in 2016 compared to 2015 (Figure 3.8).

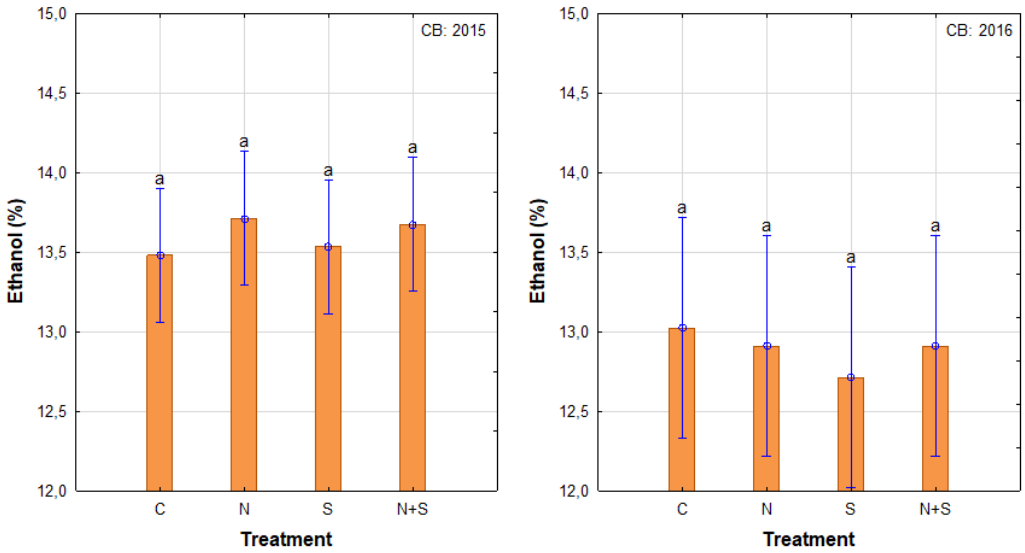


**Figure 3.11** LS means plot illustrating the treatment effect on ethanol levels of Chenin Blanc wines in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

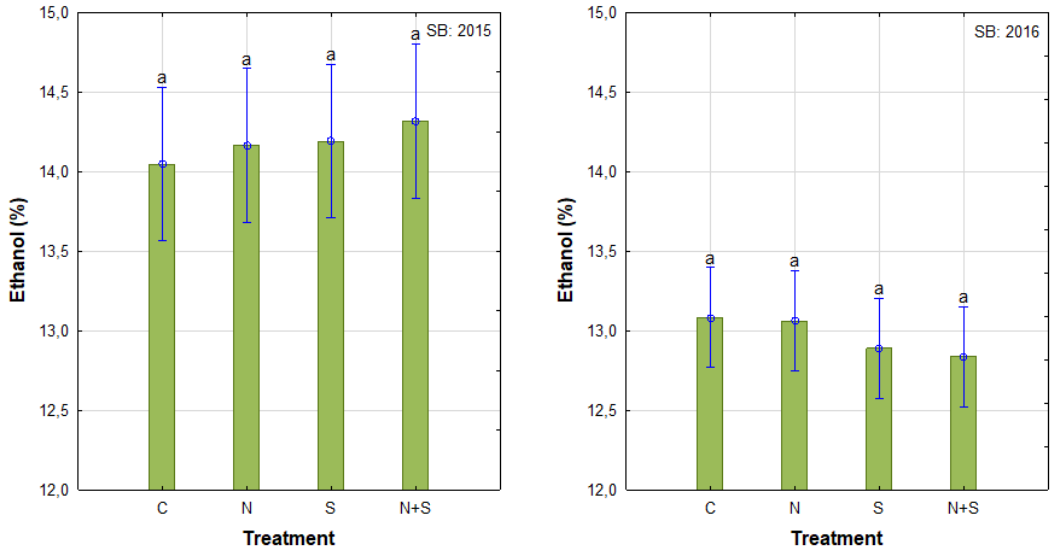


**Figure 3.12** LS means plot illustrating the treatment effect on ethanol levels of Sauvignon Blanc wines in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

### 3.3.2 Yeast assimilable nitrogen and amino acids

#### 3.3.2.1 Yeast assimilable nitrogen

Low YAN levels in the must can lead to poor and slow fermentation, low yeast populations, increased production of hydrogen sulphide ($H_2S$) and higher alcohols, and decreased production of long chain fatty acids and esters (Bell & Henschke, 2005). Generally, the transition zone for successful alcoholic fermentation is 140 mg/L in tems of YAN concentrations, whereas below this level, the risk of slow, sluggish, or stuck alcoholic fermentation could increase (Henschke & Jiranek, 1993). $NH_4^+$ and FAN are the constituents of YAN and play a major role as nitrogen sources in the yeast fermentation kinetics. Primary amino acids are the constituents of FAN, and some of them are precursors of aroma compounds (Jiranek *et al.*, 1995). Assimilable nitrogen content of must provide a good estimation of the vine nitrogen status (van Leeuwen *et al.*, 2000). YAN levels can be increased with nitrogen foliar applications at véraison or post-véraison (Lasa *et al.*, 2012; Hannam *et al.*, 2014).

The influence of the foliar fertilization treatments on the YAN content of the harvested grapes was evaluated. For both cultivars and both vintages, a similar trend occurred in YAN levels (Figure 3.13 and 3.14). An increase in YAN was noted with the N and N+S treatments for all the cultivars compared to the C and S treatments, although only the Sauvignon Blanc N+S (2015 and 2016) and N treatments (2015) were significantly higher. The average YAN levels for C treatments for the Sauvignon Blanc (2015 and 2016) and Chenin Blanc (2015) ranged from 110-152.5 mg/L N and are considered below or close to the required levels for successful alcoholic fermentation (Bell & Henschke, 2005; Dufourcq *et al.*, 2009) (Figure 3.13 and 3.14).

From this it could be concluded that most of the vineyards chosen for the project had a relatively low nitrogen status. In contrast, the Chenin Blanc vineyard in 2016 had a high nitrogen status, as shown by the YAN levels (280-370 mg/L N). Even in this case, though, the N and N+S treatments resulted in a higher YAN level. No significant increases were observed for these treatments compared to the C. The higher YAN level results agree with what is found in literature for both N and N+S treatments (Lacroux *et al.*, 2008; Hannam *et al.*, 2014; Dienes-Nagy *et al.*, 2017).

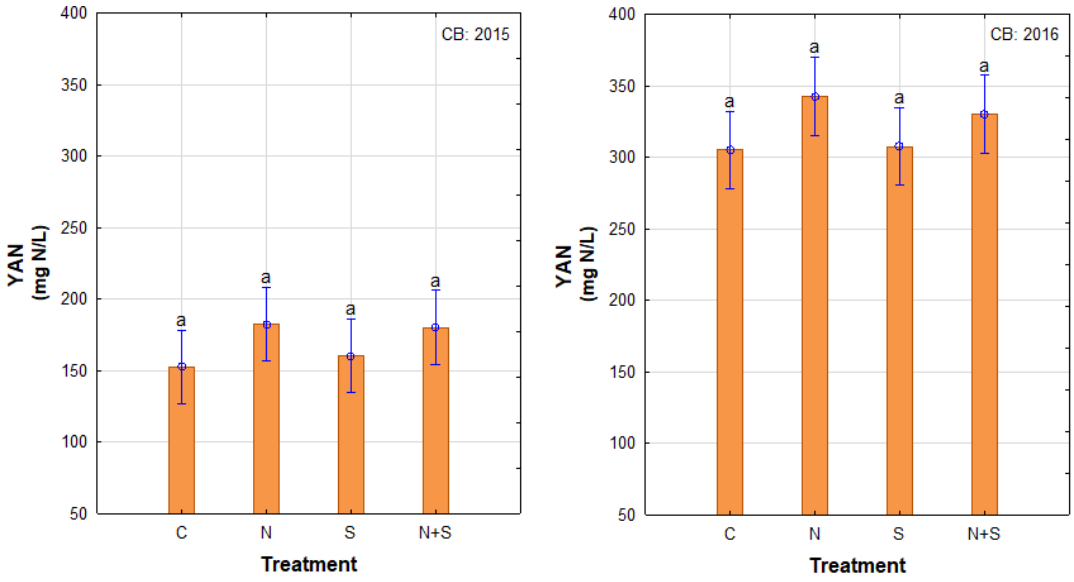


**Figure 3.13** LS means plot illustrating the treatment effect on YAN levels of Chenin Blanc juices in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

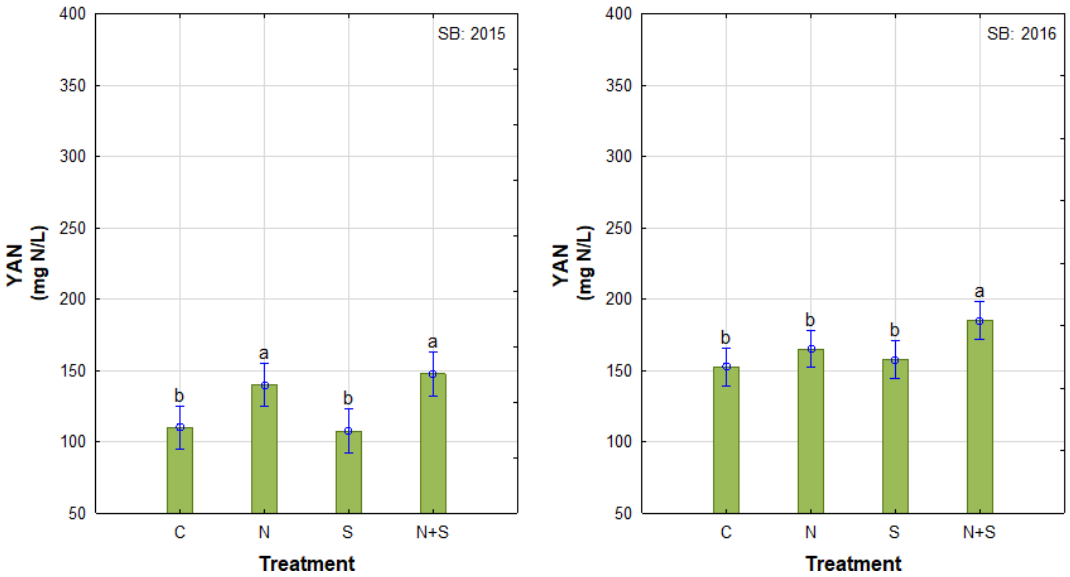


**Figure 3.14** LS means plot illustrating the treatment effect on YAN levels of Sauvignon Blanc juices in 2015 and 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

General studies performed with late-season foliar fertilization sprays have resulted in high must YAN levels (Lacroux *et al.*, 2008; Jreij *et al.*, 2009; Lasa *et al.*, 2012; Hannam *et al.*, 2014). Nitrogen foliar fertilization treatments applied at véraison (Hannam *et al.*, 2014) or a few weeks thereafter (Lasa *et al.*, 2012) can increase the YAN levels. Hannam *et al.* (2014) showed that with 2% v/w foliar urea treatment applied to four cultivars (both red and white) resulted in increased YAN concentrations over three subsequent seasons, while Lacroux *et al.* (2008) applied nitrogen and nitrogen with sulphur treatments to Sauvignon Blanc twice prior to véraison and YAN levels increased 60% compared to the Control.

In the present study, both cultivars increased for both years, but Kelly (2013) found a vintage effect for Petit Manseng. In 2011, nitrogen with sulphur treatment resulted in four times higher YAN concentrations than the C, but in 2012 no differences were found between treatments (Kelly, 2013). Differences can be possibly ascribed to variations, clones of the vines, and/or treatments not absorbed successfully due to climatic conditions (Lacroux *et al.*, 2008; Hannam *et al.*, 2014).

Both FAN and $NH_4^+$ compounds are an important component of YAN levels in the juices. FAN consists of all the free and primary amino acids, which are the most prevalent form of nitrogen in the juice and grape and are precursors of various volatile compounds of certain aroma compounds (Bell, 1994; Bell & Henschke, 2005). It is worth mentioning that HYP and PRO are secondary amino acids and are not included in FAN value. The addition of d-ammonium DAP just before the onset of alcoholic fermentation for both cultivars could have increased the $NH_4^+$ and YAN levels for successful alcoholic fermentation (Henschke & Jiranek, 1993). Only for 2016, the FAN and $NH_4^+$ were evaluated separately for both cultivars (Figure 3.15 and 3.16).

In a recent South African study, FAN levels in juices ranged from 64-221 mg N/L for Chenin Blanc and Sauvignon Blanc levels were 60-267 mg N/L (Petrovic, Kidd & Buica, 2019). Both cultivars had levels within these ranges, by Chenin Blanc were slightly higher than the maximum levels reported. The FAN levels were much higher for the Chenin Blanc juices compared to Sauvignon Blanc (Figure 3.15). The levels of N and N+S treatments for Chenin Blanc juices were 4–14% higher than the other treatments, while only the N treatment were 14% significantly higher than the control. For Sauvignon Blanc, the N+S treatments showed 20% significant increases compared to the C, while the N and S treatments mean levels were similar.

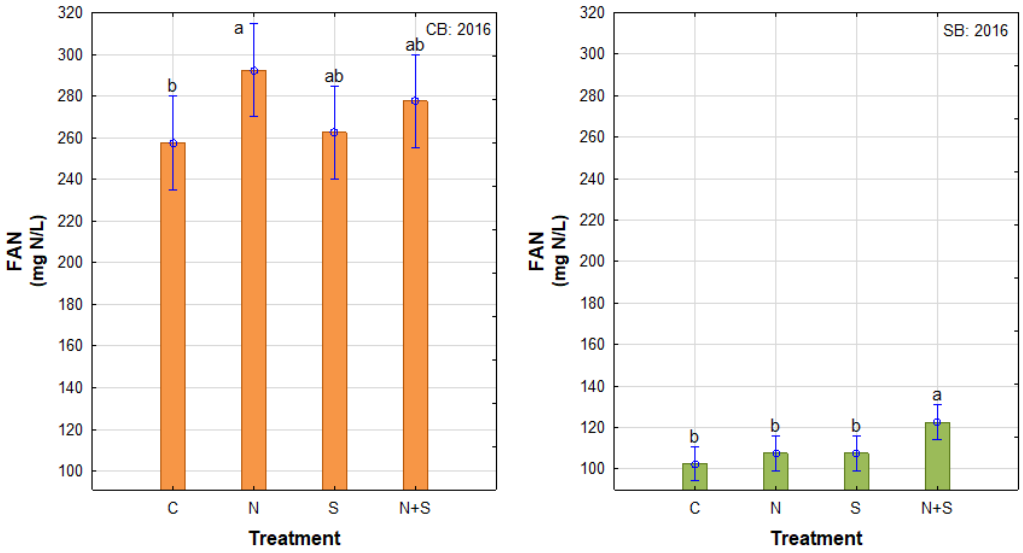


**Figure 3.15** LS means plot illustrating the treatment effect on FAN levels of Chenin Blanc and Sauvignon Blanc juices in 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

Proportionally, the $NH_4^+$ concentrations increased more than that of the FAN. The N and N+S treatments resulted in higher $NH_4^+$ levels (5-25% increase) compared to the C for both cultivars. Only significant higher levels for the N+S treatments were observed for Sauvignon Blanc compared to the C. In concentration terms, this corresponded to an increase of around 47.5-52.7 mg/L N for $NH_4^+$ and around 257-292 mg/L N for FAN, respectively. Hannam *et al.* (2016) showed that in the case of Pinot Gris, the N treatment increased the $NH_4^+$ and FAN levels significantly compared to the C in 2011, but not in 2010.

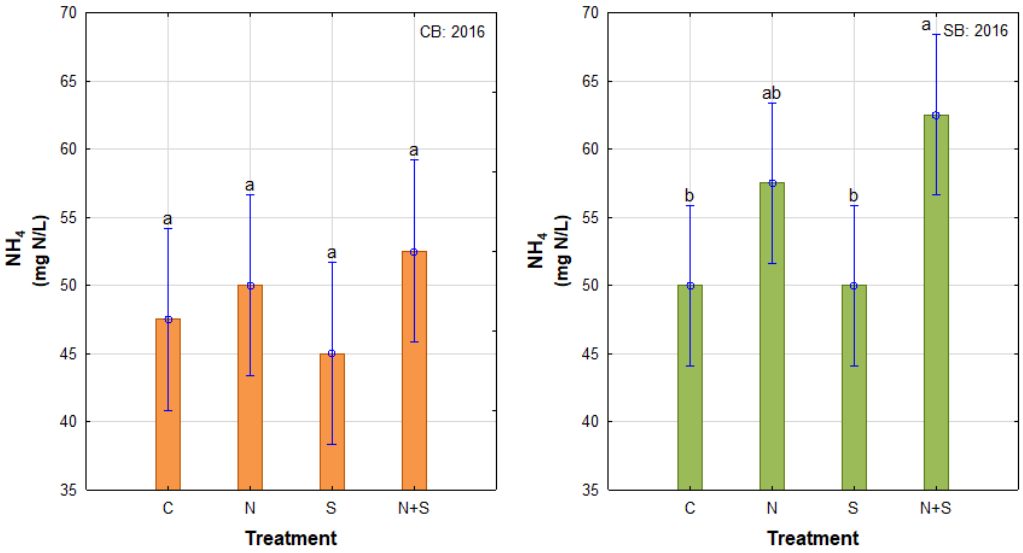


**Figure 3.16** LS means plot illustrating the treatment effect on $NH_4^+$ levels of Chenin Blanc and Sauvignon Blanc juices in 2016 with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

No scientific literature is currently available on foliar fertilization studies with Chenin Blanc. Although, two studies with soil fertilization were performed and focused on the nutrient consumption of Chenin Blanc vines under sandy soils in South Africa (Conradie, 1981; Conradie *et al.,* 1989).

### 3.3.2.2 Amino acids

Several factors can influence the amino acid composition and concentration of the grapes, such as cultivar, rootstock, site, seasonal conditions, and viticultural practices. Various volatile compounds are formed from amino acids during alcoholic fermentation, therefore amino acids can be considered precursors of aroma compounds (Bell & Henschke, 2005). It was shown in the literature that grape juices, from vines treated with foliar nitrogen and sulphur, with high amino acid content resulted in a greater synthesis of ethyl esters, which is desirable for wine quality due to their aromatic potential and fruity aromas (Dufourcq *et al.*, 2009; Lacroux *et al.*, 2008).

During alcoholic fermentation Arginine (ARG) is preferred by the yeast as a nitrogen source (Garde-Cerda & Ancin-Azpilicueta, 2008; Holzapfel *et al.*, 2015). Amino acids can be divided into different groups based on their role or structure: yeast-preferred, branched, sulphur (S)-containing, and other amino acids (Godard *et al.*, 2007; Ljungdahl & Daignan-Fornier, 2012). Yeast preferred amino acids are considered the most important, because yeast metabolizes them first (Monteiro & Bisson, 1991; Godard *et al.*, 2007). Branched amino acids are precursors of volatile esters (Antalick *et al*., 2014), while S-containing amino acids are involved in yeast metabolism under certain conditions and can result in $H_2S$ production (Henschke & Jiranek, 1991; Giudici & Kunkee, 1994). All the other amino acids are used by the yeast only in the case that other nitrogen sources are depleted first (Duteutre *et al.*, 1971). An in-depth discussion of all these amino acids can be viewed in Section 2.2.1.2.

Individual amino acids were measured only in 2016 in the grape juices of both cultivars (Figure 3.17, 3.18, 3.19, 3.20, and 3.20). Full chemical data of the amino acid groups and single amino acids can be viewed in Appendix A-Table A.3 and A.4. Also indicated with the FAN results, Chenin Blanc juice had higher total average amino acid concentrations for both N and N+S treatments (Figure 3.15 and 3.17). Even though the total amino acid levels of the Sauvignon Blanc were roughly three times lower, they followed the same trend as seen with Chenin Blanc with regards to treatment effect.

For both cultivars, the total amino acid and ARG content was higher with the N treatment compared to the C (Appendix A-Table A.3 and A.4 and Figure 3.17), in accordance with findings by Lasa *et al.* (2012), Kelly *et al.* (2017) and Verdenal *et al.* (2016). ARG, Threonine (THR), Alanine (ALA), Aminobutyric acid (GABA), and Proline (PRO) for Chenin Blanc and ARG, GABA, PRO, and Glutamic acid (GLU) for Sauvignon Blanc had the highest amino acid concentrations, while Asparagine (ASN), Tyrosine (TYR), Cysteine (CYS), Methionine (MET), Tryptophan (TRP), Isoleucine (ILE), Ornithine (ORN), Lysine (LYS), and Hydroproline (HYP) were among the least abundant (Appendix A-Table A.4). Chenin Blanc amino acid levels were proportionally 220-320% higher than Sauvignon Blanc levels, and this can be linked to the high FAN levels of the juice mentioned earlier (Section 3.3.2.1).

Even though the levels of yeast preferred amino acids were higher for all the nitrogen containing treatments (N and N+S) compared to the C, they were not significantly different (Figure 3.18, Appendix A-Table A.3 and A.4). The highest increase was observed for Chenin Blanc for the N+S treatment with an average of 1220 mg N/L compared to the C (av. 890 mg N/L). The effect on individual amino acids varied depending on cultivar and treatment. All N-containing treatments resulted in higher levels of ARG and ALA, while the level of Aspartic acid (ASP) and Serine (SER) increased only in the case of Chenin Blanc for the N+S treatment (Table A.4). Results for Glutamine (GLN) and GLU varied depending on the cultivar.

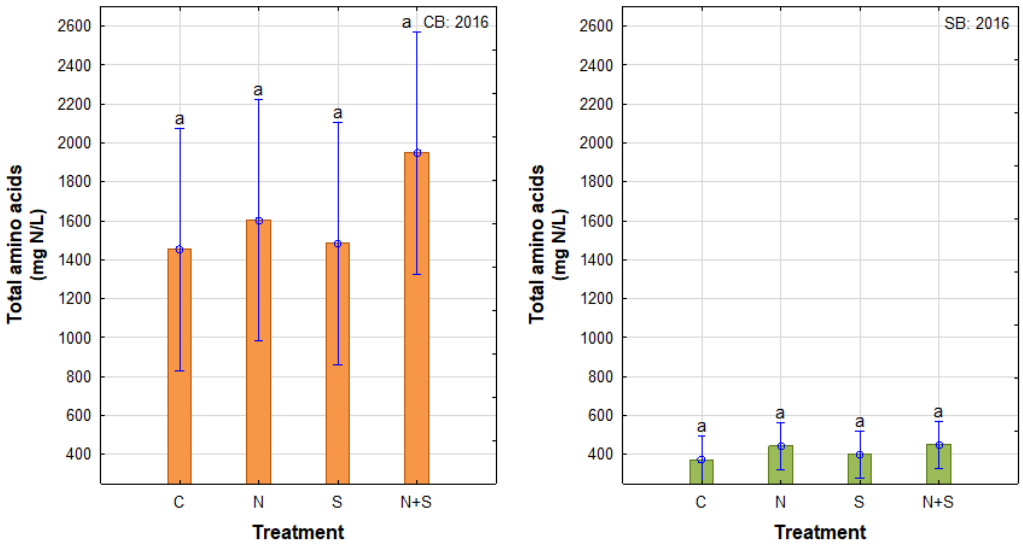


**Figure 3.17** LS means plot illustrating the treatment effect of total amino acids of Chenin Blanc and Sauvignon Blanc juices in 2016 with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

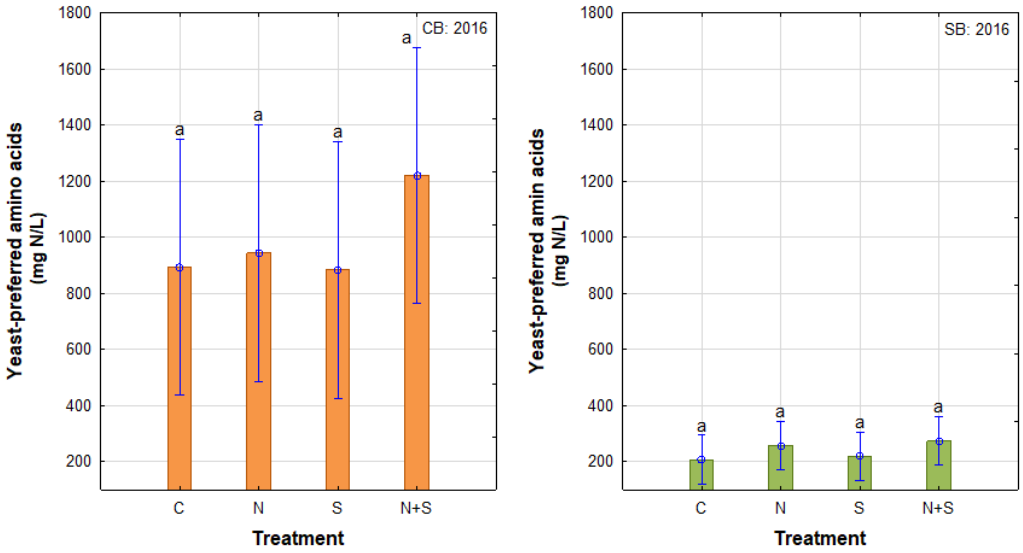


**Figure 3.18** LS means plot illustrating the treatment effect of total yeast-preferred amino acids of Chenin Blanc and Sauvignon Blanc juices in 2016 with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

Lasa *et al.* (2012) reported that the amino acid content was only significantly higher in Sauvignon Blanc juice with doses of 10 and 50 kg/ha urea applied during and after véraison. ARG, ALA, and GLU increased significantly compared to the control for both foliar applications, but with 50 kg/ha application the levels were significantly higher than for 10 kg/ha at those two ripening stages. However, 10 kg/ha application at pre-véraison showed higher increases of ALA, GLU, and ARG levels compared to the 50 kg/ha application (Lasa *et al.*, 2012). Kelly *et al.* (2017) found abundant levels of ARG and ALA compared to the control with foliar nitrogen with sulphur (15 kg/ha urea and 5 kg/ha sulphur) and nitrogen (15 kg/ha urea) treatments twice at pre-véraison.

The treatment effect on Chenin Blanc and Sauvignon Blanc for branched amino acids differed and no significant differences were observed (Figure 3.19 and Appendix A-Table A.3 and A.4). In the case of Sauvignon Blanc, none of the treatments produced an increase in this class of amino acids. For Chenin Blanc, both N and N+S treatments resulted in an increase of branched amino acids with N+S treatment having the bigger effect, but none of the differences were significant.

All the Sauvignon Blanc juices had an average of 18-20 mg N/L, while Chenin Blanc levels increased to 104 mg N/L for N and 125 mg N/L for N+S treatments compared to the C. Each individual branched amino acid increased compared to the C for Chenin Blanc (Appendix A-Table A.4). Reports from the literature show that pre-véraison applications of urea on Sauvignon Blanc result in significantly higher amino acid levels for only Valine (VAL), Leucine (LEU), and PHE (Lasa *et al.*, 2012). For Petit Manseng, Kelly *et al.* (2017) showed that nitrogen treatments, with and without sulphur, result in higher levels of VAL, LEU, and ILE compared to the control and nitrogen soil applications.

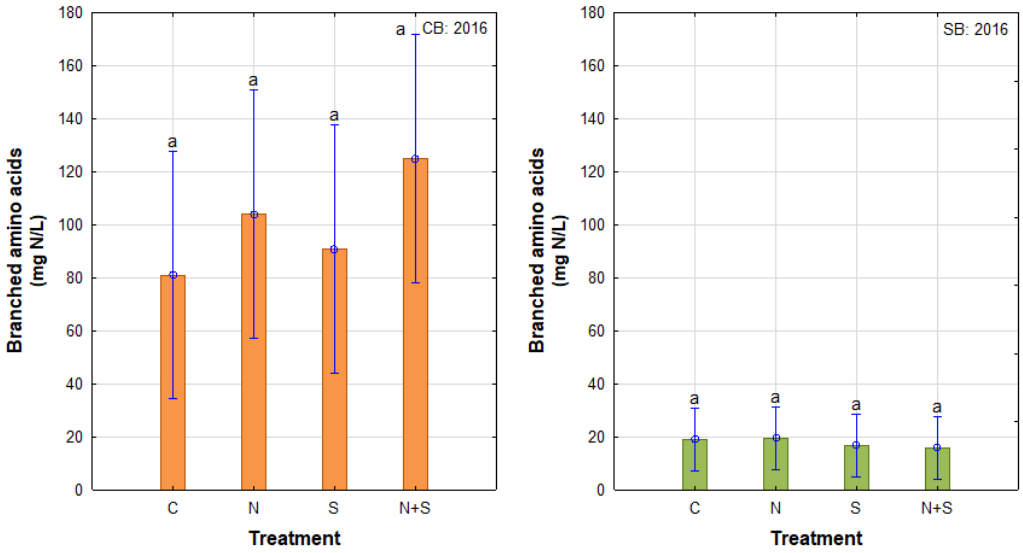


**Figure 3.19** LS means plot illustrating the treatment effect of total branched amino acids of Chenin Blanc and Sauvignon Blanc juices in 2016 with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

The results showed that S-containing amino acids were present at much lower levels compared to the other amino acid groups, 3-3.5 mg N/L for Sauvignon Blanc and 8.85-11.4 mg N/L for Chenin Blanc respectively (Figure 3.20 and Appendix A-Table A.3 and A.4). Sulphur is an integral component of the amino acids, CYS and MET, and the low levels are within ranges reported in literature (Bell & Henschke, 2005). It was relevant to see if the S-containing treatments resulted in higher levels of S-containing amino acids.

Once more, the effect of the treatments on the two cultivars was different. For Sauvignon Blanc, no trend for the treatments in S-containing amino acids occurred, while for Chenin Blanc only the N+S treatment resulted in a higher level of these compounds, but these differences were not significant (Figure 3.20 and Appendix A-Table A.3 and Table A.4). Significantly higher MET levels compared to the control were found with 5 kg/ha urea foliar applications at pre-véraison and this increase did not occur with higher urea doses at or after véraison (Lasa *et al.*, 2012). This class of amino acids is not often reported because they are at lower levels and more difficult to detect (Bell & Henschke, 2005; Kelly *et al.*, 2017).

The rest of the amino acids were grouped under “other amino acids” (Figure 3.21). Although these compounds make up the majority content of the total amino acids, they are not preferred by the yeast and are used only when the available N pool gets depleted (Monteiro & Bisson, 1991; Godard *et al.*, 2007). Chenin Blanc was the only cultivar affected by the treatments, an ongoing trend with increased amino acid levels for both nitrogen containing applications (N and N+S treatments) (Figure 3.21 and Appendix A-Table A.3 and A.4).

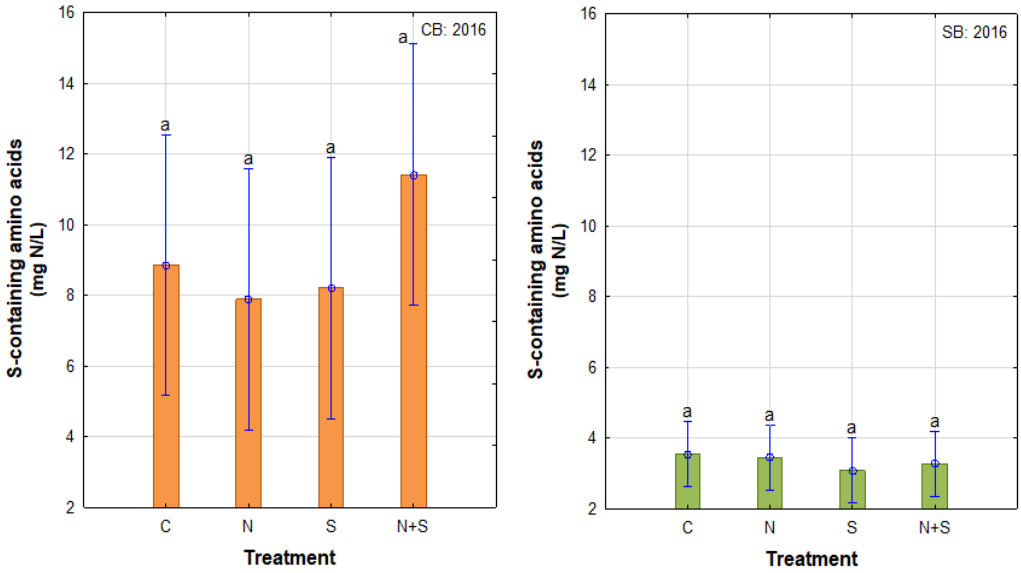


**Figure 3.20** LS means plot illustrating the treatment effect of total S-containing amino acids of Chenin Blanc and Sauvignon Blanc juices in 2016 with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

The overall effect observed differed for individual amino acids. Glycine (GLY), Histidine (HIS), ORN, LYS, and TRP levels for Chenin Blanc decreased for N and S treatments compared to the C, while for THR, GABA, and TYR increased (Appendix A-Table A.4). For Sauvignon Blanc, THR, ORN, and TRP decreased for N+S treatments, while GLY, GABA, HIS, TYR, and LYS increased compared to the C. GLY, THR, GABA, TYR, HIS, and LYS levels for Chenin Blanc is higher compared to Sauvignon Blanc and this can be linked to the higher YAN levels of Chenin Blanc (Figure 3.21 and Appendix A-Table A.4).

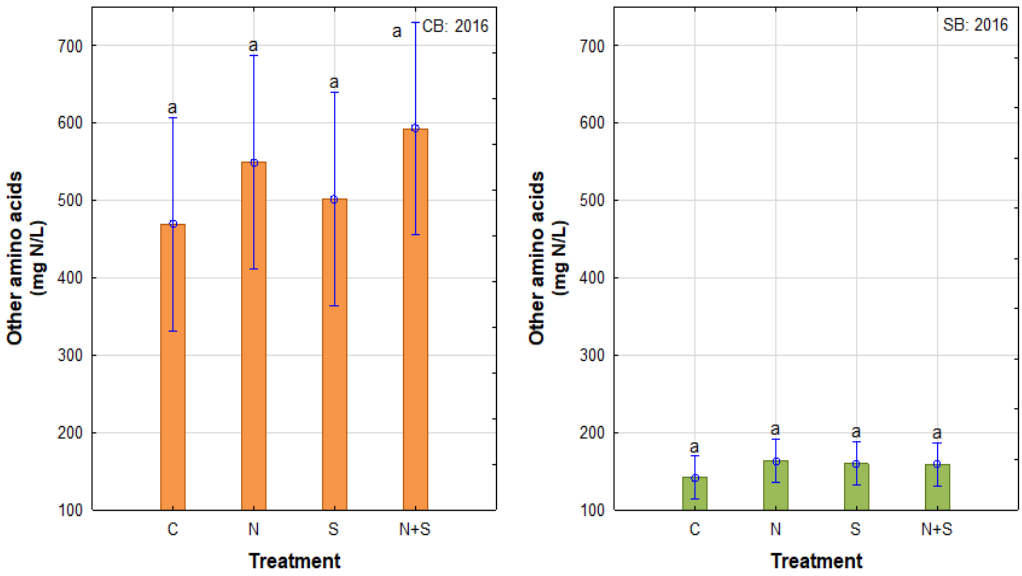


**Figure 3.21** LS means plot illustrating the treatment effect of other amino acids of Chenin Blanc and Sauvignon Blanc juices in 2016 with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

The N and N+S treatments tended to be higher compared to the C with GLY, THR, and TYR for Sauvignon Blanc, and THR, GABA, LYS, and TRP for Chenin Blanc. Increases for the S treatments compared to the C were observed with GABA, HIS, ORN, and LYS for Sauvignon Blanc, while GLY, TYR, and HIS levels increased for the Chenin Blanc. All the levels for these amino acids are within the ranges mentioned in literature (Bell & Henschke, 2005). Lasa *et al.*

(2012) reported for 10 kg/ha urea foliar applications at pre-véraison, increases compared to the Control could be seen for Sauvignon Blanc's GLY, THR, GABA, TYR, HIS, LYS, and TRP. Significantly higher levels of GLY, THR, GABA, TYR, and HIS was found with N+S and N foliar applications applied to Petit Manseng (Kelly *et al.*, 2017).

Under this category, secondary amino acids (HYP and PRO) are also included, but they do not contribute to the FAN value. PRO is among the two dominant amino acids that make up the bulk of the total amino acids (Kliewer, 1968). PRO from the juice cannot be assimilated by yeast in the absence of oxygen, therefore, when alcoholic fermentation is completed, PRO is normally at around the same level in the resulting wine (Ribéreau-Gayon *et al.*, 2006). All treatments resulted with higher PRO levels compared to the C, but they were not significantly different (Appendix A-Table A.4).

For Sauvignon Blanc, the N+S increased 38% compared to the C, while for Chenin Blanc a 9.7-28% increase for all the treatments occurred compare to the C. PRO levels in Chenin Blanc were 162-364% higher compared to Sauvignon Blanc. Kelly *et al.* (2017) reported higher PRO levels found with N-containing treatments, but the levels increased the most with both nitrogen and nitrogen with sulphur foliar treatments compared to the control and nitrogen soil treatment. In this study, HYP levels were not significantly different for both cultivars. N and N+S treatments for Chenin Blanc increased 61% and 32% respectively compared to the C.

### 3.3.3 Glutathione

GSH is a S-containing tri-peptide consisting of GLU, GLY, and CYS (Castellarin *et al.*, 2012). Being reactive to quinones, GSH plays an important role in juice by protecting varietal volatile thiols from oxidation (Choné *et al.*, 2006). GSH can be found in different forms, mainly reduced GSH (GSH-R) and oxidised GSH (GSH-O) (Figure 3.22) (Fahey, 2001; Coetzee, 2011). GSH concentrations range from 1-71 mg/L in juice and up to 35 mg/L in wine (Du Toit *et al.*, 2007; Janeš *et al.*, 2010; Fracassetti *et al.*, 2011).

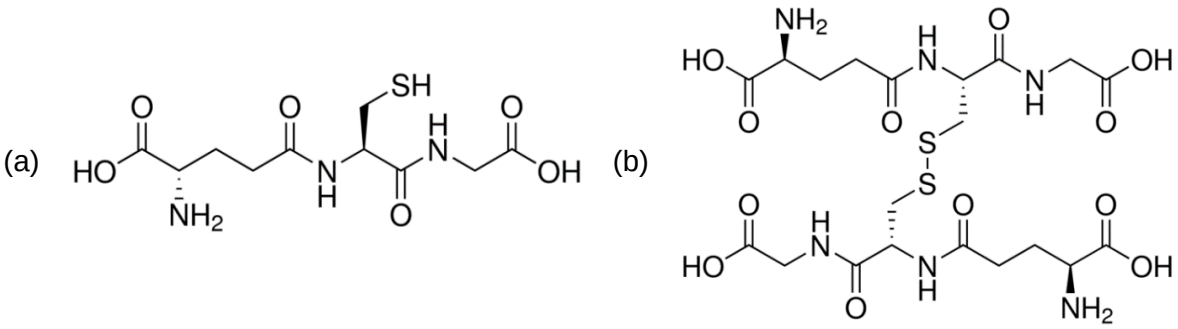


**Figure 3.22** L-Glutathione reduced form (a) and L-Glutathione oxidized form (b).

Only three recent fertilization studies focused on the effect of nitrogen fertilization on GSH levels in the juices and/or wines (Lacroux *et al.*, 2008; Juhasz, 2015, Helwi *et al.*, 2017). In plants, S uptake are driven by the demand for S-containing compounds, such as CYS and GSH, if S depletion has occurred (Davidian and Kopriva, 2010). It is of interest to see if the S and N+S treatments will lead to increased GSH levels compared to the C. Both forms of GSH were measured at various stages of the winemaking process during this project. Unlike YAN, there were no obvious trends between treatments, in other words, treatment effect was not as evident as for YAN. The treatment effect and evolution of GSH levels are discussed separately below.

### 3.3.3.1 Treatment effect

GSH-R concentrations were within the range of levels reported in other South African studies (Du Toit *et al.*, 2007; Janeš *et al.*, 2010; Fracassetti *et al.*, 2011). Generally, the differences between the treatments were not significant (Appendix A-Table A.5, A.6, and A.7). Various factors including UV light exposure, temperature stress, origin, and vintage could have influenced these GSH levels (Cheynier *et al.*, 1989; Coetzee, 2011; Castellarin *et al.*, 2012).

Even in the cases when significant differences were found, the differences were small in real terms. In 2015, the initial GSH-R levels for N treatments (N and N+S) were significantly higher than the C for the Chenin Blanc racked juices, while in 2016 the N and S treatments were significantly higher (Figure 3.23). For Sauvignon Blanc in 2015 and Chenin Blanc in 2016 the GSH-R levels reduced from racked juice to after cold stabilization for all the foliar treatments (Figure 3.23 and 3.24 and Appendix A-Table A.5). The GSH-R levels of Sauvignon Blanc (2015) ranged at racked juice from 9.73-13.11 mg/L and from 9.22-10.77 mg/L after cold stabilisation, while for Chenin Blanc (2016) at racked juice the levels were 8.12-13.38 mg/L and 6.14-8.81 mg/L after cold stabilisation.

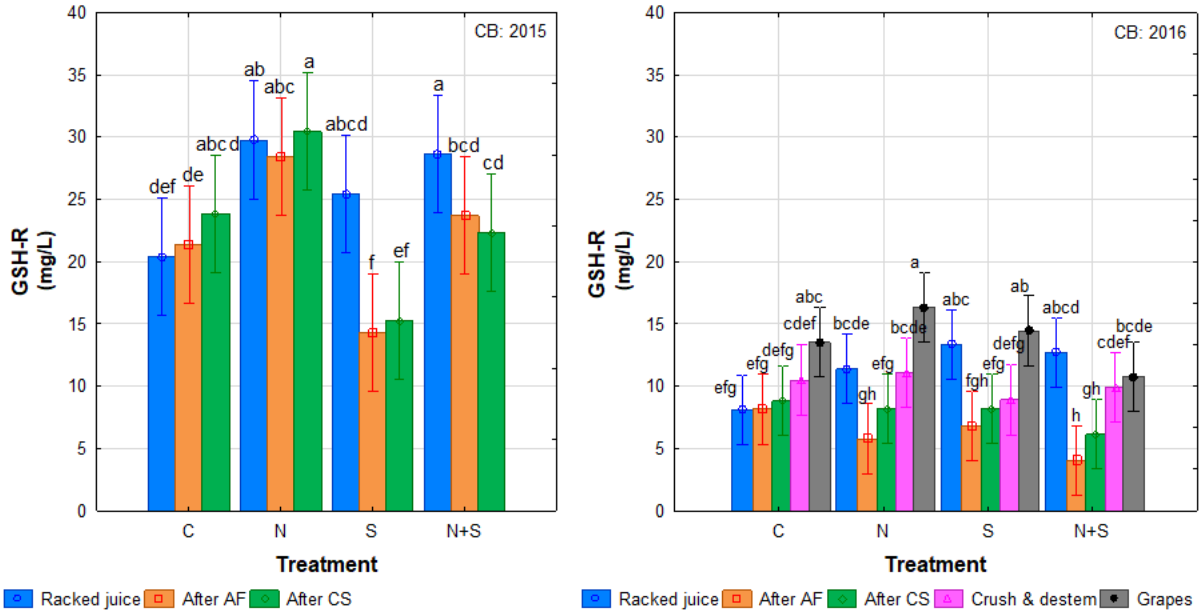


**Figure 3.23** LS means plot illustrating the treatment*time interaction of GSH-R for Chenin Blanc in 2015 and 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

Conversely, for Chenin Blanc in 2015 and Sauvignon Blanc in 2016, the differences between the treatments increased significantly for most Chenin Blanc treatments from the racked juice stage during the winemaking. For Sauvignon Blanc (2016) the GSH-R levels ranged at racked juice from 4.61-7.06 mg/L and from 5.15-11.58 mg/L after cold stabilisation, while for Chenin Blanc (2015) at racked juice the levels were 20.38-29.78 mg/L and 15.23-30.44 mg/L after cold stabilisation (Figure 3.23 and 3.24 and Appendix A-Table A.5). At juice racking, N+S treatment usually resulted in the highest level of GSH-R, with the exception of Sauvignon Blanc in 2016, when the N treatment had the highest level. By the end of the sampling stages, after cold stabilization, the order had generally changed, and only in the case of Sauvignon Blanc both 2015 and 2016, N+S treatment levels were the highest.

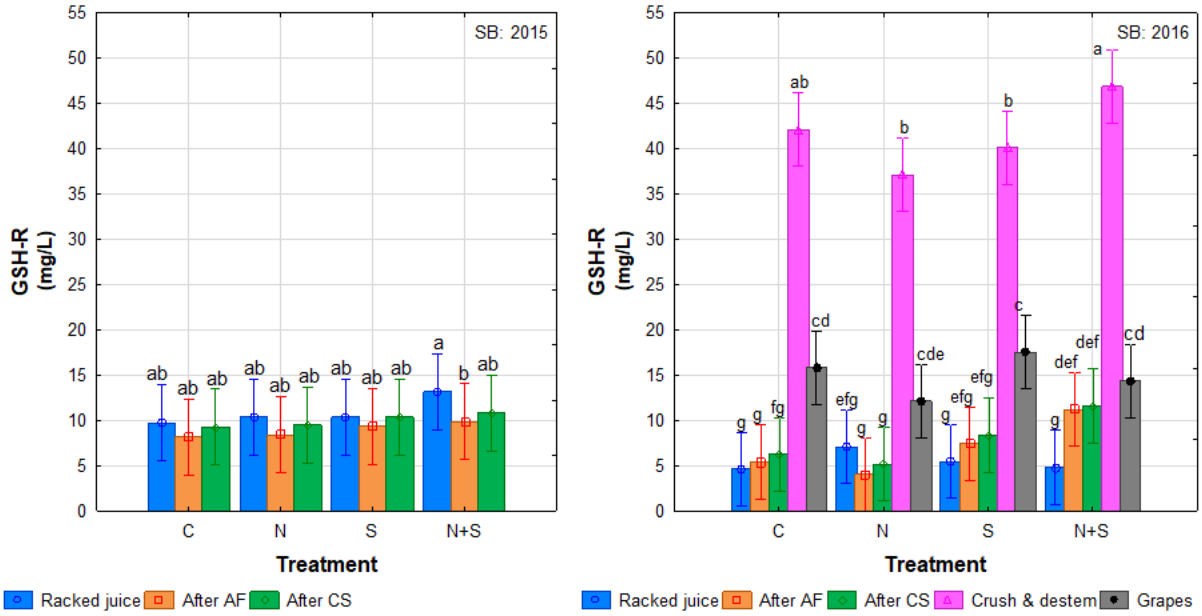


**Figure 3.24** LS means plot illustrating the treatment*time interaction of GSH-R for Sauvignon Blanc in 2015 and 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

GHS-O levels found in a South African study on Sauvignon Blanc ranged from 0.46-2.93 mg/L (Du Toit *et al.*, 2007). In this research study, the levels were between 0-6.17 mg/L (Figure 3.25 and 3.25 and Appendix A-Table A.6). In most cases, there were no differences between the treatments at any sampling stage with two exceptions. Sauvignon Blanc juice in 2016 had the highest GSH-O for the N+S treatment (6.17 mg/L) and the lowest for S (4.55 mg/L), but by the second sampling stage, after alcoholic fermentation, there were no differences between treatments. In the case of Chenin Blanc in 2015, at the end of alcoholic fermentation, there were also significant differences between C and N (lowest) and S and N+S (highest) but these differences reduced by the final sampling stage. The C and N treatments ranged from 3.49-3.76 mg/L and S and N+S treatments from 4.16-4.48 mg/L after alcoholic fermentation. Differences observed for Chenin Blanc racked juice in 2015, even though statistically significant, were not relevant in real terms.

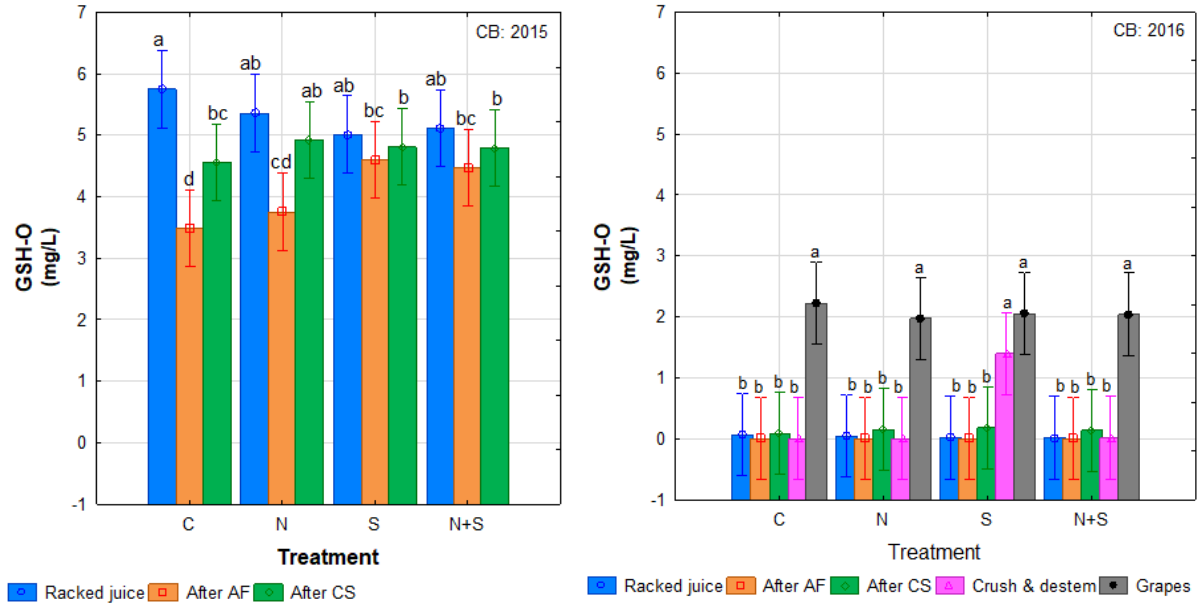


**Figure 3.25** LS means plot illustrating the treatment*time interaction of GSH-O for Chenin Blanc in 2015 and 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

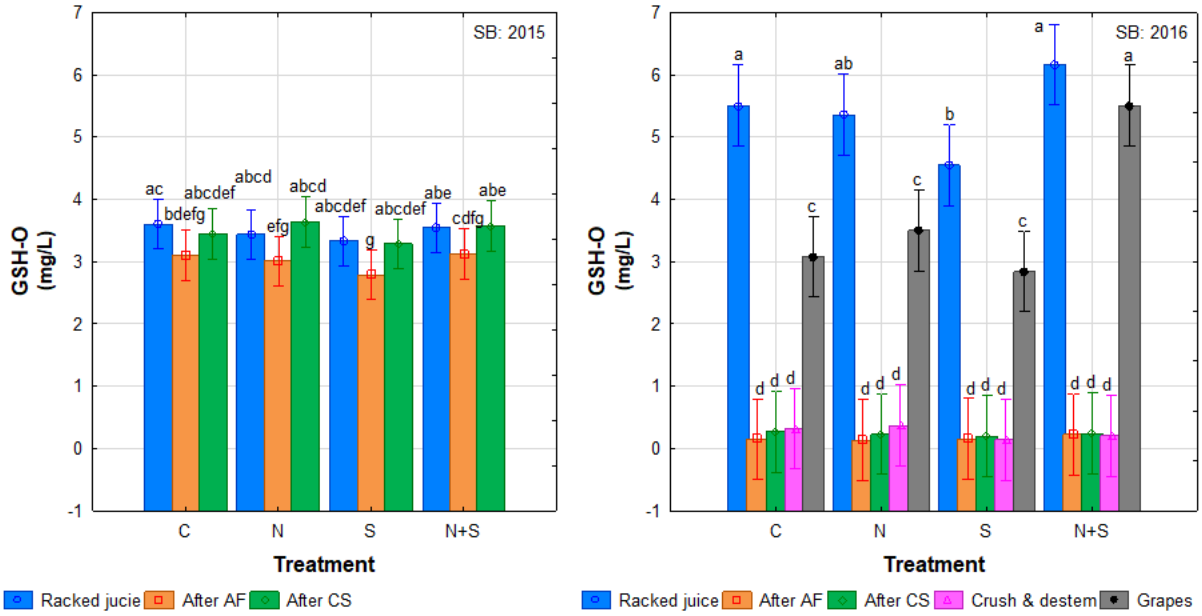


**Figure 3.26** LS means plot illustrating the treatment*time interaction of GSH-O for Sauvignon Blanc in 2015 and 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

The overall treatment effect on the total GSH reflects the variability in the results obtained for the reduced and oxidised forms (Appendix A-Table A.7). Chenin Blanc in 2015 had the highest GSH levels, racked juice levels ranged from 26.13-35.14 mg/L and after cold stabilisation the levels were between 20.04-35.37 mg/L. Sauvignon Blanc GSH levels were similar for the two seasons, but higher in 2015 with juice levels ranging from 13.33-16.66 mg/L and in 2016 from 9.98-12.42 mg/L. For Sauvignon Blanc in 2015 and Chenin Blanc in 2016, the differences between the treatments decreased during the sampling stages, while for Chenin Blanc in 2015 and Sauvignon Blanc in 2016 the differences increased. There was no observable trend regarding the highest or lowest treatment effect. Generally, C had the lowest levels, but there were exceptions, which can possibly be explained by errors in measurement or by the deviation in measurement for the various biological repeats.

Only a few fertilization studies focused on GSH content in must or wines. Lacroux *et al.* (2008) studied the impact of soil nitrogen, foliar nitrogen and foliar nitrogen with sulphur applications on low YAN containing Sauvignon Blanc vines, but only measured GSH-R in wine. They found that both foliar treatments had significantly higher GSH-R content in the wines compared to the Control and soil application, with the nitrogen with sulphur treatment not significantly different from the nitrogen only treatment. Helwi *et al.* (2017) performed a similar study on Sauvignon Blanc and the GSH levels increased with all the treatments, including soil applications, compared to the Control in both juices and wines. Choné *et al.* (2006) applied ammonium nitrate to Sauvignon Blanc at bloom and the juice GSH was seven times higher compared to the Control. Therefore, an increase in nitrogen supply can have a positive effect on the GSH levels.

### 3.3.3.2 Evolution of GSH

Generally, GSH-R levels followed a similar trend, with an initial decrease followed by a small increase from the racked juice to after cold stabilisation. In Figure 3.23, the GSH-R levels decreased and thereafter increased with the N and S treatments in 2015 and with N, S, and N+S treatments in 2016 for Chenin Blanc. In the cases that this trend was not observed, most situations can be explained by the differences between the biological repeats, which were higher than the

differences between the treatments and the sampling stages. The exception was for Sauvignon Blanc in 2016 (Figure 3.24). In this case, N+S treatment resulted in increasing levels of GSH-R throughout the sampling stages, with the final level at significantly higher concentration than the other treatments.

GSH-O followed the same trend throughout the sampling stages for both cultivars and both seasons, with an initial decrease followed by a small (not statistically significant) increase (Figure 3.25 and 3.26). The initial decrease, from juice to after alcoholic fermentation, was different depending on the season and cultivar, Sauvignon Blanc in 2016 having the highest decrease, and Chenin Blanc 2016 the lowest. The high decrease might be ascribed to enzymatic and redox reactions in the juice, by the metabolism of yeast during alcoholic fermentation, or my human error during analysis (Du Toit *et al.*, 2007). For the last stages, from alcoholic fermentation to after cold stabilization, GSH-O levels in Sauvignon Blanc increased more than for Chenin Blanc in both seasons, maybe due to oxidation. From alcoholic fermentation stage, GSH-O levels in Chenin Blanc were close to 0 mg/L.

Juhasz (2015) found a significant increase in the GSH levels with the nitrogen with sulphur treatments compared to the Control during alcoholic fermentation. Various factors, including yeast strain, initial GSH level, must composition and oxidation can play a role with GSH evolution during vinification (Du Toit *et al.*, 2007; Coetzee, 2011; Kritzinger, 2012). In literature, contradictory results have been reported for GSH levels and evolution during alcoholic fermentation. Park *et al.* (2000); Dubourdieu & Lavigne (2004); Tirelli *et al.* (2010); Kritzinger (2012); Kritzinger & Du Toit, (2013) have reported that GSH levels increased with the onset of alcoholic fermentation, while Du Toit *et al.* (2007); Lavigne *et al.* (2007); and Coetzee (2011) reported a decrease in GSH levels, in accordance to the results found in the present study. Kritzinger (2012) reported that with QA23 yeast strain, higher GSH levels were obtained after alcoholic fermentation compared to other industry yeasts used. A decrease in GSH levels during alcoholic fermentation can be due to low levels of CYS present in all the musts leading to an higher uptake of GSH during alcoholic fermentation (Choné *et al.*, 2006). Oxidation of GSH during alcoholic fermentation could also be another explanation, but due to $CO_2$ being released regularly this is unlikely.

## 3.4 Conclusions

The aim of this research study was to investigate the effect of different foliar applications of sulphur and nitrogen before and during véraison on the composition of must, juice, and wines for Sauvignon Blanc and Chenin Blanc. The results obtained for the non-volatile compounds measured suggest that the grape composition of both cultivars was influenced by foliar N, S, and N+S applications. The effect of the treatments was minimal for certain non-volatile compounds. To our knowledge, this is the first study to examine the effect of nitrogen, sulphur and nitrogen with sulphur foliar fertilization on Chenin Blanc in South Africa.

From the standard oenological parameters, homogeneity within the treatments and between treatments were observed. In other words, the foliar fertilization did not influence pH, TA, ˚B, and ethanol levels for the various winemaking stages measured. Some small differences noticed between treatments could be ascribed to variations in measurement.

On the other hand, for both years and both cultivars, N and N+S treatments resulted in significantly increased levels of YAN compared to the C. Although not significant, both cultivars

had higher total amino acids and ARG content with the N treatment compared to the C, and is in accordance with findings by Lasa *et al.* (2012). The resulting wines have the potential to be more aromatic than the control wines, because of the higher amount of amino acid precursors in the must (Garde-Cerda & Ancin-Azpilicueta, 2008).

Only a few fertilization studies focused on GSH content in must or wines. In the present study, it was shown that N and N+S treatments resulted in increased levels of GSH compared to the C for both cultivars in 2015 and 2016. The results and trends were not as evident as for YAN, though. Therefore, in some cases, an increase in nitrogen supply can have a positive effect on the GSH levels and can therefore better protect the volatile thiols during vinification.

Chenin Blanc is regarded as a neutral grape variety and lacks in primary aroma compounds (Marais, 2006). Foliar fertilization practices have shown to increase the levels of aroma precursors in the musts and juices of Chenin Blanc. The wine quality depends on the aroma and flavour and these precursors can be optimized to produce Chenin Blanc wines with increased complexity.

This study highlighted the influence of different foliar applications to Chenin Blanc and Sauvignon Blanc. The non-volatile compounds such as YAN, amino acids, and GSH have been influenced by these foliar applications. Only with the YAN levels, significant higher levels were observed with the nitrogen containing treatments, while with the amino acids and GSH similar trends were observed. Nitrogen- and sulphur containing compounds were influenced by the foliar treatments which consists of the same chemical compounds. This research study can guide viticulturists and winemakers by being able to influence the non-volatile content in their musts, juices, and wines by applying foliar fertilization. A greater understanding of the effect of foliar fertilization on the volatile content and aromatic expression of juice and aged can aid researchers and winemakers to understand these compounds. Also, to determine to what extend the fertilization influenced the juice and wine's chemical and sensory content.

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# Chapter 4

# Research Results

## Effect of foliar Nitrogen and Sulphur fertilization applications on Chenin Blanc and Sauvignon Blanc juices and wines: Volatile chemistry and sensory expression

# Chapter 4 Effect of foliar Nitrogen and Sulphur fertilization applications on Chenin Blanc and Sauvignon Blanc juices and wines: Volatile chemistry and sensory expression

## 4.1 Introduction

Wine is a complex medium and various processes and interactions between many chemical compounds present can contribute and influence the wine's aromatic expression (Marais, 1994). Currently Chenin Blanc and Sauvignon Blanc are of great interest to researchers and various studies have investigated the contribution of chemical compounds to the aromatic expression of the wines (Swiegers *et al.*, 2005; Malherbe *et al.*, 2013; Van Wyngaard, 2013; Von Mollendorff, 2013; Weightman, 2014; Botha, 2015; Wilson, 2017).

Chenin Blanc is considered a neutral grape cultivar (Clarke, 2007; WOSA, 2017b) and in the past few years the focus has been on producing high quality wines with a variety of complex aromas (Loubser, 2008; WOSA, 2017a; Buica *et al.*, 2018). Different Chenin Blanc wine styles such as 'fresh and fruity' (FF), 'rich and ripe-unwooded' (RRUW), and 'rich and ripe-wooded' (RRW) has been identified (CBA, 2016). FF wines have been described as having 'floral', 'fresh fruit', 'pineapple', 'lemon', 'tropical', 'vegetative', and 'sweet' aromas, while RRUW wines have 'citrus', 'earthy', 'fruity', 'floral', 'green', and 'tropical' aromas, and RRW wines have 'buttery', 'caramel', 'grapefruit', 'guava', 'honey', 'marmalade', 'nutty', 'oak', 'peach', 'ripe fruits', 'rich fruit', 'spicy', 'sweet', 'toasted bread', 'vanilla', and 'wood' aroma descriptors (Bester, 2011; Hanekom, 2012; Van Antwerpen, 2012; Botha, 2015; Wilson, 2017).

Sauvignon Blanc plays a very important role locally and internationally (SAWIS, 2016). This cultivar can be made into two wine styles: a 'green style' and 'tropical/fruity style'. The 'green style' include aroma compounds such as 'asparagus', 'capsicum', 'grassy', 'green pepper', 'herbaceous', 'tomato leaf', and 'vegetative', while the 'tropical/fruity style' have 'gooseberry', 'grapefruit', 'pineapple', and 'passion fruit' aromas (Treurnicht, 2011).

These different aromas contribute to the overall expression of the wines and originate from grape-derived compounds, fermentation-derived compounds, or are produced during bottle ageing (Rapp, 1998). Compounds such as methoxypyrazines are present in the grapes, while major volatiles (esters, fatty acids, and higher alcohols) and volatile thiols are formed during alcoholic fermentation and malolactic fermentation, and various chemical reactions between esters, fatty acids, higher alcohols, and volatile thiols can occur during bottle or barrel maturation (Rapp, 1998; Ferreira *et al.*, 2000). These volatile compounds are discussed in detail in Section 2.2.2 regarding their structure, purpose, and aroma expression. The link between the different Chenin Blanc and Sauvignon Blanc wines styles, volatile chemistry composition, and related aromas are discussed in Section 2.3. Various factors such as cultivar heredity, viticulture practices, yeast strain, temperature of fermentation, lees contact, and malolactic fermentation can influence the formation and evolution of these chemical compounds.

Vine nitrogen fertilization has been shown to have a positive impact on the composition of grapes (Mengel, 2002; Oosterhuis, 2009; Lasa *et al.*, 2012). Foliar fertilization applications are mostly used in cases where small nutrient corrections in deficient vineyards are required and are more cost effective. Focus and interest in foliar fertilization has increased the past decade, research studies have looked into applying fertilization at various doses and different times during the

ripening season, and various farmers have started incorporating these practices in their canopy management practices (Christensen & Peacock, 2000; Lacroux *et al.*, 2008; Linsenmeier *et al.*, 2008; Jreij *et al.*, 2009; Lasa *et al.*, 2012; Geffroy *et al.*, 2016b; Gutiérrez-Gamboa *et al.*, 2017; Helwi *et al.*, 2017). Foliar fertilization studies executed under South African climate can provide new and additional information regard for researchers and producers in the industry.

Commonly Sauvignon Blanc has been included in soil and foliar fertilization studies, while only two soil fertilization studies focused on Chenin Blanc (Section 2.4). Results obtained from foliar fertilization studies can contribute to a better understanding of the impact these practices have on the levels and perceptions of the various classes of aroma compounds through chemical and sensory profiling of the resulting wines. This knowledge could ultimately aid researchers and winemakers to understand these compounds, produce a specific wine style, and produce better quality wines with complexity.

The aim of this study was to assess the effect of various fertilization treatments on the volatile content and aromatic expression of the wines at two stages after bottling (three and nine-month-aged wines). This chapter will focus on volatile compounds such as major volatiles, methoxypyrazines, and volatile thiols and will thereafter evaluate the overall volatile content of the wines. The second part will present the effect of the fertilization on the sensory expression of the wines. Lastly, the overall aroma attributes will be investigated and see how the aromatic expression of wines were affected during maturation and from season to season.

## 4.2 Materials and methods

Different foliar fertilization applications were applied to specific Chenin Blanc and Sauvignon Blanc vines in 2014/2015 and 2015/2016. A full description of the experiment layout and treatments can be found in Section 3.2. After the vinification process was complete, the bottled wines were stored in a 15˚C temperature-controlled room for bottle maturation.

### 4.2.1 Storage and wine sampling

Wines underwent sensory and chemical analysis after bottle maturation of three and nine months (Figure 4.1).

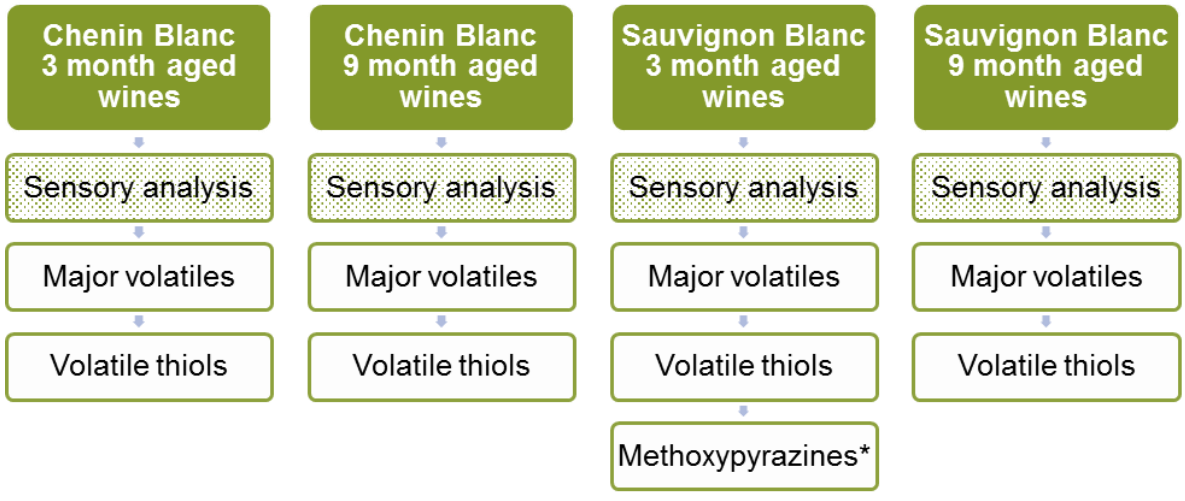


**Figure 4.1** Chemical compound analyses and sampling stages at different maturation stages.

Wine samples for the different chemical analyses were taken on the same day as the sensory analysis (Figure 4.1). For the analysis of methoxypyrazines and volatile thiols, a 250 mL sample of each wine was taken and transferred into a plastic bottle filled with carbon dioxide ($CO_2$) and stored in the -20°C fridge-freezer until analysis. For major volatile analysis, a 15 mL sample was poured into a plastic tube filled with $CO_2$ and stored in the -20°C fridge-freezer until analysis.

### 4.2.2 Chemical and statistical data analysis

The chemical analyses were performed immediately after the sensory sessions. Prior to the specific chemical analysis, the wine samples were left at room temperature to defreeze and were sonicated to homogenise the samples.

#### 4.2.2.1 Major volatiles

A method described by Louw *et al.*, (2009) was used to determine the major volatile compounds. The analysis was performed in the Chemical Analytical Laboratory, Department of Viticulture and Oenology, Stellenbosch University. Major volatiles (32 compounds) were quantified by gas chromatography with a flame ionization detector (GC-FID) with an Agilent GC system HP 6890 series. 100 µL internal standard solution (4-methyl-2-pentanol) were added to five mL wine. The liquid-liquid extraction procedure followed by adding one mL of diethyl ether to the wine sample to extract the volatile compounds. The ether layer was removed and dried by using anhydrous sodium sulphate ($Na_2SO_4$). The dry extract was injected into the GC-FID in duplicate and the averages of each sample were calculated and reported. The major volatiles quantified include ethyl acetate, methanol, ethyl butyrate, propanol, isobutanol, isoamyl acetate, butanol, isoamyl alcohol, ethyl hexanoate, pentanol, hexyl acetate, acetoin, 3-methyl-1-pentanol, ethyl lactate, hexanol, 3-ethoxy-1-propanol, ethyl caprylate, acetic acid, octanoic acid, ethyl-3-hydroxy-butanoate, propionic acid, isobutyric acid, butyric acid, ethyl caprate, iso-valeric acid, diethyl succinate, valeric acid, ethyl phenyl-acetate, 2-phenylethyl acetate, hexanoic acid, 2-phenyl-ethanol, and decanoic acid.

#### 4.2.2.2 Methoxypyrazines

Methoxypyrazine analysis was outsourced and performed by an independent laboratory, VinLAB (ISO17025 and B-BBEE accredited), situated in Stellenbosch, South Africa. The two compounds quantified were 2-methoxy-3-isobutylpyrazine (IBMP) and 2-methoxy-3-isopropyl-pyrazine (IPMP). The wine samples were extracted by using C18 solid phase extraction (SPE) cartridges, concentrated under nitrogen, and injected into the gas chromatography coupled with a mass spectrometry (MS). A full description of the method was published by Coetzee (2014).

#### 4.2.2.3 Volatile thiols

Two volatile thiol compounds, 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) were quantified with a multistep method. Sample preparation was done in the Chemical Analytical Laboratory, Department of Viticulture and Oenology and the instrumental analysis at the Central Analytical Facility of the Stellenbosch University. The wine samples underwent liquid/liquid extraction, followed by concentration and derivatization with *o*-phthaldialdehyde. The extract was injected into an ultra-performance liquid chromatography–tandem mass spectrometer (UPLC-MS/MS). A full description of the method was published by Piano *et al.* (2015).

#### 4.2.2.4 Statistical data analysis

For the statistical analysis of the chemical data, Statistica® software version 13 (Dell Inc., Tulsa, USA) was used. Differences between the treatments were tested for significance by applying the analysis of variance (ANOVA). Separate statistical tests were performed for each cultivar and vintage. Even though the same vineyard was used for Sauvignon Blanc for both years, it was decided to do separate tests as with Chenin Blanc. For total major volatile content and volatile thiols, a treatment*time interaction was done, while for the mean total major volatiles, esters, fatty acids, higher alcohols, and methoxypyrazines the treatment effect was tested. With the presence of a treatment*time interaction or treatment effect, a mixed repeated ANOVA model, Variance Estimation, Precision and Comparison (VEPAC), was performed. The least squares means (LS Means) were calculated form the linear model, ANOVA. Fisher's least significance difference (LSD) tests were used for post-hoc analysis and a p value threshold of 0.05 (different letters account for the significance level at $p<0.05$) was used for the determination of statistical significance. If a sample was considered as an outlier in the ANOVA's linear regression, the data was excluded from the LS Means and VEPAC computed graphs.

For the overall volatile content, a multivariate analysis technique (Principal Component Analysis, PCA) was performed using SIMCA® 14.1 software (Umetrics, Sweden) to obtain a better overview of the relationship differences between samples with their volatile compounds (Wold *et al.*, 1987). Volatile thiols and major volatiles such as higher alcohols, fatty acids, and esters have been included, while methoxypyrazines were excluded because they were only analysed at one bottle maturation stage for Sauvignon Blanc.

### 4.2.3 Sensory and statistical data analysis

#### 4.2.3.1 Free sorting analysis

After three and nine months of bottle maturation, the wines underwent sensory analysis. A rapid method, namely free sorting, was used (Cartier *et al.*, 2006; Valentin *et al.*, 2012). Fifteen judges were used to perform the sorting exercise. The judges were not informed prior to the tasting what the nature or goal of the study was. All the wines of a specific cultivar were presented at once in a randomised order. The wines were coded with random numbers, generated by a Compusense® 5.0 computer software (Release 5.6). The free sorting was repeated and completed in two sessions (30 answers in total). The judges were given instructions and a pre-determined list of general aroma descriptors used during red and white wine tastings at the Sensory Laboratory, Department of Viticulture and Oenology, Stellenbosch University (Appendix B-Figure B.1 and B.2). The judges evaluated the wines and grouped them according to aroma. The judges were free to make as many groups as they wanted and place as many wines in the groups. The groups were described with at least three aroma attributes to characterise that specific group.

#### 4.2.3.2 Statistical data analysis

For statistical data analysis of the free sorting method, Microsoft Excel with XLSTAT® (Version 18.06, Addinsoft) was used. Two types of data can be obtained from a free sorting method: firstly the groups of samples (wines) and secondly the aroma descriptors (Cartier *et al.*, 2006; Valentin *et al.*, 2012). A contingency table with the wines in the rows and the aroma descriptors in the columns was compiled. If a descriptor was cited less than three times by judges, it was combined

or grouped together with a descriptor of similar meaning. The rules for this combination were kept constant throughout the study. The total sum of each descriptor's citation indicates the frequency of the specific descriptor by the judges. The frequency data can be used to compute correspondence analysis (CA), which is a multivariate-graphical method looking into the symmetric and correspondence association between variables (Jaeger *et al.*, 2015; Vidal *et al.*, 2015). Thereafter, multi-dimensional scaling (MDS) was performed to find the differences and similarities between the wines and attributes. Finally, scatter plots were produced to visualise the differences and similarities between the wines, and the sensory attributes (Lawless *et al.*, 1995).

Cluster analysis is a method used to divide data into several subgroups. Members situated close to each other are closely related or are similar (Brotzman *et al.*, 2015). In a similar contingency table as described above the wines are both in the rows and columns and grouped according to the groups made by the judges. To compute dendrogram of responses generated by agglomerated hierarchical cluster (AHC) analysis, Euclidean distances and Ward's linkage were used to identify groupings of similar samples on the CA plots with XLSTAT (Version 18.06, Addinsoft). Wordle® online website was used to create word clouds with the sensory attributes; the size of the word is proportional to the frequency of citation of the attribute. These analyses were done for both vintages, cultivars, and wine ages.

## 4.3 Results and discussions

### 4.3.1 Chemistry Results

#### 4.3.1.1 Major volatiles

Major volatiles, such as esters, acids, fatty acids, and higher alcohols are fermentation-derived compounds and are produced as secondary metabolites of amino acids and fatty acids (Styger *et al.*, 2011). These compounds can positively or negatively influence the aroma profile of wines. Pleasant aromas such as 'fruity, 'floral', and 'green' or unwanted aromas like 'cheesy', 'vinegar', and 'solvent-like' can be linked to these volatile compounds (Lambrechts & Pretorius, 2000; Bell & Henschke, 2005; Swiegers *et al.*, 2005).

***Total major volatile content***

The total averages of major volatiles for Chenin Blanc in 2015 ranged from 364-628 mg/L (Figure 4.2). Overall, the 3-month aged wines showed lower levels of total volatiles for all the treatments compared to the C, where the S treatment wines were the lowest (15% lower than C). After 9 months of bottle maturation, only the N+S wines showed higher levels of total major volatiles compared to the C, while the other treatments were lower than the C. The volatile content of the wines significantly increased during bottle maturation for all the treatments, but the highest increases occurred with the N+S treatment.

In 2016, the Chenin Blanc major volatile content ranged from 395-578 mg/L (Figure 4.3). Highest major volatile content was observed with the N treatment wines, while the S and N+S treatments were lower compared to C. Although no significant differences were observed between the treatments. During bottle maturation C, S, and N+S treatment wines increased in major volatile content, while the N treatment decreased. The N+S treatment significantly increased (395 to 540 mg/L) the most compared to the other treatments during bottle maturation, similar to the effect observed in 2015.

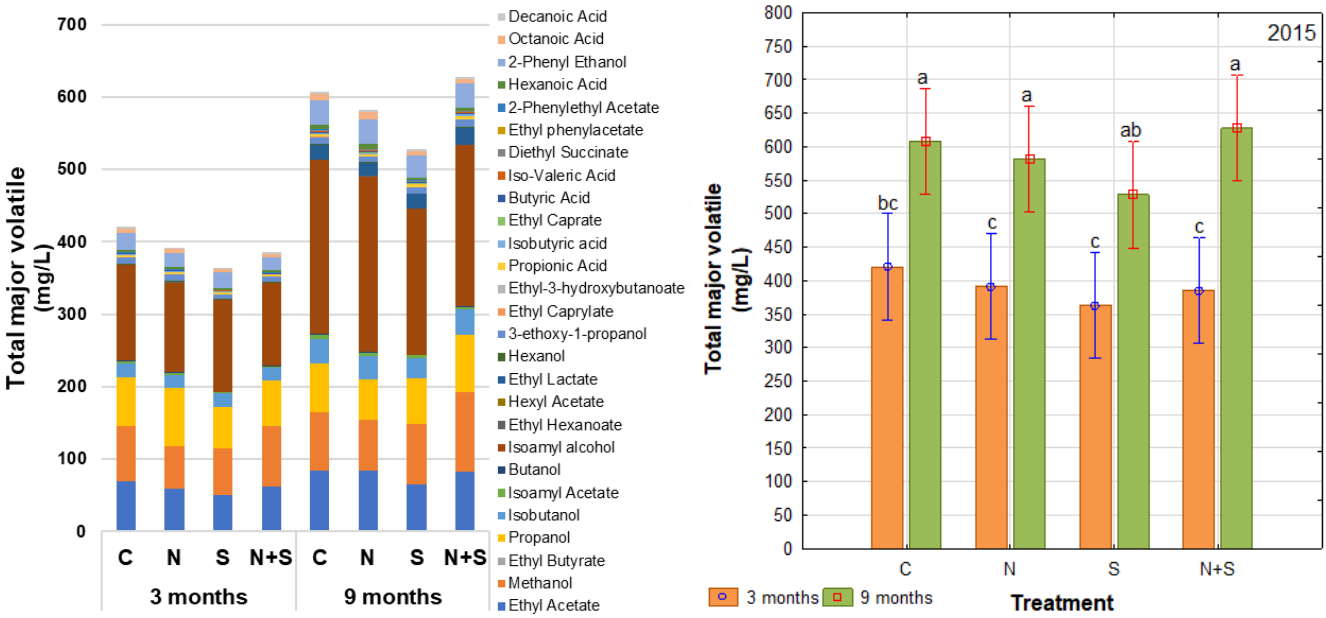


**Figure 4.2** Averages of total major volatile compounds present in different aged Chenin Blanc 2015 wines. LS means plot illustrating the treatment*time interaction of total major volatile content in aged Chenin Blanc wines in 2015 (right) for different treatments with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

During maturation, all the treatments increased for both vintages, except for the N treatment in 2016. The treatment effect had no obvious pattern between the different wines. N+S treatment wines had the highest total major volatile content for both vintages for 9-month aged wines. Wines from the N and S treatments generally had lower levels of major volatiles than C for both vintages and wine ages, except for N treatment in 3-month aged wines in 2016. The differences in major volatile levels from 2015 to 2016 cannot be assigned to a real vintage effect, since two different vineyards were used for the two seasons.

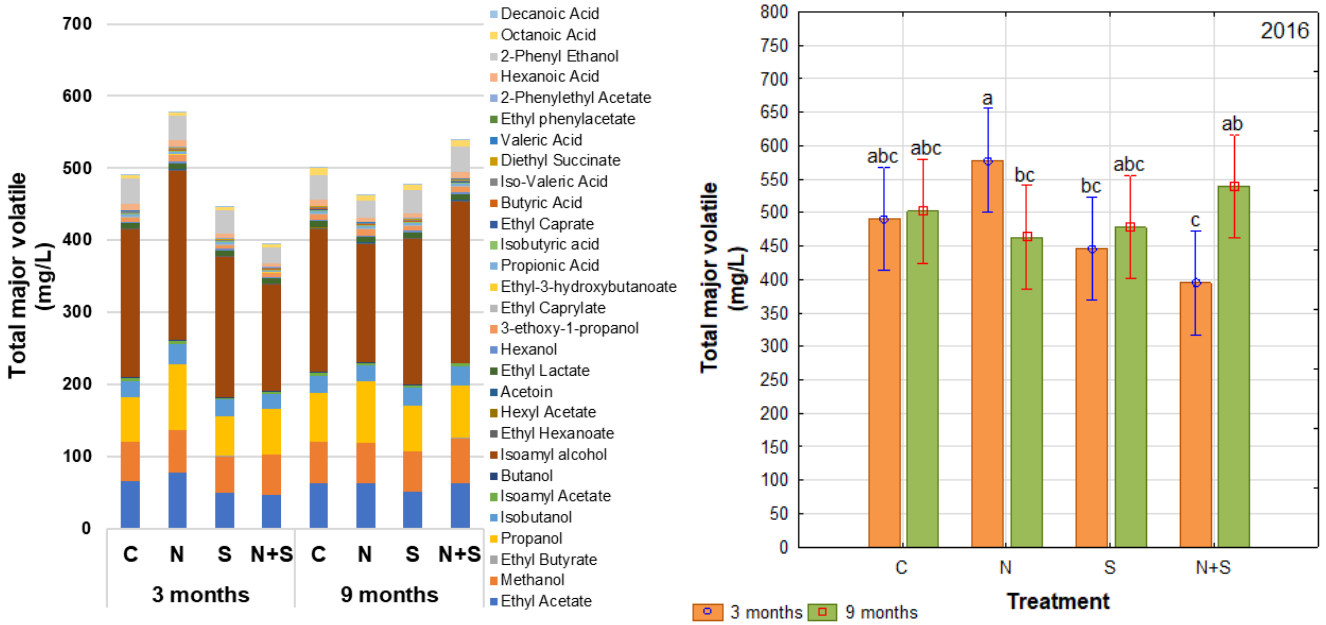


**Figure 4.3** Averages of total major volatile compounds present in different aged Chenin Blanc 2016 wines. LS means plot illustrating the treatment*time interaction of total major volatile content in aged Chenin Blanc wines in 2016 (right) for different treatments with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

The major volatile content for Sauvignon Blanc wines in 2015 ranged from 416-656 mg/L (Figure 4.4). The 3-month aged wines had higher levels of major volatiles for all the treatments compared to the C. The N+S major volatile content was 25% higher, while N treatment was 21% higher compared to the C. Only the C increased in major volatile content during bottle maturation, while all the other treatments decreased significantly compared to the C. The S treatment wines had the highest decrease of 35% during bottle maturation.

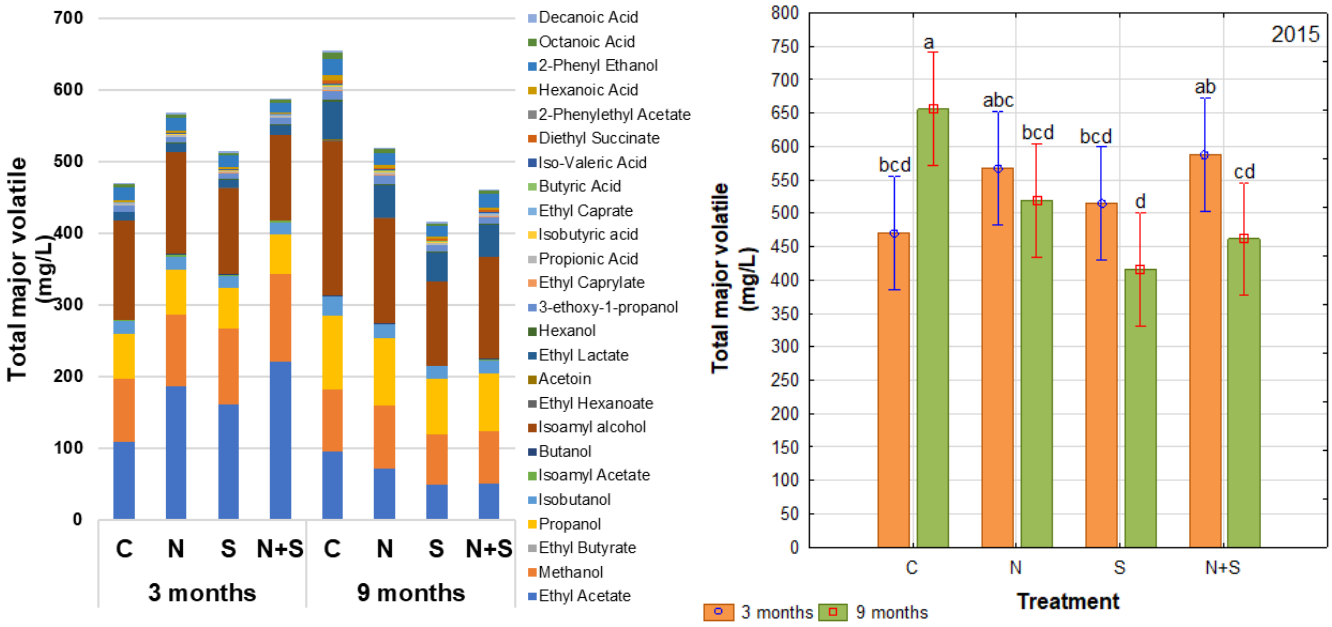


**Figure 4.4** Averages of total major volatile compounds present in different aged Sauvignon Blanc 2015 wines. LS means plot illustrating the treatment*time interaction of total major volatile content in aged Sauvignon Blanc wines in 2015 (right) for different treatments with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

In 2016, total major volatile levels ranged from 378-619 mg/L for Sauvignon Blanc (Figure 4.5). After 3 months of maturation, similar to 2015, all the treatments showed significant higher levels compared to C, but the pattern was different.

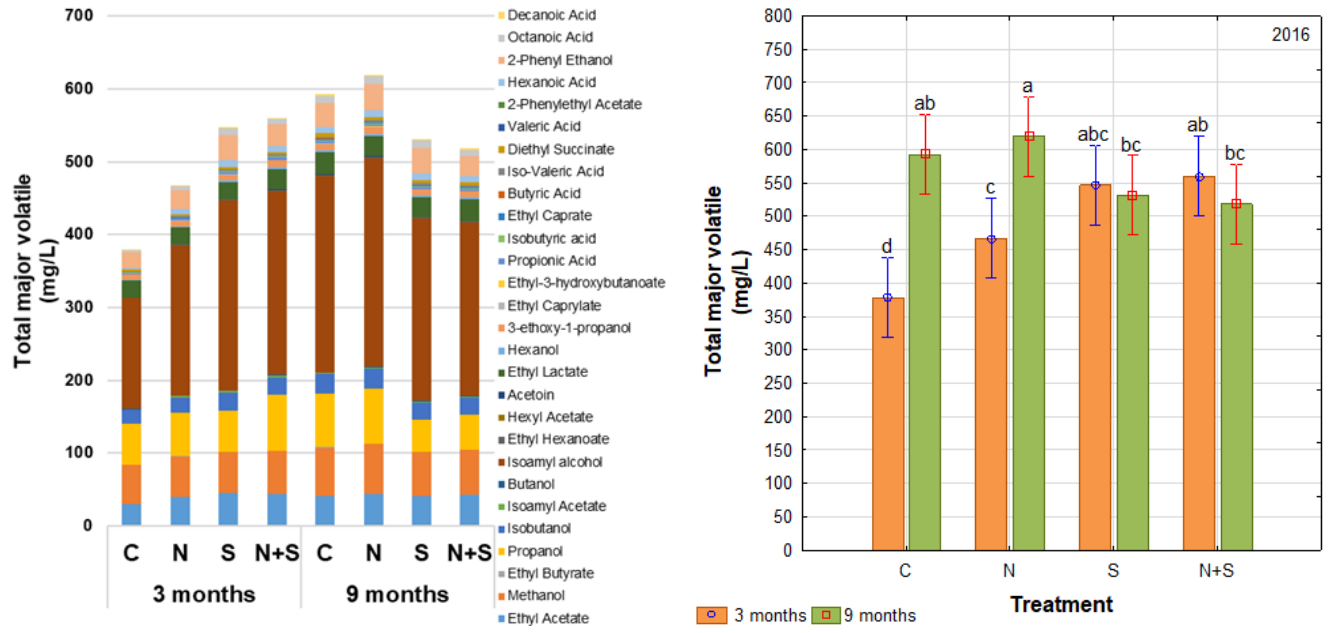


**Figure 4.5** Averages of total major volatile compounds present in different aged Sauvignon Blanc 2016 wines. LS means plot illustrating the treatment*time interaction of total major volatile content in aged Sauvignon Blanc wines in 2016 (right) for different treatments with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

The S treatment wines were 44% higher, while the N+S treatment was the highest (48%) compared to C. The 9-month aged wines showed a different trend, with the N wines higher than the C, while the S and N+S treatment wines had lower major volatile content. During bottle maturation, the major volatile content increased with C and N treatment and decreased with S and N+S treatments.

In the case of Sauvignon Blanc, the same vineyard and vines were used for both years (2015 and 2016). For the 3-month aged wines, a treatment effect occurred, and all the treatments had higher major volatile levels compared to the C for both vintages. N+S treatment was the highest (2015-25% and 2016-44%) for both vintages compared to the C at 3 months. The same treatment effect was not observed at 9 months, as both the S and N+S treatments decreased compared to the C.

***Esters***

Some major volatile compounds, such as pentanol, hexyl acetate, acetoin, 3-methyl-1-pentanol, ethyl-3-hydroxybutanoate, and ethyl phenylacetate have been excluded from the statistical results because they were either not detected, or no variation has been found between treatments. Ester compounds in Chenin Blanc and Sauvignon Blanc wines can be seen in Table B.1 (Appendix B).

Esters contribute to the pleasant fruity and floral aromas in wines (Swiegers *et al.*, 2005). Various esters were within levels found in South African wines. Ethyl acetate (‘sweet’, ‘fruity’, ‘solvent-like’, and ‘varnish’) is the most prominent ester and levels found in this study were within levels previously reported in South African Sauvignon Blanc wines (Van Wyngaard, 2013). Ethyl butyrate (‘pineapple’, ‘varnish’, and ‘balsamic’), ethyl hexanoate (‘fruity’, ‘green apple’, and ‘strawberry’), ethyl caprylate (‘pineapple’, ‘pear’, ‘floral’, and ‘fruit’), diethyl succinate (‘fruity’) and 2-phenylethyl acetate (‘fruity’) levels were within reported levels. Isoamyl acetate (‘fruity’ and ‘banana’) and ethyl caprate (‘fruity’) levels were also within reported levels found in South African wines, but were slightly lower in Sauvignon Blanc (2015 and 2016) and Chenin Blanc in 2015 respectively. Ethyl lactate (fruity’, ‘buttery’, ‘creamy’, and ‘lactic’) levels were slightly higher in Sauvignon Blanc wines from 2015 than those reported in South African Sauvignon Blanc wines.

In Chenin Blanc wines in 2015 and 2016 at both maturation stages, ethyl acetate, ethyl butyrate, isoamyl acetate, ethyl hexanoate, and 2-phenylethyl acetate, and in all Sauvignon Blanc wines ethyl acetate, ethyl butyrate, isoamyl acetate, and diethyl succinate were above their respective sensory thresholds (Appendix B-Table B1). Overall, the 3-month aged wines showed significantly higher levels of ethyl acetate with N treatments for Chenin Blanc and Sauvignon Blanc in 2016 compared to the C. Sauvignon Blanc had significant higher levels of ethyl acetate with N and S treatments compared to C in both 2015 and 2016. No similarities were found between the 9-month aged wines between cultivars. In 2016, N+S treatment wines had higher diethyl succinate levels compared to C for Chenin Blanc (9 months) and Sauvignon Blanc (3 months).

As an example, 3-month aged Chenin Blanc wines in 2015 showed higher isoamyl acetate levels for the N treatment compared to the C, while isoamyl acetate, ethyl hexanoate, ethyl caprylate, diethyl succinate, and 2-phenylethyl acetate were higher compared to the C with 9-month aged wines (Appendix B-Table B.1). Ethyl lactate was only higher with N+S treatment compared to the C. Although these increases are not significant during the bottle maturation, all the ester levels increased significantly from 3 to 9 months. Ethyl acetate (C and S), ethyl hexanoate (S and N+S), ethyl caprylate (S and N+S), and ethyl caprate (S) increased but were not significant. The total average esters for 3-month aged wines ranged from 54-74 mg/L and significantly increased to 91-114 mg/L after 9 months of maturation.

In 2015, the total average esters of Sauvignon Blanc wines ranged from 123-238 mg/L at 3 months and 94-159 mg/L at 9 months (Appendix B-Table B.1). The N treatment had higher levels for all the esters compared to the C, but only significant higher levels with ethyl acetate (72%), isoamyl acetate (36%), and 2-phenylethyl acetate (11%). The S treatment were also significantly higher with the ethyl acetate (102%) and isoamyl acetate (29%) compared to the C. The 9-month aged wines showed lower levels for all the esters compared to the C. During bottle maturation all the treatments decreased for ethyl acetate, isoamyl acetate, and phenylethyl acetate. Significant increases during maturation occurred for ethyl butyrate (C), ethyl hexanoate (C), ethyl lactate (all treatments) ethyl caprylate (C and N treatments), ethyl caprate (C, N, and N+S treatments), and diethyl succinate (all treatments). In 2015, only the C significantly increased in total major volatile content during bottle maturation, while in 2016 both C and N treatment increased significantly compared to all the treatments.

Only one foliar study has included esters in their research studies (Juhasz, 2015). Juhasz (2015) followed a similar foliar fertilization study and reported that the fertilization did not influence the ester levels significantly. Esters such as 2-phenylethyl acetate, diethyl succinate, ethyl butyrate, ethyl lactate, ethyl phenylacetate, and hexyl acetate had the highest increased levels with the N+S treatment compared to the C (Juhasz, 2015). Isoamyl acetate, ethyl caprylate, and ethyl acetate had the highest increased levels with the S treatment compared to the C (Juhasz, 2015).

Nitrogen compounds, such as amino acids, are released during yeast metabolism as ethyl esters, which are desirable for wine quality because of their positive ‘fruity’ aromas (Lacroux *et al.*, 2008). In this current research study, the foliar treatments had a positive effect on esters present in the matured wines. The overall ester content increased over time for most treatments for Chenin Blanc and Sauvignon Blanc, but in 2015 only the C increased for Sauvignon Blanc. The N+S had increased diethyl succinate levels compared to the C for Chenin Blanc (2016-9 months) and Sauvignon Blanc (2016-3 months), and this can be due to the transformation of lactic acid and succinate acids to diethyl succinate and researchers reported with white varietals an increase in ester levels over time (Selli *et al.*, 2006).

### *Fatty acids*

Fatty acids have an important impact on wine quality (Bell & Henschke, 2005). Low concentration fatty acids can positively contribute to the complexity and aroma of wines (Coetzee, 2011), while too high concentrations can contribute unwanted flavours like ‘cheesy’, ‘vinegar’, and ‘rancid’ (Lambrechts & Pretorius, 2000). Many factors can influence the fatty acid levels such as yeast strain, sugar concentration, inoculation rate, juice clarification, fermentation temperature, nitrogen, oxygen exposure, and sulphur dioxide ($SO_2$) additions (Henschke & Jiranek, 1993; Garde-Cerdán *et al.*, 2009; Coetzee, 2011).

In this current study, various fatty acids were within levels found in South African wines (Appendix B-Table B.2). Propionic acid (‘pungent’, ‘soy’, and ‘rancid’ aromas), isobutyric acid (‘cheese’, ‘butter’, and ‘rancid’ aromas), octanoic acid (‘cheese’ and ‘sweat’), and decanoic acid (‘fat’ and ‘rancid’) levels were within reported levels for both cultivars. Sauvignon Blanc wines had slightly higher levels for iso-valeric acid (‘acid’, ‘sweat’, and ‘rancid’) and hexanoic acid (‘cheese’, ‘fatty’, ‘rancid’, and ‘sweat’) compared to levels in South African Sauvignon Blanc wines. Chenin Blanc and Sauvignon Blanc have slightly lower butyric acid (‘cheese’, ‘sweat’, and ‘rancid’) levels for both vintages. South African Sauvignon Blanc wines have significantly higher fatty acids compared to Chardonnay and includes only certain acids (Louw *et al.*, 2010).

Looking at both cultivars in 2015, N treatment had higher levels than the C with isobutyric acid and butyric acid for Sauvignon Blanc (3 months) and Chenin Blanc (9 months) (Appendix B-Table B.2). In 2016, significant higher levels of valeric acid were obtained with Chenin Blanc (3 months) and Sauvignon Blanc (9 months) and isobutyric acid with Sauvignon Blanc (3 and 9 months) with the N treatment. By comparing the similar ages between the wines, Chenin Blanc 3-month aged wines showed higher levels for butyric acid for both vintages with the S treatment, while the 9-month aged wines increased with N+S treatment for isobutyric acid and iso-valeric acid. N treatment had higher levels of iso-butyric acid, hexanoic acid, octanoic acid for 3-month aged Sauvignon Blanc wines for both years. Similarly, the 3-month aged Sauvignon Blanc wines the S containing treatments had higher levels of propionic acid and iso-valeric acid. Iso-valeric acid, hexanoic acid, and octanoic acid were above the sensory threshold for both cultivars and vintages.

Total average fatty acid content of 3-month aged Chenin Blanc wines in 2016 ranged from 14-22 mg/L and from 21-28 mg/L for 9-month aged wines (Appendix B-Table B.2). The 3-month aged wines had significant higher levels for the N treatment for propionic acid and valeric acid. For the 9-month aged wines, N+S treatments showed higher levels with isobutyric acid, iso-valeric acid, and valeric acid, while N only showed increases with valeric acid. During bottle maturation all the fatty acid levels increased for all the treatments, except N treatment. During maturation S treatments increased by 22% and N+S treatments increased by 80%. Significant increases occurred with butyric acid and decanoic acid for all the treatments, while with isobutyric acid, iso-valeric acid, hexanoic acid, and decanoic acid increased significantly with N+S treatments.

Only one foliar study has included fatty acids in their research studies. Juhasz, (2015) reported that there were no significant differences between the foliar fertilization treatments. All the fatty acids were above the sensory threshold, except for hexanoic acid and valeric acid (Juhasz, 2015). The nitrogen with sulphur treatment showed the most increased levels compared to the control with butyric acid, decanoic acid, iso-valeric acid, and valeric acid, while the sulphur treatment showed increased levels with acetic acid, hexanoic acid, and octanoic acid (Juhasz, 2015). In the current study, the hexanoic acid levels were also below the sensory threshold for both cultivars. Similarly, the N+S treatments showed increased levels with iso-valeric acid compared to the C in Chenin Blanc (9 months in 2015 and 2016) and Sauvignon Blanc (3 months in 2015 and 2016).

No foliar research have focused on wine maturation, and the wine age in the study by Juhasz, (2015) was unknown. Šuklje *et al.* (2016) applied two inactive dry yeast derivative products twice to Sauvignon Blanc grapes (4 kg/ha and 3 kg/ha) one week after 100% véraison and ten days thereafter. The wines underwent two months of maturation and only octanoic acid increased significantly for the one yeast, while for the second yeast, propanoic acid, isobutyric acid, iso-valeric acid, hexanoic acid, and octanoic acid decreased significantly (Šuklje *et al.*, 2016).

### *Higher alcohols*

Higher alcohols can influence the aroma and flavour of the wine (Bell & Henschke, 2005). Higher alcohols below 300 mg/L can positively influence the complexity of wines, while levels more than 400 mg/L can have a detrimental effect and display unpleasant ‘fusel’ and ‘solvent-like’ aromas, with the exception of 2-phenyl ethanol (‘rose’ and ‘floral’ aromas) (Lambrechts & Pretorius, 2000).

Higher alcohols present in the aged Chenin Blanc and Sauvignon Blanc wines can be seen in Table B.3 (Appendix B). In this current study, various higher alcohols were within levels found in studies performed in South African wines (Van Wyngaard, 2013). Methanol (‘alcohol’), isobutanol

('ripe fruit' and 'alcohol'), butanol ('alcohol' and 'solvent'), hexanol ('resin', 'flower', and 'green cut grass'), and isoamyl alcohol ('wine', 'solvent', and 'bitter') levels were within reported levels for both cultivars. Slightly higher levels were obtained for propanol ('ripe fruit' and 'alcohol'), 3-ethoxy-1-propanol ('fruity'), 2-phenyl ethanol ('flower', 'perfume', and 'pollen'), iso-valeric acid ('acid', 'sweat', and 'rancid'), and hexanoic acid ('cheese', 'fatty', 'sweat', and 'rancid') compared to levels in South African Sauvignon Blanc wines reported by Van Wyngaard (2013).

Comparing the different ages of wines within each vintage, in 2015, the N treatment showed highest levels compared to the C with propanol, butanol, and 3-ethoxy-1-propanol with 3-month aged Sauvignon Blanc and Chenin Blanc wines (Appendix B-Table B.3). The N+S treatment showed significant higher levels of methanol compared for Chenin Blanc (3 and 9 months) and Sauvignon Blanc (3 months). In 2016 higher levels of isoamyl alcohol were obtained for 3-month aged wines, while N treatment increased propanol, butanol, and 3-ethoxy-1-propanol levels for 9-month aged wines for both cultivars. S treatment had significantly higher isobutanol levels for both cultivars at 3 months of age, while butanol was higher for both cultivars at 9 months of age.

Looking more into detail at one of the vintages as an example, the total average higher alcohol levels of 3-month aged Chenin Blanc wines in 2015 ranged from 296-329 mg/L and the 9-month aged wines ranged from 418-492 mg/L (Appendix B-Table B.3). The 3-month aged wines showed lower levels for all the treatments compared to the C for most of the higher alcohols. The N treatments showed higher levels with propanol, butanol, and 3-ethoxy-1-propanol, S treatment had higher levels of isobutanol, while N+S had higher methanol levels compared to the C. The 9-month aged wines had higher levels with N treatment with butanol, isoamyl alcohol, hexanol, and 2-phenyl ethanol, S treatment had higher levels with methanol, and N+S treatment had higher methanol, propanol, and isobutanol levels. During bottle maturation the average of total higher alcohol levels increased significantly for all the treatments. N increased by 43%, S increased by 41%, and N+S increased the most by 61%. During maturation significant increases for all the treatments occurred with isobutanol, isoamyl alcohol, and 2-phenyl ethanol.

Various wines produced from nitrogen fertilization studies resulted in lower levels of higher alcohols compared to the control (Bell & Henschke, 2005). Higher alcohols are formed by being synthesized anabolically from glucose or synthesized catabolically from branched-amino acids, such as LEU, ILE, THR, and VAL (Bell *et al.*, 1979). Higher alcohols are directly related to amino acid metabolism and are influenced directly by YAN levels. Higher alcohols reach a peak between 200-300 mg/L YAN and degrade if the YAN levels increase (Ugliano *et al.*, 2007). Chenin Blanc (2016) had significant higher levels of YAN with N treatment compared to the C and the higher alcohol levels were the highest at 3 months and decreased during maturation. Similarly, in 2016, Sauvignon Blanc wines had the highest higher alcohol levels with N+S treatment and decreased during maturation. Bell & Henschke (2005) summarized research findings with similar results.

Only one foliar fertilization study has included fatty acids in their research studies and Juhasz (2015) reported that there were no significant differences between the foliar fertilization treatments. The N+S treatment showed the highest levels compared to the C with butanol, hexanol, and methanol, while the S treatment showed increased levels with 2-phenyl-1-ethanol, isobutanol, isoamyl alcohol, and propanol (Juhasz, 2015). Similarly, in the current study, the N+S treatments showed increased levels of methanol for Chenin Blanc (3 and 9 months) and Sauvignon Blanc (3 months) in 2015. The S treatment also significantly increased isobutanol levels for 3-month aged wines for both cultivars.

### 4.3.1.2 Methoxypyrazines

The 'green' associated aromas of Sauvignon Blanc wines are known to be linked to methoxypyrazines, of which IBMP is the most important representative. Methoxypyrazines contribute to the aroma expression of wines and are described as imparting 'asparagus', 'gooseberry', 'grassy', 'green pepper', 'herbaceous', and 'vegetative' notes (Lacey *et al.*, 1991; Marais, 1998). Various factors including environmental parameters, clones, canopy management, origin, soil, and terroir can influence the accumulation of methoxypyrazines in the berries (Swiegers *et al.*, 2006). Methoxypyrazines are not present in Chenin Blanc grapes, must, or wines (Lacey *et al.*, 1991; Marais, 1998).

The IBMP levels in the Sauvignon Blanc wines varied between treatments and ranged from 1-4 ng/L (Figure 4.6). After three months of maturation, the average IBMP levels were the highest for the C and the lowest for the N+S treatment. The N+S treatment were significantly lower than the C. The IBMP levels did not vary much between the treatments. All treatments were above the odour threshold of 2 ng/L (Lacey *et al.*, 1991; Marais, 1994; Ribéreau-Gayon *et al.*, 2006), except the N+S treatment. Therefore, IBMP could have influenced the aromatic expression of the wines. In real terms, though, the levels for all the wines were very low and close to the limit of detection of the analysis method. Additionally, no IPMP was detected in the specific wine samples.

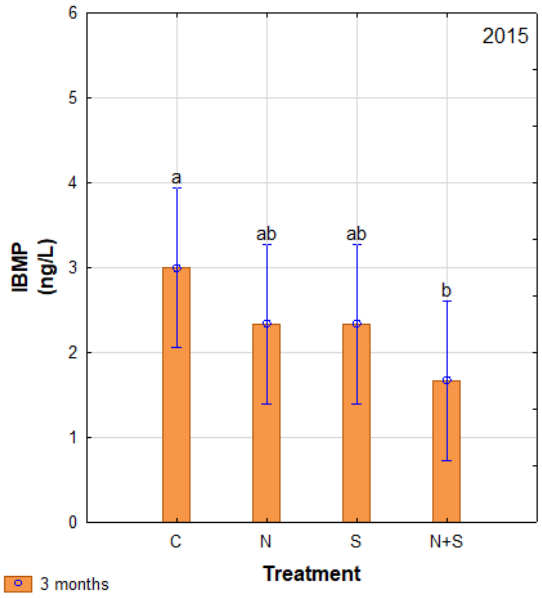


**Figure 4.6** LS means plot illustrating the treatment effect on IBMP levels of Sauvignon Blanc (2015) wines at 3 months of bottle maturation with significant letters from the LSD post-hoc test. Vertical bars denote 95% confidence intervals.

South African studies have shown that IBMP levels in wines ranged from 1.2-40 ng/L and IPMP levels were 1-13 ng/L (Alberts *et al.*, 2009; Lund *et al.*, 2009; Coetzee, 2011, 2014; Benkwitz *et al.*, 2012; Van Wyngaard, 2013; Juhasz, 2015). In the current study, IBMP levels were within the levels found in South Africa studies, but were in the lower end of the range. Even though different levels of methoxypyrazines were obtained, no obvious treatment effect occurred. Methoxypyrazines are formed by the catabolism of VAL, GLY, and MET (Cheng *et al.*, 1991), and these amino acids were present at low values in the harvested grapes (Appendix A-Table A.3 and A.4). It can be hypothesised that the amino acid catabolism was limited and low methoxypyrazine levels were to be expected. The nitrogen status of the vines in 2015 was deficient (Section 3.3.2.1), and higher IBMP levels were obtained by N compared to the N+S treatment.

Sulphur can play a role during the N+S treatment and might negatively influence the absorption of nitrogen by the vine and contribute to the methoxypyrazine levels. Light exposure with leaf or shoot thinning during ripening is known to decrease methoxypyrazine levels (Hunter *et al.*, 2004), while grapes grown in cooler climate zones have higher levels of IBMP (Coetzee & Du Toit, 2012). At this specific Sauvignon Blanc vineyard (Farm C), leaf removal practices were performed at véraison where all the leaves were removed around the bunch zone in the afternoon sun side. Therefore, low IBMP and IPMP levels could have been influenced by climate and viticultural practices such as bunch or grape exposure to the sun and leaf removal practices at véraison (Marais *et al.*, 1999; Sala et al., 2004). By applying earlier foliar applications, for instance at or after flowering, the methoxypyrazine levels could have been higher, but various factors in the vineyard can still influence these applications.

Only a limited number of samples were subjected to methoxypyrazine analysis; as the levels found were very low and no obvious treatment effect was noticed the wines were not subjected to further methoxypyrazine analyses.

Very little foliar fertilization research has been done and focused on methoxypyrazines. Only two foliar studies have measured the methoxypyrazines levels in wines, and similar results as found in this study were obtained where the control had the highest levels and the nitrogen with sulphur foliar treatment were the lowest (Juhasz, 2015). Another study has shown that a nitrogen soil application also had no significant effect on the methoxypyrazine levels of the wines, and resulted in higher IBMP level for the control (Helwi *et al.*, 2017).

#### 4.3.1.3 Volatile thiols

Volatile thiols are known to contribute to the positive 'tropical' aromas such as 'guava', 'grapefruit', and 'passion fruit' in Sauvignon Blanc and Chenin Blanc wines (Tominaga *et al.*, 1998b, 2000; Wilson, 2017). These compounds are sensitive to oxidation and degrade during maturation or ageing, especially 3MH, and 3MHA is also susceptible to hydrolysis (Figure 4.7) (Nikolantonaki *et al.*, 2010).

The odour thresholds for these volatile thiols are very low for 3MH (60 ng/L) and 3MHA (4 ng/L) respectively, compared to other volatile compounds (Tominaga *et al.*, 1996, 1998a; Francis & Newton, 2005). Factors such as terroir and winemaking processes prior to alcoholic fermentation can influence the precursors present in the grapes (Murat *et al.*, 2001; Dubourdieu *et al.*, 2006).

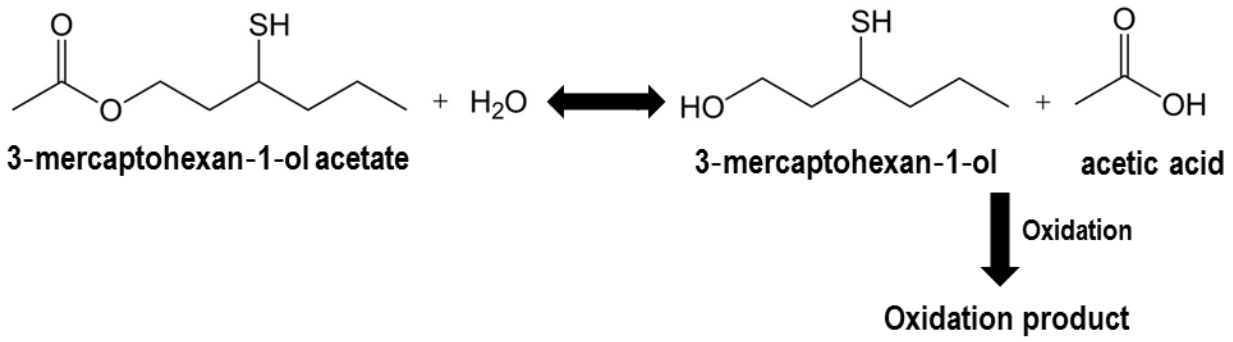


**Figure 4.7** Hydrolysis of 3MH to 3-mercaptohexan-1-ol and acetic acid (Herbst-Johnstone *et al*., 2011).

***3MHA***

In this research study, the 3MHA levels for Chenin Blanc wines ranged from 31-262 ng/L, whereas Sauvignon Blanc wines ranged from 8-167 ng/L (Figure 4.8 and 4.9). Volatile thiols are susceptible to oxidation during aging and a decrease in levels can be expected (Coetzee, 2011). 3MHA levels present in Sauvignon Blanc wines were within ranges found in studies (Coetzee & Du Toit, 2012) and were slightly higher than those reported in South African studies (Van Wyngaard, 2013; Piano *et al.*, 2015). The Chenin Blanc wines had generally higher levels compared to reported 3MHA levels in Chenin Blanc (Wilson, 2017). Both cultivars had 3MH levels above the sensory threshold of 4.2 ng/L (Tominaga *et al.*, 1996). During alcoholic fermentation 3MHA is formed by the acetylation of 3MH by the yeast ester and forms alcohol acetyltransferase with acetic acid (Figure 4.7) (Nikolantonaki *et al.*, 2010). Various yeast strains differ in their capability to convert 3MH to 3MHA, and QA23 have the highest capability (Swiegers *et al.*, 2006).

Even though different Chenin Blanc vineyards (Farm A and B) were used for 2015 and 2016, it is of interest to see how the vineyards performed with the foliar applications. The 3-month aged Chenin Blanc wines in 2015 showed higher 3MHA levels for all the treatments compared to the C (Figure 4.8). The S treatment had the highest level (85%) compared to the C. Even though differences were observed, the 3MHA increases were not significant between the treatments. After 9 months of maturation, the 3MHA levels for the N and S treatments were lower compared to the C, while the N+S treatment showed a non-significant increase of 41%. During bottle ageing, 3MHA levels increased for the C and N+S treatments and decreased for the N and S treatments.

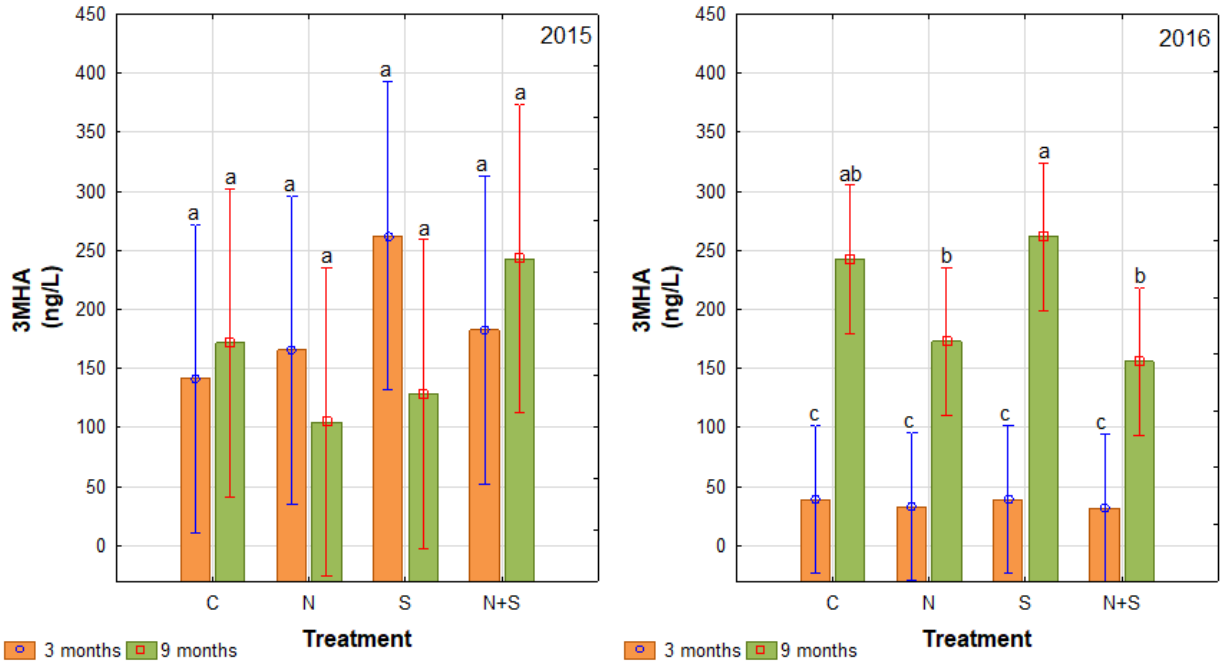


**Figure 4.8** LS means plot illustrating the treatment*time interaction of 3MHA levels present in aged Chenin Blanc wines in 2015 (left) and 2016 (right) for different treatments with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

In 2016, Farm B showed different results were different compared to Farm A in 2015 (Figure 4.8). The 3-month aged wines ranged from 32-39 ng/L and showed no significant increases or decreases between the different treatments. The S treatment had a significant increase of 51-68% compared to the N and N+S treatments after 9 months of ageing. Both nitrogen containing treatments (N and N+S) were the lowest compared to the C and S treatments. During ageing in

2016, all the treatments increased significantly. The N and N+S treatments increased five-fold. This was unexpected, since the 3MHA levels are expected to decrease during bottle ageing due to hydrolysis (forming 3MH) and oxidation (Figure 4.7). Error due to analysis was excluded as a possible source for these results, and all four wine repeats for each treatment gave consistent results. It was previously hypothesised that some precursors stay in the wine after fermentation and they might increase thiol levels in the bottle (Coetzee & Du Toit, 2012).

By comparing 3MHA levels for both Chenin Blanc vintages and wine ages, the 2015 vintage ranged from 105-262 ng/L and in 2016 the levels were 31-262 ng/L. The 2016 3MHA levels at 3-month aged wines were much lower compared to 2015's. In 2016 all the 3MHA levels increased between 396-573%, while in 2015 the C and N+S treatment showed an increase and the N and S treatments decreased. 3MHA levels varied much more in 2015 compared to 2016. The differences in 3MHA levels can be partly ascribed to the different vineyard plots used so a true vintage (vineyard) effect cannot be described.

Significant higher levels of 3MHA are observed for the 3-month aged Sauvignon Blanc wines with S (92%) and N+S (78%) treatments compared to the C in 2015 (Figure 4.9). This trend was still present after 9 months of maturation, but not significant anymore except for S *vs* N. During bottle ageing, all the 3MHA levels significantly decreased for all the treatments of the Sauvignon Blanc wines in 2015. In 2016, no significant differences were observed between the treatments for the 3-month aged Sauvignon Blanc wines (Figure 4.9), although all the treatments were 130-255% higher compared to the C for the 3MHA levels. After 9 months of maturation, the wines from the S and N+S treatments were significantly higher compared to the N treatment, similar to the trend observed in 2015. These S-containing treatments had similar levels of 3MHA. During the bottle aging, the 3MHA levels increased significantly for all treatments.

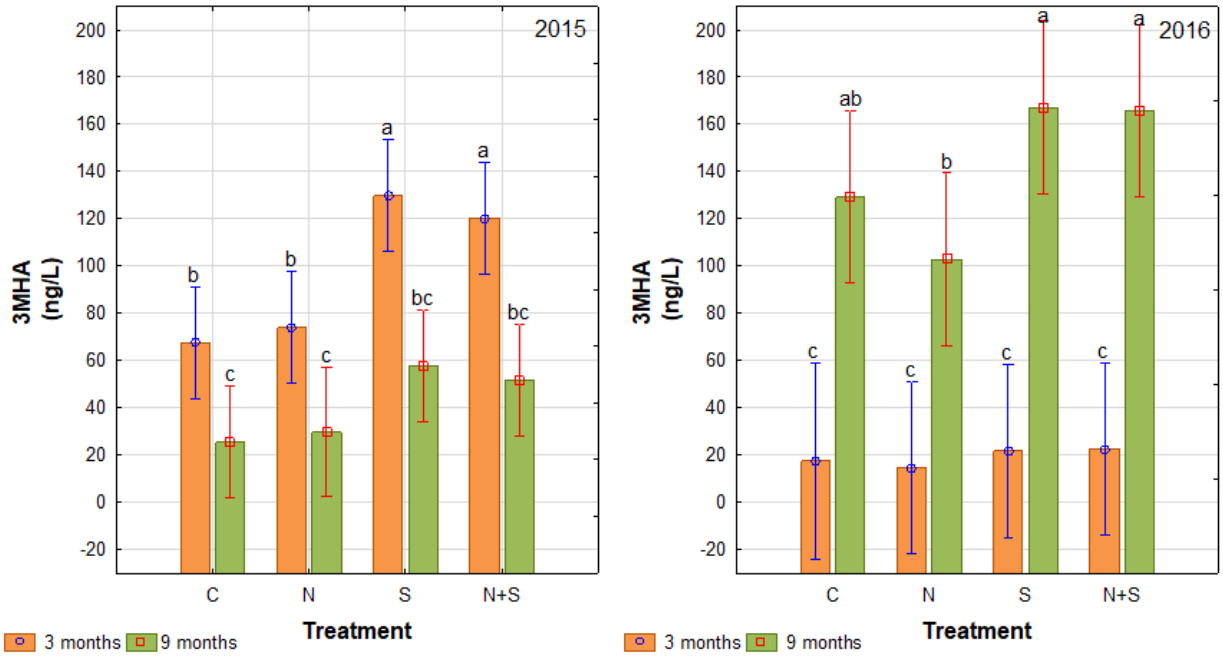


**Figure 4.9** LS means plot illustrating the treatment*time interaction of 3MHA levels present in aged Sauvignon Blanc wines in 2015 (left) and 2016 (right) for different treatments with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

By comparing the two vintages of the same Sauvignon Blanc vineyard, similar increases of all the treatments compared to the C can be observed for both vintages at 3 months. In 2016, increases

occurred for 9-month aged wines with S and N+S treatments compared to 3-month aged wines. Conversely, 2016 showed significantly higher levels of 3MHA at 9 months compared to 3 months, while 2015 showed decreases during maturation. Even though in the case of Sauvignon Blanc the same vineyard has been used over the two years, the results show a vintage effect reflected in both the levels and the evolution of 3MHA during bottle aging.

Only two foliar fertilization studies included 3MHA in their results and focused only on Sauvignon Blanc wines (Lacroux *et al.*, 2008; Juhasz, 2015), while others combined 3MHA and 3MH together and gave the total volatile thiol content. Lacroux *et al.* (2008) performed nitrogen and nitrogen with sulphur foliar fertilization (10 kg/ha urea and 10 kg/ha urea with 5 kg/ha sulphur) twice before véraison to Sauvignon Blanc vines with naturally low nitrogen status. The treatments resulted in significant increased levels of 11% and 133% for 3MHA compared to the control in the wines respectively (Lacroux *et al.*, 2008). The age of the wines was not made known in this publication.

Juhasz (2015) followed a similar foliar application as mentioned in Section 3.2, but found no significant differences between the treatments. Higher levels of 3MHA were obtained with sulphur containing treatments compared to the control, where the sulphur treatment increased the most with 166% and the nitrogen with sulphur treatment increased by 137% (Juhasz, 2015). The age of the wines was also not reported in the publication (Juhasz, 2015). In this research study, similar increases of the N+S treatments compared to the C can be seen with Chenin Blanc (2015) at 9 months and Sauvignon Blanc (2016) at 3 months (Lacroux *et al.*, 2008). Chenin Blanc (3 months-2015 and 9 months-2016) and Sauvignon Blanc (3 and 9 months-2015 and 9 months-2016) showed similar increases of S treatment compared to the C as reported by Juhasz (2015).

Herbst-Johnstone *et al.* (2011) reported 3MHA was the least stable and decreased steadily in concentration during bottle maturation. After 3 months 29-46% of 3MHA was lost and after 4 months the levels continued to decrease until 7% of the initial 3MHA levels were present (Herbst-Johnstone *et al.*, 2011). In this study, all the Sauvignon Blanc wines in 2015 had decreased levels of 3MHA during bottle maturation, while the 3MHA levels increased for both cultivars in 2016, and some treatments in Chenin Blanc in 2015. These increases in 3MH can be due to the conversion of 3MHA to 3MH via hydrolysis (Figure 4.7) (Nikolantonaki *et al.*, 2010). The losses of 3MHA can be due to the hydrolysis producing 3MH or the direct oxidation of 3MHA. An additional reaction, the direct oxidation of 3MH, will accelerate the hydrolysis of 3MHA through the removal of 3MH as a hydrolysis product (Herbst-Johnstone *et al.*, 2011).

***3MH***

3MH levels for Chenin Blanc wines ranged from 422-2025 ng/L (Figure 4.10), while Sauvignon Blanc wines ranged from 161-1039 ng/L (Figure 4.11). The 3-month aged Chenin Blanc wines (2015) showed higher levels for all the treatments compared to the C, while only the S treatment were significantly higher (127%) (Figure 4.10). The 9-month-aged wines showed higher levels compared to the C for all the treatments, where the S treatment (64%) was significantly higher. 3MH levels increased significantly for all the treatments during bottle maturation, where N treatment increased the most with 1404 ng/L and N+S treatment increased the least with 565 ng/L. For both maturation ages the S treatment was significantly higher compared to the C.

In 2016, the 3-month aged wines of Chenin Blanc had higher 3MH levels for all the treatments compared to the C (Figure 4.10). The S treatment again had the highest levels and was 43% higher than the C, while the N+S treatment levels were non-significantly 27% higher. The 9-month aged wines also showed that all the treatments were higher compared to the C for 3MH, but not

significantly. During bottle maturation, all the treatments showed significant increased levels of 3MH. Although different vineyards were used, the ranges of the 3MH levels were almost the same. 3MH levels increased the most during maturation in 2015 with Farm A with the N treatment.

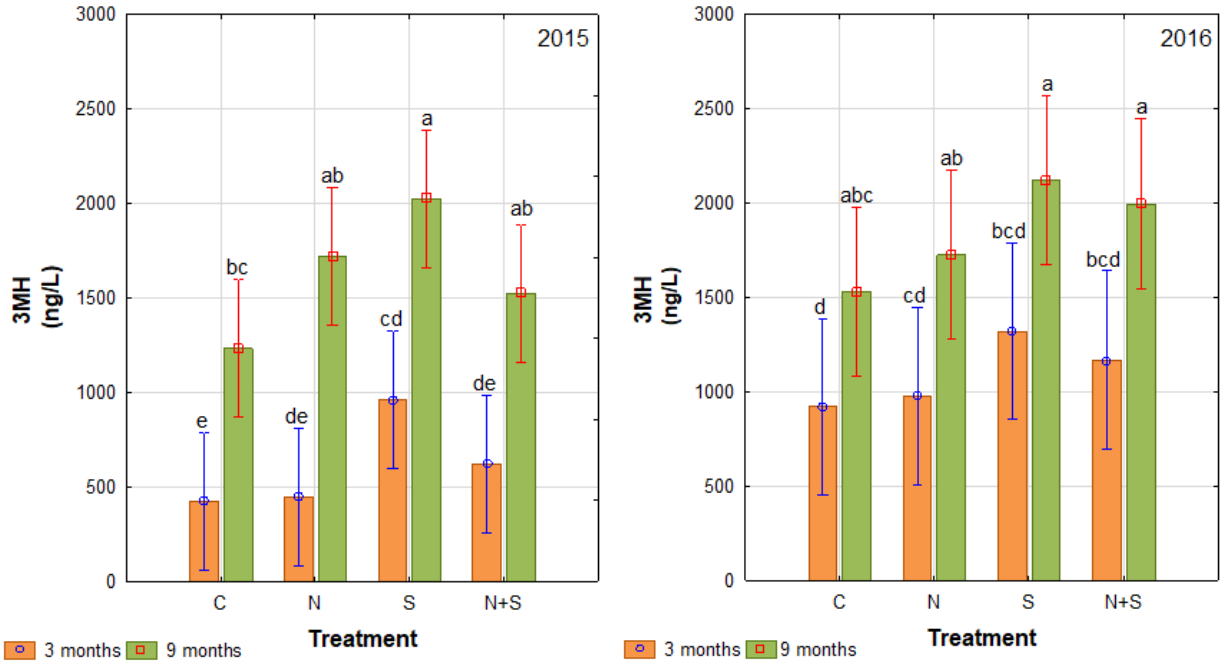


**Figure 4.10** LS means plot illustrating the treatment*time interaction of 3MH levels present in aged Chenin Blanc wines in 2015 (left) and 2016 (right) for different treatments with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

Sauvignon Blanc (2015) 3-month aged wines had higher levels of 3MH for all the treatments compared to the C, although none were significant (Figure 4.11). The S treatment was 175% higher, while N+S treatment was 168% higher than the C. All the 9-month aged wines had higher 3MH levels compared to the C and both the S treatment (223%) and N+S treatment (210%) were significantly higher compared to C. Both wine ageing stages showed similar increases for all the treatments compared to the C. 3MH levels increased by 100-134% during bottle maturation and both S and N+S treatments increased significantly over time during maturation.

The 2016 Sauvignon Blanc wines showed all the treatments had higher 3MH levels compared to the C, although none were significantly higher (Figure 4.11). The N+S (62%) and S (70%) treatment were higher compared to the C with 3-month aged wines. Only the N+S treatment was significantly higher by 65% compared to the C with the 9-month aged wines. During bottle ageing, the 3MH levels increased significantly. From 3 to 9 months, the N+S treatments increased the most by 99 ng/L. The same vineyard was used for both 2015 and 2016 and an obvious vintage effect can be observed with the 3MH levels and increases during maturation. A treatment effect can be observed with the S treatments for both years with the higher 3MH levels.

The 3MH levels of Sauvignon Blanc wines were within reported levels of 26-18000 ng/L (Coetzee & Du Toit, 2012), but were lower if compared to levels found in South African studies (Van Wyngaard, 2013). The Chenin Blanc wines had 3MH levels within those reported by Wilson (2017) and were above the sensory threshold of 60 ng/L (Tominaga *et al.*, 1998a).

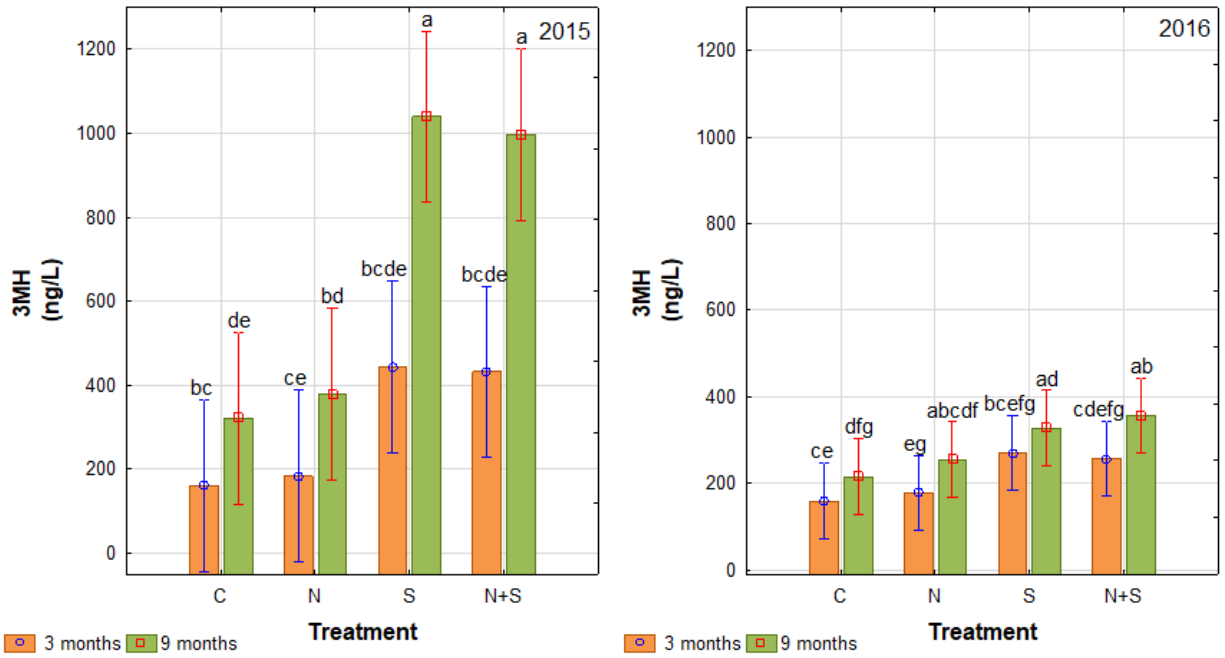


**Figure 4.11** LS means plot illustrating the treatment*time interaction of 3MH present in aged Sauvignon Blanc wines in 2015 (left) and 2016 (right) for different treatments with significant letters from LSD post-hoc test. Vertical bars denote the 95% confidence intervals.

Few foliar fertilization research studies included volatile thiols in their research and were done only on Sauvignon Blanc (Lacroux *et al.*, 2008; Helwi *et al.*, 2014; Juhasz, 2015). Lacroux *et al.* (2008) performed foliar nitrogen and nitrogen with sulphur fertilization (10 kg/ha urea and 10 kg/ha urea with 5 kg/ha sulphur) twice before véraison to Sauvignon Blanc vines with naturally low nitrogen status. The N and N+S treatments resulted in significant increases of 64% occurred with 3MH compared to the C in the wines. The age of the wines was not made known in this particular research study (Lacroux *et al.*, 2008).

In the current research study, 3MH levels increased for all the treatments compared to the C, although not all were significant increases. For all the wines aged at 3 months, the S treatment had the highest levels compared to the C, except for Chenin Blanc (2016) where N+S were the highest. At 9 months, all the S treatments had the highest levels compared to the C, except for Sauvignon Blanc (2016) where the N+S treatments were the highest. During maturation all the treatments increased significantly for Chenin Blanc (2015), and Sauvignon Blanc (2015 and 2016). These findings agreed with what has been found in literature. S treatments increased the most compared to the C has also been shown by Helwi *et al.* (2014), while Lacroux *et al.* (2008) and Juhasz (2015) have reported that N+S treatments had the most increases. In the current study, the 3MH levels in Chenin Blanc levels of 3MH were higher than those of Sauvignon Blanc (2016) and Helwi *et al.* (2014) also showed similar 3MH levels.

Helwi *et al.* (2014) applied soil fertilisation (50 kg/ha-two applications), soil (100 kg/ha-two applications), and foliar N fertilisation (15 kg/ha-three applications) to Sauvignon Blanc vines. The foliar nitrogen resulted in 100% increases of 3MH compared to the C and similar significant increases occurred for the other treatments. Juhasz (2015) followed a similar method and found no significant differences were observed between the treatments. All the treatments resulted in increased levels of 3MH compared to the control (Juhasz, 2015). The nitrogen with sulphur treatment had the highest increased levels of 212%, while sulphur was 156% higher compared to the C. The age of the wines was also not made known in this research study (Juhasz, 2015).

Herbst-Johnstone *et al.* (2011) reported that during bottle maturation 3MH levels increased during the first three months and increased more in the next four months in New Zealand Sauvignon Blanc. In the current study, all the Chenin Blanc and Sauvignon Blanc wines' levels increased from 3 to 9 months during maturation. The increase was possibly due to the hydrolysis of 3MHA to 3MH, being derived from thiol precursors present in the wines or by the breakdown of 3MH disulphide present in the wines (Capone *et al.*, 2010; Sarrazin *et al.*, 2010). QA23 yeast has been proven to have the highest capability to convert 3MHA to 3MH (Swiegers *et al.*, 2006). One of the hypotheses put forward in the literature is that elemental sulphur can be converted to $H_2S$ that can serve as a precursor for volatile thiols with C6 compounds (Hänsch *et al.*, 2006).

#### 4.3.1.4 Overall volatile content

Volatile thiols and major volatiles have been included in the overall volatile content; while methoxypyrazines were excluded due to the limited samples analysed. Additional PCA plots can be viewed in the Appendix B (Figure B.3, B.4, B.5, B.6, and B.7).

In the PCA a 60.5% explained variance (R2x [1] and R2x [2]) was represented by the first two components of the overall volatile composition of Chenin Blanc (Figure 4.12). A vintage effect can be seen with the separation along mostly component 2 and can be explained simply by the fact that the wines for the two vintages were made from grapes from different vineyards. A trend can be observed for the separation according to wine age mostly along component one. In this case, there is a more evident separation according to age in 2015 for all the samples, except for an outlier in the 2016 vintage.

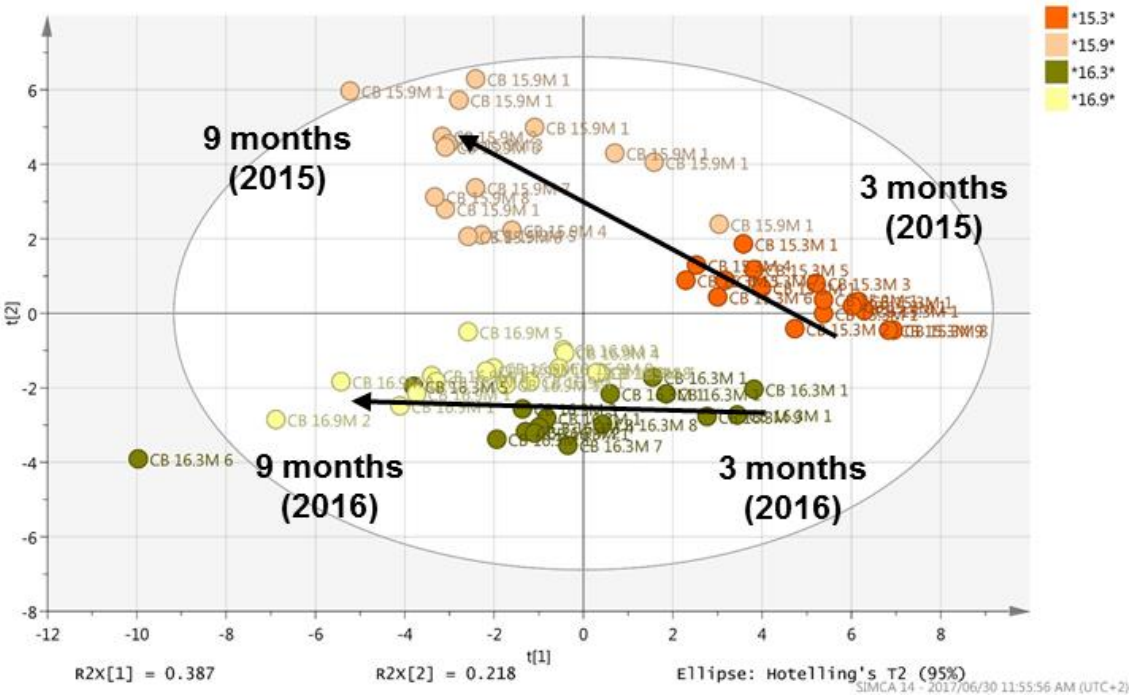


**Figure 4.12** PCA-X score plot (PC1 vs PC2) distribution of the volatile composition of 3 and 9-month aged Chenin Blanc wines of 2015 and 2016. Each sample is coded cultivar (Chenin Blanc), vintage (2015/2016), age (3/9 months), and sample number (1-16).

According to this representation, for 2015, the 3- and 9-month aged wines were more different from each other, while in 2016 the wines were more similar. Looking at the chemistry analysis, the 9-month aged Chenin Blanc wines in 2015 had higher volatile thiols and major volatiles

compared to the 3-month aged wines (Figure 4.2, 4.3, 4.8, and 4.10). Meanwhile, for 2016, even though the 9-month aged wines had higher volatile thiols levels compared to the 3-month aged wines, but these differences were not large enough to drive a separation in the PCA representation. In the ANOVA analysis, no outlier was shown and therefore it was not excluded in the PCA's.

In the figures below (Figure 4.13, 4.14, and 4.15), the treatments of the Chenin Blanc wines are colour-coded (C1-C4:1-4, N1-N4:6-8, S1-S4:9-12, and N+S1-N+S4:13-16). When comparing the 3-month aged Chenin Blanc wines from 2015 and 2016, as expected, the main effect observed was a vineyard (vintage) effect (Figure 4.13). Even though no obvious treatment effect was noted, in 2015 the grouping of all wines was closer compared to 2016. This indicates that the wines for 2015 were similar in volatile content while the wines for 2016 differed much more.

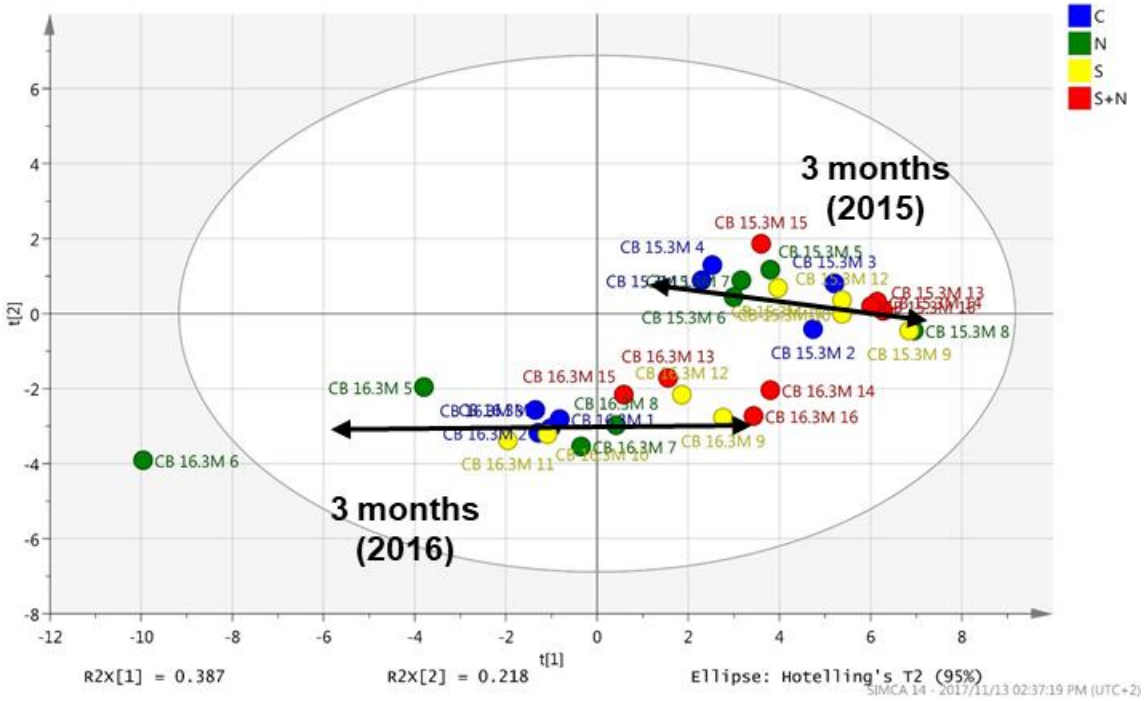


**Figure 4.13** PCA-X score plot (PC1 vs PC2) distribution of the volatile composition of 3-month aged Chenin Blanc wines of 2015 and 2016. Each sample is coded cultivar (Chenin Blanc), vintage (2015/2016), age (3 months), and sample number (1-16).

The 9-month aged Chenin Blanc wines in 2015 and 2016 had 61.5% explained variance for the first two components and showed a clear vineyard (vintage) effect in the second dimension (Figure 4.14). In 2016, the wines were more closely grouped together compared to 2015, and the S and N+S treatment wines formed tight groups in 2016. The 2016 wines were similar in volatile content and differed very little, while in 2015 the wines were much more spread out and indicate that the wines varied in volatile content.

Generally, the total overall volatile content in Chenin Blanc wines had a vineyard (vineyard) effect over the second component and age effect over the first component (Figure 4.15) with clear separation when all the wines and ages were compared. No evident grouping can be observed with the treatments.

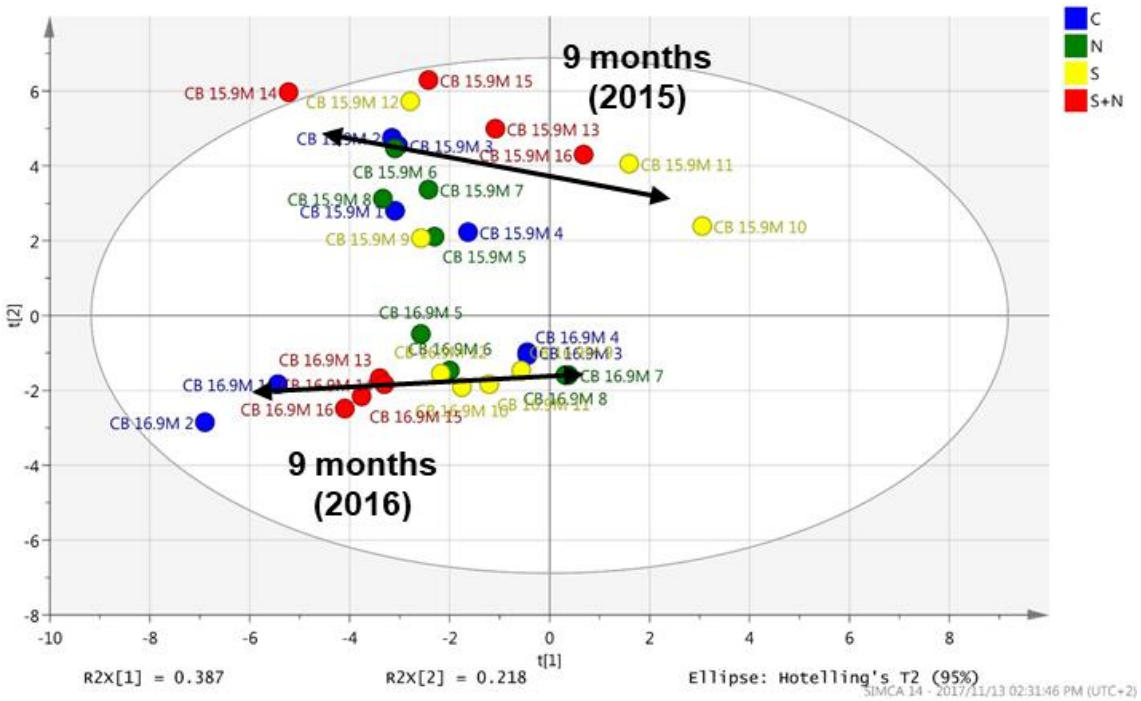


**Figure 4.14** PCA-X score plot (PC1 vs PC2) distribution of the volatile composition of 9-month aged Chenin Blanc wines of 2015 and 2016. Each sample is coded cultivar (Chenin Blanc), vintage (2015/2016), age (9 months), and sample number (1-16).

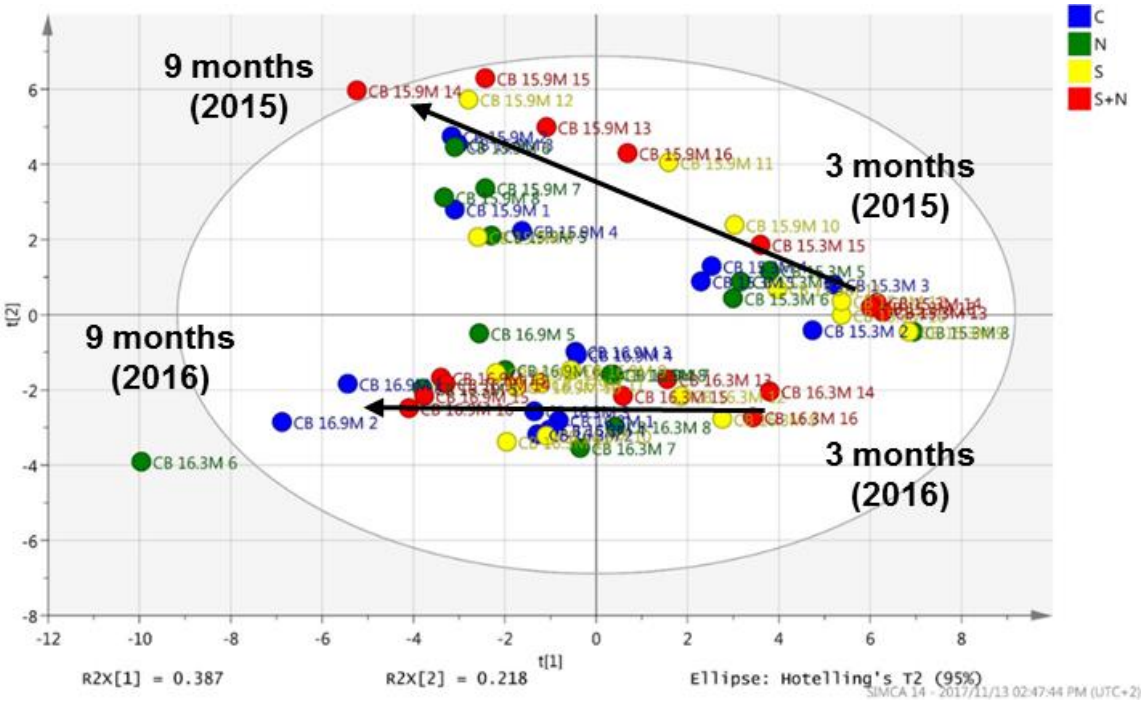


**Figure 4.15** PCA-X scatter plot (PC1 vs PC2) distribution of the volatile composition of 3 and 9-month aged Chenin Blanc wines of 2015 and 2016. Each sample is coded cultivar (Chenin Blanc), vintage (2015/2016), age (3/9 months), and sample number (1-16).

Including vintages and ages for Sauvignon Blanc wines, the explained variance of the volatile composition was 64.6% for the first two components (Figure 4.16). A similar vintage effect as seen with Chenin Blanc can be observed with Sauvignon Blanc. In this case, though, the wines were made from grapes from the same vineyard; therefore, this is a true vintage effect. The grouping according to vintage could be observed mostly along the first component, while the wine age effect was observed along the second component.

In Figure 4.16, a greater separation with ageing occurred in 2015, while in 2016 the 3 and 9-months aged wines were grouped closer, indicating more similar volatile composition (less change with age). In 2015, the 9-month aged wines had higher volatile thiols compared to the 3-month aged wines, and this drove the separation between the wines (Figure 4.4, 4.5, 4.9, and 4.11). Meanwhile, in 2016 the 9-month aged wines had higher volatile thiol levels, the separation was less between the groups, and the wines were more similar (Figure 4.4, 4.5, 4.9, and 4.11).

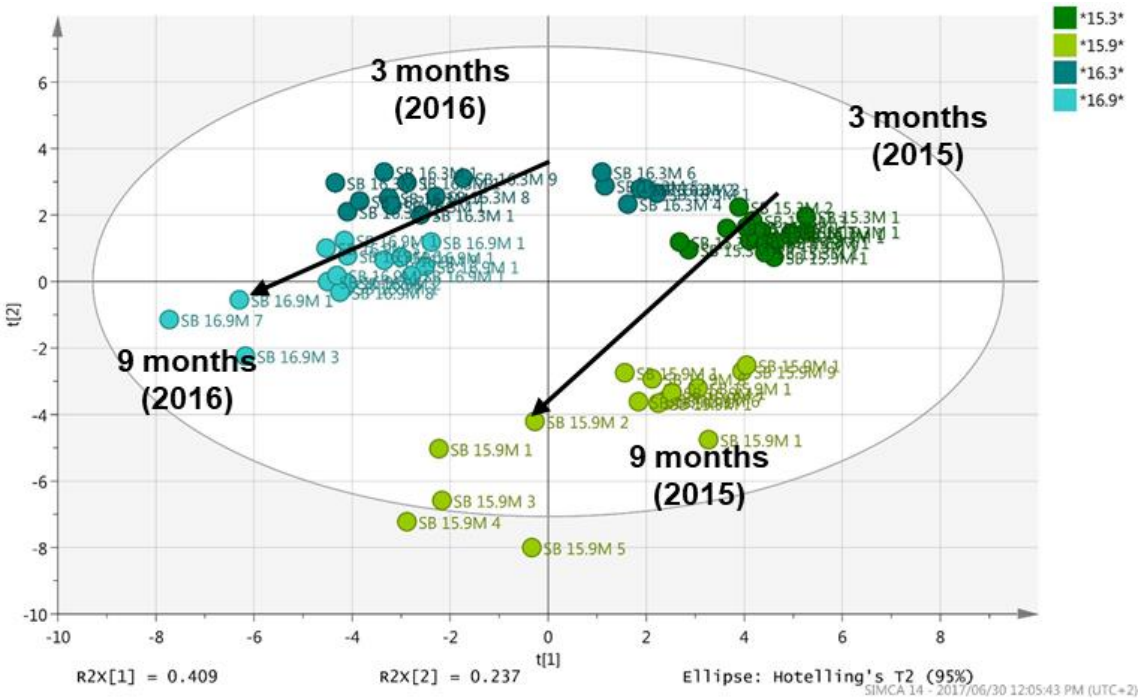


**Figure 4.16** PCA-X score plot (PC1 vs PC2) distribution of the volatile composition of 3 and 9-month aged Sauvignon Blanc wines of 2015 and 2016. Each sample is coded cultivar (Sauvignon Blanc), vintage (2015/2016), age (3/9 months), and sample number (1-16).

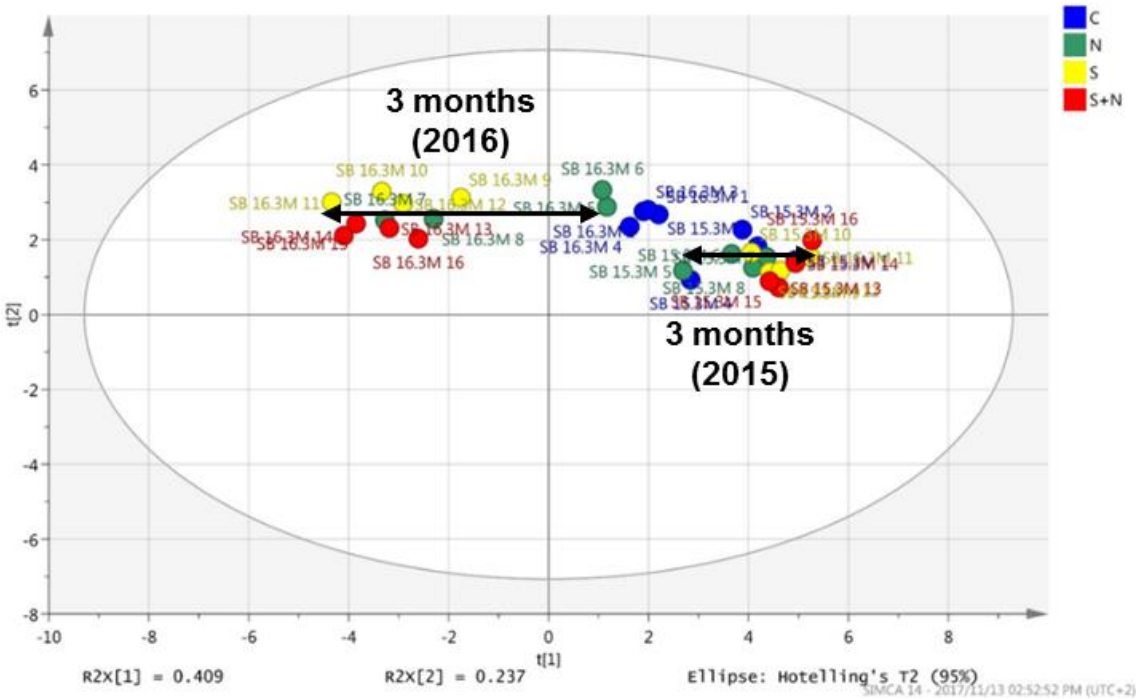


**Figure 4.17** PCA-X score plot (PC1 vs PC2) distribution of the volatile composition of 3-month aged Sauvignon Blanc wines of 2015 and 2016. Each sample is coded cultivar (Sauvignon Blanc), vintage (2015/2016), age (3 months), and sample number (1-16).

Sauvignon Blanc wines had similar effects and trends as found with Chenin Blanc wines stated previously. The effect of the different foliar fertilization treatments is illustrated colour-coded in Figure 4.17. Even though grouping can be seen with C, S, and N+S treatments, the differences between the groups are small.

The 9-month aged Sauvignon Blanc wines in 2015 and 2016 had 64% explained variance for the first two components (Figure 4.18). The 2015 wines were more spread out, while the 2016 wines were grouped closer together. This indicated that the 2015 differed more in volatile content from each other, while in 2016 the volatile content of the wines was similar after maturation. When all Sauvignon Blanc wines were represented, the vintage and age effects were the most obvious (Figure 4.19). Even though some grouping according to treatment was observed, the treatment effect was not evident.

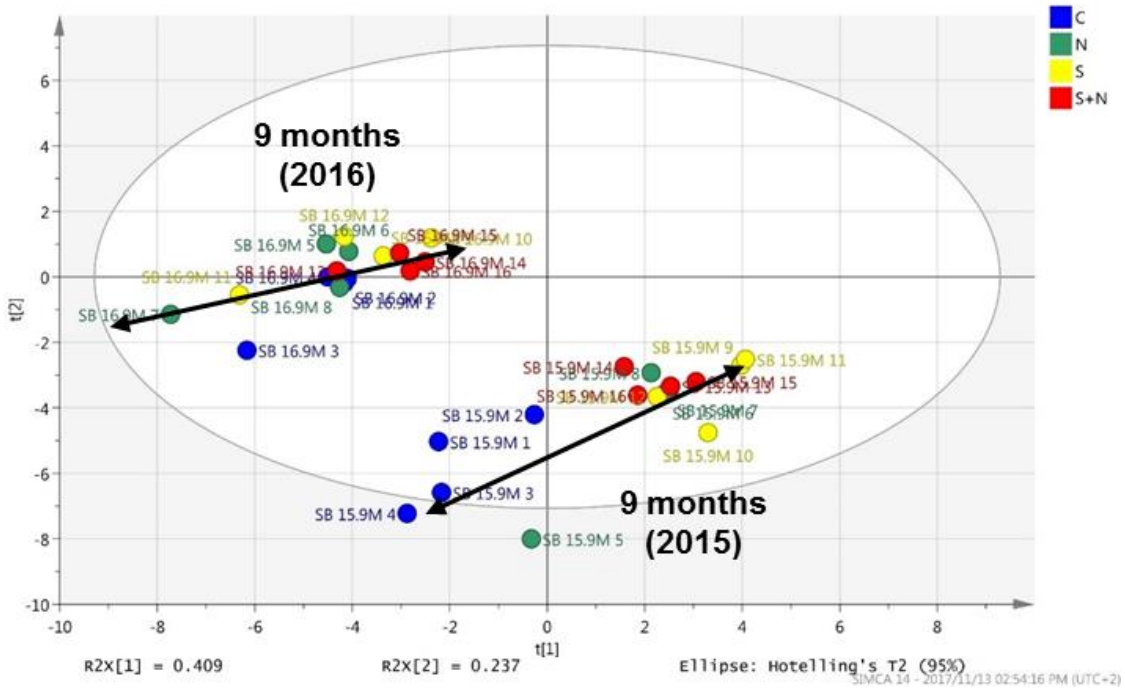


**Figure 4.18** PCA-X score plot (PC1 vs PC2) of the volatile composition of 9-month aged Sauvignon Blanc wines of 2015 and 2016. Each sample is coded cultivar (Sauvignon Blanc), vintage (2015/2016), age (9 months), and sample number (1-16).

In conclusion, for both cultivars a vintage and age effect occurred in 2015 and 2016. A clear grouping for 3-month aged wines occurred in 2015 (Chenin Blanc) and 2016 (Sauvignon Blanc) with the C, while 9-month aged wines showed closed groupings with the N+S treatments in 2016.

Hypothetically, a treatment effect should have occurred, but the results show that the wines were very similar in volatile content between the treatments. The volatile content gradually changed during bottle maturation and showed greater or smaller increases or decreases with both cultivars. For both cultivars, the 9-month aged wines had higher volatile content (major volatiles and volatile thiols) compared to the 3-month aged wines and a clearer separation occurred when the levels were more different.

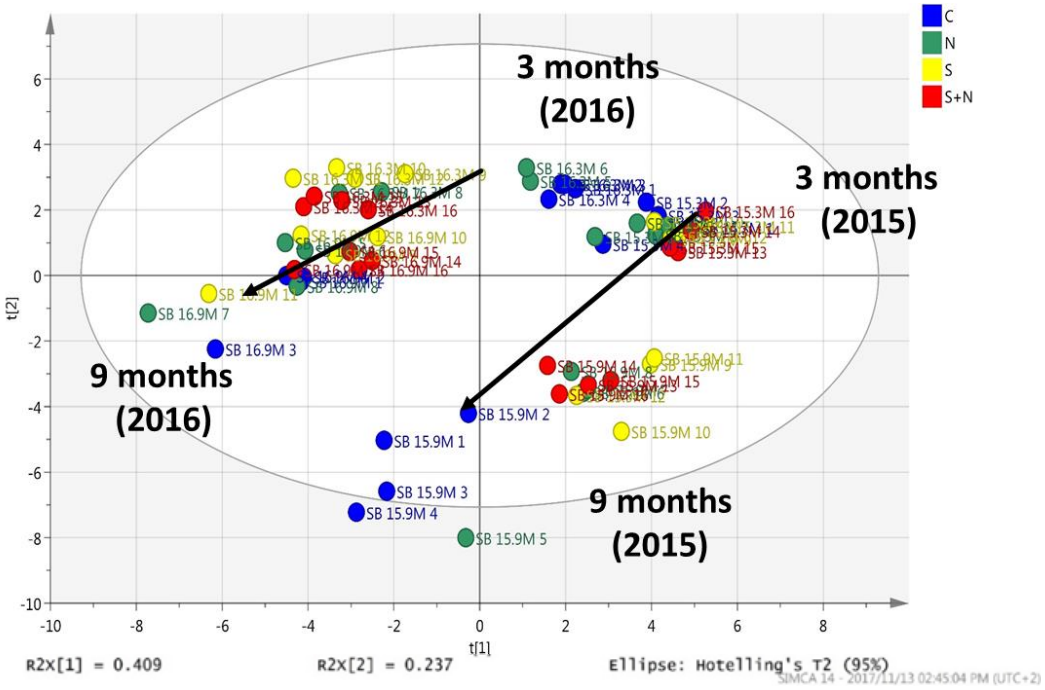


**Figure 4.19** PCA-X plot (PC1 vs PC2) distribution of the volatile composition of 3 and 9-month aged Sauvignon Blanc wines of 2015 and 2016. Each sample is coded cultivar (Sauvignon Blanc), vintage (2015/2016), age (3/9 months), and sample number (1-16).

### 4.3.2 Sensory Results

#### 4.3.2.1 Treatment effect

The effect of the treatments on the grouping and aromas are presented in this section. The results presented below can be considered illustrative for all the data obtained during this research project. The only wines discussed in detail are Chenin Blanc 2016 aged for 3 and 9 months, for they showed the best results. The scatterplots, dendrograms, and frequency tables of the other wines can be viewed in the Appendix B (Figure B.8, B.9, B.10, B.11, B.12, and B.13 and Table B.4 and B.5).

The dendrogram in Figure 4.20 shows the results generated by AHC analysis for Chenin Blanc 2016 wines aged for 3 months. The wines were not grouped according to treatments in most cases. For instance, the repeats for the N+S were in separate groups. This suggests that the wines from different foliar fertilization treatments were very similar. The scatterplots also show that the repeats of the samples were not grouped close together. The N (N1 with N3 and N2 with N4 grouped together) treatments showed a closer grouping compared to all the treatments. Also, S2, S3, and S4 were grouped closer together. This finding didn't correspond with the overall volatile content representation for these wines (Fig 4.12). This is not surprising, since only a few selected volatiles have been analyzed in this study.

Looking at the corresponding attributes, the C and N wines showed similar high frequencies of 'pineapple', 'passion fruit', and 'apple' aromas, while N also showed 'sweet associated' aromas (Table B.4). The S and N+S treatment wines were described with 'pineapple', 'passion fruit', 'apple', and 'sweet associated', but also had notes such as 'sulphur', 'cooked vegetables', and 'herbaceous'. The 'tropical fruit' aromas were present in all the treatments, but only the S wines had some negative off-flavour aromas.

The aromas can be linked to certain volatile compounds such as esters ('banana', 'honey', 'pear', 'apple', 'floral', 'fruity', and 'pineapple' aromas (Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012)), volatile thiols ('passion fruit', 'guava', 'grapefruit' 'fresh', 'herbaceous', 'sulphur', and 'cooked vegetables' aromas) (Coetzee, 2014; Wilson, 2017)). The choice of attributes and their frequencies could be considered an indication of the similarities between all the wines, regardless of the treatment applied. This can be an explanation for the lack of grouping according to treatment indicated also from the dendrogram. Since the wines were described similarly, the treatment effect supported by chemistry findings was not strong enough to lead to evident sensory groupings according to treatment.

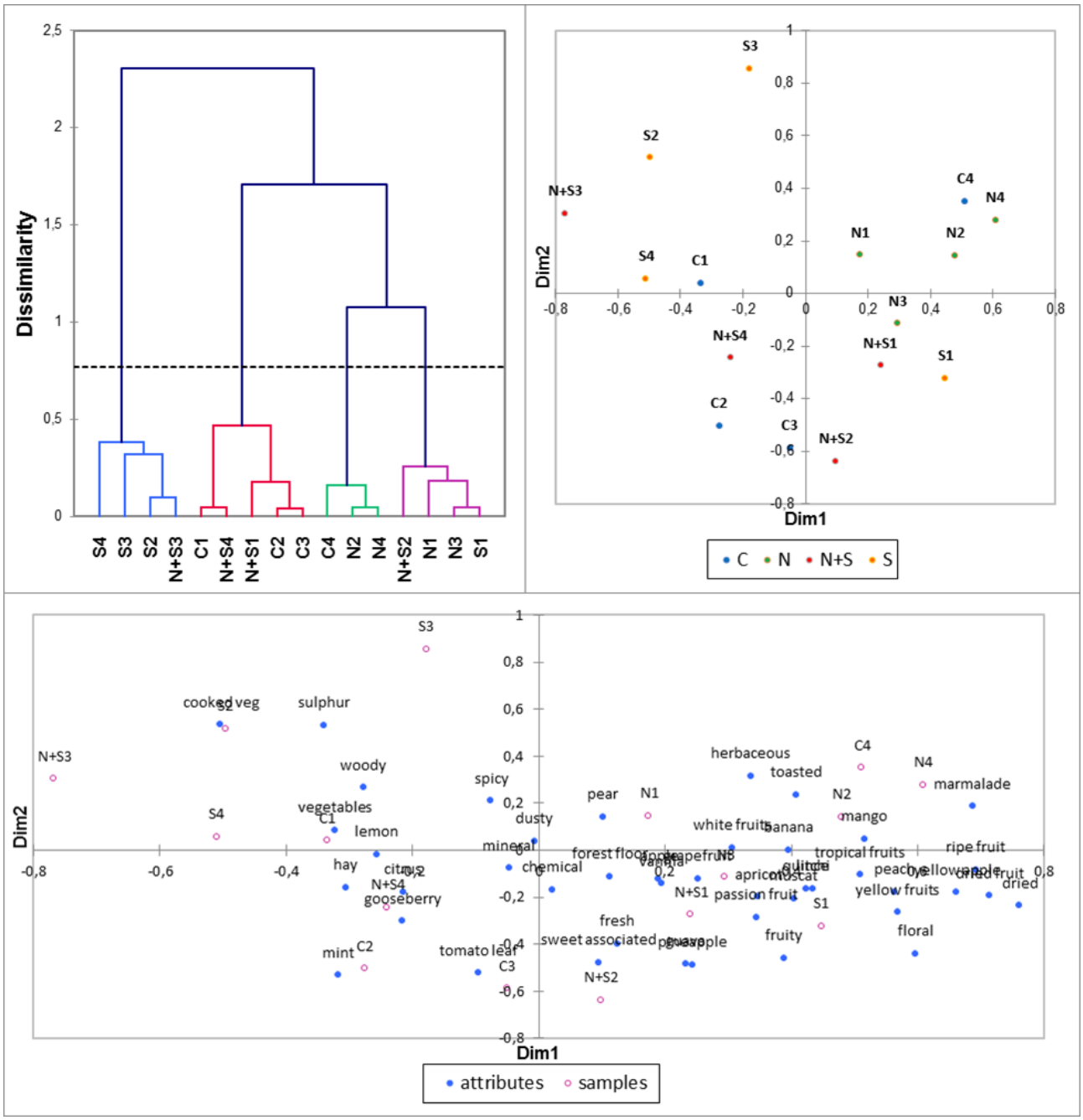


**Figure 4.20** A dendrogram (top left), scatter plot (top right), and bi-plot (below) illustrating the sensory free sorting of 3-month aged Chenin Blanc wines of 2016.

Chenin Blanc 2016 wines matured for 9 months showed a similar lack of grouping according to treatment as with the 3-months aged wines (Figure 4.21). Only two repeats of all the treatments were grouped together: C (C1 and C2), S (S2 and S3), N (N2 and N4) and N+S (N+S1 and N+S3). N1 treatment wines were missing during sensory evaluation, and therefore was not included in the analysis. N+S and S repeats were considered similar as shown by the lowest dissimilarity.

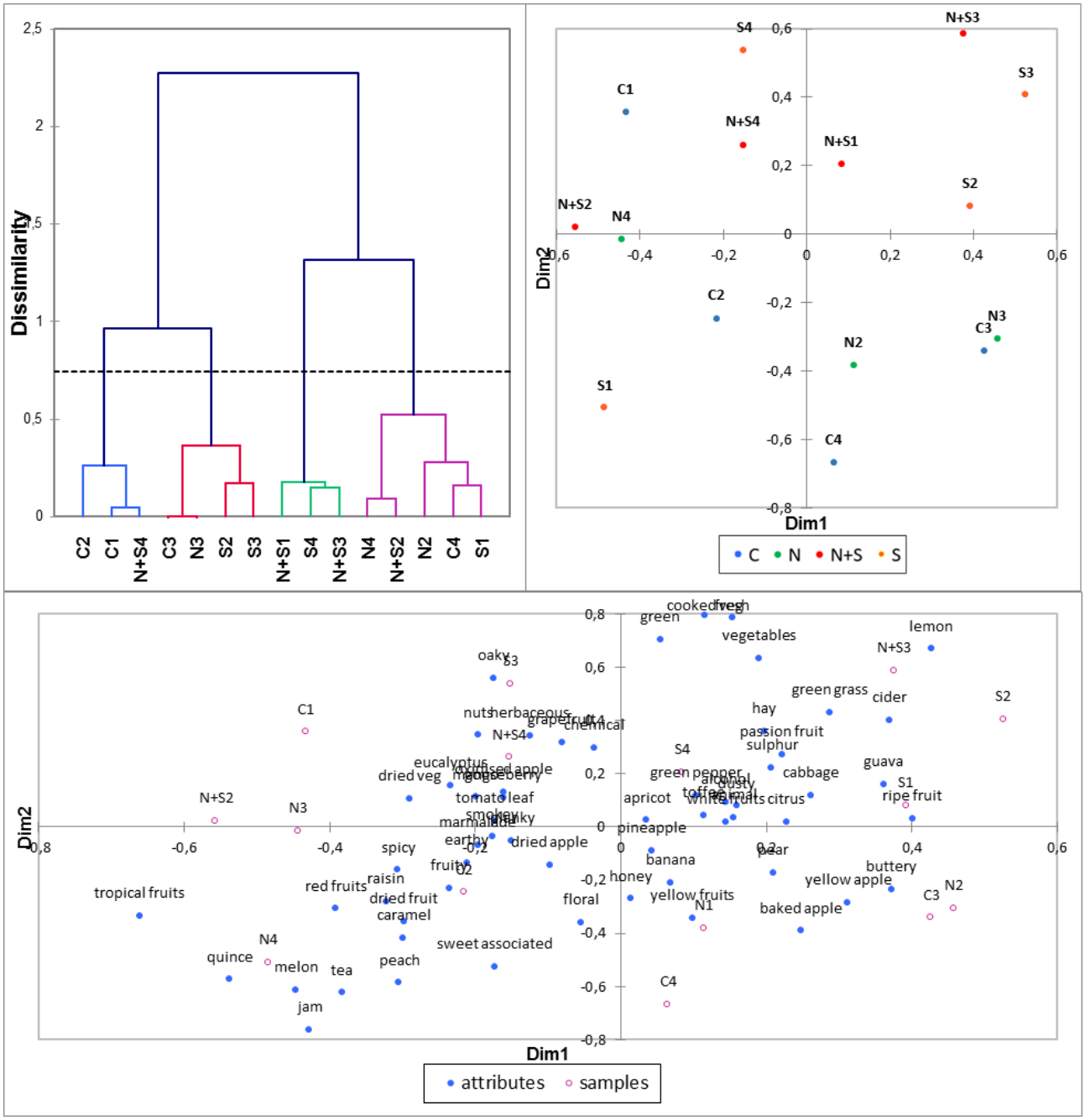


**Figure 4.21** A dendrogram (top left), scatter plot (top right), and bi-plot (below) illustrating the sensory free sorting of 9-month aged Chenin Blanc wines of 2016.

The total frequencies for 9-month aged Chenin Blanc 2016 wines showed that C and N had high citing for ‘tropical fruits’, ‘honey’, ‘caramel’, and ‘dried fruits’ for some of the repeats (Appendix B-Table B.4). While S and N+S treatments were associated with ‘tropical fruits’, ‘dried fruit’, ‘herbaceous’, and ‘cooked vegetables’ aromas. ‘Pineapple’ was the highest cited aroma, while ‘grapefruit’, ‘dried fruits’, ‘passion fruit’, and ‘guava’ aromas were present in all the treatments. Only S and N+S treatments had ‘herbaceous’ and ‘cooked vegetable’ aromas, but it seemed that

their impact and frequency was not high enough to result in a separation of these wines from the rest in the sorting exercise. These wines appeared to be driven by volatile thiol, ester, and reductive sulphur compounds.

All the other 3 and 9-month aged wines of Chenin Blanc in 2015 and Sauvignon Blanc in 2015 and 2016 were not discussed further in detail in this section. As proven in the previous section with the aged Chenin Blanc wines of 2016, no clear groupings of treatment repeats were found with the free sorting (Figure 4.20 and 4.21). According to the sorting results, all the wines were very similar and therefore the judges could not distinguish between them according to treatment. The scatterplots also showed that the repeats of the samples were not grouped close together. See the Appendix B section for all the scatterplots, dendrograms, and frequency tables of these wines the Appendix B (Figure B.8, B.9, B.10, B.11, B.12, and B.13 and Table B.4 and B.5).

The sulphur treated wines for both cultivars have more prominent 'green' associated aromas compared to the other treatments. These aromas could be associated by the reductive sulphur compounds. A similar foliar fertilization study reported that with quantitative descriptive analyse only the cooked vegetable aroma was significantly higher in the N+S treatment compared to the C (Juhasz, 2015). Lacroux *et al.* (2008) produced wines from foliar trials and the N (10 kg/ha urea-twice before véraison) with S application (5 kg/ha-twice before véraison) wines resulted in significantly higher aroma intensity (23 experts, aroma intensity rated on a scale from 0 to 5), while the N wines had decreased intensities (Lacroux *et al.*, 2008). Geffroy *et al.* (2016a) reported that the 10 and 20 kg/ha N and 5 and 10 kg/ha S foliar applications resulted in wines with more intense and increased notes of 'grapefruit' and 'tropical fruit', while no undesirable sulphur-related notes were perceived. Both research studies did not report the sensory method used for judging the wines.

#### 4.3.2.2 Wine age effect

A large group of aroma descriptors were generated from the free sorting method for the different wines at different maturation stages. Considering the results presented in the previous section (Section 4.3.2.1) and the lack of grouping of the samples according to treatment, the top ten descriptors of the wines were combined regardless of the treatment. Wordle® word clouds were generated to compare the sensory expression of the wines according to vintage and age and to see how the wines evolved during bottle maturation from a sensory perception point of view. The data presented here is a compilation of the results from Table B.5 (Appendix B).

Even though the different Chenin Blanc vineyards were used in 2015 and 2016, similarities were found with the aroma descriptors of the wines (Figure 4.22 and Appendix B-Table B.5). 'Pineapple' (8.6%), 'guava' (8.2%), 'passion fruit' (7.7%), and 'grapefruit' (7.7%) were the most cited descriptors for 3-month aged Chenin Blanc (2015), while in 2016 'pineapple' (9.1%) and 'passion fruit' (6.7%) were most prominent. The 9-month aged wines had 'pineapple' (8.1%), 'grapefruit' (7.5%), 'guava' (6.4%), and 'tropical fruits' (5.2%) in 2015, while in 2016 'pineapple' (12.2%), 'grapefruit' (6.4%), 'peach' (5.5%), and 'passion fruit' (5.5%) aromas were the most prominent.

During bottle maturation (2015), 'pineapple' and 'passion fruit' aroma intensities decreased, while 'guava', 'grapefruit', 'peach', 'lemon', 'yellow fruits', and 'herbaceous' increased. This could be due to the increases of volatile thiols and esters (ethyl butyrate, isoamyl acetate, ethyl hexanoate, ethyl lactate, ethyl caprylate, ethyl caprate, diethyl succinate, and 2-phenylethyl acetate) during

maturation (Figure 4.2, 4.3, 4.8, and 4.10). Although ethyl acetate levels increased, the overall 'pineapple' aroma intensities decreased. In 2016, the wine aromas such as 'pineapple', 'caramel', 'peach' 'grapefruit', 'guava', 'dried fruit', 'honey', and 'banana' notes increased, while 'passion fruit', 'sweet associated', 'yellow apple', and 'floral' notes decreased. Volatile thiols increased while some esters increased or decreased during maturation in 2016 (Figure 4.2, 4.3, 4.8, and 4.10).

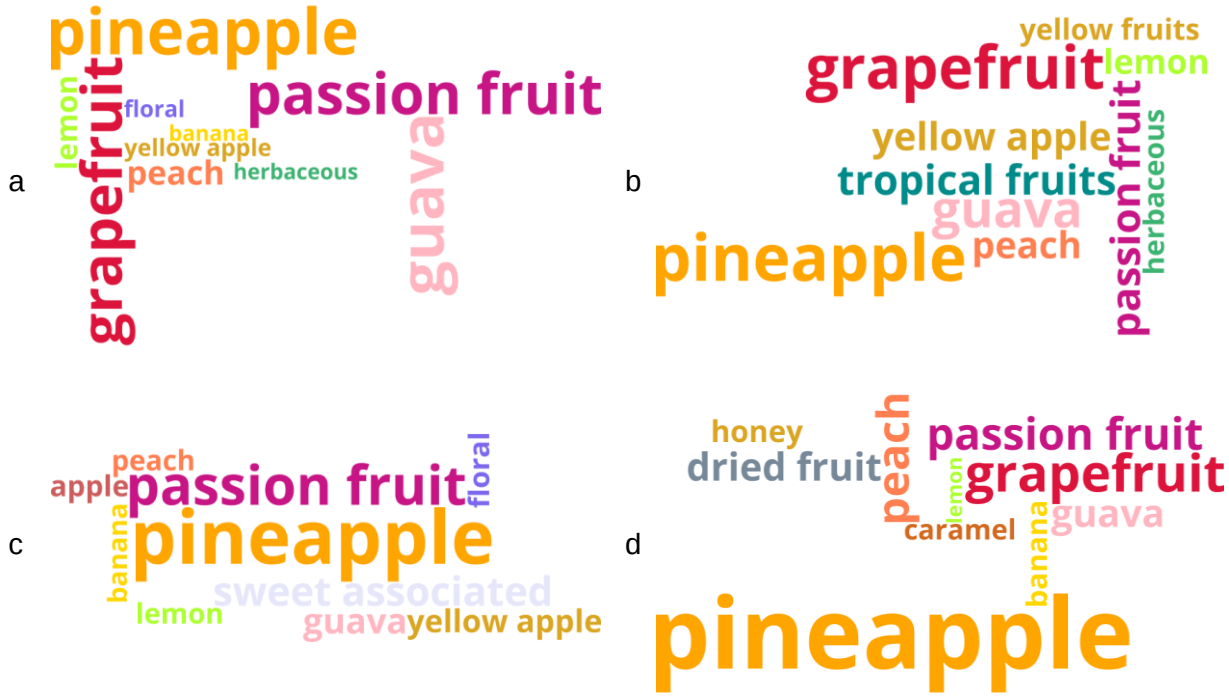


**Figure 4.22** Top ten cited aroma attributes represented in percentage of each wine. (Chenin Blanc 2015 3 months (a), Chenin Blanc 2015 9 months (b), Chenin Blanc 2016 3 months (c), and Chenin Blanc 2016 9 months (d)).

The Chenin Blanc wines were like FF and FFUW wine aroma styles. Certain volatile compounds can be linked to the aromas. Esters are associated with 'banana', 'honey', 'pear', 'apple', 'floral', 'fruity', and 'pineapple' aromas (Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012), volatile thiols are associated with 'passion fruit', 'guava', 'grapefruit', 'fresh', and 'herbaceous' aromas (Wilson, 2017), while monoterpenes are linked to 'floral' and 'citrus' aromas. RRUW wines are associated with volatile compounds such as esters, monoterpenes, and volatile thiols (Bester, 2011; Hanekom, 2012; Lawrence, 2012; Van Antwerpen, 2012; Botha, 2015; Wilson, 2017).

The Sauvignon Blanc wines had prominent aromas like a 'tropical style' Sauvignon Blanc (Figure 4.23 and Appendix B- Table B.5). In 2015, the 3-month aged wines had prominent 'passion fruit' (10.3%), 'pineapple' (9.7%), 'grapefruit' (7.3%), and 'guava' (6.3%) aromas, while in 2016 'pineapple' (11.7%), 'yellow apple' (5%), 'lemon' (4.8%), and 'passion fruit' (4.4%) aromas were prominent. The 9-month aged wines showed the highest levels of 'pineapple' for both vintages. In 2015, 'tropical fruits' (7.65%), 'lemon' (5.4%), 'passion fruit' (4.8%), and 'guava' (4.6%) aromas had the highest citations, while in 2016, 'passion fruit' (7.3%), 'grapefruit' (6.7%), 'dried fruit' (5.3%), and 'peach' (5%) aromas were the most cited.

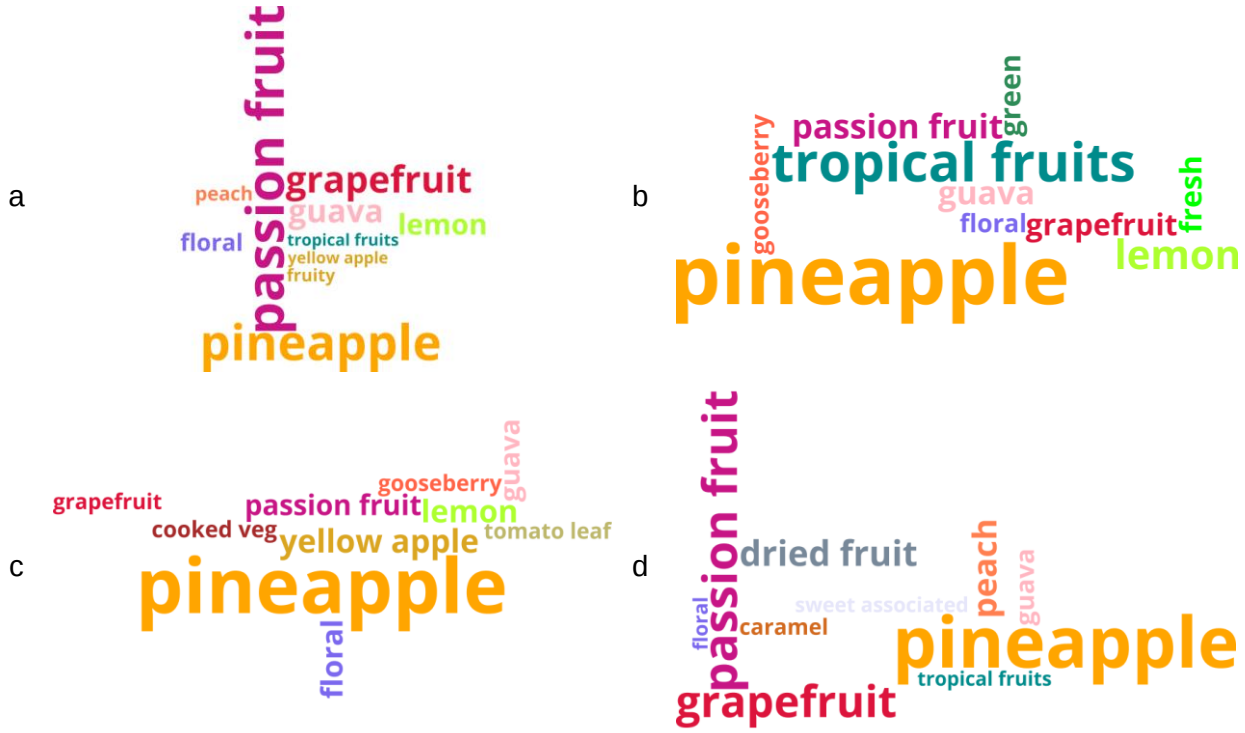


**Figure 4.23** Top ten cited aroma attributes represented in percentage of each wine. (Sauvignon Blanc 2015 3 months (a), Sauvignon Blanc 2015 9 months (b), Sauvignon Blanc 2016 3 months (c), and Sauvignon Blanc 2016 9 months (d)).

During bottle maturation in 2015, 'tropical fruits' increased the most, while 'pineapple' had the most citations (Appendix B-Table B.5). Aromas such as 'grapefruit', 'guava', and 'floral' decreased, while 'passion fruit' decreased by half. This can be due to the conversion of 3MHA to 3MH during maturation. In 2016, the 'pineapple' percentage decreased but it was still the most prominent aroma attribute. Aromas such as 'yellow apple', 'lemon', 'floral', 'guava', 'cooked vegetables', and 'tomato leaf' decreased for Sauvignon Blanc during maturation. Whereas, 'passion fruit', 'grapefruit', 'dried fruit', 'peach', 'caramel', and 'sweet associated' aromas increased during maturation. In 2016, the volatile thiols increased, while some ester levels increased or decreased during bottle maturation (Figure 4.4, 4.5, 4.9, and 4.11). The Sauvignon Blanc wine in 2015 showed the highest levels of 'pineapple', 'tropical fruits', and 'passion fruit' aromas. These wines could be classified as being made in a 'tropical/fruity' style wine. This style includes aromas such as 'gooseberry', 'grapefruit', 'pineapple', and 'passion fruit' (Treurnicht, 2011). Various volatile compounds such as esters, higher alcohols, monoterpenes, and volatile thiols are associated with these specific aromas of the aged wines (Van Wyngaard, 2013).

## 4.4 Conclusions

The formation of volatile compounds during grape ripening, alcoholic fermentation, and wines maturation is important for the aromatic expression of the final wine. Although various research studies have been done to link fertilization to chemical compounds, none have focused on foliar fertilization under South African climate conditions. This research chapter focused on the effect of various fertilization treatments on the volatile content and aromatic expression of the different wines. The compounds contributing to the aroma of Chenin Blanc and Sauvignon Blanc are complex and numerous, but only selected volatile compounds were analysed in this study.

The present study supports findings from Helwi *et al.* (2017), Juhasz (2015), Lacroux *et al.* (2008), and Herbst-Johnstone *et al.* (2011) These volatile compounds differed in their reactions to the treatments due to the metabolic formation of each volatile compound group. No clear tendencies could be observed in terms of the treatments on the total major volatile content, esters, alcohols, fatty acids, and methoxypyrazines.

Major volatiles such as esters, fatty acids and higher alcohols were affected the most by S-containing treatments (S or N+S), for both cultivars. As expected, the overall ester content increased over time for most treatments for both cultivars (Selli *et al.*, 2006). Methoxypyrazine, IBMP, levels were very low and were not influenced by the treatments in the wines. Leaf removal practices performed after véraison could have influenced the IBMP and IPMP levels in the grapes.

Volatile thiols, 3MHA and 3MH, show a vintage, vineyard, and treatment effect for the cultivars. 3MHA levels decreased significantly for Sauvignon Blanc in 2015 during ageing due to hydrolysis and oxidation (Herbst-Johnstone *et al.*, 2011). 3MH levels increased significantly for S and N+S treatments and during bottle maturation a treatment effect can be observed with all the treatments for Chenin Blanc (2015) and N+S treatments for Chenin Blanc (9 months, 2016) and Sauvignon Blanc (9 months, 2015 and 2016). The increase was possibly due to the hydrolysis of 3MHA to 3MH, being derived from thiol precursors present in the wines or by the breakdown of 3MH disulphide present in the wines (Capone *et al.*, 2010; Sarrazin *et al.*, 2010)

Furthermore, by looking at the overall volatile composition, a vintage and age effect for both cultivars in 2015 and 2016 was observed. Clear groupings were observed and when the groupings were closer grouped together the wines were very similar. The volatile content gradually changed during bottle maturation and showed greater or smaller increases or decreases with both cultivars. Treatment effect was not marked in this type of data representation.

In the Chenin Blanc wines, ‘tropical’ and ‘rich and ripe’ aromas were the most dominating aromas and are distinct of FF and RRUW Chenin Blanc wines (Bester, 2011; Van Antwerpen, 2012; CBA, 2016). On the other hand, Sauvignon Blanc wines had prominent ‘tropical fruits’, ‘passion fruit’, and ‘grapefruit’ aromas which are distinct of the ‘tropical style’ of Sauvignon Blanc wines. During bottle maturation some aromas were maintained but their frequency of citations changed.

The Chenin Blanc and Sauvignon Blanc wines are ester and major volatile driven, while the S and N+S treatments had more prominent negative associated ‘cooked vegetables’, ‘sulphur’, and ‘herbaceous’ aromas which can be associated with reductive sulphur compounds. Even though the chemistry makeup of the wines changed, the overall effect was not observed in the aromatic expression of the wines. This can be due to the matrix effect of the volatile compounds.

However, it is evident that the foliar treatments applied to the cultivars had a significant effect on the formation of some of these important volatile compounds. This research study could impact the way viticulturists can influence the nitrogen or sulphur-containing compounds in wines by applying sulphur or sulphur with nitrogen foliar fertilization at véraison. Also, winemakers can enhance the aromatic expression of the wines by increasing the major volatiles, ester, and volatile thiol levels in the wines. However, a greater understanding of the evolution of these volatile compounds during grape ripening, alcoholic fermentation, and maturation is required to understand the influence of the foliar applications and how to improve the aromatic expression of Chenin Blanc and Sauvignon Blanc wines.

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# Chapter 5

# General discussion and conclusions

# Chapter 5. General discussion and conclusions

The complexity of the various chemical compounds present in the grape berry and must can contribute to the intricate aromatic expression, flavour, and mouth-feel properties of a wine (Marais, 1994; Fischer, 2007). Many winemakers and viticulturists have experimented with various processes in the cellar or practices in the vineyard to positively influence the non-volatile and volatile compounds present in grapes and wine. Vine nitrogen fertilization has been shown to have a positive impact on the composition of grapes (Mengel, 2002; Oosterhuis, 2009; Lasa *et al.*, 2012). Due to climate change and frequent summer droughts, foliar fertilization has been widely used on various crops for small deficiencies and can lead to a quick nutrient uptake through the leaves (Christensen, 2005; Jreij *et al.*, 2009; Lasa *et al*., 2012). Positive results of foliar fertilization studies in vineyards have gained the attention of South Africa's wine industry (Section 2.4) and winemakers want to positively influence the aroma and complexity of the wines through this type of viticulture practices.

Two of the most planted white wine cultivars in South Africa, Chenin Blanc and Sauvignon Blanc, are currently of great interest to researchers locally and internationally (SAWIS, 2016). The overall aim of this research study was to investigate the effect of different foliar fertilization treatments on the chemical composition (volatile and non-volatile compounds) of the juice and wine of *Vitis vinifera* L. cultivars Sauvignon Blanc and Chenin Blanc. Furthermore, the effect of the treatments on the aroma composition and sensory expression of the wines after bottle maturation was also assessed.

The vineyards used in this research study have a history of producing low Yeast Assimilable Nitrogen (YAN) containing grapes. Grapes with low YAN can lead to low yeast populations, poor fermentation vigour and increased risk of sluggish or stuck alcoholic fermentations (Monteiro & Bisson, 1991). As expected, the different foliar fertilization applications influenced the non-volatile and volatile content of the juices and wines, but also influenced the aromatic expression of the chenin Blanc and Sauvignon Blanc wines. Generally, the composition and levels found were in agreement with the findings of the few published foliar fertilization reports (Lacroux *et al.*, 2008; Lasa *et al.*, 2012; Juhasz, 2015; Gutiérrez-Gamboa *et al.*, 2017).

Generally, all relative recent reports looked only at selected aspects and due to interest internationally more research came out in the past years on foliar fertilization. This research study was more comprehensive and investigates some aspects, but also into the overall effect (combine all volatiles) in the wines. Since a vineyard treatment will affect not only the composition of the grape, but will have a lasting effect all the way to the wine. The compounds selected for evaluation were the ones potentially affected by the treatments applied (S and N-containing compounds).

The differences of the treatments were sometimes significant and at other times minimal, but trends were observed with certain non-volatile and volatile compounds and aromatic expression of the Chenin Blanc and Sauvignon Blanc wines. The results highlighted that a cultivar, vintage, or wine age effect were sometimes more present than in other cases.

In this study, nitrogen containing foliar fertilizations (N and N+S) positively increased the YAN levels compared to the control and these findings were supported by the literature (Lacroux *et al.*, 2008; Hannam *et al.*, 2014; Dienes-Nagy *et al.*, 2017). Although only significant for Sauvignon Blanc juices. Moreover, the total amino acids and Arginine content in the juices were higher with

the N treatments compared to the control, in agreement with research by Lasa *et al.* (2012). The increase in amino acids is relevant not only for yeast metabolism, but also for the aromatic potential of a wine, as certain amino acids being precursors of aroma compounds. Di-ammonium phosphate (DAP) additions (increase to 250 mg/L) just before the onset of alcoholic fermentation to nitrogen deficient juices could have influenced the chemical and aromatic expression of the resulting wines.

On the other hand, the results and trends of glutathione (GSH) were not as evident as for YAN, though N and N+S treatments generally resulted in higher GSH levels compared to the control for both cultivars and both years in accordance with Lacroux *et al.* (2008). If the wines have the potential to be more aromatic compared to the control, and the GSH levels present play a crucial role by protecting varietal volatile thiols from oxidation, and therefore protect the aroma expression of wines (Lavigne *et al.*, 2007).

Major volatiles arise as primary metabolites of yeast and sugar and the metabolism of amino acids (Henschke & Jiranek, 1993; Swiegers *et al.*, 2005). These volatile compounds contribute to the pleasant fruity and floral aromas in wines (Swiegers *et al.*, 2005). Major volatiles such as esters, fatty acids and higher alcohols were affected the most by Sulphur-containing treatments (S or N+S), for both cultivars. As expected, the overall ester content increased over time for most treatments for both cultivars (Selli *et al.*, 2006). Methoxypyrazine, IBMP, levels were very low and were not influenced by the treatments in the wines. Leaf removal practices performed after véraison could have influenced the IBMP and IPMP levels in the grapes.

Volatile thiols, 3MHA and 3MH, show a vintage, vineyard, and treatment effect for the cultivars. 3MHA levels decreased significantly for Sauvignon Blanc in 2015 during ageing due to hydrolysis and oxidation (Herbst-Johnstone *et al.*, 2011). 3MH levels increased significantly for S and N+S treatments and during bottle maturation a treatment effect can be observed with all the treatments for Chenin Blanc (2015) and N+S treatments for Chenin Blanc (9 months, 2016) and Sauvignon Blanc (9 months, 2015 and 2016). The increase was possibly due to the hydrolysis of 3MHA to 3MH, being derived from thiol precursors present in the wines or by the breakdown of 3MH disulphide present in the wines (Capone *et al.*, 2010; Sarrazin *et al.*, 2010).

Additionally, by looking at the overall volatile composition, a vintage and age effect occurred for both cultivars. Clear groupings were observed between three and nine months of maturation, and the volatile content gradually changed during this period. Treatment effect was less evident, and not a drive for the separation of samples in the multivariate data representation.

For the sensory evaluation of the wines, a free sorting method was used. Previously, descriptive analysis (DA) has not been successful in this type of work (Juhasz, 2015), and other research reports did not mention the sensory analysis method used (Lacroux *et al.*, 2008; Geffroy *et al.*, 2016). The results showed that the wines were not grouped according to treatment, and seldom two or three of the four repeats were grouped together. This can be due to the judges not being able to differentiate between the wines because the wines were very similar in their aromatic expression. The Chenin Blanc wines had ‘tropical’ and ‘rich and ripe’ aromas specific of FF and RRUW Chenin Blanc style wines (Bester, 2011; Van Antwerpen, 2012; CBA, 2016). The Sauvignon Blanc wines had prominent ‘tropical’, ‘passion fruit’, and ‘grapefruit’ aromas which are distinct of the ‘tropical style’ of Sauvignon Blanc wines. During bottle maturation, some notes and aroma characters were maintained but their frequency of citations changed.

Overall, both cultivars were ester and volatile thiol driven (Section 4.3.1), while the S and N+S treatments had more prominent negative associated 'cooked vegetables', 'sulphur', and 'herbaceous' aromas, which can be linked to reductive sulphur compounds. Although the chemistry makeup of the wines changed, the overall effect was not observed in the aromatic expression of the wines. This can be due to the matrix effect or the interactions of the volatile and non-volatile compounds present in the wines.

A better understanding of the factors linking vineyard fertilization applications, grape heredity, climate, must, and wine composition to the sensory expression of wines need to be investigated to fully understand how these different factors can affect the sensory expression of the wines. Some suggestions for future research are: vines of the same age and cultivar planted in different South African wine regions; the effect of vine age on the outcome of the treatments; expansion to other cultivars relevant to South Africa (on nitrogen deficient red and volatile-rich cultivars, such as Sémillon, Colombard, Riesling, Cabernet Sauvignon, and Merlot); combination of soil and foliar fertilization in South African climate.

More measurements can be considered: vine measurements, such as light intensity, vine water status, and nitrogen content in the leaves and berries should be included to determine the canopy density and nitrogen content between the sprays; measuring methoxypyrazines throughout the ripening and winemaking process to see how the levels are affected by the fertilization sprays; during winemaking sulphur containing precursors, glutathionylated and cysteinylated volatile thiol precursors could be measured and possibly related back to the S foliar fertilizations treatments; major volatiles and thiols alongside oxygen levels should be measured during winemaking and maturation to compare and see how oxygen exposure affects the volatiles' levels. A different sensory method, such as Projective mapping, could be used. This is a discrimination method where products are judged according to similarity and dissimilarity on a 2D scale (Santos *et al.*, 2013).

To our knowledge, this is the first study to examine the effect of N, S, and N+S foliar fertilization on Chenin Blanc. The only research regarding foliar application in South Africa was published by Juhasz (2015) and focused only on Sauvignon Blanc. No foliar fertilization studies have focused on determining the non-volatile and volatile content present in wines during bottle maturation. This study contributed to the knowledge on South African Chenin Blanc and Sauvignon Blanc wines, but also demonstrated that foliar fertilization can be used to influence the non-volatile and volatile content of wines. This knowledge could ultimately aid researchers and winemakers to understand these compounds, produce a specific wine style, and produce better quality wines.

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# Appendices

# Appendix A

## Chemical Analyses

### *Oenological parameters*

**Table A.1** LS means plot illustrating the treatment*time interaction of pH for Chenin Blanc and Sauvignon Blanc juices and wines in 2015 and 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

| pH | | Sampling Stage | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2015 | | Crushing and destemming | | | Racked juice | | | After alcoholic fermentation | | | Sensory 3 months | | | Sensory 9 months | | |
| Cultivar | Tmt | Mean ± SD | %RSD | SF | Mean ± SD | %RSD | SF | Mean ± SD | %RSD | SF | Mean ± SD | %RSD | SF | Mean ± SD | %RSD | SF |
| SB | C | 3.06 ± 0.04 | 0.02 | efg | 3.12 ± 0.04 | 0.02 | abc | 3.02 ± 0.04 | 0.02 | hij | 3.08 ± 0.02 | 0.01 | defg | 3.02 ± 0.03 | 0.01 | ij |
| SB | N | 3.16 ± 0.02 | 0.01 | a | 3.15 ± 0.02 | 0.01 | a | 3.08 ± 0.03 | 0.02 | ce | 3.11 ± 0.02 | 0.01 | bd | 3.06 ± 0.03 | 0.02 | fgh |
| SB | S | 3.14 ± 0.01 | 0.00 | ab | 3.13 ± 0.02 | 0.01 | ab | 3.04 ± 0.03 | 0.01 | ghi | 3.07 ± 0.03 | 0.02 | ef | 3.02 ± 0.03 | 0.02 | j |
| SB | N+S | 3.15 ± 0.03 | 0.01 | a | 3.13 ± 0.01 | 0.00 | ab | 3.07 ± 0.04 | 0.02 | defg | 3.08 ± 0.03 | 0.01 | defg | 3.01 ± 0.03 | 0.02 | ij |
| CB | C | 3.58 ± 0.07 | 0.04 | ac | 3.41 ± 0.03 | 0.01 | h | 3.51 ± 0.03 | 0.01 | def | 3.50 ± 0.03 | 0.01 | def | 3.47 ± 0.04 | 0.02 | defg |
| CB | N | 3.52 ± 0.02 | 0.01 | abcde | 3.45 ± 0.01 | 0.00 | fgh | 3.57 ± 0.02 | 0.01 | abc | 3.57 ± 0.01 | 0.01 | abc | 3.53 ± 0.02 | 0.01 | abcd |
| CB | S | 3.58 ± 0.05 | 0.02 | ab | 3.42 ± 0.00 | 0.00 | gh | 3.52 ± 0.03 | 0.01 | cde | 3.52 ± 0.01 | 0.00 | cde | 3.47 ± 0.02 | 0.01 | defgh |
| CB | N+S | 3.51 ± 0.02 | 0.01 | bde | 3.46 ± 0.13 | 0.07 | efgh | 3.52 ± 0.06 | 0.03 | abcd | 3.53 ± 0.05 | 0.02 | abcd | 3.48 ± 0.05 | 0.02 | defg |
| 2016 | | | | | | | | | | | | | | | | |
| SB | C | 2.96 ± 0.05 | 0.02 | fhi | 3.23 ± 0.03 | 0.01 | a | 3.05 ± 0.02 | 0.01 | c | 2.99 ± 0.02 | 0.01 | efgh | 3.00 ± 0.01 | 0.01 | eg |
| SB | N | 2.95 ± 0.04 | 0.02 | hi | 3.23 ± 0.02 | 0.01 | a | 3.05 ± 0.04 | 0.02 | c | 3.00 ± 0.03 | 0.02 | efg | 2.99 ± 0.03 | 0.01 | efg |
| SB | S | 2.96 ± 0.02 | 0.01 | ghi | 3.21 ± 0.07 | 0.04 | a | 3.07 ± 0.01 | 0.01 | c | 3.00 ± 0.02 | 0.01 | ef | 3.00 ± 0.01 | 0.01 | de |
| SB | N+S | 2.93 ± 0.03 | 0.02 | i | 3.14 ± 0.03 | 0.02 | a | 3.05 ± 0.01 | 0.00 | cd | 2.98 ± 0.01 | 0.00 | efgh | 2.98 ± 0.01 | 0.01 | efgh |
| CB | C | 3.85 ± 0.03 | 0.02 | ij | 3.99 ± 0.01 | 0.00 | abcde | 3.83 ± 0.24 | 0.12 | jk | 3.88 ± 0.02 | 0.01 | ghij | 3.89 ± 0.02 | 0.01 | ghij |
| CB | N | 3.91 ± 0.03 | 0.02 | efghij | 4.06 ± 0.03 | 0.02 | a | 4.02 ± 0.06 | 0.03 | abcd | 3.96 ± 0.05 | 0.03 | bcdefgh | 3.96 ± 0.04 | 0.02 | bcdef |
| CB | S | 3.86 ± 0.05 | 0.02 | hij | 4.03 ± 0.02 | 0.01 | abc | 3.95 ± 0.03 | 0.01 | defghi | 3.90 ± 0.03 | 0.02 | fghij | 3.90 ± 0.04 | 0.02 | fghij |
| CB | N+S | 3.87 ± 0.04 | 0.02 | ghij | 4.05 ± 0.04 | 0.02 | ab | 3.98 ± 0.02 | 0.01 | abcdef | 3.92 ± 0.04 | 0.02 | efghij | 3.93 ± 0.04 | 0.02 | defghi |

**Table A.2** LS means plot illustrating the treatment*time interaction of the total acidity (TA) for Chenin Blanc and Sauvignon Blanc juices and wines in 2015 and 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

| Total Acidity | | Sampling Stage | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2015 | | Crushing and destemming | | | Racked juice | | | After alcoholic fermentation | | | Sensory 3 months | | | Sensory 9 months | | |
| Cultivar | Tmt | Mean ± SD (g/L) | %RSD | SF | Mean ± SD (g/L) | %RSD | SF | Mean ± SD (g/L) | %RSD | SF | Mean ± SD (g/L) | %RSD | SF | Mean ± SD (g/L) | %RSD | SF |
| SB | C | 8.66 ± 0.32 | 0.16 | b | 6.50 ± 0.09 | 0.05 | fgh | 7.44 ± 0.09 | 0.04 | c | 6.47 ± 0.08 | 0.04 | fgh | 6.21 ± 0.11 | 0.06 | i |
| SB | N | 8.64 ± 0.32 | 0.16 | b | 6.83 ± 0.40 | 0.20 | de | 7.41 ± 0.08 | 0.04 | c | 6.45 ± 0.08 | 0.04 | ghi | 6.25 ± 0.20 | 0.10 | hi |
| SB | S | 8.96 ± 0.11 | 0.06 | a | 6.57 ± 0.34 | 0.17 | efg | 7.59 ± 0.15 | 0.07 | c | 6.55 ± 0.16 | 0.08 | ghi | 6.35 ± 0.12 | 0.06 | ghi |
| SB | N+S | 8.62 ± 0.18 | 0.09 | b | 6.94 ± 0.21 | 0.10 | d | 7.62 ± 0.11 | 0.06 | c | 6.74 ± 0.10 | 0.05 | def | 6.53 ± 0.16 | 0.08 | fg |
| CB | C | 8.02 ± 0.48 | 0.24 | a | 5.75 ± 0.26 | 0.13 | de | 6.34 ± 0.06 | 0.03 | b | 5.49 ± 0.04 | 0.02 | e | 5.56 ± 0.03 | 0.01 | e |
| CB | N | 7.95 ± 0.25 | 0.12 | a | 5.67 ± 0.14 | 0.07 | d | 6.19 ± 0.06 | 0.03 | bcd | 5.37 ± 0.06 | 0.03 | e | 5.43 ± 0.05 | 0.03 | e |
| CB | S | 8.28 ± 0.28 | 0.14 | a | 5.73 ± 0.15 | 0.08 | de | 6.27 ± 0.10 | 0.05 | bd | 5.49 ± 0.12 | 0.06 | e | 5.58 ± 0.12 | 0.06 | e |
| CB | N+S | 8.40 ± 0.11 | 0.05 | a | 5.39 ± 1.42 | 0.71 | d | 6.42 ± 0.14 | 0.07 | b | 5.65 ± 0.13 | 0.07 | e | 5.76 ± 0.14 | 0.07 | cde |
| 2016 | | | | | | | | | | | | | | | | |
| SB | C | 8.18 ± 0.22 | 0.11 | a | 6.38 ± 0.15 | 0.07 | d | 7.16 ± 0.19 | 0.09 | b | 6.56 ± 0.14 | 0.07 | c | 6.5 ± 0.14 | 0.07 | d |
| SB | N | 8.43 ± 0.53 | 0.27 | a | 6.54 ± 0.46 | 0.23 | d | 7.32 ± 0.18 | 0.09 | b | 6.69 ± 0.21 | 0.11 | cd | 6.62 ± 0.21 | 0.11 | d |
| SB | S | 8.29 ± 0.56 | 0.28 | a | 6.63 ± 0.11 | 0.06 | d | 7.04 ± 0.24 | 0.12 | bc | 6.41 ± 0.16 | 0.08 | d | 6.43 ± 0.11 | 0.06 | d |
| SB | N+S | 8.50 ± 0.57 | 0.28 | a | 6.54 ± 0.08 | 0.04 | d | 7.14 ± 0.29 | 0.15 | b | 6.65 ± 0.22 | 0.11 | cd | 6.59 ± 0.22 | 0.11 | d |
| CB | C | 5.23 ± 0.33 | 0.16 | a | 3.00 ± 0.00 | 0.00 | f | 4.16 ± 0.70 | 0.35 | cde | 4.09 ± 0.10 | 0.05 | de | 4.13 ± 0.07 | 0.03 | de |
| CB | N | 5.08 ± 0.07 | 0.04 | a | 3.12 ± 0.24 | 0.12 | f | 4.43 ± 0.06 | 0.03 | bc | 4.04 ± 0.04 | 0.02 | de | 4.10 ± 0.05 | 0.02 | de |
| CB | S | 5.13 ± 0.29 | 0.15 | a | 3.10 ± 0.20 | 0.10 | f | 4.49 ± 0.05 | 0.03 | b | 4.16 ± 0.09 | 0.04 | cde | 4.18 ± 0.07 | 0.04 | cd |
| CB | N+S | 5.26 ± 0.36 | 0.18 | a | 3.00 ± 0.00 | 0.00 | f | 4.48 ± 0.06 | 0.03 | b | 4.12 ± 0.07 | 0.04 | de | 4.17 ± 0.05 | 0.02 | cde |

## *Amino acids*

**Table A.3** LS means plot illustrating the treatment effect of different amino acid groups for Chenin Blanc and Sauvignon Blanc juices in 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

| Amino Acids | | Total AA | | | Yeast-preferred AA | | | Sulphur- AA | | | Branched AA | | | Other AA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar | Tmt | Mean ± SD (mg N/L) | %RSD | SF | Mean ± SD (mg N/L) | %RSD | SF | Mean ± SD (mg N/L) | %RSD | SF | Mean ± SD (mg N/L) | %RSD | SF | Mean ± SD (mg N/L) | %RSD | SF |
| SB | C | 371.55 ± 179.53 | 89.77 | a | 207.64 ± 131.98 | 65.99 | a | 3.53 ± 1.49 | 0.74 | a | 19.07 ± 13.11 | 6.55 | a | 141.31 ± 35.59 | 17.79 | a |
| SB | N | 443.37 ± 88.59 | 44.30 | a | 256.93 ± 59.57 | 29.78 | a | 3.45 ± 0.18 | 0.09 | a | 19.54 ± 9.09 | 4.54 | a | 163.46 ± 21.8 | 10.90 | a |
| SB | S | 399.16 ± 54.55 | 27.28 | a | 219.89 ± 45.13 | 22.56 | a | 3.08 ± 0.70 | 0.35 | a | 16.67 ± 11.34 | 5.67 | a | 159.53 ± 13.14 | 6.57 | a |
| SB | N+S | 451.29 ± 80.56 | 40.28 | a | 273.41 ± 45.59 | 22.79 | a | 3.26 ± 0.30 | 0.15 | a | 15.88 ± 9.33 | 4.66 | a | 158.73 ± 26.52 | 13.26 | a |
| CB | C | 1453.36 ± 500.76 | 250.38 | a | 893.75 ± 378.31 | 189.15 | a | 8.85 ± 1.50 | 0.75 | a | 80.97 ± 38.85 | 19.42 | a | 469.79 ± 83.93 | 41.97 | a |
| CB | N | 1604.13 ± 271.08 | 135.54 | a | 943.29 ± 93.05 | 46.53 | a | 7.89 ± 3.25 | 1.63 | a | 104.05 ± 53.27 | 26.64 | a | 548.90 ± 136.24 | 68.12 | a |
| CB | S | 1483.92 ± 160.71 | 80.35 | a | 883.17 ± 114.59 | 57.30 | a | 8.20 ± 1.88 | 0.94 | a | 90.78 ± 18.49 | 9.25 | a | 501.77 ± 31.66 | 15.83 | a |
| CB | N+S | 1950.06 ± 977.50 | 488.75 | a | 1220.59 ± 733.50 | 366.75 | a | 11.41 ± 5.44 | 2.72 | a | 124.97 ± 51.69 | 25.85 | a | 593.08 ± 193.86 | 96.93 | a |

**Table A.4** LS means plot illustrating the treatment effect of different amino acids for Chenin Blanc and Sauvignon Blanc juices in 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

| Amino Acids | | ASP | | | GLU | | | ASN | | | SER | | | GLN | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar | Tmt | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF |
| SB | C | 16.39 ± 11.35 | 5.67 | b | 36.07 ± 24.51 | 12.25 | cd | 1.51 ± 0.34 | 0.17 | b | 17.16 ± 12.03 | 6.02 | b | 20.92 ± 14.95 | 7.47 | b |
| SB | N | 14.74 ± 6.49 | 3.25 | b | 37.04 ± 13.57 | 6.78 | bcd | 2.06 ± 0.46 | 0.23 | b | 20.97 ± 4.72 | 2.36 | b | 22.39 ± 11.32 | 5.66 | b |
| SB | S | 14.38 ± 9.77 | 4.89 | b | 31.68 ± 21.27 | 10.64 | cd | 1.97 ± 0.47 | 0.24 | b | 16.14 ± 10.79 | 5.40 | b | 17.91 ± 12.04 | 6.02 | b |
| SB | N+S | 12.53 ± 6.12 | 3.06 | b | 30.31 ± 15.72 | 7.86 | d | 1.88 ± 0.40 | 0.20 | b | 17.89 ± 7.02 | 3.51 | b | 22.74 ± 10.37 | 5.18 | b |
| CB | C | 23.86 ± 4.95 | 2.48 | ab | 54.16 ± 21.69 | 10.85 | abcd | 6.95 ± 5.50 | 2.75 | a | 49.35 ± 14.21 | 7.11 | a | 41.38 ± 43.30 | 21.65 | a |
| CB | N | 19.59 ± 6.14 | 3.07 | ab | 66.25 ± 9.19 | 4.60 | ab | 5.52 ± 2.72 | 1.36 | ab | 50.04 ± 18.89 | 9.44 | a | 50.16 ± 21.20 | 10.60 | ab |
| CB | S | 22.87 ± 3.74 | 1.87 | ab | 60.38 ± 11.32 | 5.66 | abc | 6.44 ± 2.89 | 1.45 | a | 50.70 ± 6.88 | 3.44 | a | 42.04 ± 17.82 | 8.91 | a |
| CB | N+S | 31.02 ± 11.85 | 5.92 | ab | 78.45 ± 32.72 | 16.36 | a | 9.24 ± 4.87 | 2.44 | a | 74.27 ± 38.91 | 19.46 | a | 83.22 ± 50.78 | 25.39 | a |
| | | HIS | | | GLY | | | TRH | | | ARG | | | ALA | | |
| Cultivar | Tmt | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF |
| SB | C | 5.60 ± 2.76 | 1.38 | b | 1.17 ± 0.51 | 0.25 | c | 18.21 ± 12.48 | 6.24 | c | 94.33 ± 54.67 | 27.34 | b | 21.26 ± 14.46 | 7.23 | c |
| SB | N | 9.02 ± 1.91 | 0.96 | b | 1.84 ± 0.31 | 0.16 | bc | 20.02 ± 7.02 | 3.51 | c | 132.67 ± 32.21 | 16.10 | b | 27.07 ± 5.59 | 2.79 | c |
| SB | S | 8.04 ± 1.55 | 0.78 | b | 1.54 ± 0.25 | 0.13 | bc | 16.12 ± 10.76 | 5.38 | c | 119.34 ± 22.52 | 11.26 | b | 18.48 ± 12.33 | 6.16 | c |
| SB | N+S | 7.81 ± 0.87 | 0.44 | b | 1.87 ± 0.25 | 0.13 | bc | 17.87 ± 7.23 | 3.62 | c | 159.16 ± 13.84 | 6.92 | b | 28.89 ± 8.28 | 4.14 | c |
| CB | C | 50.04 ± 17.38 | 8.69 | a | 6.68 ± 1.93 | 0.97 | a | 70.50 ± 29.67 | 14.83 | b | 617.38 ± 260.26 | 130.13 | a | 100.67 ± 36.75 | 18.38 | b |
| CB | N | 43.07 ± 13.60 | 6.80 | a | 4.69 ± 2.06 | 1.03 | ab | 89.50 ± 17.14 | 8.57 | ab | 618.92 ± 43.25 | 21.62 | a | 132.82 ± 21.83 | 10.92 | ab |
| CB | S | 50.22 ± 7.53 | 3.77 | a | 6.11 ± 2.49 | 1.25 | a | 80.65 ± 15.89 | 7.95 | ab | 592.82 ± 81.12 | 40.56 | a | 107.91 ± 10.95 | 5.48 | ab |
| CB | N+S | 70.29 ± 51.42 | 25.71 | a | 8.09 ± 5.60 | 2.80 | a | 110.50 ± 59.9 | 29.95 | ab | 791.18 ± 521.55 | 260.77 | a | 153.20 ± 75.30 | 37.65 | a |
| | | GABA | | | TYR | | | CY2 | | | VAL | | | MET | | |
| Cultivar | Tmt | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF | Mean ± SD (mg N/L) | SE | SF |
| SB | C | 49.79 ± 7.99 | 4.00 | b | 2.46 ± 1.73 | 0.86 | b | 3.02 ± 1.10 | 0.55 | b | 5.28 ± 3.63 | 1.81 | c | 0.51 ± 0.41 | 0.20 | b |
| SB | N | 57.33 ± 5.05 | 2.53 | b | 3.26 ± 0.75 | 0.38 | b | 3.02 ± 0.20 | 0.10 | b | 5.57 ± 2.09 | 1.04 | c | 0.43 ± 0.31 | 0.16 | b |
| SB | S | 61.13 ± 15.49 | 7.74 | b | 2.20 ± 1.47 | 0.74 | b | 2.72 ± 0.48 | 0.24 | b | 4.46 ± 3.01 | 1.50 | c | 0.36 ± 0.25 | 0.13 | b |
| SB | N+S | 58.29 ± 4.57 | 2.28 | b | 2.84 ± 1.00 | 0.50 | b | 3.11 ± 0.16 | 0.08 | b | 5.26 ± 2.38 | 1.19 | c | 0.15 ± 0.21 | 0.10 | b |
| CB | C | 129.96 ± 19.70 | 9.85 | a | 8.82 ± 0.95 | 0.48 | a | 6.88 ± 1.64 | 0.82 | a | 25.35 ± 8.07 | 4.04 | b | 1.97 ± 2.42 | 1.21 | ab |
| CB | N | 134.90 ± 16.19 | 8.09 | a | 9.77 ± 3.82 | 1.91 | a | 5.32 ± 0.97 | 0.49 | a | 34.20 ± 15.8 | 7.90 | ab | 2.57 ± 2.91 | 1.45 | ab |
| CB | S | 133.23 ± 12.17 | 6.09 | a | 9.80 ± 0.99 | 0.50 | a | 6.36 ± 1.44 | 0.72 | a | 31.38 ± 3.12 | 1.56 | ab | 1.85 ± 2.00 | 1.00 | ab |
| CB | N+S | 164.96 ± 88.56 | 44.28 | a | 11.56 ± 4.92 | 2.46 | a | 7.28 ± 2.76 | 1.38 | a | 40.13 ± 15.89 | 7.95 | a | 4.13 ± 3.13 | 1.56 | a |

| | | TRP | | | PHE | | | ILE | | | ORN | | | LEU | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **Tmt** | **Mean ± SD (mg N/L)** | **SE** | **SF** | **Mean ± SD (mg N/L)** | **SE** | **SF** | **Mean ± SD (mg N/L)** | **SE** | **SF** | **Mean ± SD (mg N/L)** | **SE** | **SF** | **Mean ± SD (mg N/L)** | **SE** | **SF** |
| SB | C | 1.23 ± 0.76 | 0.38 | b | 6.95 ± 4.85 | 2.43 | b | 2.56 ± 1.75 | 0.87 | b | 2.03 ± 4.06 | 2.03 | ab | 4.28 ± 2.92 | 1.46 | c |
| SB | N | 1.26 ± 0.57 | 0.29 | b | 7.26 ± 2.85 | 1.43 | b | 2.40 ± 1.44 | 0.72 | b | 2.11 ± 1.01 | 0.50 | ab | 4.30 ± 2.74 | 1.37 | c |
| SB | S | 0.89 ± 0.61 | 0.30 | b | 6.09 ± 4.21 | 2.10 | b | 2.18 ± 1.48 | 0.74 | b | 2.48 ± 1.67 | 0.83 | ab | 3.94 ± 2.67 | 1.33 | c |
| SB | N+S | 0.95 ± 0.70 | 0.35 | b | 5.22 ± 3.35 | 1.67 | b | 2.00 ± 1.35 | 0.68 | b | 0.98 ± 0.59 | 0.29 | ab | 3.40 ± 2.41 | 1.20 | c |
| CB | C | 9.04 ± 4.87 | 2.44 | a | 22.64 ± 11.72 | 5.86 | a | 11.07 ± 6.41 | 3.20 | a | 4.83 ± 3.21 | 1.60 | a | 21.92 ± 13.58 | 6.79 | b |
| CB | N | 9.93 ± 5.97 | 2.98 | a | 27.02 ± 13.98 | 6.99 | a | 14.45 ± 9.62 | 4.81 | a | 3.97 ± 5.17 | 2.58 | a | 28.38 ± 14.02 | 7.01 | ab |
| CB | S | 8.55 ± 2.38 | 1.19 | a | 23.53 ± 4.95 | 2.48 | a | 11.95 ± 3.55 | 1.77 | a | 0.00 ± 0.00 | 0.00 | b | 23.92 ± 7.50 | 3.75 | ab |
| CB | N+S | 11.18 ± 4.73 | 2.37 | a | 31.00 ± 11.72 | 5.86 | a | 17.35 ± 6.57 | 3.29 | a | 0.00 ± 0.00 | 0.00 | b | 36.48 ± 17.58 | 8.79 | a |

| | | LYS | | | HYP | | | PRO | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **Tmt** | **Mean ± SD (mg N/L)** | **SE** | **SF** | **Mean ± SD (mg N/L)** | **SE** | **SF** | **Mean ± SD (mg N/L)** | **SE** | **SF** |
| SB | C | 1.57 ± 1.07 | 0.53 | c | 12.24 ± 11.75 | 5.87 | ab | 47.02 ± 25.26 | 12.63 | b |
| SB | N | 1.93 ± 1.30 | 0.65 | c | 4.55 ± 3.65 | 1.82 | ab | 62.16 ± 11.48 | 5.74 | b |
| SB | S | 2.47 ± 1.12 | 0.56 | bc | 4.56 ± 3.71 | 1.86 | ab | 60.10 ± 6.19 | 3.09 | b |
| SB | N+S | 1.63 ± 1.07 | 0.54 | c | 1.25 ± 1.78 | 0.89 | b | 65.24 ± 13.09 | 6.55 | b |
| CB | C | 5.38 ± 5.27 | 2.63 | bc | 14.39 ± 17.01 | 8.51 | ab | 170.15 ± 2.54 | 1.27 | a |
| CB | N | 11.25 ± 10.19 | 5.09 | ab | 23.14 ± 28.86 | 14.43 | a | 218.69 ± 85.9 | 42.95 | a |
| CB | S | 5.35 ± 4.14 | 2.07 | ab | 10.76 ± 13.12 | 6.56 | ab | 197.09 ± 19.45 | 9.73 | a |
| CB | N+S | 10.81 ± 11.38 | 5.69 | a | 18.97 ± 15.98 | 7.99 | ab | 186.72 ± 62.62 | 31.31 | a |

## *Glutathione*

**Table A.5** LS means plot illustrating the treatment*time interaction of reduced glutathione Chenin Blanc and Sauvignon Blanc juices and wines in 2015 and 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

| **GSH: Reduced** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **2015** | | **Racked juice** | | | **After alcoholic fermentation** | | | **After cold stabilisation** | | |
| **Cultivar** | **Tmt** | **Mean ± SD (mg N/L)** | **%RSD** | **SF** | **Mean ± SD (mg N/L)** | **%RSD** | **SF** | **Mean ± SD (mg N/L)** | **%RSD** | **SF** |
| SB | C | 9.73 ± 3.61 | 2.08 | ab | 8.15 ± 5.00 | 2.89 | ab | 9.22 ± 5.87 | 3.39 | ab |
| SB | N | 10.35 ± 2.21 | 1.28 | ab | 8.39 ± 1.05 | 0.61 | ab | 9.47 ± 1.65 | 0.95 | ab |
| SB | S | 10.37 ± 4.06 | 2.34 | ab | 9.35 ± 2.19 | 1.26 | ab | 10.33 ± 2.37 | 1.37 | ab |
| SB | N+S | 13.11 ± 7.30 | 4.21 | a | 9.81 ± 4.04 | 2.33 | ab | 10.77 ± 4.53 | 2.62 | ab |
| CB | C | 20.38 ± 2.33 | 1.35 | de | 21.39 ± 3.49 | 2.01 | de | 23.85 ± 3.43 | 1.98 | abcd |
| CB | N | 29.78 ± 8.20 | 4.73 | ab | 28.45 ± 6.44 | 3.72 | abc | 30.44 ± 6.53 | 3.77 | a |
| CB | S | 25.46 ± 2.66 | 1.54 | abcd | 14.29 ± 4.38 | 2.53 | f | 15.23 ± 5.05 | 2.92 | ef |
| CB | N+S | 28.64 ± 3.70 | 2.14 | a | 23.70 ± 1.96 | 1.13 | bcd | 22.36 ± 1.26 | 0.73 | cd |
| **2016** | | | | | | | | | | |
| SB | C | 4.61 ± 0.41 | 0.24 | g | 5.39 ± 4.82 | 2.78 | g | 6.23 ± 4.63 | 2.67 | fg |
| SB | N | 7.06 ± 2.93 | 1.69 | efg | 4.03 ± 1.69 | 0.98 | g | 5.15 ± 1.91 | 1.10 | g |
| SB | S | 5.43 ± 2.28 | 1.32 | g | 7.41 ± 4.80 | 2.77 | efg | 8.33 ± 4.88 | 2.82 | efg |
| SB | N+S | 4.80 ± 1.46 | 0.84 | g | 11.19 ± 4.62 | 2.67 | def | 11.58 ± 3.89 | 2.25 | def |
| CB | C | 8.12 ± 3.04 | 1.76 | efg | 8.16 ± 3.71 | 2.14 | efg | 8.81 ± 2.82 | 1.63 | defg |
| CB | N | 11.39 ± 0.81 | 0.47 | bcde | 5.78 ± 1.63 | 0.94 | fg | 8.20 ± 1.70 | 0.98 | efg |
| CB | S | 13.38 ± 4.32 | 2.49 | abc | 6.80 ± 1.84 | 1.06 | fgh | 8.20 ± 0.50 | 0.29 | efg |
| CB | N+S | 12.73 ± 7.18 | 4.15 | abcd | 4.04 ± 1.01 | 0.58 | h | 6.14 ± 0.72 | 0.42 | gh |

**Table A.6** LS means plot illustrating the treatment*time interaction of oxidised glutathione for Chenin Blanc and Sauvignon Blanc juices and wines in 2015 and 2016. Significant letters were obtained from LSD post-hoc test and vertical bars denote the 95% confidence intervals.

| **GSH: Oxidised** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **2015** | | **Racked juice** | | | **After alcoholic fermentation** | | | **After cold stabilisation** | | |
| **Cultivar** | **Tmt** | **Mean ± SD (mg N/L)** | **%RSD** | **SF** | **Mean ± SD (mg N/L)** | **%RSD** | **SF** | **Mean ± SD (mg N/L)** | **%RSD** | **SF** |
| SB | C | 3.60 ± 0.26 | 0.15 | ac | 3.10 ± 0.24 | 0.14 | bdefg | 3.45 ± 0.41 | 0.24 | abcdef |
| SB | N | 3.44 ± 0.06 | 0.03 | abcd | 3.01 ± 0.14 | 0.08 | efg | 3.64 ± 0.19 | 0.11 | abcd |
| SB | S | 3.33 ± 0.15 | 0.09 | abcdef | 2.79 ± 0.27 | 0.15 | g | 3.29 ± 0.37 | 0.22 | abcdef |
| SB | N+S | 3.55 ± 0.40 | 0.23 | abe | 3.13 ± 0.44 | 0.26 | cdfg | 3.57 ± 0.92 | 0.53 | abe |
| CB | C | 5.75 ± 0.66 | 0.38 | a | 3.49 ± 0.22 | 0.12 | d | 4.56 ± 0.41 | 0.24 | bc |
| CB | N | 5.36 ± 1.10 | 0.64 | ab | 3.76 ± 0.44 | 0.25 | cd | 4.93 ± 0.51 | 0.29 | ab |
| CB | S | 5.02 ± 0.55 | 0.32 | ab | 4.60 ± 0.56 | 0.32 | bc | 4.81 ± 0.65 | 0.37 | b |
| CB | N+S | 5.12 ± 0.97 | 0.56 | ab | 4.48 ± 0.32 | 0.18 | bc | 4.80 ± 0.14 | 0.08 | b |
| **2016** | | | | | | | | | | |
| SB | C | 5.51 ± 1.16 | 0.67 | a | 0.15 ± 0.05 | 0.03 | d | 0.27 ± 0.12 | 0.07 | d |
| SB | N | 5.36 ± 0.45 | 0.26 | ab | 0.14 ± 0.03 | 0.01 | d | 0.23 ± 0.06 | 0.03 | d |
| SB | S | 4.55 ± 0.99 | 0.57 | b | 0.15 ± 0.10 | 0.06 | d | 0.20 ± 0.03 | 0.02 | d |
| SB | N+S | 6.17 ± 0.66 | 0.38 | a | 0.23 ± 0.05 | 0.03 | d | 0.24 ± 0.09 | 0.05 | d |
| CB | C | 0.06 ± 0.07 | 0.04 | b | 0.00 ± 0.00 | 0.00 | b | 0.09 ± 0.04 | 0.02 | b |
| CB | N | 0.05 ± 0.09 | 0.05 | b | 0.00 ± 0.00 | 0.00 | b | 0.15 ± 0.04 | 0.02 | b |
| CB | S | 0.02 ± 0.04 | 0.02 | b | 0.00 ± 0.00 | 0.00 | b | 0.18 ± 0.08 | 0.05 | b |
| CB | N+S | 0.02 ± 0.02 | 0.01 | b | 0.00 ± 0.00 | 0.00 | b | 0.13 ± 0.11 | 0.07 | b |

**Table A.7** The mean of the total glutathione content at different stages of treatments for Chenin Blanc and Sauvignon Blanc in 2015 and 2016.

| GSH: Total | | | | |
|---|---|---|---|---|
| **2015** | | **Racked juice** | **After alcoholic fermentation** | **After cold stabilisation** |
| **Cultivar** | **Tmt** | **Mean (mg N/L)** | **Mean (mg N/L)** | **Mean (mg N/L)** |
| SB | C | 13.33 | 11.25 | 12.67 |
| SB | N | 13.79 | 11.40 | 13.11 |
| SB | S | 13.70 | 12.14 | 13.62 |
| SB | N+S | 16.66 | 12.94 | 14.34 |
| CB | C | 26.13 | 24.88 | 28.41 |
| CB | N | 35.14 | 32.21 | 35.37 |
| CB | S | 30.48 | 18.89 | 20.04 |
| CB | N+S | 33.76 | 28.18 | 27.16 |
| **2016** | | | | |
| SB | C | 10.12 | 5.54 | 6.50 |
| SB | N | 12.42 | 4.17 | 5.38 |
| SB | S | 9.98 | 7.55 | 8.53 |
| SB | N+S | 10.97 | 11.42 | 11.82 |
| CB | C | 8.18 | 8.16 | 8.90 |
| CB | N | 11.44 | 5.78 | 8.35 |
| CB | S | 13.40 | 6.80 | 8.38 |
| CB | N+S | 12.75 | 4.04 | 6.27 |

# Appendix B

## Free Sorting method

**Name ……..  Judge:…..  Rep: 1  Date:  /  /**

**Tasting Instructions:**

1. Please **SMELL** the 16 wine samples presented
2. Sort the 16 given wine samples according to **SIMILARITY** into as many groups as you want
3. Provide **3-5 DESCRIPTORS** to explain and characterise the groups
   a. Please use the provided list for aroma
   b. You are allowed to give descriptors that is not on the list if you find the list insufficient

**Figure B.1** Free sorting tasting instructions.

### Aromatic Descriptors List

**FRUITY**

**WHITE FRUITS:**
Quince
Pear
Apple
Yellow apple

**YELLOW FRUITS:**
Apricot
Peach
Melon

**RED FRUITS**

**BLACK FRUITS**

**DRIED FRUITS:**
Dried Apple
Dried Peach
Dried Apricot
Dried Fig
Date
Raisin
Prune

**CITRUS:**
Lemon
Orange
Grapefruit

**TROPICAL FRUITS:**
Pineapple
Banana
Guava
Passion Fruit
Litchi
Mango
Gooseberry
Papaya/Pawpaw
Coconut

**SWEET ASSOCIATED:**
Ripe Fruit
Marmalade
Honey
Fruit Jam
Glazed/Crystallized Fruit
Muscat
Baked Apple

**TOASTED / WOOD**

**TOASTED:**
Caramel/Burnt Sugar
Toffee
Vanilla
Roasted Coffee
Toasted Bread

**WOODY:**
Planky/Wood Shavings
Oaky
Burnt/Smoked Wood

**VEGETATIVE/GREEN**

**VEGETABLES:**
Artichoke
Asparagus
Cabbage
Celery
Green Beans
Green Pepper
Olive
Celery

**FLORAL:**
Camomile
Honeysuckle
Orange Blossom
Geranium
Jasmine
Lilac
Rose
Lemon Blossom
Violets

**NUTS:**
Almond
Hazelnut
Walnut

**ANIMAL:**
Leather
Smoked
Musk
Cat Urine
Wet Dog

**MINERAL:**
Chalky
Iodine/Salty
Mineral/Flinty
Solvent/Chemical

**FRESH/PLANTLIKE:**
Eucalyptus
Herbaceous
Tomato Leaf
Green/Cut Grass
Lemon Grass
Mint

**DRIED:**
Hay/Dried Grass
Tobacco

**SPICY:**
Aniseed / Fennel
Cinnamon
Clove
Curry
Juniper
Ginger
Bay Leaf / Laurel
Nutmeg
Black Pepper
White Pepper
Liquorice
Thyme

**FOREST FLOOR:**
Humus/Earthy
Mouldy
Mushroom

**OTHER:**
Butter
Lactic
Sulphur
Stuffy
Dusty
Wet Mop
Yeast

**Figure B.2** The aromatic descriptors list provided to judges with the free sorting analysis method.

## Chemical analyses

**Table B.1** LS means table illustrating the treatment*time interaction of ethyl and acetate esters present in aged Chenin Blanc and Sauvignon Blanc with significant letters from LSD post-hoc test.

| ESTERS | | | Ethyl Acetate | | Ethyl Butyrate | | Isoamyl Acetate | | Ethyl Hexanoate | | Ethyl Lactate | | Ethyl Caprylate | | Ethyl Caprate | | Diethyl Succinate | | 2-Phenylethyl Acetate | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tmt | M | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL |
| Chenin Blanc 2015 | C | 3 | 68.87 ± 11.83 | abc | 0.10 ± 0.07 | bc | 3.42 ± 1.34 | bc | 0.51 ± 0.22 | b | 0 ± 0 | c | 0.47 ± 0.15 | b | 0.23 ± 0.11 | bc | 0 ± 0 | b | 0.49 ± 0.03 | bc |
| | C | 9 | 83.98 ± 12.62 | a | 0.31 ± 0.06 | a | 5.18 ± 0.65 | a | 0.89 ± 0.15 | a | 20.63 ± 3.64 | ab | 1.07 ± 0.14 | a | 0.49 ± 0.06 | a | 1.22 ± 0.22 | a | 0.66 ± 0.03 | a |
| | N | 3 | 59.73 ± 9.82 | c | 0.10 ± 0.08 | bc | 3.61 ± 1.03 | bc | 0.46 ± 0.25 | b | 0 ± 0 | c | 0.46 ± 0.20 | b | 0.19 ± 0.13 | bc | 0 ± 0 | b | 0.47 ± 0.08 | bcd |
| | N | 9 | 83.83 ± 16.96 | ab | 0.30 ± 0.03 | a | 5.59 ± 0.97 | a | 0.92 ± 0.09 | a | 17.59 ± 1.54 | b | 1.14 ± 0.15 | a | 0.43 ± 0.03 | a | 1.30 ± 0.18 | a | 0.70 ± 0.03 | a |
| | S | 3 | 50.44 ± 9.48 | c | 0.04 ± 0.02 | c | 1.82 ± 0.32 | d | 0.33 ± 0.09 | b | 0 ± 0 | c | 0.46 ± 0.05 | b | 0.09 ± 0.07 | c | 0 ± 0 | b | 0.38 ± 0.01 | d |
| | S | 9 | 64.34 ± 21.37 | abc | 0.15 ± 0.14 | b | 3.31 ± 1.59 | bc | 0.47 ± 0.41 | b | 19.76 ± 2.86 | ab | 0.65 ± 0.45 | b | 0.33 ± 0.22 | ab | 1.07 ± 0.35 | a | 0.53 ± 0.14 | b |
| | N+S | 3 | 62.87 ± 9.74 | bc | 0.01 ± 0.01 | c | 2.28 ± 0.49 | cd | 0.27 ± 0.11 | b | 0 ± 0 | c | 0.41 ± 0.10 | b | 0.08 ± 0.07 | c | 0 ± 0 | b | 0.41 ± 0.02 | cd |
| | N+S | 9 | 82.85 ± 13.18 | a | 0.17 ± 0.08 | b | 3.61 ± 0.83 | b | 0.49 ± 0.22 | b | 23.37 ± 4.77 | a | 0.65 ± 0.30 | b | 0.49 ± 0.14 | a | 1.12 ± 0.17 | a | 0.55 ± 0.05 | b |
| Chenin Blanc 2016 | C | 3 | 66.40 ± 7.78 | b | 0.62 ± 0.05 | ab | 4.43 ± 1.28 | ab | 1.10 ± 0.11 | abc | 7.54 ± 0.52 | bc | 0.85 ± 0.10 | bc | 0.67 ± 0.04 | ab | 1.32 ± 0.14 | abc | 0.58 ± 0.06 | ab |
| | C | 9 | 62.59 ± 5.56 | b | 0.64 ± 0.23 | ab | 4.65 ± 1.54 | ab | 1.42 ± 0.62 | a | 8.25 ± 0.62 | a | 1.74 ± 0.76 | a | 0.69 ± 0.15 | ab | 1.42 ± 0.58 | ab | 0.58 ± 0.11 | ab |
| | N | 3 | 78.08 ± 11.58 | a | 0.74 ± 0.22 | a | 5.40 ± 1.88 | ab | 1.31 ± 0.49 | ab | 7.68 ± 0.43 | abc | 0.96 ± 0.33 | bc | 0.77 ± 0.26 | a | 1.66 ± 0.51 | a | 0.59 ± 0.16 | a |
| | N | 9 | 63.47 ± 10.08 | b | 0.50 ± 0.08 | bc | 3.83 ± 1.37 | bcd | 1.02 ± 0.17 | abc | 7.78 ± 0.33 | abc | 1.34 ± 0.26 | ab | 0.57 ± 0.05 | bc | 1.13 ± 0.11 | bc | 0.49 ± 0.11 | bcd |
| | S | 3 | 49.47 ± 5.55 | c | 0.49 ± 0.11 | bc | 2.59 ± 0.72 | cd | 0.89 ± 0.24 | bc | 7.38 ± 0.17 | c | 0.88 ± 0.35 | bc | 0.57 ± 0.08 | bc | 1.30 ± 0.43 | abc | 0.44 ± 0.03 | cd |
| | S | 9 | 50.88 ± 2.66 | c | 0.50 ± 0.04 | bc | 2.51 ± 0.19 | cd | 1.04 ± 0.08 | abc | 7.99 ± 0.23 | ab | 1.28 ± 0.07 | abc | 0.60 ± 0.02 | abc | 1.53 ± 0.15 | ab | 0.43 ± 0.02 | cd |
| | N+S | 3 | 47.19 ± 5.29 | c | 0.31 ± 0.09 | c | 2.37 ± 0.17 | d | 0.67 ± 0.11 | c | 7.35 ± 0.39 | c | 0.76 ± 0.20 | c | 0.47 ± 0.07 | c | 0.89 ± 0.22 | c | 0.42 ± 0.03 | d |
| | N+S | 9 | 63.15 ± 4.54 | b | 0.62 ± 0.05 | ab | 4.06 ± 0.66 | abc | 1.26 ± 0.19 | ab | 8.23 ± 0.36 | a | 1.63 ± 0.24 | a | 0.68 ± 0.04 | ab | 1.53 ± 0.21 | ab | 0.51 ± 0.05 | abc |
| Sauvignon Blanc 2015 | C | 3 | 108.81 ± 38.42 | cd | 0.07 ± 0.09 | bc | 2.11 ± 0.78 | c | 0.32 ± 0.25 | b | 11.08 ± 0.49 | c | 0.44 ± 0.21 | c | 0.12 ± 0.11 | d | 0 ± 0 | c | 0.38 ± 0.04 | bc |
| | C | 9 | 96.41 ± 23.44 | de | 0.30 ± 0.08 | a | 1.24 ± 0.31 | d | 0.88 ± 0.53 | a | 53.15 ± 7.07 | a | 1.16 ± 0.38 | a | 0.77 ± 0.16 | a | 4.26 ± 0.88 | a | 0.36 ± 0.01 | cd |
| | N | 3 | 187.40 ± 32.14 | ab | 0.09 ± 0.03 | b | 2.86 ± 0.51 | a | 0.43 ± 0.05 | b | 11.78 ± 0.67 | c | 0.52 ± 0.03 | c | 0.17 ± 0.03 | cd | 0 ± 0 | c | 0.42 ± 0.01 | a |
| | N | 9 | 71.79 ± 30.37 | da | 0.04 ± 0.04 | c | 0.91 ± 0.25 | de | 0.53 ± 0.22 | ab | 45.68 ± 7.28 | b | 0.76 ± 0.23 | b | 0.43 ± 0.18 | b | 2.44 ± 0.15 | b | 0.34 ± 0.02 | de |
| | S | 3 | 161.55 ± 64.86 | bc | 0.04 ± 0.02 | bc | 2.20 ± 0.35 | bc | 0.3 ± 0.04 | b | 11.33 ± 0.90 | c | 0.45 ± 0.05 | c | 0.12 ± 0.04 | d | 0.06 ± 0.13 | c | 0.40 ± 0.01 | ab |
| | S | 9 | 50.21 ± 11.13 | e | 0.02 ± 0.03 | bc | 0.39 ± 0.16 | e | 0.28 ± 0.07 | b | 39.93 ± 6.12 | b | 0.54 ± 0.07 | bc | 0.21 ± 0.06 | cd | 2.62 ± 0.70 | b | 0 ± 0 | f |
| | N+S | 3 | 220.77 ± 35.91 | ab | 0.07 ± 0.04 | bc | 2.73 ± 0.30 | ab | 0.28 ± 0.04 | b | 12.65 ± 0.94 | c | 0.43 ± 0.05 | c | 0.11 ± 0.04 | d | 0.21 ± 0.14 | c | 0.39 ± 0.00 | ab |
| | N+S | 9 | 51.38 ± 8.79 | e | 0.05 ± 0.04 | bc | 0.57 ± 0.11 | e | 0.35 ± 0.07 | b | 44.76 ± 2.63 | b | 0.55 ± 0.09 | bc | 0.30 ± 0.07 | bc | 3.07 ± 0.29 | b | 0.32 ± 0.01 | e |
| Sauvignon Blanc 2016 | C | 3 | 29.51 ± 2.70 | e | 0.30 ± 0.05 | c | 0.88 ± 0.19 | c | 0.43 ± 0.08 | e | 22.61 ± 1.18 | c | 0.30 ± 0.07 | b | 0.45 ± 0.08 | c | 1.87 ± 0.23 | d | 0.35 ± 0.01 | d |
| | C | 9 | 41.11 ± 2.12 | abcd | 0.60 ± 0.08 | a | 1.34 ± 0.22 | bc | 1.36 ± 0.22 | a | 30.33 ± 1.24 | a | 1.18 ± 0.88 | a | 0.77 ± 0.12 | ab | 4.47 ± 0.56 | a | 0.38 ± 0.02 | c |
| | N | 3 | 39.20 ± 8.10 | bd | 0.47 ± 0.17 | b | 1.96 ± 0.95 | ab | 0.76 ± 0.32 | d | 23.21 ± 2.23 | c | 0.45 ± 0.23 | b | 0.63 ± 0.15 | b | 2.30 ± 0.35 | cd | 0.42 ± 0.05 | bc |
| | N | 9 | 43.70 ± 3.83 | ac | 0.62 ± 0.07 | a | 1.72 ± 0.37 | b | 1.21 ± 0.17 | abc | 27.92 ± 9.61 | abc | 0.53 ± 0.06 | b | 0.89 ± 0.12 | a | 4.88 ± 0.48 | a | 0.41 ± 0.02 | bc |
| | S | 3 | 45.42 ± 5.85 | abcd | 0.63 ± 0.05 | a | 2.51 ± 0.48 | a | 1.05 ± 0.13 | bcd | 23.92 ± 1.65 | bc | 0.66 ± 0.11 | ab | 0.77 ± 0.08 | ab | 2.72 ± 0.23 | bc | 0.46 ± 0.03 | a |
| | S | 9 | 40.92 ± 2.60 | cd | 0.63 ± 0.08 | a | 1.54 ± 0.18 | bc | 1.27 ± 0.21 | ab | 27.14 ± 2.77 | abc | 0.59 ± 0.10 | b | 0.79 ± 0.11 | a | 4.69 ± 0.75 | a | 0.4 ± 0.02 | bc |
| | N+S | 3 | 44.47 ± 2.53 | abcd | 0.61 ± 0.05 | a | 2.65 ± 0.59 | a | 0.95 ± 0.23 | cd | 27.91 ± 1.45 | abc | 0.65 ± 0.24 | b | 0.80 ± 0.04 | a | 3.11 ± 0.53 | b | 0.44 ± 0.02 | ab |
| | N+S | 9 | 42.68 ± 2.63 | abcd | 0.60 ± 0.05 | ab | 1.70 ± 0.15 | b | 1.09 ± 0.09 | abc | 29.81 ± 2.20 | ab | 0.51 ± 0.05 | b | 0.76 ± 0.04 | ab | 4.19 ± 0.58 | a | 0.39 ± 0.01 | c |

**Table B.2** LS means table illustrating the treatment*time interaction of acids and fatty acids present in aged Chenin Blanc and Sauvignon Blanc wines with significant letters from LSD post-hoc test.

| ACIDS | | | Propionic Acid | | Isobutyric acid | | Butyric Acid | | Iso-Valeric Acid | | Valeric Acid | | Hexanoic Acid | | Octanoic Acid | | Decanoic Acid | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tmt | M | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL |
| Chenin Blanc 2015 | C | 3 | 2.70 ± 0.57 | bc | 1.11 ± 0.23 | cd | 0.75 ± 0.5 | d | 0.82 ± 0.19 | b | 0 ± 0 | a | 4.12 ± 0.72 | cd | 5.79 ± 1.02 | b | 2.11 ± 0.28 | bc |
| | C | 9 | 3.07 ± 0.54 | bc | 1.43 ± 0.13 | ab | 1.57 ± 0.15 | ab | 1.50 ± 0.21 | a | 0 ± 0.00 | a | 6.20 ± 1.07 | ab | 9.15 ± 1.80 | a | 3.26 ± 0.46 | a |
| | N | 3 | 2.69 ± 0.46 | bc | 1.02 ± 0.23 | cd | 1.05 ± 0.14 | bcd | 0.68 ± 0.13 | b | 0 ± 0 | a | 3.82 ± 1.12 | cd | 5.33 ± 1.48 | b | 2.02 ± 0.34 | bc |
| | N | 9 | 2.95 ± 0.20 | bc | 1.45 ± 0.18 | ab | 1.76 ± 0.21 | a | 1.52 ± 0.19 | a | 0 ± 0 | a | 6.13 ± 0.55 | ab | 9.03 ± 0.86 | a | 3.05 ± 0.21 | a |
| | S | 3 | 2.27 ± 0.40 | c | 0.89 ± 0.16 | d | 1.16 ± 0.10 | bcd | 0.67 ± 0.13 | b | 0 ± 0 | a | 3.10 ± 0.47 | cd | 4.37 ± 0.43 | b | 1.80 ± 0.12 | bc |
| | S | 9 | 3.30 ± 0.69 | b | 1.26 ± 0.22 | bc | 1.28 ± 0.45 | abcd | 1.39 ± 0.30 | a | 0 ± 0 | a | 4.29 ± 1.93 | cd | 6.32 ± 2.95 | b | 2.32 ± 0.82 | bc |
| | N+S | 3 | 2.29 ± 0.53 | c | 0.86 ± 0.15 | cd | 0.87 ± 0.58 | cd | 0.64 ± 0.10 | b | 0 ± 0 | a | 2.90 ± 0.44 | d | 4.22 ± 0.61 | b | 1.76 ± 0.18 | c |
| | N+S | 9 | 4.60 ± 0.74 | a | 1.65 ± 0.28 | a | 1.31 ± 0.35 | abc | 1.66 ± 0.20 | a | 0 ± 0 | a | 4.56 ± 1.02 | bc | 6.47 ± 1.47 | b | 2.39 ± 0.38 | b |
| Chenin Blanc 2016 | C | 3 | 2.45 ± 0.17 | b | 1.62 ± 0.09 | abc | 0.31 ± 0.46 | b | 1.63 ± 0.26 | a | 0.44 ± 0.05 | b | 8.65 ± 0.58 | ab | 4.55 ± 0.14 | cd | 0.44 ± 0.02 | c |
| | C | 9 | 2.65 ± 0.13 | ab | 1.59 ± 0.26 | abc | 1.44 ± 0.49 | a | 1.56 ± 0.41 | ab | 0.51 ± 0.07 | ab | 8.25 ± 3.26 | abc | 10.03 ± 4.24 | a | 1.67 ± 0.68 | a |
| | N | 3 | 2.90 ± 0.68 | a | 1.83 ± 0.50 | a | 0.15 ± 0.30 | b | 1.66 ± 0.45 | a | 0.58 ± 0.17 | a | 9.41 ± 2.80 | a | 4.68 ± 1.34 | cd | 0.45 ± 0.04 | c |
| | N | 9 | 2.56 ± 0.13 | ab | 1.39 ± 0.25 | bc | 1.27 ± 0.14 | a | 1.20 ± 0.15 | b | 0.54 ± 0.08 | ab | 5.86 ± 0.71 | cd | 7.00 ± 1.01 | bc | 1.36 ± 0.19 | ab |
| | S | 3 | 2.51 ± 0.12 | ab | 1.47 ± 0.18 | abc | 0.34 ± 0.39 | b | 1.46 ± 0.27 | ab | 0.43 ± 0.02 | b | 6.56 ± 1.95 | bcd | 4.49 ± 1.72 | cd | 0.53 ± 0.15 | c |
| | S | 9 | 2.53 ± 0.08 | ab | 1.50 ± 0.12 | abc | 1.02 ± 0.18 | a | 1.47 ± 0.18 | ab | 0.49 ± 0.03 | ab | 6.36 ± 0.17 | bcd | 7.23 ± 0.56 | abc | 1.08 ± 0.24 | b |
| | N+S | 3 | 2.39 ± 0.33 | b | 1.26 ± 0.21 | c | 0.48 ± 0.32 | b | 1.17 ± 0.19 | b | 0.46 ± 0.10 | ab | 4.31 ± 0.56 | d | 3.71 ± 0.89 | d | 0.53 ± 0.08 | c |
| | N+S | 9 | 2.63 ± 0.08 | ab | 1.65 ± 0.05 | ab | 1.06 ± 0.15 | a | 1.65 ± 0.04 | a | 0.52 ± 0.05 | ab | 8.05 ± 1.17 | abc | 9.04 ± 1.74 | ab | 1.09 ± 0.21 | b |
| Sauvignon Blanc 2015 | C | 3 | 2.54 ± 0.27 | d | 0.87 ± 0.09 | bc | 0.25 ± 0.50 | cd | 0.74 ± 0.10 | b | 0 ± 0 | a | 2.88 ± 0.94 | cd | 4.17 ± 1.37 | bc | 1.86 ± 0.38 | b |
| | C | 9 | 4.51 ± 0.68 | a | 1.13 ± 0.21 | a | 1.33 ± 0.25 | a | 1.31 ± 0.20 | a | 0 ± 0 | a | 7.21 ± 1.19 | a | 8.75 ± 1.17 | a | 3.19 ± 0.59 | a |
| | N | 3 | 2.86 ± 0.17 | cd | 0.94 ± 0.11 | ab | 0.48 ± 0.56 | bcd | 0.92 ± 0.11 | b | 0 ± 0 | a | 3.14 ± 0.25 | cd | 4.52 ± 0.29 | bc | 1.93 ± 0.09 | b |
| | N | 9 | 3.74 ± 0.79 | b | 0.77 ± 0.15 | c | 1.24 ± 0.14 | a | 0.98 ± 0.21 | b | 0 ± 0 | a | 4.22 ± 1.14 | b | 5.32 ± 1.38 | b | 2.20 ± 0.46 | b |
| | S | 3 | 2.80 ± 0.32 | cd | 0.82 ± 0.13 | bc | 0.23 ± 0.46 | cd | 0.79 ± 0.12 | b | 0 ± 0 | a | 2.74 ± 0.17 | cd | 3.99 ± 0.25 | c | 1.80 ± 0.08 | b |
| | S | 9 | 3.04 ± 0.39 | cd | 0.75 ± 0.15 | bc | 1.05 ± 0.04 | ab | 0.86 ± 0.20 | b | 0 ± 0 | a | 3.03 ± 0.26 | cd | 3.91 ± 0.27 | c | 1.83 ± 0.10 | b |
| | N+S | 3 | 2.79 ± 0.08 | cd | 0.87 ± 0.03 | bc | 0 ± 0 | d | 0.85 ± 0.12 | b | 0 ± 0 | a | 2.56 ± 0.10 | d | 3.84 ± 0.21 | c | 1.77 ± 0.06 | b |
| | N+S | 9 | 3.25 ± 0.26 | bc | 0.80 ± 0.06 | bc | 0.81 ± 0.55 | abc | 0.95 ± 0.18 | b | 0 ± 0 | a | 3.46 ± 0.47 | bc | 4.63 ± 0.66 | bc | 2.02 ± 0.17 | b |
| Sauvignon Blanc 2016 | C | 3 | 2.21 ± 0.18 | e | 0.98 ± 0.05 | d | 0 ± 0 | c | 1.07 ± 0.11 | d | 0.15 ± 0.01 | cd | 3.44 ± 0.56 | c | 3.04 ± 0.40 | e | 0.47 ± 0.02 | c |
| | C | 9 | 2.74 ± 0.16 | abcd | 1.40 ± 0.10 | bc | 1.55 ± 1.29 | a | 1.68 ± 0.15 | abc | 0.16 ± 0.01 | bcd | 7.57 ± 1.21 | ab | 9.74 ± 1.34 | abc | 1.98 ± 0.45 | a |
| | N | 3 | 2.47 ± 0.28 | de | 1.26 ± 0.20 | c | 0.31 ± 0.35 | bc | 1.48 ± 0.25 | c | 0.18 ± 0.03 | abc | 6.29 ± 2.54 | b | 6.01 ± 2.62 | d | 0.60 ± 0.23 | c |
| | N | 9 | 2.96 ± 0.32 | ab | 1.59 ± 0.19 | a | 0.75 ± 0.05 | bc | 1.91 ± 0.21 | a | 0.17 ± 0.03 | abcd | 8.73 ± 1.18 | a | 10.99 ± 1.70 | ab | 1.63 ± 0.32 | ab |
| | S | 3 | 2.61 ± 0.13 | cd | 1.43 ± 0.04 | abc | 0.29 ± 0.34 | bc | 1.78 ± 0.12 | ab | 0.19 ± 0.01 | ab | 8.90 ± 0.97 | a | 8.83 ± 1.30 | bc | 0.66 ± 0.06 | c |
| | S | 9 | 2.76 ± 0.30 | abcd | 1.36 ± 0.14 | bc | 0.81 ± 0.08 | ab | 1.65 ± 0.17 | bc | 0.14 ± 0.02 | de | 9.01 ± 1.27 | a | 11.25 ± 1.25 | a | 1.71 ± 0.21 | ab |
| | N+S | 3 | 2.90 ± 0.21 | ac | 1.51 ± 0.12 | ab | 0.23 ± 0.3 | bc | 1.71 ± 0.06 | abc | 0.19 ± 0.02 | a | 8.40 ± 0.74 | a | 7.93 ± 1.33 | cd | 0.66 ± 0.09 | c |
| | N+S | 9 | 2.67 ± 0.14 | bd | 1.37 ± 0.06 | c | 0.72 ± 0.04 | bc | 1.60 ± 0.08 | bc | 0.12 ± 0.01 | e | 7.90 ± 0.32 | ab | 9.56 ± 0.52 | ab | 1.47 ± 0.13 | b |

**Table B.3** LS means table illustrating the treatment*time interaction of higher alcohols present in aged Chenin Blanc and Sauvignon Blanc with significant letters from LSD post-hoc test.

| HIGHER ALCOHOLS | | | Methanol | | Propanol | | Isobutanol | | Butanol | | Isoamyl alcohol | | Hexanol | | 3-ethoxy-1-Propanol | | 2-Phenyl Ethanol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tmt | M | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL | Mean ± SD (mg N/L) | SL |
| Chenin Blanc 2015 | C | 3 | 77.13 ± 17.09 | bcd | 67.09 ± 25.37 | a | 18.77 ± 3.77 | b | 0.80 ± 0.13 | bc | 132.14 ± 25.31 | b | 1.34 ± 0.20 | a | 8.64 ± 2.46 | ab | 23.08 ± 5.91 | bc |
| | C | 9 | 80.17 ± 14.32 | bc | 68.21 ± 17.33 | a | 33.64 ± 4.60 | a | 1.24 ± 0.16 | a | 240.33 ± 17.24 | a | 1.42 ± 0.05 | a | 8.13 ± 1.65 | ab | 34.00 ± 5.21 | a |
| | N | 3 | 58.52 ± 7.04 | d | 79.68 ± 17.11 | a | 17.99 ± 3.59 | b | 0.84 ± 0.17 | bc | 123.63 ± 24.26 | b | 1.30 ± 0.30 | a | 9.46 ± 2.62 | ab | 18.96 ± 4.96 | c |
| | N | 9 | 70.23 ± 10.89 | bcd | 55.71 ± 8.17 | a | 31.74 ± 1.73 | a | 1.26 ± 0.24 | a | 241.91 ± 13.67 | a | 1.46 ± 0.14 | a | 6.86 ± 1.20 | ab | 34.96 ± 5.21 | a |
| | S | 3 | 64.33 ± 10.50 | cd | 56.92 ± 12.94 | a | 18.81 ± 3.83 | b | 0.69 ± 0.10 | c | 126.41 ± 28.3 | b | 1.32 ± 0.31 | a | 6.45 ± 0.68 | b | 20.89 ± 4.00 | c |
| | S | 9 | 84.6 ± 23.89 | b | 61.82 ± 19.16 | a | 29.15 ± 4.33 | a | 1.08 ± 0.28 | ab | 201.18 ± 48.71 | a | 1.21 ± 0.34 | a | 8.12 ± 3.19 | ab | 30.14 ± 8.70 | ab |
| | N+S | 3 | 82.19 ± 9.48 | bc | 62.89 ± 18.87 | a | 17.72 ± 5.07 | b | 0.71 ± 0.13 | c | 114.66 ± 28.25 | b | 1.20 ± 0.25 | a | 7.20 ± 1.56 | ab | 18.85 ± 3.32 | c |
| | N+S | 9 | 110.23 ± 8.39 | a | 78.31 ± 14.19 | a | 35.02 ± 6.51 | a | 1.21 ± 0.18 | a | 222.75 ± 43.67 | a | 1.24 ± 0.19 | a | 9.78 ± 1.81 | a | 33.14 ± 6.49 | a |
| Chenin Blanc 2016 | C | 3 | 53.70 ± 4.00 | ab | 61.42 ± 16.89 | c | 22.67 ± 1.57 | abcd | 0.98 ± 0.07 | bc | 204.5 ± 18.34 | abc | 2.45 ± 0.19 | ab | 6.02 ± 0.96 | bcd | 35.31 ± 8.33 | abcd |
| | C | 9 | 57.13 ± 4.99 | ab | 68.07 ± 20.78 | bc | 23.55 ± 2.61 | abcd | 1.02 ± 0.05 | abc | 197.98 ± 44.69 | abc | 2.24 ± 0.63 | abc | 7.05 ± 1.23 | abcd | 33.94 ± 12.68 | abcd |
| | N | 3 | 57.91 ± 11.47 | ab | 91.33 ± 18.31 | a | 27.18 ± 9.13 | ac | 1.23 ± 0.31 | abc | 234.71 ± 80.01 | a | 2.80 ± 1.02 | a | 8.17 ± 2.36 | a | 34.32 ± 12.85 | ac |
| | N | 9 | 55.48 ± 3.16 | ab | 84.15 ± 11.16 | ab | 22.41 ± 3.28 | bd | 1.07 ± 0.16 | bc | 163.59 ± 23.65 | bc | 1.75 ± 0.27 | bc | 7.90 ± 1.72 | ab | 23.31 ± 4.15 | bd |
| | S | 3 | 50.74 ± 4.39 | b | 55.02 ± 8.38 | c | 23.03 ± 3.79 | abcd | 0.88 ± 0.07 | bc | 195.18 ± 53.47 | abc | 2.29 ± 0.92 | abc | 5.18 ± 0.47 | cd | 31.71 ± 7.97 | abcd |
| | S | 9 | 55.91 ± 1.68 | ab | 63.03 ± 9.93 | bc | 25.65 ± 2.74 | abcd | 0.96 ± 0.04 | bc | 202.10 ± 27.53 | abc | 2.15 ± 0.29 | abc | 6.16 ± 0.70 | abcd | 32.52 ± 5.05 | abcd |
| | N+S | 3 | 54.96 ± 3.14 | b | 63.57 ± 15.78 | bc | 21.10 ± 5.94 | cd | 0.88 ± 0.18 | c | 148.42 ± 39.42 | c | 1.49 ± 0.33 | c | 5.72 ± 1.27 | d | 23.22 ± 5.79 | cd |
| | N+S | 9 | 61.84 ± 3.88 | a | 72.42 ± 11.84 | abc | 26.88 ± 3.21 | ab | 1.07 ± 0.09 | ab | 223.49 ± 16.71 | ab | 2.56 ± 0.40 | ab | 6.84 ± 0.73 | abc | 35.68 ± 3.49 | ab |
| Sauvignon Blanc 2015 | C | 3 | 88.53 ± 20.72 | bc | 61.93 ± 9.51 | b | 17.64 ± 2.02 | b | 0.67 ± 0.05 | c | 138.02 ± 17.92 | b | 1.38 ± 0.15 | b | 8.03 ± 1.17 | c | 17.89 ± 2.26 | ab |
| | C | 9 | 85.57 ± 10.80 | bc | 103.04 ± 13.49 | a | 25.98 ± 3.10 | a | 1.51 ± 0.22 | a | 214.21 ± 25.27 | a | 1.91 ± 0.23 | a | 12.77 ± 2.64 | a | 23.26 ± 7.63 | ab |
| | N | 3 | 98.77 ± 4.80 | ab | 63.06 ± 7.81 | b | 18.28 ± 2.63 | b | 0.72 ± 0.04 | c | 141.48 ± 22.32 | b | 1.32 ± 0.26 | bc | 8.09 ± 1.09 | c | 17.80 ± 3.99 | ab |
| | N | 9 | 88.76 ± 17.47 | bc | 92.66 ± 21.96 | a | 19.64 ± 4.11 | b | 1.33 ± 0.34 | a | 146.35 ± 26.23 | b | 1.23 ± 0.22 | bc | 12.10 ± 3.66 | ab | 16.04 ± 2.57 | b |
| | S | 3 | 105.10 ± 23.16 | ab | 57.46 ± 6.15 | b | 15.98 ± 2.06 | b | 0.64 ± 0.03 | c | 120.42 ± 15.47 | b | 1.16 ± 0.13 | bc | 7.83 ± 0.82 | c | 16.07 ± 1.47 | b |
| | S | 9 | 69.37 ± 5.16 | c | 77.54 ± 21.98 | ab | 17.37 ± 3.15 | b | 0.88 ± 0.17 | bc | 117.49 ± 17.46 | b | 1.04 ± 0.16 | c | 8.97 ± 2.36 | c | 14.51 ± 3.93 | b |
| | N+S | 3 | 122.05 ± 27.33 | a | 56.13 ± 37.74 | b | 16.01 ± 2.18 | b | 0.76 ± 0.06 | c | 118.94 ± 18.68 | b | 1.18 ± 0.20 | bc | 9.17 ± 0.81 | bc | 13.11 ± 2.18 | b |
| | N+S | 9 | 72.56 ± 9.92 | c | 80.52 ± 13.20 | ab | 18.69 ± 2.32 | b | 1.07 ± 0.09 | b | 142.37 ± 20.78 | b | 1.20 ± 0.17 | bc | 9.31 ± 1.40 | bc | 18.29 ± 5.19 | ab |
| Sauvignon Blanc 2016 | C | 3 | 53.86 ± 4.23 | ef | 56.97 ± 4.39 | abc | 18.26 ± 2.47 | d | 0.73 ± 0.05 | e | 152.75 ± 14.84 | d | 1.49 ± 0.11 | b | 6.44 ± 0.63 | c | 19.37 ± 1.54 | d |
| | C | 9 | 66.66 ± 7.09 | ab | 73.51 ± 7.04 | ab | 26.54 ± 3.83 | ab | 0.97 ± 0.06 | abcd | 268.85 ± 30.67 | ab | 3.00 ± 0.30 | a | 8.47 ± 0.73 | ab | 33.95 ± 2.65 | ab |
| | N | 3 | 56.16 ± 4.29 | cdef | 59.14 ± 13.73 | abc | 21.30 ± 2.32 | cd | 0.88 ± 0.13 | cde | 206.64 ± 43.01 | c | 2.12 ± 0.62 | b | 6.69 ± 1.53 | c | 25.75 ± 2.96 | c |
| | N | 9 | 68.80 ± 3.77 | a | 75.00 ± 19.17 | a | 27.88 ± 3.90 | a | 1.09 ± 0.15 | ab | 286.24 ± 43.73 | ab | 3.17 ± 0.64 | a | 8.98 ± 2.32 | a | 35.94 ± 5.27 | a |
| | S | 3 | 55.90 ± 1.83 | df | 56.67 ± 1.71 | abc | 24.25 ± 1.92 | abc | 0.89 ± 0.08 | d | 260.89 ± 21.75 | ab | 2.91 ± 0.25 | a | 6.64 ± 0.35 | c | 35.36 ± 3.82 | a |
| | S | 9 | 60.70 ± 2.63 | bce | 43.29 ± 34.96 | c | 23.58 ± 2.28 | bc | 0.97 ± 0.13 | bc | 250.23 ± 27.78 | abc | 2.99 ± 0.42 | a | 7.26 ± 0.77 | bc | 34.31 ± 4.11 | ab |
| | N+S | 3 | 58.59 ± 6.66 | cdef | 75.83 ± 5.74 | a | 23.71 ± 1.35 | bc | 1.12 ± 0.07 | a | 252.63 ± 18.95 | ab | 2.96 ± 0.33 | a | 8.66 ± 0.55 | ab | 29.17 ± 3.13 | bc |
| | N+S | 9 | 61.76 ± 6.06 | abcd | 48.10 ± 19.99 | bc | 23.16 ± 1.65 | bc | 1.08 ± 0.07 | ab | 237.87 ± 23.33 | bc | 2.84 ± 0.46 | a | 8.43 ± 0.48 | ab | 27.29 ± 3.49 | c |

### *PCA's of the overall volatile content*

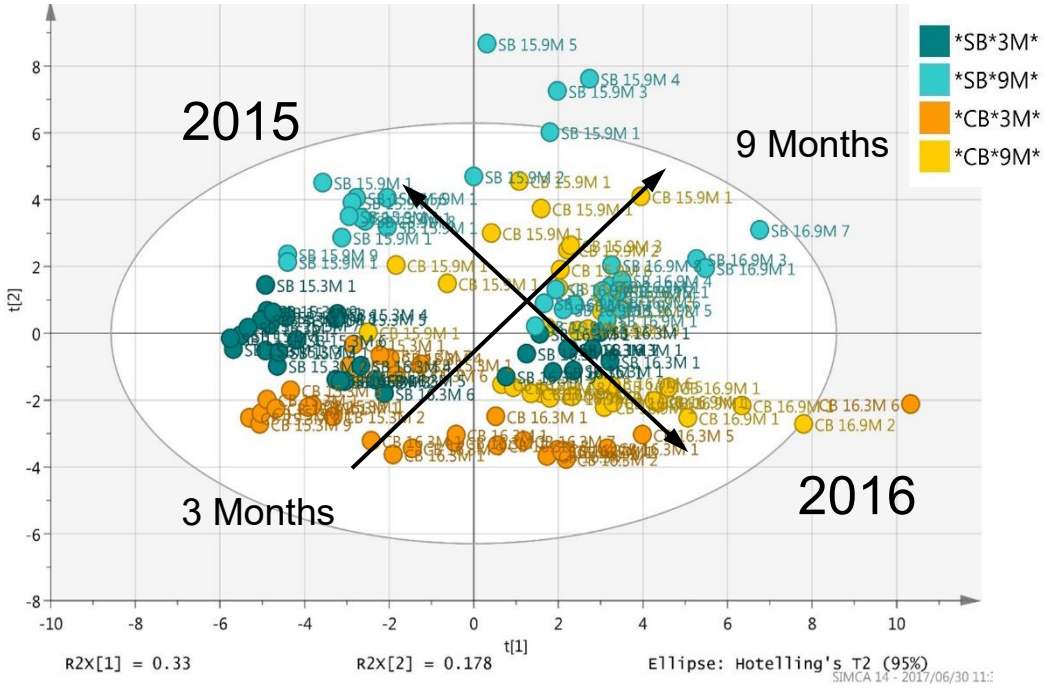


**Figure B.3** PCA-X plot distribution of the volatile composition of 3 and 9-month old Chenin Blanc and Sauvignon Blanc wines of 2015 and 2016.

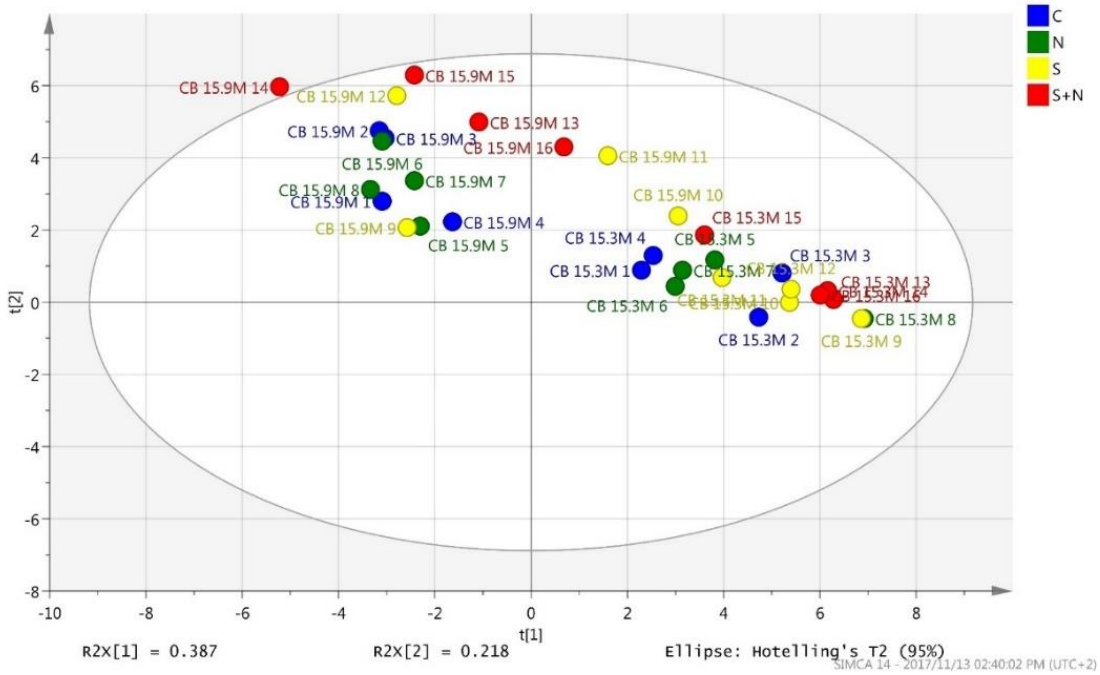


**Figure B.4** PCA-X plot distribution of the volatile composition of 3 and 9-month old Chenin Blanc wines of 2015.

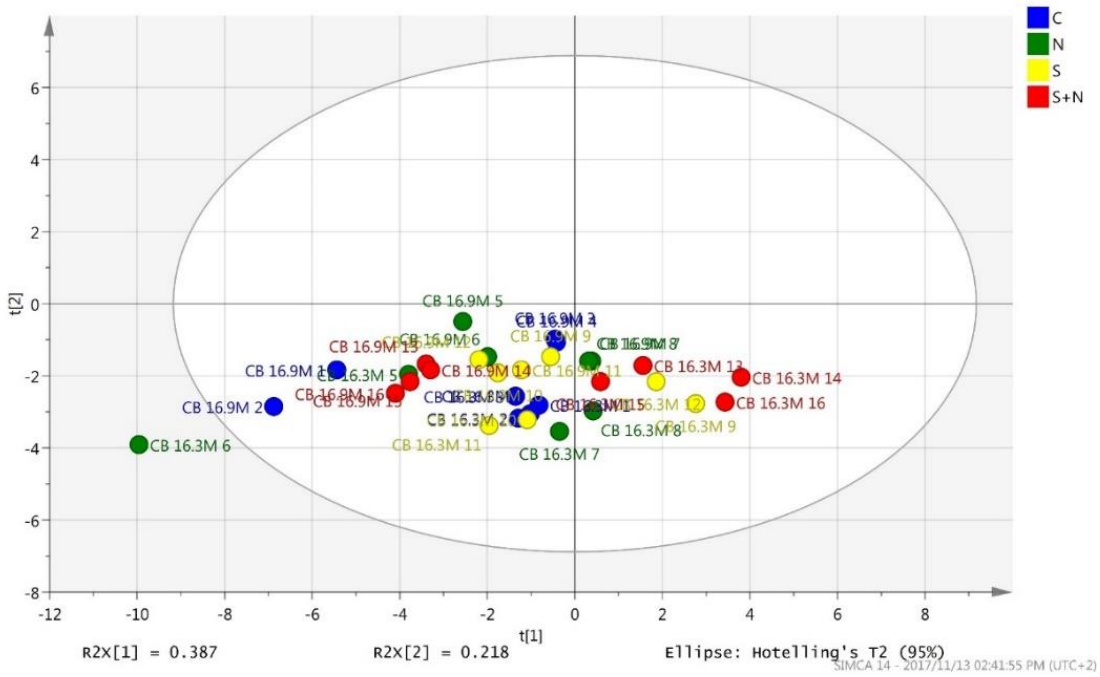


**Figure B.5** PCA-X plot distribution of the volatile composition of 3 and 9-month old Chenin Blanc wines of 2016.

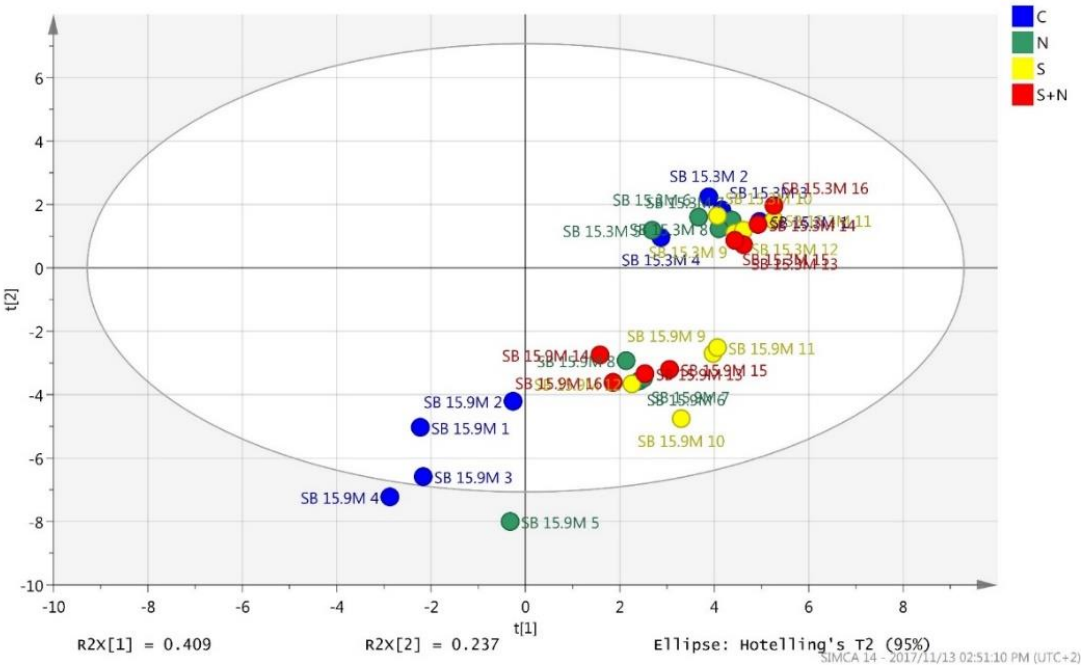


**Figure B.6** PCA-X plot distribution of the volatile composition of 3 and 9-month old Sauvignon Blanc wines of 2015.

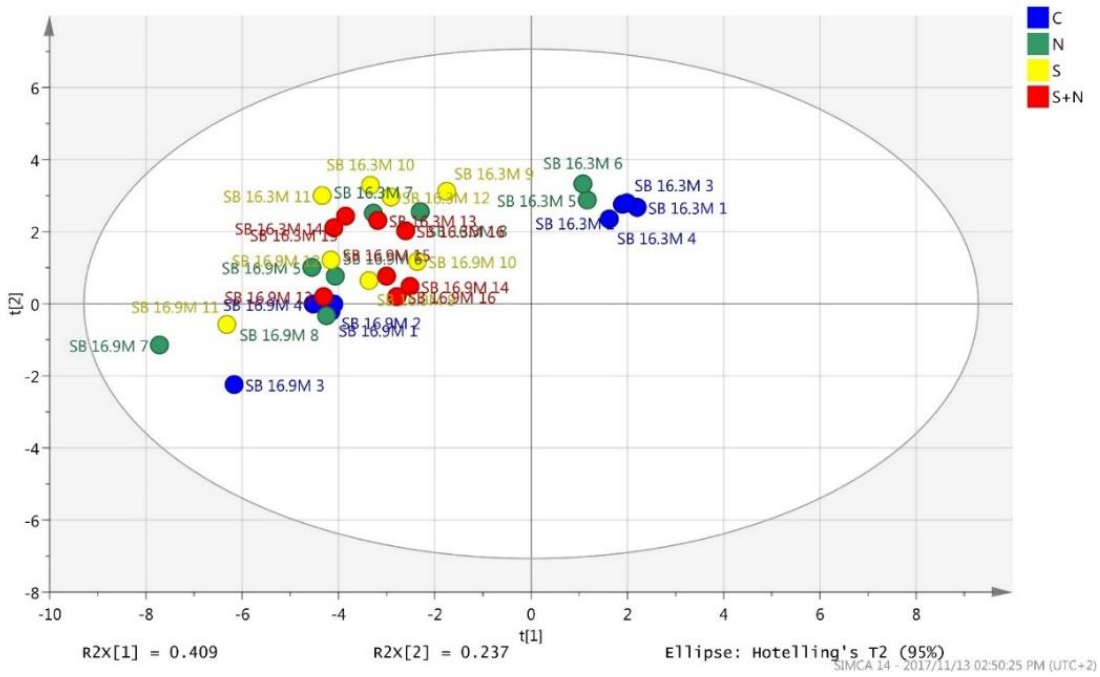


**Figure B.7** PCA-X plot distribution of the volatile composition of 3 and 9-month old Sauvignon Blanc wines of 2016.

## Sensory analysis

### *Dendrograms, scatter plots and bi-plots*

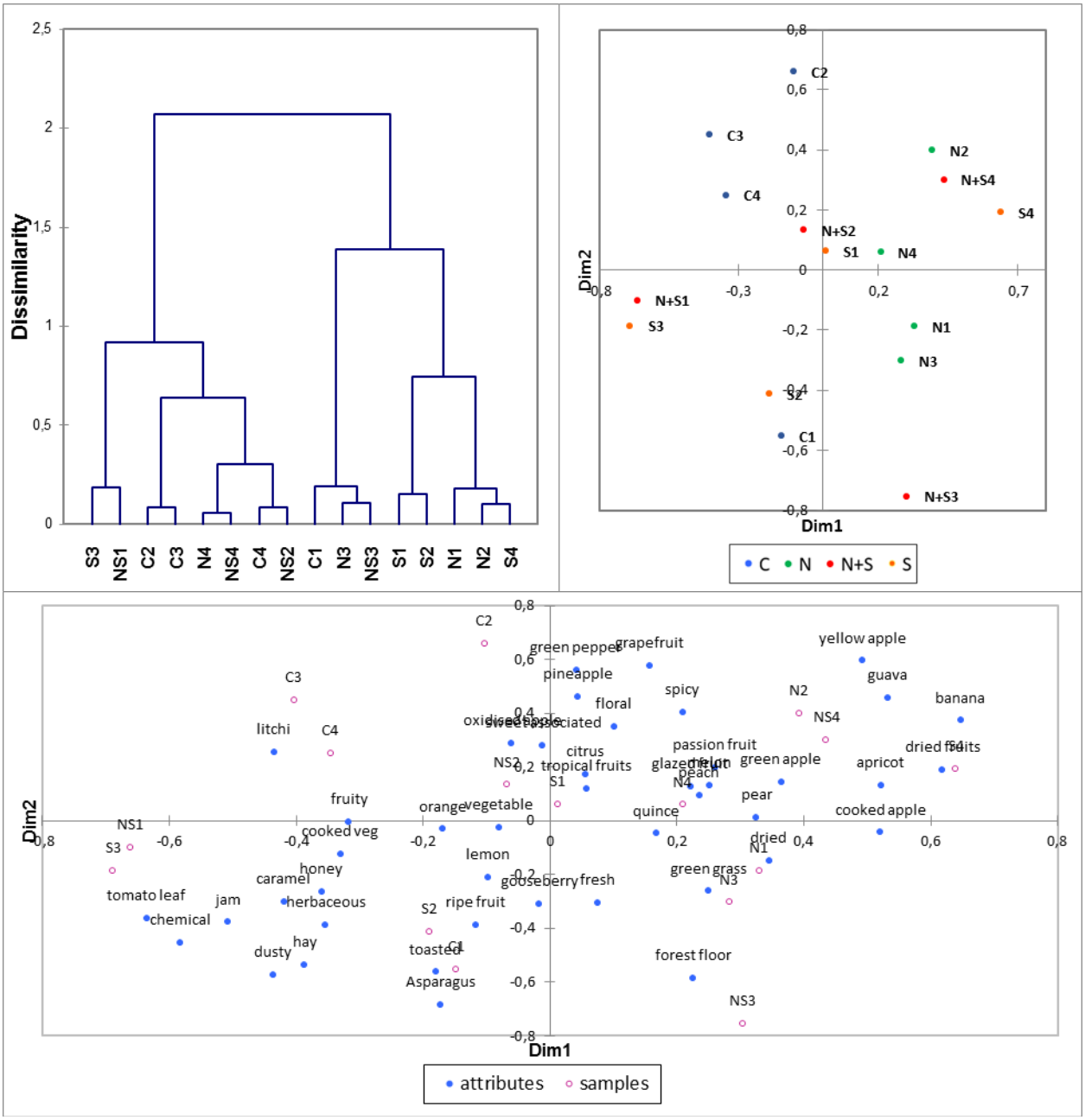


**Figure B.8** A dendrogram (left top), scatter plot (right top), and bi-plot (below) illustrating the sensory free sorting of 3-month aged Chenin Blanc wines of 2015.

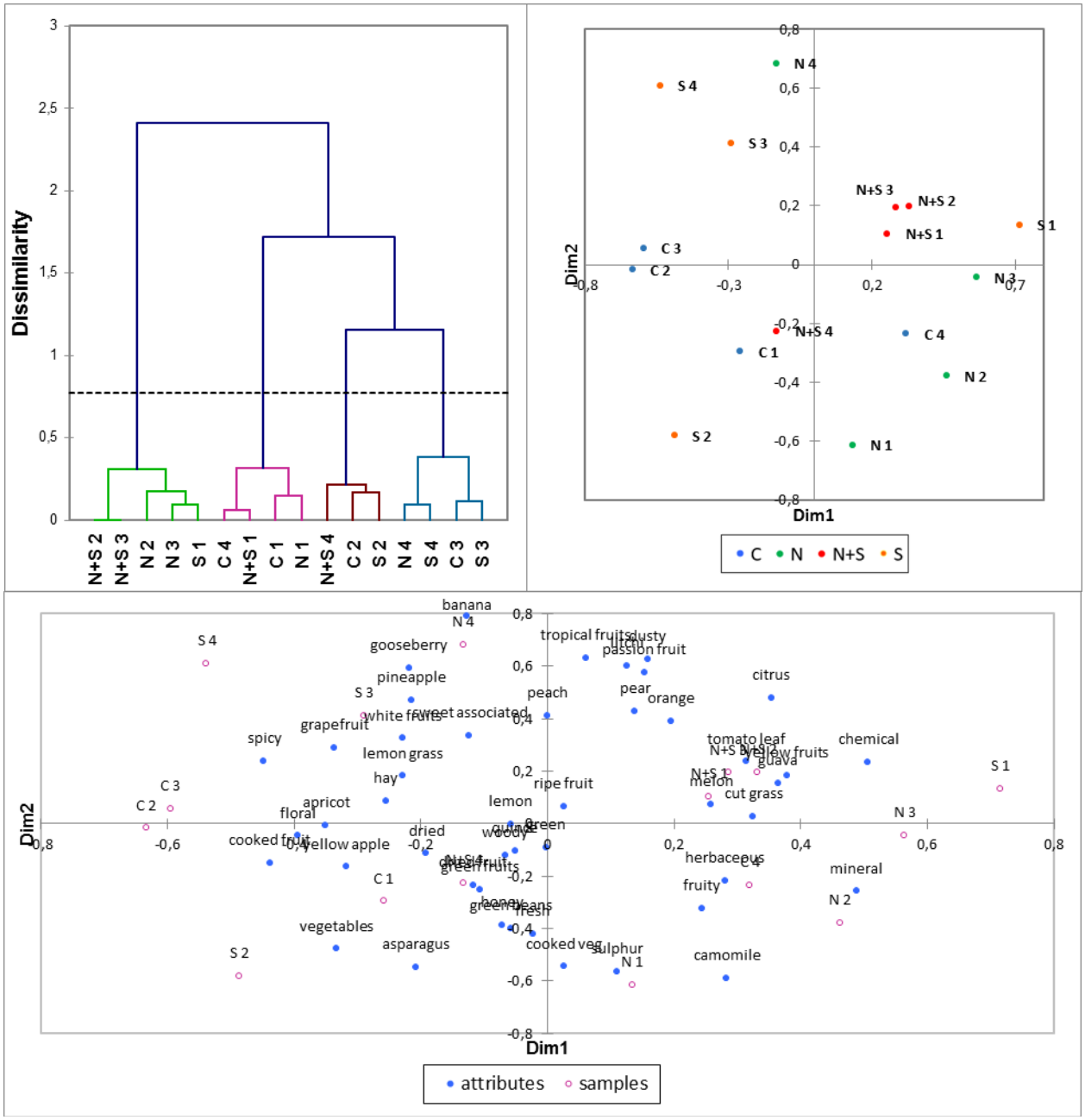


**Figure B.9** A dendrogram (left top), scatter plot (right top), and bi-plot (below) illustrating the sensory free sorting of 9-month aged Chenin Blanc wines of 2015.

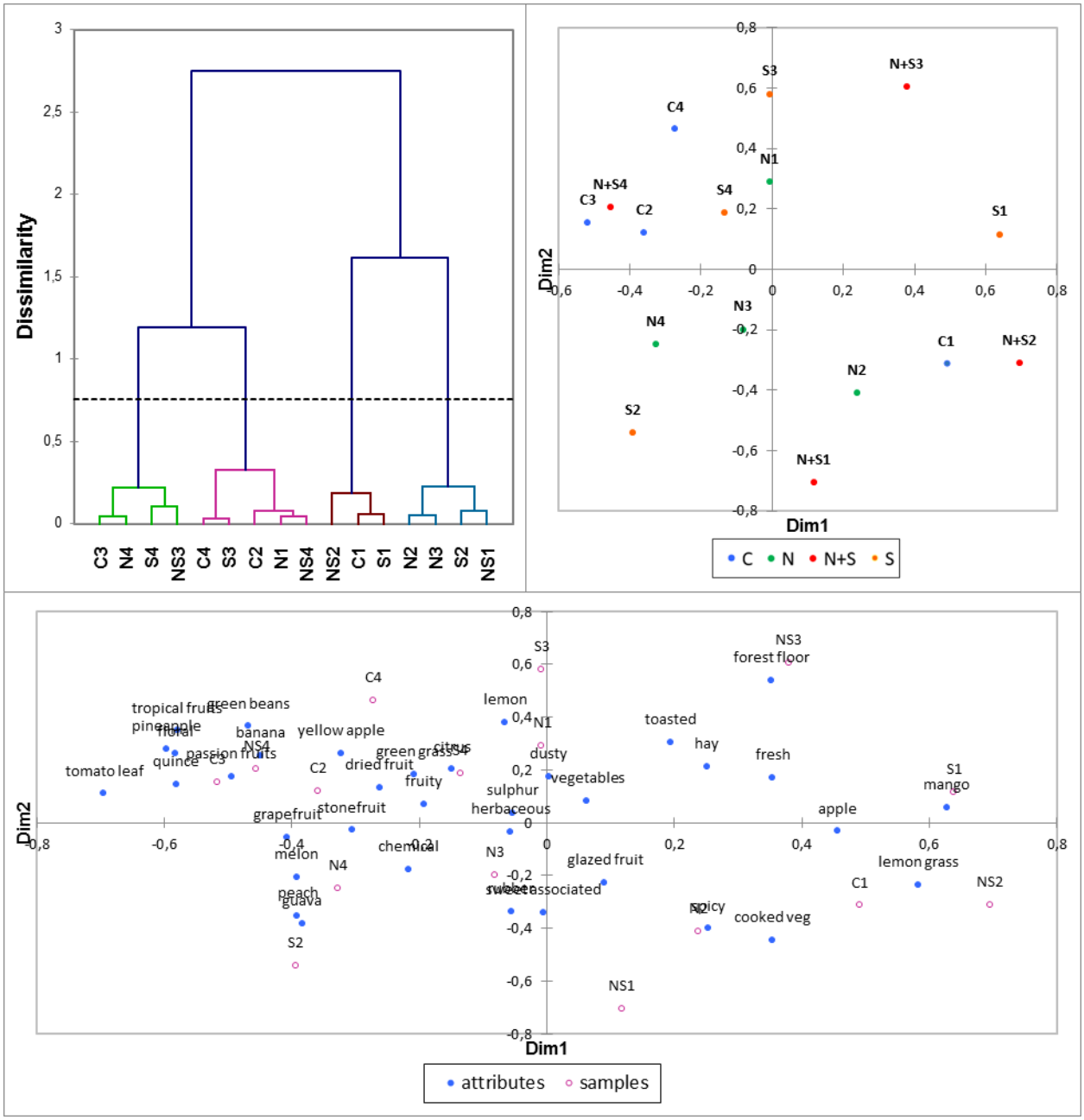


**Figure B.10** A dendrogram (top left), scatter plot (top right), and bi-plot (below) illustrating the sensory free sorting of 3-month aged Sauvignon Blanc 2015.

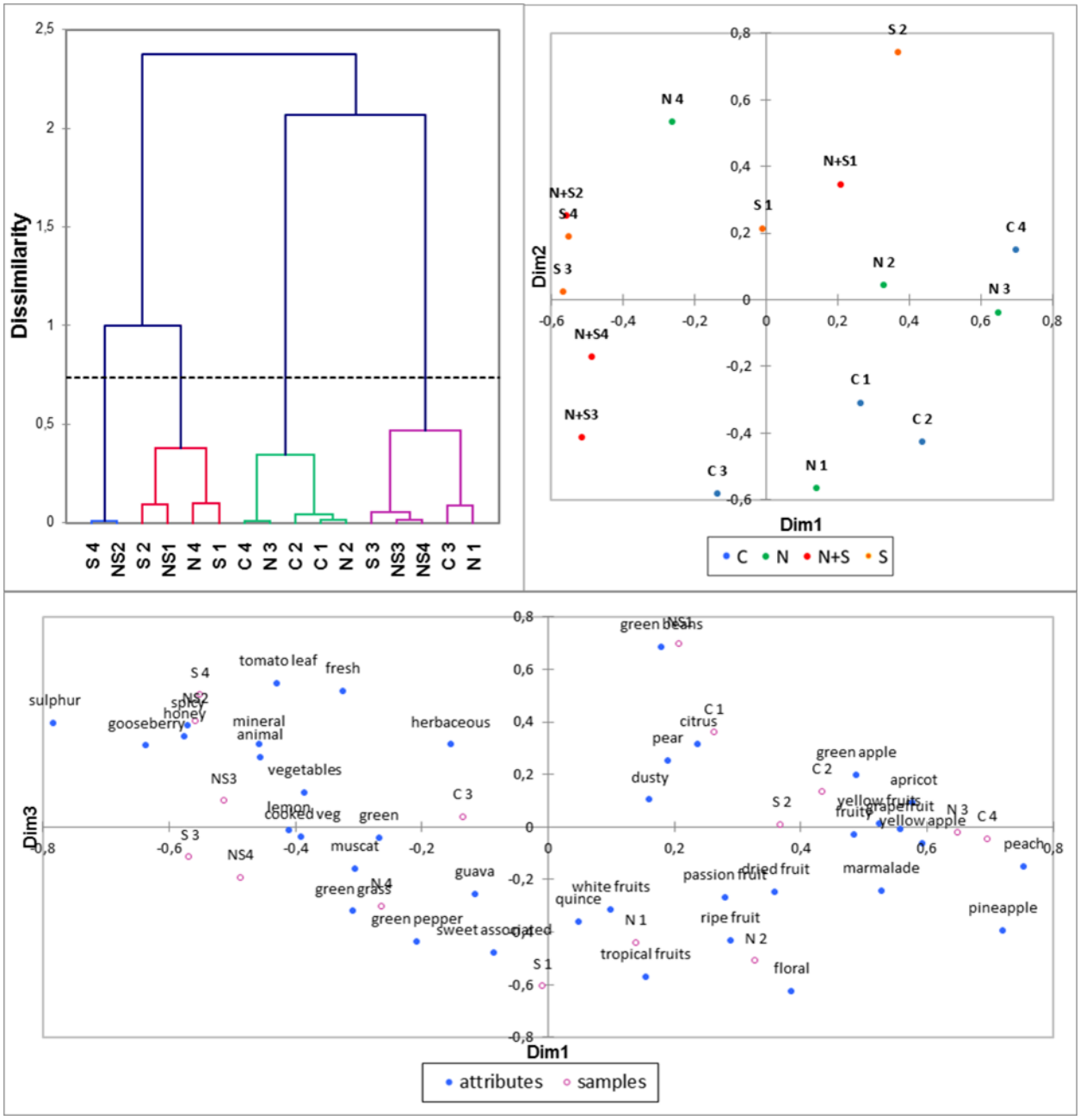


**Figure B.11** A dendrogram (top left), scatter plot (top right), and bi-plot (below) illustrating the sensory free sorting of 9-month aged Sauvignon Blanc 2015.

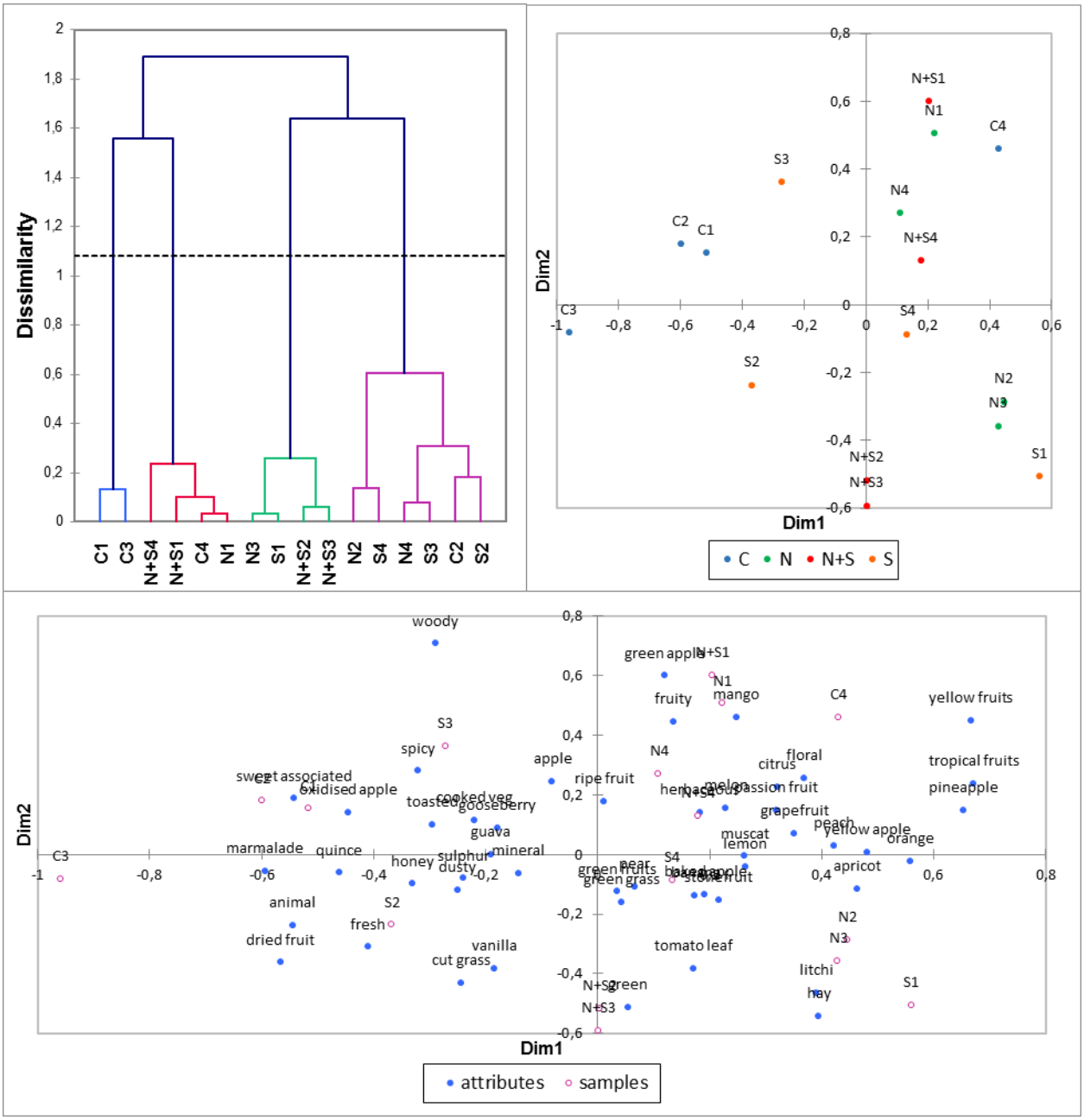


**Figure B.12** A dendrogram (left top), scatter plot (right top), and bi-plot (below) illustrating the sensory free sorting of 3-month aged Sauvignon Blanc wines of 2016.

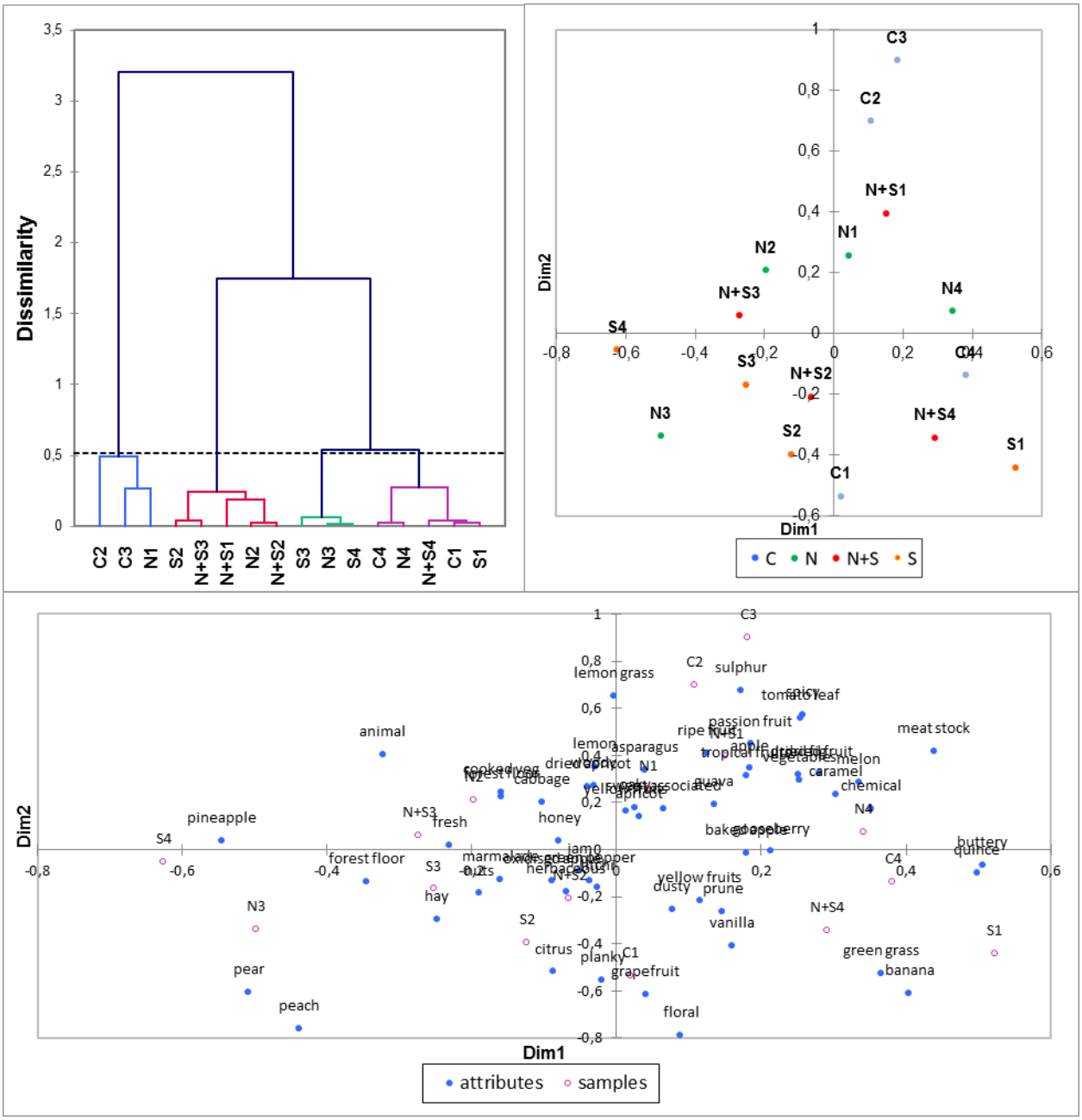


**Figure B.13** A dendrogram (left top), scatter plot (right top), and bi-plot (below) illustrating the sensory free sorting of 9-month aged Sauvignon Blanc wines of 2016.

## *Frequency of aroma citations*

**Table B.4** Top ten aromas of Chenin Blanc and Sauvignon Blanc matured wines made from different foliar fertilization treatments.

| Chenin Blanc 2015 - 3-month aged wines | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C 1** | | **C 2** | | **C 3** | | **C 4** | | **N 1** | | **N 2** | | **N 3** | | **N 4** | |
| **Aroma** | **F** | **Aroma** | **F** | **Aroma** | **F** | **Aroma** | **F** | **Aroma** | **F** | **Aroma** | **F** | **Aroma** | **F** | **Aroma** | **F** |
| passion fruit | 14 | pineapple | 13 | pineapple | 12 | grapefruit | 14 | guava | 14 | grapefruit | 14 | pineapple | 11 | passion fruit | 16 |
| pineapple | 10 | guava | 13 | passion fruit | 12 | pineapple | 13 | grapefruit | 11 | guava | 11 | passion fruit | 11 | pineapple | 15 |
| grapefruit | 9 | grapefruit | 11 | guava | 11 | guava | 12 | pineapple | 10 | pineapple | 10 | guava | 10 | peach | 10 |
| guava | 9 | passion fruit | 11 | grapefruit | 10 | passion fruit | 12 | passion fruit | 9 | peach | 9 | grapefruit | 9 | guava | 10 |
| lemon | 6 | lemon | 7 | peach | 8 | lemon | 6 | pear | 7 | yellow apple | 7 | lemon | 7 | yellow apple | 8 |
| peach | 5 | peach | 6 | floral | 6 | floral | 6 | peach | 7 | passion fruit | 7 | peach | 6 | grapefruit | 8 |
| sweet associated | 5 | green pepper | 5 | yellow apple | 5 | fruity | 5 | lemon | 7 | cooked veg | 6 | pear | 4 | lemon | 6 |
| fresh | 5 | yellow apple | 4 | banana | 5 | yellow apple | 5 | yellow apple | 6 | apricot | 5 | yellow apple | 4 | sweet associated | 6 |
| herbaceous | 5 | green apple | 4 | quince | 4 | banana | 5 | green apple | 6 | banana | 5 | apricot | 4 | dried fruits | 5 |
| floral | 5 | apricot | 4 | lemon | 4 | fresh | 5 | dried fruits | 5 | fruity | 4 | banana | 4 | green grass | 5 |
| **S 1** | | **S 2** | | **S 3** | | **S 4** | | **N+S 1** | | **N+S 2** | | **N+S 3** | | **N+S 4** | |
| pineapple | 15 | cooked veg | 10 | pineapple | 15 | pineapple | 17 | pineapple | 11 | passion fruit | 12 | guava | 10 | guava | 15 |
| guava | 15 | herbaceous | 10 | passion fruit | 13 | passion fruit | 17 | grapefruit | 9 | pineapple | 11 | passion fruit | 10 | pineapple | 12 |
| grapefruit | 14 | guava | 9 | grapefruit | 11 | guava | 15 | herbaceous | 8 | guava | 11 | pineapple | 9 | grapefruit | 11 |
| passion fruit | 11 | grapefruit | 8 | guava | 11 | grapefruit | 13 | peach | 7 | grapefruit | 10 | grapefruit | 8 | passion fruit | 11 |
| green apple | 6 | pineapple | 7 | hay | 5 | banana | 9 | lemon | 7 | lemon | 8 | peach | 7 | banana | 7 |
| peach | 6 | vegetable | 7 | chemical | 5 | floral | 9 | guava | 6 | yellow apple | 5 | lemon | 6 | yellow apple | 6 |
| floral | 6 | peach | 6 | fruity | 4 | yellow apple | 5 | fruity | 5 | dried fruits | 5 | fresh | 6 | spicy | 6 |
| yellow apple | 5 | chemical | 6 | peach | 4 | peach | 5 | cooked veg | 5 | green grass | 5 | green grass | 6 | green apple | 5 |
| citrus | 5 | lemon | 5 | lemon | 4 | melon | 4 | dusty | 5 | floral | 5 | apricot | 5 | peach | 5 |
| lemon | 5 | toasted | 4 | sweet associated | 4 | lemon | 4 | passion fruit | 4 | peach | 4 | banana | 5 | lemon | 5 |
| **Chenin Blanc 2015 - 9-month aged wines** | | | | | | | | | | | | | |
| **C 1** | | **C 2** | | **C 3** | | **C 4** | | **N 1** | | **N 2** | | **N 3** | | **N 4** | |
| grapefruit | 11 | pineapple | 12 | pineapple | 14 | pineapple | 13 | grapefruit | 13 | grapefruit | 8 | pineapple | 11 | grapefruit | 14 |
| pineapple | 9 | grapefruit | 9 | grapefruit | 11 | guava | 10 | pineapple | 12 | guava | 8 | grapefruit | 8 | pineapple | 14 |
| guava | 8 | yellow apple | 8 | yellow apple | 11 | grapefruit | 9 | yellow apple | 10 | pineapple | 7 | herbaceous | 8 | tropical fruits | 10 |
| lemon | 7 | gooseberry | 6 | tropical fruits | 9 | lemon | 9 | peach | 8 | sulphur | 7 | passion fruit | 8 | guava | 9 |
| green | 6 | guava | 6 | peach | 8 | passion fruit | 8 | tropical fruits | 8 | yellow fruits | 6 | tropical fruits | 8 | yellow fruits | 9 |
| herbaceous | 6 | lemon | 6 | gooseberry | 6 | tropical fruits | 7 | guava | 7 | cooked veg | 5 | citrus | 7 | gooseberry | 8 |
| yellow apple | 6 | passion fruit | 6 | guava | 6 | cut grass | 5 | passion fruit | 7 | herbaceous | 5 | lemon | 7 | banana | 7 |
| fresh | 5 | peach | 6 | banana | 5 | peach | 5 | gooseberry | 6 | peach | 5 | banana | 6 | passion fruit | 7 |
| green beans | 5 | yellow fruits | 6 | lemon | 5 | yellow fruits | 5 | lemon | 5 | yellow apple | 5 | guava | 6 | peach | 7 |
| peach | 5 | banana | 5 | passion fruit | 5 | banana | 4 | yellow fruits | 5 | green beans | 4 | peach | 6 | yellow apple | 5 |
| **S 1** | | **S 2** | | **S 3** | | **S 4** | | **N+S 1** | | **N+S 2** | | **N+S 3** | | **N+S 4** | |
| grapefruit | 10 | cooked veg | 10 | pineapple | 14 | grapefruit | 12 | guava | 11 | grapefruit | 10 | peach | 11 | pineapple | 13 |
| guava | 10 | grapefruit | 10 | tropical fruits | 11 | pineapple | 12 | grapefruit | 9 | guava | 10 | guava | 9 | guava | 10 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pineapple | 10 | guava | 9 | grapefruit | 10 | passion fruit | 11 | pineapple | 8 | pineapple | 10 | pineapple | 9 | passion fruit | 10 |
| tropical fruits | 9 | sulphur | 9 | passion fruit | 10 | guava | 8 | cut grass | 7 | passion fruit | 9 | grapefruit | 8 | lemon | 9 |
| herbaceous | 7 | green beans | 7 | banana | 9 | tropical fruits | 8 | passion fruit | 7 | lemon | 7 | sweet associated | 8 | grapefruit | 7 |
| passion fruit | 7 | fresh | 5 | guava | 9 | lemon | 7 | yellow fruits | 7 | peach | 6 | passion fruit | 7 | herbaceous | 7 |
| yellow apple | 7 | herbaceous | 5 | gooseberry | 8 | peach | 7 | tropical fruits | 6 | tropical fruits | 6 | tropical fruits | 6 | yellow apple | 7 |
| gooseberry | 5 | vegetables | 5 | lemon | 8 | banana | 6 | yellow apple | 6 | yellow apple | 6 | yellow apple | 5 | peach | 6 |
| lemon | 5 | yellow apple | 5 | peach | 7 | yellow apple | 6 | peach | 5 | yellow fruits | 6 | banana | 4 | tropical fruits | 6 |
| peach | 5 | cut grass | 4 | yellow apple | 6 | citrus | 5 | cooked veg | 4 | herbaceous | 5 | camomile | 4 | green | 4 |

## Chenin Blanc 2016 - 3-month aged wines

| C 1 | | C 2 | | C 3 | | C 4 | | N 1 | | N 2 | | N 3 | | N 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pineapple | 12 | pineapple | 12 | pineapple | 15 | passion fruit | 9 | pineapple | 19 | pineapple | 8 | pineapple | 11 | pineapple | 10 |
| passion fruit | 9 | passion fruit | 10 | passion fruit | 9 | banana | 7 | passion fruit | 17 | quince | 7 | floral | 7 | passion fruit | 8 |
| lemon | 8 | guava | 9 | floral | 8 | pineapple | 7 | banana | 14 | dried fruit | 6 | passion fruit | 7 | peach | 6 |
| guava | 6 | gooseberry | 6 | sweet associated | 7 | yellow apple | 7 | peach | 13 | passion fruit | 5 | tropical fruits | 7 | yellow apple | 6 |
| sweet associated | 5 | sweet associated | 6 | fresh | 6 | guava | 6 | yellow apple | 12 | ripe fruit | 5 | sweet associated | 6 | apple | 5 |
| gooseberry | 5 | apple | 5 | guava | 6 | peach | 6 | floral | 11 | sweet associated | 5 | dried fruit | 5 | dried | 5 |
| fresh | 5 | fresh | 5 | apple | 5 | floral | 5 | sweet associated | 10 | yellow apple | 5 | mango | 5 | floral | 5 |
| citrus | 5 | lemon | 5 | banana | 5 | ripe fruit | 5 | guava | 9 | apple | 4 | peach | 5 | herbaceous | 5 |
| yellow apple | 4 | citrus | 4 | citrus | 5 | apple | 4 | tropical fruits | 9 | guava | 4 | apple | 4 | lemon | 5 |
| quince | 4 | floral | 4 | gooseberry | 5 | dried | 4 | apple | 7 | lemon | 4 | citrus | 4 | ripe fruit | 5 |
| **S 1** | | **S 2** | | **S 3** | | **S 4** | | **N+S 1** | | **N+S 2** | | **N+S 3** | | **N+S 4** | |
| pineapple | 22 | pineapple | 11 | pineapple | 10 | pineapple | 8 | pineapple | 22 | pineapple | 13 | pineapple | 8 | pineapple | 15 |
| passion fruit | 16 | passion fruit | 8 | passion fruit | 7 | apple | 6 | passion fruit | 17 | passion fruit | 9 | passion fruit | 6 | sweet associated | 10 |
| tropical fruits | 12 | sweet associated | 7 | lemon | 6 | fresh | 6 | sweet associated | 13 | guava | 8 | sweet associated | 6 | gooseberry | 7 |
| peach | 11 | lemon | 6 | cooked veg | 5 | lemon | 6 | citrus | 10 | sweet associated | 8 | apple | 5 | passion fruit | 6 |
| yellow apple | 11 | sulphur | 6 | guava | 5 | passion fruit | 6 | fresh | 10 | yellow apple | 7 | citrus | 5 | banana | 5 |
| floral | 10 | citrus | 5 | sulphur | 5 | citrus | 5 | apple | 9 | peach | 6 | cooked veg | 5 | hay | 5 |
| guava | 10 | cooked veg | 5 | apple | 4 | sweet associated | 5 | floral | 9 | banana | 5 | hay | 5 | lemon | 5 |
| dried | 9 | gooseberry | 5 | banana | 4 | tropical fruits | 5 | peach | 9 | dried fruit | 4 | sulphur | 5 | ripe fruit | 5 |
| dried fruit | 9 | guava | 5 | citrus | 4 | cooked veg | 4 | dried fruit | 8 | floral | 4 | woody | 5 | apple | 4 |
| sweet associated | 9 | apple | 4 | herbaceous | 4 | floral | 4 | guava | 8 | fruity | 4 | guava | 4 | citrus | 4 |

## Chenin Blanc 2016 - 9-month aged wines

| C 1 | | C 2 | | C 3 | | C 4 | | N 1 | N 2 | | N 3 | | N 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pineapple | 21 | pineapple | 18 | pineapple | 17 | pineapple | 18 | | pineapple | 21 | pineapple | 21 | pineapple | 16 |
| grapefruit | 10 | honey | 10 | grapefruit | 9 | dried fruit | 12 | | peach | 14 | guava | 9 | peach | 9 |
| passion fruit | 10 | peach | 10 | honey | 8 | peach | 10 | | grapefruit | 9 | passion fruit | 9 | grapefruit | 8 |
| guava | 8 | dried fruit | 8 | peach | 8 | grapefruit | 9 | | honey | 8 | grapefruit | 8 | passion fruit | 8 |
| peach | 8 | grapefruit | 8 | caramel | 7 | passion fruit | 9 | | passion fruit | 8 | banana | 6 | guava | 7 |
| honey | 7 | guava | 8 | dried fruit | 7 | guava | 8 | | banana | 7 | lemon | 6 | banana | 6 |
| banana | 6 | passion fruit | 7 | passion fruit | 7 | banana | 7 | | dried fruit | 6 | peach | 6 | caramel | 6 |
| oaky | 6 | caramel | 6 | guava | 6 | caramel | 6 | | guava | 6 | caramel | 5 | honey | 6 |
| dried fruit | 5 | banana | 5 | baked apple | 5 | baked apple | 4 | | tropical fruits | 6 | dried fruit | 5 | dried fruit | 5 |
| fresh | 5 | citrus | 4 | floral | 5 | citrus | 4 | | citrus | 5 | dusty | 5 | dusty | 5 |

| S 1 | | S 2 | | S 3 | | S 4 | | N+S 1 | | N+S 2 | | N+S 3 | | N+S 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pineapple | 23 | pineapple | 16 | pineapple | 18 | pineapple | 15 | pineapple | 18 | pineapple | 18 | pineapple | 17 | pineapple | 22 |
| grapefruit | 12 | grapefruit | 12 | grapefruit | 11 | grapefruit | 11 | peach | 11 | dried fruit | 13 | passion fruit | 8 | grapefruit | 10 |
| passion fruit | 10 | guava | 12 | guava | 8 | passion fruit | 10 | dried fruit | 9 | grapefruit | 13 | banana | 7 | passion fruit | 10 |
| peach | 7 | passion fruit | 9 | dried fruit | 7 | guava | 8 | grapefruit | 9 | passion fruit | 9 | caramel | 7 | peach | 10 |
| dried fruit | 6 | peach | 9 | cooked veg | 6 | banana | 7 | caramel | 8 | peach | 9 | dried fruit | 7 | honey | 7 |
| dusty | 6 | honey | 8 | lemon | 6 | caramel | 6 | banana | 6 | caramel | 8 | grapefruit | 7 | banana | 6 |
| guava | 6 | dried fruit | 7 | passion fruit | 6 | dried fruit | 6 | guava | 6 | herbaceous | 6 | citrus | 6 | dried fruit | 6 |
| herbaceous | 6 | lemon | 7 | citrus | 5 | cooked veg | 5 | baked apple | 5 | guava | 5 | cooked veg | 6 | caramel | 5 |
| honey | 6 | fresh | 6 | dusty | 5 | oaky | 5 | floral | 5 | tropical fruits | 5 | guava | 6 | floral | 5 |
| lemon | 6 | herbaceous | 5 | peach | 5 | peach | 5 | honey | 5 | dusty | 4 | baked apple | 5 | guava | 5 |

## Sauvignon Blanc 2015 - 3-month aged wines

| C 1 | | C 2 | | C 3 | | C 4 | | N 1 | | N 2 | | N 3 | | N 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| passion fruit | 11 | pineapple | 14 | passion fruit | 25 | pineapple | 15 | passion fruit | 15 | passion fruit | 13 | pineapple | 14 | guava | 9 |
| pineapple | 10 | passion fruit | 13 | pineapple | 24 | passion fruit | 10 | pineapple | 13 | pineapple | 11 | passion fruit | 12 | passion fruit | 9 |
| guava | 9 | grapefruit | 11 | grapefruit | 20 | lemon | 8 | lemon | 11 | grapefruit | 7 | grapefruit | 10 | pineapple | 8 |
| grapefruit | 8 | floral | 7 | guava | 15 | guava | 7 | guava | 8 | guava | 7 | lemon | 7 | floral | 8 |
| lemon | 8 | guava | 6 | yellow apple | 10 | grapefruit | 6 | grapefruit | 7 | lemon | 6 | guava | 6 | grapefruit | 7 |
| apple | 5 | sweet associated | 5 | lemon | 10 | fruity | 5 | tropical fruits | 6 | floral | 6 | sweet associated | 6 | lemon | 7 |
| fresh | 5 | green beans | 5 | floral | 10 | yellow apple | 5 | floral | 6 | yellow apple | 5 | floral | 6 | herbaceous | 6 |
| herbaceous | 5 | fruity | 4 | peach | 9 | hay | 5 | fruity | 5 | melon | 4 | yellow apple | 5 | fruity | 5 |
| fruity | 4 | quince | 4 | banana | 9 | floral | 5 | quince | 4 | banana | 4 | fruity | 4 | peach | 5 |
| peach | 4 | yellow apple | 4 | fruity | 8 | peach | 4 | yellow apple | 4 | sweet associated | 4 | quince | 4 | tropical fruits | 4 |

| S 1 | | S 2 | | S 3 | | S 4 | | N+S 1 | | N+S 2 | | N+S 3 | | N+S 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| passion fruit | 11 | passion fruit | 14 | passion fruit | 14 | pineapple | 9 | passion fruit | 13 | pineapple | 9 | passion fruit | 13 | passion fruit | 16 |
| grapefruit | 8 | grapefruit | 13 | pineapple | 13 | lemon | 8 | guava | 12 | grapefruit | 8 | pineapple | 9 | pineapple | 13 |
| pineapple | 7 | pineapple | 11 | lemon | 10 | grapefruit | 7 | peach | 9 | guava | 6 | grapefruit | 8 | guava | 8 |
| guava | 6 | guava | 8 | grapefruit | 8 | guava | 7 | pineapple | 9 | fruity | 5 | lemon | 7 | peach | 7 |
| sweet associated | 6 | sweet associated | 6 | floral | 7 | passion fruit | 7 | grapefruit | 7 | peach | 5 | banana | 5 | grapefruit | 7 |
| fruity | 5 | floral | 6 | tropical fruits | 6 | vegetables | 7 | lemon | 6 | lemon | 5 | fresh | 5 | floral | 6 |
| yellow apple | 5 | fruity | 5 | guava | 5 | sulphur | 6 | tropical fruits | 4 | passion fruit | 5 | floral | 5 | banana | 5 |
| lemon | 5 | peach | 5 | green grass | 5 | green beans | 5 | quince | 3 | vegetables | 5 | citrus | 4 | quince | 4 |
| apple | 4 | lemon | 5 | yellow apple | 4 | fresh | 5 | yellow apple | 3 | fresh | 5 | guava | 4 | yellow apple | 4 |
| citrus | 4 | citrus | 4 | peach | 4 | fruity | 4 | banana | 3 | floral | 5 | green beans | 4 | tropical fruits | 4 |

## Sauvignon Blanc 2015 - 9-month aged wines

| C 1 | | C 2 | | C 3 | | C 4 | | N 1 | | N 2 | | N 3 | | N 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pineapple | 16 | pineapple | 16 | pineapple | 19 | pineapple | 21 | pineapple | 19 | pineapple | 17 | pineapple | 19 | pineapple | 16 |
| tropical fruits | 12 | tropical fruits | 12 | tropical fruits | 12 | tropical fruits | 12 | tropical fruits | 13 | tropical fruits | 12 | tropical fruits | 11 | lemon | 11 |
| lemon | 11 | lemon | 8 | lemon | 9 | grapefruit | 8 | guava | 9 | floral | 9 | passion fruit | 10 | tropical fruits | 11 |
| herbaceous | 7 | passion fruit | 8 | passion fruit | 9 | passion fruit | 7 | fresh | 7 | lemon | 8 | citrus | 8 | floral | 8 |
| fresh | 6 | grapefruit | 7 | fresh | 8 | peach | 7 | grapefruit | 7 | grapefruit | 7 | fresh | 7 | passion fruit | 8 |
| green | 6 | citrus | 6 | guava | 8 | citrus | 6 | passion fruit | 7 | guava | 7 | guava | 7 | gooseberry | 7 |
| mineral | 6 | floral | 6 | green | 6 | fresh | 6 | floral | 6 | peach | 6 | peach | 7 | guava | 7 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| gooseberry | 5 | green | 6 | sweet associated | 6 | guava | 6 | fruity | 6 | yellow apple | 6 | apricot | 6 | green grass | 5 |
| grapefruit | 5 | guava | 5 | floral | 5 | floral | 5 | green | 6 | passion fruit | 5 | grapefruit | 6 | sulphur | 5 |
| passion fruit | 5 | ripe fruit | 5 | fruity | 5 | green | 5 | lemon | 6 | yellow fruits | 5 | green | 6 | vegetables | 5 |
| **S 1** | | **S 2** | | **S 3** | | **S 4** | | **N+S 1** | | **N+S 2** | | **N+S 3** | | **N+S 4** | |
| pineapple | 15 | pineapple | 21 | pineapple | 16 | pineapple | 11 | pineapple | 14 | pineapple | 13 | tropical fruits | 14 | pineapple | 14 |
| tropical fruits | 10 | passion fruit | 10 | tropical fruits | 12 | fresh | 9 | lemon | 9 | sulphur | 10 | pineapple | 13 | lemon | 11 |
| herbaceous | 9 | guava | 9 | lemon | 10 | sulphur | 8 | herbaceous | 8 | fresh | 9 | guava | 9 | tropical fruits | 11 |
| lemon | 8 | tropical fruits | 9 | fresh | 7 | gooseberry | 7 | gooseberry | 7 | green | 8 | lemon | 8 | gooseberry | 8 |
| grapefruit | 7 | grapefruit | 8 | passion fruit | 7 | herbaceous | 7 | sulphur | 7 | guava | 8 | grapefruit | 6 | green | 8 |
| green | 7 | floral | 7 | citrus | 6 | lemon | 7 | fresh | 6 | tropical fruits | 8 | green | 6 | passion fruit | 8 |
| sulphur | 6 | lemon | 7 | sulphur | 6 | tomato leaf | 7 | grapefruit | 6 | grapefruit | 7 | floral | 5 | guava | 7 |
| floral | 5 | green | 6 | floral | 5 | vegetables | 7 | tomato leaf | 6 | passion fruit | 7 | fresh | 5 | herbaceous | 7 |
| gooseberry | 5 | citrus | 5 | gooseberry | 5 | citrus | 6 | tropical fruits | 6 | tomato leaf | 7 | gooseberry | 5 | sulphur | 7 |
| passion fruit | 5 | fresh | 5 | green | 5 | grapefruit | 6 | citrus | 5 | gooseberry | 6 | herbaceous | 5 | mineral | 6 |
| **Sauvignon Blanc 2016 - 3-month aged wines** | | | | | | | | | | | | | | | |
| **C 1** | | **C 2** | | **C 3** | | **C 4** | | **N 1** | | **N 2** | | **N 3** | | **N 4** | |
| pineapple | 13 | sulphur | 11 | sweet associated | 11 | pineapple | 20 | pineapple | 11 | pineapple | 15 | pineapple | 11 | pineapple | 16 |
| yellow apple | 7 | guava | 9 | honey | 9 | floral | 8 | mineral | 6 | passion fruit | 6 | lemon | 8 | floral | 9 |
| banana | 6 | pineapple | 9 | oxidised apple | 9 | passion fruit | 7 | gooseberry | 5 | sulphur | 6 | floral | 7 | guava | 8 |
| floral | 6 | cooked veg | 8 | marmalade | 8 | grapefruit | 6 | herbaceous | 5 | yellow apple | 6 | mineral | 6 | passion fruit | 8 |
| passion fruit | 5 | gooseberry | 7 | floral | 6 | lemon | 6 | lemon | 5 | cooked veg | 5 | passion fruit | 6 | yellow apple | 8 |
| cooked veg | 4 | lemon | 7 | ripe fruit | 5 | yellow apple | 6 | cooked veg | 4 | floral | 5 | yellow apple | 6 | fruity | 5 |
| fruity | 4 | mineral | 5 | dried fruit | 4 | fruity | 4 | dusty | 4 | lemon | 5 | hay | 5 | grapefruit | 5 |
| gooseberry | 4 | tomato leaf | 5 | grapefruit | 4 | ripe fruit | 4 | floral | 4 | fruity | 4 | gooseberry | 4 | ripe fruit | 4 |
| guava | 4 | yellow apple | 5 | mineral | 4 | banana | 3 | grapefruit | 4 | guava | 4 | grapefruit | 4 | sweet associated | 4 |
| mineral | 4 | passion fruit | 4 | toasted | 4 | gooseberry | 3 | sulphur | 4 | tomato leaf | 4 | guava | 4 | citrus | 3 |
| **S 1** | | **S 2** | | **S 3** | | **S 4** | | **N+S 1** | | **N+S 2** | | **N+S 3** | | **N+S 4** | |
| pineapple | 13 | pineapple | 13 | pineapple | 14 | pineapple | 16 | pineapple | 17 | pineapple | 17 | pineapple | 14 | pineapple | 16 |
| floral | 7 | lemon | 9 | cooked veg | 8 | yellow apple | 7 | lemon | 8 | lemon | 6 | guava | 7 | floral | 7 |
| yellow apple | 7 | passion fruit | 9 | lemon | 7 | floral | 6 | yellow apple | 7 | mineral | 6 | lemon | 7 | lemon | 7 |
| grapefruit | 6 | guava | 8 | yellow apple | 6 | grapefruit | 6 | cooked veg | 6 | tomato leaf | 5 | sulphur | 7 | passion fruit | 7 |
| tomato leaf | 5 | cooked veg | 7 | gooseberry | 5 | guava | 5 | floral | 6 | cooked veg | 4 | yellow apple | 7 | guava | 5 |
| guava | 4 | gooseberry | 7 | guava | 5 | lemon | 5 | tomato leaf | 6 | ripe fruit | 4 | cooked veg | 6 | yellow apple | 5 |
| lemon | 4 | yellow apple | 7 | passion fruit | 5 | fresh | 4 | guava | 5 | yellow apple | 4 | tomato leaf | 6 | fruity | 4 |
| passion fruit | 4 | grapefruit | 6 | floral | 4 | gooseberry | 4 | passion fruit | 5 | apple | 3 | gooseberry | 5 | grapefruit | 4 |
| ripe fruit | 4 | tomato leaf | 6 | grapefruit | 4 | passion fruit | 4 | gooseberry | 4 | banana | 3 | mineral | 4 | banana | 3 |
| banana | 3 | floral | 5 | sulphur | 4 | tomato leaf | 4 | mineral | 4 | dusty | 3 | passion fruit | 4 | citrus | 3 |
| **Sauvignon Blanc 2016 - 9-month aged wines** | | | | | | | | | | | | | | | |
| **C 1** | | **C 2** | | **C 3** | | **C 4** | | **N 1** | | **N 2** | | **N 3** | | **N 4** | |
| pineapple | 19 | pineapple | 16 | dried fruit | 14 | pineapple | 20 | dried fruit | 14 | pineapple | 20 | pineapple | 16 | pineapple | 20 |
| passion fruit | 16 | grapefruit | 9 | sweet associated | 13 | dried fruit | 12 | pineapple | 11 | passion fruit | 14 | passion fruit | 14 | grapefruit | 12 |
| grapefruit | 13 | guava | 9 | caramel | 10 | grapefruit | 11 | sweet associated | 9 | grapefruit | 13 | peach | 13 | passion fruit | 12 |
| guava | 8 | lemon | 9 | marmalade | 7 | passion fruit | 10 | baked apple | 7 | dried fruit | 9 | dried fruit | 8 | tropical fruits | 9 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| dried fruit | 7 | tomato leaf | 7 | lemon | 6 | caramel | 9 | caramel | 7 | peach | 9 | grapefruit | 8 | dried fruit | 7 |
| peach | 7 | cooked veg | 6 | peach | 6 | floral | 8 | marmalade | 7 | honey | 8 | guava | 6 | floral | 7 |
| floral | 6 | passion fruit | 6 | pear | 5 | tropical fruits | 8 | grapefruit | 6 | tropical fruits | 6 | floral | 5 | peach | 7 |
| banana | 5 | chemical | 5 | spicy | 5 | baked apple | 7 | lemon | 6 | caramel | 5 | lemon | 5 | guava | 6 |
| dusty | 4 | dried fruit | 4 | baked apple | 4 | peach | 6 | passion fruit | 6 | floral | 5 | sweet associated | 5 | marmalade | 6 |
| green grass | 4 | marmalade | 4 | oxidised apple | 4 | banana | 5 | apple | 4 | guava | 5 | caramel | 4 | baked apple | 5 |
| **S 1** | | **S 2** | | **S 3** | | **S 4** | | **N+S 1** | | **N+S 2** | | **N+S 3** | | **N+S 4** | |
| pineapple | 17 | pineapple | 16 | pineapple | 18 | passion fruit | 13 | pineapple | 14 | pineapple | 22 | pineapple | 20 | pineapple | 15 |
| grapefruit | 11 | grapefruit | 12 | passion fruit | 16 | pineapple | 13 | grapefruit | 10 | grapefruit | 14 | passion fruit | 14 | grapefruit | 11 |
| passion fruit | 11 | passion fruit | 11 | peach | 12 | grapefruit | 10 | dried fruit | 8 | passion fruit | 14 | grapefruit | 10 | passion fruit | 11 |
| peach | 8 | peach | 10 | grapefruit | 9 | peach | 9 | passion fruit | 8 | peach | 9 | guava | 10 | dried fruit | 9 |
| dried fruit | 7 | caramel | 6 | caramel | 6 | dried fruit | 7 | cooked veg | 7 | dried fruit | 7 | peach | 8 | peach | 9 |
| guava | 7 | cooked veg | 6 | floral | 6 | guava | 7 | tomato leaf | 6 | guava | 6 | dried fruit | 6 | floral | 8 |
| floral | 6 | dried fruit | 6 | guava | 6 | floral | 6 | animal | 5 | floral | 5 | cooked veg | 5 | banana | 7 |
| baked apple | 5 | banana | 5 | tropical fruits | 6 | pear | 6 | green pepper | 5 | honey | 5 | herbaceous | 5 | caramel | 7 |
| banana | 5 | floral | 5 | dried fruit | 5 | baked apple | 5 | peach | 5 | lemon | 5 | honey | 5 | guava | 6 |
| tropical fruits | 5 | guava | 5 | baked apple | 4 | tropical fruits | 5 | vegetables | 5 | pear | 5 | lemon | 5 | sweet associated | 6 |

**Table B.5** Top ten sensory attributes of the different Chenin Blanc and Sauvignon Blanc wines.

| Chenin Blanc | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 2015 | | | | | | 2016 | | | | |
| 3-month aged wines | | | 9-month aged wines | | | 3-month aged wines | | | 9-month aged wines | |
| Aroma | F | % | Aroma | F | % | Aroma | F | % | Aroma | F | % |
| pineapple | 191 | 8.62 | pineapple | 172 | 8.08 | pineapple | 203 | 9.14 | pineapple | 279 | 12.22 |
| guava | 182 | 8.22 | grapefruit | 159 | 7.46 | passion fruit | 149 | 6.71 | grapefruit | 146 | 6.39 |
| passion fruit | 171 | 7.72 | guava | 136 | 6.38 | sweet associated | 110 | 4.95 | peach | 126 | 5.52 |
| grapefruit | 170 | 7.67 | tropical fruits | 110 | 5.16 | guava | 96 | 4.32 | passion fruit | 125 | 5.47 |
| peach | 99 | 4.47 | passion fruit | 109 | 5.12 | yellow apple | 86 | 3.87 | dried fruit | 109 | 4.77 |
| lemon | 90 | 4.06 | yellow apple | 103 | 4.84 | apple | 79 | 3.56 | guava | 108 | 4.73 |
| yellow apple | 71 | 3.21 | peach | 99 | 4.65 | floral | 78 | 3.51 | honey | 85 | 3.72 |
| floral | 69 | 3.12 | lemon | 91 | 4.27 | lemon | 77 | 3.47 | banana | 81 | 3.55 |
| banana | 65 | 2.93 | yellow fruits | 78 | 3.66 | peach | 77 | 3.47 | caramel | 79 | 3.46 |
| herbaceous | 65 | 2.93 | herbaceous | 76 | 3.57 | banana | 73 | 3.29 | lemon | 60 | 2.63 |
| **Sauvignon Blanc** | | | | | | | | | | |
| 2015 | | | | | | 2016 | | | | |
| 3-month aged wines | | | 9-month aged wines | | | 3-month aged wines | | | 9-month old | |
| Aroma | F | % | Aroma | F | % | Aroma | F | % | Aroma | F | % |
| passion fruit | 201 | 10.27 | pineapple | 260 | 11.56 | pineapple | 217 | 11.72 | pineapple | 261 | 10.72 |
| pineapple | 189 | 9.65 | tropical fruits | 171 | 7.60 | yellow apple | 92 | 4.97 | passion fruit | 177 | 7.27 |
| grapefruit | 142 | 7.25 | lemon | 129 | 5.74 | lemon | 89 | 4.81 | grapefruit | 162 | 6.66 |
| guava | 123 | 6.28 | passion fruit | 108 | 4.80 | floral | 85 | 4.59 | dried fruit | 130 | 5.34 |
| lemon | 110 | 5.62 | guava | 104 | 4.62 | passion fruit | 81 | 4.38 | peach | 123 | 5.05 |
| floral | 91 | 4.65 | fresh | 96 | 4.27 | guava | 79 | 4.27 | guava | 95 | 3.90 |
| peach | 75 | 3.83 | grapefruit | 96 | 4.27 | cooked veg | 65 | 3.51 | caramel | 82 | 3.37 |
| fruity | 67 | 3.42 | green | 92 | 4.09 | gooseberry | 64 | 3.46 | sweet associated | 76 | 3.12 |
| tropical fruits | 63 | 3.22 | floral | 81 | 3.60 | tomato leaf | 63 | 3.40 | floral | 74 | 3.04 |
| yellow apple | 62 | 3.17 | gooseberry | 80 | 3.56 | grapefruit | 62 | 3.35 | tropical fruits | 73 | 3.00 |