Cape Peninsula
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**CRYOGENIC PRE-TREATMENT DURING WINEMAKING PRACTICES: EFFECT ON WHITE WINE SENSORY AND CHEMICAL PROFILES**

**by**

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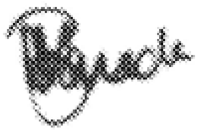
**DECLARATION**

I, VALMARY MICHELLE VAN BREDA, declare that the contents of this thesis represent my unaided work and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

**Signed**  **Date 15 November 2025**

**ABSTRACT**

Sauvignon blanc (*Vitis vinifera*), one of the most extensively cultivated white wine grape varieties globally, is renowned for producing wines with characteristic “grassy” and “tropical” varietal aromas. These aromas result from aroma compounds, such as methoxypyrazines and varietal thiols, present in the grape skin and pulp, the latter being released by yeast during the alcoholic fermentation process. Similarly, Chenin blanc, another popular white wine cultivar that shares a genetic origin with Sauvignon blanc (both originating from Savagnin blanc or Traminer), was also shown to possess varietal thiols. Several anthropogenic factors have been investigated to increase varietal aroma compounds, specifically the varietal thiols; however, the reported results varied. Alternative technologies, such as low-temperatures and cryogenic pre-treatment, have been researched and shown promise. Additionally, the impact of harvesting technique (hand versus machine-harvested) has been researched, reporting that grape juice and wines from mechanically harvested grapes had higher levels of varietal thiol precursors and varietal thiols in the final wines. Therefore, this research aimed to investigate the effect of pre-fermentative cryogenic freezing (-20 ºC and -4 ºC) at four production stages (whole grapes [WG], macerated grapes [MG], turbid must [TM] and clear juice [CJ]), immediately (T0) and for a four-month (T4) storage period on the standard physicochemical properties, varietal aroma compounds (volatile thiols and methoxypyrazines) and the sensory profiles of two popular South African (SA) white wine varieties (Sauvignon blanc and Chenin blanc). Following complete defrosting, a standard white winemaking protocol was followed. The control wines were not subjected to any cryogenic treatments. Subsequently, all grape musts were analysed for physicochemical properties, and wines were analysed for physicochemical properties, varietal aroma compounds, and sensory properties.

Physicochemical parameters in the grape must were generally unaffected by the cryogenic pre-treatments (total sugar, glucose/fructose ratio, and total soluble solids), except for the pH (higher in cryogenically pre-treated grape must) and total acidity (TA) (lower in cryogenically pre-treated grape must) when compared to the unfrozen control. Furthermore, the yeast assimilable nitrogen (YAN) was higher in the unfrozen control and cryogenically pre-treated grape must for all Producers in 2020 compared to 2021, suggesting that the differences were vintage-related. The physicochemical parameters of the final wines were generally within the legal limits for SA white wines, except for pH, which was slightly higher (> 3.4) in the wines made from the cryogenic pre-treatments. The concentrations of the varietal thiol, 3-sulfanylhexyl acetate (3-SHA), detected in the Sauvignon blanc wines exceeded the aroma perception threshold (4 ng $L^{-1}$) and the reported range for SA Sauvignon blanc wines

(23-151 ng $L^{-1}$), whilst the concentrations of 3-sulfanylhexan-1-ol (3-SH) were generally within the aroma perception threshold (60 ng $L^{-1}$) and reported range (178-904 ng $L^{-1}$). Moreover, the concentrations of 4-methyl-4-sulfanylpentan-2-one (4-MSP) were found to be below the aroma perception threshold (0.8 ng $L^{-1}$) and the typically reported range (0-21.9 ng $L^{-1}$). For the Chenin blanc wines, the concentrations of 3-SHA were found to be higher than the typical reported range for SA Chenin blanc (5-253 ng $L^{-1}$) and above the aroma perception threshold (4 ng $L^{-1}$), whilst the concentration of 3-SH were generally below its reported range (99-1124 ng $L^{-1}$) and aroma perception threshold (60 ng $L^{-1}$). Furthermore, the concentrations of 4-MSP were detected above the reported range, not detected (n.d.), but below the aroma perception threshold (0.8 ng $L^{-1}$). Methoxypyrazines were detected at concentrations above the reported aroma perception threshold (2-16 ng $L^{-1}$) and range for 3-isobutyl-2-methoxypyrazine (ibMP) (2-30 ng $L^{-1}$) and 2-methoxy-3-sec-butylpyrazine (sbMP) (< 10 g $L^{-1}$) in Sauvignon blanc wines, for most cryogenic treatments compared to the control wines. Differences were also observed between producers from different regions and between vintages (2020 and 2021), with overall methoxypyrazine levels higher in 2021.

Moreover, from a sensory perspective, the wines made from WG and MG subjected to cryogenic pre-treatment technologies yielded wines with higher tropical, thiol-type, pineapple and banana aromas as well as higher body, general quality and overall intensity when compared to most wines made from the control grapes as well as the remaining cryogenic treatments. This study highlighted that the production stage at which the cryogenic treatment was applied had the most prominent effect, whilst the effects of the cryogenic temperatures and storage times were negligible. Furthermore, vintage and regional differences also influenced the final wine sensory profiles. Differences in varietal thiol concentrations for Sauvignon blanc wines resulted mainly from wine region and vintage for the T4 wines made from cryogenically pre-treated WG and MG (-4 ºC). Although no definite trends were observed in terms of which cryogenic temperature, stage of production, or storage time yielded the most favourable levels in final wines, indications are that the region from which the grapes originated, the harvesting method, and the vintage contributed the most. Therefore, the industry recommendation would be to apply cryogenic pre-fermentative treatments to WG or MG at -4 ºC (energy efficient) for T0 (more economical), considering the region and vintage to achieve the desired outcome.

Keywords: aroma compounds, Chenin blanc, cryogenic technologies, methoxypyrazines, Sauvignon blanc, varietal thiols, sensory profiles

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## DEDICATION

This Thesis is dedicated to the Lord Almighty for providing me with strength, my husband, Neal van Breda, my mother, Mary Daniels and in loving memory of my late father, Vivian Daniels.

Thank you for your continuous support and patience throughout my studies.

"I can do all things through Christ who strengthens me"
Philippians 4:13

"You will succeed in whatever you choose to do, and light will shine on the road ahead of you"
Job 22:28

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## ABBREVIATIONS

**Abbreviations**

| | |
|---|---|
| CJ | Clear juice |
| CM | Carbonic maceration |
| FT-IR ATR | Fourier Transform Infrared spectroscopy - Attenuated Total Reflectance |
| ibMP | 3-isobutyl-2-methoxypyrazine |
| ipMP | 3-isopropyl-2-methoxypyrazine |
| MAE | Microwave-Assisted Extraction |
| MG | Macerated grapes |
| MPs | Methoxypyrazines |
| NTU | Nephelometric Turbidity Unit |
| p.a. | purissimum ad analysim |
| PEF | Pulsed Electric Field |
| puriss. | purissimum |
| SA | South Africa |
| sbMP | 2-methoxy-3-sec-butylpyrazine |
| SIDA | Stable Isotope Dilution Assay |
| 3-SH | 3-sulfanylhexan-1-ol |
| 3-SHA | 3-sulfanylhexyl acetate |
| 4-MSP | 4-methyl-4-sulfanylpentan-2-one |
| TA | Total acidity |
| TM | Turbid must |
| TSS | Total soluble solids |
| $UPC^2$-MS/MS | Ultra Performance Convergence Chromatography coupled with Tandem Mass Spectrometry |
| US | Ultrasound |
| WG | Whole grapes |
| YAN | Yeast Assimilable Nitrogen |
| YNAN | Yeast Non-Assimilable Nitrogen |

## GLOSSARY

### Terms

| | |
|---|---|
| Aroma of wine | Refers to the smells unique to the grape variety and are most readily demonstrated in a varietal wine. |
| Agronomic practices | Agronomic practices are the techniques, strategies, and practices used in the cultivation of crops, including planting, fertilisation, pest control, irrigation, and harvest. |
| *Botrytis* | *Botrytis cinerea* is a necrotrophic fungus that affects many plant species, although its most notable hosts may be wine grapes. In Viticulture, it is commonly known as "botrytis bunch rot"; in Horticulture, it is known as "grey mould". |
| Chenin blanc | Chenin blanc is a white wine grape variety from the Loire Valley of France. Its high acidity means it can be used for a variety of wine styles, from sparkling wines to well-balanced dessert wines. |
| Cold maceration | The process of cold maceration, also known as cold soak, involves keeping the temperatures of the fermenting must low to encourage extraction by water and added sulfur dioxide, rather than relying principally on heat and alcohol to act as a solvent. |
| Cryogenic agents | A cryogenic agent is a solid, liquid, or gas that can be used to cool an object reaching temperatures even below -150 °C (-238 °F). |
| Cryogenic extraction | The production and behaviour of materials at very low temperatures. Cryogenics in Sauvignon Blanc winemaking refers to the use of very low temperatures during grape processing, such as cryomaceration or chilling the must, to preserve delicate aromatics, enhance varietal expression, and minimise oxidation. |
| Cryoscopic decrease | Also known as freezing point depression, refers to the phenomenon where the freezing point of a solvent is lowered when a non-volatile solute is added, a colligative property dependent on solvent properties, not the solute |
| Diemersdal Winter Ferment | After harvest, the juice for the Winter Ferment Sauvignon blanc is frozen at -20 ºC and kept in this state for five months. It is then thawed and fermented in the heart of the Cape winter, producing a uniquely tropical style of Sauvignon blanc. |
| Flavour profile | A wine's flavour profile is a combination of its aromas, tastes, and textures can be categorised into three main groups: primary, secondary, and tertiary. |

| | |
|---|---|
| FTIR–ATR | Fourier Transform Infrared spectroscopy – Attenuated Total Reflectance provides information related to the presence or absence of specific functional groups, as well as the chemical structure of polymer materials. |
| Four-month wines/treatments | Pre-fermentative cryogenic freezing of WG, MG, TM and CJ over four months, followed by defrosting, processing and winemaking. Will be referred to as T4 wines. |
| Grape juice | Grape juice is the liquid extracted from freshly harvested grapes, typically obtained through crushing and pressing. It serves as the base material for fermentation in winemaking. Unlike must, grape juice may refer specifically to the clarified portion used in winemaking. |
| Grape must | In white winemaking, must is the juice obtained after pressing grapes, which typically contains suspended solids such as pulp fragments, tiny skin particles, and other organic matter. |
| Maceration | Maceration is the winemaking process where the phenolic materials of the grape tannins, colouring agents (anthocyanins) and flavour compounds are leached from the grape skins, seeds and stems into the must. |
| Methoxypyrazines | Methoxypyrazines are a class of chemical compounds that produce odours. Two methoxypyrazine compounds, 3-isobutyl-2-methoxypyrazine (ibMP) and 3-isopropyl-2-methoxypyrazine (ipMP), are considered to be important determinants of "green" (i.e., herbal, grassy, or vegetal) flavours in Sauvignon blanc wines and is also present in Cabernet Sauvignon. |
| Phenolic content of wine | The phenolic content in wine refers to the phenolic compounds, natural phenol and polyphenols in wine. |
| Postponed fermentation | Fermentation takes place later than the first scheduled. |
| puriss. (purissimum) | "Purissimum" is a Latin adjective meaning "most pure" or "of the highest purity". It's the superlative form of the Latin word "purus," meaning "pure". Used in scientific and technical contexts to describe the highest quality or purity of a substance or material. |
| Racemic mixture | A 50:50 mixture of two enantiomers, which are mirror-image molecules that have identical chemical and physical properties but differ in their spatial arrangement. |
| Sulfur nomenclature | The International Union of Pure and Applied Chemistry (IUPAC) sets the international standard for naming chemical compounds to ensure unambiguous communication and sets the rules for naming chemical compounds globally. IUPAC uses the "f" spelling for the element (sulfur) and its derived functional groups (e.g., sulfanyl, sulfide, sulfate). |
| $UPC^2$-MS/MS | In $UPC^2$-MS/MS, the "2" in UPC2 stands for "UltraPerformance Convergence Chromatography," where the "2" indicates the second generation or an advanced form of convergence chromatography technology that uses sub-2-micron particle columns for higher resolution and faster separations. This technique combines supercritical fluid chromatography principles with ultra-performance capabilities, enhancing |

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| | separation efficiency before detection by tandem mass spectrometry (MS/MS) |
| Varietal thiols | Volatile” or “varietal” thiols are a specific class of sulfur-containing chemicals present in wine at very low concentrations. |
| Vinification | The conversion of grape juice into wine by fermentation. |
| *Vitis vinifera* | The common grapevine. *Vitis vinifera* is the species of grapevine native to the Mediterranean region, central Europe, and southwestern Asia, and is the primary species used in the production of fine wines worldwide. It encompasses thousands of cultivated varieties (cultivars), including well-known wine grapes such as Sauvignon blanc and Chenin blanc. |
| YAN | Yeast Assimilable Nitrogen (YAN) is a vital nutrient for yeast growth and fermentation in wine. |
| YNAN | Yeast-Non-Assimilable Nitrogen is the remaining component of total nitrogen, which includes proline and hydroxyproline, larger molecular weight peptides and protein. |
| Zero-month wines/treatments | Pre-fermentative cryogenic freezing and defrosting of WG, MG, TM and CJ immediately after harvesting, before winemaking. Will be referred to as T0 wines. |

**CHAPTER 1**
**MOTIVATION FOR AND DESIGN OF THE STUDY**

## 1.1 Introduction

Sauvignon blanc is one of the most widely planted white wine grape varieties in the world, with different terroirs resulting in the production of wines with an extensive array of styles and flavours (Anonymous, 2021a; b). Initially, Sauvignon blanc gained popularity because of its relatively simple flavour profile, i.e., fresh-cut grass and pyrazine-focused style (Marais, 1994:43-44; Allen et al., 1991:110-111; Anonymous, 2021c). However, these wines have since developed into more complex and regionally derived styles described as either "green" (grassy, asparagus, vegetative, herbaceous, green pepper, tomato leaf) or "tropical" (pineapple, guava, gooseberry, grapefruit and passion fruit) (Augustyn et al., 1982:55-59; Marais, 1994:43-44; 2001:47-50; Baiano et al., 2012:2697-2700; Coetzee & du Toit, 2012:287-290; Coetzee, 2018:182-184, Anonymous, 2021a; d). Similarly, Chenin blanc, one of the most planted wine grape cultivars in SA, gained popularity for its perceived "Guava" aroma. Chenin blanc, which shares a genetic origin with Sauvignon blanc, both originating from Savagnin blanc or Traminer, was previously shown to possess varietal thiols. This was confirmed when Du Plessis & Augustyn (1981:101-102) attributed the guava aroma to a sulfur compound, i.e, 4-MSP. Moreover, Wilson (2017:28-38) and Wilson et al. (2018:1-13) further confirmed the presence of varietal thiols by detecting 3-SH and 3-SHA in commercial SA Chenin blanc wines at levels as high as 23 ng $L^{-1}$ and 893 ng $L^{-1}$, respectively. Additionally, both thiols exceeded their respective odour threshold values and were considered odour-active (Wilson, 2017:28-38).

Aromas result primarily from compounds such as methoxypyrazines (MP's) ("green") and volatile thiols ("tropical") but include other compounds such as esters, higher alcohols, fatty acids and monoterpenes present in the grape (Allen et al., 1991:110-111; Glória & Vieira, 2007:263-264; Allen et al., 2011:10648-10649; Roland et al., 2011a:7356-7359; Robinson et al., 2014:2-7; Jeffery 2016:1323-1324). These compounds are often present in the grape skin and pulp as non-volatile precursors released during the winemaking process (Swiegers et al., 2009:207-208; Coetzee, 2011:73; Roland et al., 2011b:143, Cosme et al., 2016:188-189; Chen et al., 2018:4674; 4679-4680; Lan et al., 2019:195). The three major varietal thiols responsible for the tropical aromas in Sauvignon blanc wines are 3-mercaptohexan-1-ol (3-MH), 3-mercaptohexyl acetate (3-MHA) and 4-mercapto-4-methylpentan-2-one (4-MMP) (Augustyn et al., 1982:55-59; Tominaga et al., 1998:161-162; Chen & Li, 2022:316). The abovementioned nomenclature has subsequently been amended, where the "mercapto" prefix has been replaced with "sulfanyl", i.e., 3-SH, 3-SHA and 4-MSP (Roland et al., 2011a:7356; Chen et al., 2019a:3-4). Even though these varietal thiols contribute significantly to the characteristic aromas in Sauvignon blanc, they are also present in other white and red *Vitis vinifera* cultivars (Roland et al., 2011a:7358; Coetzee & du Toit, 2012:287-290). Moreover, these varietal flavour compounds are influenced by factors such as climate, agricultural, viticultural and oenological practices which hugely influence these compounds (Marais, 2001:47-50; Jussier et al., 2006:224-226; Kilmartin, 2012:81-86; Olejar et al., 2015:187-188; Hart et al., 2017:153; Hart et al., 2019:8). Research conducted in New Zealand, Italy, China and Australia, focused on only one or two of the following aspects per study (Sacchi et al., 2005:197-203; Lund et al., 2009:18-25, Swiegers et al., 2006:34-41; Parr et al., 2013:472-474; Varela, 2016:9870), i.e., machine vs hand harvesting techniques, cryo-maceration at different temperatures (4 ºC, 6 ºC, 8 ºC and 10 ºC), sunlight and UV radiation exposure and using single or combinations of yeast strains (Molina et al., 2009:683; 685; Allen et al., 2011:10648-10649; Jouanneau, 2012:341-342; Nicolini et al., 2011:132; Kilmartin 2012:81-86; Sadoudi et al., 2012:252; Steyer et al., 2012:10; Song et al., 2015:430; Naviglio et al., 2018:8-13). Methoxypyrazines (3-alkyl-2-methoxypyrazines) are aroma compounds primarily responsible for the "green" aroma in the Sauvignon blanc grape and wine. The most essential MP found in grapes and wines is ibMP. However, two additional MP's present in must and wine, at lower concentrations, are ipMP and sbMP, contributing to the earthy, asparagus aromas (Marais & Rapp, 1988:29; Marais 1994:43-44; Ruiz et al., 2019:7425-7450).

White wines are traditionally fermented directly after harvest and crushing and are produced during the typical South African harvest period (February-April) each year. However, some winemakers have been investigating the effect of chilling or freezing juice for several months before fermentation, to produce wines during the SA winter months (June-August), resulting in a postponed fermentation (Parenti et al., 2004:365; Salinas et al., 2005:1532-1535; Threlfall et al., 2006:168-169; Carillo et al., 2011:11-12; Chen et al., 2019b:642-643). This approach results in the production of fresh white wines throughout the year with enhanced varietal aroma profiles and superior quality, as well as wines with considerably higher thiol levels (Marais, 2001:50; Peinado et al., 2004:589; Baiano et al., 2012:2700; Benkwitz et al., 2012a:6296-6300; b:69-71; Dias Araujo et al., 2017:133-136). Moreover, a typical SA tropical-styled Sauvignon blanc wine produced directly after harvesting, has thiol concentrations less than 2500 ng $L^{-1}$, whereas the "Winter Ferment" Sauvignon blanc thiol concentration measures in the region of 5000 ng $L^{-1}$ which is comparable to Marlborough Sauvignon blanc wine produced from machine harvested grapes (Carillo et al., 2011:11-12; Kilmartin 2012:81-86; Chen et al., 2019b:642-643; Coetzee et al., 2018:1-11; Van Breda et al., 2024:1-14). However, to date, no formal scientific studies on the effects of freezing grape must, nor on comparing the quality of postponed-fermentation (winter-fermented) wines to summer-fermented wines for SA Sauvignon blanc and Chenin blanc, have been reported. Hence, the effects of freezing for different storage intervals for whole grapes (WG), macerated grapes (MG), turbid must (TM) and clear juice (CJ) at the various winemaking stages, machine versus hand-harvesting, and the effect of different climatic regions on varietal thiol levels and wine quality, for both Sauvignon blanc and Chenin blanc, were investigated in this study. Moreover, this investigation of cryogenic treatments, stage of production and storage period may lead to optimised vinification techniques that can provide the SA wine industry with the necessary tools to produce wines with enhanced varietal sensory profiles.

## 1.2 Statement of the research problem

In SA, the first Sauvignon blanc with enhanced tropical fruit expression was produced in 2017. Since its release, this wine, i.e., "Diemersdal Winter Ferment" Sauvignon blanc has won numerous South African accolades including the First National Bank (FNB) Top 10 Sauvignon blanc awards and best Sauvignon blanc at the National Young Wine Show. This widely acclaimed, unique wine is produced by freezing (-20 ºC) grape must immediately after the grapes are harvested, typically in February. The grape must is stored for four months until June, defrosted and fermented (i.e., during the South African winter). These wines display very high thiol levels of 5000 ng $L^{-1}$ which were above the average SA levels of between 1000-2500 ng $L^{-1}$, resulting in wines with superior tropical character. These very high thiol levels are unusual for SA and comparable with those observed in wines from the Marlborough wine region in New Zealand. This then elicited questions and keen interest from the Wine Industry in understanding more about winter wine fermentations and the effect of freezing juice on final wine quality. Reported factors affecting thiol levels include yeasts, UV radiation, origin and vintage influences, and the impact of harvesting methods and storage temperature. The first year of this study focused on wine production from Sauvignon blanc WG, MG, TM and CJ following thawing and freezing for different periods (T0 and T4). The same treatments were applied in year two, along with the effect of hand-harvested grapes, grapes from a different climatic region, and the inclusion of an additional cultivar (Chenin blanc).

## 1.3 Objectives of the research

### 1.3.1 Broad Objectives

The objective of this study was to produce wines with increased volatile flavour compounds and optimal wine quality by freezing (-4 ºC and -20 ºC) followed by defrosting to 15 ºC, of WG, MG, TM and CJ, immediately after processing (0 months) (T0), and after a four-month storage period (T4), before vinification. The specific physicochemical parameters (Brix, malic acid, pH, total acid, total sugar, glucose, fructose, haze, and YAN) serve as the standard chemical indices during the must stage, while, in the final wine, they function as foundational quality markers that determine structural balance.

The aims of this study were:

- To compare the effects of freezing (-4 ºC and -20 ºC) of Sauvignon blanc WG, MG, TM and CJ for T0 and T4 on the various chemical and sensorial indices of wine quality, to identify the treatment that will deliver a wine with optimal quality and aroma flavour compounds.
- To compare the effects of freezing (-4 ºC and -20 ºC) of Chenin blanc WG, MG, TM and CJ for T0 and T4 on the various chemical and sensorial indices of wine quality, to identify the treatment that will deliver a wine with optimal quality and aroma flavour compounds.

### 1.3.2 Specific objectives

The specific objectives were:

- To assess the effects of freezing (-4 ºC and -20 ºC) on the standard chemical indices (Brix, malic acid, pH, total acid, total sugar, glucose, fructose, haze and YAN) of Sauvignon blanc grape must from WG, MG, TM and CJ, after grape processing at T0 and T4.
- To determine the effects of freezing (-4 ºC and -20 ºC) on the standard chemical indices (volatile acidity, alcohol, fructose, glucose, malic acid, total acidity, pH and total sugar) and the aroma flavour compounds (varietal thiols and methoxypyrazines) of Sauvignon blanc wine made from WG, MG, TM and CJ, after grape processing and small-scale wine production at T0 and T4.
- To assess the effects of freezing (-4 ºC and -20 ºC) on the standard chemical indices (Brix, malic acid, pH, total acid, total sugar, glucose, fructose, haze and YAN) of Chenin blanc grape must from WG, MG, TM and CJ, after grape processing at T0 and T4.
- To determine the effects of freezing (-4 ºC and -20 ºC) on the standard chemical indices (volatile acidity, alcohol, fructose, glucose, malic acid, total acidity, pH and total sugar) and the aroma flavour compound (varietal thiols) of Chenin blanc wine made from WG, MG, TM and CJ, after grape processing and small-scale wine production at T0 and T4.
- To compare sensory profiles and quality of Sauvignon blanc and Chenin blanc wines produced from the WG, MG, TM and CJ subjected to freezing (-4 ºC and -20 ºC) at T0 and T4 to unfrozen control wines.

## 1.4 Hypotheses

It is hypothesised that freezing (-4 ºC and -20 ºC) Sauvignon blanc and a second white wine cultivar (Chenin blanc) at different production stages (WG, MG, TM and CJ) for T0 and T4 will result in wines with increased levels of aroma flavour compounds (i.e., varietal thiols and potentially methoxypyrazines). It is also hypothesised that the standard chemical profile of the frozen wines (residual sugar, ethanol, volatile acidity, total sulfur, and pH) will be comparable to that of the control wine. It is anticipated that there will be minor shifts in fixed acidity and pH due to tartrate precipitation at sub-zero temperatures. It is hypothesised that the standard chemical profile of the frozen wines will remain oenologically comparable to the control, staying within the parameters of a balanced, commercially sound wine. Furthermore, it is hypothesised that the wines with higher levels of aroma flavour compounds (i.e., varietal thiol and methoxypyrazines) and improved or novel sensory attributes compared to the control wine (standard vinification practice, without freezing or any other special treatment).

## 1.5 Delineation of the research

This study was conducted using two popular white wine grape cultivars, known to produce wines with “Tropical” and “Fruity” aromas, i.e., Sauvignon blanc and Chenin blanc. The experimental design was conducted on a small scale over two vintages only, using grapes from three different wine-grape growing regions within the Western Cape. Subsequently, conducting it on grapes from a single region or vintage only might not truly reflect actual circumstances within the wine industry, therefore, a second vintage and more than one region were also included to address this deficiency. A second popular SA white wine cultivar, i.e., Chenin blanc was included in the second year of the study because it is also known to contain varietal thiol compounds.

## 1.6 Importance of the study

This investigation of delayed winemaking strategies, including cryogenic treatments, stage of production and storage period, may lead to optimised vinification techniques that can provide the SA wine industry with the necessary tools to produce wines with enhanced varietal sensory profiles.

## 1.7 Thesis Overview

The results of the research presented in this thesis were conducted in the Post-Harvest and Agro-processing Technologies Division, at the Agricultural Research Council; ARC Infruitec-Nietvoorbij (Fruit, Wine and Vine Institute), Western Cape, South Africa. The thesis is composed of 5 (five) chapters as highlighted below:

**Chapter 1:** Introduction: General introduction and background to the research project, objectives and the significance of the research.

**Chapter 2:** Literature review.

**Chapter 3:** Pre-fermentative cryogenic treatment of Sauvignon blanc and Chenin blanc grapes and must: Impact on chemical and sensory profiles of wine.

**Chapter 4:** Impact of cryogenic pre-treatment technologies on aroma compounds and organoleptic properties of Sauvignon blanc and Chenin Blanc wine.

**Chapter 5:** General Summary, Conclusions, and Recommendations

## 1.8 References

Allen, M.S., Lacey, M.J., Harris, R.L. & Brown, W.V. 1991. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *American Journal of Enology and Viticulture*, 42(2):109-112.

Allen, T., Herbst-Johnstone, M., Girault, M., Butler, P., Logan, G., Jouanneau, S., Nicolau, L. & Kilmartin, P.A. 2011. Influence of grape-harvesting steps on varietal thiol aromas in Sauvignon blanc wines. *Journal of Agricultural and Food Chemistry*, 59(19):10641-10650. https://doi.org/10.1021/jf2018676.

Anonymous 2021a. White-wine varieties grown in South Africa (Wine of South Africa). [WWW document] URL http://www.wosa.co.za/The-Industry/Varieties-and-Styles/White-Wine-Varieties [31 March 2021].

Anonymous 2021b. White grape varieties from South Africa. [WWW document] URL https://capreo.com/en/white-grape-varieties [31 March 2021].

Anonymous 2021c. Methoxypyrazines and greenness in wines: myth or reality? A few perspectives [WWW document] URL https://www.wineland.co.za/ methoxypyrazines-greenness wines myth reality perspectives [20 May 2021].

Anonymous 2021d. Sauvignon blanc [WWW document] URL https://southafrica.co.za/sauvgnon blanc.html [02 June 2021].

Augustyn, P.H., Rapp, A. & Van Wyk, C.J. 1982. Some volatile aroma components of *Vitis vinifera* L. cv. Sauvignon blanc. *South African Journal of Enology and Viticulture*, 3(2):52-60. https://doi.org/10.21548/3-2-2382.

Baiano, A., Terracone, C., Longobardi, F., Ventrella, A., Agostiano, A. & Del Nobile, M.A. 2012. Effects of different vinification technologies on physical and chemical characteristics of Sauvignon blanc wines. *Food Chemistry*, 135(4):2694-2701. https://doi.org/10.1016/j.foodchem.2012.07.075.

Benkwitz, F., Nicolau, L., Lund, C., Beresford, M., Wohlers, M. & Kilmartin, P.A. 2012a. Evaluation of key odorants in Sauvignon blanc wines using three different methodologies. *Journal of Agricultural and Food Chemistry*, 60(25):6293-6302. https://doi.org/10.1021/jf300914n.

Benkwitz, F., Tominaga, T., Kilmartin, P.A., Lund, C., Wohlers, M. & Nicolau, L. 2012b. Identifying the chemical composition related to the distinct aroma characteristics of New Zealand Sauvignon blanc wines. *American Journal of Enology and Viticulture*, 63(1):62-72. https://doi.org/10.5344/ajev.2011.10074.

Carillo, M., Formato, A., Fabiani, A., Scaglione, G., & Pucillo, G. P. 2011. An inertizing and cooling process for grapes cryomaceration. *Electronic Journal of Biotechnology*, 14(6):8-8. https://doi.org/10.2225/vol14-issue6-fulltext-10.

Chen, L., Capone, D.L., Tondini, F.A. & Jeffery, D.W. 2018. Chiral polyfunctional thiols and their conjugated precursors upon winemaking with five *Vitis vinifera* Sauvignon blanc clones. *Journal of Agricultural and Food Chemistry*, 66:4674-4282. https://doi.org/10.1021/acs.jafc.8b01806.

Chen, L., Capone, D.L. & Jeffery, D.W. 2019a. Analysis of potent odour-active volatile thiols in foods and beverages with a focus on wine. *Molecules*, 24(13):2472. https://doi.org/10.3390/molecules24132472.

Chen, L., Capone, D.L., Nicholson, E.L. & Jeffery, D.W. 2019b. Investigation of intraregional variation, grape amino acids, and pre-fermentation freezing on varietal thiols and their precursors for *Vitis vinifera* Sauvignon blanc. *Food Chemistry*, 295:637-645. https://doi.org/10.1016/j.foodchem.2019.05.126.

Chen, K. & Li, J. 2022. A glance into the aroma of white wine. In Morata, A. (ed.). White Wine Technology. Spain: Elsevier Academic Press; 313-326. https://doi.org/10.1016/B978-0-12-823497-6.00018-1.

Coetzee, C. 2011. Oxygen and sulfur dioxide additions to Sauvignon blanc: effect on must and wine composition. Unpublished PhD Thesis, University of Stellenbosch, Stellenbosch.

Coetzee, C. 2018. Grape-derived fruity volatile thiols - Adjusting Sauvignon blanc aroma and flavor complexity. *Wines and Vines*:1-11.

Coetzee, C. & du Toit, W.J. 2012. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. *Food Research International*, 45:287-298. https://doi.org/10.1016/j.foodres.2011.09.017.

Coetzee, C., Schulze, A., Mokwena, L., Du Toit, W. J., & Buica, A. 2018. Investigation of thiol levels in young commercial South African Sauvignon Blanc and Chenin Blanc wines using propiolate derivatization and GC-MS/MS. *South African Journal of Enology and Viticulture*, 39(2):180-184. https://doi.org/10.21548/39-2-2683.

Cosme, F., Gonçalves, B., Inês, A., Jordão, A. M., & Vilela, A. 2016. Grape and wine metabolites: Biotechnological approaches to improve wine quality. *Grape and Wine Biotechnology*:187-214. http://dx.doi.org/10.5772/64822.

Dias Araujo, L., Vannevel, S., Buica, A., Callerot, S., Fedrizzi, B., Kilmartin, P.A. & du Toit, W.J. 2017. Indications of the prominent role of elemental sulfur in the formation of the varietal thiol 3-mercaptohexanol in Sauvignon blanc wine. *Food Research International*, 98:79-86. https://doi.org/10.1016/j.foodres.2016.12.023.

Du Plessis, C.S. & Augustyn, O.P.H. 1981. Initial study on the guava aroma of Chenin blanc and Colombar wines. *South African Journal of Enology and Viticulture*, 2(2):101-103. https://doi.org/10.21548/2-2-2401.

Glória, M.B.A. & Vieira, S.M. 2007. Technological and toxicological significance of bioactive amines in grapes and wines. *Food, Global Science Books, Reino Unido*, 1(2):258-270.

Hart, R.S., Ndimba, B.K. & Jolly, N.P. 2017. Characterisation of thiol-releasing and lower volatile acidity-forming intra-genus hybrid yeast strains for Sauvignon blanc wine. *South African Journal of Enology and Viticulture*, 38(2):144-155. https://doi.org/10.21548/38-2-1322.

Hart, R.S., Jolly, N.P. & Ndimba, B.K. 2019. Characterisation of hybrid yeasts for the production of varietal Sauvignon blanc wine-A review. *Journal of Microbiological Methods*, 165:105699. https://doi.org/10.1016/j.mimet.2019.105699.

Jeffery, D.W. 2016. Spotlight on varietal thiols and precursors in grapes and wines. *Australian Journal of Chemistry*, 69:1323-1330. https://doi.org/10.1071/CH16296.

Jouanneau, S., Weaver, R.J., Nicolau, L., Herbst-Johnstone, M., Benkwitz, F. & Kilmartin, P.A. 2012. Subregional survey of aroma compounds in Marlborough Sauvignon Blanc wines. *Australian Journal of Grape and Wine Research*, 18(3):329-343. https://doi.org/10.1111/j.1755-0238.2012.00202.x.

Jussier, D., Morneau, A.D. & de Orduna, R.M. 2006. Effect of simultaneous inoculation with yeast and bacteria on fermentation kinetics and key wine parameters of cool-climate Chardonnay. *Applied and Environmental Microbiology*, 72(1):221-227. https://doi.org/10.1128/AEM.72.1.221-227.2006.

Kilmartin, P. 2012. Machine harvesting versus handpicking: impacts on tropical and green characters in Sauvignon blanc wines. *The Australian & New Zealand Grape grower and Winemaker*, 81-86.

Lan, Y., Xiang, X., Qian, X., Wang, J., Ling, M., Zhu, B., Liu, T., Sun, L., Shi, Y., Reynolds, A.G. & Duan, C. 2019. Characterization and differentiation of key odor-active compounds of 'Beibinghong' icewine and dry wine by gas chromatography-olfactometry and aroma reconstitution. *Food Chemistry*, 287:186-196. https://doi.org/10.1016/j.foodchem.2019.02.074.

Lund, C.M., Nicolau, L., Gardner, R.C. & Kilmartin, P.A. 2009. Effect of polyphenols on the perception of key aroma compounds from Sauvignon blanc wine. *Australian Journal of Grape and Wine Research*, 15(1):18-26. https://doi.org/10.1111/j.1755-0238.2008.00028.x.

Marais, J. 1994. Sauvignon blanc cultivar aroma-a review. *South African Journal of Enology and Viticulture*, 15(2):41-45.

Marais, J. 2001. Effect of grape temperature and yeast strain on Sauvignon blanc wine aroma composition and quality. *South African Journal of Enology and Viticulture*, 22(1):47-50.

Marais, J., & Rapp, A. 1988. Effect of skin-contact time and temperature on juice and wine composition and wine quality. *South African Journal of Enology and Viticulture*, 9(1):22-30.

Molina, A.M., Guadalupe, V., Varela, C., Swiegers, J.H., Pretorius, I.S. & Agosin, E. 2009. Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. *Food Chemistry*, 117(2):189-195. https://doi.org/10.1016/j.foodchem.2009.03.116.

Nicolini, G., Moser, S., Roman, T., Mazzi, E. & Larcher, R. 2011. Effect of juice turbidity on fermentative volatile compounds in white wines. *Vitis*, 50(3):131-135.

Naviglio, D., Formato, A., Scaglione, G., Montesano, D., Pellegrino, A., Villecco, F. & Gallo, M. 2018. Study of the grape cryo-maceration process at different temperatures. *Foods*, 7(7):107. https://doi.org/10.3390/foods7070107.

Olejar, K.J., Fedrizzi, B. & Kilmartin, P.A. 2015. Influence of harvesting technique and maceration process on aroma and phenolic attributes of Sauvignon blanc wine. *Food Chemistry*, 183:181-189. https://doi.org/10.1016/j.foodchem.2015.03.040.

Parenti, A., Spugnoli, P., Calamai, L., Ferrari, S., & Gori, C. 2004. Effects of cold maceration on red wine quality from Tuscan Sangiovese grape. *European Food Research and Technology*, 218(4):360-366. http://doi.org/10.1007/s00217-003-0866-1.

Parr, W.V., Schlich, P., Theobald, J.C. & Harsch, M.J. 2013 Association of selected vini viticultural factors with sensory and chemical characteristics of New Zealand Sauvignon blanc wines. *Food Research International*, 53:464-475. https://doi.org/10.1016/j.foodres.2013.05.028.

Peinado, R.A., Moreno, J., Bueno, J.E., Moreno, J.A. & Mauricio, J.C. 2004. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chemistry*, 84(4):585-590. https://doi.org/10.1016/S0308-8146(03)00282-6.

Robinson, A.L., Boss, P.K., Solomon, P.S., Trengove, R.D., Heymann, H. & Ebeler, S.E. 2014. Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture*, 65(1):1-24. https://doi.org/10.5344/ajev.2013.12070.

Roland, A., Schneider, R., Razungles, A. & Cavelier, F. 2011a. Varietal thiols in wine: discovery, analysis and applications. *Chemical Reviews*, 111(11):7355-7376. https://doi.org/10.1021/cr100205b.

Roland, A., Schneider, R., Charrier, F., Cavelier, F., Rossignol, M. & Razungles, A. 2011b. Distribution of varietal thiol precursors in the skin and the pulp of Melon B. and Sauvignon Blanc grapes. *Food Chemistry*, 125(1):139-144. https://doi.org/10.1016/j.foodchem.2010.08.050.

Ruiz, J., Kiene, F., Belda, I., Fracassetti, D., Marquina, D., Navascués, E., Calderón, F., Benito, A., Rauhut, D., Santos, A. & Benito, S. 2019. Effects on varietal aromas during winemaking: A review of the impact of varietal aromas on the flavor of wine. *Applied Microbiology and Biotechnology*, 103(18):7425-7450. https://doi.org/10.1007/s00253-019-10008-9.

Sacchi, K.L., Bisson, L.F. & Adams, D.O. 2005. A review of the effect of winemaking techniques on phenolic extraction in red wines. *American Journal of Enology and Viticulture*, 56(3):197-206.

Sadoudi, M., Tourdot-Maréchal, R., Rousseaux, S., Steyer, D., Gallardo-Chacón, J.J., Ballester, J., Vichi, S., Guérin-Schneider, R., Caixach, J. & Alexandre, H. 2012. Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon blanc wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts. *Food Microbiology*, 32(2):243-253. https://doi.org/10.1016/j.fm.2012.06.006.

Salinas, M. R., Garijo, J., Pardo, F., Zalacain, A., & Alonso, G. L. 2005. Influence of pre-fermentative maceration temperature on the colour and the phenolic and volatile composition of rosé wines. *Journal of the Science of Food and Agriculture*, 85(9):1527-1536. https://doi.org/10.1002/jsfa.2133.

Steyer, D., Ambroset, C., Brion, C., Claudel, P., Delobel, P., Sanchez, I., Erny, C., Blondin, B., Karst, F. & Legras, J.L. 2012. QTL mapping of the production of wine aroma compounds by yeast. *BMC Genomics*, 13(1):1-15. https://doi.org/10.1186/1471-2164-13-573.

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A. & Qian, M.C. 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of *Vitis vinifera* L. cv. Pinot noir wine. *Food Chemistry*, 173:424-431. https://doi.org/10.1016/j.foodchem.2014.09.150.

Swiegers, J.H., Francis, I.L., Herderich, M.J. & Pretorius, I.S. 2006. Meeting consumer expectations through management in vineyard and winery. *Wine Industry Journal*, 21(1):34-43.

Swiegers, J.H., Kievit, R.L., Siebert, T., Lattey, K.A., Bramley, B.R., Francis, I.L., King, E.S. & Pretorius, I.S. 2009. The influence of yeast on the aroma of Sauvignon blanc wine. *Food Microbiology*, 26(2):204-211. https://doi.org/10.1016/j.fm.2008.08.004.

Threlfall, R., Main, G. & Morris, J. 2006. Effect of freezing grape berries and heating must samples on extraction of components and composition parameters of red wine grape varieties. *Australian Journal of Grape and Wine Research*, 12(2):161-169. https://doi.org/10.1111/j.1755-0238.2006.tb00056.x.

Tominaga, T., Peyrot des Gachons, C. & Dubourdieu, D. 1998. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: S-cysteine conjugates. *Journal of Agricultural and Food Chemistry*, 46(12):5215-5219. https://doi.org/10.1021/jf980481u.

van Breda, V.M., van Jaarsveld, F.P. & van Wyk, J. 2024. Pre-Fermentative Cryogenic Treatments: The Effect on Aroma Compounds and Sensory Properties of Sauvignon Blanc and Chenin Blanc Wine - A Review. *Applied Sciences*, 14(4):1-14. https://doi.org/10.3390/app14041483.

Varela, C. 2016. The impact of non-Saccharomyces yeasts in the production of alcoholic beverages. *Applied Microbiology and Biotechnology*, 100(23): 9861-9874. https://doi.org/10.1007/s00253-016-7941-6.

Wilson, C. L. 2017. *Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines* (Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch). http://hdl.handle.net/10019.1/101250.

Wilson, C., Brand, J., du Toit, W. & Buica, A. 2018. Interaction effects of 3-mercaptohexan-1-ol (3MH), linalool and ethyl hexanoate on the aromatic profile of South African dry Chenin blanc wine by descriptive analysis (DA). *South African Journal of Enology and Viticulture*, 39(2):1-13. http://dx.doi.org/10.21548/39-2-3165.

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Introduction

Wine complexity and sensory quality can be attributed to the many flavour compounds originating from several origins and wine production practices, i.e., grape variety, viticulture, oenology and climatic conditions (Neethling et al., 2017:799; Pons et al., 2017:145; Kemp et al., 2022:350; Reynolds, 2022:510-513). These factors influence the development and concentration of flavour compounds within the grape berry during and after ripening and throughout processing. (Alem et al., 2019:978-982; Pinu et al., 2019:13-15; Cataldo et al., 2021:12; Chen & Li, 2022:313). The grapevine species, *Vitis vinifera* has between 5000 and 10000 different varieties, however, only a few varieties have commercial significance for wine and table grape production (Wine and Spirit Education Trust, 2012:2-5; Anonymous, 2021a; Robinson, 2021). Although each grape variety has its unique varietal characteristics, the wine producer can use agricultural practices and winemaking technologies as a tool to influence the chemical composition of the grape berry and organoleptic profiles of the wine (Capone & Jeffery, 2011:4663-4664; Ghantous, 2016:67; Neethling et al., 2017:799; Kemp et al., 2022:350; Prusova et al., 2022:12).

Viticultural and oenological practices are of great importance since previous research has shown that grape handling, processing, and yeast strains used for fermentation influence the levels of aroma compounds in the grape berry and final wine (Capone & Jeffery, 2011:4663-4665; Makhotkina et al., 2013:211-212; Parr et al., 2013:474; Casassa & Sari, 2015:1044-1055; Hart et al., 2019:165; Coetzee & du Toit, 2012:292-293; Prusova et al., 2022:12). Moreover, grape growers and winemakers are continuously confronted with new challenges as climate change affects grape physiology and viticultural practices. These, in addition to advances in winemaking technologies, influence the development of varietal aroma and flavour metabolites in final wines (Coetzee & du Toit, 2012:292-293; Cosme et al., 2016:178-208; Pons et al., 2017:145; Tomasevic et al., 2019:349-350; 355; Reynolds, 2022:451-459; 510-513). Numerous volatile aroma compounds are formed during the processing of grapes and grape juice (must) which contribute to the aroma of the wine together with the primary aromas to form the typical aromas of the variety and terroir from which it originates (Roland et al., 2011a:7371; b:142; Zhang et al., 2015:21622; Cosme et al., 2016:178-208; Prusova et al., 2022:2-9). Therefore, winemakers increasingly emphasise interactions between viticultural (climate, soil, water, cultivar, and grape growing practices) and oenological (pre- and post-fermentation treatments) practices to produce wines with sought-after organoleptic quality, as opposed to exclusively focusing on the contribution of cultivar type (Vilanova et al., 2013:126-130; Casassa & Sari, 2015:1047-1053; Pons et al., 2017:145; Chen et al., 2019b:639; 642; Ruiz et.al., 2019:7425-7450). In this regard, studies have shown that applying low-temperature, pre-fermentative maceration to white wine grapes enhances the extraction of desirable compounds during skin-juice contact. This allowed for an increased extraction of aroma compounds and their precursors that are mainly located in the skin of the grape berries (Baiano et al., 2012:2700; Chen et al., 2019b:642; Jagatić Korenika et al., 2019:467; Ruiz-Rodríguez et al., 2020:1-2; 11; Bestulić et al., 2022:6; Malićanin et al., 2022:2-17; Pedrosa-López et al., 2022:2-13).

The cold maceration process is used during white wine production to enhance the varietal character of the wines and also commonly used during red wine production to increase the extraction of phenolic compounds, which contribute to the colour and flavour intensity of red wine (Casassa et al., 2015:114; de Santis & Frangipane, 2010:51-52; Lukíc et al., 2017:259-260; Aleixandre-Tudo & du Toit, 2018:204-206; Benucci et al., 2018:261-264; Ruiz-Rodríguez et al., 2020:6; Korenika et al., 2020:52). Although cold maceration increases the concentration of aromatic compounds, it also extracts components that can cause undesirable characteristics (Aragón-García et al., 2021:1). A modification of this technique, known as cryofreezing or cryoextraction, involves the freezing of grapes (Zhang et al., 2016:39-40; 45; Tomaz et al., 2018:1-10; Ruiz-Rodríguez et al., 2020:1-13). Wines produced using this method exhibited higher aroma intensity and stability of taste properties compared to wines prepared using traditional maceration (Casassa et al., 2015:114; Dias Araujo et al., 2017:133-136; Lasanta et al., 2023:16). The wines perceived enhanced varietal taste and aromas may have resulted from the cryogenically frozen grapes experiencing reduced enzyme activity and oxidation, thereby preserving more of the grape's varietal character. Wines were well-balanced and better-rounded, with a fuller mouthfeel (Baiano et al., 2012:2700; Dias Araujo et al., 2017:133-136; Chen et al., 2019b:643-644; Ruiz-Rodríguez et al., 2020:6-11; Bestulić et al., 2022:9-10; Malićanin et al., 2022:11; 14; Pedrosa-López et al., 2022:10-11). Consequently, vineyard and/or wine-making technologies for enhancing such aroma compounds in wines are required. Moreover, the use of low-temperature treatment of grapes using cryogenic technologies has the potential to be a useful option for the wine industry, based on the use of cryogenic processing technologies applied in other beverage industries. Therefore, the present literature review aims to provide a comprehensive overview of the wine technological processes, i.e., viticultural and winemaking technologies, with an emphasis on pre-fermentative cryogenic treatment and its influence on grape aroma compounds, in particular the key varietal aromas of interest in this study, i.e., varietal thiols and methoxypyrazines linked to wine aroma and organoleptic quality of white wine.

### 2.2 Factors affecting grape and wine aroma compounds

### 2.2.1 Viticulture

#### 2.2.1.1 Agronomic practices

Agronomic practices such as soil and vineyard management, in combination with climatic conditions, are known to greatly influence the quality of the grapevine as well as concentrations of primary and secondary metabolites formed in the grape berry (Song et al., 2015:424-431; Alem et al., 2019:982; Pinu et al., 2019:15; Rice et al., 2019:1-12; Cataldo et al., 2021:8-11; Kemp et al., 2022:339; 349). The winemaker should have a good understanding of the abovementioned practices and how they can be controlled to enhance grape aroma metabolites (Reynolds & Wardle, 1997:3-17). Furthermore, practices such as pruning, irrigation, fertilisation, fungicide and foliar spray applications in the vineyard from one season to the next and harvesting time and methods directly impact grape metabolite formation (González-Barreiro et al., 2015:208-214; Song et al., 2015:426-430; Pinu et al., 2019:15; Rice et al., 2019:1-12; Kemp et al., 2022:349; Suklje et al., 2016a:1082; Rodrigues et al., 2023:2). Parr et al. (2013:474) demonstrated the association between viticultural and oenological factors and the ultimate effect on the final expression of Sauvignon blanc wines from New Zealand particularly related to vineyard location, vineyard row orientation, and grape processing operation (hand vs machine harvesting).

The application of foliar sprays in the vineyard has been shown to increase the concentrations of volatile thiols (3-sulfanylhexan-1-ol [3-SH] and 3-sulfanylhexyl acetate [3-SHA]) but not the grape-derived aromatic compounds (methoxypyrazines) (Suklje et al., 2016a:1082). Similarly, Lacroux et al. (2008:126;129-130) showed how the application of foliar nitrogen combined with sulfur increased the concentration of the volatile thiol 4-methyl-4-sulfanylpentan-2-one (4-MSP) and improved the overall aromatic profile of Sauvignon blanc wines. Cerreti et al. (2015:413-415) illustrated how the S-cysteinylated and S-glutathionylated thiol precursors increased with total soluble solids (TSS) during véraison, especially in a high-flavour cultivar, such as Sauvignon blanc. Similarly, this was also found for a second moderate-flavour cultivar (Grechetto); however, no differences in precursor compounds related to berry ripening were observed in a low-flavour cultivar (Malvasia del Lazio). Moreover, they were the first to identify and quantify S-30-(4-methyl-4-sulfanyl-pentan-2-ol)-glutathione in all three cultivars as a glutathione-conjugated precursor of 4-methyl-4-sulfanylpentan-2-ol (4-MSPOH). Suklje et al. (2012:9458-9460; 2016b:922;924) reported a similar correlation between methoxypyrazines (3-isobutyl-2-methoxypyrazine [ibMP] and 3-isopropyl-2-methoxypyrazine [ipMP]) and TSS concentration during berry ripening in a leaf removal trial. The concentrations of ibMP were found to be below the LOD when TSS concentrations were high, but present in berries with low TSS. Furthermore, it was shown that defoliation decreased the concentrations of ipMP and ibMP in the grape berries compared to the control berries. These results can be used as a valuable tool by wine producers in the harvesting and winemaking processes, for managing and possibly increasing the thiol varietal character of wines (Cerreti et al., 2015:413-415).

Research conducted by Rice et al. (2019:1-12) reported on the differences found in the aroma profiles of white grape varieties, Brianna and Frontenac Gris wines when harvested at different degrees of ripeness. Aroma profiles of the Brianna wines changed from "cotton candy" and "floral" to "banana" and "butterscotch" and eventually "honey", "caramel", and an "unknown neutral" aroma. The aroma profiles of Frontenac Gris wines changed from an "unknown neutral" aroma to "fruity" to "rose". Harvest time accounted for between 68-98% of the variation in key odour-active compounds for both cultivars (Rice et al., 2019:4-11). These studies, therefore, highlight the importance of agricultural practices, especially in the case of white wine grape production as the aromas originating from the metabolites in the grapes directly influence the varietal profile associated with the cultivar as well as the organoleptic profile of the wine (Aleixandre-Tudo & du Toit, 2018:204-206; Pinu et al., 2019:15; Cataldo et al., 2021:11-12).

#### 2.2.1.2 Fungal disease

Wine grapes are prone to spoilage by fungal diseases that affect the vine, i.e., downy mildew and powdery mildew (Steel et al., 2013:5189-5200). Research conducted by Dzedze et al. (2019:265-276) on Chenin blanc grapes treated with different chemical sprays, i.e., Methyl-1H-pyrazole carboxylic acid phenylethyl amide, boscalid and penconazole with extract of *Gelania africana,* highlighted that the different sprays affected wine yeast protein expression. This is important to note since yeast-derived proteins are needed to release certain wine aroma-enhancing compounds (metabolites), particularly volatile thiols during fermentation, thus influencing the aroma profile of the wine (Hart et al., 2017:147, 2019:165). Differences in metabolite levels were observed, which meant that the fungicide treatments affected the yeast's ability to express proteins, influencing the wine's aroma and flavour (Dzedze et al., 2019:273-275).

Although fungi are generally associated with the spoilage of wine grapes, *Botrytis cinerea* (grey mould) causes noble rot under favourable conditions producing botrytised wines. Noble rot is a unique process that, under specific conditions, greatly enhances the grape quality and produces desirable wines (Roland et al., 2011a:7370; Steel et al., 2013:5193; Magyar & Soos, 2016:29-37; Avizcuri-Inac et al., 2018:15-28; Dankó et al., 2021:1-5; Santos et al., 2022:1-12). The noble rot process involves the decomposition of grape compounds, while new compounds are produced by the fungus. Interestingly, previous studies found that wines made from botrytised grapes had significant amounts of volatile thiols, especially 3-SH (Dzedze et al., 2019:265-276; Dankó et al., 2021:1-5). This is largely due to the concentration effect that *Botrytis* has on grape berries. Furthermore, *Botrytis*-infected Sauvignon blanc is commonly used to produce noble late-harvest wines known for their aromatic profiles and sweet taste (Roland et al., 2011a:7370; Avizcuri-Inac et al., 2018:16; Dankó et al., 2021:1-5).

#### 2.2.1.3 Harvesting methods

Wine grapes are commonly harvested by hand (manually) or by machine (mechanically). Hand harvesting has long been the traditional and acceptable method for harvesting grapes for wine production. However, in recent years, wine producers have frequently used mechanical harvesters to increase yield and reduce time and cost (Allen et al., 2011:10641-10650; Anonymous, 2022a). It was also found, in many instances, that the grape juice and wines resulting from mechanically harvested grapes had higher levels of varietal thiol precursors 3-S-cysteinylhexan-1-ol (Cys-3-SH) and 3-S-glutathionylhexan-1-ol (Glut-3-SH) as well as varietal thiols in the final wines. This attracted the attention of the wine industry and numerous studies have been conducted, although results vary (Allen et al., 2011:10641-10642; Kilmartin, 2012:81-83; Jeffery, 2016:1323-1330; Coetzee, 2020a; b). Hence, the type of harvesting method implemented, hand-picking, mechanical harvesting, or a combination of the two, depends mainly on the style of wine that the winemaker wishes to produce (Parenti et al., 2015:20-21; Brillante et al, 2018:261-271).

##### 2.2.1.3.1 Hand-harvesting

Globally and in SA, wine grapes were traditionally harvested manually, where grapes were handpicked into crates and transported to the winery for vinification (Allen et al., 2011:10649; Jeffery, 2016:1323-1330; Coetzee, 2020b). The advantage of hand-harvesting is the ability of the worker to distinguish between ripe and unripe grape berries, as well as healthy and diseased grape berries, which will impact the quality of the wine produced (Parenti et al., 2015:21-22). Previous studies showed that the levels of thiol precursors from hand-harvested grape bunches were lower because of less damage to the grape berry, resulting in less extraction of the precursors from the grape skin (Allen et al., 2011:10641-10650; Coetzee & du Toit, 2012:288-289; Coetzee, 2020b; Jeffery, 2016:1328-1329). Hand-harvesting is a gentler process than mechanical-harvesting and therefore reduces the potential for oxidation of the juice (Creasy & Creasy, 2002:218; Olejar et al., 2015a:189; Coetzee, 2020b).

#### 2.2.1.3.2 Mechanical-harvesting

Larger producers often use mechanical harvesting to increase harvest speed, especially in large areas and hot wine regions. However, due to the inability of the mechanical harvester to differentiate between rotten and healthy grapes, there is an increased risk of oxidation that results in poor grape juice quality (Creasy & Creasy, 2002:218-219; Kilmartin, 2012:81-82; 86; Coetzee, 2020a; b). Consequently, it is important to protect the must from oxygen by adding sulfur dioxide ($SO_2$) and limiting oxidation (Makhotkina et al., 2013:211-212). Studies have reported a possible correlation between an increase in the concentrations of varietal thiol precursors, the type of thiols, and the concentration of 3-sulfanylhexan-1-ol (3-SH) and 3-sulfanylhexyl acetate (3-SHA) concentrations in the final wines with mechanically harvested grapes (Allen et al., 2011:10641-10650; Kilmartin, 2012:82; Coetzee, 2020a; b; Jeffery, 2016:1328-1329). This is believed to result from a pre-fermentative pathway between (*E*)-2-hexenal and the cysteine or glutathione present in the grape must by way of the enzymatic oxidation of grape fatty acids, thus leading to the formation of the 3-SH precursors as a result of the grape berry being damaged (Jeffery, 2016:1327-1329; Ferreira & Lopez, 2019:8; 12; Tirelli et al., 2021:66-67).

Harvesting time and duration of transportation of the machine-harvested grapes to the cellar were also shown to play a role in significantly increasing concentrations of the Cys-3-SH (tenfold) and Glut-3-SM (doubled) thiol precursors (Allen et al., 2011:10641-10650; Jeffery, 2016:1328-1329). Research conducted by Jouanneau (2011:117-118) and Sun et al. (2022:2-3) found that commercial Sauvignon blanc produced from machine-harvested grapes had 5-10 times higher concentrations of 3-SH than wines produced from hand-picked grapes. It should also be noted that an increase in the levels of thiols is not necessarily a measure of the quality of the wine as it is known that at high levels they could contribute to undesirable aromas (Swiegers et al., 2006:36; Kilmartin, 2012:81-86; Olejar et al., 2015a:188; Ferreira & Lopez, 2019:2-35; Coetzee, 2020a; b).

## 2.3 Wine technological processes affecting aroma compounds

Dating back to 6000 BC, winemaking has been around for thousands of years. A process that was accidentally discovered has over the last three decades, seen a huge shift in technological development. These advances in winemaking technologies have led to the production of novel wine styles with enhanced quality from the same grape cultivar (Allen et al., 2011:10641-10650; Baiano et al., 2012:2694-2701; Muñoz García et al., 2022:3-16). Amongst these technologies, specifically, pre-fermentative cold maceration techniques to produce wines with enhanced varietal and sensory profiles show promise and will be discussed in more detail in this thesis (Aleixandre-Tudo & du

Toit, 2018:204-207; Benucci et al., 2018:257-266; Korenika et al., 2020:49-53; Bestulić et al., 2022:1-11).

### 2.3.1 Traditional winemaking practices

#### 2.3.1.1 Crushing and destemming

Following harvesting (hand or machine), grapes are delivered to the cellar and either crushed immediately or allowed to cool overnight. Whole grape bunches are loaded into a crushing and destemming machine which separates the grape berries from the stalks (Anonymous, 2022b; Guerrini et al., 2022:2; Kemp et al., 2022:339). During the crushing process, the grape berries burst open, allowing for the extraction of precursors and aroma compounds from the grape skins into the juice, resulting in a redistribution of the grape components amongst the different segments of the disrupted berries (Darias-Martín et al., 2004:336-338; Guerrini et al., 2022:2). This is of importance because the different components contain specific compounds, i.e., the grape skin contains the highest concentrations of minerals, aromatic compounds, and phenols, whilst the grape flesh is abundant in sugars and acids. The seeds contain essential oils and catechins for bitter and herbaceous notes (Darias-Martín et al., 2004:335-340).

Research conducted by Guerrini et al. (2022:13) illustrated that during the production of rosé wines, the pressure applied during pressing, in conjunction with destemming and crushing, determined the characteristics of the final wine. Moreover, their research showed that the application of various crushing and pressing techniques can change crucial properties such as colour, acidity, and astringency of wines and highlighted how these pre-fermentation processes influence the style and quality of the final wine (Guerrini et al., 2022:13).

#### 2.3.1.2 Grape skin maceration

Skin maceration or skin contact is not standard practice during the white wine process however, it has been used to allow for maximum extraction of aroma compounds from the grape skins (Maggu et al., 2007:10287; Petropulos et al., 2014:513-514). Crushed grapes and juice are placed in a tank at a cool temperature (~10 ºC) to macerate for a few hours. This process will depend on the style of wine that the winemaker intends to produce (Petropulos et al., 2014:506-514; Olejar et al., 2015b:181-189, Anonymous, 2022b). During skin maceration, the colour and chemical composition of the grape juice is affected, resulting from the distribution of volatile and phenolic compounds from the solid to the liquid portion of the juice (Maggu et al., 2007:10287; Guerrini et al., 2022:4-13; Kemp et al., 2022:344). Selli et al. (2006:76) investigated the effect of skin contact on the concentrations of free and bound aroma compounds in Narince wines. It was found that the concentrations of both the free and bound aroma compounds in skin-contact wines had much higher levels of volatiles compared to the control wines. However, for the free aroma compounds, concentrations of the nitrogenous and acetal compounds were lower (Selli et al., 2006:77-80).

Maggu et al. (2007:10287) conducted a trial using commercial Sauvignon blanc grape must collected from pressing before skin contact, after 1 hour of skin contact, followed by pressing at 0.4, 1.2 and 2 bar pressure. A concurrent laboratory-scale trial was also conducted on Sauvignon blanc grape must to evaluate the effects of pressing (0 or free run, 0.4, and 2 bar) in combination with skin contact time (0, 4, 16 and 32 hours) on the concentration of varietal aroma compounds (Maggu et al., 2007:10285). In general, it was found that concentrations of the Cys-3-SH precursor increased as pressing time and pressure increased, for both trials. However, the levels of the methoxypyrazine 3-isobutyl-2-methoxypyrazine (ibMP) were not significantly affected during the cellar trial but showed a consistent increase during the laboratory-scale trial, especially in juices pressed after prolonged skin contact (Maggu et al., 2007:10284-10285). Moreover, it was seen that although the increased skin contact time increased concentrations of varietal aroma compounds in Sauvignon blanc wines, the juice oxidative potential also increased, leading to a decrease in the wine organoleptic quality (Marais, 1998:14; Maggu et al., 2007:10287).

Maceration technologies such as thermovinification, carbonic maceration (CM) and flash release systems are currently being used to facilitate the maceration process. However, these techniques are often time-consuming, costly and the high temperatures have been shown to negatively affect aroma compounds and the aroma profile of the wine (Ayestarán et al., 2019:187-194; Casassa et al., 2019:2-14; Romero-Díez et al., 2019:258-266; Fanzone et al., 2022:13-14; Casassa et al., 2022:2-27; Muñoz García et al., 2022:2). During CM, whole grape bunches are placed in a tank which is subsequently filled with carbon dioxide to create an anaerobic atmosphere and left for a few days (Mozzon et al., 2016:198). During this process, grapes are subjected to enzymatic reactions rather than yeast interactions. The grape structure is broken down and thereafter the yeast fermentation process begins. The flavour profile of CM grapes can be associated with a biochemical pathway regarding specific amino acids which allows the distinctive must aromas to remain after the fermentation process. Aromatic profiles of wines produced by CM were shown to exhibit pleasant fruity notes, rather than herbaceous notes due to the production of $C_6$ aldehydes and alcohols in the absence of oxygen and due to the lack of crushing of the grapes (Mozzon et al., 2016:198). Furthermore, the aroma of CM white wines is a complex combination of grape varietal aromas, volatile compounds and fermentative aromas which greatly improve the wine quality when compared to traditionally produced white wines (Ayestarán et al., 2019:189-190; 193).

Cold maceration is yet another technique used during white wine production to enhance the varietal character of the wine (Zhang et al., 2015:21622; Chen et al., 2019b:643-644; Pedrosa-López et al., 2022:1-12). Although numerous studies have been conducted, literature suggests that maceration, and particularly the impact of cold maceration, on the extraction and stability of phenolic compounds still requires further investigation (Zhang et al., 2015:21622; Aleixandre-Tudo & du Toit, 2018:204-207; Chen et al., 2019b:643-644; Pedrosa-López et al., 2022:1-12).

#### 2.3.1.3 Pressing

Pressing is a critical step during the winemaking process and can affect the resultant yield, overall quality and flavour profile of the wine (Maggu et al., 2007:10287; Ferreira-Lima et al., 2016:956; Catania et al., 2019:159; 164; Day et al., 2019:194-198; Fracassetti et al., 2021:2-6). Pressing generally follows the crushing and destemming process, however, depending on the style, whole grape bunches can also be pressed. This process allows for the extraction of the juice from the grape flesh and skins (berry). Moreover, the pressing process (batch or continuous), type of press (vertical basket, pneumatic membrane, horizontal screw press and continuous screw press), as well as the duration of the pressing process will positively or negatively affect the extraction of compounds from the pips, skins and in some instances stems (Creasy & Creasy, 2002:219-2020; Anonymous, 2022c; Kemp et al., 2022:339). During pressing, pressures can range from less than one bar to a maximum of six bars and can be performed for a duration of one to two hours or longer, depending on the wine style (Maggu et al., 2007:10284).

As previously mentioned, Maggu et al. (2007:10284) conducted laboratory-scale trials to investigate the effects of pressing in combination with skin contact time on the concentration of varietal aroma compounds in Sauvignon blanc grape must. The study found that the pressure (0.4, 1.2 and 2 bar) applied during the pressing process increased the levels of the Cys-3-SH precursor, responsible for the passion fruit aroma associated with Sauvignon blanc. This result was confirmed in a study conducted by Tirelli et al. (2021:67), who found that most of the G-3SHal and Cys-3-SH were produced after the grapes were pressed. Maggu et al. (2007:10284) made similar observations when comparing pressed and free-run juice and found that the levels of the 3-SH-cys precursor increased 6.2 times when pressed at an atmospheric (atm) pressure of 2 atm, compared to only increasing 4.4 times when pressed at 1.3 atm. The abovementioned research also considered the effect of pressing on the methoxypyrazine compound, ibMP and found that, unlike the thiol precursors, which increased, ibMP levels increased with prolonged skin contact, and tended to decrease with increasing pressure during pressing (Maggu et al., 2007:10284-10286). It was further seen that the pressed juice without any skin contact had high concentrations of glutathione and caftaric acid, which were similar to those observed for the free run juice used in the winery trials (Maggu et al., 2007:10287; Ferreire-Lima et al., 2016:954).

Moreover, during pressing, oxygen can be introduced to the grape must, which can substantially affect the chemical composition, organoleptic properties and overall quality of the wine. Excessive or harsh pressing could result in high levels of herbaceous notes, and increased juice oxidative potential, which is observed as a deterioration in the protective glutathione content and an increase in oxidizable polyphenol compounds (Marais, 1998:14; Maggu et al., 2007:10287; Catania et al., 2019:161-164; Day et al., 2019:194-198; Anonymous, 2022c; Kemp et al., 2022:345). Boselli et al. (2010:1499) investigated the effect of pressing Chardonnay, Grechetto and Orvieta (white wine cultivars) under nitrogen and found that the phenolic compounds in the Chardonnay and Grechetto grape must were well protected against oxidation. Similarly, Pons et al. (2015:188-189; 193) investigated the effect of pressing Sauvignon blanc grapes in the presence of nitrogen and found that the concentration of glutathione was increased when pressing at low pressure (< 1 bar). These studies highlight the effects that pre-fermentative winemaking practices can have on the chemical composition and overall quality of white wines and illustrate the importance of pressing under inert conditions (Boselli et al., 2010:1499; Pons et al., 2015:193; Ferreira-Lima et al., 2016:96; Catania et al., 2019:164).

#### 2.3.1.4 Settling/clarification

Following pressing, the turbid grape must, containing suspended solids, is transferred to a settling tank or barrel. Settling or clarification processes used during white wine production involve the removal of solid particles from the must by either sedimentation, filtration or flotation (Mierczynska-Vasilev et al., 2015:618; Casalta et al., 2019:148; Ridge et al., 2021:10-11; Vázquez-Pateiro et al., 2022:1). However, excessive clarification or settling of the must can lead to the reduction of volatile compound concentrations and, consequently, modify the sensorial properties of the wines (Nicolini et al., 2011:133; Ma et al., 2020:14; Ridge et al., 2021:2-3; Vázquez-Pateiro et al., 2022:1;14). These processes are used to remove compounds responsible for undesirable flavours and aromas without negatively affecting the fermentation process as well as suspended and colloidal particles which could cause turbidity and haziness in the final wine (Mierczynska-Vasilev et al., 2015:615; Vázquez-Pateiro et al., 2022:1). Extensive settling or clarification, however, removes essential nutrients needed by the yeast and could lead to stuck fermentations and wine with a poor flavour profile and could affect the levels of varietal aroma compounds such as methoxypyrazines in final wines (Ma et al., 2020:14; Anonymous 2022b; Kemp et al., 2022:343).

Vázquez-Pateiro et al. (2022:14) studied the effect of two clarification techniques, i.e., flotation and traditional static sedimentation on an industrial scale on the volatile composition of two white wine cultivars, Albariño and Treixadura. Most concentrations of volatile compounds in the wines, for both cultivars, were found to be similar irrespective of the clarification method employed, except for the concentrations of 1-hexanol, octanoic acid and furfural in Albariño wines and that of benzyl alcohol in Treixadura wines, all of which increased when using flotation. The sensory evaluation showed that the panel preferred wines produced using the flotation technique for both Albariño and Treixadura varieties (Vázquez-Pateiro et al., 2022:3; 8; 14). Their study suggested that must clarification through flotation was favoured over clarification by static settling because it saved time, decreased the costs, did not change the physicochemical parameters (alcoholic content, pH, etc.) of the wines and did not reduce the concentrations of volatile compounds. In addition, the study suggested that wines made by using flotation for clarification produced wines with better quality; however, additional studies are necessary (Vázquez-Pateiro et al., 2022:14). Gil et al. (2019:497) investigated the effect of Polyvinylpolypyrrolidone (PVPP) on the colour, polyphenol content and thiol aroma compounds of rosé wines. Results revealed an increase in the thiol content with an increasing dose of PVPP, furthermore, improved colour and increased polyphenol content were also observed (Gil et al., 2019:495-497). Moreover, although numerous other studies investigated the effect of the many clarification techniques and fining agents currently available, it is not the focus of this review and will not be discussed further (Matthews et al., 2004:5715–5731; Mierczynska-Vasilev et al., 2015:624; Gil et al., 2019:495-497; Prodanov et al., 2019:736-737; Ma et al., 2020:14; Vázquez-Pateiro et al., 2022:1).

#### 2.3.1.5 Alcoholic fermentation

During the alcoholic fermentation process, yeast converts the sugars present in the grape juice into alcohol and carbon dioxide ($CO_2$). Alcoholic fermentations are predominantly conducted using the wine yeast strain *Saccharomyces cerevisiae*, although the yeasts naturally occurring on the grapes also play an important role (Jussier et al., 2006:224-226; Swiegers & Pretorius, 2007:954-960; van Breda et al., 2013:87; Varela, 2016:9869; Coetzee, 2022b). White wine fermentations are generally conducted at ~15 ºC for 14 days. The choice of the yeast and inoculation method, i.e., inoculated or uninoculated, choice of the fermentation vessel, i.e., stainless steel tank, oak barrel or concrete eggs, fermentation temperature, juice contact with grape skins and seeds, the type of yeast nutrient, malolactic fermentation (MLF), and the use of oak for ageing wines, all influence the aroma profile, flavour and appearance of white wine. For example, low temperatures (10 ºC or 15 ºC) increase the production of volatile aroma compounds (esters, acetates, medium-chained fatty acids) in white wines. However, the presence and concentration of aroma compounds in white wines are also highly influenced by the number of aroma precursors located in the grape skin and pulp (Roland et al., 2011b:142-144; Zhang et al., 2015:21622).

During the fermentation process, aroma-inactive, non-volatile precursors in the grape must are converted to volatile aroma compounds by the $\beta$-lyase enzymatic activity of the yeast (Hart et al., 2016:2077; 2079; Průšová et al., 2018:892; 896; Hart et al., 2019:165; Dimopoulou et al., 2020:751; Coetzee, 2020c; Coetzee, 2022b). This enzyme activity allows for the conversion of the cysteine and glutathione precursor conjugates from non-aromatic to aromatic volatile thiols (e.g., resulting from the cleavage of the carbon-sulfur linkage (Murat et al., 2001:136-139). This process is therefore vital in releasing fermentation flavours that contribute to the aroma complexity and quality of wines and will be discussed in more detail later (Hart et al., 2016:2079; 2017:153; Visan et al., 2018:425; Williams 2018:13-16; Hart et al., 2019:165; Dimopoulou et al., 2020:751; 757; Červinka et al., 2021:488; 492).

#### 2.3.1.6 Maturation

Maturation refers to all the changes that occur in the wine after fermentation and before bottling. During this process, the wine may be subjected to various treatments, including malolactic fermentation, clarification, stabilisation, and bulk storage (Anonymous, 2022d; Kemp et al., 2022:339). Depending on the winemaking style, this process could last for a period of four months up to two years. Tertiary aromas develop during maturation, altering wine aromas from fresh fruity to oxidative, dried fruit and spicy aromas (Anonymous, 2022e; Fracassetti et al., 2020:11; Kemp et al., 2022:339). Sauvignon blanc wines are generally matured in stainless steel tanks or during bottle storage and could also be blended with other white grape varieties or aged in oak. Chenin blanc, on the other hand, is not generally matured in stainless steel tanks, but rather in oak barrels, imparting smoother, buttery characteristics and adding complexity to the wine (Anonymous, 2022d; e; Kemp et al*.,* 2022:339). Furthermore, during the maturation process, Sauvignon Blanc and Chenin blanc wines experience a significant decrease in volatile thiols due to oxidation (Bailly et al., 2006:7233; Ugliano et al., 2011:2570-2571; Coetzee & du Toit, 2012:293; Wilson, 2017:30-31). Methoxypyrazines, however, are more resistant to oxidation, but still diminish over time (Marais, 1994:43; Coetzee, 2011:20). Therefore, prolonged ageing or excessive oxygen exposure during maturation can lead to a loss of the desirable tropical, citrus, and green-vegetative aromas and varietal characters typically associated with Sauvignon blanc and Chenin blanc wines (Ugliano et al., 2011:2570-2571; Coetzee & du Toit, 2012:293; Wilson, 2017:30-31).

#### 2.3.1.7 Bottling and ageing

The final step in the winemaking process is the bottling of the wine. During bottling, the wine is subjected to multiple processes, i.e., pumping, filtration, filling and closure with corking or capping (Anonymous, 2022b; Kemp et al., 2022:339). These processes must be controlled as wines are often susceptible to oxygen exposure, resulting in undesirable acetaldehyde formation (Silva et al., 2011:905-907; October, 2020:41-45). Furthermore, following bottling, wines could also further be exposed to oxygen in the headspace of the bottle, intake into the bottle via the lining of the closure and from the closure into the bottle, due to compression during bottling (Bailly et al., 2009:8561; Silva et al., 2011:905-907; Anonymous, 2022b). Bottle ageing occurs when wines are left in the bottle for prolonged periods. During ageing, the properties of the wine change, i.e., the fruity and fermentation flavours evolve into developed characteristics resulting from numerous acid-catalysed reactions, therefore affecting the wine aroma and quality (Bailly et al., 2009:8560-8561; Silva et al., 2011:909-911; Fracassetti et al., 2020:11; Kemp et al., 2022:339).

## 2.4 Non-typical winemaking practices

### 2.4.1 Cryogenic technologies

Pre-fermentative cold maceration is a technique used during white wine production to enhance the varietal character of the wine (Peinado et al., 2004:586-589; Zhang et al., 2015:21609-21610; Chen et al., 2019b:637-644; Pedrosa-López et al., 2022:1-12). However, in the last two decades, this technology has also been applied as a treatment during the red winemaking process to improve the extraction of colour and stabilisation of phenolic compounds (de Santis & Frangipane, 2010:49-52; Baiano et al., 2012:2697; Aleixandre-Tudo & du Toit, 2018:204-207; Benucci et al., 2018:261-265; Korenika et al., 2020:49-53). The treatment involves subjecting whole grapes and macerated grapes to rapid cooling (5 ºC and below) and holding them at the desired temperature for hours or days, thus improving the extraction of compounds (phenolic compounds and primary aroma) contained within the grape skins (Fragasso et al., 2010:20-25; Aleixandre-Tudo & du Toit, 2018:204-205; Benucci et al., 2018:261-265; Korenika et al., 2020:49-53; Bestulić et al., 2022:1-10; Pedrosa-López et al., 2022:1-12). Cryo-extraction or cryo-freezing is a modification of this conventional cold maceration process and involves the freezing of grape berries (Sacchi et al., 2005:199-200; Naviglio et al., 2018:1-2; Tomaz et al., 2018:1-10). During the freezing process, grapes are frozen and ice crystals are formed which tear the pectocellulose cell walls, disorganizing the tissues, thus facilitating the extraction of compounds from the grape skin (Heydarov et al., 2020:46-47; Ruiz-Rodríguez et al., 2020:2). Cryogenic treatments have been applied using various methods, i.e., large capacity refrigerators/chambers, freeze-concentration technology, blast-freezing or by adding cryogenic agents in a solid or liquid state, i.e., nitrogen ($N_2$) or carbon dioxide ($CO_2$) (Sacchi et al., 2005:199-200; Carillo et al., 2011:1-14; Schmid & Jiranek, 2011:26; Zhang et al., 2016:39-40; Ruiz-Rodríguez et al., 2020:1-13, Coetzee, 2022a). Consequently, the techniques mentioned above have advantages and disadvantages which will be discussed below.

#### 2.4.1.1 Large capacity refrigerators

Conventional freezing in large refrigerators/chambers leads to increased costs and energy consumption due to cooling taking place at a much slower rate, ranging from hours to days (Schmid & Jiranek, 2011:26; Ruiz-Rodríguez et al., 2020:2; Coetzee, 2022a). Consequently, this increases the risk of developing undesirable flavours due to continuous biochemical changes within the grape berry and the risk of oxidation (Santesteban et al., 2013:3011; 3015). In addition, during conventional or slow freezing, large ice crystals are formed, which damage cells in the berry, hence the speed of freezing should be considered when applying this technique. However, conditions can be controlled when using large freezing chambers that reduce the extraction of bitter tannins transferred to the wine (Santesteban et al., 2013:3011-3015).

#### 2.4.1.2 Freeze concentration

Freeze concentration is a technology used in the food industry involving solid-liquid phase separation at low temperatures (< 0 ºC) for recovering a food solute from a solution based on the separation of pure ice crystals from a freeze-concentrated liquid phase (Petzold et al., 2013:357-361; 2015:192-197). This technology has been used to concentrate products, such as fruit juices, coffee, tea extracts and aroma extracts (Deshpande et al., 2009:189-195; Petzold et al., 2013:357-361; 2015:192-197). The process occurs at low temperatures, producing high-quality concentrates since heat-sensitive volatile compounds are protected. The process involves three steps, i.e., ice crystal formation, ice crystal growth, and the separation of ice crystals from the solution. Based on ice crystal formation, two methods of freeze concentration exist, i.e., suspension freeze concentration and progressive freeze concentration (Deshpande et al., 1984:189-195; Miyawaki, 2012:377-382). Following suspension freeze-concentration, fewer soluble solids remain in the ice crystal, making this technology easier to use on an industrial scale (Deshpande et al., 1984:189-195; Ye et al., 2014:276; 283).

Moreover, this technology can prove to be valuable for the wine industry because the process is conducted at a low temperature where no vapour/liquid interface exists, therefore resulting in minimal loss of volatiles (Deshpande et al., 1984:222-223; Petzold et al., 2013:357-361). Most wineries have fermentation tanks with cooling systems in which ice can be crystallised and therefore have the potential to perform freeze concentration without purchasing new equipment (Miyawaki, 2012:378-379). Zhang et al. (2016:21621-21622) and Keyzers & Boss (2011:1162) used suspension freeze technology to concentrate grape juice and discovered that wines produced from the concentrated grape juice were superior to control wines in terms of chemical and sensory profiles. Deshpande et al. (1984:222-223) illustrated that freeze concentration could also be applied to wine to be exported to prevent deterioration, however, two major drawbacks are the loss of alcohol to the ice and the precipitation of tannins. This can, however, be overcome by modifying the processes. Another drawback of the older technology is the high energy consumption, but with the more modern technologies, this can also be overcome to make it a very energy-efficient process (Deshpande et al., 1984:244).

#### 2.4.1.3 Blast-freezing

Blast-freezing is a rapid freezing technique often used in the food industry to preserve food before transportation and has also been used as a pre-fermentation treatment in the wine industry (Schmid & Jiranek, 2011:25-30; Santesteban et al., 2013:3010-3015). During the conventional freezing process, water within the food structure crystallises and forms large ice crystals, causing the food material cell walls to burst, affecting the food quality and flavour (Dempsey & Bansal, 2012:71-72). The speed of freezing is, therefore, an important aspect to consider, since it is directly related to the degree of disorganisation of the food and in the case of grapes, the berry structure (Dempsey & Bansal, 2012:71-72; Santesteban et al., 2013:3010-3011). Rapid freezing within a blast-freezer causes the formation of very small ice crystals which cause less damage and preserve food at a higher quality (Dempsey & Bansal, 2012:71-72). Blast-freezing used as a pre-fermentative cryogenic treatment for wine grapes is conducted by blasting cold air, with temperatures ranging between -10 ºC to -120 °C directly onto whole grape bunches. This freezing method allows all individual berries to be frozen within 1-2 hours (Schmid & Jiranek, 2011:26). Equipment used can either be hired or bought, making it more cost-effective than building an external refrigeration unit (Schmid & Jiranek, 2011:26; Dempsey & Bansal, 2012:71-83; Santesteban et al., 2013:3010).

#### 2.4.1.4 Cryogenic agents

Cryogenic agents, i.e., carbon dioxide ($CO_2$) and nitrogen ($N_2$) are commonly added to whole grapes before crushing or during crushing in a solid state (dry ice or solid $CO_2$)

or using direct injection of the cryogenic agent in liquid form (Carillo et al*.,* 2011:1-14; Schmid & Jiranek, 2011:26; Ruiz-Rodríguez et al., 2020:2-3). The use of both these cryogens, solid $CO_2$ and liquid $N_2$, induces a thermal shock, leading to an increase in the degradation of the cell structure of grape berries (Santesteban et al., 2013:3015; Ruiz-Rodríguez et al., 2020:5-9). This is advantageous as it prolongs the contact time between the grape pulp and must in the absence of oxygen, thus protecting the grapes from oxidation (Santesteban et al., 2013:3015). If dry ice is used for the pre-fermentation skin contact, tissues in the cell walls are disrupted and "disorganised", which enables the extraction of compounds located within the grape skin (Schmid & Jiranek, 2011:25-30; Ruiz-Rodríguez et al., 2020:2). The freezing process causes an increase in the intracellular liquids, therefore, disrupting the membranes, allowing for the release of aromatic and phenolic compounds (Baiano et al., 2012:2697-2698). Cryomaceration with cryogens was found to be the most effective method because they are heavier than oxygen, they displace the air and create an inert atmosphere, protecting the grapes from oxidation. However, the cost implications associated with cryogenic agents could be a limiting factor (Carillo et al., 2011:1-14; Baiano et al., 2012:2699-2700; Mencarelli & Bellincontro, 2020:5051; 5053).

### 2.4.2 Effect of cryogenic treatments on physicochemical parameters of grape must and wine

Total soluble solids (TSS) of must were shown to be least affected by freezing, as no difference was observed for storage periods less than six months (Threlfall et al., 167; Olarte Mantilla et al., 2013:352; Santesteban et al., 2013:3011, Zhang et al., 2015:21611; Pedrosa-López et al., 2022:4-5). However, a study conducted by Ruiz-Rodríguez et al. (2020:6; 13) found that different freezing techniques, i.e., liquid nitrogen as opposed to ultra-fast mechanical freezing, resulted in significant differences in TSS values. The total acidity (TA) of grape must was found to be lower in cryogenically treated samples, which confirmed the earlier findings of Olarte Mantilla et al. (2013:352), Santesteban et al. (2013:3011) and Zhang et al. (2015:21611). This was related to the precipitation of potassium ($K^+$) salts during the freezing process and the lower solubility of acidic salts during defrosting of the grapes. Additionally, higher pH levels observed in musts obtained after freezing and defrosting confirmed previous studies (Schmid & Jiranek, 2011:28-29; Olarte Mantilla et al., 2013:352-353; Santesteban et al., 2013:3012; Zhang et al., 2015:21611; Ruiz-Rodríguez et al., 2020:5; Pedrosa-López et al., 2022:4-5). Moreover, the chemical parameter most affected by freeze storage was found to be titratable acidity (Pedrosa-López et al., 2022:4-5).

Research conducted by Carillo et al. (2011:1-14), Naviglio et al. (2018:1-13) and Naranjo et al. (2021:1-13) involved the rapid cooling of grapes by sparging the grapes with liquid $CO_2$ before crushing and destemming. It was seen that the wines produced from the Chardonnay, Maturana Blanca and Bianchello del Metauro (white) grape cultivars were not statistically different from the control wines in terms of certain physicochemical parameters, i.e., alcohol, pH, titratable acidity and volatile acidity (Carillo et al., 2011:9-10; Naviglio et al., 2018:8; Naranjo et al., 2021:5). This was similar to findings observed in the earlier research of Antonelli et al. (2010:88). However, significant differences for seen for malic acid levels which increased from 1.08 g $L^{-1}$ in traditional winemaking when compared to 1.7 g $L^{-1}$ in wine made from $CO_2$ pretreated grapes (Carillo et al., 2011:10; Santesteban et al., 2013:3012). Previous research showed that the physicochemical parameters of wine produced from fresh and frozen grapes and grape juice had significant differences in tartaric acid, but not the alcohol content (Schmid & Jiranek, 2011:28-29; Olarte Mantilla et al., 2013:352; Santesteban et al., 2013:3012). Moreover, studies conducted by Zhang et al. (2015:21611) and Pedrosa-López et al. (2022:4-5) found differences in alcohol levels in final wines produced from previously frozen whole grapes and macerated grapes. These differences were attributed to the extraction of the compound, which affected the fermentation process. Therefore, the freezing method influences the quantity and type of compounds extracted from the grapes, which in turn influence how well the fermentation proceeds, and the alcohol concentrations in the final wine. Zhang et al. (2015:21610-21611) further found that wines produced from cold-macerated grapes had higher pH and glycerol levels compared to the control and skin-macerated treatments. This was similar to previous findings (Olarte Mantilla et al., 2013:352; Santesteban et al., 2013:3012), although it differed from the findings of Carillo et al. (2011:10) and other research which showed that wines produced from grapes subjected to cold maceration did not show significant differences in chemical parameters when compared to the control (Schmid & Jiranek, 2011:25-30; Korenika et al., 2020:49-53; Naranjo et al., 2021:5; Pedrosa-López et al., 2022:1-12).

It should, however, be noted that these effects were not necessarily only due to the freezing treatment but resulted from storage time, duration of skin contact time and grape cultivar (Santesteban et al., 2013:3010-3015; Zhang et al., 2015:21621-21622; Ruiz-Rodríguez et al., 2020:1-13). Furthermore, it should be noted that the type of cryomaceration treatment applied, as well as whether applied to whole berries, macerated grapes or juice, also influences must and wine physicochemical parameters as conflicting results were observed between studies where the application of these parameters varied considerably (Zhang et al., 2015:21611; 21621-21622; Ruiz-Rodríguez et al., 2020:1-13).

### 2.4.3 Effect of pre-fermentative cryogenic treatments on sensory properties of wine

Wine quality and consumer acceptance of wine are frequently determined by its organoleptic properties (aroma, colour, and taste), particularly the aroma profile (Vilanova et al., 2013:126-130; Robinson et al., 2014b:32-35; Lund et al., 2009a:295-303; b:20-25; c:5-11; Ruiz et al., 2019:7441-7442; Karabagias et al., 2020:2235; 2238-2242). In most cases, wines produced from grapes subjected to pre-fermentative cryomaceration treatments had a higher aroma intensity, improved mouthfeel, improved colour, oxygen stability and enhanced varietal aroma characteristics related to the cultivar (Carillo et al., 2011:1-14, Schmid & Jiranek, 2011:29-30; Baiano et al., 2012:2700; Chen et al., 2019b:642-643; Ruiz-Rodríguez et al., 2020:1-13; Bestulić et al., 2022:1-11; Malićanin et al., 2022:11; 14). This is due to an increase in the extraction of the aroma and flavour compounds, i.e., terpenes, thiols, esters, and phenols present in the grape skin (Aleixandre-Tudo & du Toit, 2018:204-207; Benucci et al., 2018:261-265; Lan et al., 2019:186-196; Korenika et al., 2020:49-53; Bestulić et al., 2022:1-11; Pedrosa-López et al., 2022:1-12).

## 2.5 Aroma compounds

### 2.5.1 Grape aroma compounds

Aroma compounds or odorants can be classified according to the different stages of wine grape and/or juice processing that they originated from (Table 2.1), namely varietal (cultivar) aroma (from the untreated grape berry), pre-fermentative aroma (from processing, i.e., crushing and pressing), fermentation (from yeast and bacteria during alcoholic and malolactic fermentation), and post-fermentation [from ageing (maturation) in wood, wine bottle storage, and preservation] aromas (Roland et al., 2011a:7372; Jeffery, 2016:1323-1324; Cataldo et al., 2021:11-12; Chen & Li 2022:31 3; 318-319).

These compounds are often present in grapes as odourless or non-volatile precursors and are released during winemaking, specifically during the alcoholic fermentation process (Roland et al., 2011a:7371; Robinson et al., 2014a:7-8; Jeffery, 2016:1323-1324). The three major classes of odourants that contribute significantly to varietal characteristics in wines are monoterpenes, methoxypyrazines and volatile thiols (Augustyn et al., 1982:53-59; Glória & Vieira, 2007:263-264; Benkwitz et al., 2012a:6296-6298; Ruiz et al., 2019:7426; 7433-7435; Chen & Li, 2022:313-315). Moreover, volatile thiol compounds, also considered impact odorants, can either have a positive (tropical, passionfruit, guava–like) or negative influence (rotten egg, cooked vegetables, onion, cabbage), depending on their concentration in wines (Swiegers et al., 2006:36; Coetzee & du Toit, 2012:287-298). Other compounds, also present in wine and found to play a significant role in its aroma, are esters, fatty acids, higher alcohols, and aldehydes (Roland et al., 2011a:7355; Ruiz et al., 2019:7438-7440).

Table 2.1: Aroma development stage, compounds, and their origin.

| Aroma development stage | Compounds | Origin |
|---|---|---|
| Varietal | Precursors (free or bound) | Grape berry (skin and pulp) |
| Pre-fermentative | $C_6$ compounds | Enzymatic/catalytic reactions due to processing (crushing of berries) |
| Fermentation | Ethyl esters, fusel alcohols, fatty acids, thiols | Microorganism metabolism (yeast and bacterial) |
| Post-fermentation | Oxidation of volatile aroma compounds, increase in fatty acids, esters, aldehydes, ketones and polyphenols | Wine aging/storage (bottle, barrel, storage, aging on lees) |

Adapted from Roland et al. (2011a:7355); Ruiz et al. (2019:7425-7450); Ferreira & Lopez (2019:1-35).

However, over the past three decades, there has been considerable interest in and research into the volatile thiol aroma compounds and their precursors (Roland et al., 2011a:7355-7356; Coetzee & du Toit, 2012:287-298; Pinu et al., 2014:570; Jeffery, 2016:1324). Grape aroma compounds are predominantly located in the grape skin and require an extraction process to be released. The extraction of a compound is dependent on the nature of the compound, the concentration in the berry, the location within the berry and the method used during processing (Moreno-Pérez et al., 2013:770; Aleixandre-Tudo et al., 2015:366-376; Olejar et al., 2015a:181-189).

Winemakers typically achieve this extraction of compounds by using a maceration step, during which the components are transferred from the solid to the juice. Pre-fermentative cold maceration is another technique that has gained popularity during the white wine production process and was shown to enhance the varietal character of the wines produced (Chen et al., 2019b:643-644; Pedrosa-López et al., 2022:2-12). Research conducted by Carillo et al. (2011:11) showed significant differences in phenolic (gallic, caftaric, coutaric, caffeic and syringic acid) and polyphenol concentrations between wines produced from frozen and fresh grapes. Zhang et al. (2015:21611-21613), Naviglio et al. (2018:8) and Ruiz-Rodríguez et al. (2020:6) had similar observations regarding the polyphenol concentrations, which confirms the findings of Carillo et al. (2011:11). Schmid & Jiranek (2011:28) found significant differences in acetaldehyde concentrations between their wines which were confirmed by Ruiz-Rodríguez et al. (2020:9) and October (2020:28). Ouellet & Pedneault (2016:1-8) investigated the impact of frozen storage on 22 free volatile compounds of two table grape varieties, i.e., Thompson seedless and Flame seedless. The free volatile compound profile of the frozen grapes and juice was significantly different from the fresh juice (Ouellet & Pedneault, 2016:8). It was further shown that the different freezing treatments affected the volatile profiles of juice differently and that grape variety also played a role (Threlfall et al., 2006:161-169). Moreover, when analysing the volatile profiles of grape juice, fresh juice is preferable (Ouellet & Pedneault, 2016:8). However, should long-term storage be required, storage under liquid $N_2$ or at a temperature of -80 ºC is advised to reduce biochemical reactions from altering the free volatile compounds (Ouellet & Pedneault, 2016:8). Pedrosa-López et al., (2022:7) found that extractable anthocyanins, total phenolics and terpenic alcohols were among the compounds most affected by the freezing process.

#### 2.5.1.1 Pre-fermentation aromas

Pre-fermentation aromas, also known as primary aromas, are grape-derived volatiles originating from biosynthetic pathways (Conde et al., 2007:2; Ebeler & Thorngate, 2009:8099; Kapaklis, 2014:17; Robinson et al., 2014a:2-8; 11; González-Barreiro et al., 2015:202-208; Greyling, 2019:4-5). These include the grape-derived precursors, i.e., carotenoids, glycosidically bound terpenes, lipids, volatile thiol precursors, and volatiles such as methoxypyrazines, monoterpenes, $C_6$ and $C_{13}$-norisoprenoid compounds (Coetzee & du Toit, 2012:287-288; Robinson et al., 2014a:1-7; Casalta et al., 2015:3-6;13-14; Greyling, 2019:4-5). These compounds develop between harvest and before the alcoholic fermentation through enzymatic reactions occurring during the processing stages of the grapes (Reynolds & Wardle, 1997:3-17; Conde et al., 2007:1-22; Ebeler & Thorngate, 2009:8099; Kapaklis, 2014:17-20; Robinson et al., 2014a:1-7; González-Barreiro et al., 2015:202-208; Greyling, 2019:4-5).

#### 2.5.1.2 Fermentation aromas

The alcoholic fermentation process and yeast strain used to conduct the alcoholic fermentation play an important role in the aroma profiles of wines (Hart et al., 2016:2077; 2079; 165; Williams et al., 2021:18; 28). Wine is a complex medium with aromas emanating from thousands of non-aromatic compounds present in the grape. Grape juice generally is odourless and requires yeast, through the fermentation process, to convert these compounds from non-volatile to aromatic compounds (Murat et al., 2001:136-139; Howell et al., 2004:125-129; Dubourdieu et al., 2006:82; Kapaklis, 2014:22; Hart et al., 2016:2076-2079; 2019:165; Coetzee, 2020c). Moreover, the yeast, in combination with the grape variety, climate, viticultural and winemaking practices, enhances the character of the final wine (Murat et al., 2001:136-139; Dubourdieu et al., 2006:82; 85-87).

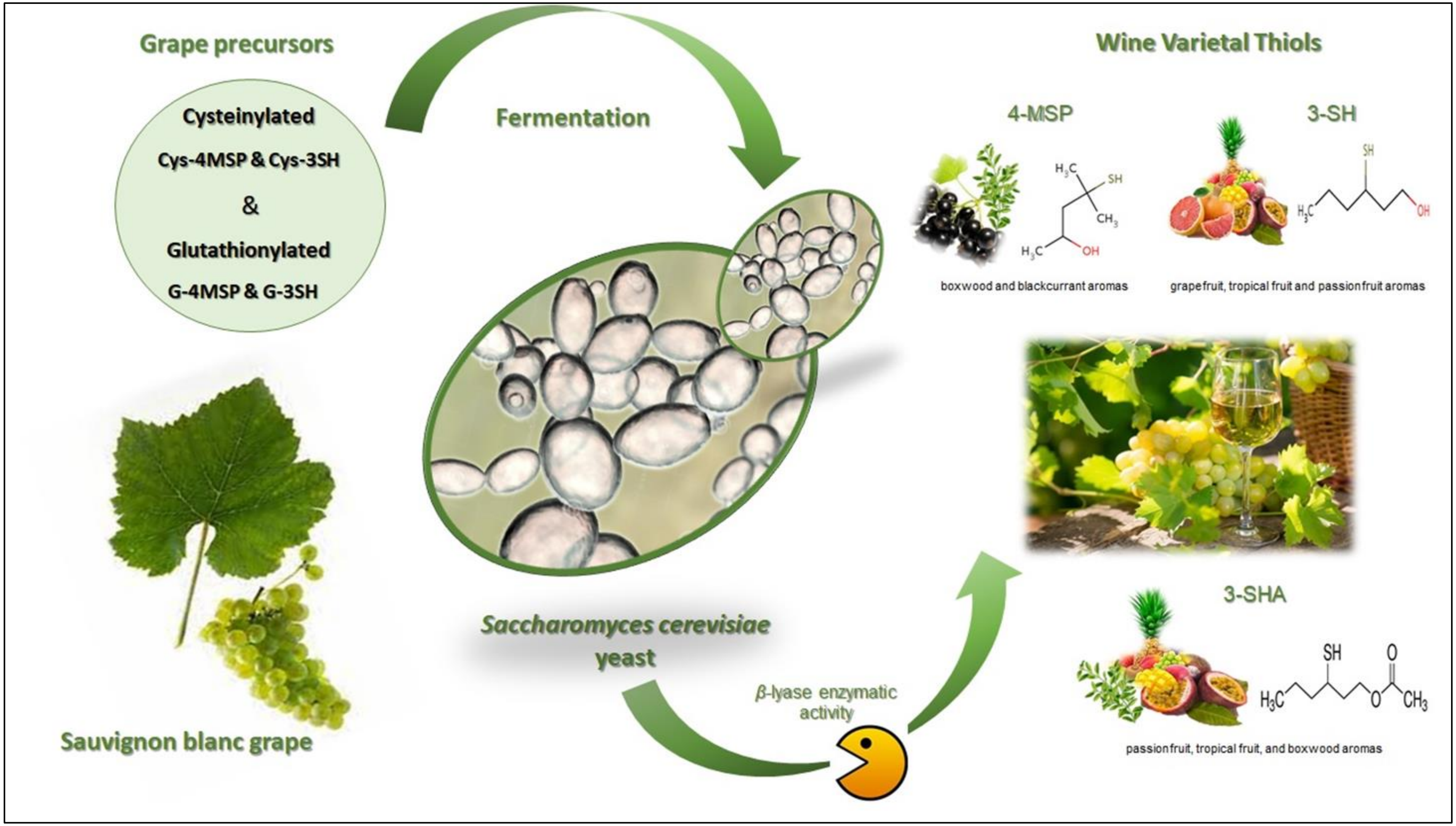



Figure 2.1. Schematic illustration of the conversion of Cysteinylated and Glutathionylated precursors to 4-MSP (1), 3-SH (3), and 3-SHA (2) during alcoholic fermentation.

Notably, fermentation aromas are not solely a product of yeast metabolism but are also shaped by pre-fermentative conditions and practices. Therefore, wine yeast selection plays an essential role in the production of wines with characteristic varietal aromas - (Fig. 2.1) (Swiegers & Pretorius, 2007:954-960; Swiegers et al., 2009:204-211; Roland et al., 2011a:7371; van Breda et al., 2013:80-88; Hart et al., 2019:165; Ruiz et al., 2019:7430; 7432). Yeasts release varietal thiols by cleaving conjugated thiol precursors in the grape juice through beta-lyase activity ($\beta$-lyase) (Roland et al., 2011a:7356-7358; Hart et al., 2017:2079; Ruiz et al., 2019:7432). During fermentation, cysteine and glutathione precursor conjugates can generate aromatic volatile thiols resulting from the cleavage of the carbon-sulfur linkage (Murat et al., 2001:136-139). Whilst most of these studies involved *Saccharomyces cerevisiae* yeasts, since these are most widely used for wine production, extensive research has also been conducted to investigate the impact of non-*Saccharomyces* yeasts, together with inter-genus hybrid yeast strains, to produce wines with enhanced varietal aromas (Dubourdieu et al., 2006:85-87; Molina et al., 2009:683; Swiegers et al., 2009:204-211; Hart et al., 2016:2077; Belda et al., 2017:183-191; Hart et al., 2019:6-8; Williams et al., 2021:18; 28). Regardless of the success of these non-*Saccharomyces* yeast strains in conducting fermentations, it was accompanied by high concentrations of acetic acid, acetaldehyde, acetoin and ethyl acetate. Additionally, off-flavours linked to the presence of vinyl and ethyl phenols have also been reported (Chatonnet et al., 1995:465-467; Dubourdieu et al., 2006:85-87; Ciani et al., 2010:125; October, 2020:42-45).

Moreover, the importance of specific genes ,i.e., *BNA3*, *CYS3*, *STR3*, *GLO1* and *IRC7* in *S. cerevisiae* and *Torulaspora delbrueckii* were shown to be directly responsible for the conversion of non-volatile pre-cursors into specific varietal thiols (Howell et al., 2004:128; Thibon et al., 2008:1076-1086; Sadoudi et al., 2012:243-253; Steyer et al., 2012:2-15; van Breda et al., 2013:80-88; Belda et al., 2017:184; 189-190; Ruiz et al., 2019:7433-7436). Copious investigations involving *S. cerevisiae* and non-*Saccharomyces* yeast strains highlight the significance of selecting the appropriate yeast to convert non-aromatic precursors into varietal thiols and producing wines with desired sensory profiles. Moreover, yeast strain, in combination with various factors (region, viticultural practices, grape variety and winemaking practices), can be utilised by winemakers to create desired wine styles.

#### 2.5.1.3 Post-fermentation aromas

Post-fermentative aromas are usually associated with bottle storage as well as barrel ageing of wine. During the storage process, varietal fermentative aromas are lost, resulting in the formation of a new aroma profile (Pérez-Coello et al., 2003:303-304;

Ugliano et al., 2011:2567; Roland et al., 2011a:7355; 7372; Del Caro et al., 2014:128-138; Coetzee, 2019; Ruiz et al., 2019:7440-7441). The post-fermentative profile results from oxidation (ageing process) and contact with lees as well as oak, if aged in barrels (Ruiz et al., 2019:7440). However, depending on the style of wine produced, oxygen may be beneficial to some extent, because during the maturation of red wine, oxygen enhances the colour and decreases astringency. In white wines, oxygen is detrimental because it causes browning and the loss of "fruity" aromas. Moreover, as mentioned above, these newly formed oxidative aromas could therefore either positively or negatively affect overall wine quality (Pérez-Coello et al., 2003:303-305; Ugliano et al., 2011:2567; Roland et al., 2011a:7372; Del Caro et al., 2014:134; Coetzee, 2019; Ruiz et al., 2019:7440-7441).

Varietal thiols are chemically unstable and easily oxidised when in contact with oxygen (Tsai et al., 2022:7-8). Particularly, the concentration of 3-SH formed during the alcoholic fermentation process greatly decreases due to dissolved oxygen. It is, therefore, important to work reductively during the wine bottling process. To minimise contact with oxygen, low oxygen transmission rate (OTR) closures and antioxidants (e.g., $SO_2$) are used during the bottling process (Roland et al., 2011a:7372; Coetzee, 2019). Sulfur dioxide is most commonly used, however, regulations limit the amounts allowed in wines (Tsai et al., 2022:7-8). Therefore, alternative biological antioxidants such as glutathione (GSH) are also used. Glutathione (GSH) protects thiol oxidation, as well as decreases the development of volatile esters and terpenes during storage (Ugliano et al., 2011:2570-2571; Coetzee, 2019; Ruiz et al., 2019:7441). Moreover, a study conducted by Pérez-Coello et al. (2003:303-305) highlighted the importance of storage temperatures on white wine aroma profiles and found that white wine could retain its "young wine bouquet" by storage at 0 ºC for several years. Additionally, it was found that storing wines at temperatures lower than 20 ºC could lead to the formation of "bottle maturation bouquets" (Pérez-Coello et al., 2003:303-305).

### 2.5.2 Grape varietal impact compounds

#### 2.5.2.1 Varietal thiols

Varietal thiols are sulfur compounds found in grapes in a bound form that originates from fatty acids (Kapaklis, 2014:21; Pinu et al., 2014:570; Hart et al., 2017:147-148; Cataldo et al., 2021:2). They are considered the main compounds involved in the aroma of wine and responsible for its archetypal flavour (Chen et al., 2019b:642-644). Thiols are "bound" with glutathione or cysteine and released by the yeast during the fermentation process, through the carbon-sulfur lyase (C-S) enzyme. However, the quantification of their natural precursors in the must is important and can help the wine producer determine the aromatic potential of the grapes. Synthesis of

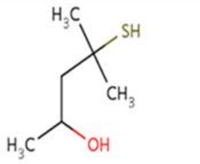
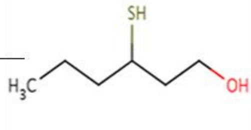
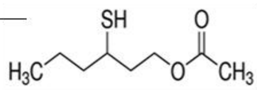
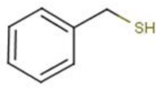
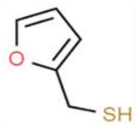
4-mercapto-4-methylpentan-2-one precursors, i.e., S-4-(4-methylpentan-2-one)-L-cysteine and-S-4-(4-methylpentan-2-one)-glutathione were reported as natural and deuterated compounds, with accurate quantification of trace levels in grapes achieved by stable isotope dilution assay (SIDA) that involves labelled analogs (Bonnaffoux et al., 2018:127; Cataldo et al., 2021:8). Key thiols present in Sauvignon blanc and responsible for its varietal aromas are 4-MSP, 3-SH and 3-SHA, with perception thresholds of 0.8 ng $L^{-1}$, 60 ng $L^{-1}$ and 4 ng $L^{-1}$, respectively (Table 2.2) (Van Wyngaard, 2013:65). They are predominantly responsible for the "tropical" (gooseberry, grapefruit, and passion fruit) characters associated with Sauvignon blanc (Roland et al., 2011a:7358-7359; b:139).

However, when present in excessive concentrations, they often impart less desirable, strong, sweaty aromas resembling "cat urine" (Roland et al., 2011a:7359; Coetzee & du Toit, 2012:289; Benkwitz et al., 2012a:6294). Furthermore, research conducted on SA Chenin blanc revealed the presence of the varietal thiols 3-SH and 3-SHA in concentrations above their aroma thresholds indicating that these two compounds also contribute significantly to the aroma of Chenin blanc wines (Coetzee et al., 2018:180-183; Wilson, 2017:27-38; Wilson et al., 2019:635-640). Varietal thiols are present in grape juice in the form of aroma-inactive, non-volatile precursors and are released by yeast enzymes during the fermentation process (Coetzee & du Toit, 2012:290; Hart et al., 2016:2072; Visan et al., 2018:425; Williams, 2018:13-16; Hart et al., 2019:5; 6; 8; Dimopoulou et al., 2020:751; 757; Korenika et al., 2020:49-53). Pinu et al. (2014:564-566; 570) showed that the formation of varietal thiols and other aroma compounds in Sauvignon blanc wines is not necessarily only dependent on nitrogenous and sulfur compounds but is also influenced by other juice metabolites such as carboxylic and fatty acids. These discoveries enable the wine industry to produce different wine styles from the same grape varietal based on the metabolic profile of the juice. In addition, juice modulation through new winemaking practices, i.e., metabolite supplementation or blending, could be seen as a useful tool to create new wine styles (Pinu et al., 2014:570). As previously mentioned, due to the location (skin of the berry) of these volatile compounds and their precursors, an extraction process is required to release it into the juice (Moreno-Pérez et al., 2013:770-776; Aleixandre-Tudo et al., 2015:373; Olejar et al., 2015a:181-189).

#### 2.5.2.2 Precursors

Volatile thiol compounds are considered non-existent in grape juice, although they have been detected in low concentrations (~100 ng $L^{-1}$) (Esti & Tamborra, 2006:176-178; Roland et al., 2011a:7371; Coetzee & du Toit, 2012:289-294; Ruiz et al., 2019:7433-7436).

Table 2.2: Varietal thiols present in Sauvignon blanc and Chenin blanc wines: aroma description, perception, and range in wine.

| Cultivar | Compound & Chemical structure | Aroma description | Aroma perception in wine ($ng\ L^{-1}$) | Range in wine ($ng\ L^{-1}$) | Range in SA wine ($ng\ L^{-1}$) |
|---|---|---|---|---|---|
| Sauvignon blanc | **4MSP[1]**   | Boxwood, blackcurrant  | 0.8 | 0–88 | 0 – 21.9 |
| Chenin blanc | | | | 0–23 | n.d.* |
| Sauvignon blanc | **3SHA[2]**   | Passionfruit, tropical, boxwood  | 4 | 0–106 | 23–151 |
| Chenin blanc | | | | 0–100 | 5–253 |
| Sauvignon blanc | **3SH[3]**   | Grapefruit, tropical, passionfruit  | 60 | 350–5664 | 178–904 |
| Chenin blanc | | | | 10–1368 | 99–1124 |
| Sauvignon blanc | **BM[4]**   | Smoke, toasty, struck flint  | 0.3 | 0.6–5.5 | n.d.* |
| Chenin blanc | | | | 30–40 | n.d.* |
| Sauvignon blanc | **FFT[5]**   | Roasted Coffee  | 0.4 | 1–36 | n.d.* |
| Chenin blanc | | | | 14 | n.d.* |

[1]4-methyl-4-sulfanylpentan-2-one
[2]3-sulfanylhexyl acetate
[3]3-sulfanylhexan-1-ol
[4]benzyl mercaptan
[5]2-furfurylthiol
*Not detected
Adapted from Capone et al. (2015:1227); Jeffery (2016:1324); Coetzee et al. (2018:180-184).

Thiols are released from odourless precursors usually present in the grape skin and pulp (des Gachons et al., 2002:144-146; Roland et al., 2011b:139; 142; Coetzee & du Toit, 2012:289; Ruiz et al., 2019:7433-7436). Moreover, the alcoholic fermentation process through the *β*-lyase activity of yeast is responsible for the release of the cysteine and glutathione conjugate precursors (Fig. 2.2) (Tominaga et al., 1998:161-162; Peyrot des Gachons, et al., 2002:4077-4078; Roland et al., 2011a:7371; b:142-143; Coetzee & du Toit, 2012:289; Ruiz et al., 2019:7433-7435).

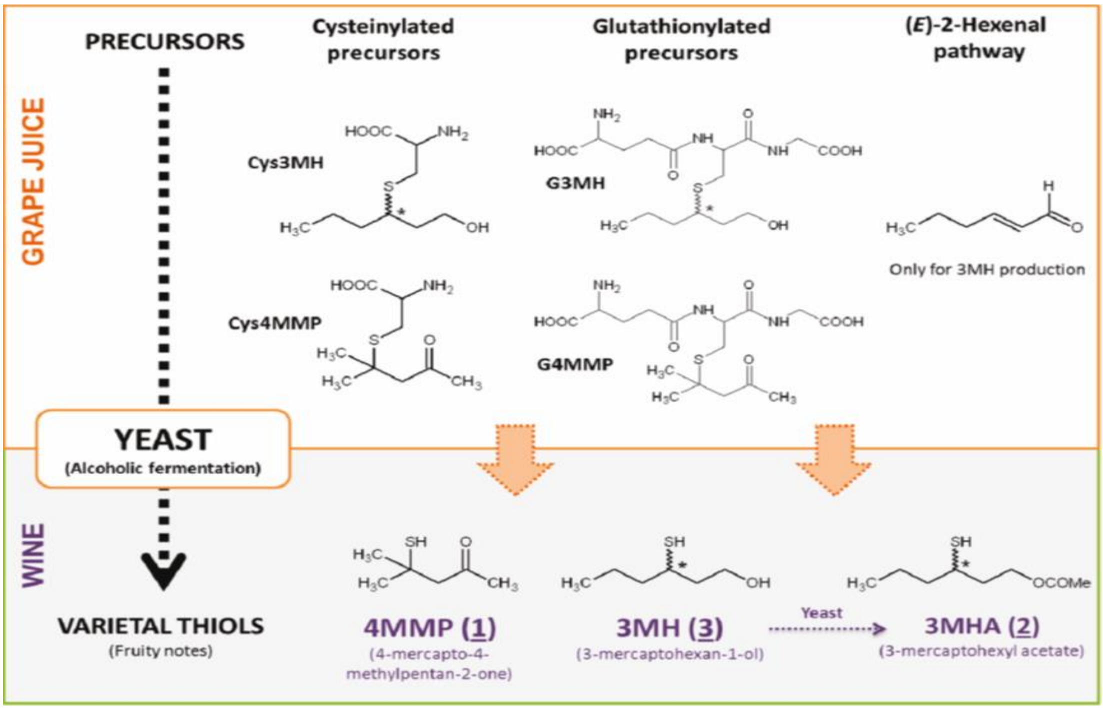



Figure 2.2. Different biogenesis pathways to produce 4-MSP (1), 3-SH (3), and 3-SHA (2) during alcoholic fermentation (Roland et al., 2011a:7358).

Precursors of volatile thiols in Sauvignon blanc grapes are detected in both the skin and the pulp (Roland et al., 2011a:7356; b:141-143). Moreover, the origin of the Sauvignon blanc grapes influences the distribution and abundance of the precursors. Roland et al. (2011a:7356; b:139-140) demonstrated that precursor extraction was increased when using high pressure during the pressing process, therefore resulting in increased thiol levels in the final wines. This was also found in subsequent studies (Aleixandre-Tudo et al., 2015:373; Olejar et al., 2015a:186-188). Furthermore, the location of the cysteinylated precursors of 3-SH and 4-MSP has already been researched for Sauvignon blanc grapes. Peyrot des Gachons et al. (2002:4077-4078) demonstrated that during harvesting, the S-3-(1-hexanol) cysteine (Cys-3SH) was primarily located in the skin, whereas the S-4-(4-methyl-4-mercapto-2-pentanone) cysteine (Cys-4MSP) was found both in the skin and the pulp. Murat et al. (2001:136-139) showed that Cys-3SH concentrations, present at 60% in the skin, increased in Merlot and Cabernet Sauvignon grape juices with prolonged skin contact and by using higher maceration temperatures (up to 25 ºC). In addition, the concentration was also influenced by the process of pressing in the winery, which showed that the Cys-3SH precursor increased significantly in grape juices where skin contact had been prolonged (Maggu et al., 2007:10287; Larcher et al., 2013:1196-1202). Pre-fermentative cold maceration has gained popularity in white wine production as it was shown to enhance the varietal character of the wines (Vanzo et al., 2017:3-8; Chen et al., 2019b:643-644; Pedrosa-López et al., 2022:2-12).

Capone et al. (2011:4656-4657) illustrated that the two volatile precursors of 3-SH, Cys-3-SH and 3-Glut-3-SH were significantly higher in freeze-thawed berries than in the juice of fresh berries, as well as in the frozen juice of fresh berries. This could suggest that the precursors that were released from the grape skins during the freezing and thawing process were significantly higher in the freeze-thawed berries than in the juice of fresh berries, and in the frozen juice. Glut-3-SH was fourfold higher in frozen grapes stored at -20 ºC for two months compared to that found in frozen or fresh juices (Capone et al., 2011:4656-4657). Although numerous studies have been conducted using cryogenic pre-treatment techniques on whole grapes and grape juice, the focus has been on the overall aroma compounds. However, the effect of such treatments on varietal thiols and their precursors still requires extensive research (Capone et al., 2011:4656-4657; Carillo et al., 2011:1-14; Wang et al., 2016:2360-2366; Chen et al., 2018:4674-4682; 2019a:1-24; b:639-641; Pedrosa-López et al., 2022:2-12).

#### 2.5.2.3 Biogenesis pathways of varietal thiols

Varietal thiols are released into wine from their precursors through three proposed biogenesis pathways (Tominaga et al., 1998:161-162; Ebeler & Thorngate, 2009:8101; Coetzee & du Toit, 2012:289-290; Kapaklis, 2014:22). The first pathway involves the cleavage of the cysteinylated precursors of 3-SH and 4-MSP by the *β*-lyase enzyme activity of the *S. cerevisiae* yeast (Hart et al., 2016:276-277; 2017:146-147; Williams et al., 2021:15-22). The second pathway involves the glutathionylated precursors. It is known that S-glutathione conjugates are involved in the cell detoxification of living organisms, both plants and animals (Peyrot des Gachons et al., 2002:4077-4078; Chen et al., 2019a:1-24; Künstler et al., 2020:5-6). Moreover, S-cysteine conjugates are commonly found in conjunction with the corresponding S-glutathione conjugates. The toxic compound to be removed is conjugated with glutathione by the S-glutathione transferase enzyme. The toxin is broken down by γ-glutamyltranspeptidase, which eliminates glutamic acid, and carboxypeptidase, which eliminates glycine, therefore forming the S-cysteine conjugate (Tominaga et al., 1998:161-162; Peyrot des Gachons et al., 2002:4077-4078; Ebeler & Thorngate, 2009:8101). Thibon et al. (2009:714-715; 2011:1347; 2016:714-715) demonstrated that the cells of *Vitis vinifera* can produce Cys-3SH from G-3SH, and in the presence of *Botrytis cinerea* stimulate the production of Cys-3SH increasing it by a thousandfold. The third biogenesis pathway involves the conjugated carbonyl compounds, (*E*)-2-hexanal and mesityl oxide. Through the addition of sulfur and conversion by the yeast during the fermentation process, this pathway results in the production of 3-SH and 4-MSP (Schneider et al., 2006:58; Ebeler & Thorngate, 2009:8101; Roland et al., 2011a:7356-7358).

Moreover, it was found that the addition of glutathione or hexenal to Sauvignon blanc juice increased the production of 3-SH and 3-S-HA by 25% and 41% (Schneider et al., 2006:58). These observations were similar to a laboratory-scale trial performed on the same grape variety with the same conditions. This highlights the role of glutathione during pre-fermentative winemaking processes (Thibon et al., 2011:1347-1348; 2016:714-715). In addition, glutathione was also involved in varietal thiol production through the production of G-3SH. Oxidation compounds such as hexenal are often considered to be off-flavours, but it was shown to play an important role in G-3MH formation when present at low concentrations. The production of 3-SH in wines seems to originate from two different pathways, i.e., from precursors naturally occurring in the grapes and secondly linked to the winemaking technologies (hexenal and G-3SH pathways). The hexenal pathway described by Schneider et al. (2006:62-63) associated sulfur contributors during winemaking to glutathione which contributed to the production of the G-3SH pro-precursor.

#### 2.5.2.4 Varietal thiol characterisation

Varietal thiols and their precursors are present in grape must and wine at very low concentrations, i.e., parts per billion and can only be quantified and detected by highly sensitive and accurate analytical methods (Coetzee & du Toit, 2012:287-298; Piano et al., 2015:43-46; Coetzee et al., 2018:182-183; Mafata et al., 2018:5-9; Chen et al., 2019a:4-24). Furthermore, despite extensive research, the most critical issue is the lack of a rapid, accurate, and sensitive analytical detection method for these compounds (Herbst-Johnstone et al., 2013:104-110). Over the past three decades, considerable progress has been made toward developing novel analytical technologies for sample preparation (derivatisation, isolation, separation) and detection of thiols and their precursors (Vanzo et al., 2017:1-11; Coetzee et al., 2018:182-183; Mafata et al., 2018:5-9; Chen et al., 2019a:4-24).

Moreover, volatile thiols are highly reactive, low-molecular-weight compounds susceptible to oxidation. Therefore, they require a selective extraction or pre-concentration technique with selective detection methods (Roland et al., 2011a:7361-7362; de Villiers et al., 2012:2-23; Roland et al., 2012:267; Coetzee et al., 2018:182-183). Additionally, due to the complexity and diversity of the grape must and wine matrices, the sample preparation procedure is vital (Roland et al., 2011a:7361-7362; Roland et al., 2012:267; Chen et al., 2019a:1-24). Tominaga et al. (1998:159-160) were the first to report on sample preparation, which involved the selective extraction of thiols from a dichloromethane extract of wine using a reversible interaction between the SH function and sodium *p*-hydroxymercuribenzoate. Analysis of these extracts by GC-MS allowed for the successful quantification of 3-SH, 3-SHA, 4-MSP, 4-MSPOH, and 3-mercapto-3-methylbutan-1-ol in Sauvignon blanc wines (Darriet et al. 1995:120-127; Tominaga et al., 1998:161-162). However, this method had poor specificity for 4-MSP due to the type of detection and the possible degradation of 3-SHA and 3-SH resulting from the alkalinity of the extraction step (Darriet et al. 1995:120-127; Tominaga et al., 1998:159-160; Roland et al., 2012:267; Coetzee et al., 2018:182-183). Subsequently, advanced methods were developed using ethyl propiolate and pentafluorobenzyl bromide as derivatisation agents which protected the thiols from oxidation and provided thermal stability (Capone et al., 2015:1228-1230).

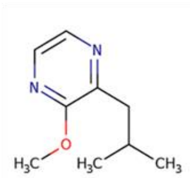
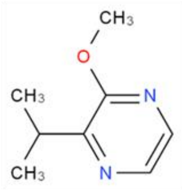
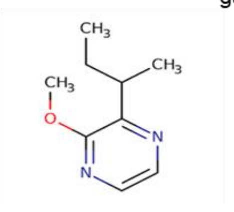
Chen et al. (2019a:1-24) provided a comprehensive updated summary of the current technologies in a review based on information extracted from 395 publications dating from 1990 to 2019. The major observations regarding the current analysis of thiols are that derivatisation-based extraction methods remain popular due to their efficiency and simplicity. The application of a GC coupled to different detectors (e.g., olfactometry, sulfur selective detectors, and mass spectrometry) is still considered valuable in detecting new thiol compounds even though novel LC-MS/MS approaches with thiol-specific derivatisation and precursor ion scan have been investigated (Capone et al., 2015:1228-1230; Piano et al., 2015:43-46; Chen et al., 2018:4675-4681; Mafata et al., 2018:5-9). Subsequently, the development of quantitative LC-MS methods was shown to outperform existing GC-MS analysis when comparing the entire protocol, from sample extraction to analysis. Furthermore, the application of SIDA methods has also been applied more frequently for their reliable quantification of volatile thiols (Kotseridis et al.,1998:78; Bencomo-Rodriguez, 2011; Capone et al., 2015:1228-1230; Piano et al., 2015:43-46; Chen et al., 2018:4672-4684). Emerging trends include novel stationary phases compatible with HPLC, MS and LC instruments for the separation of volatile thiol compounds in combination with QqQ and high resolution (Q-TOF or Orbitrap) MS for identification and quantification (Ali et al., 2012:3235-3249; Chen et al., 2018:4672-4684; Carlin et al., 2022:5-6). However, considering all the advances made, there is still room for improvement and the development of faster, more cost-effective, greener technologies that can provide more comprehensive information (Bencomo-Rodriguez, 2011; Coetzee et al., 2018:180-184; Mafata et al., 2018:5-9; Chen et al., 2019a:1-24).

### 2.5.3 Methoxypyrazines

Methoxypyrazines (MP’s) (3-alkyl-2-methoxypyrazines) are volatile nitrogen-containing heterocyclic compounds found in plants, insects, fungi and bacteria (Suklje et al., 2013; Sidhu et al., 2015:485). They are primarily responsible for the vegetative, grassy, green pepper, capsicum and asparagus aromas present in Sauvignon blanc and Bordeaux red wine cultivars, i.e., Cabernet Franc, Merlot, Carménère and Cabernet Sauvignon

(Lacey et al., 1991:105; Sidhu et al., 2015:485-487; Lei et al., 2018:1142; Ruiz et al., 2019:7426-7469; Cataldo et al., 2021:2,4,8,10; Kemp et al., 2022:341-342). Although the perception of green attributes is seen as positive and adds complexity to certain varieties, they could also be perceived as an undesirable aroma in red wines (Augustyn et al., 1982:53-59; Bogart & Bison, 2006:13-20; Ruiz et al., 2019:7426-7429). The most essential MP found in grapes and wines is 3-isobutyl-2-methoxypyrazine (ibMP), the main contributor to the vegetative, grassy, green pepper, capsicum and asparagus aromas present in Sauvignon blanc (Lacey et al., 1991:105; 107; Marais, 1994:41; Ruiz et al., 2019:7426-7429; Kemp et al., 2022:341). It is typically present in wine as free volatile compounds in concentrations ranging from 2-30 ng $L^{-1}$ (Table 2.3).

Table 2.3: Methoxypyrazines present in Sauvignon blanc wines: aroma description, perception, and range in wine.

| Compound & Chemical structure | Aroma description | Aroma perception in water (ng $L^{-1}$) | Aroma perception in wine (ng $L^{-1}$) | Range in wine (ng $L^{-1}$) |
|---|---|---|---|---|
| **ibMP**[1]  | vegetative, green pepper  | 1–2 | 2–16 | 2–30 |
| **ipMP**[2]  | earthy, mushroom, cooked, or canned asparagus, green beans  | 1–2 | 2–16 | <10 |
| **sbMP**[3]  | green (peas, bell pepper, galbanum), ivy leaves, bell pepper  | 1–2 | 2–16 | <10 |

[1]ibMP-2-methoxy-3-*iso*butylpyrazine
[2]ipMP-2-methoxy-3-*iso*propylpyrazine
[3]sbMP-2-methoxy-3-*sec*-butylpyrazine
Adapted from Sidhu et al. (2015:485-487); Lei et al. (2018:1142).

Moreover, two additional MP's present in must and wine, at lower concentrations, are 3-isopropyl-2-methoxypyrazine (ipMP) and 2-methoxy-3-sec butyl pyrazine (sbMP) and they contribute to the earthy, asparagus aromas (Marais

1994:41-44; Ruiz et al., 2019:7426-7429; Anonymous, 2021c). Sensory detection thresholds for ibMP, ipMP and sbMP are typically very low (1-2 ng $L^{-1}$) (Allen et al., 1991:110-111; Marais, 1994:41-44; Roujou de Boubée et al., 2000:4830-4834; Ruiz et al., 2019:7426-7429). Pinu et al. (2014:564-566,570) demonstrated that concentrations of wine aroma compounds can be modified by pre-fermentative treatments of the juice, thus altering the metabolite levels. Studies have found that ibMP in the berry is produced by the berry itself and not transported from either the leaves or the shoots (Koch et al., 2010:2192; 2194-2195; Kuhn et al., 2014:4547; 4553). At harvest, 95% of the ibMP are found in the grape skins, 4% in the seeds and 1% in the pulp (Roujou de Boube et al., 2000:4830-4834). Methoxypyrazines are not very sensitive to oxidation, although they are light-sensitive during grape maturation (Suklje et al., 2012:9458-9460; 2016b:922;924; Tsai et al., 2022:8). Additionally, viticultural and winemaking practices, i.e., leaf removal, foliar sprays, pressing of the juice and skin contact, either increase or decrease the concentration of ibMP (Roujou de Boube et al., 2000:4830-4834; Maggu et al., 2007:10284; Coetzee, 2011:22-24; Suklje et al., 2012:9458-9460; 2016b:922;924). Furthermore, ibMP is shown to reach the maximum concentrations, two to three weeks before véraison, thereafter, it declines (Marais, 1994:41-44; Roujou de Boubée et al., 2000:4830-4834; Suklje et al., 2012:9458-9460; 2016b:922;924). The concentration of MPs at harvest are largely dependent on the origin of the grapes, clonal differences and climate. Moreover, MPs degrade in warm climates, while cooler climates result in higher concentrations (Maggu et al., 2007:10287; Benkwitz et al., 2012b:67-69; Suklje et al., 2012:9458-9460; 2016b:922;924; Kemp et al., 2022:343).

### 2.5.4 Other aroma compounds

#### 2.5.4.1 Terpenoids

Terpenoids, also known as isoprenoids, are a class of naturally occurring organic plant chemicals that play a role in phytohormones and photosynthetic pigments (Baron et al., 2017:42-50; Mele et al., 2021:1429-1447). Terpenoids consist of various compounds including monoterpenes, diterpenes and triterpenes that play a role in the plant defence and stress response by exuding volatile compounds. While these may act as repellents to herbivores and pathogens, they also contribute floral and fruity aromas appreciated in grapes and wine (Reynolds & Wardle, 1997:3-17; Baron et al., 2017:46-49; Mele et al., 2021:1429-1447). In wine grapes, monoterpenes ($C_{10}$) and monoterpene alcohols are mainly present in the grape skin in free and glycosidically bound (non-volatile) forms. The sugar moieties involved in these glycosides typically include arabinose, glucose, apiose, and rhamnose (Liu et al., 2017:249; Ruiz et al., 2019:7430-7432; Cataldo et al., 2021:7; Červinka et al., 2021:490; Mele et al., 2021:1429-1447). Although found in all grape cultivars, they are more commonly associated with the cultivar Muscat of Alexandria (Coetzee & du Toit, 2012:295; Hjelmeland & Ebeler, 2015:4; 8). Terpene glycosides were the first glycosides or glycosidic compounds detected in grapes. While terpene glycosides are not considered impact odorants in Sauvignon blanc, whose aroma is primarily shaped by volatile thiols, they may contribute more noticeably to the floral and citrus aromatic profile of Chenin blanc, depending on grape maturity and winemaking practices. (Liu et al., 2017:249; Cataldo et al., 2021:7; Mele et al., 2021:1429-1447). During winemaking, the bound terpenes can be released by glycosidase enzymes produced by grapes, bacteria and yeast, thus increasing the volatile terpenoid composition. The most important terpenoids in wine are linalool, (*E*)-hotrienol, citronellol, geraniol, nerol, (-)-*cis*-rose oxide, and α-terpineol which typically contribute to the flowery, elderflower, green lemon, rose, geranium, citrus, and the floral aromas in wine, respectively (Ruiz et al., 2019:7430-7432; Cataldo et al., 2021:4).

Sesquiterpenes ($C_{15}$) is another class of terpenes commonly associated with the spicy, herbaceous, wood and peppery aromas found in red wine. Rotundone is the most known and imparts the black pepper aroma to Shiraz wine. Additionally, norisoprenoid ($C_{13}$) compounds are derived from grape carotenoids and are equally important in contributing to the floral and fruity aromas in wine (Hjelmeland & Ebeler, 2015:1-9; Ruiz et al., 2019:7430-7432). Norisoprenoid glycosides produce odourless compounds after hydrolysis and are only converted to aroma-active compounds by acid-catalysed conversion during grape ripening and alcoholic fermentation (Liu et al., 2017:249-252; Cataldo et al., 2021:5). The most important norisoprenoids for wine flavour are actinidol, *β*-ionone, *β*-damascenone, vitispirane, 1,1,6-trimethyl-1,2-dihydr onaphthalene (TDN), 4-(2,3,6-trimethylphenyl) buta-1,3-diene (TPB), and 2,2,6-trimet hylcyclohexanone (TCH) (Ouellet & Pedneault, 2016:7-8; Ferreira & Lopez, 2019:7; Cataldo et al., 2021:5; Parish-Virtue et al., 2021:7-8).

Terpene and norisoprenoid concentrations in grape must and wines are dependent on and influenced by grape cultivar, winegrowing region and winemaking technologies, with cryomaceration increasing the terpenic compounds (Baron et al., 2017:48-50; Mele et al., 2021:1432). Zhang et al. (2015:21611-21613) conducted a study using the wine grape variety, Solaris, subjected to different pre-fermentative cold maceration and skin fermentation treatments. It was observed that the final wines produced from the combined cold maceration and skin fermentation treatment had increased rose and elderflower notes which are aromas associated with *β*-damascenone and linalool, respectively. Ouellet & Pedneault (2016:5-8) investigated the effect of frozen storage conditions on the free volatile compound profile of table grapes with a focus on the volatile compounds including thirteen $C_6$ compounds, five monoterpenes, two $C_{13}$-norisoprenoids and two benzene derivatives. Results showed that monoterpenes (α-terpineol, linalool, (+)-limonene) were higher in the frozen juice treatments than in the fresh juice for the Thompson Seedless cultivar. Similarly, it was observed that for the Flame Seedless cultivar, levels of *α*-terpineol were higher in the frozen juices when compared to the fresh juice (Ouellet & Pedneault, 2016:7-8). The levels of hexanal and hexanol were lower, whilst other monoterpenes were unaffected. Frozen juice of both cultivars showed significantly higher levels of *β*-damascenone and 2-ethylhexanol than fresh juice. It was interesting to note that the freezing treatments yielded better results for the Thompson Seedless cultivar when compared to the Flame Seedless cultivar (Ouellet & Pedneault, 2016:5-8).

Baron et al. (2017:48-50) studied the effect of maceration time (0, 5, 12 and 24 hours) at 14 ºC on terpenes and found that wines produced after 24 hours of maceration showed the highest concentrations. Maceration temperature, and in particular cryomaceration increased the concentration of terpenic compounds in wines produced from Malvasia. Ruiz-Rodríguez et al. (2020:7-9) applied different cryoextraction procedures to whole grape berries of Muscat of Alexandria, followed by 3 hours of maceration and found significant increases in volatile terpenoid concentrations. Pedrosa-López et al. (2022:7) froze whole grape berries, as well as crushed grapes of Muscat of Alexandria and found significant increases in terpenic alcohol concentrations which corroborated previous studies. Wines produced from grapes that were instantly frozen with cryogenic agents were found to be more aromatic than wines produced from grapes using the chamber freezing process (Ruiz-Rodríguez et al., 2020:1-13). Furthermore, the wines produced from grapes pre-treated with liquid nitrogen and ultra-fast freezing showed higher concentrations of all terpenoid compounds when compared to the reference wine (Ruiz-Rodríguez et al., 2020:5-9).

#### 2.5.4.2 Higher alcohols, esters and fatty acids

Higher alcohols, esters, and fatty acids are produced by yeast during fermentation and contribute to young wines' fresh, fruity, and even tropical aromas (Prusova et al., 2022:2-4). Several factors affect the production of these compounds, i.e., grape temperature, fermentation conditions, juice clarification, oxygen ($O_2$) addition and the yeast strain used (Ruiz et al., 2019:7438-7439; Cataldo et al., 2021:4; Červinka et al., 2021:490). Higher alcohols refer to alcohols that have more than two carbon atoms in their structure with a higher molecular weight and boiling point than ethanol, namely butanol, pentanol, isoamyl alcohol, isobutyl alcohol and 2-phenyl ethanol (Coetzee & du Toit, 2012:294-295; Belda et al., 2017:187-188). They are secondary metabolites produced via amino acid metabolism during the alcoholic fermentation process. Felix Ehrlich (1905) first proposed that their formation in the catabolic pathway was derived from amino acids whilst their formation during the anabolic pathway was derived from sugars (Prusova et al., 2022:2-4). In addition, higher alcohols are important precursors for ester formation and have an impact on the aromatic composition of wines as they often display intense and pleasant flavours when present at low concentrations. However, in excess (concentrations > 400 mg $L^{-1}$), they can contribute to strong, overpowering flavours (Coetzee & du Toit, 2012:294-295; Moreno-Pérez et al., 2013:771; Belda et al., 2017:187-188). Studies also showed that isoamyl alcohol levels were found to be higher in South African Sauvignon blanc wines compared to Chardonnay wines (Weldegergis et al., 2011:1101-1102).

Esters are compounds formed by the condensation of a hydroxyl and carboxyl group of a phenol or alcohol and organic acid, respectively (Moreno-Pérez et al. 2013:776; Ruiz et al., 2019:7439-7440). These compounds are produced by the yeast during the alcoholic fermentation and are considered important volatile aroma compounds in wine, following higher alcohols, directly influencing the aroma profiles and sensory perception of wines (Moreno-Pérez et al. 2013:771; Ruiz et al., 2019:7439-7440). These fermentation-derived esters (acetate esters) are primarily responsible for the fresh, fruity, and tropical aromas of young wines while esters of fatty acids contribute to the complexity at low concentrations (Coetzee & du Toit, 2012:294-295; Ruiz et al., 2019:7439-7440). Wine contains more than 150 different esters, present in trace concentrations. Acetate esters consist of two main groups, i.e., an alcohol group from ethanol or higher alcohol derived from yeast amino acid metabolism and an acid group (acetate) (Ruiz et al., 2019:7439-7440; Cataldo et al., 2021:4). Esters with pleasant odour fragments include isoamyl acetate and ethyl hexanoate (banana aroma), 2-phenylethylacetate (rose aroma), ethyl octanoate (pineapple), ethyl 2-methyl-butanoate (strawberry), and ethyl butanoate and ethyl decanoate (fruity and floral aromas) (Ruiz et al., 2019:7439-7440). Although pleasurable at low concentrations, esters are considered undesirable when they dominate the wine aroma and are found to mask some of the green notes associated with methoxypyrazines, especially when high ester-producing yeast strains were used for Sauvignon blanc wine production (Coetzee & du Toit, 2012:294-295; Ruiz et al., 2019:7439-7440). In addition, excessively high concentrations of esters can produce undesirable aromas as well as mask desirable grape varietal aromas. Therefore, it is important to manage fermentation conditions to maintain ester concentrations at an optimal level (Ruiz et al., 2019:7439-7440).

Volatile acidity in wine is primarily composed of acetic acid, which accounts for over 90 % of the total volatile acids. Although not a fatty acid in the classical sense, acetic acid is the dominant volatile acid and can impart an undesirable vinegar-like aroma when its concentration exceeds the sensory threshold of 0.8 g $L^{-1}$ (Hart et al., 2017:148-149; Ruiz et al., 2019:7440). Other fatty acids also present include propionic acid, isobutyric acid, 2-methylbutanoic acid, isovalerianic acid, phenylacetic acid, hydroxyphenyl acid and methylthiopropionic acid (Visan et al., 2018:426). These are typically responsible for less desirable aromas (sour, apple rot and sweat), however, also to a lesser extent positive aromas (fruity, floral and green notes) (Visan et al., 2018:426). These compounds are present in the form of saturated straight-chain fatty acids with varying lengths ranging from short to medium to long-chain carbon atoms ($C_2$-$C_{18}$) (Ruiz et al., 2019:7438-7439). Volatile fatty acids are typically produced in small amounts by yeast during the alcoholic fermentation process, with higher levels often associated with bacterial spoilage (Hart et al., 2016:2072-2078; 2017:148; Kelly et al., 2020:7-9). Moreover, when present in lower concentrations, below the detection threshold, it imparts desirable varietal and fruity characteristics to the wine.

The aforementioned volatile aroma compounds are produced during the fermentation process, however, concentrations in final wines are also influenced by pre-fermentative winemaking practices (Fragasso et al., 2010:20-25; Aleixandre-Tudó et al., 2016:368-375). Cryo-maceration with dry ice was shown to increase the concentration of certain esters, although the increase was cultivar-dependent as no changes were observed in wines produced from a second cultivar (Fragasso et al., 2010:20-25; Aleixandre-Tudó et al., 2016:368-375). Moreno-Pérez et al. (2013:771-776) and Cai et al. (2014:219-229) also demonstrated that three varietal red wines, i.e., Cabernet Sauvignon, Syrah and Monastrell, produced from whole grapes subjected to freezing before processing, followed by the addition of dry ice during processing and/or maceration resulted in wines with higher acetate, esters, fatty acids and minor alcohol levels when compared to wines produced from conventional winemaking. Mihnea et al. (2015:37-40) made a similar discovery, whereby wines made from cryo-macerated grapes had higher levels of fusel and other alcohols when compared to control wines, with their results confirmed by the findings of more recent studies (Aleixandre-Tudó et al., 2016:368-375; Korenika et al., 2020:49; Ruiz-Rodríguez et al., 2020:9; Naranjo et al., 2021:5-7; Pedrosa-López et al., 2022:10-12). It was, however, noted that although the volatile compounds produced could be used as a tool to differentiate between varieties and wine age, it could not distinguish between the type of cold pre-treatment used (Moreno-Pérez et al., 2013:776; Aleixandre-Tudó et al., 2016:368-375; Ruiz-Rodríguez et al., 2020:11).

## 2.6 White-wine grape varieties in South Africa

The SA wine industry boasts a remarkable selection of white wine grape cultivars, producing a wide range of white wine styles. Chenin blanc and Sauvignon blanc, the most planted vines in SA, produce wines that are described as fresh and crisp. In addition, grape varieties such as Chardonnay and Semillon also prove to be popular and are more renowned for their smoothness. Additionally, lesser-known white wine varieties include Gewürztraminer, Viognier, Bukettraube, Chenel, Clairette blanche, Colombar(d), Crouchen Blanc (Cape Riesling), Emerald Riesling, Grenache (Blanc), Muscat d'Alexandrie (Hanepoot), Nouvelle Palomino (White French Grape), Pinot Gris (Grigio), Riesling (Rhine or Weisser Riesling), Roussanne and Semillon (Green Grape) (Anonymous, 2021a).

In recent years, globally, including the New World wine regions and particularly in SA, Sauvignon blanc has gained popularity because of the diverse styles of wine produced from this cultivar. Sauvignon blanc was first planted in SA at Groot Constantia during the late 1880s. Moreover, the quality of the planting material was poor, therefore, resulting in many of these vines being deracinated in the 1940s. The variety once again gained popularity in the 1970s, after virus-free material became available from the Agricultural Research Council, Infruitec-Nietvoorbij. Today, it is one of the five most planted varieties in the country (Anonymous, 2021e; g).

### 2.6.1 Sauvignon blanc

Sauvignon blanc is one of the most widely used white varieties of *Vitis vinifera*, originating from the Loire and Bordeaux vineyards in France since the 18th century. It is a genetic descendant of Savagnin Blanc (or Traminer), also a common descendant of other varieties, such as Chenin blanc, Verdejo, Verdohro and Gruner Veltliner. Therefore, based on the aforementioned, Sauvignon blanc and Chenin blanc share common ancestry (Jouanneau, 2011:1). Sauvignon blanc was also formerly referred to as 'Blanc Fumé or Fumé Blanc, but this terminology is not commonly used anymore (Anonymous 2021a; e; Robinson, 2021:3,8-10,12-13). The grape variety is now grown globally in most of the major wine-growing regions, including USA, Chile, South Africa, East Europe, Australia and New Zealand (Jackson, 2008:1-9). In New Zealand, Sauvignon blanc appeared 40 years ago with the first wine trials dating back to 1973. Moreover, in New Zealand's Marlborough region, tropical fruit-styled Sauvignon blanc wines are considered their flagship wines (Pinu et al., 2014:570; Robinson, 2021:8; 12).

In SA, Chenin blanc dominated Sauvignon blanc, however, there is a shift where the two cultivars are equally prevalent. An increase in Sauvignon blanc plantings was seen between 2014 and 2021, increasing from 9.3% to 10.9% of the region's vineyards, respectively. Chenin blanc only increased by 0.6% in the same time frame, whilst Colombard and Chardonnay decreased (Anonymous, 2023; WOSA, 2023a; b). Although the increase in Sauvignon blanc is negligible, the major change is not in numbers but in quality. South African Sauvignon blanc is getting attention as the area's winemaking pioneers push the category to new heights. South African Sauvignon blanc wines can range from "tropical" to more "green" aromas, usually influenced by climate, viticultural and oenological factors (Allen et al., 1991:110-111; Marais, 1994:41-44; Roujou de Boubée et al., 2000:4830-4834; Anonymous, 2021c). Sauvignon blanc gained popularity because of its fresh-cut grass and pyrazine-focused style. While Sauvignon blanc was initially thought of as simple, nonetheless developed into more complex and regionally derived styles (Anonymous, 2021a; e). Wines originating from the Cape Point and Overstrand areas in SA have characteristic fynbos and black currant aromas (Anonymous, 2021a; e). Sauvignon blanc wines are produced reductively to protect their delicate aromas, however, there has been a move towards a more oxidative and textured style, similar to the wines from Sancerre, in the Loire Valley of France (Robinson, 2021). Wines produced had relatively low floral and white peach notes with a mineral palate. Moreover, another characteristic style has dusty, saline notes, typical of Sauvignon blanc from the Darling and Elim wine regions (Anonymous, 2021a; e). In addition, the popular SA Sauvignon blanc, has also been made with the use of ageing in oak barrels, adding complexity and increasing the ageing potential of the wines (Anonymous 2021a; e; Robinson, 2021).

### 2.6.2 Chenin blanc

Chenin blanc remains the most widely planted white wine grape variety in SA, with 16,827 ha planted, representing 18.6% of the total wine grape area and is of great importance to the SA wine industry (SAWIS, 2020; SAWIS, 2021; WOSA, 2023b). Chenin blanc is a neutral grape cultivar with resulting wines having aromas ranging from fresh and crisp to rich and heavy (Wilson, 2017:6-20). Du Plessis & Augustyn (1981:102) were the pioneers in making the correlation between sulfur compounds, i.e., mercaptans (at low concentrations) and their impact on wine aroma and quality. Moreover, Chenin blanc has previously been profiled for volatile compounds such as fatty acids, ethyl and acetate esters, terpenes, and higher alcohols, but knowledge of thiol levels in Chenin blanc wines is still extremely limited (Lawrence, 2012:55; Wilson, 2017:12-13; Wilson et al., 2018:2; 11). Furthermore, research conducted by Wilson (2017:28-38) and Wilson et al. (2018:1-13) aimed to elucidate the typical levels and perception of volatile thiol compounds in commercial SA Chenin blanc wines. Their study found that the varietal thiols, i.e., 3-SH and 3-SHA were detected in SA Chenin blanc wines in concentrations high enough to influence the aroma of the wines at 23 ng $L^{-1}$ and 893 ng $L^{-1}$, respectively. Both thiols exceeded their respective odour threshold values and were considered odour-active (Wilson, 2017:28-38). In addition, their research substantiated the hypothesis of Du Plessis & Augustyn (1981:102), namely that a sulfur compound was responsible for the 'guava' aroma of Chenin blanc wines, confirming the association of the 'guava' aroma to the 4-MSP thiol compound.

Wang et al. (2016:2362-2365) investigated the effect of various maceration techniques on the volatile composition of Chenin blanc. They found that separate fermentation before blending (SFB), and extended skin contact during fermentation (ESF) treatments significantly increased the polyphenol content and transformed the aromatic components of the Chenin blanc wines. Furthermore, the cryogenic maceration (CR) technique increased the levels of esters and terpenes in the Chenin blanc wines and improved their overall quality (Wang et al., 2016:2360-2366). Coetzee et al. (2018:180-184) investigated the thiol levels in young commercial SA Sauvignon blanc and Chenin blanc wines using a propiolate derivatisation technique with GC-MS/MS detection and found that 4-MSP was present in Sauvignon blanc, but not in the Chenin blanc wines analysed. Additional research is required to determine whether 4-MSP is absent in SA Chenin blanc wines as it has not been detected to date. Further research focusing on the detection and quantification of thiols in Chenin blanc is still needed to support the previous findings (Wilson, 2017:38; Coetzee et al., 2018:180-184).

### 2.6.3 Other white wine grape cultivars

Sauvignon blanc has been researched extensively because of its distinctive aroma profile linked to the presence of varietal thiols (3-SH, 3-SHA and 4-MSP) (Roland et al., 2011a:7355-7376; b:139-144; Coetzee & du Toit, 2012:295, Hart et al., 2017:148-149). However, it has been shown that other white wine grape varieties also contain these thiols, but in lower concentrations (Guth, 1997a:3024; b:3030-3031; Roland et al., 2011b:142-143). Guth (1997a:3024-3025; b:3030-3031) demonstrated the olfactory impact of 4-MSP in Scheurebe wines, detecting the thiol at concentrations of 400 ng $L^{-1}$. Concentrations of 3-SH and 3-SHA were also present well above its perception threshold in wines made from other white varieties, i.e., Petit Manseng, Gros Manseng, Colombard, and botrytized Semillon (Tominaga et al., 2000:1799-1801; Dubourdieu & Tominaga., 2009:275-293; van Rooyen et al., 2023:38). In addition, it was found that 3-SH was present in small quantities, but still above the perception threshold in the wine of the white grape varieties Melon de Bourgogne and Chardonnay (Schneider, 2006:58-64). These volatile thiols were also identified in wines from the Canary Islands and those made from Petite Arvine, a Swiss grape variety (Lopez et al., 2003:3420-3424; Fretz et al., 2005:407-410).

Furthermore, research conducted by Fracassetti et al. (2018:125-133) demonstrated the essential role of 3-SH and 3-SHA in the Italian white varieties, i.e., Catarratto Bianco Comune (CBC) and Grillo by analytical and sensory approaches. However, the 4-MSP precursors were not detected in the grape must varieties and final wine. This extensive loss of glutathionyl precursors was thought to have occurred during the juice extraction process, negatively affecting the sensory properties of the wine (Fracassetti et al., 2018:129-130). Van Rooyen et al. (2023:35-37) investigated the concentrations of varietal thiols in twenty-four commercial SA Colombard wines, before bottling through chemical and sensory analysis. Their research also found the levels of varietal thiols present at concentrations above their sensory threshold (60 ng $L^{-1}$ for 3-SH, 4.2 ng $L^{-1}$ for 3-SHA and 0.8 ng $L^{-1}$ for 4-MSP) and to be similar to that found in young SA Chenin blanc and Sauvignon blanc wines (van Rooyen et al., 2023:35). Dournes et al. (2022:494-496) investigated the effect of cultivation practices, i.e., organic vs conventional vineyard practices on thiol precursors from Colombard and Gros Manseng cultivars. Their research found no detectable Cys-4MSP and Glut-4MSP precursors, as well as no 4-MSP in the final wines which was consistent with the findings of Tominaga et al. (2000:1799-1801). The concentration of the Glut-3SH precursor was found to be relatively high for both Colombard and Gros Manseng. This once again highlighted the impact of winemaking processes and their effect on wine aroma and quality (Fracassetti et al., 2018:129-130; 132).

## 2.7 South African wine regions

### 2.7.1 Wine regions and terroir

South Africa has seven geographical wine-producing regions, i.e., Cape West Coast (Subregion), Cape Coastal (Overarching regions), Breede River Valley, Cape South Coast, Coastal Region, Klein Karoo and the Olifants River region (Fig. 2.3) (Anonymous, 2021a; b; f; g; WOSA, 2023a). Based on their geographical location, the regions range from cooler to warmer climates. In addition, the climate in viticulture can be further subdivided into three levels, macroclimate, meso-climate (also named topo climate) and microclimate. Macroclimate refers to the climate of a region, whilst the meso-climate of the region differs from the macroclimate due to differences in altitude, slope inclination, aspect, or distance from large bodies of water, and usually describes the climate of a particular vineyard. Microclimate is the climate surrounding grape bunches within the vine canopy as modified by vine and canopy management characteristics and can differ (Anonymous, 2021a; b; f; g). The Western Cape region is the largest wine-producing geographical unit comprising smaller sub-regions, but still broad-spanning, such as the Cape South Coast. These regions are further divided into districts, e.g., Walker Bay, which is further subdivided into wards, e.g., Elgin. For this study, all grapes received were from wine Producers originating from the Coastal and Cape South Coast wine regions (Anonymous, 2021a; b; f; g).

Stellenbosch, known as one of the oldest winemaking towns, boasts a winemaking tradition that stretches back to the end of the 17th century. The town's

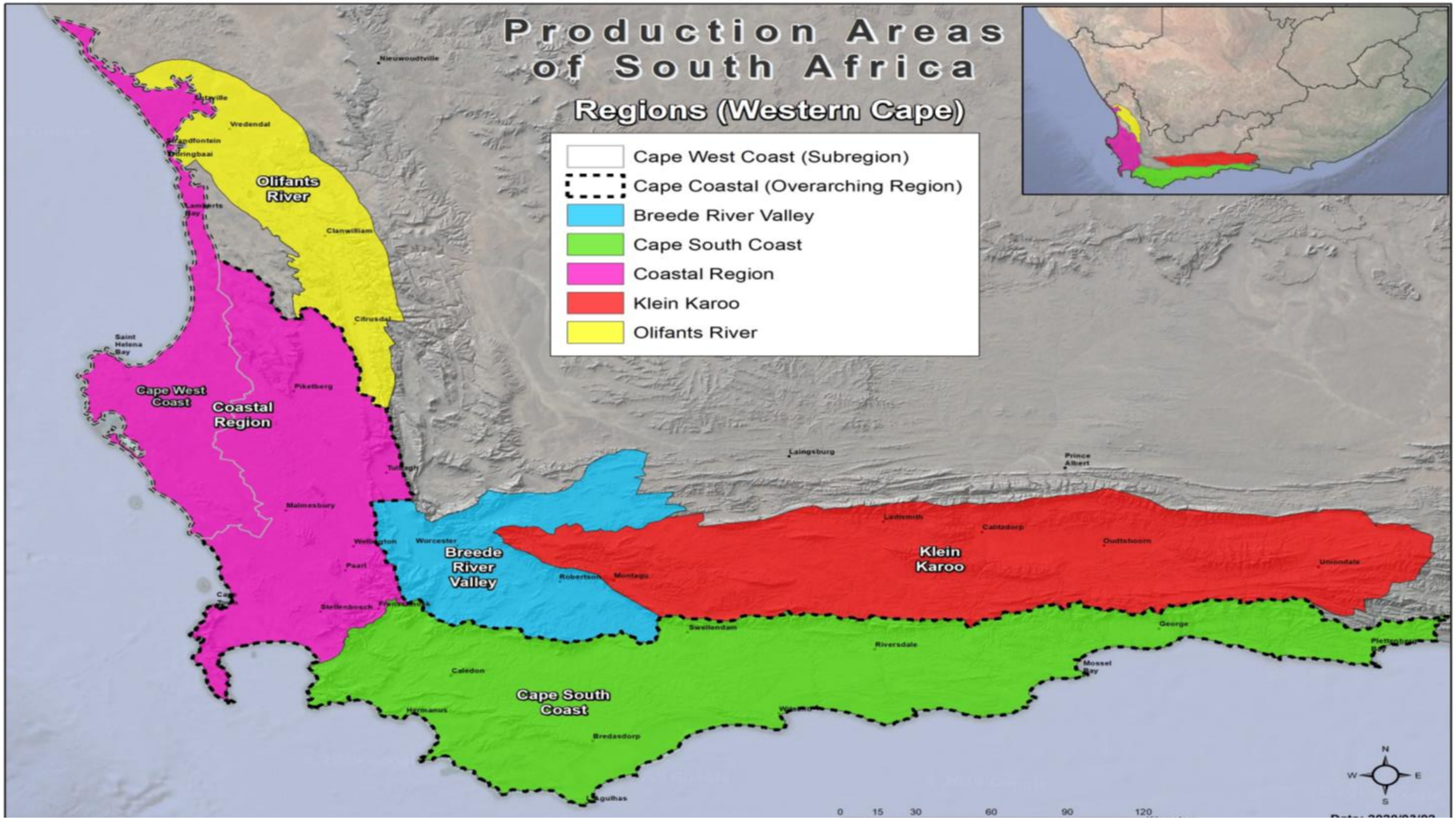



Figure 2.3: South African wine regions (SAWIS, accessed 02.06.2021)

diverse terroir makes it a sought-after viticultural area, with the number of wine estates and producers rapidly escalating, presently exceeding 200. Within the region, a combination of historic estates and wineries yields premium grape varieties, establishing a reputation for high-quality white and red wines (Anonymous, 2021a; b; f; g). Stellenbosch, often referred to as the 'town of oaks', serves as the focal point for education and research within the wine-producing area. Stellenbosch University is currently the sole institution in South Africa offering a specialised degree in Viticulture and Oenology, boasting a successful number of winemakers among its alumni. Adjacent to Stellenbosch lies the Elsenburg School of Agriculture, along with the Nietvoorbij Institute of Viticulture and Oenology. Moreover, this institute houses a state-of-the-art experimental cellar, where significant research is conducted on new grape varieties, clones, and rootstocks across its experimental farms situated in various wine-growing regions. The viticultural region of Stellenbosch has further been subdivided into multiple smaller sub-regions, each with its distinct characteristics, including Banghoek, Bottelary, Devon Valley, Jonkershoek Valley, Papegaaiberg, Polkadraai Hills, Simonsberg-Stellenbosch, and Vlottenburg (Anonymous, 2021a; b; f; g).

A newly established district, Wine of Origin (W.O.) Cape Town is a fairly recent designation created in 2017 and named after Cape Town, a prominent tourism destination, which incorporates the regions of Constantia, Hout Bay, Durbanville, and Philadelphia (Anonymous, 2025). At its farthest point, this district is located 36 km away from the central business district of Cape Town. Positioned on the southern inclines of the Table Mountain range within the renowned floral kingdom, lies the historic Constantia Valley, which is considered the birthplace of winemaking in the Cape region (Anonymous, 2021a, b, f, g). This valley, falling under the Cape Peninsula district, was the location of Simon van der Stel's wine farm in the 17th century and the birthplace of the famous Constantia dessert wines that gained popularity across Europe in the 18th century. Situated in ancient soils, the vineyards ascend the east-facing slopes of the Constantiaberg, benefiting from the refreshing sea breezes originating from False Bay (Anonymous, 2021a, b, f, g). With an average annual rainfall of about 1,000 mm, irrigation is unnecessary in this ward, which also boasts an average February temperature of 20.6°C. The premier ward hosts only a few cellars, where the cool climate is conducive to producing high-quality white wines, particularly Sauvignon blanc, continuing a tradition of exceptional wine production since 1685. The well-regarded Cape Point vineyards, some located just 1.2 km from the shoreline, are found on the western perimeters of the Cape Peninsula. Recognised for their Sauvignon blanc and Semillon varieties, these vineyards in the cool maritime climate of the Cape Town district are highly esteemed. Similar to Constantia, the vineyards in

Durbanville are near Cape Town and extend towards the Northern suburbs. Numerous estates and wineries on the undulating hills with varying aspects and elevations continuously produce a diverse range of wine styles. Some vineyards are situated as high as 380 m above sea level. Wines from this ward include Sauvignon blanc, Chardonnay, Merlot, and Cabernet Sauvignon. Factors such as deep soils, cooling sea breezes, misty nights, and the proximity to the ocean all contribute to the grape quality. A recently established ward north of Durbanville, Philadelphia benefits from the cooling influences of the Atlantic Ocean. The mountainous landscape of this region results in some vineyards being elevated up to 260 m above sea level, leading to a significant day-night temperature variation and slower grape ripening. This unique environment has already produced high-quality Cabernet Sauvignons, Merlots, and red blends, showcasing the potential of this emerging appellation (Anonymous, 2021a; b; f; g).

Most of the maritime vineyards in this region are located within the ward of Elim, near the southernmost point of Africa, Cape Agulhas. Elim, a village with historical significance, was established in 1824 as a Moravian mission settlement and is now designated as a national monument. The strong, cooling winds during the summer create an optimal environment for the gradual and cool ripening process, particularly beneficial for Sauvignon blanc and promising for Semillon and Shiraz (Anonymous, 2021a; b; f; g). The limited yet burgeoning land area dedicated to viticulture in this coastal area has captured significant attention within the wine industry, highlighting the considerable potential it holds. Sauvignon blanc vines flourish in medium-potential soils in cooler climatic regions. However, they are produced in all the wine-producing regions of South Africa, with Stellenbosch accounting for the biggest area under production, followed by Robertson, the Swartland and Worcester. Moreover, it is the most planted variety in the Cape South Coast (Anonymous, 2021d; e). The diversity of the South African wine growing regions allows for Sauvignon blanc wines that have either “vegetative/grassy”, “citrus/grapefruit”, “tropical” or “green apple skin” aromas. Sauvignon blanc grapes grown under warm climatic regions (Stellenbosch, Robertson and Paarl) generally produce wine with flavours of tropical and yellow fruit, while cooler climatic (Elgin and Cape South Coast) conditions produce wines with more spicy and herbaceous tones, such as grass, asparagus and green figs (Marais, 1994:41-44; Coetzee & du Toit, 2012:288-295; Ruiz et al., 2019:7425-7450; Anonymous, 2021a).

Methoxypyrazines and varietal thiols are present in different quantities when the grapes originate from different wine-growing regions (Marais, 1994:41-44). In regions with lower temperatures such as Elim, wines tend to exhibit pronounced spicy characteristics. Conversely, in the western part of the Western Cape, Sauvignon Blanc is often described as having more "tropical" and "fruity" attributes. In contrast, the Elgin wine region is recognised for its lively style, showcasing a unique jalapeño-like quality that sets it apart (Anonymous, 2021a; b; f; g). It has also previously been shown that due to the complexity of the wine matrix, there are major interactions between compounds, leading to suppression or masking of aromas and flavour, i.e., when thiols are present in high enough concentrations, they can suppress the sensory effect of methoxypyrazine compounds (King et al., 2011:179).

## 2.8 Other novel winemaking technologies applied in winemaking to enhance wine aroma

Industries and technologies are continuously evolving, and over the last decade, various emerging thermal and non-thermal technologies have been proposed, in addition to traditional methods, for enhancing the varietal aroma, flavour profiles, and quality of wines (Rodríguez-Rojo et al., 2012:98-103; Chandrasekaran et al., 2013:244-259; Chemat et al, 2017:551-555; 558; Comuzzo et al., 2018:14-18; Romero-Díez et al., 2019:258-266; Comuzzo et al., 2020:2; 13; Maza et al., 2020:12). Technologies that have been successfully used in the food and pharmaceutical industries and show promise for its application in the wine industry include Ultrasound (US) technology, Microwave-assisted extraction (MAE) and Pulsed electric field (PEF) (Rodríguez-Rojo et al., 2012:98-103; Chandrasekaran et al., 2013:244 259; Chemat et al, 2017:551-555; 558; Comuzzo et al., 2018:14-18; Romero-Díez et al., 2019:258-266; Comuzzo et al., 2020:2; 13; Maza et al., 2020:12). Moreover, the International Organisation of Vine and Wine (OIV) has now approved the use of US as a new technology to increase the extraction of compounds from grape tissues during winemaking (International Organisation of Vine and Wine, 2019). The abovementioned technologies have typically been applied to red grape cultivars for the extraction of polyphenolic compounds, enhancing colour, wine pasteurization and to speed up the maturation of wines (Rodríguez-Rojo et al., 2012:98-103; Chandrasekaran et al., 2013:244-259; Yang et al., 2016:29; Chemat et al, 2017:551-555; 558; Comuzzo et al., 2018:14-18; Romero-Díez et al., 2019:258-266; Comuzzo et al., 2020:2; 13; Maza et al., 2020:12). The use of PEF on white wine grape cultivars, such as Sauvignon blanc is gaining popularity and have been investigated as a prefermentative extraction step to increase the yield of precursors of aroma compounds (Leong et al., 2016). However, to date, no literature has reported on the use of these technologies during Chenin blanc wine production which could be a valuable technique to enhance the varietal character of wines made from this wine grape variety.

### 2.8.1 Ultrasound (US) technology

Ultrasound (US) technology has been used across the pharmacology and food industry sectors (fresh, frozen, dried products and liquid products) with numerous applications, i.e., cooking, cutting, freezing, thawing, degassing, filtration, extraction, defoaming, emulsification, drying, microbial inactivation and fermentation (Rodríguez-Rojo et al., 2012:98-103; Ferraretto et al., 2013:160-168; Bautista-Ortín et al., 2017:1314-1323; Roman et al., 2020:6-8; Das et al., 2022:1-4; Gómez-Plaza et al., 2022:73; 74-75). Different US systems are used for food applications, depending on the food medium and the desired effect, however, US through a liquid medium is the most widely used (Chemat et al., 2017:549-552; Gómez-Plaza et al., 2022:73-83). The technology uses mechanical waves with frequencies ranging from 20 kHz-10 MHz (Ferraretto et al., 2013:160-168; Bautista-Ortín et al., 2017:1316; Chemat et al., 2017:542-546; Roman et al., 2020:2; 4; Coetzee, 2021). Ultrasound treatments for the food industry can be divided into two frequencies, i.e., high frequency or low power intensity (100 kHz-1 MHz and <1 W/cm$^2$) used as a non-destructive analytical technique for quality control and power ultrasound (16-100 kHz and 10-1000 W/cm$^2$) which generates physical or chemical interactions, used for enhancing food processing (Martín & Sun, 2013:40-53; Tao & Sun, 2015:570-794; Chemat et al., 2017:542-546; Romero-Díez et al., 2019:263-265; Das et al., 2022:1-26; Gómez-Plaza et al., 2022:74-75).

Although US has been used widely in the food industry, its application in the wine industry is relatively new (Ferraretto et al., 2013:160-168; Bautista-Ortín et al., 2017:1314-1323; Roman et al., 2020:6-11; Das et al., 2022:1-26; Gómez-Plaza et al., 2022:74-83). Ferraretto et al. (2013:160-168) investigated the use of US for the extraction of phenolic compounds from nine red grape varieties. The treatment was applied to the yeast lees to enhance the lytic effect of yeast cell structures to speed up ageing on the lees. Results showed an increase in the extraction of polyphenolics as well as an increase in the release of colloids, polysaccharides and mannoproteins from yeast cells. Furthermore, Ferraretto & Celotti (2016:531-533) applied US technology to young red wines, four months after racking to speed up the ageing reactions to reduce the time between production and consumption. The results showed an increase in the extraction of phenolic compounds and an acceleration of the ageing process making it suitable for use on young, well-coloured wines (Maza et al., 2020:12). In a study conducted by Romero-Díez et al. (2019:264-265), the effects of microwave-assisted extraction (MAE) were compared to those of US. It was found that MAE increased the internal mass transfer from wine lees, thereby enhancing anthocyanin extraction, whereas US only influenced the processing time, but not the extraction yield (Romero-Díez et al., 2019:264). Oliver Simancas et al. (2021:17) also demonstrated that the application of US led to an increase in most free varietal compounds in grape must with resultant wines showing an increase in the $C_6$ alcohols, terpenes and norisoprenoids (all varietal compounds) at both frequencies (20 kHz and 28 kHz), but at the shorter maceration time. Similarly, Aragón-García et al. (2021:3-7) applied US to grape must of the Muscat of Alexandria variety, treated at two time periods, i.e., 40 min and 80 min and showed that the concentration of terpenes was substantially increased at 80 min. These results illustrated that two different wines could be produced from the same cultivar by applying US at different periods (Aragón-García et al., 2021:4-7; 9-11).

Bautista-Ortín et al. (2017:1314-1323) used US as an alternative to maceration by applying it as a pre-treatment process to crushed red grapes, before pressing, to improve the extraction of phenolic compounds and varietal aromas. The results showed promise, as wines produced retained their quality characteristics, while reducing the skin maceration time, thus optimising the winery capacity (Bautista-Ortín et al., 2017:1314-1323; Gómez-Plaza et al., 2022:74-75; 77-81). Roman et al. (2020:1-13) investigated the effect of US as a potential technology for the extraction of sulfur aroma precursors in Sauvignon blanc by applying it to grape must and a synthetic medium. An increase in catechins and total phenols was observed in grape must, however, no significant differences were observed in thiol precursors. However, an increase was observed for both 3-SH and 4-MSP in the model wine compared to the untreated samples. It should be noted that further investigations are still needed to identify the mechanism of production of thiol precursors due to US treatment (Roman et al., 2020:10).

Ultrasound technology shows promise as a novel technology in wine production and can be used for the optimisation of wine technological processes during white and red wine production. Furthermore, US-extracted compounds are free from residual solvents and contaminants, offering extracts with better quality, a process with increased yield and improved extraction time, making it an environmentally friendly, green technique (Chemat et al, 2017:551-555; 558). However, current studies have only been conducted on a small or laboratory scale and further optimisation on an industrial scale is needed to verify the reliability of results. Moreover, the treatment also showed promise in the acceleration of aroma extraction from oak chips, for the reduction in microbiological populations, reducing $SO_2$ usage, as well as the acceleration of ageing on the lees (Ferraretto et al., 2013:165; Martín & Sun, 2013:40-53; Bautista-Ortín et al., 2017:1318-1323; Roman et al., 2020:6-13; Romero-Díez et al., 2019:258-266; Das et al., 2022:25-26; Gómez-Plaza et al., 2022:76-81).

### 2.8.2 Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) is another technology widely applied in the food industry for the extraction of active compounds, drying, pasteurisation, sterilisation, thawing, tempering, and baking of food (Rodríguez-Rojo et al., 2012:98-103; Chandrasekaran et al., 2013:244-259; Romero-Díez et al., 2019:258-266). Microwaves

(MW) are electromagnetic waves with frequencies varying between 300 MHz and 300 GHz. Industrial MW heating occurs at frequencies ranging from 915 MHz to 2.45 GHz, whilst domestic MW operates at a frequency of 2.45 GHz (Chandrasekaran et al., 2013:244-259). Microwave heating results from two mechanisms, i.e., dipolar rotation and ionic conduction (Rodríguez-Rojo et al., 2012:98-103; Chandrasekaran et al., 2013:244-259). During the microwave process, the rapid heating and evaporation cause intracellular water as well as compounds to leach from cellular tissue, speeding up the extraction process. MAE is also much more efficient than conventional maceration/extraction techniques because the heating occurs in a few seconds (Romero-Díez et al., 2019:264).

During the winemaking process, maceration is an important step for the extraction of compounds from the grape skin (Muñoz García et al., 2021:2; 13; 2022:12). Various technologies, i.e., thermovinification, cryomaceration and flash release systems are currently being used to facilitate this process. However, these techniques are often time-consuming, and the high temperatures have been shown to negatively alter the aroma compounds and aroma profile of the wine (Casassa et al., 2019:3; Fanzone et al., 2022:13-14; Casassa et al., 2022:24; Muñoz García et al., 2022:2). MAE is a feasible, ecologically friendly alternative as it does not require water or any chemical aids and does not generate any additional winery waste (Casassa et al., 2022:2-27; Fanzone et al., 2022:2-14). Romero-Díez et al. (2019:258-266) compared the effects of MAE and ultrasonic-assisted extraction (UAE) to conventional solvent extraction to increase the yield of anthocyanins from yeast lees. The outcomes of the study showed that MAE increased the internal transfer of mass from the yeast cells, thus increasing the extraction yield of the anthocyanins at a shorter time, whilst UAE did not affect anthocyanin yield, but reduced processing time. Casassa et al. (2019:2-16) examined the effects of MAE on the phenolic composition and colour of Merlot wines. Grapes were harvested at three different maturity levels, i.e., unripe, optimally ripe and overripe and the musts were subjected to MAE after crushing. Results showed that MAE improved the extraction of anthocyanins, tannins, total phenolics, polymeric pigments and colour when treatments were applied to unripe grapes as opposed to the control, optimally ripe and overripe treatments (Casassa et al., 2019:2-16).

Muñoz García et al. (2021:9-10; 13-14) investigated the effect of MAE at a laboratory scale on the extraction of the free and glycosidically bound fraction of volatile compounds of Cabernet Sauvignon must and wine and the overall aroma of the wine. Wines were produced with and without $SO_2$ to possibly reduce the addition during vinification. Results showed an increase in the concentrations of varietal compounds of the must for both free and glycosidically bound fractions which was due to greater extraction of these compounds from the grape skin due to the MAE treatment (Muñoz García et al., 2021:8; 13). Furthermore, it was seen that, in the presence of $SO_2$, wines had higher concentrations of $C_6$ compounds, alcohols, terpenes, and norisoprenoids (free form) whilst few changes in the bound fraction and benzenic compounds were observed (Makhotkina et al., 2013:211-212). In the absence of $SO_2$, MAE wines showed changes in the concentrations of some volatile compounds, such as a decrease in some esters or an increase in linalool or 2-phenylethanol. These findings, therefore, suggest that MAE can be used to increase the aromatic potential of wines by reducing $SO_2$ levels during production (Muñoz García et al., 2021:13-14). In another study, Muñoz García et al. (2022:12) also investigated the effect of microwave technology on the natural yeast population of crushed grapes, fermentation kinetics and the amino acid and polysaccharide composition of the wine. Microwave technology did not modify the total bacterial and yeast population in the must, however, it reduced the number of species. Furthermore, an improvement in the fermentation kinetics was observed with little change to the basic chemical composition. It was further shown that MAE-treated wines had higher polyphenols, major amino acids and total assimilable nitrogen levels (Muñoz García et al., 2022:9;13).

Microwave technology was also applied to Cabernet Sauvignon, Merlot and Syrah must, to which stems were added (Casassa et al., 2022:2-27). The most notable effect observed was increased flavanols, which positively contributed to the wine colour, mouthfeel and bitterness. Wines also had decreased herbaceous aromas, although jammy aromas and flavours increased. Microwave technology could, therefore, be a useful process to decrease herbaceous aromas in wines and reduce coarse mouthfeel (Casassa et al., 2022:23-24). Similar observations were made in a study by Fanzone et al. (2022:2-14), whereby wines produced from MAE grape must had improved colour stability and an improved organoleptic profile. Although promising results have been obtained and MAE is considered a green technology, most studies were conducted on red cultivars at a small scale. Further applications are required on white grape varieties as well as on an industrial scale. Moreover, MAE can add value to the winemaking industry and can be used as a valuable tool to create novel wine styles (Casassa et al., 2019:14; Romero-Díez et al., 2019:264-265; Fanzone et al., 2022:2-14; Casassa et al., 2022:2-27; Muñoz García et al., 2021:1-16; 2022:6-12).

### 2.8.3 Pulsed electric field (PEF)

Pulsed electric field (PEF) is another novel non-thermal processing technology used in the food industry for food dehydration, sterilisation, extraction, reduction in pesticide residues and inactivation of enzymes (Yang et al., 2016:28-38). This technology involves the application of short-duration (μs to ms) electric field pulses of high intensity

(10-80 kV $cm^{-1}$) to materials or food products placed between two electrodes (Clodoveo et al., 2016:43; 50; Ozturk & Anli, 2017:2-3; Delso et al., 2022:62). The PEF technique causes the formation of pores in cell membranes (electroporation), which increases cell membrane permeability and allows for the leaching of intracellular compounds (López-Giral et al., 2023:278-279; 281). Additionally, PEF is considered a mild processing technique, which is beneficial for the extraction of heat-sensitive compounds. Furthermore, PEF is a low-cost technology with a shorter processing time than conventional maceration (Ricci et al., 2020:2). Therefore, this technology will be beneficial to the wine industry as a novel pre-treatment technique to increase the extraction of phenolic compounds, nutraceutical components and varietal aroma compounds from grape skins (Clodoveo et al., 2016:50-51; Comuzzo et al., 2018:17; Delso et al., 2022:65-66; 69; Feng et al., 2022:3-4). It can further be used as an alternative technique to reduce the natural microbial population in grape juice and thereby lower the amount of $SO_2$ required during the winemaking process (Yang et al., 2016:29; 34-35; Ozturk & Anli, 2017:2-3). Additionally, PEF was also found to accelerate the release of mannoproteins during yeast autolysis (Comuzzo et al., 2018:4).

Previous research demonstrated the effectiveness of PEF technology as a pre-treatment technique for the extraction of colour and phenolic compounds from, mostly, red wine grape varieties (Clodoveo et al., 2016:51; Yang et al., 2016:32; Ozturk & Anli, 2017:1-3; Ricci et al., 2020:8-9). Leong et al. (2016) successfully applied PEF to macerated Sauvignon blanc grapes and found that the wines produced had a different metabolomic profile in terms of the acids which were higher and sugars which were lower than the control wines. Although PEF have been applied during white grape processing its effect on the composition of white wine is still limited and requires further investigation (Comuzzo et al., 2018:14-18). Though numerous studies have already been conducted with PEF, its application in the wine industry is recent with most studies being pilot scale and further optimisation at an industrial scale is needed (Delso et al., 2022:69). With the wine industry searching for and developing innovative technologies, the results achieved with PEF applications in winemaking indicate that it is a promising alternative tool to enahnce traditional processing techniques, however, it still requires further investigation (Clodoveo et al., 2016:51-53; Yang et al., 2016:28-38; Ozturk & Anli, 2017:1-3; Comuzzo et al., 2018:17-18).

## 2.9 Future trends

Various scientific and wine technological developments have been made within the wine industry to improve the varietal aroma, flavour profiles and the quality of wines. These advances have given rise to novel wine styles being produced from the same grape varieties by either direct manipulation of the grape berry before the winemaking process or a combination of processes during wine production. However, the ability to manage the development of flavour compounds, i.e., varietal thiols and their association with the sensory quality of wine using viticultural or oenological practices, is yet to be demonstrated. Literature reported that a combination of vineyard practices, grape cultivar, processing and fermentation techniques could influence varietal thiols and their precursors. Most procedures demonstrated varied results, with the number of precursors in grapes not directly reflected in the varietal thiols quantified in the final wine. Therefore, specific vineyard and winemaking practices for increasing thiol and aroma compound concentrations in wines are still needed. Moreover, the cryogenic treatment of grapes as a pre-fermentation treatment could be a useful alternative based on cryogenic processing technologies already applied in the beverage industry.

Capone et al. (2011:4656-4657) demonstrated its potential for thiol management in Sauvignon blanc, with thiol precursors 3-S-cysteinylhexan-1-ol (Cys-3-SH) and 3-S-glutathionylhexan-1-ol (Glut-3-SH), whereby Glut-3-SH was increased four-fold in frozen grapes stored at -20 °C for two months compared to that found in frozen or fresh juices. Moreover, studies using dry ice for pre-fermentative cryomaceration of Sauvignon blanc grape must also show an increase in 3-SH and 3-SHA concentrations in the resultant wine. However, the effect of cryogenic storage on thiol production during fermentation remains to be further investigated, and the influences of cryogenic treatments on grape precursors and wine thiols have never been conducted simultaneously. It should also be noted that, although these new winemaking technologies greatly contribute to an increase in the extraction of aroma compounds, the grape variety used is still largely responsible for the aroma profile of the final wine. The absence of information on the effect of these treatments on MP's also remains to be addressed, as they are also important compounds contributing to the varietal aroma of Sauvignon blanc wine. Therefore, the present study investigated the effect of wine technological processes, i.e., viticultural and winemaking technologies with an emphasis on pre-fermentative cryogenic treatment and its influence on grape aroma compounds linked to wine aroma and organoleptic quality of white wine.

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## 2.10 References

Alem, H., Rigou, P., Schneider, R., Ojeda, H. & Torregrosa, L. 2019. Impact of agronomic practices on grape aroma composition: a review. *Journal of the Science of Food and Agriculture*, 99(3):975-985. https://doi.org/10.1002/jsfa.9327.

Aleixandre-Tudo, J. L. & du Toit, W. 2018. Cold maceration application in red wine production and its effects on phenolic compounds: A review. *LWT-Food Science and Technology* 95:200-208. https://doi.org/10.1016/j.lwt.2018.04.096.

Aleixandre-Tudo, J. L., Weightman, C., Panzeri, V., Nieuwoudt, H. H.& Du Toit, W. J. 2015. Effect of skin contact before and during alcoholic fermentation on the chemical and sensory profile of South African Chenin blanc white wines. *South African Journal of Enology and Viticulture*, 36(3):366-377. http://www.scielo.org.za/scielo.php?script=sci_arttextandpid=S2224-79042015000300007andlng=enandtlng=es.

Aleixandre-Tudo, J. L., Alvarez, I., Lizama, V., Nieuwoudt, H., Garcia, M. J., Aleixandre, J. L. & Du Toit, W. J. 2016. Modelling phenolic and volatile composition to characterize the effects of pre-fermentative cold soaking in Tempranillo wines. *LWT-Food Science and Technology*, 66:193-200. https://doi.org/10.1016/j.lwt.2015.10.033.

Ali, I., AL-Othman, Z.A., Nagae, N., Gaitonde, V.D. & Dutta, K.K. 2012. Recent trends in ultra-fast HPLC: New generation superficially porous silica columns. *Journal of Separation Science*, 35(23):3235-3249.

Allen, M.S., Lacey, M.J., Harris, R.L. & Brown, W.V. 1991. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *American Journal of Enology and Viticulture*, 42(2):109-112.

Allen, T., Herbst-Johnstone, M., Girault, M., Butler, P., Logan, G., Jouanneau, S., Nicolau, L. & Kilmartin, P.A. 2011. Influence of grape–harvesting steps on varietal thiol aromas in Sauvignon blanc wines. *Journal of Agricultural and Food Chemistry*, 59(19):10641-10650. https://doi.org/10.1021/jf2018676.

Anonymous, 2021a. White-wine varieties grown in South Africa (Wine of South Africa). http://www.wosa.co.za/The–Industry/Varieties–and–Styles/White-Wine-Varieties/. [31 March 2021].

Anonymous, 2021b. White grape varieties from South Africa. [WWW document] URL https://capreo.com/en/white-grape-varieties [31 March 2021].

Anonymous, 2021c. Methoxypyrazines and greenness in wines: myth or reality? A few perspectives [WWW document] URL https://www.wineland.co.za/methoxypyrazines greenness wines-myth-reality-perspectives [20 May 2021].

Anonymous, 2021d. Making sense of warm and cool climates. [WWW document] URL https://www.wineland.co.za/making-sense-of-warm-and-cool-climates/ [02 June 2021].

Anonymous, 2021e. Sauvignon blanc [WWW document] URL https://southafrica.co.z a/sauvgnon-blanc.html [02 June 2021].

Anonymous, 2021f. Map of South African Wine Regions. [WWW document] URL http://www.sawis.co.za/cert/download/Regions_-_Mrt2020.pdf [02 June 2021].
Anonymous, 2021g. Grape Varieties SA, Sauvignon blanc. [WWW document] URL https://topwinesa.com/about-wine/wine-grape-varieties/#SauvignonBlanc. [03 June 2021].

Anonymous, 2022a. Wine grape Harvest. Retrieved from https://www.thespruceeats. com/wine–grape–harvest–3511325/. [08 March 2022].

Anonymous, 2022b. How white wine is made: the stages of vinification. Retrieved from https://www.bestheim.com/en/news/268-stages-vinification-white-wines/. [04 July 2022].

Anonymous, 2022c. Wine production: Choosing a wine press - Gravity Wine House. Retrieved from https://gravitywinehouse.com/blog/wine-production-choosing-a-wine-press/. [05 July 2022].

Anonymous, 2022d. How white wine is made. Retrieved from https://www.winemag.com/2019/09/24/how-white-wine-is-made/. [06 July 2022].

Anonymous, 2022e. Do You Confuse Primary, Secondary, and Tertiary Wine Aromas? Here Are 3 Ways To Keep Them Straight. Retrieved from https://mag.somm tv.com/2021/06/understanding-wine-aromas/. [06 July 2022].

Anonymous, 2023. Is South African Sauvignon Blanc on the Precipice of Global Demand? Retrieved from https://mag.sommtv.com/2023/01/south-african-sauvignon-blanc/. [25 July 2023].

Anonymous, 2025. New wine of origin Cape Town flies flag for South African Wine Industry. Retrieved from https://https://www.wosa.co.za/Wosa-News/Press-Releases/2017/New-Wine-of-Origin-Cape-Town-Flies-Flag-for-South-African-Wine-Industry/. [10 September 2025].

Antonelli, A., Arfelli, G., Masino, F. & Sartini, E. 2010. Comparison of traditional and reductive winemaking: influence on some fixed components and sensorial characteristics. *European Food Research and Technology*, 231:85-91.

Aragón-García, F., Ruíz-Rodríguez, A. & Palma, M. 2021. Changes in the Aromatic Compounds Content in the Muscat Wines as a result of the Application of Ultrasound during Pre-Fermentative Maceration. *Foods*, 10(7):1462. https://doi.org/10.3390/foods10071462.

Augustyn, P.H., Rapp, A. & Van Wyk, C.J. 1982. Some volatile aroma components of *Vitis vinifera L. cv*. Sauvignon blanc. *South African Journal of Enology and Viticulture*, 3(2):52-60.

Avizcuri-Inac, J. M., González-Hernández, M., Rosáenz-Oroz, D., Martínez-Ruiz, R. & Vaquero-Fernández, L. 2018. Chemical and sensory characterisation of sweet wines obtained by different techniques. *Ciência e Técnica Vitivinícola*, 33(1):15-30. https://doi.org/10.1051/ctv/20183301015.

Ayestarán, B., Martínez-Lapuente, L., Guadalupe, Z., Canals, C., Adell, E. & Vilanova, M. 2019. Effect of the winemaking process on the volatile composition and aromatic profile of Tempranillo Blanco wines. *Food Chemistry*, 276:187-194. https://doi.org/10.1016/j.foodchem.2018.10.013.

Baiano, A., Terracone, C., Longobardi, F., Ventrella, A., Agostiano, A. & Del Nobile, M.A. 2012. Effects of different vinification technologies on physical and chemical characteristics of Sauvignon blanc wines. *Food Chemistry*, 135(4):2694–2701. https://doi.org/10.1016/j.foodchem.2012.07.075.

Bailly, S., Jerkovic, V., Marchand-Brynaert, J. & Collin, S. 2006. Aroma extraction dilution analysis of Sauternes wines. Key role of polyfunctional thiols. *Journal of Agricultural and Food Chemistry*, 54(19):7227-7234. https://doi.org/10.1021/jf060814k.

Bailly, S., Jerkovic, V., Meuree, A., Timmermans, A. & Collin, S. 2009. Fate of key odorants in Sauternes wines through aging. *Journal of Agricultural and Food Chemistry*, 57(18):8557-8563. https://doi.org/10.1021/jf901429d.

Baron, M., Prusova, B., Tomaskova, L., Kumsta, M. & Sochor, J. 2017. Terpene content of wine from the aromatic grape variety 'Irsai Oliver' (*Vitis vinifera* L.) depends on maceration time. *Open Life Sciences*, 12(1):42-50. https://doi.org/10.1515/biol-2017-0005.

Bautista-Ortín, A. B., Jiménez-Martínez, M. D., Jurado, R., Iniesta, J. A., Terrades, S., Andrés, A. & Gómez-Plaza, E. 2017. Application of high-power ultrasounds during red wine vinification. *International Journal of Food Science and Technology*, 52(6):1314-1323. https://doi.org/10.1111/ijfs.13411.

Belda, I., Ruiz, J., Esteban-Fernández, A., Navascués, E., Marquina, D., Santos, A. and & Moreno-Arribas, M. V. 2017. Microbial contribution to wine aroma and its intended use for wine quality improvement. *Molecules*, 22(2):189. https://doi.org/10.3 390/molecules22020189.

Bencomo-Rodriguez, J.J., Gambetta, J., Rigou, P., Canelo, N., Roland, A., Salmon, J.M. & Bouvier, N. 2011, June. Quantitative determination of varietal disulfides in wine and their behavior during alcoholic fermentation. Paper presented at *9e Symposium international d'œnologie de Bordeaux-Oeno 2011*. Bordeaux, France: Dunod. https://hal.archives-ouvertes.fr/hal-01762389 [07 June 2021].

Benkwitz, F., Nicolau, L., Lund, C., Beresford, M., Wohlers, M. & Kilmartin, P. A. 2012a. Evaluation of key odorants in Sauvignon blanc wines using three different methodologies. *Journal of Agricultural and Food Chemistry*, 60(25):6293-6302. https://doi.org/10.1021/jf300914n.

Benkwitz, F., Tominaga, T., Kilmartin, P. A., Lund, C., Wohlers, M. & Nicolau, L. 2012b. Identifying the chemical composition related to the distinct aroma characteristics of New Zealand Sauvignon blanc wines. *American Journal of Enology and Viticulture*, 63(1):62-72. https://doi.org/10.5344/ajev.2011.10074.

Benucci, I., Cerreti, M., Liburdi, K., Nardi, T., Vagnoli, P., Ortiz-Julien, A. & Esti, M. 2018. Pre-fermentative cold maceration in presence of non-*Saccharomyces* strains: Evolution of chromatic characteristics of Sangiovese red wine elaborated by sequential inoculation. *Food Research International*, 107:257-266. https://doi.org/10.1016/j.foodres.2018.02.029.

Bestulić, E., Rossi, S., Plavša, T., Horvat, I., Lukić, I., Bubola, M., Peršurić, A.S.I., Jeromel, A. & Radeka, S. 2022. Comparison of different maceration and non-maceration treatments for enhancement of phenolic composition, colour intensity, and taste attributes of Malvazija istarska (*Vitis vinifera* L.) white wines. *Journal of Food Composition and Analysis*, 109:104472. https://doi.org/10.1016/j.jfca.2022.104472.

Bogart, K., & Bisson, L. 2006. Persistence of vegetal characters in wine grapes and wine. *Practical Winery and Vineyard*, 86:13-20. https://www.burgundy-report.com/wp/wp-content/uploads/2005/09/vegetal-characters-Practical-Winery.pdf.

Boselli, E., Di Lecce, G., Alberti, F. & Frega, N.G. 2010. Nitrogen gas affects the quality and the phenolic profile of must obtained from vacuum-pressed white grapes. *LWT-Food Science and Technology*, 43(10):1494-1500. https://doi.org/10.1016/j.lwt.2010.03.006.

Bonnaffoux, H., Delpech, S., Rémond, E., Schneider, R., Roland, A. & Cavelier, F. 2018. Revisiting the evaluation strategy of varietal thiol biogenesis. *Food Chemistry*, 268:126-133. https://doi.org/10.1016/j.foodchem.2018.06.061.

Brillante L, Martínez-Lüscher J, Kurtural SK. 2018. Applied water and mechanical canopy management affect berry and wine phenolic and aroma composition of grapevine (*Vitis vinifera* L., cv. Syrah) in Central California. *Scientia Horticulturae*, 3(227):261-71.

Cai, J., Zhu, B. Q., Wang, Y. H., Lu, L., Lan, Y. B., Reeves, M. J. & Duan, C. Q. 2014. Influence of pre-pre-fermentation cold maceration treatment on aroma compounds of Cabernet Sauvignon wines fermented in different industrial scale fermenters. *Food Chemistry*, 154:217-229. https://doi.org/10.1016/j.foodchem.2014.01.003.

Capone, D.L. & Jeffery, D.W. 2011. Effects of transporting and processing Sauvignon blanc grapes on 3-mercaptohexan-1-ol precursor concentrations. *Journal of Agricultural and Food Chemistry*, 59(9):4659-4667. https://doi.org/10.1021/jf200119z.

Capone, D.L., Sefton, M.A. & Jeffery, D.W. 2011. Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. *Journal of Agricultural and Food Chemistry*, 59(9):4649-4658. https://doi.org/10.1021/jf200116q.

Capone, D. L., Ristic, R., Pardon, K. H. & Jeffery, D. W. 2015. Simple quantitative determination of potent thiols at ultra-trace levels in wine by derivatization and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. *Analytical chemistry*, 87(2):1226-1231. https://doi.org/10.1021/ac503883s.

Carlin, S., Piergiovanni, M., Pittari, E., Lisanti, M.T., Moio, L., Piombino, P., Marangon, M., Curioni, A., Rolle, L., Segade, S.R. & Versari, A. 2022. The contribution of varietal thiols in the diverse aroma of Italian monovarietal white wines. *Food Research International*, 157:111404. https://doi.org/10.1016/j.foodres.2022.111404.

Carillo, M., Formato, A., Fabiani, A., Scaglione, G. & Pucillo, G. P. 2011. An inertizing and cooling process for grapes cryomaceration. *Electronic Journal of Biotechnology*, 14(6):8-8. https://doi.org/10.2225/vol14-issue6-fulltext-10.

Casalta, E., Salmon, J.M., Picou, C. & Sablayrolles, J.M. 2019. Grape solids: Lipid composition and role during alcoholic fermentation under enological conditions. *American Journal of Enology and Viticulture*, 70(2):147-154. https://doi.org/10.5344/aj ev.2018.18049.

Casalta, E., Vernhet, A., Sablayrolles, J.M., Tesniere, C. & Salmon, J.M. 2015. Characterization and role of grape solids during alcoholic fermentation under enological conditions. *American Journal of Enology and Viticulture*, 67(2):133-138. https://doi.org /10.5344/ajev.2015.15060.

Casassa, L.F. & Sari, S.E. 2015. Sensory and chemical effects of two alternatives of pre-fermentative cold soak in Malbec wines during winemaking and bottle ageing. *International Journal of Food Science & Technology*, 50(4):1044-1055. https://doi.org/ 10.1111/ijfs.12572.

Casassa, L.F., Bolcato, E.A. & Sari, S.E. 2015. Chemical, chromatic, and sensory attributes of 6 red wines produced with prefermentative cold soak. *Food Chemistry*, 174:110–118. https://doi.org/10.1016/j.foodchem.2014.10.146.

Casassa, L. F., Sari, S. E., Bolcato, E. A. & Fanzone, M. L. 2019. Microwave-assisted extraction applied to Merlot grapes with contrasting maturity levels: effects on phenolic chemistry and wine color. *Fermentation*, 5(1):15. https://doi.org/10.3390/fer mentation5010015.

Casassa, L. F., Gannett, P. A., Steele, N. B. & Huff, R. 2022. Multi-Year Study of the Chemical and Sensory Effects of Microwave-Assisted Extraction of Musts and Stems in Cabernet Sauvignon, Merlot and Syrah Wines from the Central Coast of California. *Molecules*, 27(4):1270. https://doi.org/10.3390/molecules27041270.

Catania, P., Bono, F., De Pasquale, C. & Vallone, M. 2019. Closed tank pneumatic press application to improve Sauvignon Blanc wine quality and nutraceutical properties. *Journal of Agricultural Engineering*, 50(4):159-165. https://doi.org/10.4081/ jae.2019.896.

Cataldo, E., Salvi, L., Paoli, F., Fucile, M. & Mattii, G. B. 2021. Effect of Agronomic Techniques on Aroma Composition of White Grapevines: A Review. *Agronomy*, 11(10):2027. https://doi.org/10.3390/agronomy11102027.

Cerreti, M., Esti, M., Benucci, I., Liburdi, K., De Simone, C. & Ferranti, P. 2015. Evolution of S-cysteinylated and S-glutathionylated thiol precursors during grape ripening of *Vitis vinifera* L. cv. Grechetto, Malvasia del Lazio and Sauvignon blanc. *Australian Journal of Grape and Wine Research*, 21(3):411-416. https://doi.org/ 10.1111/ajgw.12152.

Červinka, L., Burg, P., Soural, I., Mašán, V., Čížková, A., Souček, J., Višacki, V., Ponjičan, O. & Sedlar, A. 2021. Effects of different vinification technologies and yeasts on qualitative parameters and terpene compounds of Sauvignon Blanc wines. *Acta Alimentaria*, 50(4):484-493. https://doi.org/10.1556/066.2021.00045.

Chandrasekaran, S., Ramanathan, S. & Basak, T. 2013. Microwave food processing-A review. *Food Research International*, 52(1):243-261. https://doi.org/10.1 016/j.foodres.2013.02.033.

Chatonnet, P., Dubourdieu, D. & Boidron, J.N. 1995. The influence of Brettanomyces/Dekkera sp. yeasts and lactic acid bacteria on the ethylphenol content of red wines. *American Journal of Enology and Viticulture*, 46(4):463-468. https://doi.org/10.5344/ajev.1995.46.4.463.

Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S. & Abert-Vian, M. 2017. Ultrasound-assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics sonochemistry*, 34:540-560. https://doi.org/10.1016/j.ultsonch.2016.06.035.

Chen, K. & Li, J. 2022. In White Wine Technology. A. Morata (ed.). *A glance into the aroma of white wine*: Academic Press; 313 326. https://doi.org/10.1016/B978-0-12-823497-6.00018-1.

Chen, L., Capone, D.L., Tondini, F.A. & Jeffery, D.W. 2018. Chiral polyfunctional thiols and their conjugated precursors upon winemaking with five *Vitis vinifera* Sauvignon blanc clones. *Journal of Agricultural and Food Chemistry*, 66(18):4674-4282. https://doi.org/10.1021/acs.jafc.8b01806.

Chen, L., Capone, D.L. & Jeffery, D.W. 2019a. Analysis of potent odour-active volatile thiols in foods and beverages with a focus on wine. *Molecules*, 24(13):2472. https://doi.org/10.3390/molecules24132472.

Chen, L., Capone, D.L., Nicholson, E.L. & Jeffery, D.W. 2019b. Investigation of intraregional variation, grape amino acids, and pre-fermentation freezing on varietal thiols and their precursors for *Vitis vinifera* Sauvignon blanc. *Food Chemistry*, 295:637-645. https://doi.org/10.1016/j.foodchem.2019.05.126.

Ciani, M., Comitini, F., Mannassu, I. & Domizio, P. 2010. Controlled mixed culture fermentation: A new perspective on the use of non-*Saccharomyces* yeasts in winemaking. *FEMS Yeast Research*. 10:123-133. https://doi.org/10.1111/j.1567-1364.2009.00579.x.

Clodoveo, M. L., Dipalmo, T., Rizzello, C. G., Corbo, F. & Crupi, P. 2016. Emerging technology to develop novel red winemaking practices: An overview. *Innovative Food Science and Emerging Technologies*, 38:41-56. https://doi.org/10.1016/j.ifset.2016.08.020.

Coetzee, C. 2011. Oxygen and sulfur dioxide additions to Sauvignon blanc: effect on must and wine composition Unpublished Doctoral dissertation, University of Stellenbosch, Stellenbosch.

Coetzee, C. 2018. Grape-derived fruity volatile thiols - Adjusting Sauvignon blanc aroma and flavor complexity. *Wines and Vines*:1-11. https://winebusinessanalytics.com/sections/printout_article.cfm?content=197002&article=feature.

Coetzee, C. 2019. The evolution of Sauvignon blanc's friend (3MH) and foe ($H_2S$) during bottle storage. [Blog, 22 July]. https://sauvignonblanc.com/the-evolution-of-sauvignon-blancs-friend-3mh-and-foe-h2s-during-bottle-storage/ [04 June 2021].

Coetzee, C. 2020a. *Effect of mechanical harvesting on Sauvignon blanc protein content*. [Blog, 21 January]. https://sauvignonblanc.com/effect-of-mechanical-harvesting-on-sauvignon-blanc-protein-content/ [8 June 2021].

Coetzee, C. 2020b. *Hand harvest vs Machine harvesting? The effect on the volatile thiols*. [Blog, 12 February]. https://sauvignonblanc.com/hand-harvest-vs-mechanical-harvesting-the-effect-on-the-volatile-thiols/ [8 June 2021].

Coetzee, C. 2020c. *Yeast and its ability to release thiols.* [Blog, 01 August] https://www.wineland.co.za/yeast–and–its–ability–to–release–thiols/ [5 March 2021].

Coetzee, C. 2021. *Enhancing volatile thiols through ultrasound*. [Blog 01 May]. https://www.wineland.co.za/enhancing-volatile-thiols-through-ultrasound/ [29 July 2021].

Coetzee, C. 2022a. *Cooling Sauvignon blanc grapes before processing - is it worth the effort, time and energy?* [Blog, 01 January]. https://www.wineland.co.za/cooling-sauvignon-blanc-grapes-before-processing-is-it-worth-the-effort-time-and-energy/ [17 May 2022].

Coetzee, C. 2022b. Thiol-forming yeasts – it's all in the genes [Blog, 01 January]. https://sauvignonblanc.com/thiol-forming-yeasts-its-all-in-the-genes/ [25 May 2024].

Coetzee, C. & du Toit, W.J. 2012. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. *Food Research International*, 45:287-298. https://doi.org/10.1016/j.foodres.2011.09.017.

Coetzee, C., Schulze, A., Mokwena, L., Du Toit, W. J. & Buica, A. 2018. Investigation of thiol levels in young commercial South African Sauvignon Blanc and Chenin Blanc wines using propiolate derivatization and GC-MS/MS. *South African Journal of Enology and Viticulture*, 39(2):180-184. http://dx.doi.org/10.21548/39-2-2683.

Comuzzo, P., Marconi, M., Zanella, G. & Querzè, M. 2018. Pulsed electric field processing of white grapes (cv. Garganega): Effects on wine composition and volatile compounds. *Food Chemistry*, 264:16-23. https://doi.org/10.1016/j.foodchem.2018.04.116.

Comuzzo, P., Voce, S., Grazioli, C., Tubaro, F., Marconi, M., Zanella, G. & Querzè, M. 2020. Pulsed electric field processing of red grapes (cv. Rondinella): modifications of phenolic fraction and effects on wine evolution. *Foods*, 9(4):1-15.. https://doi.org/10.3390/foods9040414.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S. & Gerós, H. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. *Food*, 1(1):1-22. https://hdl.handle.net/1822/6820.

Creasy, G.L. & Creasy, L.L. 2002. Grape-derived wine flavonoids and stilbenes. In Sandler, M. & Pinder, R. (eds.), *In Wine: A Scientific Exploration*. London: CRC Press; 199-227.

Cosme, F., Gonçalves, B., Inês, A., Jordão, A. M. & Vilela, A., 2016. Grape and wine metabolites: Biotechnological approaches to improve wine quality. *Grape and wine biotechnology*, 187-214. http://dx.doi.org/10.5772/64822.

Dankó, T., Szelényi, M., Janda, T., Molnár, B. P. & Pogány, M. 2021. Distinct volatile signatures of bunch rot and noble rot. *Physiological and Molecular Plant Pathology*, 114:101626. https://doi.org/10.1016/j.pmpp.2021.101626.

Darriet, P., Tominaga, T., Lavigne, V., Boidron, J.N. & Dubourdieu, D. 1995. Identification of a powerful aromatic component of Vitis vinifera L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour and Fragrance Journal*, 10(6):385-392. https://doi.org/10.1002/ffj.2730100610.

Darias-Martín, J., Díaz-González, D. & Díaz-Romero, C. 2004. Influence of two pressing processes on the quality of must in white wine production. *Journal of Food Engineering*, 63(3):335-340. https://doi.org/10.1016/j.jfoodeng.2003.08.005.

Das, K., Zhang, M., Bhandari, B., Chen, H., Bai, B. & Roy, M. C. 2022. Ultrasound generation and ultrasonic application on fresh food freezing: Effects on freezing parameters, physicochemical properties and final quality of frozen foods. *Food Reviews International*:1-31. https://doi.org/10.1080/87559129.2022.2027436.

Day, M. P., Schmidt, S. A., Pearson, W., Kolouchova, R. & Smith, P. A. 2019. Effect of passive oxygen exposure during pressing and handling on the chemical and sensory attributes of Chardonnay wine. *Australian Journal of Grape and Wine Research*, 25(2):185-200. https://doi.org/10.1111/ajgw.12384.

Dempsey, P. & Bansal, P. 2012. The art of air blast freezing: Design and efficiency considerations. *Applied Thermal Engineering*, 41:71-83. https://doi.org/10.1016/j.applthermaleng.2011.12.013.

De Santis, D. & Frangipane, M. T. 2010. Effect of pre-fermentative cold maceration on the aroma and phenolic profiles of a Merlot red wine. *Italian Journal of Food Science*, 22(1):47. ISSN: 1120-1770.

Del Caro, A., Piombino, P., Genovese, A., Moio, L., Fanara, C. & Piga, A. 2014. Effect of bottle storage on colour, phenolics and volatile composition of Malvasia and Moscato white wines. *South African Journal of Enology and Viticulture*, 35(1):128-138. https://doi.org/10.21548/35-1-992.

Delso, C., Martínez, J.M., Aguilar-Machado, D., Maza, M., Morata, A., Álvarez, I. & Raso, J. 2022. Use of pulsed electric fields in white grape processing. In White Wine Technology (pp. 61-71). Academic Press. https://doi.org/10.1016/B978-0-12-823497-6.00005-3

Des Gachons, C.P., Tominaga, T. & Dubourdieu, D. 2002. Localization of S-cysteine conjugates in the berry: Effect of skin contact on aromatic potential of *Vitis vinifera* L. cv. Sauvignon blanc must. *American Journal of Enology and Viticulture*, 53(2):144-146. https://doi.org/10.5344/ajev.2002.53.2.144.

De Villiers, A., Alberts, P., Tredoux, A.G. & Nieuwoudt, H.H. 2012. Analytical techniques for wine analysis: An African perspective; a review. *Analytica Chimica Acta*, 730:2-23. https://doi.org/10.1016/j.aca.2011.11.064.

Dias Araujo, L., Vannevel, S., Buica, A., Callerot, S., Fedrizzi, B., Kilmartin, P.A. & du Toit, W.J. 2017. Indications of the prominent role of elemental sulfur in the formation of the varietal thiol 3-mercaptohexanol in Sauvignon blanc wine. *Food Research International*, 98:79-86. https://doi.org/10.1016/j.foodres.2016.12.023.

Dimopoulou, M., Troianou, V., Toumpeki, C., Gosselin, Y., Dorignac, É. & Kotseridis, Y. 2020. Effect of strains from different Saccharomyces species used in different inoculation schemes on chemical composition and sensory characteristics of Sauvignon blanc wine. *OENO One*, 54(4):745-759. https://doi.org/10.20870/oeno-one.2020.54.4.3240.

Deshpande, S.S., Cheryan, M., Sathe, S.K., Salunkhe, D.K. & Luh, B.S. 1984. Freeze concentration of fruit juices. *Critical Reviews in Food Science & Nutrition*, 20(3):173-248. https://doi.org/10.1080/10408398409527389.

Dournes, G., Verbaere, A., Lopez, F., Dufourcq, T., Mouret, J.R. & Roland, A. 2022. First characterisation of thiol precursors in Colombard and Gros Manseng: comparison of two cultivation practices. *Australian Journal of Grape and Wine Research*, 28(3):492-499. https://doi.org/10.1111/ajgw.12547.

Dubourdieu, D. & Tominaga, T. 2009. Polyfunctional thiol compounds. In *Wine Chemistry and Biochemistry*:275-293. Springer, New York, NY.

Dubourdieu, D., Tominaga, T., Masneuf, I., des Gachons, C.P. & Murat, M.L. 2006. The role of yeasts in grape flavor development during fermentation: the example of Sauvignon blanc. *American Journal of Enology and Viticulture*, 57(1):81-88. https://doi.org/10.5344/ajev.2006.57.1.81.

Du Plessis, C.S. & Augustyn, O.P.H. 1981. Initial study on the guava aroma of Chenin blanc and Colombar wines. *South African Journal of Enology and Viticulture*, 2(2):101-103. https://doi.org/10.21548/2-2-2401.

Dzedze, N., Van Breda, V., Hart, R.S. & Van Wyk, J. 2019. Wine chemical, sensory, aroma compound and protein analysis of wines produced from chemical and biological fungicide treated Chenin blanc grapes. *Food Control*, 105:265-276. https://doi.org/10.1016/j.foodcont.2019.06.007.

Ebeler, S.E. & Thorngate, J.H. 2009. Wine chemistry and flavor: looking into the crystal glass. *Journal of Agricultural and Food Chemistry*, 57(18):8098-8108. https://doi.org/10.1021/jf9000555.

Esti, M. & Tamborra, P. 2006. Influence of winemaking techniques on aroma precursors. *Analytica Chimica Acta*, 563(1-2):173-179. https://doi.org/10.1016/j.aca.2005.12.025

Fanzone, M., Coronado, I., Sari, S., Catania, A., Cortiella, M. G., Assof, M., Jofré, V., Ubeda, C. & Peña-Neira, A. 2022. Microwave-assisted maceration and stems addition in Bonarda grapes: Effects on wine chemical composition over two vintages. *Food Research International*, 156:111169. https://doi.org/10.1016/j.foodres.2022.111169.

Feng, Y., Yang, T., Zhang, Y., Zhang, A., Gai, L. & Niu, D. 2022. Potential applications of pulsed electric field in the fermented wine industry. *Frontiers in Nutrition*, 9:1-15. https://doi.org/10.3389/fnut.2022.1048632.

Ferraretto, P., Cacciola, V., Batllo, I. F. & Celotti, E. 2013. Ultrasounds application in winemaking: grape maceration and yeast lysis. *Italian Journal of Food Science*, 25(2):160-168. ISSN:1120-1770.

Ferraretto, P. & Celotti, E. 2016. Preliminary study of the effects of ultrasound on red wine polyphenols. *CyTA-Journal of Food*, 14(4):529-535. https://doi.org/10.1080/19476337.2016.1149520.

Ferreira, V. & Lopez, R., 2019. The actual and potential aroma of winemaking grapes. *Biomolecules*, 9(12):818. https://doi.org/10.3390/biom9120818.

Ferreira-Lima, N.E., Burin, V.M., Caliari, V. & Bordignon-Luiz, M.T. 2016. Impact of pressing conditions on the phenolic composition, radical scavenging activity and glutathione content of Brazilian Vitis vinifera white wines and evolution during bottle ageing. *Food and Bioprocess Technology*, 9:944-957. https://doi.org/10.1016/0950-3293(94)P4210-W.

Fracassetti, D., Stuknytė, M., La Rosa, C., Gabrielli, M., De Noni, I. & Tirelli, A. 2018. Thiol precursors in Catarratto Bianco Comune and Grillo grapes and effect of clarification conditions on the release of varietal thiols in wine. *Australian Journal of Grape and Wine Research*, 24(1):125-133. https://doi.org/10.1111/ajgw.12311.

Fracassetti, D., Camoni, D., Montresor, L., Bodon, R. & Limbo, S. 2020. Chemical characterization and volatile profile of Trebbiano di Lugana wine: A case study. *Foods*, 9(7):956. https://doi.org/10.3390/foods9070956.

Fracassetti, D., De Noni, I., Stuknytė, M., Pica, V. & Tirelli, A. 2021. Influence of pre-fermentative steps on varietal thiol precursors. *Internet Journal of Viticulture and Enology*, 9:1-7. https://hdl.handle.net/2434/903504.

Fragasso, M., Antonacci, D., Pati, S., La Gatta, B., La Gatta, M., Coletta, A. & La Notte, E., 2010. Pre-fermentative cold maceration for the aroma enhancement of Aglianico and Montepulciano wines. In *33rd World Congress of Vine and Wine. 8th General Assembly of the OIV* , 20-25.

Fretz, C.B., Luisier, J.L., Tominaga, T. & Amadò, R. 2005. 3-Mercaptohexanol: An aroma impact compound of Petite Arvine wine. *American Journal of Enology and Viticulture*, 56(4):407-410. https://doi.org/10.5344/ajev.2005.56.4.407.

Gil, M., Louazil, P., Iturmendi, N., Moine, V., Cheynier, V. & Saucier, C. 2019. Effect of polyvinylpolypyrrolidone treatment on rosés wines during fermentation: Impact on color, polyphenols and thiol aromas. *Food Chemistry,* 295:493-498. https://doi.org/10. 1016/j.foodchem.2019.05.125.

Glória, M.B.A. & Vieira, S.M. 2007. Technological and toxicological significance of bioactive amines in grapes and wines. *Food, Global Science Books, Reino Unido*, 1(2):258-270.

Ghantous, G. 2016. Cultivation of Malbec in Lebanon. Unpublished Doctoral dissertation, Lebanese University, Beirut, Lebanon.

Gómez-Plaza, E., Pérez-Prieto, L. J., Pérez-Porras, P. & Bautista-Ortín, A. B., 2022. Ultrasound to process white grapes. In *White Wine Technology* (Chapter 7) (pp. 73-85). Academic Press. https://doi.org/10.1016/B978-0-12-823497-6.00002-8.

González-Barreiro, C., Rial-Otero, R., Cancho-Grande, B. & Simal-Gándara, J. 2015. Wine aroma compounds in grapes: A critical review. *Critical Reviews in Food Science and Nutrition*, 55(2):202-218. https://doi.org/10.1080/10408398.2011.650336.

Greyling, I. 2019. Extraction and bioconversion of aroma impact compounds from Sauvignon Blanc grapes to wine matrices during white wine production. Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch.

Guerrini, L., Corti, F., Angeloni, G., Masella, P., Spadi, A., Calamai, L. & Parenti, A. 2022. The Effects of Destemming/Crushing and Pressing Conditions in Rosé Wine Production. *Australian Journal of Grape and Wine Research*, (1):1-14. https://doi.org/10.1155/2022/9853264.

Guth, H. 1997a. Identification of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry*, 45(8):3022-3026. https://doi.org/10.1021/jf9608433.

Guth, H. 1997b. Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry*, 45(8):3027-3032. https://doi.org/10.1021/jf970280a.

Hart, R.S., Jolly, N.P. & Ndimba, B.K. 2019. Characterisation of hybrid yeasts for the production of varietal Sauvignon blanc wine–A review. *Journal of Microbiological Methods*, 165:105699. https://doi.org/10.1016/j.mimet.2019.105699.

Hart, R. S., Ndimba, B. K., & Jolly, N. P. 2017. Characterisation of thiol-releasing and lower volatile acidity-forming intra-genus hybrid yeast strains for Sauvignon blanc wine. *South African Journal of Enology and Viticulture*, 38(2):44-155. https://doi.org/10.21548/38-2-1322.

Hart, R.S., Jolly, N.P. Mohamed, G. Booyse, M. & Ndimba, B.K. 2016. Characterisation of Saccharomyces cerevisiae hybrids selected for low volatile acidity formation and the production of aromatic Sauvignon blanc wine. *African Journal of Biotechnology*, 15(38):2068-2081. https://doi.org/10.5897/AJB2016.15388.

Herbst-Johnstone, M., Piano, F., Duhamel, N., Barker, D. & Fedrizzi, B. 2013. Ethyl propiolate derivatisation for the analysis of varietal thiols in wine. *Journal of Chromatography A*, 1312:104-110. https://doi.org/10.1016/j.chroma.2013.08.066.

Heydarov, E. E., Mammadov, B. A., Fataliyev, H. K., Alekberov, A. M., Qadimova, N. S. & İmanova, K. F. 2020. Substantiation of Cryoprocessing regimes of white and red wine materials. *Vitivinícola*,*35*(5), 40-48. ISSN:2416-3953.

Hjelmeland, A. K. & Ebeler, S. E. 2015. Glycosidically bound volatile aroma compounds in grapes and wine: a review. *American Journal of Enology and Viticulture*, 66(1):1-11. https://doi.org/10.5344/ajev.2014.14104.

Howell K.S., Swiegers J.H., Elsey G.M., Siebert T.E., Bartowsky E.J., Fleet G.H., Pretorius I.S. & de Barros Lopes M.A. 2004. Variation in 4-mercapto-4-methyl-pentan-2-one release by *Saccharomyces cerevisiae* commercial wine strains. *FEMS Microbiology Letters*., 240(2):125-9. https://doi.org/10.1016/j.femsle.2004.09.022.

International Organisation of Vine and Wine (OIV) 2019. Resolution OIV-OENO 616 2019; Geneva, Switzerland: OIV.

Jagatić Korenika, A. M. J., Maslov, L., Jakobović, S., Palčić, I. & Jeromel, A. 2019. Comparative study of aromatic and polyphenolic profiles of Croatian white wines produced by cold maceration. *Czech Journal of Food Sciences*, 36(6):459-469. https:/ /doi.org/10.17221/448/2017-CJFS.

Jackson, R.S. 2008. Introduction. In Jackson, R.S. (ed.). *Wine science: principles and applications*. 3rd ed. Elsevier Academic Press, 1-13.

Jeffery, D.W. 2016. Spotlight on varietal thiols and precursors in grapes and wines. *Australian Journal of Chemistry*, 69:1323-1330. https://doi.org/10.1071/CH16296.

Jouanneau, S., 2011. Survey of aroma compounds in Marlborough Sauvignon blanc wines-Regionality and small-scale winemaking. Unpublished Doctoral dissertation, The University of Auckland, Auckland, New Zealand.

Jouanneau, S., Weaver, R.J., Nicolau, L., Herbst-Johnstone, M., Benkwitz, F. & Kilmartin, P.A. 2012. Subregional survey of aroma compounds in Marlborough Sauvignon Blanc wines. *Australian Journal of Grape and Wine Research*, 18(3):329-343. https://doi.org/10.1111/j.1755-0238.2012.00202.x.

Jussier, D., Morneau, A.D. & de Orduna, R.M. 2006. Effect of simultaneous inoculation with yeast and bacteria on fermentation kinetics and key wine parameters of cool-climate Chardonnay. *Applied and Environmental Microbiology*, 72(1):221-227. https:// doi.org/10.1128/AEM.72.1.221-227.2006.

Karabagias, I. K., Sykalia, D., Mannu, A. & Badeka, A. V., 2020.Physico-chemical parameters complemented with aroma compounds fired up the varietal discrimination of wine using statistics. *European Food Research and Technology*, 246(11):2233–2248. https://doi.org/10.1007/s00217-020-03568-y.

Kapaklis, A. 2014. Impact of specific volatile thiols on varietal aroma of wines produced from Greek and some international grape varieties. Unpublished Doctoral dissertation, Institute for Phytopathology, Justus Liebig University of for Phytopathology, Justus Liebig University of Giessen.

Kelly, J. M., Inglis, D. L. & Pickering, G. J. 2020. Sensorial and Volatile Analysis of Wines Made from Partially Dehydrated Grapes: An Ontario Case Study. *Journal of Food Quality*, (1):1-12. https://doi.org/10.1155/2020/8861185.

Kemp, B., Botezatu, A., Charnock, H., Inglis, D., Marchal, R., Pickering, G., Yang, F. & Willwerth, J. 2022. White winemaking in cold climates. In *White Wine Technology* (Chapter 26) (pp. 339-354). Academic Press. https://doi.org/10.1016/B978-0-12-823497-6.00007-7.

Keyzers, R.A. & Boss, P.K. 2010. Changes in the volatile compound production of fermentations made from musts with increasing grape content. *Journal of Agricultural and Food Chemistry*, *58*(2):1153-1164. https://doi.org/10.1021/jf9023646.

Kilmartin, P. 2012. Machine harvesting versus handpicking: impacts on tropical and green characters in Sauvignon blanc wines. *The Australian and New Zealand Grapegrower and Winemaker*:81-86.

King, E.S., Osidacz, P., Curtin, C., Bastian, S.E.P. & Francis, I.L. 2011. Assessing desirable levels of sensory properties in Sauvignon blanc wines–consumer preferences and contribution of key aroma compounds. *Australian Journal of Grape and Wine Research*, 17(2):169-180. https://doi.org/10.1111/j.1755-0238.2011.00133.x.

Koch A, Doyle C, Matthews M, Williams L. & Ebeler S. 2010. 2-Methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype. *Phytochemistry*, 71:2190-2198. https://doi.org/10.1016/j.phytochem.2010.09.006.

Korenika, A. M., Prusina, T. & IviÄ, S. 2020. Influence of cold maceration treatment on aromatic and sensory properties of Vugava wine (*Vitis vinifera* L.). *Journal of*

*Microbiology, Biotechnology and Food Sciences*, 10(1):49-53. https://doi.org/10.15414/jmbfs.2020.10.1.49-53.

Kotseridis, Y.S., Spink, M., Brindle, I.D., Blake, A.J., Sears, M., Chen, X., Soleas, G., Inglis, D. & Pickering, G.J. 2008. Quantitative analysis of 3-alkyl-2-methoxypyrazines in juice and wine using stable isotope labelled internal standard assay. *Journal of Chromatography A*, *1190*(1-2):294-301. https://doi.org/10.1016/j.chroma.2008.02.088.

Kuhn, N., Guan, L., Dai, Z. W., Wu, B. H., Lauvergeat, V., Gomès, E. & Delrot, S., 2013. Berry ripening: recently heard through the grapevine. *Journal of Experimental Botany*, *65*(16), 4543-4559. https://doi.org/10.1093/jxb/ert395.

Künstler, A., Gullner, G., Ádám, A.L., Kolozsváriné Nagy, J. & Király, L. 2020. The versatile roles of sulfur-containing biomolecules in plant defense-A road to disease resistance. *Plants*, 9(12):1705. https://doi.org/10.3390/plants9121705.

Lacey, M.J., Allen, M.S., Harris, R.L. & Brown, W.V. 1991. Methoxypyrazines in Sauvignon blanc grapes and wines. *American Journal of Enology and Viticulture*, 42(2):103-108. https://doi.org/10.5344/ajev.1991.42.2.103.

Lacroux, F., Trégoat, O., Van Leeuwen, C., Pons, A., Tominaga, T., Lavigne-Cruege, V. and Dubourdieu, D. 2008. Effect of foliar nitrogen and sulphur application on aromatic expression of Vitis vinifera L. cv. Sauvignon blanc. *Oeno One*, 42(3):125-132. https://doi.org/10.20870/oeno-one.2008.42.3.816.

Larcher, R., Tonidandel, L., Nicolini, G. & Fedrizzi, B. 2013. First evidence of the presence of S-cysteinylated and S-glutathionylated precursors in tannins. *Food Chemistry*, 141(2):1196-1202. https://doi.org/10.1016/j.foodchem.2013.04.037.

Lan, Y., Xiang, X., Qian, X., Wang, J., Ling, M., Zhu, B., Liu, T., Sun, L., Shi, Y., Reynolds, A.G. & Duan, C. 2019. Characterization and differentiation of key odor-active compounds of'Beibinghong' icewine and dry wine by gas chromatography-olfactometry and aroma reconstitution. *Food Chemistry*, 287:186-196. https://doi.org/10.1016/j.foo dchem.2019.02.074.

Lasanta, C., Cejudo, C., Gómez, J. & Caro, I. 2023. Influence of pre-fermentative cold maceration on the chemical and sensory properties of red wines produced in warm climates. *Processes*, 11(2):374. https://doi.org/10.3390/pr11020374.

Lawrence, N. 2012. Volatile metabolic profiling of SA Chenin blanc fresh and fruity and rich and ripe wine styles: Development of analytical methods for flavour compounds (aroma and flavour) and application of chemometrics for resolution of complex analytical measurements. Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch.

Lei, Y., Xie, S., Guan, X., Song, C., Zhang, Z. & Meng, J. 2018. Methoxypyrazines biosynthesis and metabolism in grape: A review. *Food Chemistry*, 245:1141-1147. htt ps://doi.org/10.1016/j.foodchem.2017.11.056.

Leong, S.Y., Rozhkova, A., Oey, I., Weinert, C.H., Egert, B., Mayer-Miebach, E., Greiner, R., Kulling, S.E., Hofmann, R.W. & Burritt, D.J. 2016. Effect of Pulsed Electric Field-assisted vinification on New Zealand Sauvignon Blanc grapes: Using GCxGC-qMS analysis as an untargeted global metabolomics approach. Paper presented at *Max Rubner Conference 2016 – Karlsruhe, Germany*: *Food Metabolomics*. https://agris.fao.org/search/en/providers/125097/records/67a0b71620 478411b0265487. [17 September 2025].

Liu, J., Zhu, X. L., Ullah, N. & Tao, Y. S. 2017. Aroma glycosides in grapes and wine. *Journal of Food Science*, 82(2):248-259. https://doi.org/10.1016/j.foodchem.2017.11. 056.

López, R., Ortín, N., Pérez-Trujillo, J.P., Cacho, J. & Ferreira, V. 2003. Impact odorants of different young white wines from the Canary Islands. *Journal of Agricultural and Food Chemistry*, 51(11):3419-3425. https://doi.org/10.1021/jf026045w.

López-Giral, N., López, R., Santamaría, P. 2023. Phenolic and colour characteristics of must and wine obtained from red grapes treated by pulsed electric fields. Efficacy of PEF to reduce maceration time in elaboration of red wines. *European Food Research and Technology*, 249:273-282). https://doi.org/10.1007/s00217-022-04114-8.

Lukić, I., Budić-Leto, I., Bubola, M., Damijanić, K. & Staver, M. 2017. Pre-fermentative cold maceration, saignée, and various thermal treatments as options for modulating volatile aroma and phenol profiles of red wine. *Food Chemistry*, 224:251-261. https://doi.org/10.1016/j.foodchem.2016.12.077.

Lund, C.M., Jones, V.S. & Spanitz, S. 2009a. Effects and influences of motivation on trained panelists. *Food Quality and Preference*, 20(4):295-303. https://doi.org/10.1016/j.foodqual.2009.01.004.

Lund, C.M., Nicolau, L., Gardner, R.C. & Kilmartin, P.A. 2009b. Effect of polyphenols on the perception of key aroma compounds from Sauvignon blanc wine. *Australian Journal of Grape and Wine Research*, 15(1):18-26. https://doi.org/10.1111/j.1755-0238.2008.00028.x.

Lund, C.M., Thompson, M.K., Benkwitz, F., Wohler, M.W., Triggs, C.M., Gardner, R., Heymann, H. & Nicolau, L. 2009c. New Zealand Sauvignon blanc distinct flavor characteristics: Sensory, chemical, and consumer aspects. *American Journal of Enology and Viticulture*, 60(1):1-12. https://doi.org/10.5344/ajev.2009.60.1.1.

Ma, T.Z., Gong, P.F., Lu, R.R., Zhang, B., Morata, A. & Han, S.Y. 2020. Effect of different clarification treatments on the volatile composition and aromatic attributes of ‘Italian Riesling’icewine. *Molecules*, *25*(11):2657. https://doi.org/10.3390/molecules25112657.

Mafata, M., Stander, M.A., Thomachot, B. & Buica, A. 2018. Measuring thiols in single cultivar South African red wines using 4, 4-dithiodipyridine (DTDP) derivatization and ultraperformance convergence chromatography-tandem mass spectrometry. *Foods*, 7 (9):138. https://doi.org/10.3390/foods7090138.

Maggu, M., Winz, R., Kilmartin, P.A., Trought, M.C. & Nicolau, L. 2007. Effect of skin contact and pressure on the composition of Sauvignon Blanc must. *Journal of Agricultural and Food Chemistry*, 55(25):10281-10288. https://doi.org/10.1021/jf072192o.

Magyar, I. & Soos, J. 2016. Botrytized wines–current perspectives. *International Journal of Wine Research*,8*:*29-39. https://doi.org/10.2147/IJWR.S100653.

Makhotkina, O., Herbst-Johnstone, M., Logan, G., du Toit, W. & Kilmartin, P.A. 2013. Influence of sulfur dioxide additions at harvest on polyphenols, C6-compounds, and varietal thiols in Sauvignon blanc. *American Journal of Enology and Viticulture*, 64(2):203-213. https://doi.org/10.5344/ajev.2012.12094.

Malićanin, M., Danilović, B., Stamenković Stojanović, S., Cvetković, D., Lazić, M., Karabegović, I. & Savić, D. 2022. Pre-Fermentative Cold Maceration and Native Non-*saccharomyces* Yeasts as a Tool to Enhance Aroma and Sensory Attributes of Chardonnay Wine. *Horticulturae*, 8(3):212. https://doi.org/10.3390/horticulturae80302 12.

Matthews, A., Grimaldi, A., Walker, M., Bartowsky, E., Grbin, P. & Jiranek, V. 2004. Lactic Acid Bacteria as a Potential Source. *Applied and Environmental Microbiology*, 57:15-5731. https://doi.org/10.1128/AEM.70.10.5715-5731.2004.

Marais, J. 1994. Sauvignon blanc cultivar aroma-a review. *South African Journal of Enology and Viticulture*, 15(2):41-45.

Marais, J. 1998. Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. *South African Journal of Enology and Viticulture*, 19(1):10-16.

Marais, J. 2001. Effect of grape temperature and yeast strain on Sauvignon blanc wine aroma composition and quality. *South African Journal of Enology and Viticulture*, 22(1):47-50. https://doi.org/10.21548/22-1-2168.

Martín, J. F. G. & Sun, D. W. 2013. Ultrasound and electric fields as novel techniques for assisting the wine ageing process: The state-of-the-art research. *Trends in Food Science and Technology*, 33(1):40-53. https://doi.org/10.1016/j.tifs.2013.06.005.

Maza, M.A., Martínez, J.M., Cebrián, G., Sánchez-Gimeno, A.C., Camargo, A., Álvarez, I. & Raso, J. 2020. Evolution of polyphenolic compounds and sensory properties of wines obtained from grenache grapes treated by pulsed electric fields during aging in bottles and in oak barrels. *Foods*,9(5):1-14. https://doi:10.3390/foods9 050542.

Mele, M. A., Kang, H. M., Lee, Y. T. & Islam, M. Z. 2021. Grape terpenoids: Flavor importance, genetic regulation, and future potential. *Critical Reviews in Food Science and Nutrition*, 61(9):1429-1447. https://doi.org/10.1080/10408398.2020.1760203.

Mencarelli, F. & Bellincontro, A. 2020. Recent advances in postharvest technology of the wine grape to improve the wine aroma. *Journal of the Science of Food and Agriculture*, 100(14):5046-5055. https://doi.org/10.1002/jsfa.8910.

Mierczynska-Vasilev, A. & Smith, P.A. 2015. Current state of knowledge and challenges in wine clarification. *Australian Journal of Grape and Wine Research*, 21:615-626. https://doi.org/10.1111/ajgw.12198.

Mihnea, M., González-SanJosé, M. L., Ortega-Heras, M. & Pérez-Magariño, S. 2015. A comparative study of the volatile content of Mencía wines obtained using different pre-fermentative maceration techniques. *LWT-Food Science and Technology*, 64(1):32-41. https://doi.org/10.1016/j.lwt.2015.05.024.

Miyawaki, O., Kato, S. & Watabe, K. 2012. Yield improvement in progressive freeze-concentration by partial melting of ice. *Journal of Food Engineering*, 108 (2012):377-382. https://doi.org/10.1016/j.jfoodeng.2011.09.013.

Molina, A.M., Guadalupe, V., Varela, C., Swiegers, J.H., Pretorius, I.S. & Agosin, E. 2009. Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. *Food Chemistry*, 117(2):189-195. https://doi.org/10.1016/j.foodchem.2009.03.116.

Moreno-Pérez, A., Vila-López, R., Fernández-Fernández, J. I., Martínez-Cutillas, A. & Gil-Muñoz, R. 2013. Influence of cold pre-fermentation treatments on the major volatile compounds of three wine varieties. *Food Chemistry*, 139(1-4):770-776. https://doi.org/10.1016/j.foodchem.2013.01.052.

Mozzon, M., Savini, S., Boselli, E. & Thorngate, J.H. 2016. The herbaceous character of wines. *Italian Journal of Food Science*, 28(2):190-207. https://doi: 10.14674/1120-1770/IJFS.V304.

Muñoz García, R., Oliver Simancas, R., Díaz-Maroto, M. C., Alañón Pardo, M. E. & Pérez-Coello, M. S. 2021. Effect of microwave maceration and $SO_2$ free vinification on volatile composition of red wines. *Foods*, 10(6):1164. https://doi.org/10.3390/foods10061164.

Muñoz García, R., Oliver-Simancas, R., Arévalo Villena, M., Martínez-Lapuente, L., Ayestarán, B., Marchante-Cuevas, L., Díaz-Maroto, M. C. & Pérez-Coello, M. S. 2022. Use of Microwave Maceration in Red Winemaking: Effect on Fermentation and Chemical Composition of Red Wines. *Molecules,* 27(9):3018. https://doi.org/10.3390/molecules27093018.

Murat, M.L., Masneuf, I., Darriet, P., Lavigne, V., Tominaga, T. & Dubourdieu, D. 2001. Effect of *Saccharomyces cerevisiae* yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. *American Journal of Enology and Viticulture*, 52(2):136-139. https://doi.org/10.5344/ajev.2001.52.2.136.

Naranjo, A., Martínez-Lapuente, L., Ayestarán, B., Guadalupe, Z., Pérez, I., Canals, C. & Adell, E. 2021. Aromatic and sensory characterization of Maturana Blanca wines made with different technologies. *Beverages*, 7(1):10. https://doi.org/10.3390/beverag es7010010.

Naviglio, D., Formato, A., Scaglione, G., Montesano, D., Pellegrino, A., Villecco, F. & Gallo, M., 2018. Study of the grape cryo-maceration process at different temperatures. *Foods*, 7(7):107. https://doi.org/10.3390/foods7070107.

Neethling, E., Petitjean, T., Quénol, H. & Barbeau, G. 2017. Assessing local climate vulnerability and winegrowers' adaptive processes in the context of climate change. *Mitigation and Adaptation Strategies for Global Change*, 22(5):777-803. https://doi.org/10.1007/s11027-015-9698-0.

Nicolini, G., Moser, S., Roman, T., Mazzi, E. & Larcher, R. 2011. Effect of juice turbidity on fermentative volatile compounds in white wines. *Vitis*, 50(3):131-135. https://doi.org/10.5073/vitis.2011.50.131-135.

October, F.M. 2020. Effect of yeasts and oenological parameters on acetaldehyde production during alcoholic fermentation of South African grape musts. Unpublished thesis, Stellenbosch University, Stellenbosch. https://scholar.sun.ac.za.

Olarte Mantilla, S. M., Collins, C., Iland, P. G., Kidman, C. M., Jordans, C. & Bastian, S. E. P., 2013. Comparison of sensory attributes of fresh and frozen wine grape berries using Berry Sensory Assessment. *Australian Journal of Grape and Wine Research*, *19*(3), 349-357. https://doi.org/10.1111/ajgw.12041.

Olejar, K.J., Fedrizzi, B. & Kilmartin, P.A. 2015a. Influence of harvesting technique and maceration process on aroma and phenolic attributes of Sauvignon blanc wine. *Food Chemistry*, 183:181-189. https://doi.org/10.1016/j.foodchem.2015.03.040.

Olejar, K.J., Fedrizzi, B. & Kilmartin, P.A. 2015b. Antioxidant activity and phenolic profiles of Sauvignon blanc wines made by various maceration techniques. *Australian*

*Journal of Grape and Wine Research*, 21(1):57-68. https://doi.org/10.1111/ajgw.12119.

Oliver Simancas, R., Díaz-Maroto, M. C., Alañón Pardo, M. E., Pérez Porras, P., Bautista-Ortín, A. B., Gómez-Plaza, E. & Pérez-Coello, M. S. 2021. Effect of power ultrasound treatment on free and glycosidically-bound volatile compounds and the sensorial profile of red wines. *Molecules*, 26(4):1193. https://doi.org/10.3390/molecules26041193.

Ouellet, É. & Pedneault, K. 2016. Impact of frozen storage on the free volatile compound profile of grape berries. *American Journal of Enology and Viticulture*, 67(2):239-244. https://doi.org/10.5344/ajev.2015.15087.

Ozturk, B. & Anli, E. 2017. Pulsed electric fields (PEF) applications on wine production: A review. In BIO Web of Conferences (Vol. 9, p. 02008). *EDP Sciences*. https://doi.org/10.1051/bioconf/20170902008.

Parenti, A., Spugnoli, P., Masella, P., Guerrini, L., Benedettelli, S. & Di Blasi, S. 2015. Comparison of grape harvesting and sorting methods on factors affecting the must quality. *Journal of Agricultural Engineering*, 46(1):19-22. https://hdl.handle.net/2158/1001550.

Parish–Virtue, K., Herbst–Johnstone, M., Bouda, F., Fedrizzi, B., Deed, R.C. & Kilmartin, P. A. 2021. Aroma and Sensory Profiles of Sauvignon Blanc Wines from Commercially Produced Free Run and Pressed Juices. *Beverages*, 7(2):29. https://doi.org/10.3390/beverages7020029.

Parr, W.V., Schlich, P., Theobald, J.C. & Harsch, M.J. 2013. Association of selected viniviticultural factors with sensory and chemical characteristics of New Zealand Sauvignon blanc wines. *Food Research International*, 534:64-475. https://doi.org/10.1016/j.foodres.2013.05.028.

Pedrosa-López, M. D. C., Aragón-García, F., Ruíz-Rodríguez, A., Piñeiro, Z., Durán-Guerrero, E. & Palma, M. 2022. Effects from the freezing of either whole or crushed grapes on the volatile compounds contents in Muscat Wines. *Foods*, 11(12): 1782. https://doi.org/10.3390/foods11121782.

Peinado, R.A., Moreno, J., Bueno, J.E., Moreno, J.A. & Mauricio, J.C. 2004. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chemistry*, 84(4):585-590. https://doi.org/10.1016/S0308-8146(03)00282-6.

Pérez-Coello, M.S., González-Viñas, M.A., Garcıa-Romero, E., Dıaz-Maroto, M.C. & Cabezudo, M.D. 2003. Influence of storage temperature on the volatile compounds of young white wines. *Food Control*, 14(5):301-306. https://doi.org/10.1016/S0956-7135(02)00094-4.

Petropulos, V.I., Bogeva, E., Stafilov, T., Stefova, M., Siegmund, B., Pabi, N. & Lankmayr, E. 2014. Study of the influence of maceration time and oenological practices on the aroma profile of Vranec wines. *Food Chemistry*, 165:506-514. https://doi.org/10.1016/j.foodchem.2014.05.144.

Petzold, G., Niranjan, K. & Aguilera, J. M., 2013. Vacuum-assisted freeze concentration of sucrose solutions. *Journal of Food Engineering*, *115*(3), 357-361. https://doi.org/10.1016/j.jfoodeng.2012.10.048.

Petzold, G., Moreno, J., Lastra, P., Rojas, K. & Orellana, P., 2015. Block freeze concentration assisted by centrifugation applied to blueberry and pineapple juices. *Innovative Food Science and Emerging Technologies, 30*, 192-197. https://doi.org/10.1016/j.ifset.2015.03.007.

Peyrot des Gachons, C., Tominaga, T. & Dubourdieu, D. 2002. Sulfur aroma precursor present in S-glutathione conjugate form: identification of S-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon blanc. *Journal of Agricultural and Food Chemistry*, 50(14):4076-4079. https://doi.org/10.1021/jf020002y.

Piano, F., Fracassetti, D., Buica, A., Stander, M., Du Toit, W.J., Borsa, D. & Tirelli, A. 2015. Development of a novel liquid/liquid extraction and ultra-performance liquid chromatography tandem mass spectrometry method for the assessment of thiols in South African Sauvignon blanc wines. *Australian Journal of Grape and Wine Research*, 21(1):40-48. https://doi.org/10.1111/ajgw.12117.

Pinu, F. R., Jouanneau, S., Nicolau, L., Gardner, R. C. & Villas-Boas, S. G. 2012. Concentrations of the volatile thiol 3-mercaptohexanol in Sauvignon blanc wines: no correlation with juice precursors. *American Journal of Enology and Viticulture*, 63(3), 407-412. https://doi.org/10.5344/ajev.2012.11126.

Pinu, F.R., Edwards, P.J., Jouanneau, S., Kilmartin, P.A., Gardner, R.C. & Villas-Boas, S.G. 2014. Sauvignon blanc metabolomics: Grape juice metabolites affecting the development of varietal thiols and other aroma compounds in wines. *Metabolomics*, 10(4), 556-573. https://doi.org/10.1007/s11306-013-0615-9.

Pinu, F.R., Tumanov, S., Grose, C., Raw, V., Albright, A., Stuart, L., Villas-Boas, S.G., Martin, D., Harker, R. & Greven, M., 2019. Juice Index: An integrated Sauvignon blanc grape and wine metabolomics database shows mainly seasonal differences. *Metabolomics*, 15(1), 1-18. https://doi.org/10.1007/s11306-018-1469-y.

Pons, A., Lavigne, V., Darriet, P. & Dubourdieu, D. 2015. Glutathione preservation during winemaking with *Vitis vinifera* white varieties: Example of Sauvignon blanc grapes. *American Journal of Enology and Viticulture*, 66(2):187-194. https://doi.org/10 .5344/ajev.2014.14053.

Pons, A., Allamy, L., Schüttler, A., Rauhut, D., Thibon, C. & Darriet, P. 2017. What is the expected impact of climate change on wine aroma compounds and their precursors in grape?. *OENO one*, 51(2-3):141-146. https://doi.org/10.20870/oeno-one.2017.51.2. 1868.

Prodanov, M., Aznar, M., Cabellos, J.M., Vacas, V., López, F., Hernández, M.T. & Estrella, M.I. 2019. Tangential-flow membrane clarification of Malvar (*Vitis vinifera* L.) wine: Incidence on chemical composition and sensorial expression. *OENO one*, 4:725-739. http://doi.org/10.20870/oeno-one.2019.53.4.2480.

Průšová, B., Sochor, J., Baroň, M. & Kumšta, M. 2018. Effect of yeasts on the aroma profile of Sauvignon Blanc varietal wine. *Acta Universitatis Agriculturae et Silviculturae Mendelianae* Brunensis:889-896. https://doi.org/10.11118/actaun201866040889.

Prusova, B., Humaj, J., Sochor, J. & Baron, M. 2022. Formation, Losses, Preservation and Recovery of Aroma Compounds in the Winemaking Process. *Fermentation,* 8(3): 93. https://doi.org/10.3390/fermentation8030093.

Reynolds, A.G. 2022. Viticultural and vineyard management practices and their effects on grape and wine quality. In Managing wine quality (2nd ed.). Woodhead Publishing, 443-539. https://doi.org/10.1016/B978-0-08-102067-8.00012-9.

Reynolds, A.G. & Wardle, D.A. 1997. Flavour development in the vineyard: impact of viticultural practices on grape monoterpenes and their relationship to wine sensory response. *South African Journal of Enology and Viticulture*, 18(1):3-18. https://pdfs.se manticscholar.org/6f03/b8fad901d74612a34684bbf66f9e69c68468.pdf.

Ricci, A., Parpinello, G. P., Banfi, B. A., Olivi, F., & Versari, A. 2020. Preliminary Study of the Effects of Pulsed Electric Field (PEF) Treatments in Wines Obtained from Early-Harvested Sangiovese Grapes. *Beverages*, 6(2):34. https://doi.org/10.3390/bev erages6020034.

Rice, S., Tursumbayeva, M., Clark, M., Greenlee, D., Dharmadhikari, M., Fennell, A. & Koziel, J. A. 2019. Effects of harvest time on the aroma of white wines made from cold-hardy Brianna and Frontenac Gris grapes using headspace solid-phase microextracti on and gas chromatography-mass spectrometry-olfactometry. *Foods*, 8(1):29. https:// doi.org/10.3390/foods8010029.

Ridge, M., Sommer, S. & Dycus, D.A. 2021. Addressing Enzymatic Clarification Challenges of Muscat Grape Juice. *Fermentation*, 7(3),198:1-12. https://doi.org/10.33 .90/fermentation7030198.

Robinson, J. 2021. Sauvignon blanc wine grapes. [Blog, 30 April] URL https://www.ja ncisrobinson.com/learn/grape-varieties/white/sauvignon-blanc [30 April 2022].

Robinson, A.L., Boss, P.K., Solomon, P.S., Trengove, R.D., Heymann, H. & Ebeler, S.E. 2014a. Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture*, 65(1):1-24. https://do i.org/10.5344/ajev.2013.12070.

Robinson, A.L., Boss, P.K., Solomon, P.S., Trengove, R.D., Heymann, H. & Ebeler, S.E. 2014b. Origins of grape and wine aroma. Part 2. Chemical and sensory analysis. *American Journal of Enology and Viticulture*, 65(1):25-42. https://doi.org/10.5344/ajev .2013.13106.

Rodrigues, M., Forestan, C., Ravazzolo, L., Hugueney, P., Baltenweck, R., Rasori, A., Cardillo, V., Carraro, P., Malagoli, M., Brizzolara, S., Quaggiotti, S., Porro, D., Meggio, F., Bonghi, C., Battista, F., & Ruperti, B. 2023. Metabolic and Molecular Rearrangements of Sauvignon Blanc (*Vitis vinifera* L.) Berries in Response to Foliar Applications of Specific Dry Yeast. *Plants*, 12(19):3423. https://doi.org/10.3390/plants 12193423.

Rodríguez-Rojo, S., Visentin, A., Maestri, D. & Cocero, M. J. 2012. Assisted extraction of rosemary antioxidants with green solvents. *Journal of Food Engineering*, 109(1):98 -103. https://doi.org/10.1016/j.jfoodeng.2011.09.029.

Roland, A., Schneider, R., Razungles, A. & Cavelier, F. 2011a. Varietal thiols in wine: discovery, analysis and applications. *Chemical Reviews*, 111(11):7355-7376. https://d oi.org/10.1021/cr100205b.

Roland, A., Schneider, R., Charrier, F., Cavelier, F., Rossignol, M. & Razungles, A. 2011b. Distribution of varietal thiol precursors in the skin and the pulp of Melon B. and Sauvignon blanc grapes. *Food Chemistry*, 125(1):139-144. https://doi.org/10.1016/j.fo odchem.2010.08.050.

Roland, A., Cavelier, F. & Schneider, R. 2012. How organic and analytical chemistry contribute to knowledge of the biogenesis of varietal thiols in wine. A review. *Flavour and Fragrance Journal*, 27(4):266-272. https://doi.org/10.1002/ffj.3100.

Roman, T., Tonidandel, L., Nicolini, G., Bellantuono, E., Barp, L., Larcher, R. & Celotti, E. 2020. Evidence of the possible interaction between ultrasound and thiol precursors. *Foods*, 9(1):104. https://doi.org/10.3390/foods9010104.

Romero-Díez, R., Matos, M., Rodrigues, L., Bronze, M. R., Rodríguez-Rojo, S., Cocero, M. J. & Matias, A. A. 2019. Microwave and ultrasound pre-treatments to enhance anthocyanins extraction from different wine lees. *Food Chemistry,* 272:258-266. https://doi.org/10.1016/j.foodchem.2018.08.016.

Roujou de Boubée, D., Van Leeuwen, C. & Dubourdieu, D. 2000. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *Journal of Agricultural and Food Chemistry*, 48(10):4830-4834. https://doi.org/10.1021/jf000181o.

Ruiz, J., Kiene, F., Belda, I., Fracassetti, D., Marquina, D., Navascués, E., Calderón, F., Benito, A., Rauhut, D., Santos, A. & Benito, S. 2019. Effects on varietal aromas during winemaking: A review of the impact of varietal aromas on the flavor of wine. *Applied Microbiology and Biotechnology*, 103(18):7425-7450. DOI10.1007/s00253-019-10008-9.

Ruiz-Rodríguez, A., Durán-Guerrero, E., Natera, R., Palma, M. & Barroso, C. G. 2020. Influence of two different cryoextraction procedures on the quality of wine produced from muscat grapes. *Foods*, 9(11):1529. https://doi.org/10.3390/foods9111529.

Sacchi, K.L., Bisson, L.F. & Adams, D.O. 2005. A review of the effect of winemaking techniques on phenolic extraction in red wines. *American Journal of Enology and Viticulture*, 56(3):197-206. https://doi.org/10.5344/ajev.2005.56.3.197.

Sadoudi, M., Tourdot-Maréchal, R., Rousseaux, S., Steyer, D., Gallardo-Chacón, J.J., Ballester, J., Vichi, S., Guérin-Schneider, R., Caixach, J. & Alexandre, H. 2012. Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon blanc wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts. *Food Microbiology*, 32(2):243-253. https://doi.org/10.1016/j.fm.2012.06.006.

Santesteban, L. G., Miranda, C. & Royo, J. B. 2013. Influence of the freezing method on the changes that occur in grape samples after frozen storage. *Journal of the Science of Food and Agriculture*, 93(12):3010-3015. https://doi.org/10.1002/jsfa.6133.

Santos, H., Augusto, C., Reis, P., Rego, C., Figueiredo, A. C. & Fortes, A. M. 2022. Volatile metabolism of wine grape Trincadeira: impact of infection with *Botrytis cinerea*. *Plants*, 11(1):141. https://doi.org/10.3390/plants11010141.

SAWIS, 2020. South African Wine Harvest report 2020. [WWW document] URL http://www.sawis.co.za/info/download/Book_2020_statistics_Final_1.pdf (accessed 18 April 2022).

SAWIS, 2021. South African Wine Harvest report 2020. [WWW document] URL https://vinpro.co.za/wp-content/uploads/2021/05/South-African-Wine-Harvest-report-2021_full.pdf [04 June 2022].

Selli, S., Canbas, A., Cabaroglu, T., Erten, H., Lepoutre, J.P. & Gunata, Z. 2006. Effect of skin contact on the free and bound aroma compounds of the white wine of *Vitis* *vinifera* L. cv Narince. *Food Control*, 17:75-82. https://doi.org/10.1016/j.foodcont.2004.09.005.

Schmid, F. & Jiranek, V. 2011. Use of fresh versus frozen or blast-frozen grapes for small-scale fermentation. *International Journal of Wine Research*. 3:25-30. https://doi.org/ 10.2147/IJWR.S23325.

Schneider, R., Charrier, F., Razungles, A. & Baumes, R. 2006. Evidence for an alternative biogenetic pathway leading to 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one in wines. *Analytica Chimica Acta*, 563(1-2):58-64. https://doi.org/10.1016/j.aca.2006.01.057.

Sidhu, D., Lund, J., Kotseridis, Y. & Saucier, C. 2015. Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. *Critical Reviews in Food Science and Nutrition*, 55(4):485-502. https://doi.org/10.1080/10408398.2012.658587.

Silva, M. A., Julien, M., Jourdes, M. & Teissedre, P. L. 2011. Impact of closures on wine post-bottling development: A review. *European Food Research and Technology*, 233(6):905-914. https://doi.org/10.1007/s00217-011-1603-9.

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A. & Qian, M.C. 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of *Vitis vinifera* L. cv. Pinot noir wine. *Food Chemistry*, 173:424-431. https://doi.org/10.1016/j.foodchem.2014.09.150.

Steel, C.C., Blackman, J.W. & Schmidtke, L.M. 2013. Grapevine bunch rots: impacts on wine composition, quality, and potential procedures for the removal of wine faults. *Journal of Agricultural and Food Chemistry*, 61(22):5189-5206. https://doi.org/10.1021/jf400641r.

Steyer, D., Ambroset, C., Brion, C., Claudel, P., Delobel, P., Sanchez, I., Erny, C., Blondin, B., Karst, F. & Legras, J.L. 2012. QTL mapping of the production of wine aroma compounds by yeast. *BMC Genomics*, 13(1):1-15. DOI10.1186/1471-2164-13-573.

Šuklje, K., Lisjak, K., Baša Česnik, H., Janeš, L., Du Toit, W., Coetzee, Z., Vanzo, A. & Deloire, A. 2012. Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon blanc (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry*, 60(37):9454-9461. https://doi.org/10.1021/jf3020766.

Suklje, K., Gobler, N.; Coetzee, Z., Lisjak, K. & Deloire, A., 2013. *Methoxypyrazines and greenness in wines: myth or reality?* A few perspectives. [Blog, 01 February]. https://www.wineland.co.za/methoxypyrazines-greennes-wines-myth-reality-perspectives/ [02 December 2021].

Šuklje, K., Antalick, G., Buica, A., Coetzee, Z.A., Brand, J., Schmidtke, L.M. and Vivier, M.A., 2016a. Inactive dry yeast application on grapes modify Sauvignon Blanc wine aroma. *Food Chemistry*, 197:1073-1084. https://doi.org/10.1016/j.foodchem.2015.11.105.

Šuklje, K., Antalick, G., Buica, A., Langlois, J., Coetzee, Z.A., Gouot, J., Schmidtke, L.M. & Deloire, A. 2016b. Clonal differences and impact of defoliation on Sauvignon blanc (*Vitis vinifera* L.) wines: A chemical and sensory investigation. *Journal of the Science of Food and Agriculture*, 96(3):915-926. https://doi.org/10.1002/jsfa.7165.

Sun, Q., Ebersole, C., Wong, D.P. & Curtis, K. 2022. The Impact of Vineyard Mechanization on Grape and Wine Phenolics, Aroma Compounds, and Sensory Properties. *Fermentation*, 8(7):318. https://doi.org/10.3390/fermentation8070318.

Swiegers, J.H. & Pretorius, I.S. 2007. Modulation of volatile sulfur compounds by wine yeast. *Applied Microbiology and Biotechnology*, 74(5):954-960. https://doi.org/10.1007/s00253-006-0828-1.

Swiegers, J.H., Francis, I.L., Herderich, M.J. & Pretorius, I.S. 2006. Meeting consumer expectations through management in vineyard and winery. *Wine Industry Journal*, 21(1):34-43.

Swiegers, J.H., Kievit, R.L., Siebert, T., Lattey, K.A., Bramley, B.R., Francis, I.L., King, E.S. & Pretorius, I.S. 2009. The influence of yeast on the aroma of Sauvignon blanc wine. *Food Microbiology*, 26(2):204-211. https://doi.org/10.1016/j.fm.2008.08.004.

Tao, Y. & Sun, D. W. 2015. Enhancement of food processes by ultrasound: a review. *Critical Reviews in Food Science and Nutrition*, 55(4):570-594. https://doi.org/10.1080 /10408398.2012.667849.

Thibon, C., Marullo, P., Claisse, O., Cullin, C., Dubourdieu, D. & Tominaga, T. 2008. Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisiae* during wine fermentation. *FEMS Yeast Research*, 8(7):1076-1086. https://doi.org/10.1111/j.1567-1364.2008.00381.x.

Thibon, C., Dubourdieu, D., Darriet, P. & Tominaga, T. 2009. Impact of noble rot on the aroma precursor of 3-sulfanyl hexanol content in *Vitis vinifera* L. cv Sauvignon blanc and Semillon grape juice. *Food Chemistry*, 114(4):1359-1364. https://doi.org/10.1016/ j.foodchem.2008.11.016.

Thibon, C., Cluzeet, S., Mérillon, J. M., Darriet, P., & Dubourdieu, D. 2011. 3-Sulfanylhexanol precursor biogenesis in grapevine cells: The stimulating effect of *Botrytis cinerea*. *Journal of Agricultural and Food Chemistry*, 59:1344-1351. https://doi.org/10.1021/jf103915y.

Thibon, C., Böcker, C., Shinkaruk, S., Moine, V., Darriet, P. & Dubourdieu, D. 2016. Identification of S-3-(hexanal)-glutathione and its bisulfite adduct in grape juice from Vitis vinifera L. cv. Sauvignon blanc as new potential precursors of 3SH. *Food Chemistry*, 199:711-719. https://doi.org/10.1016/j.foodchem.2015.12.06.

Threlfall, R., Main, G. & Morris, J. 2006. Effect of freezing grape berries and heating must samples on extraction of components and composition parameters of red wine grape varieties. *Australian Journal of Grape and Wine Research*, 12(2):161-169. https://doi.org/10.1111/j.1755-0238.2006.tb00056.x.

Tirelli, A., De Noni, I., Stuknyté, M., Pica, V. & Fracassetti, D. 2021. Role of extraction procedures on the concentration of varietal thiol precursors in Grillo white grape must. *Australian Journal of Grape and Wine Research*, 28(1):61-69. https://doi.org/10 .1111/ajgw.12514.

Tomasevic, M., Lisjak, K., Vanzo, A., Basa Cesnik, H., Gracin, L., Curko, N. & Kovacevic Ganic, K. 2019. Changes in the composition of aroma and phenolic compounds induced by different enological practices of Croatian white wine. *Polish*

*Journal of Food and Nutrition Sciences*, 69(4):343-358. http://dx.doi.org/10.31883/pjfns/112328.

Tominaga, T., Peyrot des Gachons, C. & Dubourdieu, D. 1998. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: S-cysteine conjugates. *Journal of Agricultural and Food Chemistry*, 46(12):5215-5219. https://doi.org/10.1021/jf980481u.

Tominaga, T., Baltenweck-Guyot, R., Des Gachons, C.P. & Dubourdieu, D. 2000. Contribution of volatile thiols to the aromas of white wines made from several Vitis vinifera grape varieties. *American Journal of Enology and Viticulture*, 51(2):178-181. https://doi.org/10.5344/ajev.2000.51.2.178.

Tomaz, I., Šeparović, M., Štambuk, P., Preiner, D., Maletić, E. & Karoglan Kontić, J. 2018. Effect of freezing and different thawing methods on the content of polyphenolic compounds of red grape skins. *Journal of Food Processing and Preservation*,42(3):13550. https://doi.org/10.1111/jfpp.13550.

Tsai, P.C., Araujo, L.D. & Tian, B. 2022. Varietal Aromas of Sauvignon Blanc: Impact of Oxidation and Antioxidants Used in Winemaking. *Fermentation*, 8(12):686. https://doi.org/10.3390/fermentation8120686.

Ugliano, M., Kwiatkowski, M., Vidal, S., Capone, D., Siebert, T., Dieval, J.B., Aagaard, O. & Waters, E.J. 2011. Evolution of 3-mercaptohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. *Journal of Agricultural and Food Chemistry*, 59(6):2564-2572. https://doi.org/10.1021/jf1043585.

Van Breda, V., Jolly, N. & van Wyk, J. 2013. Characterisation of commercial and natural *Torulaspora delbrueckii* wine yeast strains. *International Journal of Food Microbiology*, 163(2-3):80-88. https://doi.org/10.1016/j.ijfoodmicro.2013.02.011.

Van Rooyen, R., Brand, J. & du Toit, W. 2023. Varietal thiols levels and sensory effects in South African Colombard wines. *OENO One*, 57(1):3-40. https://doi.org/10.20870/oeno-one.2023.57.1.7121.

Van Wyngaard, E. 2013. Volatiles playing an important role in South African Sauvignon blanc wines (Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch). http://hdl.handle.net/10019.1/80274.

Vanzo, A., Janeš, L., Požgan, F., Velokonja Bolta, Š., Sivilotti, P. & Lisjak, K. 2017. UHPLC-MS/MS determination of varietal thiol precursors in Sauvignon blanc grapes. *Scientific reports*, 7(13122):1-11.

Varela, C. 2016. The impact of non-*Saccharomyces* yeasts in the production of alcoholic beverages. *Applied microbiology and biotechnology*, 100(23):9861-9874. https://doi.org/10.1007/s00253-016-7941-6.

Vázquez-Pateiro, I., Mirás-Avalos, J.M. & Falqué, E. 2022. Influence of must clarification technique on the volatile composition of Albariño and Treixadura wines. *Molecules*, 27(3):810. https://doi.org/10.3390/molecules27030810.

Vilanova, M., Escudero, A., Graña, M. & Cacho, J. 2013. Volatile composition and sensory properties of Northwest Spain white wines. *Food Research International*, 54(1):562-568. https://doi.org/10.1016/j.foodres.2013.07.036.

Visan, L., Tamba–Berehoiu, R.M., Popa, C.N., Danaila–Guidea, S.M. & Culea, R. 2018. Aromatic compounds in wines. *Scientific papers*, 18(4):423-430. E-ISSN 2285-3952.

Wang, J., Huo, S., Zhang, Y., Liu, Y. & Fan, W. 2016. Impact of various maceration techniques on the phenolic and volatile composition of Chenin Blanc wines. *International Journal of Food Science & Technology*, 51(11):2360-2366.

Weldegergis, B. T., de Villiers, A. & Crouch, A. M. 2011. Chemometric investigation of the volatile content of young South African wines. *Food Chemistry*, 128(4):1100-1109. https://doi.org/10.1016/j.foodchem.2010.09.100.

Williams, M. T. 2018. *Characterisation of wine yeasts for varietal red wine production by using chemical, sensory and metabolomic tools* (Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch). http://hdl.handle.net/10019.1/103370.

Williams, M., Khan, W., Ntushelo, N. & Hart, R. 2021. An indigenous *Saccharomyces cerevisiae* yeast strain isolated from Paarl regional Shiraz grapes to enhance Shiraz wine typicity. *OENO One*, 55(2):209-225. https://doi.org/10.20870/oeno-one.2021.55.2.4552.

Wilson, C. L. 2017. *Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines* (Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch). http://hdl.handle.net/10019.1/101250.

Wilson, C., Brand, J., du Toit, W. & Buica, A. 2018. Interaction effects of 3-mercaptohexan-1-ol (3MH), linalool and ethyl hexanoate on the aromatic profile of South African dry Chenin blanc wine by descriptive analysis (DA). *South African Journal of Enology and Viticulture*, 39(2):1-13. http://dx.doi.org/10.21548/39-2-3165.

Wilson, C., Brand, J., du Toit, W. & Buica, A. 2019. Matrix effects influencing the perception of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) in different Chenin Blanc wines by Projective Mapping (PM) with Ultra Flash profiling (UFP) intensity ratings. *Food Research International*, 121:633-640. htt ps://doi.org/10.1016/j.foodres.2018.12.032.

Wine and Spirits Education Trust, 2012. *Wine and spirits*: Understanding wine quality (2nd ed.):2-5, London, ISBN 978-1-905819-15-7.

WOSA, 2023a. https://www.wosa.co.za/The-Industry/Winegrowing-Areas/Winelands-of-South-Africa/ [17 July 23].

WOSA, 2023b. South African Wine Harvest report 2020. [WWW document] URL https://www.wosa.co.za/The-Industry/Statistics/SA-Wine-Industry-Statistics/ [25 July 2023].

Yang, N., Huang, K., Lyu, C. & Wang, J. 2016. Pulsed electric field technology in the manufacturing processes of wine, beer, and rice wine: A review. *Food Control*, 61:28-38. https://doi.org/10.1016/j.foodcont.2015.09.022.

Ye, D., Zhang, L., Sun, S., Chen, J. & Fang, T. 2014. Production of high-aroma instant tea powder using various novel technologies. *Journal of Food Process Engineering*, 37(3):273-284. https://doi.org/10.1111/jfpe.12083.

Young, P.R., Eyeghe-Bickong, H.A., du Plessis, K., Alexandersson, E., Jacobson, D.A., Coetzee, Z., Deloire, A. and Vivier, M.A. 2016. Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. *Plant Physiology*, 170(3):1235-1254. https://doi.org/10.1104/pp.15.01775.

Zhang, S., Petersen, M. A., Liu, J. & Toldam-Andersen, T. B. 2015. Influence of pre-fermentation treatments on wine volatile and sensory profile of the new disease tolerant cultivar Solaris. *Molecules*, 20(12):21609-21625. https://doi.org/10.3390/molecules 201219791.

Zhang, Q., Sun, X., Sheng, Q., Chen, J., Huang, W. & Zhan, J. 2016. Effect of suspension freeze-concentration technology on the quality of wine. *South African Journal of Enology and Viticulture*, 37(1):39-46. https://doi.org/10.21548/37-1-757.

## CHAPTER THREE: RESEARCH CHAPTER 1

**Cryogenic pre-treatment of Sauvignon blanc and Chenin blanc grapes and must: Impact on chemical and varietal profiles of must and wine.**

### Abstract

Sauvignon blanc, one of the most extensively cultivated white wine grape varieties globally, is renowned for producing wines with characteristic "grassy" and "tropical" varietal aromas. These aromas result from aroma compounds, such as methoxypyrazines and varietal thiols, present in the grape skin and pulp. Similarly, Chenin blanc, also a popular white wine cultivar and widely planted in SA, gained popularity for its perceived "Guava" aroma. Chenin blanc shares a genetic origin with Sauvignon blanc, and was previously shown to also possess varietal thiols, which was subsequently confirmed. Several anthropogenic factors have been investigated to increase varietal aroma compounds in these cultivars, specifically the varietal thiols; however, inconsistent results have been reported. Alternative technologies, such as low-temperature cryogenic pre-treatment of whole grapes and grape must, have also been researched and shown promise.

Therefore, this study investigated the effect of pre-fermentative cryogenic treatments at four production stages (whole grapes [WG], macerated grapes [MG], turbid must [TM] and clear juice [CJ]) on two popular white wine *Vitis vinifera* L. cv. cultivars, i.e., Sauvignon blanc and Chenin blanc. Two cryogenic temperatures (-20 ºC and -4 ºC) were applied to four production stages (WG, MG, TM & CJ) and T0 and T4 storage periods. Following thawing, the typical white wine-making procedure was followed. The control wines were not subjected to any cryogenic treatments. Subsequently, all grape must were subjected to chemical analysis and all wines produced were analysed for standard physicochemical properties and the varietal aroma compounds of interest, i.e., volatile thiols (3-SH, 3-SHA and 4-MSP) and methoxypyrazines (ibMP and sbMP).

The results showed that the physicochemical parameters of the grape must least affected by the cryogenic pre-treatments were the total sugar, glucose/fructose ratio and total soluble solids (TSS). The parameters most affected were the pH, which was higher in the grape must subjected to cryogenic treatments compared to the unfrozen control and the total acidity (TA), which was lower in the grape must subjected to cryogenic pre-treatments when compared to the unfrozen control. Furthermore, YAN and haze levels for all treatments and Producers were significantly higher in 2020 than in 2021, indicating that the differences observed were vintage dependent rather than resulting from the treatments. The physicochemical parameters of the final wines were generally all within the specified limits for SA white wines, except for the pH, which was slightly higher in the wines made from the cryogenic pre-treatments.

Differences in varietal thiol and methoxypyrazine concentrations for the Sauvignon blanc wines from the various wine regions were influenced by harvesting method and vintage. The concentrations of 3-SHA, 4-MSP and 3-SH detected in the wines exceeded the aroma perception threshold and ranges commonly reported in SA Sauvignon blanc. Furthermore, in the Chenin blanc wines, the concentrations of 3-SHA (5-253 ng $L^{-1}$) and 4-MSP (n.d.) were above the generally reported ranges for SA Chenin blanc wines, whilst 3-SH fell below (99-1124 ng $L^{-1}$). Moreover, for the aroma threshold, 3-SHA (4 ng $L^{-1}$) and 3-SH (60 ng $L^{-1}$) were detected above the typically reported levels whilst the 4-MSP varied above and below reported levels (0.8 ng $L^{-1}$) over the vintages and between regions. Overall, regarding the desired levels of the specific aroma compounds, i.e., varietal thiols and methoxypyrazines, no definite trends in terms of cryogenic temperature, stage of production, or storage time were observed that would yield the most favourable levels in final wines.

## 3.1 Introduction

Climate change and market development are among the challenges facing the wine industry at present; therefore, the implementation of novel winemaking techniques that are easily accessible to producers is essential. Climatic conditions have a huge influence on viticulture and grape physiology and influence the development of varietal aroma and flavour metabolites in final wines (Allen et al., 1991:111; Marais, 1998:10; 16; Marais, 2001:47; 51; Capone & Jeffery, 2011:4665; Coetzee & du Toit, 2012:295; Cosme et al., 2016:191; 193). During the processing of grapes and grape must, numerous volatile compounds are formed which, together with the primary aromas, contribute to the aroma of the wine, typical of the variety and terroir from which it originates (Roland et al., 2011a:7355; Roland et al., 2011b:142-143; Coetzee & du Toit, 2012:295; Cosme et al., 2016:208). Therefore, to produce a good quality wine, the winemaker cannot simply rely on cultivar type, but must consider numerous interactions between factors, including viticultural (climate, soil, water, cultivar, and grape growing practices) and oenological practices (pre-fermentation treatments of grapes, fermentation, and post-fermentation treatments) (Vilanova et al., 2012:129-130; Steel et al., 2013:5193; Hart et al., 2019:5; Ruiz et.al., 2019:7433-7434; Karabagias et al., 2020:2234).

The application of low temperature pre-fermentative maceration techniques during white wine production has been used to increase the contact time between the grape skins and juice, leading to an increase in the extraction of aroma compounds and their precursors mainly located in the skin of the grape berries (Peinado et al., 2004:587-589; Salinas et al., 2005:1527; 1535; Baiano et al., 2012:2700; Blesic et al., 2016:118-119; Ruiz-Rodríguez et al., 2020:6;11; Bestulić, et al., 2022:2;9-10; Malićanin et al., 2022:11; 14; Pedrosa-López et al., 2022:8). Furthermore, cold maceration has not only shown promise during white wine production to enhance the varietal character of the wines but also during red wine production to increase the extraction of phenolic compounds which contribute to the colour and flavour intensity of the wine (de Santis & Frangipane, 2010:52; Lukíc et al., 2017:257-259; Aleixandre-Tudo & du Toit 2018:204-207; Benucci et al., 2018:265; Ruiz-Rodríguez et al., 2020:6-10; Korenika et al., 2020:52; Bestulić et al., 2022:5-7; 10). Although cold maceration increases the concentration of aromatic compounds, it can also extract components that contribute to undesirable characteristics (Aragón-García et al., 2021:1; Pedrosa-López et al., 2022:8-9).

A modification of this technique, known as cryofreezing or cryoextraction, involves the freezing of grape berries and juice (Blesic et al., 2016:118-119; Tomaz et al., 2018:3; Vilar-Bustillo et al., 2023:8;12; Van Breda et al., 2024:2-5). Previous research showed that wines produced using this method had a higher aroma intensity and stability of taste properties than wines prepared using traditional maceration (Carillo et al., 2011:5-6; Baiano et al., 2012:2695; Cai et al., 2014:228; Zhang et al., 2016:21610; 21617; Tomaz et al., 2018:7-8; Chen et al., 2019:638; 644; O'Kennedy, 2020; Ruiz-Rodríguez et al., 2020:10). The perceived enhanced varietal taste and aromas in wines made from cryogenically frozen grapes were effectively protected from oxidation, and therefore from the formation of undesirable odour compounds. Wines were also shown to be acceptable, well-balanced, and better-rounded, with a fuller mouthfeel (Baiano et al., 2012:2700; Chen et al., 2019:644; Ruiz-Rodríguez et al., 2020:11; Bestulić et al., 2022:10; Malićanin et al., 2022:14; Pedrosa-López et al., 2022:6-7). The treatment involves subjecting WG and MG to rapid cooling/chilling (5 ºC and below) and holding it at the desired temperature for hours or days, thus improving the extraction of compounds (phenolic and primary aroma) contained within the grape skins (Fragasso et al., 2010:3-4; Aleixandre-Tudo & du Toit, 2018:200-201; 207; Benucci et al., 2018:258; 264-265; Korenika et al., 2019:467; Korenika et al., 2020:52; Bestulić et al., 2022:5-6; Pedrosa-López et al., 2022:6-11). During the freezing process, ice crystals that form in the grapes tear the pectocellulosic walls, disorganising the tissues, thus facilitating the extraction of compounds from the grape skin (Heydarov et al., 2020:46-47; Ruiz-Rodríguez et al., 2020:6-11; Van Breda et al., 2024:5-9). Arora & Chen (2025:3-12) investigated the impact of short-term and long-term freezing on the profiles of cellular metabolites from spinach leaves, demonstrating that leakage increased during short-term freeze storage compared to long-term. Their research also highlighted that different clusters of metabolites leak at different times during the storage period (Arora, 2018:302; Arora & Chen, 2025:3-12). Metabolites in cluster 1 comprised 13 metabolites (including sugars, organic acids and inorganic acids) which were higher in concentration at long-term storage. The cluster 2 metabolites (comprising 34 metabolites), including all 20 amino acids, six organic acids (involved in primary and secondary metabolism), four sugar molecules and four others leaked in higher concentrations during short-term storage (Arora & Chen, 2025:5). In particular, malic acid, which is one of the six organic acids mentioned for the spinach leaves above is also known to be an important grape quality parameter, generally higher in frozen grape must compared to fresh (García et al., 2011:163). Based on the aforementioned findings, it can be hypothesised that freeze/thaw treatments applied to wine grapes, particularly frozen at different stages (WG, MG, TN and CJ) and freeze storage durations (short vs long) could lead to improved wine quality and sensory parameters. This information will prove valuable when deciding on the optimal freeze storage conditions to apply to enhance the sensory properties and quality of wines (García et al., 2011:167). Cryogenic treatments have been applied using various methods, i.e., large capacity refrigerators/chambers, freeze-concentration technology, blast freezing, freeze concentration or by adding cryogenic agents in a solid or liquid state, i.e., nitrogen ($N_2$) or carbon dioxide ($CO_2$) (Parenti et al., 2004:361; 365; Carillo et al., 2011:6; Schmid & Jiranek, 2011:26; Zhang et al., 2016:21620; Ruiz-Rodríguez et al., 2020:6-10; Coetzee, 2022; Vilar-Bustillo et al., 2023:8).

The use of external refrigeration methods, such as large refrigeration chambers with temperatures ranging between -18 ºC and -28 ºC, although less aggressive, does not protect the macerated must from oxygen. It does, however, allow for better control of the freezing process and reduces the level of bitter tannins transferred to the final wine (Tian et al., 2009:217; 219-220; Ruiz-Rodríguez et al., 2020:2). Solid carbon dioxide (freezing point of -78.5 ºC) and liquid nitrogen (freezing point of -195.8 ºC) induce a thermal shock, leading to greater degradation of the grape berries because of the contact time between the pulp and must as well as the added benefit of protecting the grapes from oxidation (Santesteban et al., 2013:3014-3015). Moreover, when considering the various cryogenic techniques, the speed at which the grapes were frozen and the duration of the freezing treatments was an important factor to consider as it was directly related to the degree of disorganization of the berry's structure and extraction of compounds (Heydarov et al., 2020:46; Ruiz-Rodríguez et al., 2020:11). Casassa & Sari (2015:1052-1053) investigated the effect of external refrigeration as well as solid $CO_2$ as a cryogenic treatment on Malbec grapes and found that the wines produced from the grapes treated with solid $CO_2$ had a higher colour intensity than the control wines which were comparable to the findings of Gil-Muñoz et al. (2009:787). Their research found that wines produced from Cabernet Sauvignon grapes exposed to pre-fermentative cold maceration had higher anthocyanin levels than Shiraz wines subjected to the same treatment, however, Shiraz grapes exposed to dry ice (solid

$CO_2$) showed higher anthocyanin levels. This was attributed to differences in the physiological structure of the Cabernet Sauvignon and Shiraz grape skins. Furthermore, the study showed that, for both varieties, the darkest wines with the highest phenolic and tannin concentrations were obtained from grapes or must that were frozen before fermentation (Gil-Muñoz et al., 2009:787). Chemical profiles of the wines only showed moderate differences with improved sensory properties found in both earlier and subsequent research (Parenti et al., 2004:364-365, Carillo et al., 2011:11-12; Casassa et al., 2015:114).

Santesteban et al. (2013:3011) explored the effect of three different cryofreezing methods, i.e., ultra-freezing, blast-freezing and standard freezing on wine chemical parameters (total soluble solids (TSS), pH, titratable acidity (TA), malic (MA) and tartaric acid (TarA) concentrations and yeast available nitrogen (YAN) of Cabernet Sauvignon, Tempranillo and Grenache. The fastest rates of freezing were reached when the ultra-freezer was used (< 15 min), followed by the blast freezer (< 25 min), while much slower and less homogeneous freezing was accomplished with the standard freezer (between 1 to 9 hr) (Santesteban et al., 2013:3011). Their research showed that increasing the speed of freezing did not necessarily improve the correlation between fresh and frozen grapes for most of the analytical parameters considered in the study, except for Tartaric Acid, which is known to precipitate at low temperatures. This is due to the precipitation of potassium salts during the freezing process and the lower solubility of the acidic salts when the grapes were defrosted (Zhang et al., 2015:21611). Moreover, low-temperature cold stabilisation is a conventional method for wine tartaric stabilisation (Santesteban et al., 2013:3011; Ruiz-Rodríguez et al., 2020:5).

White wines are commonly fermented at ~14-15 °C during the summer months. However, Diemersdal Wine Estate in the Durbanville wine region fermented a Sauvignon blanc grape must that had been frozen immediately after harvest and stored for four months. This practice produced wines that won several accolades such as the Winery Innovator in the International Wine and Spirits Competition, two FNB Top 10 Sauvignon blanc awards and best Sauvignon blanc at the National Young Wine Show. While a high thiol concentration in a tropical-styled South African (SA) Sauvignon blanc is less than 2500 ng $L^{-1}$, the “Winter Ferment” Sauvignon blanc’s thiol concentration measures in the range of 5 000 ng $L^{-1}$. This, therefore, warrants further investigation to understand the observed differences in aroma (specifically, thiol) profiles and the demonstrated superior quality of the winter-produced wines, which will be the focus of this study. To date, no formal scientific studies have compared the effects of freezing SA Sauvignon blanc WG, MG, TM and CJ simultaneously, nor have they compared the quality of winter (postponed fermentations) fermented wines to summer fermented wines. This prompted an interest in understanding more about winter wine fermentations and the impact of freezing on final wine quality by the wine industry. The outcomes of this research may lead to vinification techniques that can provide the SA wine industry with the necessary tools to produce wines with enhanced chemical and varietal sensory profiles.

## 3.2 Materials and methods

### 3.2.1 Grapes

Sauvignon blanc grapes (2000 kg) were obtained from three producers in 2020 and 2021, i.e., Producer A (Stellenbosch), Producer B (Durbanville) and Producer C (Napier), located within the Coastal and Cape South Coast (Napier) wine regions. All grapes were hand-harvested, except for 540 kg of Sauvignon blanc from the Durbanville producer, which was both machine-harvested (2020 vintage only) and hand-harvested to simulate their harvesting conditions. Chenin blanc grapes (2000 kg) were obtained from one producer, Producer D (Stellenbosch), in 2021 only. Following harvesting in February for 2020 and between February and March for 2021, grapes were delivered to the Agricultural Research Council (ARC) Nietvoorbij Research cellar and crates were weighed before processing. Additionally, grapes were sorted before processing to remove any rotten or undesirable bunches, as grey mould (*Botrytis cinerea*) was visible on some of the Chenin blanc grapes. It is important to note that for this research, the effects of cryo-freezing post-harvest on Sauvignon blanc grapes (primary cultivar), regardless of the Clone, and Chenin blanc as an additional white wine cultivar, were the main focus, therefore, the Clones used by the producers will not be investigated as a part of this study. Furthermore, the Sauvignon blanc grapes originated from the same blocks for both vintages for consistency.

### 3.2.2 Cryogenic pre-treatment and thawing procedures

Crates containing Sauvignon blanc (40 kg) and Chenin blanc grapes (40 kg) from the respective producers were randomised to ensure that batches were homogeneous before the grape bunches were divided into batches for the respective production stages, i.e., WG, MG, TM and CJ (Figure 3.1). Dry ice (solid 3 mm carbon dioxide ($CO_2$) pellets) and $SO_2$ (50 mg $L^{-1}$) were added to all the production stages to minimise oxidation, before storing them in large freezing chambers at -20 ºC and -4 ºC, at T0 and T4. All treatments were performed in triplicate. The temperatures of the WG were monitored daily using a digital thermometer until the desired cryogenic temperatures were reached. This was only done for the T0 treatments since the T4 treatments were stored away. The desired temperatures were reached within three days. Thawing of the T0 -20 ºC and -4 ºC WG and MG treatments was conducted at 24 ºC (for one day), whereafter it was moved to 15 ºC (for two days) for complete thawing, before further processing and vinification. Thawing of all TM and CJ treatments occurred at 15 ºC for three days (Figure 3.1). The same protocol was followed for complete thawing of the cryogenically pre-treated WG, MG, TM and CJ frozen (-4 ºC and -20 ºC) at T4. The wines made from grapes not subjected to cryogenic pre-treatments will be referred to as the control wines, the wines made from cryogenic pre-treatments immediately after harvesting (defrosted immediately after freezing) will be referred to as the T0 wines, whilst wines made from the four-month cryogenic pre-treatments will be referred to as T4 wines.

### 3.2.3 Wine production

Small-scale (20 L) production of Sauvignon blanc and Chenin blanc wines from the treatments in 3.2.2. were conducted, with the process repeated separately over two harvest seasons (2020 for Sauvignon blanc, and 2021 for Sauvignon blanc and Chenin blanc) (Figure 3.1). Following defrosting, crushing and pressing, 50 mg $L^{-1}$ $SO_2$ was added to all must to reduce the natural microbial population and to prevent oxidation. Furthermore, pectolytic enzyme (0.5 g $L^{-1}$) (EnartisZym RS, San Martino, Italy) was added to improve the sedimentation of the grape must. Settling took place over forty-eight hours at 14 ºC, followed by racking of CJ from the lees. Subsequently, the CJ was transferred to new fermentation vessels (20 L). A commercially available active dried wine yeast (ADWY), i.e., *Saccharomyces cerevisiae* (*S. cerevisiae*) (CKS102, Fermentis, Belgium) (20 g $hL^{-1}$, per the manufacturer's instructions) was used to conduct all alcoholic fermentations at 14 ºC. Following the addition of the ADWY, diammonium phosphate (D.A.P) was added at a concentration of 50 g $hL^{-1}$ to stimulate yeast growth and fermentation activity and to prevent the possible formation of hydrogen sulphide.

All small-scale wines were produced in triplicate to ensure that enough data points were available for statistical analysis. Once the fermentations were complete, the wines were racked, cold stabilised (two weeks at 0 ºC) and clarified before bottling. Wines were frozen at -20 ºC in 5 L containers and the remainder bottled and stored at 15 ºC. The aforementioned 5 L containers were defrosted at 15 ºC for 24 hrs and bottled after the wines produced from the T4 treatments were ready for bottling. Sensory and chemical analyses were conducted on all final wines.

### 3.2.4 Physicochemical analysis

All must and wines produced were subjected to standard chemical analysis using the ALPHA II FT-IR ATR Wine Analyser (Bruker, South Africa) and analysed at the

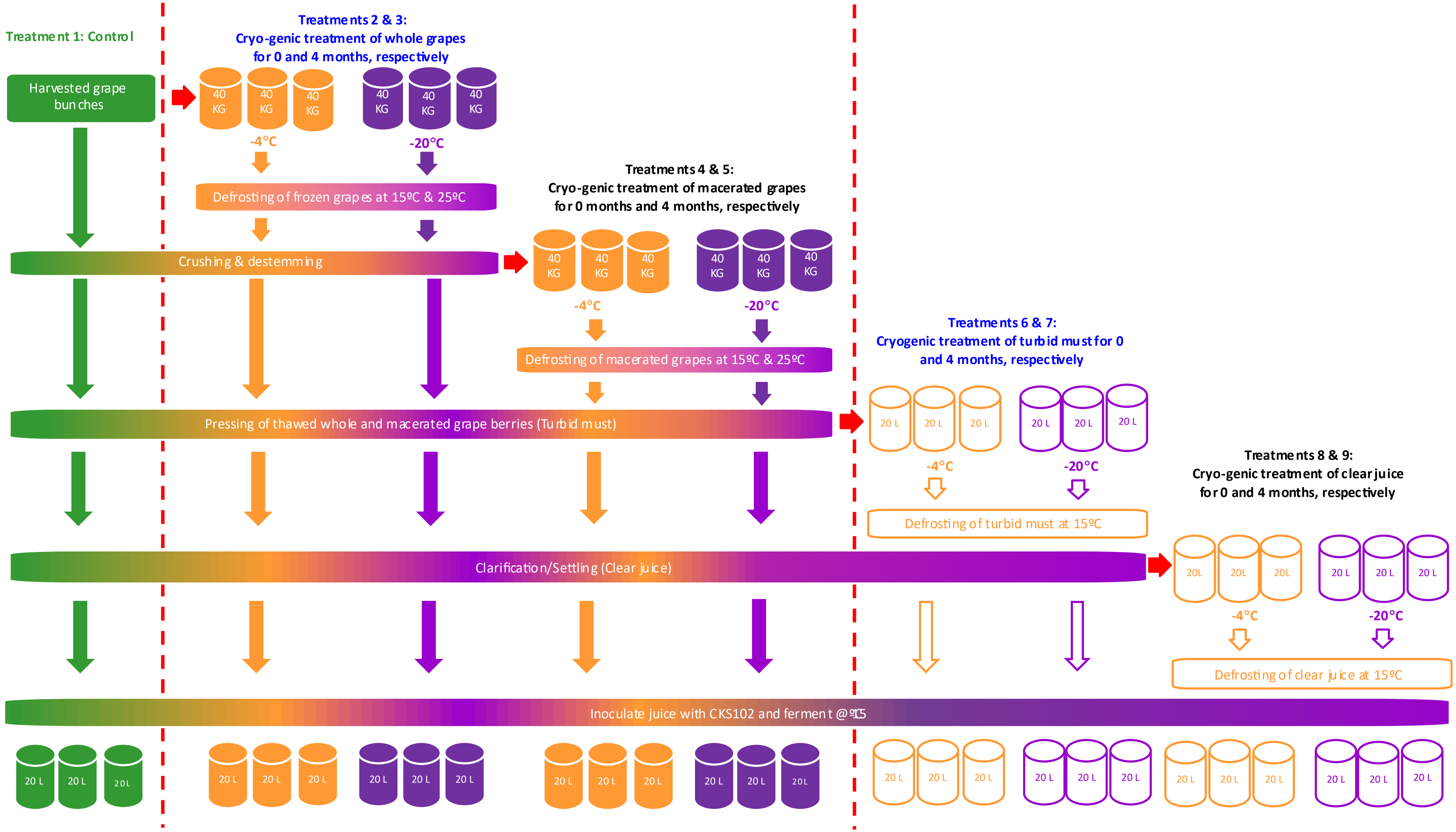



Figure 3.1. Schematic illustration of the ARC Nietvoorbij standard winemaking protocol using untreated grapes, and cryogenic (-4 °C and -20 °C) pre-treated whole grapes (WG), macerated grapes (MG), turbid must (TB) and clear juice (CJ) originating from the grapevine *Vitis vinifera* L. cv Sauvignon blanc and Chenin blanc for T0 and T4, respectively. Following the cryogenic pre-treatment of the WG, MG, TM and CJ at -4 ºC and -20 ºC, containers were thawed at 15 ºC and 25 ºC, respectively (as illustrated in the above schematic). The same protocol was followed for all producers for 2020 and 2021.

AGRI-Food Analytics Laboratory of the ARC Infruitec-Nietvoorbij. Fourier Transform Infrared (FT-IR) analyses were performed on both the grape juice samples (immediately after pressing) and the finished wine samples. Juice samples (50 mL) were added to a Falcon tube and centrifuged at 4200 rpm (4467 ×g) for 10 min (BioBase, Benchmark Scientific, Sayreville, New Jersey, USA) to remove impurities and optimize clarity. Analyses by FT-IR measurements were then carried out on the Alpha II FT-IR ATR Wine Analyser (Bruker, South Africa) following sampling of the juice or the wine from a 40 mL vial closed with a septa cap using the instrument's autosampler probe. Single measurements were performed on each of the three biological replicates using the "grape" and "finished wine" programs for grape juice and wine samples, respectively. The final output value was expressed as the average of the three measurements. The following parameters were evaluated: ethanol, pH, total and volatile acidity, total and free sulfur dioxide, glucose and fructose, reducing sugar, tartaric acid, malic acid, lactic acid, glycerol. In addition, grape must samples were analysed for YAN, glucose/fructose and turbidity following the methods of the South African Wine Laboratory Association.

***Must***

All Sauvignon blanc and Chenin blanc grape must samples were analysed for standard chemical parameters (pH, Brix, total acidity (TA), malic acid), yeast assimilable nitrogen (YAN), glucose, fructose and turbidity (haze). Must samples were taken after the grapes were crushed, destemmed and pressed. Subsequently, samples were frozen and stored at -20 ºC until analysed.

***Wines***

Bottled wines were analysed for standard wine chemical parameters (pH, volatile acidity (VA), total acidity (TA), residual sugar (RS), alcohol, and sulfur dioxide ($SO_2$). In addition, wines were analysed for volatile thiols (3-SH, 3-SHA & 4-MSP) and methoxypyrazines as described under 3.2.5.

### 3.2.5 Analysis of volatile aroma compounds

#### *3.2.5.1 Varietal thiol analysis*

***Reagents and standards***

All samples were prepared and analysed by the Central Analytical Facility (CAF) at Stellenbosch University according to the method used by Mafata et al. (2018:2). All solutions prepared were expressed in terms of volume percentage ($^{v}/_{v}$%) and made up with Milli-Q water, unless otherwise specified. All reagents and standards, including 3-SH, 3-SHA, 4-MSP and 98% 4,4′-Dithiodipyridine (98% DTDP), ethylenediaminetetra acetic acid disodium salt (EDTA-$Na_2$), methanol, 96% ethanol, sodium hydroxide (NaOH), tartaric acid, anhydrous acetaldehyde ≥ 98%, and 37% hydrochloric acid (HCl) were purchased from Sigma-Aldrich (Louisville, MO, USA), and the solid phase extraction (SPE) cartridges (Supelclean ENVI-18 SPE) from Supelco (Bellefonte, PA, USA).

***Sample preparation***

The wine samples (20 mL) were prepared according to the method of Mafata et al. (2018:2) by adding 100 μL of a 0.05 mg $L^{-1}$ acetic acid solution of the internal standard (IS, 6-MH). This was followed by adding 20 mg of EDTA-$Na_2$, 80 μL of 50% acetaldehyde (in ethanol) and 200 μL of aqueous DTDP (10 mM). The mixture was stirred at 500 rpm for 30 min at room temperature. The SPE cartridge was conditioned with 6 mL of methanol followed by 6 mL of water. The sample was loaded onto the cartridge and washed with 12 mL of 50% methanol and dried under vacuum for 5 min. The derivatives were eluted with 3 mL of methanol and injected directly into the liquid chromatography-mass spectrometry (LC-MS) instruments, described below, without further concentration.

***Instrumentation, settings, procedures and conditions***

Quantitative analysis was performed using a Waters Acuity Ultraperformance Convergence Chromatograph ($UPC^2$) device using a Waters Viridis BEH 2EP Column (130 Å, 1.7 µm, 3 mm x 100 mm; Waters, Milford, MA, USA). The column temperature and automated back-pressure regulator (ABPR) were set to 60 °C and 137.89 bar, respectively. The solvents were $CO_2$ and methanol, with a total flow rate of 1.5 $mL^{-1}$, with the gradient shown in Table 3.1. The injection volume was set at 1 μL with the total run time at 7 min, including the equilibration step.

Table 3.1. Gradient conditions for $UPC^2$-MS/MS analysis of DTDP-derivatised thiols.

| | **Time (min)** | **Flow (mL/min)** | **% A ($CO_2$)** | **% B (MeOH)** | **Gradient curve** |
|---|---|---|---|---|---|
| 1 | Initial | 1.5 | 99 | 1 | - |
| 2 | 2.7 | 1.5 | 92 | 8 | 5 |
| 3 | 4.5 | 1.5 | 90 | 10 | 8 |
| 4 | 5.0 | 1.5 | 70 | 30 | 6 |
| 5 | 5.5 | 1.5 | 70 | 30 | 6 |
| 6 | 5.7 | 1.5 | 99 | 1 | 6 |
| 7 | 7.0 | 1.5 | 99 | 1 | 6 |

Adapted from Mafata et al. (2018;3).

Quantitative mass spectrometry detection was carried out using a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, USA). A makeup pump was attached to the coupler that fed 1% (v/v) formic acid in methanol into the mixer preceding the MS line at a constant flow rate of 0.2 mL $min^{-1}$. Thiol-DTDP derivatives were analysed in multiple reaction monitoring (MRM) mode using an electrospray probe in the positive ionization mode (ESI+). The following settings were used: capillary voltage 3.8 kV, source temperature 120 ºC, desolvation temperature 500 ºC, desolvation gas 1000 L $h^{-1}$, and cone gas 150 L $h^{-1}$.

### *3.2.5.2 Methoxypyrazine Analysis*

***Reagents and standards***

All samples were prepared and analysed by the Central Analytical Facility (CAF) at Stellenbosch University according to the methods described by Kotseridis et al. (1998:72) and Godelmann et al. (2008:450). Reference standards of ibMP (purity 99%), ipMP (purity 97%) and sbMP (purity 99%) were obtained from Sigma-Aldrich, South Africa. The isotopically labelled internal standards were synthesized according to the procedures described by Kotseridis et al. (1998:72) and Godelmann et al. (2008:450). The purity was verified by a full-scan GC-MS analysis.

Stock solutions of each pyrazine of 2000 μg $mL^{-1}$ were prepared in analytical grade absolute ethyl alcohol and stored at 4 °C. The standard solutions were diluted as required for calibration standards (ibMP and sbMP) with the concentration ranges shown in Table 3.2. All reagents $H_2SO_4$-$D_2$ (in $D_2O$) had a purity of 96%, deuterium oxide ($D_2O$), ethyl alcohol abs. pro analysis (p.a.), sodium carbonate p.a., sodium chloride purissimum (puriss). p.a., sodium sulphate p.a., were purchased from Merck Life Sciences, South Africa.

Table 3.2. The concentration range of the sbMP and ibMP standards used for the calibration curve.

| Concentration (ng $L^{-1}$) | | |
|---|---|---|
| **Range** | **SBMP** | **IBMP** |
| Level 1 | 1.00 | 1.00 |
| Level 2 | 2.50 | 2.50 |
| Level 3 | 5.00 | 5.00 |
| Level 4 | 10.00 | 10.00 |
| Level 5 | 25.00 | 25.00 |
| Level 6 | 50.00 | 50.00 |

***Sample preparation***

The wine (10 mL) was transferred into an SPME vial. ibMP d3 (100 μL) was added as an internal standard. Three millilitres of a 30% sodium chloride (NaCl) solution were added. Both the samples and calibration standards were treated similarly. The mixture was vortexed for 60 seconds. The headspace of the sample was analysed with a PDMS/DVB SPME fibre (Pink).

The SPME vial with the sample was equilibrated for 5 min at 50 °C in the TriPlus RSH autosampler incubator shaken at 250 rpm. Subsequently, a coated fibre was exposed to the sample headspace for 20 min at 50 °C. After extraction, desorption of the volatile compounds from the fibre coating was carried out in the injection port of the gas chromatography-mass spectrometry instrument, i.e., Thermo TSQ 8000 triple quadrupole mass spectrometer (MS/MS) with separation conducted on a Thermo TRACE™ 1310 gas chromatograph (GC) for 10 min.

***Chromatographic separations***

The sample (1 µL) was injected on a Thermo TSQ 8000 triple quadrupole MS/MS operated in a selected reaction monitoring (SRM) mode. Separation of the methoxypyrazine compounds were performed on a Thermo TRACE™1310 gas GC coupled with a non-polar ZB-5Ms (30 m, 0.25 mm ID, 0.25 µm film thickness) capillary column. Helium was used as the carrier gas at a flow rate of 1 mL $min^{-1}$. The injector was operated in splitless mode and the temperature was maintained at 250 °C. The oven temperature was programmed as follows: 60 °C for 1 min; and finally ramped up to 250 °C at a rate of 15 °C/min and held for 2 min. The ionization source temperature was set at 250 °C and emission current of 50 µA was used with Argon collision.

### 3.2.6 Experimental design and Statistical analysis

The experiment was conducted on Sauvignon blanc (from three regions) and Chenin blanc grapes (from one region). For each region, the experimental design was completely random with nine treatment combinations replicated three times. The treatment structure was comprised of four-wine-making stages (WG, MG, TM and CJ), two cryogenic temperatures (-20 ºC and -4 ºC) and a winemaking control. Sensory and chemical evaluations were conducted after T0 and T4 storage.

One ANOVA assumption is that the residuals must follow a normal distribution, however, residuals (deviation of an observation from the predicted model value) are standardised to normal (0, 1) (by subtracting the mean and dividing by the standard deviation) to be able to detect outliers. It is expected that 99% of the data should fall within three standard deviations from the mean (0) when the data is normally distributed. Therefore, values with standardised residuals > 3 are identified as outliers. In addition, standardised residuals from the model were tested for deviation from normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965:610). Observations deviating by more than three standard deviations from the model value were identified as outliers and removed. Following the confirmation of panel reliability and normality, all subsequent statistical analyses of sensory data were conducted on means over judges for the twenty-seven wines, according to the experimental design.

All analyses were performed separately for each region and storage period (T0 and T4). Univariate analysis of variance (ANOVA) was performed on sensory and instrumental data using the General Linear Models (GLM) procedure of SAS software (Version 9.4; SAS Institute Inc., Cary, NC, USA). Fisher's least significant difference was calculated at the 5% level to compare treatment means. A probability level of 5% was considered significant for all significance tests. Principal component analysis (PCA) of the sensory and instrumental data, using the correlation matrix, was performed to visualise and elucidate the association between the treatments and observed variables. Discriminant analysis (DA) was done to determine whether treatments can be mathematically distinguished based on the observed variables. Multivariate analyses were conducted using XLStat (Addinsoft (2022). XLSTAT statistical and data analysis solution. New York, USA. https://www.xlstat.com/en).

## 3.3 Results and discussion

### 3.3.1 Effect of cryogenic treatments on the physicochemical parameters of grape must

The pre-fermentation chemical analyses performed on both the Sauvignon blanc and Chenin blanc grape must fell within the ranges expected (Table 3.3) for South African Sauvignon blanc and Chenin blanc grape must, with values from the two vintages being quite similar (Tables 3.4 to 3.10). A total soluble solid (TSS) of 22 °Brix was the desired ripeness for both cultivars, but harvesting depended on when the commercial vineyards were ready to harvest their grapes. At the time of harvesting the Brix values ranged from 18-22 ºB for the Sauvignon blanc and 20 ºB for the Chenin blanc.

Table 3.3: Typical ranges of physicochemical parameters for Sauvignon blanc and Chenin blanc grape must in SA.

| Grape cultivar | Brix (ºB) | Malic acid (g $L^{-1}$) | pH | Total Acid (g $L^{-1}$) | Total Sugar (g $L^{-1}$) | Haze (NTU) | YAN (mg N $L^{-1}$) |
|---|---|---|---|---|---|---|---|
| Sauvignon blanc | 20-24 | 2-6.5 | 3.2-3.4 | 6-9 | 200-240 | 50-150 | 140-150 |
| Chenin blanc | 20-24 | 2-6.5 | 3.2-3.4 | 6-9 | 200-240 | 50-150 | 140-150 |

Ribereau-Gayon et al., 2006:5; Volschenk et al.,2006:126; Nicolini et al., 2011:89; Miller, 2023:5

The concentration of TSS in this study was not affected by the cryogenic freezing treatments, which were similar to the findings of previous research which showed that TSS is the parameter least affected by freezing, especially for storage periods less than six months (Cynkar et al., 2004:240-241; Cynkar et al., 2009:657; Garcia et al., 2011:164; Olarte Mantilla et al., 2013:352; Santesteban et al., 2013:3011, Zhang et al., 2015:21611; Pedrosa-López et al., 2022:5). However, in studies conducted by Ruiz-Rodríguez et al. (2020:5-6) and Modesti et al., 2021:5, it was found that different freezing techniques, i.e., liquid nitrogen as opposed to ultra-fast mechanical freezing, resulted in significant differences in TSS values. Furthermore, the total sugar levels and glucose/fructose ratios for the control and cryogenically treated Sauvignon blanc and Chenin blanc grape and must samples for all the producers and both vintages (2020 and 2021) compared well with the TSS values for the control and cryogenic treatments (Tables 3.4-3.10). The glucose and fructose levels were also unaffected by the cryogenic treatments and remained within acceptable ratios, which were similar to those found in previous studies (Threlfall et al., 2006:164-167; Garcia et al., 2011:164; Olarte Mantilla et al., 2013:352). The consistency between the measured sugar levels, glucose/fructose ratios, and TSS values is expected, as cryogenic treatment did not alter sugar content, and glucose and fructose together account for the largest portion of TSS in grape must.

Malic acid levels in mature grapes can range between 4-6.5 g $L^{-1}$ in cooler climate regions and between 1-2 g $L^{-1}$ in warmer climate regions (Ribereau-Gayon et al., 2006:5; Volschenk et al., 2006:126; Miller, 2023:5). The prevalence of malic acid in must from vineyards in cooler climate regions is directly related to the temperature, with the levels of malic acid being lower in grapes from warmer regions (Ribereau-Gayon et al., 2006:12; Volschenk et al., 2006:126; Miller, 2023:5). The malic acid levels in this study were found to vary between treatments, vintages and the Producers from the different regions. In grape must from Producer A, malic acid levels ranged from 3.42-4.90 and 2.67-6.28 g $L^{-1}$ for the 2020 and 2021 vintages, respectively (Tables 3.4 and 3.5). During the 2020 vintage, it was observed that the malic acid levels for the WG and MG stored for T4 were slightly lower than all the other treatments, ranging from 3.42-3.57 g $L^{-1}$. These findings agree with previous research that showed grape skins to be rich in malic acid, and even after processing, most of the total malic acid remains in the skin (Garcia et al., 2011:166). However, during the 2021 vintage, all the TM and CJ treatments were found to be lower. Furthermore, all the must originating from the cryogenic treatments during the subsequent vintage were found to be lower than the levels found in the control. These findings highlight that differences in malic acid content are influenced not only by the type of cryogenic treatment applied but also by the winemaking stage and vintage, indicating a complex interaction between treatment conditions and seasonal variation. Malic acid levels for grape must originating from Producer B ranged between 3.28-5.08 and 3.09-4.64 g $L^{-1}$ for hand and machine-harvested grapes, respectively during 2020 and between 2.93-6.04 g $L^{-1}$

during 2021 (Tables 3.6-3.7). Overall, for Producer B it was seen that the malic acid levels for must from the cryogenic treatments were lower than that of the control must for both vintages.

The grape must that originated from Producer C had malic acid levels between 3.75-5.45 and 3.54-5.6 g $L^{-1}$ for the 2020 and 2021 vintages, respectively (Tables 3.8-3.9). Overall, the malic acid levels for Producer C were quite similar for both vintages. However, despite this general similarity, malic acid levels differed among specific treatments, notably during the 2020 vintage, where the malic acid levels were lower for the T4 cryogenic treatments, particularly WG, whilst during the 2021 vintage, it was higher than all the other treatments. For Producer D the malic acid levels ranged between 2.67-4.75 g $L^{-1}$ and were similar when comparing the control to the treatments, however, it was observed that all the must derived from the cryogenic treatments (-4 ºC and -20 ºC) stored for T4 had slightly higher malic acid levels than the T0 treatments. In some cases, especially for certain WG and MG samples stored at T4, higher malic acid levels were observed, which may be attributed to prolonged skin contact and potential cellular damage caused by ice crystal formation during the cryogenic treatment. However, this trend was not consistent across all producers or treatments, suggesting that other factors may also influence malic acid retention. It was also observed that malic acid levels in the control must were higher for the 2021 vintage than the 2020 vintage, highlighting the potential role that vintage plays in the formation of grape components. The results obtained in this research differed from a previous study where no change in MA levels for fresh and frozen grape must was detected (Garcia et al., 2011:165). Chidi et al. (2018:11) illustrated the importance of rapid, sensitive and accurate analytical techniques to provide winemakers with a better understanding of acid development and how cellar practices could be used to modulate acid profiles. This is important because the organic acids contribute to the flavour profile and organoleptic properties of wines (Ribereau-Gayon et al., 2000:8-13).

Higher pH levels were observed in grape must samples for all Producers for the Sauvignon blanc and Chenin blanc taken after freezing and defrosting (Tables 3.4-3.10) for most treatments, but more so in the whole grape and macerated grape treatments that were frozen at T4 during both vintages. The pH levels for the Sauvignon blanc ranged between 3.07-3.84 for the cryogenically treated grape must compared to the unfrozen controls which ranged between 3.12-3.42. pH values for the Chenin blanc ranged between 3.22-3.67 for the cryogenically treated grapes and grape must compared to the unfrozen control which had a pH of 3.39. The results obtained were similar to the findings of previous studies which found the increase in pH to be due to the precipitation of potassium ($K^+$) salts during the freezing process and the lower solubility of the acidic salts when the grapes were defrosted, both effects have previously been reported in literature (Darius-Martín et al., 2000:484; Schmid & Jiranek, 2011:29; Olarte Mantilla et al., 2013:352-353; Santesteban et al., 2013:3011;3015; Zhang et al., 2015:21611, Ruiz-Rodríguez et al., 2020:5; Pedrosa-López et al., 2022:5).

Total acidity (TA) is a chemical parameter most affected by freezing and frozen storage. This makes sense considering that cold temperature treatments are commonly used for wine tartaric acid stabilisation during the white winemaking process (Pedrosa-López et al., 2022:5; Threlfall et al., 2006:164-166; Garcia et al., 2011:164; Olarte Mantilla et al., 2013:353; Santesteban et al., 2013:3011; Zhang et al., 2015:21610-21611). In this study, in agreement with the observations by the aforementioned authors, the TA levels in the grape must subjected to pre-fermentative cryogenic treatments were lower than the TA levels in the unfrozen control must (Tables 3.4-3.10). The TA levels in the unfrozen control grape must from Producer A, ranged from 5.96 to 9.89 g $L^{-1}$ for 2020 and 2021, respectively (Tables 3.4-3.5). Grape must from the WG, MG, TM and CJ subjected to cryogenic treatments (-4 ºC and -20 ºC) generally had lower TA levels ranging from 3.67-7.73 and 2.43-9.01 g $L^{-1}$ for 2020 and 2021, respectively. During the 2020 vintage, it was seen that the TA levels from the TM (6.99-7.73 g $L^{-1}$) and CJ (7.27-7.38 g $L^{-1}$) treatments frozen at T4 were higher than those of the T0 TM (5.94-5.98 g $L^{-1}$) and CJ (5.11-5.49 g $L^{-1}$) treatments (Table 3.4). However, during the 2021 vintage, the inverse was observed for the TM and CJ treatments, where at T0, higher TA levels were observed (Table 3.5).

Total acidity levels for grape must originating from Producer B for the hand and machine-harvested control grapes were 8.36 and 6.07 g $L^{-1}$, respectively during the 2020 vintage and 9.35 g $L^{-1}$ for the hand-harvested grapes from the 2021 vintage (Tables 3.6 and 3.7). For the cryogenic treatments, it was seen that the total acidity levels for must from the hand-harvested grapes ranged between 4.42-4.47 g $L^{-1}$ and 4.72-6.52 g $L^{-1}$ during the 2020 and 2021 vintages, respectively and between 3.93-6.26 g $L^{-1}$ for the machine-harvested grapes during the 2020 vintage. It was observed that the TA levels were generally lower for the must obtained from the machine-harvested cryogenic treatments, except for the TM treatment stored at T4 at -4 ºC during the 2020 vintage, where the TA level was 6.26 g $L^{-1}$ and similar to the machine-harvested control. Hand-harvested grapes, therefore, retained higher acidity than machine-harvested grapes. For Producer C, the TA levels for the grape must from the unfrozen control ranged between 7.31 and 6.45 $L^{-1}$ in 2020 and 2021, respectively, whilst the TA levels for the grape must from the cryogenic treatments ranged from 3.94-7.46 and 4.57-7.05 g $L^{-1}$, for the 2020 and 2021 vintages, respectively (Tables 3.6-3.7).

Furthermore, the TA level for the unfrozen control from Producer D was 6.88 g $L^{-1}$ whilst the TA levels from the cryogenic treatments were once again lower and ranged between 3.77-6.03 g $L^{-1}$. Most TA levels for Producer D were similar for all treatments except for the WG and TM, frozen for T0, with the lowest TA levels ranging between 3.94-4.26 g $L^{-1}$ and 3.77-3.81 g $L^{-1}$, respectively. Overall, it was seen that TA levels for the unfrozen control must for Producers A and B were generally higher during the 2021 vintage for both Sauvignon blanc and Chenin blanc grape must when compared to grape must from the 2020 vintage. However, for Producer C, the TA levels for the unfrozen control must was higher for the 2020 vintage when compared to 2021. Moreover, the TA levels for all must subjected to cryogenic treatments were generally lower, with differences observed between vintages and regions.

Haze in wine is caused by pathogenesis-related (PR) proteins, specifically thaumatin-like proteins and chitinases which are synthesised in the grape berry during véraison (Pocock et al., 2000:1637). These proteins are nitrogenous compounds that contribute significantly to the undesirable yeast non-assimilable nitrogen (YNAN) concentration in grape must and wine and are predominantly responsible for white wine's haze formation (Bell & Henschke, 2005:280; Casalta et al., 2015:3-6;13-14; Casalta et al., 2019:152). Additionally, the application of nitrogen in the vineyard could increase the levels of protein accumulated during the ripening of the grape berries and subsequently increase the undesirable YNAN component also potentially increasing the risk of haze formation in the wine (Pocock et al., 2000:1641). Furthermore, the rate at which the nitrogen is applied in the vineyard should be taken into consideration for different grape varieties as it was observed that Sauvignon blanc berries accumulated twice the amount of protein when compared to Sultana grape berries cultivated under the same conditions (Pocock et al., 2000:1641; Bell & Henschke, 2005:280).

The haze levels obtained from the cryogenically pre-treated Sauvignon blanc grape must from Producer A ranged between 850.33-853.67 nephelometric turbidity units (NTU) for most treatments during the 2020 vintage when compared to the unfrozen control must (4.32 NTU). However, the haze levels for must obtained from the cryogenically treated WG (108.27-141.87 NTU) and MG (103.60-108.27 NTU) stored at both -4 ºC and -20 ºC at T4 were much lower. During the 2021 vintage, haze levels for all the grape must were significantly lower, ranging from 2.58 to 194.33 NTU for the unfrozen control and cryogenically pre-treated must (Fig. 3.2 and Tables 3.4-3.5). The same observations were made for Producer B during the 2020 vintage where the haze levels for both the hand-harvested and machine-harvested unfrozen control must were 851.67 and 849.67 NTU, respectively (Tables 3.6-3.7). The haze levels for the grape must obtained from the cryogenically pre-treated hand-harvested WG ranged between 17.65-78.36 NTU and for the machine-harvested MG, TM and CJ the levels ranged from 17.20-110.10, 1.61-4.17 and 2.64-3.95 NTU, respectively. No noteworthy differences were observed between the haze levels from the hand and machine-harvested grape must. However, once again, during the 2021 vintage, the haze levels for the unfrozen control must was lower (138.00 NTU) when compared to the levels obtained from the control during the 2020 vintage. The haze levels for the remaining must from the cryogenically pre-treated WG, MG, TM and CJ ranged between 121.33 and 156.80 NTU across treatments (Fig. 3.2).

The haze levels for must from the unfrozen control and the cryogenically pre-treated WG, MG, TM and CJ from Producer C were once again higher during the 2020 vintage, ranging from 850.67-867.00 NTU compared to the levels obtained for the control and all treatments during the 2021 vintage which ranged from 84.00-166.13 NTU (Tables 3.8-3.9). The exception for the 2020 vintage was seen for the cryogenically pre-treated WG, frozen at both -4 ºC and -20 ºC at T4 ranging from 168.00 to 147.00 NTU, respectively. The haze levels for the Chenin blanc from Producer D were 98.00 NTU for the unfrozen control must and between 56.00 and 141.86 NTU for the cryogenically pre-treated must. The same trend was observed for all the producers across vintages in terms of higher haze levels being reported during the 2020 vintage when compared to the 2021 vintage, regardless of the wine-producing region that the grapes originated from. Moreover, it was noted that during the settling process, the Sauvignon blanc grape must did not adequately settle after twenty-four hours following the addition of a pectolytic enzyme at 14 ºC and the grape must for all treatments was allowed to settle for a further twenty-four hours. This yielded sufficiently clarified juice for the fermentations to commence. No problems were encountered with the settling of the Chenin blanc grape must.

Grape must composition is primarily dependent on the grape berry composition and can significantly influence the wine quality (Casalta et al., 2015:3-6; 13-14 Casalta et al., 2019:152). Nitrogen is an important macronutrient that plays a major role in many biological functions and processes of the grapevine and influences yeast growth, fermentation kinetics and flavour metabolism (Bell & Henschke, 2005:244; Helwi et al., 2016:9-23, Losada et al., 2011:890). If the nitrogen levels of the grape must are not optimal, it can be manipulated by adding nitrogen before the fermentation process (Bell & Henschke, 2005:244). Choné et al. (2006:4-5) confirmed that the addition of nitrogen after bloom significantly increased the concentrations of the cysteine precursor in Sauvignon blanc grapes. Furthermore, the increased concentration of nitrogen in the grape must influences the formation of volatile and non-volatile varietal compounds during alcoholic fermentation (Bell & Heschke, 2005:255; Vilanova et al., 2007:147;149-151; Roland et al., 2011a:7373; Vilanova et al., 2012:127; 2015:5-6; Helwi et al., 2016:9-13).

Sauvignon blanc and Chenin blanc grape must was supplemented with DAP at a concentration of 50 gh $L^{-1}$ as part of the standard winemaking practices. The overall

YAN levels for grape must from Producer A ranged from 1.61 to 1722.00 mg N $L^{-1}$ and 4.83 to 262.50 mg N $L^{-1}$ for the cryogenically pre-treated WG, MG, TM and CJ for the 2020 and 2021 vintages, respectively (Fig. 3.2 and Tables 3.4-3.5). The YAN levels for the unfrozen controls were 162.40 mg N $L^{-1}$ and 52.55 mg N $L^{-1}$ for the 2020 and 2021 vintages, respectively. The treatments with the lowest YAN levels during the 2020 vintage were the WG and MG stored at -4 ºC and -20 ºC at T4, ranging between 1.34-2.49 and 1.61-3.16 mg N $L^{-1}$, respectively. During the 2021 vintage, the treatments with the lowest YAN levels were from the cryogenically pre-treated CJ ranging from 4.83 to 6.64 mg N $L^{-1}$.

Yeast assimilable nitrogen levels in grape must originating from Producer B during the 2020 vintage ranged from 1838.67 to 2090.67 mg N $L^{-1}$ for the unfrozen machine and hand-harvested control grapes, respectively, whilst the levels were lower for the hand-harvested control grapes (76.67 mg N $L^{-1}$) during the 2021 vintage (Tables 3.6-3.7). The YAN levels for the cryogenically pre-treated hand-harvested WG and machine-harvested MG, TM and CJ for the 2020 vintage were 158.67-159.6; 168.00-166.13; 84.93-94.27 and 142.80-154.00 mg N $L^{-1}$, respectively. During 2021, the YAN values from the cryogenically pre-treated hand-harvested WG, MG, TM and CJ were 61.07-190.00; 52.73-149.33; 13.83-5.88 and 7.84-22.53 mg N $L^{-1}$, respectively. Moreover, for Producer B, it was observed that the YAN values were similar for both vintages, except for the unfrozen control must, which was much higher for the hand and machine-harvested grapes during the 2020 vintage (Fig. 3.2).

For Producer C, the YAN levels for the unfrozen control must were 1666.00 and 104.80 mg N $L^{-1}$ for the 2020 and 2021 vintages, respectively (Tables 3.8-3.9). The YAN levels for the cryogenically pre-treated WG, MG, TM and CJ for the 2020 vintage ranged from 1598.80 to 2128.00 mg N $L^{-1}$, except for the cryogenically pre-treated WG stored for a T4 period, which was lower and ranged from 8.29-21.64 mg N $L^{-1}$. During the 2021 harvest, the YAN levels for the cryogenically pre-treated WG, MG, TM and CJ ranged from 1.02-156.00 mg N $L^{-1}$. The treatments with the lowest YAN levels were observed for the grape must from the cryogenically pre-treated CJ stored at -4 ºC and -20 ºC for T0. Once again, for this producer, the YAN levels for the 2021 vintage were much lower compared to the 2020 vintage. Furthermore, for the Chenin blanc grapes from Producer D the YAN levels for the unfrozen control grape must was 36.34 mg N $L^{-1}$ for the 2021 vintage whilst the YAN levels for the cryogenically pre-treated WG, MG, TM and CJ ranged from 0.48-94.55 mg N $L^{-1}$ (Fig. 3.2 and Table 3.10). Although lower YAN levels were reported for some treatments, most levels were above the critical levels (140 to 150 mg N $L^{-1}$), and no stuck fermentations were reported. Moreover, a correlation was seen between the YAN levels and haze levels. During the 2020 vintage, the haze and YAN levels were higher for most producers compared to the 2021 vintage (Fig. 3.2 and Tables 3.4-3.10). Furthermore, the high levels of YAN reported were not unusual as Henschke & Jiranek (1993:80-83) reported that nitrogen levels of grape juice varied widely between viticultural regions (60-2400 mg N $L^{-1}$). Moreover, when comparing the YAN and haze results for the two vintages, the levels for both parameters were generally much higher for the 2020 vintage than for the 2021 vintage for all the producers, regardless of the treatments (Fig. 3.2). This indicates that the differences observed were not a result of the cryogenic treatments, but rather wine region and vintage differences. Previous research, however, found that YAN levels can be influenced by cryogenic treatments, where the YAN levels in the treated grape juice were higher than in the unfrozen control grape juice. Zhang et al. (2015:21610) reported that the cold maceration process led to increased YAN levels from moderate (274 g $L^{-1}$) in whole pressed grapes to high (326-327 g $L^{-1}$) in grapes pre-treated by cold maceration for 6 and 24 hours. This increase was attributed to the increased skin contact during the cryogenic treatments and prolonged storage periods. Nitrogen is primarily located in the pulp and skin of the grape berry, therefore increasing the extraction of the YAN which was reported to influence the formation of volatile and non-volatile varietal compounds during the alcoholic fermentation (Bell & Heschke, 2005:255;256; Vilanova et al.; 2007:147;149-151; Roland et al., 2011a:7373; Vilanova et al., 2012:127; 2015:5-6). Similar observations were made by Helwi et al. (2016:13), which demonstrated that higher vine and berry nitrogen levels led to increased levels of the glutathionylated precursor of 3-SH in Sauvignon blanc grape berries, must and wines, but not the cysteinylated precursor.

Additionally, this research also showed that the effect of the nitrogen levels was in combination with the grape processing technologies, i.e., immediate freezing with liquid nitrogen before processing, pressing grapes at room temperature and the method of pressing (Helwi et al., 2016:10). Overall, the chemical parameters following the cryogenic pre-treatments and defrosting remained stable, with the pH and total acidity being the most affected (Tables 3.5-3.10). These observations were similar to previous research that found the increase in pH to be due to the precipitation of potassium (K+) salts during the freezing process and the lower solubility of the acidic salts when the grapes were defrosted (Schmid & Jiranek, 2011:29; Olarte Mantilla et al., 2013:352-353; Santesteban et al., 2013:3011;3015; Zhang et al., 2015:21611; Ruiz-Rodríguez et al., 2020:5; Pedrosa-López et al., 2022:5). Furthermore, the TA levels for the WG, MG, TM and CJ, subjected to the T4 cryogenic treatments were generally, but not always lower than the T0 treatments for both vintages (Tables 3.4-3.10). Most differences observed in the measured wine chemical parameters from the WG, MG, TM, and CJ subjected to cryogenic treatment were attributed to the vintages.

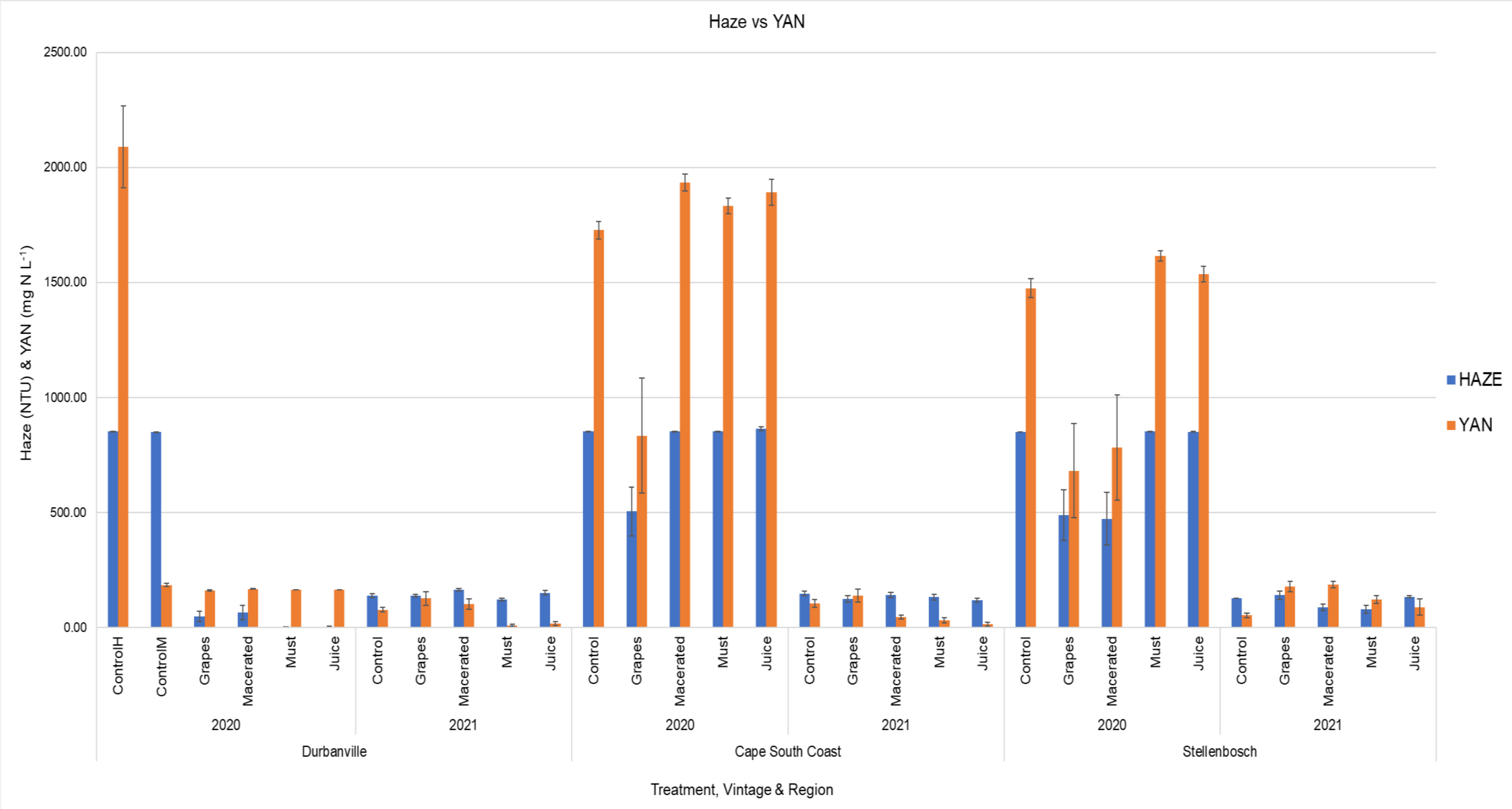



Figure 3.2. Graphical illustration of the total Haze (NTU) and YAN (mg N $L^{-1}$) measured in Sauvignon blanc grape must (means ± standard deviation [n=3]) from cryogenically pre-treated WG, MG, TM and CJ (T0 and T4) originating from Producers A, B & C from the Stellenbosch, Durbanville and Cape South Coast wine regions(2020 and 2021), respectively. Error bars represent the standard error of the mean (SEM).

Table 3.4: Chemical profiles of Sauvignon blanc must from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer A from the Stellenbosch wine region) in 2020.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Brix (ºB) | Malic acid ($g L^{-1}$) | pH | Total acid ($g L^{-1}$) | Total sugar ($g L^{-1}$) | Glucose ($g L^{-1}$) | Fructose ($g L^{-1}$) | Haze (NTU) | YAN ($mg N L^{-1}$) |
| **Control** | na[3] | na | 22.43±0.71 | 4.41±0.07 | 3.42±0.04 | 5.96±0.27 | 235.96±6.44 | 115.95±5.43 | 105.16±4.54 | 4.32±0.76 | 162.40±0.00 |
| **WG[4]** | -4 | 0 | 21.08±0.37 | 4.18±0.13 | 3.57±0.02 | 5.22±0.27 | 221.65±3.82 | 177.04±25.56 | 163.73±23.84 | **852.33±3.51**[5] | **1344.00±0.00** |
| | -20 | 0 | 20.70±0.92 | 4.19±0.12 | 3.68±0.04 | 4.59±0.39 | 218.97±10.54 | 164.09±16.47 | 151.56±16.04 | **852.33±1.15** | **1330.00±19.80** |
| | -4 | 4 | **17.91±0.77** | 3.42±0.32 | 3.55±0.03 | 4.05±0.57 | 182.42±8.24 | 148.89±24.94 | 133.23±16.49 | 141.87±3.23 | 1.61±0.81 |
| | -20 | 4 | **17.83±0.94** | 3.53±0.25 | 3.51±0.01 | 4.39±0.22 | 180.32±10.25 | 61.01±1.29 | 80.76±17.40 | 108.27±39.33 | 3.16±3.53 |
| **MG** | -4 | 0 | 22.15±0.95 | 4.54±0.08 | 3.45±0.02 | 5.49±0.16 | 234.14±10.74 | 171.86±10.51 | 161.99±13.59 | **851.00±0.00** | **1620.00±84.85** |
| | -20 | 0 | 21.77±0.28 | 4.81±0.26 | 3.49±0.02 | 5.75±0.14 | 229.58±2.44 | 184.81±14.27 | 164.31±6.14 | **852.33±2.31** | **1596.00±0.00** |
| | -4 | 4 | 20.01±1.08 | 3.57±0.10 | 3.55±0.03 | 4.11±0.32 | 203.69±11.49 | 76.06±19.36 | 109.54±7.38 | 108.27±6.47 | 1.34±0.33 |
| | -20 | 4 | 17.37±0.69 | 3.46±0.07 | 3.51±0.05 | 3.67±0.06 | 176.95±7.02 | 77.16±10.30 | 81.29±1.85 | 103.60±11.88 | 2.49±2.28 |
| **TM** | -4 | 0 | 21.08±0.70 | 4.72±0.25 | 3.41±0.07 | 5.94±0.61 | 222.26±7.98 | 180.93±7.93 | 141.27±0.62 | **850.33±0.58** | **1652.00±39.60** |
| | -20 | 0 | 21.36±0.45 | 4.73±0.14 | 3.45±0.01 | 5.98±0.56 | 225.10±4.99 | 164.52±4.27 | 148.23±3.08 | **851.33±0.58** | **1652.00±39.60** |
| | -4 | 4 | 19.15±0.13 | 4.64±0.14 | **3.19±0.02** | **6.99±0.21** | 197.49±1.50 | 196.48±0.62 | 179.96±2.45 | **850.33±0.58** | **1722.00±59.40** |
| | -20 | 4 | 20.76±0.15 | 4.90±0.04 | **3.27±0.03** | **7.73±0.30** | 214.28±1.67 | 189.11±7.04 | 171.21±7.33 | **853.67±5.51** | **1498.00±59.40** |
| **CJ** | -4 | 0 | 21.07±1.11 | 4.70±0.19 | 3.47±0.04 | 5.49±0.09 | 216.09±3.69 | 158.05±8.55 | 141.71±3.68 | **850.67±1.53** | **1470.00±19.80** |
| | -20 | 0 | 21.38±1.74 | 4.41±0.19 | 3.47±0.05 | 5.11±0.09 | 237.43±0.62 | 163.66±1.83 | 149.97±3.08 | **851.00±0.00** | **1582.00±59.40** |
| | -4 | 4 | 20.84±0.42 | 4.78±0.28 | **3.28±0.04** | **7.38±0.29** | 215.94±0.18 | 168.98±14.04 | 165.83±23.97 | **852.52±0.00** | **1458.80±0.00** |
| | -20 | 4 | 20.80±0.27 | 4.86±0.52 | **3.28±0.03** | **7.27±1.04** | 217.50±1.85 | 133.70±11.72 | 179.93±13.49 | **850.67±0.58** | **1540.00±0.00** |

[1]Means ± standard deviation (n=3) for all samples.
[2]Cryogenic temperature treatment.
[3]Not applicable – na.
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[5]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.

Table 3.5: Chemical profiles of Sauvignon blanc must from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer A from the Stellenbosch wine region) in 2021.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Brix (ºB) | Malic acid ($g L^{-1}$) | pH | Total scid ($g L^{-1}$) | Total sugar ($g L^{-1}$) | Glucose ($g L^{-1}$) | Fructose ($g L^{-1}$) | Haze (NTU) | YAN ($mg N L^{-1}$) |
| **Control** | na[3] | na | 20.06±0.54 | 6.16±0.24 | 3.37±0.03 | **9.89±0.37**[5] | 198.21±5.73 | 83.77±8.52* | 98.67±23.97* | 126.00±0.00* | 52.55±22.84* |
| **WG[4]** | -4 | 0 | **19.05±1.70** | 3.75±0.40 | 3.40±0.02 | 5.09±0.28 | 192.56±17.68 | 94.42±10.80 | 128.66±23.05 | 112.00±14.00 | 130.66±23.63 |
| | -20 | 0 | **18.93±1.62** | 4.23±0.49 | 3.44±0.04 | 5.42±0.47 | 191.92±16.46 | 88.55±19.99 | 101.15±12.43 | 112.00±0.00 | **262.50±79.90** |
| | -4 | 4 | 20.47±0.32 | 4.15±0.27 | **3.81±0.05** | 4.58±0.56 | 207.11±3.57 | 98.74±13.66 | 90.70±12.05 | 63.80±13.29* | 144.66±8.08 |
| | -20 | 4 | 20.53±0.24 | 3.64±0.75* | **3.84±0.03** | 3.43±1.07 | 206.43±2.51 | 100.75±5.75 | 93.60±6.75 | 194.33±25.32 | 141.86±14.37 |
| **MG** | -4 | 0 | 19.75±1.15 | 4.29±0.61 | 3.41±0.01 | 5.47±0.64 | 200.37±11.73 | 106.23±16.99 | 115.48±25.52 | 118.66±11.55 | 209.66±60.14 |
| | -20 | 0 | 20.71±0.84 | 4.36±0.22 | 3.47±0.01 | 5.47±0.12 | 210.28±8.42 | 102.34±16.01 | 112.41±8.03 | 109.33±14.74 | 231.00±40.78 |
| | -4 | 4 | 22.85±0.28 | 4.01±0.44 | **3.82±0.02** | 4.16±0.31 | 233.05±3.65 | 95.57±16.66 | 87.80±14.62 | 9.17±3.18 | 163.33±8.08 |
| | -20 | 4 | 22.05±0.42 | 3.75±0.15 | **3.77±0.02** | 3.45±0.06 | 225.12±5.12 | 90.97±4.75 | 87.37±3.06* | 91.85±5.86 | 140.00±28.00 |
| **TM** | -4 | 0 | 19.63±0.75 | 5.52±0.18 | 3.42±0.04 | **8.60±0.41** | 194.92±7.41 | 93.39±0.72 | 103.74±.55 | 112.00±0.00 | **176.33±49.37** |
| | -20 | 0 | 20.50±0.87 | 5.77±0.22 | 3.46±0.05 | **8.43±0.67** | 204.06±9.09 | 105.01±14.80 | 102.75±5.17 | 124.33±10.78 | 100.33±8.33 |
| | -4 | 4 | **18.36±1.02*** | 2.78±0.26 | 3.55±0.03 | **2.88±0.59** | 185.52±10.14* | 61.32±13.49* | 55.92±11.57 | 2.58±0.53 | 138.13±25.25* |
| | -20 | 4 | **17.92±0.22*** | 2.67±0.05* | 3.55±0.00* | **2.43±0.14*** | 180.91±2.16* | 67.36±7.32* | 60.42±6.75 | 112.00** | 36.90±3.82* |
| **CJ** | -4 | 0 | 20.46±0.23 | 6.28±0.84 | 3.45±0.06 | **8.85±1.21** | 204.14±3.56 | 97.84±13.69 | 109.03±22.17 | 133.33±13.01 | 114.00±15.55 |
| | -20 | 0 | 20.11±0.44 | 6.14±0.23 | 3.46±0.02 | **9.01±0.26** | 199.39±4.17 | 107.83±15.67 | 98.89±6.52 | 119.33±11.55 | 120.66±26.27 |
| | -4 | 4 | 21.21±0.19 | 3.52±0.27 | 3.56±0.04 | 4.04±0.78 | 213.41±3.23 | 73.69±8.02 | 66.94±5.28 | 146.53±19.05 | 4.83±1.97 |
| | -20 | 4 | 20.34±0.28 | 3.18±0.49 | 3.57±0.03 | 3.34±0.55 | 204.80±2.84 | 96.15±10.12 | 93.02±15.38 | 135.33±21.38 | 6.64±1.51 |

[1]Means ± standard deviation (n=3) for all samples.
[2]Cryogenic temperature treatment
[3]Not applicable – na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[5]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
*Means ± standard deviation (n=2), outliers were removed.

Table 3.6: Chemical profiles of hand-harvested and machine-harvested Sauvignon blanc grape must from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer B in the Durbanville wine region) in 2020.

| Treatment | Harvesting methods | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Brix (ºB) | Malic acid (g $L^{-1}$) | pH | Total acid (g $L^{-1}$) | Total sugar (g $L^{-1}$) | Glucose (g $L^{-1}$) | Fructose (g $L^{-1}$) | Haze (NTU) | YAN (mg N $L^{-1}$) |
| **Control** | H[4] | na[3] | na | 22.17±0.12 | 5.08±0.66 | 3.25±0.12 | **8.36±1.37**[7] | 223.45±1.95 | 165.82±11.68 | 149.82±14.71 | **851.67±0.58** | **2090.67±308.42** |
| **WG[6]** | H | -4 | 4 | 14.48±2.38 | 3.29±0.14 | **3.40±0.06** | 4.42±0.28 | 148.99±22.82 | 98.27±2.02 | 90.62±2.31 | 17.65±3.70 | 158.67±8.08 |
| | | -20 | 4 | 15.33±1.31 | 3.28±0.19 | **3.39±0.01** | 4.47±0.27 | 157.21±12.28 | 173.17±2.81 | 163.42±4.57 | 78.36±67.00 | 159.60±7.41 |
| **Control** | M[5] | na[3] | na | 21.93±0.10 | 4.64±0.03 | **3.63±0.03** | **6.07±0.24** | 224.11±1.42 | 149.98±21.05 | 123.49±49.07 | **849.67±0.58** | **1838.67±113.16** |
| **MG** | M | -4 | 4 | 15.61±0.56 | 3.37±0.11 | **3.51±0.02** | 4.41±0.10 | 160.14±5.55 | 85.39±4.82 | 78.57±2.19 | 17.20±5.08 | 168.00±0.00 |
| | | -20 | 4 | 15.79±1.30 | 3.34±0.08 | **3.50±0.02** | 4.27±0.20 | 162.45±13.12 | 108.33±2.70 | 93.22±3.59 | 110.10±88.29 | 166.13±3.23 |
| **TM** | M | -4 | 4 | 21.81±0.29 | 4.21±0.11 | **3.51±0.18** | **6.26±0.38** | 223.23±4.32 | 53.18±2.70 | 68.43±2.11 | 4.17±1.01 | 84.93±23.81 |
| | | -20 | 4 | 19.30±4.64 | 4.22±0.19 | **3.58±0.09** | **5.65±1.26** | 202.34±40.90 | 101.20±8.11 | 100.43±0.37 | 1.61±0.55 | 94.27±35.01 |
| **CJ** | M | -4 | 4 | 15.45±6.08 | 3.09±0.40 | 3.45±0.13 | 3.93±0.99 | 159.00±56.47 | 110.82±5.58 | 107.19±4.06 | 2.64±2.11 | 142.80±49.54 |
| | | -20 | 4 | 17.23** | 3.36 | 3.54 | 4.27 | 174.33 | 118.72 | 106.47 | 3.95±0.57 | 154.00±19.80 |

[1]Means ± standard deviation (n=3) for all samples, except the mechanical-harvested juice treatment at -20ºC which only had a single sample for the Brix, density, malic acid, pH, total acid
[2]Cryogenic temperature treatment.
[3]Not applicable (na).
[4]Hand-harvested grapes.
[5]Machine-harvested grapes.
[6]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[7]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
**Single values without standard deviation had outliers statistically removed.

Table 3.7: Chemical profiles of hand-harvested Sauvignon blanc grape must from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer B from the Durbanville wine region) in 2021.

| Treatment | Harvesting methods | Temp[2] (ºC) | Storage Time (Months) | Chemical Analyses[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Brix (ºB) | Malic acid (g L$^{-1}$) | pH | Total acid (g L$^{-1}$) | Total sugar (g L$^{-1}$) | Glucose (g L$^{-1}$) | Fructose (g L$^{-1}$) | Haze (NTU) | YAN (mg N L$^{-1}$) |
| **Control** | H[4] | na[3] | na | 22.11±0.40 | 6.04±0.32 | 3.32±0.01 | **9.35±0.21**[6] | 227.47±4.31 | 100.21±17.69 | 107.19±4.04 | 138.00±14.42 | 76.67±16.22 |
| **WG**[5] | H | -4 | 4 | 22.67±0.13 | 3.27±0.26 | **3.45±0.03** | **5.42±0.27** | 231.85±2.91 | 102.06±24.89 | 130.25±28.05 | 135.33±21.39 | 61.07±28.42 |
| | | -20 | 4 | 22.84±0.86 | 4.17±0.57 | **3.54±0.06** | **5.93±0.40** | 237.31±7.08 | 117.45±39.30 | 112.44±33.68 | 140.00±14.00 | 190.00±3.61 |
| **MG** | H | -4 | 4 | 24.22±0.93 | 4.36±0.22 | **3.47±0.02** | **6.21±0.15** | 249.01±10.20 | 95.58±1.32 | 79.25±11.56 | 169.87±3.23 | 52.73±21.00 |
| | | -20 | 4 | 23.78±0.76 | 4.18±0.19 | **3.47±0.01** | **5.55±0.18** | 246.65±7.98 | 121.49±18.14 | 117.36±12.54 | 155.87±13.81 | 149.33±14.19 |
| **TM** | H | -4 | 4 | 19.00±1.36 | 3.00±0.20 | 3.26±0.02 | 4.72±0.28 | 193.61±14.02 | 51.82±23.29 | 45.78±12.67 | 121.33±16.17 | 12.83±13.18 |
| | | -20 | 4 | 17.72±1.47 | 2.93±0.35 | 3.20±0.04 | 4.78±0.37 | 180.62±13.84 | 61.32±20.24 | 50.76±20.76 | 121.33±17.11 | 5.88±1.94 |
| **CJ** | H | -4 | 4 | 22.62±0.62 | 3.89±0.20 | 3.22±0.02 | **6.49±0.13** | 231.02±6.97 | 87.80±31.19 | 84.33±56.68 | 156.80±14.82 | 7.84±2.10 |
| | | -20 | 4 | 23.59±0.23 | 3.96±0.21 | 3.23±0.01 | **6.52±0.25** | 241.24±2.63 | 92.69±34.12 | 102.30±42.39 | 143.33±31.39 | 22.53±30.72 |

[1]Means ± standard deviation (n=3) for all samples, except for the Juice M_(-20ºC) treatment which had n=2.
[2]Cryogenic temperature treatment
[3]Not applicable - na
[4]Hand-harvested grapes.
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[6]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.

Table 3.8: Chemical profiles of Sauvignon blanc must from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer C from the Cape South Coast wine region) in 2020.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Brix (ºB) | Malic acid (g L$^{-1}$) | pH | Total acid (g L$^{-1}$) | Total sugar (g L$^{-1}$) | Glucose (g L$^{-1}$) | Fructose (g L$^{-1}$) | Haze (NTU) | YAN (mg N L$^{-1}$) |
| **Control** | na[3] | na | 18.95±0.41 | 4.89±0.25 | 3.12±0.03 | 7.31±0.64 | 193.09±2.28 | 192.38±4.60 | 169.96±0.62 | **853.00±1.00**[5] | **1666.00±19.80** |
| **WG[4]** | -4 | 0 | 20.09±0.99 | 4.24±0.23 | 3.42±0.03 | 7.35±0.13 | 200.55±3.25 | 180.72±6.10 | 160.74±2.46 | **852.14±0.00** | **1598.80±98.99** |
| | -20 | 0 | 18.96±0.34 | 4.27±0.43 | 3.51±0.01 | 6.33±0.36 | 188.23±0.64 | 155.46±12.21 | 146.92±9.84 | **862.33±11.68** | **1708.00±39.60** |
| | -4 | 4 | 13.13±2.11 | **3.84±0.09** | 3.43±0.06 | **3.97±0.31** | 123.20±7.31 | 61.75±1.84 | 65.20±1.23 | 168.00±0.00 | 8.29±3.19 |
| | -20 | 4 | 14.53±1.37 | **3.75±0.28** | 3.48±0.01 | **3.94±0.39** | 151.73±15.09 | 68.66±0.61 | 67.38±1.85 | 147.00±9.90 | 21.64±1.84 |
| **MG** | -4 | 0 | 19.18±1.31 | 5.45±0.25 | 3.31±0.02 | 7.06±0.27 | 207.96±1.77 | 179.57±7.23 | 154.759.84 | **854.00±1.73** | **1834.00±59.40** |
| | -20 | 0 | 18.49±0.99 | 4.24±0.23 | 3.42±0.03 | 7.35±0.13 | 200.55±3.25 | 180.72±6.10 | 160.74±2.46 | **852.14±0.00** | **1598.80±98.99** |
| | -4 | 4 | 17.95±1.24 | 4.50±0.38 | 3.31±0.01 | 6.21±0.48 | 178.23±8.87 | 139.04±9.77 | 130.84±11.68 | **851.50±0.71** | **1919.00±18.38** |
| | -20 | 4 | 18.76±0.83 | 4.25±0.01 | 3.31±0.06 | 6.65±0.43 | 189.10±5.82 | 130.25±5.11 | 123.72±4.47 | **852.67±1.15** | **2128.00±158.39** |
| **TM** | -4 | 0 | 19.61±1.05 | 5.35±0.18 | 3.28±0.01 | 7.08±0.33 | 196.15±2.83 | 128.77±12.09 | 134.75±2.46 | **853.00±2.65** | **1862.00±19.80** |
| | -20 | 0 | 16.25±1.05 | 4.56±0.66 | 3.34±0.04 | 5.99±0.65 | 202.13±8.63 | 176.24±15.89 | 178.64±1.82 | **851.67±3.06** | **1848.00±79.20** |
| | -4 | 4 | 18.01±0.80 | 4.28±0.01 | 3.34±0.01 | 5.83±0.15 | 190.83±2.26 | 140.55±16.78 | 132.56±1.82 | **852.67±0.58** | **1988.00±39.60** |
| | -20 | 4 | 18.76±1.27 | 4.31±0.01 | 3.35±0.01 | 6.06±0.38 | 186.97±1.65 | 156.00±17.61 | 175.60±18.47 | **852.00±0.00** | **1750.00±59.40** |
| **CJ** | -4 | 0 | 19.51±1.00 | 4.91±0.95 | 3.30±0.03 | 6.64±0.85 | 208.80±6.80 | 174.89±5.49 | 162.57±7.38 | **867.00±12.73** | **1848.00±39.60** |
| | -20 | 0 | 17.67±0.62 | 4.81±0.61 | **3.07±0.03** | 7.46±1.01 | 178.52±1.71 | 140.77±6.11 | 132.58±9.23 | **852.67±3.06** | **1904.00±118.79** |
| | -4 | 4 | 20.06±0.75 | 4.34±0.01 | 3.35±0.02 | 6.19±0.11 | 205.26±3.54 | 124.36±1.49 | 114.07±2.76 | **851.33±2.31** | **1820.00±0.00** |
| | -20 | 4 | 20.26±0.56 | 4.37±0.01 | 3.35±0.02 | 6.45±0.16 | 208.05±2.25 | 127.90±1.80 | 126.62±11.50 | **850.67±1.15** | **2058.00±98.99** |

[1]Means ± standard deviation (n=3) for all samples.
[2]Cryogenic temperature treatment
[3]Not applicable – na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[5]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.

Table 3.9: Chemical profiles of Sauvignon blanc must from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer C from the Cape South Coast wine region) in 2021.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Brix (ºB) | Malic acid (g L$^{-1}$) | pH | Total acid (g L$^{-1}$) | Total sugar (g L$^{-1}$) | Glucose (g L$^{-1}$) | Fructose (g L$^{-1}$) | Haze (NTU) | YAN (mg N L$^{-1}$) |
| **Control** | na[3] | na | 20.29±0.59 | 4.10±0.44 | 3.27±0.06 | 6.45±0.78 | 204.97±6.38 | 101.51±26.79 | 88.08±23.05 | 147.33±24.68 | 104.80±48.01 |
| **WG[4]** | -4 | 0 | 19.27±0.56 | 3.84±0.31 | 3.49±0.02 | 6.45±0.47 | 192.28±5.99 | 50.38±23.72 | 46.08±25.61 | 86.00±14.42 | 48.80±19.72 |
| | -20 | 0 | 20.70±0.83 | 3.71±0.06 | 3.59±0.04 | 5.26±0.14 | 209.41±9.05 | 69.09±19.46 | 70.42±29.56 | 84.00±0.00 | 126.00±32.51 |
| | -4 | 4 | 22.91±0.89 | **5.60±0.47**[5] | **3.68±0.03** | 5.77±0.54 | 216.17±4.13 | 67.08±16.05 | 83.75±11.48 | 158.67±16.17 | 156.00±26.87 |
| | -20 | 4 | 23.60±0.14 | **5.06±0.17** | **3.68±0.04** | 6.04±0.67 | 217.30±6.55 | 90.68±15.35 | 82.59±16.24 | 166.13±50.19 | 140.17±24.06 |
| **MG** | -4 | 0 | 20.16±0.13 | 3.54±0.54 | 3.47±0.04 | 5.43±0.41 | 204.18±1.43 | 111.98±35.41 | 104.61±34.78 | 118.67±42.91 | 63.77±34.96 |
| | -20 | 0 | 20.77±0.13 | 3.94±0.08 | 3.49±0.03 | 5.46±0.40 | 211.08±1.35 | 118.38±47.82 | 110.70±43.68 | 98.00±8.00 | 26.30±1.91 |
| | -4 | 4 | 21.25±0.21 | 4.52±0.03 | **3.63±0.02** | 7.05±0.53 | 233.66±9.55 | 86.07±10.23 | 75.34±7.03 | 182.00±14.00 | 21.70±1.64 |
| | -20 | 4 | 20.52±0.62 | 4.19±0.36 | **3.63±0.01** | 6.03±0.17 | 240.22±1.86 | 77.44±17.71 | 77.37±7.43 | 165.20±22.22 | 65.23±21.32 |
| **TM** | -4 | 0 | 21.38±0.31 | 3.67±0.32 | 3.35±0.01 | **4.57±0.40** | 215.88±3.69 | 163.85±15.61 | 150.69±13.65 | 149.33±16.17 | 9.36±2.73 |
| | -20 | 0 | 20.86±0.16 | 3.75±0.29 | 3.29±0.04 | **4.77±0.03** | 210.30±1.96 | 158.62±13.28 | 145.47±14.14 | 144.67±21.39 | 13.18±14.61 |
| | -4 | 4 | 20.62±0.31 | 3.90±0.33 | 3.34±0.04 | 5.71±0.51 | 207.28±3.61 | 65.92±23.45 | 53.47±25.21 | 126.00±59.40 | 7.99±5.81 |
| | -20 | 4 | 20.47±0.68 | 3.77±0.20 | 3.31±0.02 | 5.68±0.15 | 206.89±6.92 | 88.95±12.09 | 79.11±10.57 | 144.67±29.14 | 53.93±23.07 |
| **CJ** | -4 | 0 | 20.77±0.40 | 4.02±0.30 | 3.32±0.03 | 5.88±0.38 | 209.21±3.61 | 59.33±23.82 | 50.85±23.50 | 95.33±14.74 | **1.02±0.38** |
| | -20 | 0 | 20.43±0.14 | 4.41±0.53 | 3.28±0.02 | 6.69±1.07 | 206.43±3.39 | 85.79±46.39 | 71.00±44.28 | 100.00±36.06 | **1.42±0.93** |
| | -4 | 4 | 20.85±0.59 | 3.92±0.43 | 3.37±0.01 | 5.74±0.68 | 210.24±6.47 | 55.85±48.80 | 64.62±27.44 | 128.47±5.16 | **3.17±1.91** |
| | -20 | 4 | 20.64±0.27 | 3.64±0.07 | 3.34±0.00 | 5.55±0.30 | 207.51±2.56 | 97.01±15.93 | 87.80±16.97 | 144.67±21.39 | 14.09±17.94 |

[1]Means ± standard deviation (n=3) for all samples.
[2]Cryogenic temperature treatment
[3] Not applicable – na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[6]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.

Table 3.10: Chemical profiles of Chenin blanc must from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer D from the Stellenbosch wine region) in 2021.

| **Treatment** | **Temp[2] (ºC)** | **Storage time (Months)** | **Chemical Analyses[1]** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Brix (ºB)** | **Malic acid (g $L^{-1}$)** | **pH** | **Total acid (g $L^{-1}$)** | **Total sugar (g $L^{-1}$)** | **Glucose (g $L^{-1}$)** | **Fructose (g $L^{-1}$)** | **Haze (NTU)** | **YAN (mg N $L^{-1}$)** |
| **Control** | na[3] | na | 19.89±0.24 | 3.94±0.32 | 3.39±0.02 | 6.88±0.07 | 197.16±1.54 | 104.94±23.85 | 134.46±34.91 | 98.00±0.00 | 36.34±25.11 |
| **WG[4]** | -4 | 0 | 21.27±0.90 | 3.44±0.12 | 3.53±0.04 | **4.26±0.16**[5] | 215.42±10.11 | 93.56±15.67 | 91.57±12.48 | 84.00±14.00 | 62.83±34.81 |
| | -20 | 0 | 20.71** | 3.35 | 3.56 | **3.94** | 210.39 | 81.18 | 66.94 | 70.00 | 67.20 |
| | -4 | 4 | 19.88±0.95 | 3.78±0.39 | **3.62±0.03** | 4.56±0.52 | 200.20±10.04 | 90.39±14.47 | 92.44±10.62 | 121.33±16.16 | 28.06±2.66 |
| | -20 | 4 | 21.42±0.1.61 | 4.45±0.11 | **3.66±0.01** | 5.66±0.13 | 217.11±1.96 | 143.36±39.97 | 139.47±38.21 | 126.00±14.00 | 94.55±52.96 |
| **MG** | -4 | 0 | 19.63±0.09 | 3.91±0.14 | 3.42±0.01 | 5.34±0.21 | 197.86±0.91 | 92.45±37.92 | 96.71±42.10 | 84.00±0.00 | 43.80 |
| | -20 | 0 | 20.02±0.65 | 4.05±0.23 | 3.45±0.01 | 5.67±0.28 | 202.67±7.00 | 86.92±22.16 | 91.28±36.77 | 74.66±8.08 | 85.43±14.62 |
| | -4 | 4 | 23.31±1.01 | 4.55±0.27 | **3.67±0.01** | 5.44±0.11 | 237.02±10.78 | 69.09±25.31 | 68.97±23.74 | 141.86±3.23 | 47.16±40.05 |
| | -20 | 4 | 24.01±0.58 | 4.75±0.17 | **3.67±0.01** | 6.03±0.24 | 244.42±6.04 | 62.47±9.93 | 62.59±7.43 | 126.00±17.03 | 47.80±25.32 |
| **TM** | -4 | 0 | 12.11±1.63 | 2.68±0.10 | 3.22±0.02 | **3.81±0.40** | 124.91±15.40 | 117.68±7.65 | 144.30±7.39 | 91.00±29.69 | 5.87±0.36 |
| | -20 | 0 | 12.07 | 2.67 | 3.23 | **3.77** | 124.47 | 135.86 | 126.92 | 98.00 | 8.16 |
| | -4 | 4 | 20.94±1.60 | 4.06±0.22 | 3.48±0.02 | 5.73±0.43 | 211.70±17.24 | 52.97±9.23 | 58.54±29.23 | 106.40±8.40 | 4.94±0.38 |
| | -20 | 4 | 20.88±0.72 | 3.82±0.18 | 3.49±0.01 | 5.07±0.18 | 210.03±7.41 | 85.21±37.70 | 87.80±33.90 | 94.26±9.00 | 10.19±11.01 |
| **CJ** | -4 | 0 | 18.44 | 3.67 | 3.34 | 5.84 | 185.22 | 27.63 | 26.95 | 56.00 | 1.20 |
| | -20 | 0 | 19.84 | 3.59 | 3.37 | 5.68 | 198.71 | 82.04 | 74.76 | 70.00 | 0.48 |
| | -4 | 4 | 22.27±0.22 | 4.06±0.02 | 3.47±0.01 | 5.36±0.22 | 224.50±2.34 | 63.91±20.76 | 61.28±20.28 | 112.00±7.92 | 19.92±25.57 |
| | -20 | 4 | 22.72±0.12 | 4.04±0.07 | 3.46±0.00 | 5.61±0.06 | 229.23±1.26 | 116.58±74.51 | 109.53±70.08 | 133.00±9.89 | 16.67±17.72 |

[1]Means ± standard deviation (n=3) for all samples.
[2]Cryogenic temperature treatment
[3]Not applicable – na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[5]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
**Single values without standard deviation had outliers statistically removed

#### 3.3.1.1 The effect of stage of production, cryogenic temperature and storage time on must chemical profiles

The grape matrix used for wine production determines the aromatic profile and quality of the final wine. Increased contact between the grape skins and juice, increase the extraction of aroma compounds, their precursors and chemical compounds located in the skin of grape berries (Peinado et al., 2004:587-589; Salinas et al., 2005:1527; 1535; Baiano et al., 2012:2700; Blesic et al., 2016:118-119; Ruiz-Rodríguez et al., 2020:6;11; Bestulić, et al., 2022:2;9-10; Malićanin et al., 2022:11; 14; Pedrosa-López et al., 2022:8). Cold maceration as a technique to increase the extraction of aromatic compounds from grape skins and the period over which maceration is applied is vital in preventing oxidation and the extraction of undesirable compounds (Aragón-García et al., 2021:1; Pedrosa-López et al., 2022:8-9). Moreover, pre-fermentative and prolonged macerations have been previously investigated to determine the influence on white wine's physicochemical and sensory properties (Darias-Martıń et al., 2000:484; 486; Gómez-Míguez et al., 2007:763; Olejar et al., 2016:152; 154; 157; Carbone & Fiordiponti, 2016:2-3; 8; Bestulić, et al., 2022:9). To date, no previous research has focused on the effect of cryogenic pre-treatment of Sauvignon blanc and Chenin blanc at four stages of production, simultaneously. This research was therefore undertaken to elucidate the effect that the production stage (WG, MG, TM and CJ) cryogenic temperatures (-4 ºC and -20 ºC), and storage periods (T0 and T4) had on grape must chemical profiles (Figs. 3.3-3.8).

*Stage of production:*

The PCA biplots indicate that the stage of production had the greatest influence on the observed clusters. This applied to all Producers (A, B, C and D) and both cultivars (Sauvignon blanc and Chenin blanc) (Figs. 3.3-3.8). Two distinct groupings, i.e., WG and MG and TM and CJ, were observed for most producers in 2020 and to some extent in 2021, with the T4 grape must having more pronounced clusters. It was, however, noted that for Producers A and B, in 2021, three clusters were observed, the WG and MG clustered, whilst the TM and CJ each formed individual clusters (Figs 3.4-3.5). This could be related to the vintage rather than the treatments applied. Furthermore, the unfrozen control grape must clustered with or was closer to the grape must from the cryogenically pre-treated TM and CJ for most producers and across vintages (2020 and 2021) (Figs 3.3-3.8). For Producer B (Fig 3.5), the control grape must from hand-harvested and machine-harvested grapes clustered together in 2020, indicating that the harvesting method did not affect the chemical profiles of the grape must. Furthermore, the WG and MG, for most producers, over both vintages, are associated with the chemical parameters, i.e., pH, YAN, haze, Density, glucose, fructose and Brix.

The TM and CJ treatments were closely associated with the chemical parameters, i.e., total acidity and malic acid, which are less desirable. Furthermore, this study demonstrated that the process of production at which the cryogenic pre-treatment was applied had a significant impact on the grape must in terms of its chemical profiles. Winemakers can, therefore, produce wine with a desired chemical profile by applying cryogenic pre-treatments in combination with the stage of production before the winemaking process.

*Cryogenic Temperature:*

The two cryogenic temperatures used in this research, i.e., -4 ºC and -20 ºC, did not have a significant impact on the chemical profiles of the grape must since the clusters observed in the PCA biplots included a combination of the two temperatures (Figs. 3.3-3.8). This was seen for all producers (A, B, C, and D) originating from different wine regions, across the two vintages (2020 and 2021), and for two *Vitis vinifera* cultivars (Sauvignon blanc and Chenin blanc) (Figs. 3.3-3.8). Therefore, it can be concluded that the cryogenic temperatures applied will not have a notable effect on the grape must chemical profiles, but when combined with the stage of production, it could result in a wine with a unique chemical profile. Furthermore, if winemakers wanted to use cryogenic treatments as a tool to alter the grape must and enhance wine chemical profiles, the type of cryogenic treatment, e.g., large capacity freezers, cryogenic agents (nitrogen or carbon dioxide), blast freezing or whether freeze concentration was used and application method will be the deciding factors, as had been previously reported (Parenti et al., 2004:361; 365; Carillo et al., 2011:6; Schmid & Jiranek, 2011:26; Zhang et al., 2016:21620; Ruiz-Rodríguez et al., 2020:6-10; Coetzee, 2022; Vilar-Bustillo et al., 2023:8).

*Storage time:*

The effect of the storage period over which the cryogenic treatments were applied is illustrated by the PCA biplots (Figs. 3.3-3.8). Similar clusters, i.e., WG and MG and TM and CJ, were observed, however, after the T4 storage period, the clusters became more distinct and well-defined compared to those observed at T0. For T0, the unfrozen control wines clustered with the TM and CJ treatments for most Producers (A, C and D), becoming more distinct and well-defined after a T4 storage period (Figs 3.3-3.4, 3.6-3.8). Producer B had two harvesting methods and only a T4 storage period in 2020, but the clusters were similar to that of the other Producers (Fig. 3.5). Although only a T4 storage period was applied in 2020 with two harvesting methods for Producer B, the resulting clusters were similar to those of other producers (Fig. 3.5). This suggests that while cryogenic treatment had a noticeable effect on grape must composition compared to the controls, the harvesting technique alone, in combination with storage duration, did not notably influence the must chemical grouping patterns, suggesting that the major driver of the observed grouping was the cryogenic treatment, not the harvesting method. Slight differences in observations were made for the clusters in 2021 for Producers A and B, because three clusters were seen, i.e., WG and MG, TM and CJ (Figs 3.4-3.5). This observation could once again be a result of the vintage rather than the treatments applied. From this study, it could be concluded that the storage period over which the cryogenic temperatures were applied had an impact on the grape must chemical profiles of grapes from different geographical regions and across vintages. Furthermore, the results obtained corroborate the findings of previous research, which showed that pre-fermentative and prolonged macerations can influence white wine chemical properties (Darias Martıń et al., 2000:484; 486; Gómez-Míguez et al., 2007:763; Olejar et al., 2016:152; 154; 157; Carbone & Fiordiponti, 2016:2-3; 8; Bestulić, et al., 2022:9).

### 3.3.2 Wine

#### 3.3.2.1 Effect of cryogenic treatments on the physicochemical parameters of wines

Previous research conducted by Carillo et al. (2011:1-14), Naviglio et al. (2018:6-10) and Naranjo et al. (2021:1-13) involved the rapid cooling of grapes by sparging with liquid $CO_2$ before crushing and destemming. It was found that the wines produced from the Chardonnay and Maturana Blanca (white) grape cultivars were not statistically different from the control wines in terms of specific physicochemical parameters, i.e., alcohol content, pH, titratable acidity, and volatile acidity (Naviglio et al., 2018:6-10; Naranjo et al., 2021:4-5). In the present study, the physicochemical parameters of Sauvignon blanc (2020 and 2021) and Chenin blanc (2021) control wines and wines from WG, MG, TM and CJ subjected to pre-fermentative cryogenic freezing were analysed eight months after bottling using the Bruker Alpha II (FT-IR ATR) Wine Analyser (Tables 3.11-3.17). The VA concentration for all Sauvignon blanc and Chenin blanc unfrozen control and cryogenically pre-treated wines ranged between 0.14 and 0.48 g $L^{-1}$ (Tables 3.11-3.17), which was expressed as acetic acid. Additionally, it was noted that most wines produced from cryogenic pre-treated CJ had slightly higher acetic acid levels ranging from 0.37-0.48 g $L^{-1}$. All values were well below the legal limit of 1.2 g $L^{-1}$ for SA white wines, which indicates that good winemaking practices were followed and were similar to previously reported values for wines made from cryogenically pre-treated grapes (Peinado et al., 2004:586; Salinas et al., 2005:1529-1530; Carillo et al., 2011:10; Naviglio et al., 2018:8; Naranjo et al., 2021:4-5; Anonymous, 2025a). Similar observations were made by Radeka et al. (2023:6) and Gu et al. (2025:3-4) in terms of the VA concentration of reference wines being

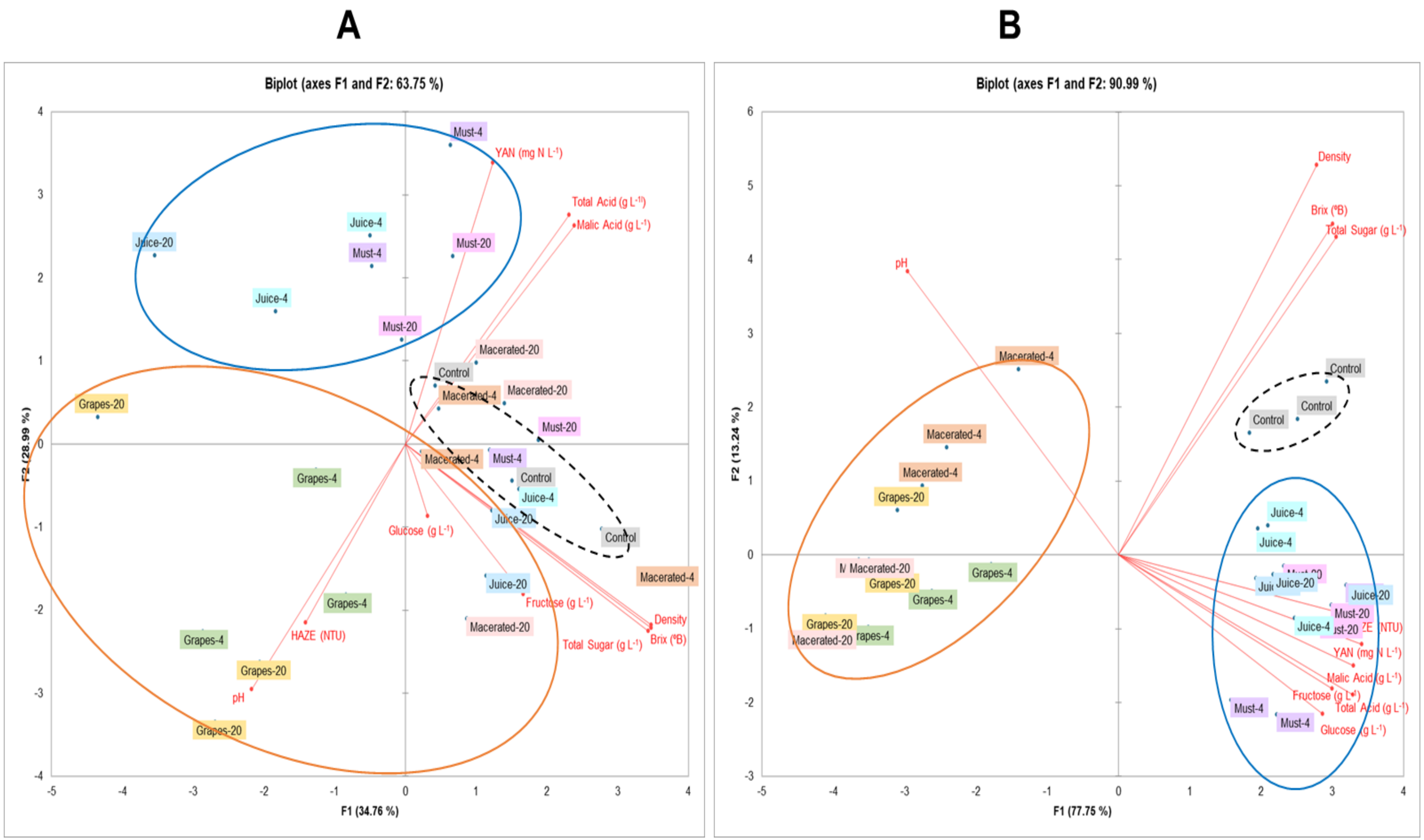



Figure 3.3. PCA bi-plots showing groupings (indicated by circles around groupings) based on grape must chemical parameters for Producer A (Stellenbosch wine region), for the control must and must from cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-4 ºC and -20 ºC) at T0 (A) and T4 (B) storage period in 2020.

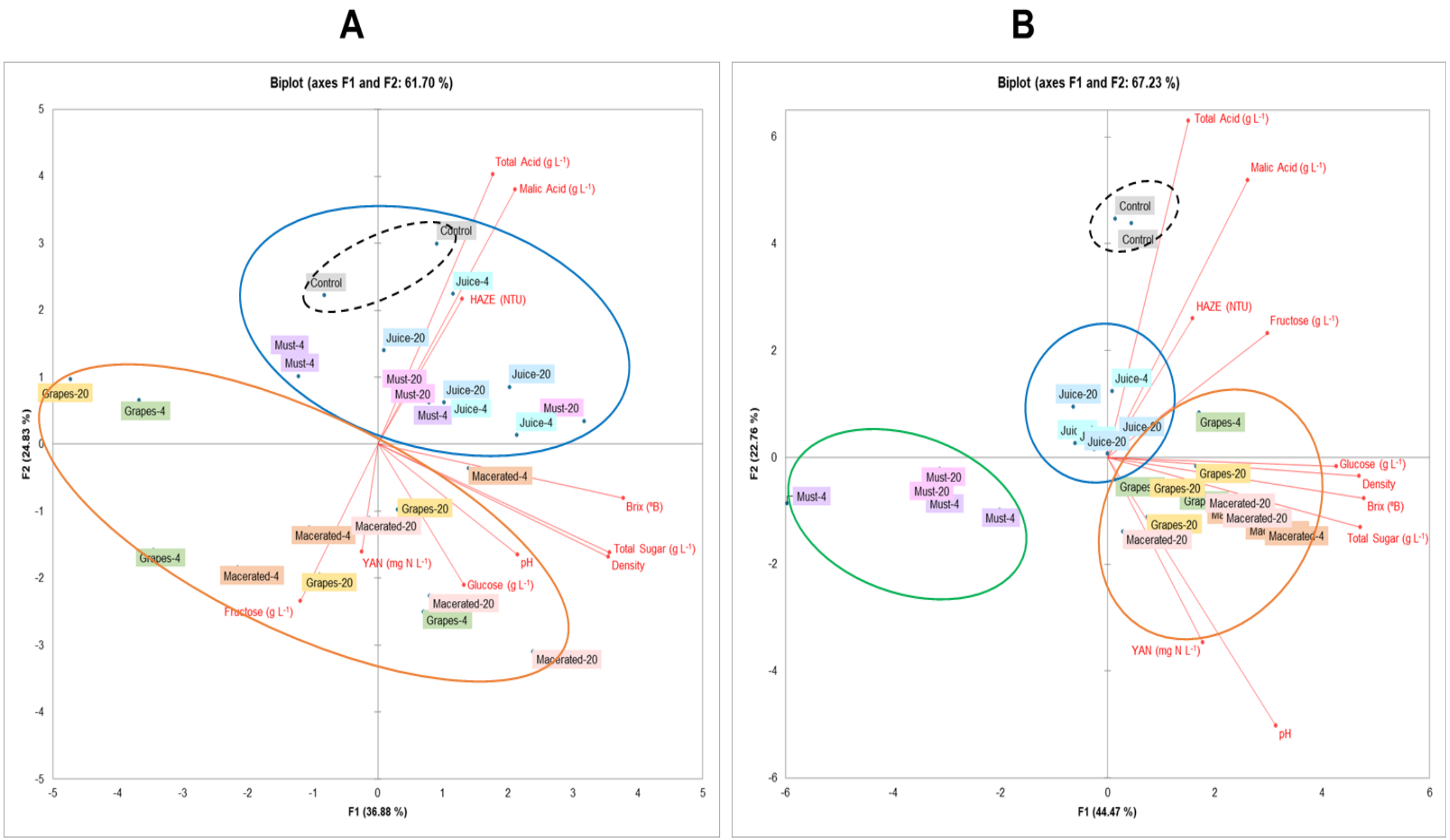



Figure 3.4. PCA biplots showing groupings (indicated by circles around groupings) based on grape must chemical parameters for Producer A (Stellenbosch wine region), for control must and must from cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-4 ºC and -20 ºC) at T0 (A) and T4 (B) storage period in 2021.

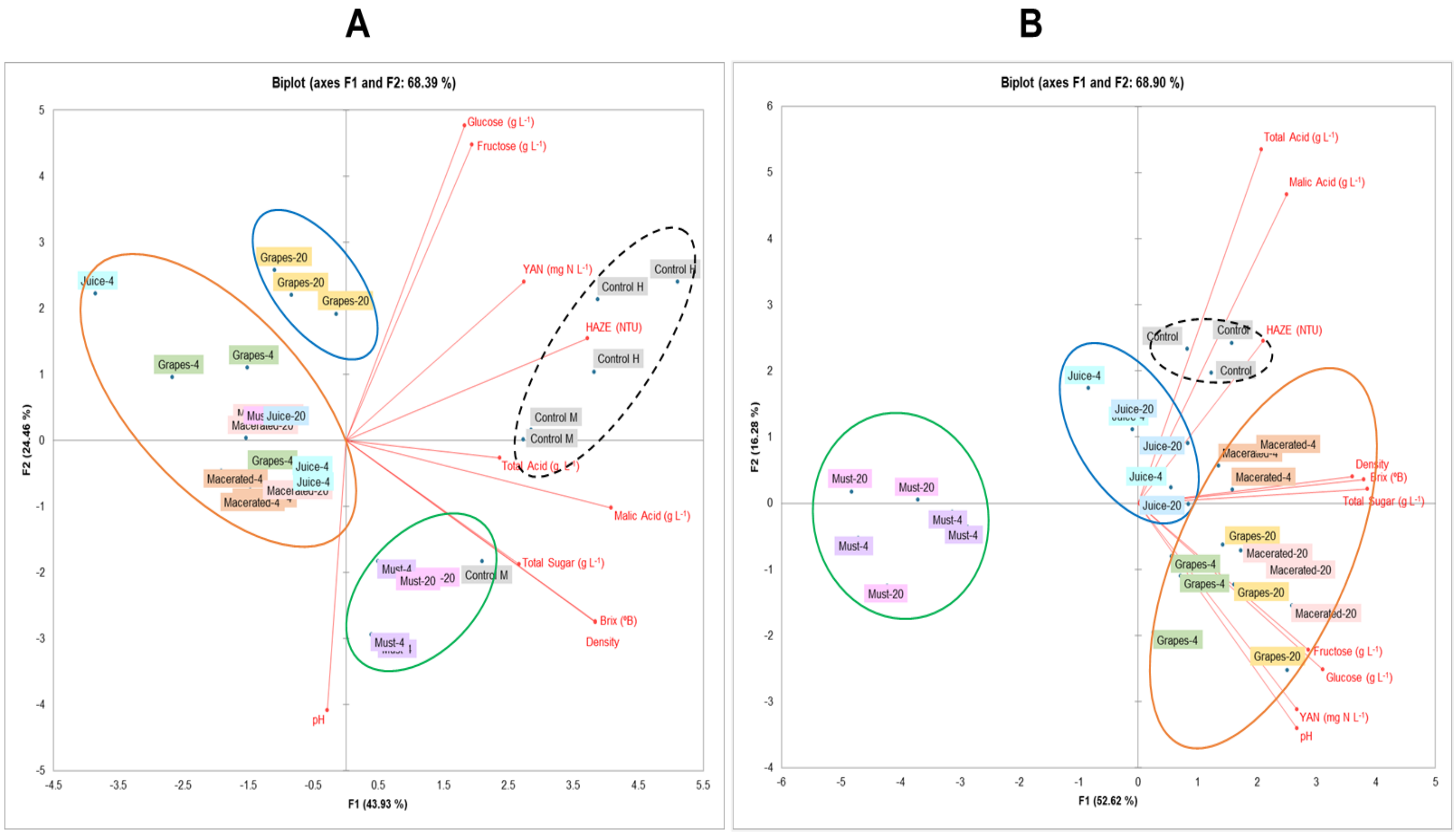



Figure 3.5. PCA biplots showing groupings (indicated by circles around groupings) based on grape must chemical parameters for Producer B (Durbanville wine region), for hand-harvested and machine-harvested control and cryogenically pre-treated Sauvignon blanc must from WG, MG, TM and CJ subjected to freezing (-4 ºC and -20 ºC) (B) at T4 in 2020 (A) and 2021 (B).

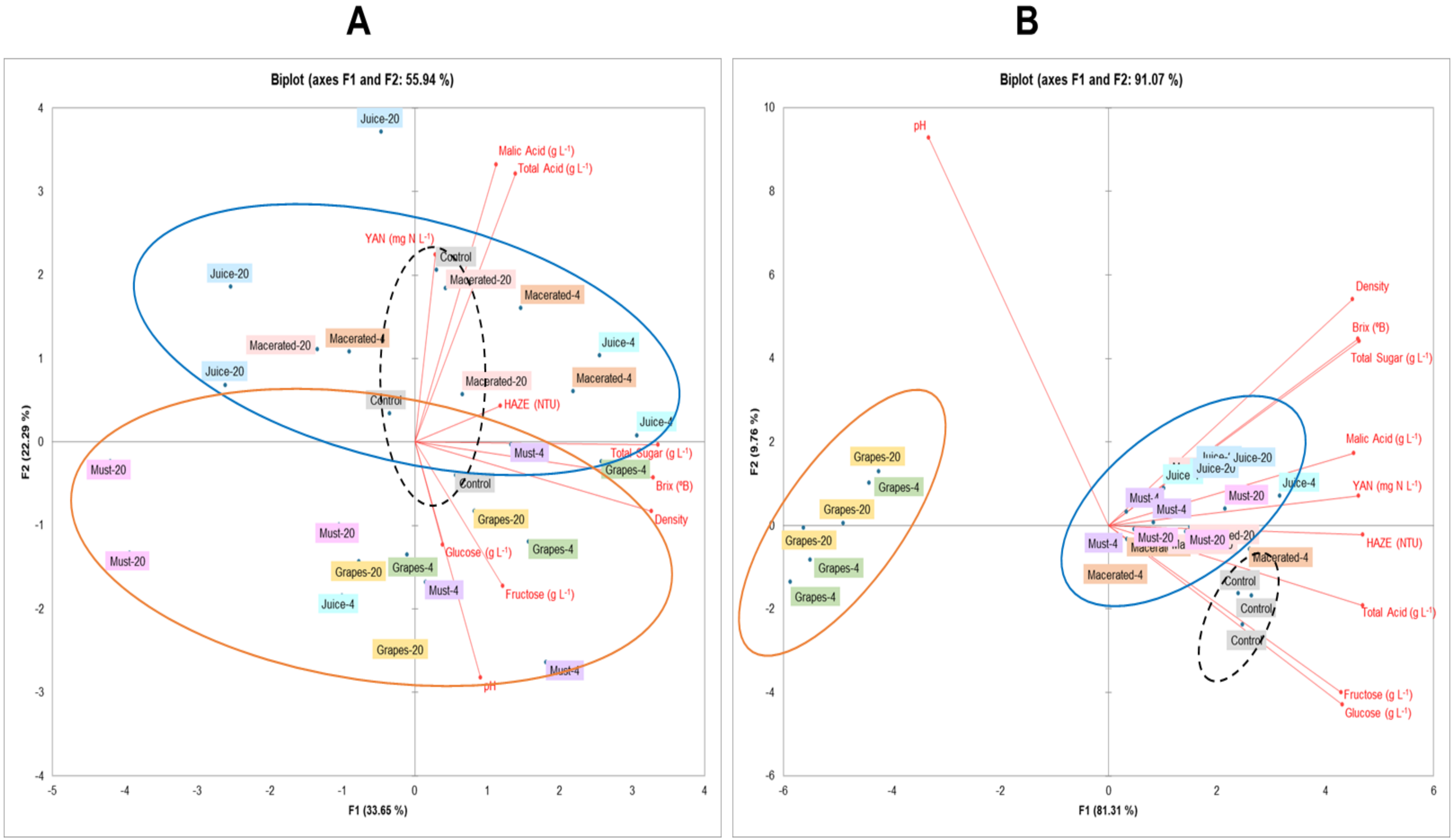



Figure 3.6. PCA biplots showing groupings (indicated by circles around groupings) based on grape must chemical parameters for Producer C (Cape South Coast wine region), for control must and must cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-4 ºC and -20 ºC) at T0 (A) and T4 (B) storage period in 2020.

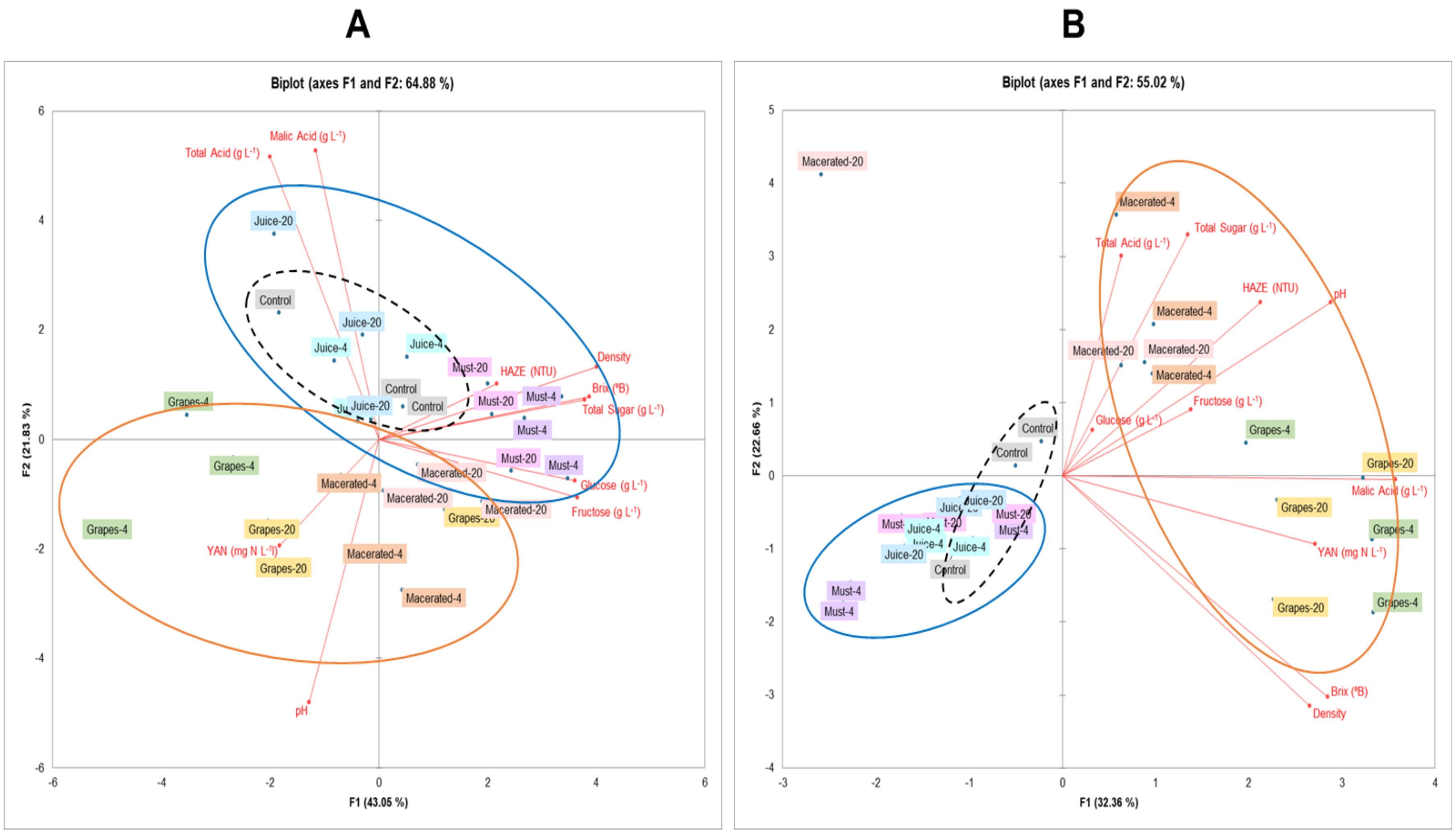



Figure 3.7. PCA biplots groupings (indicated by circles around groupings) based on grape must chemical parameters for Producer C (Cape South Coast wine region), for control must and must from cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-4 ºC and -20 ºC) at T0 (A) and T4 (B) storage period in 2021.

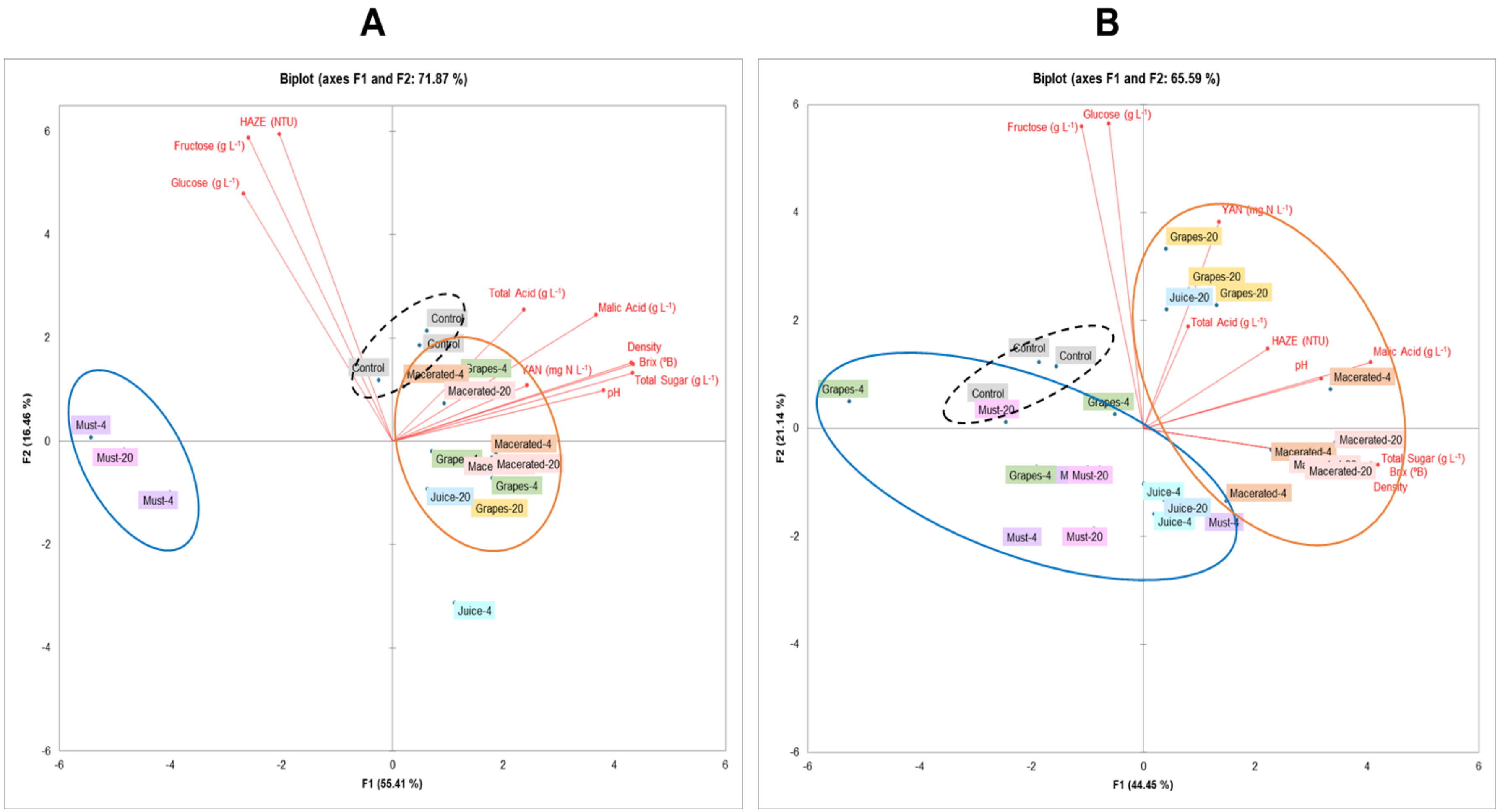



Figure 3.8. PCA biplots showing groupings (indicated by circles around groupings) based on grape must chemical parameters for Producer D (Stellenbosch wine regions), for control must and must from cryogenically pre-treated Chenin blanc WG, MG, TM and CJ subjected to freezing (-4 ºC and -20 ºC) at T0 (A) and T4 (B) storage period in 2021.

equivalent to wines made from Malvazija istarska (Croatian white grape) and Syrah grapes subjected to cryomaceration treatments, respectively.

The alcohol content of all Sauvignon blanc and Chenin blanc wines ranged between 7-15% ($^{v}/_{v}$) (Tables 3.11-3.17). The lowest alcohol value may be attributed to an anomaly or possible measurement error, as the corresponding Brix value of the base must (21.81 ºB) was comparable to those of the other samples (Table 3.6). In contrast, the highest alcohol content corresponded with a must Brix of 24.22 ºB (Table 3.7), which is unusually high for white grapes and could plausibly explain the elevated alcohol level. Overall, the alcohol content of the wines from all treatments and the unfrozen control ranged within the typical legal limit [12.5-14.5% ($^{v}/_{v}$)] for SA dry white wines (Anonymous, 2025a). These results confirmed previous research which reported that the physicochemical parameters of wine produced from fresh and frozen grapes and grape juice had significant differences in tartaric acid, but not alcohol content (Carillo et al., 2011:10; Schmid & Jiranek, 2011:29; Olejar et al., 2016:154; Naviglio et al., 2018:8; Naranjo et al., 2021:4; Radeka et al., 2023:6; Guerrini et al., 2024:5-6). However, studies conducted by Zhang et al. (2015:21621) using Solaris (white grape) MG and Pedrosa-López et al. (2022:5) using Muscat of Alexandria WG and MG, both previously frozen, found that alcohol levels in final wines were higher compared to the reference wines. These differences were attributed to the extraction of compounds, which affected the yeast performance during the fermentation process (Zhang et al., 2015:21621; Pedrosa-López et al.,2022:5). Moreover, previous research by Bavčar et al. (2011:193-194) investigating the effect of alternative skin contact techniques, including freezing (-20 ºC), found the ethanol content to be lower than that of the control wine. This was also found in a recent study conducted by Giametta et al. (2025:6), investigating the effects of two cooling techniques on Bombino nero grapes (red Italian wine grape), found that wines produced from grapes cooled with $CO_2$ had significantly lower alcohol levels than the wines cooled using a heat exchanger. This, therefore, illustrated that the type of cryogenic method used could influence wine chemical parameters.

Malic acid levels in wines made from unfrozen control grapes were generally similar to the MA levels in wines made from cryogenically pre-treated TM and CJ for all producers across vintages, with MA levels in the WG and TM being slightly higher (Tables 3.11-3.17). These observations were made for all wines, for both vintages and cultivars. Therefore, the cryogenic pre-treatment of WG, MG, TM, and CJ did not significantly affect MA levels in the final wines, however, the differences observed were due to the production stage. Furthermore, the influence of the harvesting method on MA levels in the wines was negligible. These results confirmed that of Naranjo et al. (2021:4-5), who also found no significant difference in MA levels between wines made from conventional winemaking and pre-fermentative cryomaceration. However, a study by Carillo et al. (2011:10) reported significant increases in MA levels in wines made from cryo-macerated Bianchello del Metauro wine grapes (white wine) compared to the untreated control, with the increase attributed to the type of cryogenic treatment (i.e., $CO_2$ cryomaceration or prolonged cold maceration) being used and previously reported (Zhang et al., 2015:21611; 21621-21622; Ruiz-Rodríguez et al., 2020:1-13).

The TA levels measured ranged between 3.73-6.8 g $L^{-1}$ for all wines made from cryogenically pre-treated WG, MG, TM and CJ. TA levels in control wines ranged between 5.53-6.67 g $L^{-1}$ for Sauvignon blanc and 4.03 g $L^{-1}$ for Chenin blanc wines (Tables 3.11-3.17). For most treated wines, the measured levels fell below or above the typical reported TA levels for SA Sauvignon blanc (5.5-6.5 g $L^{-1}$) and Chenin blanc (5.5-7 g $L^{-1}$) wines (Anonymous, 2025a). It was further seen that the TA levels in the hand-harvested control were higher (6.67 g $L^{-1}$) than the machine-harvested control (5.17 g $L^{-1}$) (Table 3.13), indicating that the harvesting technique affected the TA levels. Furthermore, no trend regarding the impact of the production, cryogenic temperature and storage period on the TA levels was observed (Tables 3.11-3.17). These findings contradicted previous research, which showed that wines produced from cryogenically pre-treated WG and MG had lower TA levels than reference wines (Darias-Martıń et al., 2000:484; Zhang et al., 2015:21611; Pedrosa-López et al., 2022:5; Radeka et al., 2023:6; Gu et al., 2025:3).

The pH values were found to range between 2.97-3.41 for the control wines and between 3.06-3.91 for wines made from cryogenically pre-treated WG, MG, TM and CJ, for both cultivars, regions and vintages (Tables 3.11-3.17). Wines made from cryogenically pre-treated Sauvignon blanc and Chenin blanc WG, MG, TM, and CJ generally had higher pH values than the control wines. Furthermore, it was noted that the treatments stored at T4 had slightly higher pH values than those stored for T0 (Tables 3.11-3.17). For most treatments, the pH values for the WG and MG were also higher than those of the TM and CJ. The pH values for the Sauvignon blanc control wines (2.97-3.28) and Chenin blanc control wine (3.41) reported in this research were slightly lower than ranges typically reported for SA Sauvignon blanc wines (3.18-3.69) which could be because of differences in wine regions and vintage, whilst the Chenin blanc wines fell within the typical levels (3.27-3.48) for SA Chenin blanc (Anonymous, 2025a). Moreover, pH values obtained from all cryogenically pre-treated WG, MG, TM and CJ fell within the typically reported ranges, mentioned above, except for a few MG treatments that had slightly higher pH values (Tables 3.11-3.17). This was observed for both cultivars, wine regions and vintages. These findings were similar to results reported by Darias-Martıń et al. (2000:484); Bavčar et al. (2011:193-194); Zhang et al. (2015:21610-21611); Radeka et al. (2023:6) and Gu et al. (2025:3), who found that wines produced from cold-macerated grapes had higher pH values compared to the reference wines. This, however, differed from the research by Carillo et al. (2011:10), Naviglio et al. (2018:8), Vernarelli (2018:54) and Naranjo et al. (2021:5), who found no difference in pH values for wines from cryogenically pre-treated grapes and control wines.

The glucose and fructose levels for most wines were similar (0-3 g $L^{-1}$), except for a few wines that had higher fructose levels ranging between 2-8 g $L^{-1}$ (Tables 3.11-3.17). These higher fructose levels were observed in the T4 wines made from the pre-treated Sauvignon blanc CJ and Chenin blanc MG. It is hypothesised that this was due to the prolonged cryogenic freezing process (T4), which altered the structure of the CJ and MG, thus increasing the fructose concentration in the final wine. However, when comparing the grape must glucose/fructose ratios of these wines, they were present at a ratio of approximately 1:1, indicating no imbalances in the starting grape must following the cryogenic pre-fermentative freezing treatments (Tables 3.4-3.10). Total sugar (TS) levels for all the Sauvignon blanc and Chenin blanc wines made from control and cryogenically pre-treated grape must typically range between 0-5 g $L^{-1}$, indicating that most wines were fermented to dryness, except for a few wines that had TS values > 5 g $L^{-1}$ (Tables 3.11-3.17). The Brix values of the Sauvignon blanc grape must (18-22ºB), and the Chenin blanc grape must (20 ºB) (Tables 3.4-3.10) before the alcoholic fermentation process, which were within acceptable levels for white wine production. Moreover, the TS levels that were higher were due to the higher fructose levels observed in some of the wines. Furthermore, the TS levels in the control wines were generally comparable to levels in wines made from cryogenically pre-treated WG, MG, TM and CJ (Tables 3.11-3.17). These observations applied to all producers, both cultivars and across vintages. Moreover, these results were similar to those reported by Antonelli et al. (2010:88) and Carillo et al. (2011:10), who also did not find significant differences in the TS levels of reference wines when compared to wines made from cryogenically pre-treated Sauvignon blanc, Trebbiano Romagnolo and Bianchello del Metauro grapes. This observation was also made by Radeka et al. (2023:6) on Malvazija istarska grapes, which also showed comparable results between wines. Ruiz-Rodríguez et al. (2020:6) found, however, that wines made from grapes frozen using liquid nitrogen (LN) had a higher total soluble solids (TSS) content than those made from grapes frozen by ultra-fast mechanical freezing (UF). The difference in sugar content was believed to be due to the faster defrosting process in the case of ultra-fast mechanical freezing. In contrast, when grapes were frozen using liquid nitrogen, the effect of the cryoscopic decrease was more evident, and subsequently, the must obtained had a higher sugar content.

The overall observations made regarding the effect of the cryogenic

Table 3.11: Chemical profiles of Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer A from the Stellenbosch wine region) in 2020.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Volatile acidity ($g L^{-1}$) | Alcohol (%) | Fructose ($g L^{-1}$) | Glucose ($g L^{-1}$) | Malic acid ($g L^{-1}$) | Total acidity ($g L^{-1}$) | pH | Total sugar ($g L^{-1}$) |
| **Control** | na[3] | na | 0.25±0.04 | **14.10±0.10**[5] | 0.83±0.55 | 0.00±0.00 | 2.20±0.10 | 5.53±0.06 | 3.17±0.06 | 0.00±0.00 |
| **WG**[4] | -4 | 0 | 0.28±0.05 | 13.57±0.31 | 1.37±0.40 | 0.00±0.00 | 2.87±0.25 | 5.07±0.32 | 3.40±0.10 | 1.27±2.19 |
| | -20 | 0 | 0.27±0.06 | 13.53±0.21 | 1.40±0.10 | 0.00±0.00 | 3.40±0.17 | 5.23±0.15 | 3.47±0.06 | 0.00±0.00 |
| | -4 | 4 | 0.31±0.05 | 13.63±0.12 | 1.27±0.49 | 0.77±0.67 | 3.40±0.30 | 5.33±0.35 | 3.70±0.08 | 1.33±2.31 |
| | -20 | 4 | 0.31±0.01 | 13.43±0.21 | 2.97±2.77 | 0.27±0.46 | 3.50±0.53 | 5.47±0.31 | 3.70±0.01 | 4.33±3.97 |
| **MG** | -4 | 0 | 0.28±0.06 | **14.13±0.3** | 0.63±0.06 | 0.00±0.00 | 2.90±0.17 | 4.40±0.30 | 3.63±0.12 | 0.00±0.00 |
| | -20 | 0 | 0.28±0.05 | **14.03±0.32** | 0.60±0.10 | 0.00±0.00 | 3.33±0.12 | 4.67±0.21 | 3.63±0.06 | 0.00±0.00 |
| | -4 | 4 | 0.32±0.04 | 13.60±0.53 | 1.40±1.04 | 0.70±0.62 | 2.93±0.23 | 4.90±0.44 | 3.76±0.12 | 1.60±2.77 |
| | -20 | 4 | 0.33±0.02 | 13.83±0.15 | 0.83±0.42 | 0.30±0.44 | 3.27±0.31 | 4.90±0.00 | 3.79±0.07 | 1.30±2.25 |
| **TM** | -4 | 0 | 0.31±0.06 | 12.80±0.66 | 0.70±0.26 | 0.00±0.00 | 2.67±0.40 | 5.23±0.57 | 3.33±0.23 | 1.67±1.53 |
| | -20 | 0 | 0.24±0.01 | 10.77±0.57 | 0.77±0.12 | 0.00±0.00 | 2.20±0.36 | 4.93±0.55 | 3.20±0.00 | 0.00±0.00 |
| | -4 | 4 | 0.35±0.03 | 10.30±2.70 | 2.60±2.86 | 0.00±0.00 | 1.27±0.59 | 4.93±0.50 | 3.26±0.05 | 2.63±4.56 |
| | -20 | 4 | 0.38±0.02 | 12.23±0.21 | 2.30±0.87 | 0.07±0.06 | 1.80±0.17 | 5.27±0.12 | 3.37±0.01 | 4.67±1.18 |
| **CJ** | -4 | 0 | 0.30±0.05 | **13.87±0.15** | 2.03±0.86 | 0.00±0.00 | 2.07±0.38 | 5.23±0.23 | 3.27±0.06 | 3.97±0.61 |
| | -20 | 0 | 0.32±0.04 | **14.13±0.38** | 1.90±1.08 | 0.00±0.00 | 1.80±0.17 | 4.83±0.15 | 3.23±0.06 | 4.53±1.37 |
| | -4 | 4 | **0.46±0.02** | **14.13±0.15** | **6.97±5.03** | 0.23±0.40 | 1.97±0.15 | 5.13±0.06 | 3.41±0.06 | **8.03±3.26** |
| | -20 | 4 | **0.41±0.04** | **13.57±0.15** | **7.93±4.86** | 1.00±0.17 | 2.23±0.06 | 5.33±0.12 | 3.34±0.04 | **9.40±3.45** |

[1] Means ± standard deviation (n=3) for all samples between treatments within columns.
[2] Cryogenic temperature treatment
[3] Not applicable – na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[5]Bold script highlights values that fall above or below the typical range for that physicochemical parameter

Table 3.12: Chemical profiles of Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer A from the Stellenbosch wine region) in 2021.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Volatile acidity ($g\ L^{-1}$) | Alcohol (%) | Fructose ($g\ L^{-1}$) | Glucose ($g\ L^{-1}$) | Malic acid ($g\ L^{-1}$) | Total acidity ($g\ L^{-1}$) | pH | Total sugar ($g\ L^{-1}$) |
| **Control** | na[3] | na | 0.27±0.06 | 12.90±0.36 | 1.17±0.50 | 1.03±0.23 | 2.77±0.51 | 5.67±0.25 | 3.12±0.04 | 2.30±0.46 |
| **WG[4]** | -4 | 0 | 0.24±0.11 | 12.70±0.30 | 1.33±0.85 | 0.83±0.29 | 2.90±0.56 | 4.40±0.56 | 3.55±0.09 | 2.47±0.76 |
| | -20 | 0 | 0.28±0.07 | 12.43±0.31 | 1.13±0.50 | 0.63±0.21 | 2.80±0.52 | 4.40±0.46 | 3.61±0.09 | 2.10±0.56 |
| | -4 | 4 | 0.36±0.08 | 12.60±0.40 | 1.37±0.47 | 0.70±0.40 | 2.90±0.36 | 4.57±0.31 | 3.75±0.09 | 2.43±0.71 |
| | -20 | 4 | 0.26±0.07 | 12.27±0.23 | 1.47±0.72 | 0.77±0.31 | 2.77±0.31 | 4.50±0.26 | 3.74±0.01 | 2.47±0.47 |
| **MG** | -4 | 0 | 0.28±0.10 | 12.83±0.40 | 1.43±0.59 | 1.07±0.15 | 3.10±0.10 | 4.87±0.64 | 3.59±0.03 | 2.53±0.85 |
| | -20 | 0 | 0.33±0.09 | 12.80±0.20 | 1.30±0.44 | 0.90±0.36 | 3.10±0.20 | 5.03±0.06 | 3.71±0.08 | 2.37±0.74 |
| | -4 | 4 | 0.36±0.05 | 13.23±0.29 | 1.37±0.40 | 1.30±0.20 | 2.80±0.26 | 4.50±0.10 | 3.77±0.02 | 2.77±0.65 |
| | -20 | 4 | 0.32±0.12 | 12.73±0.35 | 1.27±0.55 | 0.63±0.12 | 2.43±0.29 | 4.83±0.15 | 3.70±0.06 | 2.37±0.55 |
| **TM** | -4 | 0 | 0.26±0.10 | 11.07±0.72 | 1.23±0.58 | 0.30±0.26 | 1.57±0.15 | 4.43±0.55 | 3.25±0.02 | 2.03±0.67 |
| | -20 | 0 | 0.28±0.08 | 11.50±0.46 | 1.40±0.66 | 0.43±0.35 | 1.70±0.36 | 4.53±0.55 | 3.33±0.04 | 2.40±0.72 |
| | -4 | 4 | 0.27±0.07 | 11.13±0.71 | 1.30±0.20 | 0.23±0.25 | 2.10±0.44 | 5.20±0.56 | 3.29±0.09 | 2.23±0.51 |
| | -20 | 4 | 0.34±0.07 | 11.60±0.57 | 1.05±0.21 | 0.45±0.35 | 2.40±0.57 | 4.85±0.07 | 3.52±0.21 | 2.00±0.14 |
| **CJ** | -4 | 0 | 0.31±0.08 | 12.97±0.23 | 1.53±0.67 | 0.83±0.12 | 2.47±0.84 | 4.90±0.78 | 3.32±0.14 | 2.73±0.67 |
| | -20 | 0 | 0.28±0.05 | 12.83±0.60 | 1.80±0.87 | 1.00±0.10 | 2.27±0.21 | 4.87±0.40 | 3.27±0.04 | 3.13±0.84 |
| | -4 | 4 | 0.30±0.06 | 12.47±0.23 | 1.60±0.28 | 0.25±0.07 | 2.87±0.35 | 5.37±0.59 | 3.30±0.04 | 2.70±0.00 |
| | -20 | 4 | 0.30±0.08 | 12.50±0.36 | 1.57±0.81 | 0.47±0.45 | 2.07±0.40 | 5.17±0.38 | 3.31±0.03 | 1.80±0.14 |

[1] Means ± standard deviation (n=3) for all samples between treatments within columns.
[2] Cryogenic temperature treatment
[3] Not applicable – na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).

Table 3.13: Chemical profiles of Sauvignon blanc wines produced from hand and machine-harvested cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer B from the Durbanville wine region) in 2020.

| Treatment | Harvesting methods | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Volatile acidity (g $L^{-1}$) | Alcohol (%) | Fructose (g $L^{-1}$) | Glucose (g $L^{-1}$) | Malic acid (g $L^{-1}$) | Total acidity (g $L^{-1}$) | pH | Total sugar (g $L^{-1}$) |
| **Control** | H[3] | na[4] | na | 0.31±0.08 | 12.83±0.31 | 0.63±0.32 | 0.00±0.00 | 2.73±0.25 | 6.67±0.32 | 2.97±0.012 | 1.00±1.73 |
| **WG[6]** | H | -4 | 4 | 0.33±0.06 | 12.80±0.10 | 0.47±0.42 | 0.00±0.00 | 2.77±0.21 | 4.53±0.29 | 3.51±0.05 | 0.00±0.00 |
| | | -20 | 4 | 0.35±0.07 | 12.87±0.12 | 1.00±0.52 | 0.00±0.00 | 2.87±0.49 | 4.80±0.35 | 3.49±0.03 | 0.97±1.67 |
| **Control** | M[5] | na[3] | na | 0.27±0.10 | 12.43±0.15 | 0.90±0.10 | 0.03±0.06 | 2.70±0.10 | 5.17±1.00 | 3.28±0.19 | 1.97±1.74 |
| **MG** | M | -4 | 4 | 0.39±0.02 | 13.10±0.36 | 1.20±0.17 | 0.00±0.00 | 3.47±0.21 | 4.83±0.29 | 3.77±0.06 | 2.00±1.73 |
| | | -20 | 4 | **0.43±0.01**[7] | 13.07±0.21 | 0.97±0.21 | 0.00±0.00 | 3.70±0.20 | 5.07±0.23 | 3.76±0.04 | 0.00±0.00 |
| **TM** | M | -4 | 4 | 0.23±0.05 | 7.97±1.40 | 1.07±0.21 | 0.00±0.00 | 1.70±0.26 | 3.73±0.55 | 3.23±0.05 | 2.87±0.15 |
| | | -20 | 4 | 0.32±0.06 | 10.97±1.80 | 1.23±0.12 | 0.00±0.00 | 2.30±0.56 | 4.47±0.47 | 3.33±0.06 | 0.00±0.00 |
| **CJ** | M | -4 | 4 | **0.42±0.06** | 12.97±0.47 | 1.17±0.06 | 0.00±0.00 | 2.50±0.36 | 4.53±0.31 | 3.48±0.06 | 1.07±1.85 |
| | | -20 | 4 | 0.32±0.08 | 11.30±1.70 | 1.25±0.07 | 0.00±0.00 | 2.30±0.15 | 4.45±0.35 | 3.37±0.10 | 3.10±1.18 |

[1]Means ± standard deviation (n=3) for all samples between treatments within columns.
[2]Cryogenic temperature treatment
[3]Hand-harvested grapes
[4]Not applicable – na
[5]Machine-harvested grapes
[6]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[7]Bold script highlights values that fall above or below the typical range for that physicochemical parameter

Table 3.14: Chemical profiles of Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer B from the Durbanville wine region) in 2021.

| Treatment | Harvesting methods | Temp[2] (ºC) | Storage Time (Months) | Chemical Analyses[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Volatile acidity ($g L^{-1}$) | Alcohol (%) | Fructose ($g L^{-1}$) | Glucose ($g L^{-1}$) | Malic acid ($g L^{-1}$) | Total acidity ($g L^{-1}$) | pH | Total sugar ($g L^{-1}$) |
| **Control** | H[3] | na[4] | na | 0.14±0.01 | **14.67±0.72**[6] | **5.00±2.26** | 1.60±0.96 | 2.23±0.21 | 5.70±0.36 | 3.11±0.05 | **7.20±2.72** |
| **WG[5]** | H | -4 | 4 | 0.16±0.02 | **14.75±1.06** | 1.57±0.49 | 1.30±0.89 | 2.00±0.17 | 4.77±0.64 | 3.46±0.07 | 3.37±0.21 |
| | | -20 | 4 | 0.15±0.11 | **14.43±0.71** | 0.87±0.81 | 1.43±0.75 | 2.50±0.53 | 4.83±0.51 | 3.60±0.13 | 2.50±0.92 |
| **MG** | H | -4 | 4 | 0.27±0.11 | **15.00±0.62** | 1.70±1.93 | 1.33±0.76 | 2.20±0.53 | 5.37±0.45 | 3.52±0.05 | 3.30±2.82 |
| | | -20 | 4 | 0.28±0.05 | **14.53±0.61** | 0.37±0.55 | 1.00±0.79 | 1.87±0.15 | 5.13±0.70 | 3.54±0.08 | 1.80±1.01 |
| **TM** | H | -4 | 4 | 0.26±0.06 | 13.60±0.75 | 1.53±1.79 | 1.00±1.04 | 1.43±0.35 | 5.80±0.52 | 3.20±0.06 | 3.07±2.01 |
| | | -20 | 4 | 0.29±0.08 | 12.87±0.55 | 3.00±4.24 | 0.15±0.21 | 1.80±0.14 | 6.20±0.61 | 3.12±0.10 | 4.60±4.95 |
| **CJ** | H | -4 | 4 | 0.31±0.04 | **14.37±0.81** | 2.03±1.11 | 1.07±1.07 | 1.63±0.21 | 6.80±0.69 | 3.11±0.09 | 3.57±0.93 |
| | | -20 | 4 | 0.33±0.07 | **14.43±0.67** | **4.87±0.15** | 0.97±0.64 | 1.37±0.49 | 6.63±0.49 | 3.06±0.08 | **6.33±0.42** |

[1]Means ± standard deviation (n=3) for all samples between treatments within columns.
[2]Cryogenic temperature treatment
[3]Hand harvesting method
[4]Not applicable – na
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[6]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.

Table 3.15: Chemical profiles of Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer C from the Cape South Coast wine region) in 2020.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Volatile acidity (g $L^{-1}$) | Alcohol (%) | Fructose (g $L^{-1}$) | Glucose (g $L^{-1}$) | Malic acid (g $L^{-1}$) | Total acidity (g $L^{-1}$) | pH | Total sugar (g $L^{-1}$) |
| **Control** | na[3] | na | 0.26±0.08 | 12.83±0.12 | 0.27±0.06 | 0.57±0.06 | 1.70±0.36 | 6.37±0.42 | 3.15±0.03 | 2.60±0.17 |
| **WG[4]** | -4 | 0 | 0.27±0.03 | 12.07±0.15 | 0.57±0.12 | 0.43±0.38 | 2.03±0.25 | 6.07±0.38 | 3.34±0.06 | 2.93±0.25 |
| | -20 | 0 | 0.28±0.05 | 12.13±0.21 | 0.67±0.21 | 0.37±0.06 | 2.67±0.49 | 5.40±0.30 | 3.62±0.03 | 3.23±0.06 |
| | -4 | 4 | 0.30±0.02 | 12.50±0.20 | 0.23±0.25 | 0.03±0.06 | 2.63±0.40 | 4.20±0.17 | 3.68±0.05 | 0.00±0.00 |
| | -20 | 4 | 0.35±0.04 | 12.80±0.26 | 0.53±0.21 | 0.00±0.00 | 2.57±0.21 | 4.50±0.26 | 3.64±0.04 | 0.00±0.00 |
| **MG** | -4 | 0 | 0.34±0.04 | 12.80±0.36 | 0.40±0.10 | 0.20±0.35 | 2.47±0.32 | 5.50±0.10 | 3.56±0.18 | 2.17±1.89 |
| | -20 | 0 | 0.32±0.01 | 12.47±0.51 | 0.63±0.12 | 0.20±0.17 | 2.17±0.21 | 5.40±0.26 | 3.55±0.05 | 3.30±0.10 |
| | -4 | 4 | **0.40±0.04**[5] | 12.83±0.21 | 1.13±0.49 | 0.00±0.00 | 2.33±0.12 | 4.30±0.10 | 3.65±0.02 | 0.00±0.00 |
| | -20 | 4 | 0.39±0.06 | 12.70±0.26 | 1.43±1.50 | 0.00±0.00 | 2.13±0.06 | 4.23±0.12 | 3.60±0.12 | 2.20±3.81 |
| **TM** | -4 | 0 | 0.37±0.04 | 12.43±0.67 | 0.63±0.15 | 0.17±0.15 | 1.23±0.55 | 5.80±0.35 | 3.25±0.14 | 3.23±0.12 |
| | -20 | 0 | 0.33±0.02 | 10.87±1.01 | 0.60±0.36 | 0.00±0.00 | 0.97±0.12 | 5.80±0.10 | 3.20±0.00 | 3.13±0.35 |
| | -4 | 4 | 0.36±0.06 | 10.70±1.74 | 1.17±0.40 | 0.00±0.00 | 1.17±0.12 | 5.30±0.44 | 3.11±0.08 | 2.40±2.12 |
| | -20 | 4 | **0.41±0.03** | 12.50±0.26 | 1.53±0.12 | 0.00±0.00 | 1.67±0.67 | 5.43±0.72 | 3.30±0.31 | 3.93±0.31 |
| **CJ** | -4 | 0 | **0.40±0.05** | 12.37±0.15 | 0.57±0.21 | 0.00±0.00 | 1.47±0.40 | 6.60±0.26 | 3.11±0.10 | 2.93±0.31 |
| | -20 | 0 | **0.40±0.03** | 12.77±0.49 | 0.43±0.45 | 0.00±0.00 | 1.07±0.15 | 6.33±0.58 | 3.13±0.12 | 3.23±0.59 |
| | -4 | 4 | **0.48±0.01** | 12.97±0.25 | 2.73±1.10 | 0.00±0.00 | 1.13±0.25 | 5.97±0.23 | 3.09±0.05 | 5.20±1.21 |
| | -20 | 4 | **0.40±0.05** | 13.07±0.21 | 1.40±0.20 | 0.00±0.00 | 1.20±0.10 | 5.80±0.26 | 3.06±0.08 | 3.83±0.15 |

[1]Means ± standard deviation (n=3) for all samples between treatments within columns.
[2]Cryogenic temperature treatment
[3]Not applicable – na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[5]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.

Table 3.16: Chemical profiles of Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer C from the Cape South Coast wine region) in 2021.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Volatile acidity (g L$^{-1}$) | Alcohol (%) | Fructose (g L$^{-1}$) | Glucose (g L$^{-1}$) | Malic acid (g L$^{-1}$) | Total acidity (g L$^{-1}$) | pH | Total sugar (g L$^{-1}$) |
| **Control** | na[3] | na | 0.25±0.09 | 13.40±0.50 | 1.17±0.83 | 0.73±0.31 | 2.77±0.29 | 5.63±0.96 | 3.26±0.06 | 2.67±0.81 |
| **WG[4]** | -4 | 0 | 0.21±0.11 | 13.33±0.70 | 1.17±0.49 | 0.83±0.38 | 2.57±0.31 | 4.30±0.17 | 3.49±0.02 | 2.73±0.31 |
| | -20 | 0 | 0.19±0.11 | 13.57±0.75 | 1.07±0.72 | 0.67±0.32 | 2.97±0.06 | 4.03±0.15 | 3.58±0.04 | 2.70±0.80 |
| | -4 | 4 | 0.31±0.11 | 13.07±0.25 | 1.13±0.31 | 0.57±0.42 | 3.67±0.32 | 5.30±0.26 | 3.67±0.02 | 2.53±0.32 |
| | -20 | 4 | 0.26±0.09 | 13.17±0.29 | 1.00±0.26 | 0.50±0.46 | 2.93±0.06 | 4.63±0.15 | 3.68±0.04 | 2.63±0.25 |
| **MG** | -4 | 0 | 0.23±0.04 | 13.57±0.50 | 0.67±0.15 | 0.70±0.17 | 2.87±0.32 | 4.63±0.21 | 3.46±0.04 | 2.27±0.40 |
| | -20 | 0 | 0.28±0.09 | 13.50±0.50 | 0.73±0.45 | 0.47±0.25 | 2.90±0.66 | 4.27±0.21 | 3.49±0.03 | 2.67±0.55 |
| | -4 | 4 | 0.39±0.06 | 13.63±0.31 | 1.07±0.47 | 0.53±0.46 | 3.30±0.53 | 4.83±0.58 | 3.63±0.01 | 2.80±0.36 |
| | -20 | 4 | 0.36±0.14 | 13.13±0.76 | 0.83±0.31 | 0.23±0.21 | 3.10±0.53 | 5.27±0.46 | 3.62±0.01 | 2.17±0.35 |
| **TM** | -4 | 0 | 0.27±0.11 | 13.33±0.42 | 0.73±0.64 | 0.50±0.17 | 2.67±0.12 | 5.07±0.65 | 3.34±0.01 | 2.13±0.42 |
| | -20 | 0 | 0.22±0.07 | 12.77±0.35 | 0.57±0.67 | 0.50±0.26 | 2.73±0.23 | 5.23±0.21 | 3.29±0.04 | 2.27±0.38 |
| | -4 | 4 | 0.35±0.05 | 13.33±0.49 | 0.87±0.06 | 0.30±0.30 | 2.97±0.74 | 5.53±0.45 | 3.34±0.04 | 2.20±0.36 |
| | -20 | 4 | 0.29±0.11 | 12.77±0.06 | 0.97±0.49 | 0.33±0.21 | 2.53±0.15 | 5.47±0.23 | 3.31±0.02 | 2.27±0.67 |
| **CJ** | -4 | 0 | 0.29±0.12 | 13.67±0.29 | 0.93±0.46 | 0.53±0.47 | 2.43±0.21 | 4.93±0.42 | 3.32±0.03 | 2.57±0.32 |
| | -20 | 0 | 0.28±0.04 | 13.63±0.31 | 1.43±0.15 | 0.43±0.42 | 3.00±0.53 | 4.90±0.50 | 3.28±0.02 | 3.00±0.35 |
| | -4 | 4 | **0.39±0.11**[5] | 13.60±0.53 | 1.30±0.40 | 0.40±0.36 | 2.33±0.42 | 5.90±0.75 | 3.37±0.01 | 2.87±0.67 |
| | -20 | 4 | **0.37±0.05** | 13.40±0.10 | 1.20±0.62 | 0.40±0.36 | 2.47±0.29 | 5.47±0.76 | 3.34±0.00 | 2.67±1.14 |

[1]Means ± standard deviation (n=3) for all samples between treatments within columns.
[2]Cryogenic temperature treatment
[3]Not applicable – na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[5]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.

Table 3.17: Chemical profiles of Chenin blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer D from the Stellenbosch wine region) in 2021.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Chemical Analyses[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Volatile acidity ($g\ L^{-1}$) | Alcohol (%) | Fructose ($g\ L^{-1}$) | Glucose ($g\ L^{-1}$) | Malic acid ($g\ L^{-1}$) | Total acidity ($g\ L^{-1}$) | pH | Total sugar ($g\ L^{-1}$) |
| **Control** | na[3] | na | 0.20±0.05 | **14.03±0.78**[5] | 1.63±0.06 | 0.53±0.15 | 2.27±0.25 | 4.03±0.47 | 3.41±0.06 | 3.17±0.29 |
| **WG[4]** | -4 | 0 | 0.19±0.10 | 13.90±0.87 | 1.97±0.49 | 0.63±0.15 | 2.80±0.10 | 4.33±0.50 | 3.65±0.12 | 3.10±0.92 |
| | -20 | 0 | 0.23** | 14.10 | 1.50 | 0.60. | 2.90 | 4.50 | 3.72 | 3.00 |
| | -4 | 4 | 0.25±0.11 | 13.47±0.78 | 2.57±1.60 | 0.93±0.35 | 3.40±0.10 | 4.83±0.55 | 3.81±0.04 | 3.83±2.01 |
| | -20 | 4 | 0.25±0.09 | 13.67±0.35 | 1.40±0.62 | 0.43±0.29 | 3.47±0.12 | 5.03±0.21 | 3.85±0.12 | 2.60±0.44 |
| **MG** | -4 | 0 | 0.30±0.06 | 13.90±0.99 | 1.15±0.21 | 0.70±0.42 | 3.15±0.49 | 5.05±0.21 | 3.76±0.09 | 2.40±0.28 |
| | -20 | 0 | 0.26±0.03 | 13.85±1.20 | 0.80±0.42 | 0.45±0.07 | 3.40±0.42 | 4.90±0.00 | 3.73±0.09 | 2.05±0.49 |
| | -4 | 4 | 0.30±0.10 | **14.30±0.80** | **3.97±4.38** | 1.00±0.36 | 3.50±0.50 | 4.97±1.00 | 3.91±0.05 | **5.63±5.27** |
| | -20 | 4 | 0.22±0.14 | **14.27±0.23** | **3.60±2.86** | 1.07±0.46 | 3.33±0.40 | 4.67±0.59 | 3.87±0.09 | **5.13±3.61** |
| **TM** | -4 | 0 | 0.16±0.01 | 12.60±0.85 | 0.55±0.07 | 1.25±0.64 | 2.75±0.21 | 4.30±0.28 | 3.47±0.16 | 2.20±0.85 |
| | -20 | 0 | 0.07 | 13.40 | 1.60 | 0.40 | 2.60 | 4.00 | 3.39 | 1.90 |
| | -4 | 4 | 0.19±0.14 | 12.70±0.96 | 0.93±0.31 | 0.53±0.76 | 1.77±1.18 | 4.50±0.89 | 3.44±0.17 | 1.73±0.64 |
| | -20 | 4 | 0.24±0.13 | 13.47±0.40 | 1.10±0.95 | 0.67±0.06 | 2.30±0.10 | 4.63±0.47 | 3.56±0.12 | 2.43±1.01 |
| **CJ** | -4 | 0 | 0.31 | **14.20** | 0.80 | 0.20 | 2.80 | 4.80 | 3.48 | 2.20 |
| | -20 | 0 | 0.26 | 13.40 | 0.70 | 0.40 | 2.70 | 4.00 | 3.63 | 2.30 |
| | -4 | 4 | 0.28±0.16 | 13.75±0.92 | 0.75±0.49 | 0.35±0.35 | 2.70±0.28 | 4.70±0.57 | 3.73±0.06 | 1.65±0.35 |
| | -20 | 4 | 0.21±0.15 | 13.80±1.13 | 0.45±0.07 | 0.45±0.07 | 2.10±1.27 | 4.50±0.85 | 3.56±0.10 | 1.40±0.00 |

[1]Means ± standard deviation (n=3) for all samples between treatments within columns.
[2]Cryogenic temperature treatment
[3]Not applicable – na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[5]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
**Single values without standard deviation due to outliers being statistically removed

pre-treatment on the physicochemical parameters of the final wines were comparable to previous research, which found that alcohol, VA, MA, TS, glucose and fructose levels were generally similar in wines made from the unfrozen control and cryogenically pre-treated WG, MG, TM and CJ (Sauvignon blanc and Chenin blanc) and across vintages (Peinado et al., 2004:586; Salinas et al., 2005:1529 1530; Carillo et al., 2011:11-12; Naviglio et al., 2018:6-10; Naranjo et al., 2021:1-13). The physicochemical parameters most affected were the pH, which was higher for wines made from the cryogenic treatments. No trend was observed regarding the impact of the stage of production, cryogenic temperature and storage period on the TA levels, which fluctuated higher or lower than the levels found in the control wines and contradicted findings reported in previous studies (Darias-Martıń et al., 2000:484; Zhang et al., 2015:21610-21611; Radeka et al., 2023:6; Gu et al., 2025:3). It was also seen that the harvesting technique had an impact on the TA levels because higher levels were seen in wines from hand-harvested control grapes compared to the machine-harvested control grapes. Furthermore, previous research showed that the type of cryomaceration treatment applied and the stage of production, i.e., WG, MG or CJ influenced must and wine physicochemical parameters (Zhang et al., 2015:21611; 21621-21622; Naviglio et al., 2018:6-10; Ruiz-Rodríguez et al., 2020:1-13; Naranjo et al., 2021:1-13; Radeka et al., 2023:6; Gu et al., 2025:3). In summary, while cryogenic freezing at different stages does not broadly affect routine chemical parameters, pH is the most sensitive parameter showing an increase, and harvesting technique can influence TA. No clear grouping by stage, storage time, or temperature was observed for other parameters.

### 3.3.3 Aroma compounds

#### 3.3.3.1 Varietal thiols

Varietal thiols, well known for their impact on Sauvignon blanc wine aroma, were also analysed. Thiols are compounds that contribute greatly to the perceived varietal aroma of Sauvignon blanc wines and are responsible for the “grapefruit”, “passion fruit”, and “tropical aromas” and the green aromas, i.e., “broom”, “box tree” and “cat urine” (Benkwitz et al., 2012a:6296-6298; Ruiz et al., 2019:7426; 7433-7435; Chen et al., 2019:637; Chen & Li, 2022:313-315). The three main varietal thiols quantified in this study were 3-SHA, 4-MSP and 3-SH. A large variation in the concentrations of these compounds was observed in wines made from the unfrozen control grapes and the cryogenically pre-treated WG, MG, TM and CJ over the two vintages (2020 and 2021) and between the different wine regions (Tables 3.18-3.21).

*3-SHA*

For Producer A, it was seen that in 2020, the highest concentrations of 3-SHA were found in the T0 wines made from TM (-4 ºC) (970.70 ng $L^{-1}$) followed by the T0 wines made from MG (-20 ºC) (905.53 ng $L^{-1}$) (Table 3.18). For the 2021 harvest, the highest concentrations were found in T4 wines made from CJ (-4 ºC) (841.54 ng $L^{-1}$) and CJ (-20 ºC) (659.50 ng $L^{-1}$), followed by T4 wines made from TM (-20 ºC) (652.26 ng $L^{-1}$) and TM (-4 ºC) (618.27 ng $L^{-1}$) (Table 3.18). The levels of 3-SHA measured were found to be higher than the aroma perception threshold (4 ng $L^{-1}$) and range (23-151 ng $L^{-1}$) (Table 2.2) previously reported in SA Sauvignon blanc wines (Benkwitz et al., 2012b:66-67; Van Wyngaard, 2013:65; Piano et al., 2015:46; Coetzee et al., 2018:182). Concentrations of 3-SHA detected in wines made from grapes from Producer B were generally higher than those observed for all the other producers. In 2020, the highest concentrations were detected in wines made from hand-harvested WG (-20 ºC) (2377.47 ng $L^{-1}$), followed by the unfrozen control (678.21 ng $L^{-1}$) (Table 3.19). It was further observed that when grapes from the same vineyard were harvested by machine and subjected to cryogenic pre-fermentative treatments, the levels of 3-SHA were substantially higher (490.82-3355.15 ng $L^{-1}$) for all stages of production and cryogenic treatments (-4 ºC and -20 ºC) (Table 3.19). Wines made from the MG (-20 ºC) had the highest level of 3-SHA (3355.15 ng $L^{-1}$) whilst wines made from the unfrozen control had the lowest levels (490.82 ng $L^{-1}$).

These findings in terms of harvesting technique were comparable to previous studies that showed the levels of thiols to be higher in machine-harvested grapes compared to hand-harvested grapes, without including cryogenic treatments (Jouanneau et al., 2011:218-220; Kilmartin, 2012:81-82; Olejar et al., 2015:187; Coetzee, 2020b). The abovementioned levels of 3-SHA far exceeded levels measured in previous studies for SA Sauvignon blanc and are more aligned to the levels found in Sauvignon blanc wines from New Zealand (Jouanneau, 2011:119; Benkwitz et al., 2012a:6298; 2012b:66; Coetzee et al., 2018:182). In 2021, only hand-harvested grapes were obtained from the same producer, and the concentrations of 3-SHA were generally lower for all treatments, including the control (106.74 to 472.45 ng $L^{-1}$), however, still higher than the ranges commonly reported for SA Sauvignon blanc wines (Table 2.2). These findings supported previous research that showed the harvesting technique had an impact on the aroma compounds detected in the final wines (Jouanneau, 2011:239; Coetzee et al., 2018:182).

For Producer C, the highest concentrations of 3-SHA in 2020 were measured in the T0 wines made from MG (-4 ºC) (1229.96 ng $L^{-1}$), CJ (-4 ºC) (1068.88 ng $L^{-1}$), WG (-20 ºC) (999.40 ng $L^{-1}$), T4 wines made from WG (-20 ºC) (915.63 ng $L^{-1}$) and T0 wines made from TM (-4 ºC) (858.34 ng $L^{-1}$) (Table 3.20). The concentration of 3-SHAin the unfrozen control was substantially lower (231.47 ng $L^{-1}$), with the T0 wines made from WG (-4 ºC) having the lowest concentrations (2.1 ng $L^{-1}$).

Table 3.18: Varietal thiols detected in Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer A from the Stellenbosch wine region) in 2020 and 2021.

| Treatment | Temp[2] (ºC) | Storage Time (Months) | Varietal Thiols[1] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 3-SHA (ng L$^{-1}$) | | 4-MSP (ng L$^{-1}$) | | 3-SH (ng L$^{-1}$) | |
| | | | 2020 | 2021 | 2020 | 2021 | 2020 | 2021 |
| **Control** | na[3] | na | 36.01±37.89c | 280.31±67.97ef | 0.20±0.00a[4] | 0.20±0.00f | 430.56±175.46bcd | 204.75±169.21ab |
| **WG[5]** | -4 | 0 | 431.93±665.23abc | 206.94±58.77f | **11.57±19.69a**[6] | 0.19±0.02b | 409.37±400.40bcd | 93.82±28.96b |
| | -20 | 0 | 296.17±236.16bc | 300.27±20.91ef | 0.20±0.00a | 0.51±0.05efd | **859.67±460.35ab** | 175.86±110.84ab |
| | -4 | 4 | 119.73±52.24c | 320.46±81.10ef | **7.73±2.36a** | 0.67±0.12cde | **1320.81±514.70a** | 67.54±8.03b |
| | -20 | 4 | 108.49±49.94c | 486.79±33.32bcde | **4.77±1.82a** | 0.83±0.30cd | **778.94±209.09abc** | 162.83±76.81ab |
| **MG** | -4 | 0 | 526.20±264.47abc | 352.71±185.62ef | 0.20±0.00a | 0.79±0.42cde | **706.20±113.77abcd** | 225.19±200.09ab |
| | -20 | 0 | **905.53±1283.14ab** | 296.06±190.60ef | **9.80±16.63a** | 0.92±0.12bc | **718.93±694.40abcd** | 73.35±50.57b |
| | -4 | 4 | 100.67±47.57c | 287.19±31.82ef | 1.34±1.97a | **1.25±0.46b** | **777.02±846.34abc** | **282.36±15.43ab** |
| | -20 | 4 | 95.30±71.21c | 434.26±216.27de | 0.20±0.00a | **2.11±0.11a** | 547.94±169.53bcd | 271.64±193.74ab |
| **TM** | -4 | 0 | **970.70±389.20a** | 331.41±39.02ef | **8.07±13.62a** | 0.20±0.00f | 531.80±354.87bcd | 184.65±55.60ab |
| | -20 | 0 | 309.63±325.73bc | 394.89±16.29ef | **7.30±12.30a** | 0.20±0.00f | 359.97±441.86bcd | 273.12±4.64ab |
| | -4 | 4 | 21.12±18.39c | **618.27±0.83bcd** | 0.20±0.00a | 0.20±0.00f | 154.00±27.32d | **375.52±215.38a** |
| | -20 | 4 | 49.49±57.77c | **652.26±276.17abc** | 0.20±0.00a | 0.20±0.00f | 181.97±67.81cd | 340.79a |
| **CJ** | -4 | 0 | 350.73±275.74abc | 443.87±84.35cde | 2.77±4.44a | 0.40±0.23ef | 357.30±132.94bcd | 197.10±103.51ab |
| | -20 | 0 | 109.77±57.09c | 341.07±69.55ef | 0.20±0.00a | 0.42±0.40ef | **599.00±221.61bcd** | 248.93±192.74ab |
| | -4 | 4 | 11.82±9.54c | **841.54±173.66a** | 0.20±0.00a | 0.45±0.43efd | 190.43±55.53cd | **361.50±131.18a** |
| | -20 | 4 | 314.43±51.86bc | **659.50±183.76ab** | 0.20±0.00a | 0.20±0.00f | 270.53±156.30bcd | **283.89±166.20ab** |

[1]Means ± standard deviation (n=3).
[2]Storage temperature.
[3]Not applicable - na
[4]Limit of detection (LOD) for 4-MSP in white wine according (Mafata *et al*., 2018).
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[6]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

In 2021, wine made from the T0 MG (-20 ºC) had the highest concentration of 3-SHA (1010.81 ng $L^{-1}$), whereas the concentration in the unfrozen control was lower (399.46 ng $L^{-1}$) (Table 3.20). These measured levels were once again above the reported aroma perception threshold (4 ng $L^{-1}$) and range (23-151 ng $L^{-1}$) (Table 2.2) (Coetzee et al., 2018:182). For Producer D, wines were only made from unfrozen Chenin blanc grapes (control) and Chenin blanc grapes subjected to cryogenic treatments (-4 ºC and -20 ºC). The highest concentrations of 3-SHA were found in T0 wines made from MG (-20 ºC) (638.01 ng $L^{-1}$), T4 wines made from MG (-4 ºC) (559.15 ng $L^{-1}$) and MG (-20 ºC) (614.61 ng $L^{-1}$) (Table 3.21). The unfrozen control was lower (355.70 ng $L^{-1}$) and the concentrations of 3-SH in the remaining treatments ranged from 111.27 to 400.34 ng $L^{-1}$ (Table 3.21). These levels were higher than the previously reported aroma perception thresholds (4 ng $L^{-1}$) for SA Sauvignon blanc and Chenin blanc wines and their ranges (23-151 ng $L^{-1}$) and (5-253 ng $L^{-1}$), respectively (Table 2.2) (Wilson, 2017:28-29; Coetzee et al., 2018:182).

*4-MSP*

For the varietal thiol, 4-MSP, for Producer A (2020), the highest concentrations were detected in the T0 wines made from WG (-4 ºC) (11.57 ng $L^{-1}$), MG (-20ºC) (9.80 ng $L^{-1}$), TM (-4 ºC) (8.07 ng $L^{-1}$), the T4 wine made from WG (-4 ºC) (7.73 ng $L^{-1}$), the T0 wine made from TM (-20 ºC) (7.30 ng $L^{-1}$), the T4 wine made from WG (-20 ºC) (4.77 ng $L^{-1}$), whilst the concentrations detected in the remaining wines and the control ranged between the limit of detection (0.2 ng $L^{-1}$) and 2.77 ng $L^{-1}$ (Table 3.18). In 2021, the concentration of 4-MSP for all treatments was substantially lower than in 2020. The highest concentration was detected in the T4 wine made from MG (-20 ºC) (2.11 ng $L^{-1}$) with the concentrations in the remaining treatments ranging between the limit of detection (0.2 ng $L^{-1}$) and 1.25 ng $L^{-1}$ (Table 3.18). Moreover, for Producer A, the concentrations of 4-MSP were found to be below the aroma perception threshold (0.8 ng $L^{-1}$) and below the typical ranges commonly reported for SA Sauvignon blanc (0-21.9 ng $L^{-1}$) (Table 2.2) (Van Wyngaard, 2013:65; Capone et al., 2015:1227; Jeffery, 2016:1324; Coetzee et al., 2018:180-184).

The highest concentration of 4-MSP for Producer B (2020) was detected in wines made from hand-harvested WG (-4 ºC) (4.33 ng $L^{-1}$), whilst for the remaining WG (-4 ºC and -20 ºC) treatments and the control, it fell below the limit of detection (0.2 ng $L^{-1}$) (Table 3.19). Furthermore, for the machine-harvested unfrozen control and all treatments subjected to cryogenic pre-fermentative freezing, the concentrations were once again lower than the limit of detection (0.2 ng $L^{-1}$). In 2021, the highest concentrations of 4-MSP were detected in T4 wines made from WG (-4 ºC) (6.43 ng $L^{-1}$), followed by the control (4.53 ng $L^{-1}$) and WG (-20 ºC) (3.22 ng $L^{-1}$), with

Table 3.19: Varietal thiols detected in Sauvignon blanc wines produced from cryogenically pre-treated hand-harvested WG and machine-harvested MG, TM and CJ (supplied by Producer B from the Durbanville wine region) in 2020 and 2021.

| Treatment | Harvesting[2] technique | Temp[3] (ºC) | Storage time (Months) | Varietal Thiols[1] 3-SHA ($ng\ L^{-1}$) 2020 | 3-SHA ($ng\ L^{-1}$) 2021 | 4-MSP ($ng\ L^{-1}$) 2020 | 4-MSP ($ng\ L^{-1}$) 2021 | 3-SH ($ng\ L^{-1}$) 2020 | 3-SH ($ng\ L^{-1}$) 2021 |
|---|---|---|---|---|---|---|---|---|---|
| **Control** | H | na[4] | na | **678.21±75.83bcd**[7] | 278.08±83.71ab | 0.20±0.00b[5] | **4.53±0.72b** | **899.63±149.49cd** | **212.59±127.37bcd** |
| **WG[6]** | H | -4 | 4 | 107.60±68.20d | 305.14±52.26ab | **4.33±3.79a** | **6.43±1.86a** | **1195.10±76.05d** | 74.11±25.65e |
| | H | -20 | 4 | **2377.47±1098.21abc** | **472.45±64.82a** | 0.20±0.00b | 3.22±0.94bc | 252.98±87.57cd | **260.43±48.78b** |
| **MG** | H | -4 | 4 | na | **421.29±50.79a** | na | 2.14±0.43cd | na | **237.51±44.12bc** |
| | H | -20 | 4 | na | 316.25±155.10ab | na | 1.48±0.63d | na | 143.59±44.85cde |
| **TM** | H | -4 | 4 | na | **436.37±291.10a** | na | 2.13±0.28cd | na | **524.43±0.25a** |
| | H | -20 | 4 | na | 294.90±230.17ab | na | 2.72±1.00cd | na | 99.50±60.38e |
| **CJ** | H | -4 | 4 | na | 318.63±58.17ab | na | 2.59±0.16cd | na | 113.63±51.05de |
| | H | -20 | 4 | na | 106.74±22.87b | na | 2.34±0.65cd | na | 127.62±42.75cde |
| **Control** | M | na | na | 490.82±158.30cd | na | 0.20±0.00b | na | 549.46±256.10cd | na |
| **MG** | M | -4 | 4 | **2619.97±541.26ab** | na | 0.20±0.00b | na | **2632.96±192.97b** | na |
| | M | -20 | 4 | **3355.15±2435.37a** | na | 0.20±0.00b | na | **4137.70±2297.65a** | na |
| **TM** | M | -4 | 4 | **1575.37±683.73abcd** | na | 0.20±0.00b | na | **1067.40±166.67cd** | na |
| | M | -20 | 4 | **2643.07±1082.68ab** | na | 0.20±0.00b | na | **1334.93±541.03bcd** | na |
| **CJ** | M | -4 | 4 | **2661.06±2031.95ab** | na | 0.20±0.00b | na | **1258.20±584.50bcd** | na |
| | M | -20 | 4 | **2744.35±141.35ab** | na | 0.20±0.00b | na | **1692.30±562.00bc** | na |

[1]Means ± standard deviation (n=3) for all samples, except for the Juice M_(-20ºC) treatment which had n=2.
[2]Hand-harvested or machine-harvested grapes.
[3]Storage temperature
[4]Not applicable - na
[5]Limit of detection (LOD) for 4-MSP in white wine according (Mafata *et al*., 2018).
[6]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[7]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

concentrations in the remaining treatments ranging from 1.48 ng $L^{-1}$ to 2.72 ng $L^{-1}$ (Table 3.19). From the results, it appears that the cryogenic treatments did not have a significant impact on the levels of 4-MSP detected in the final wines, but the harvesting technique and vintage may have had an effect. Moreover, in the current research, for this producer, it was found that the concentration of 4-MSP in wines made from machine-harvested grapes mostly fell below the aroma perception threshold (0.5 ng $L^{-1}$) and range (0-21.9 ng $L^{-1}$) typically reported for SA Sauvignon blanc wines whilst for wines made from hand-harvested grapes, 4-MSP was present in concentrations above the aroma perception threshold and within the typically reported range (Table 2.2) (Jeffery, 2016:1324; Coetzee et al. 2018:182). The abovementioned results differed from those of Allen et al. (2011:10646), Kilmartin (2012:86) and Coetzee (2020b:3), who found that compounds such as the methoxypyrazines and 4-MSP were similar in wines made from hand and machine-harvested grapes. It can be hypothesised that because machine harvesting is a much more "aggressive" technique, it tends to break berries and increase skin contact time during transport. However, due to prolonged travel time to the cellar, 4-MSP was susceptible to oxidation because volatile thiols and specifically 4-MSP, are very reactive compounds.

The concentrations of 4-MSP in wines made from the control and most cryogenic pre-treated WG, MG, TM & CJ from Producer C (2020) ranged between the limit of detection (0.2 ng $L^{-1}$) and 0.62 ng $L^{-1}$, except for the T0 CJ (-4 ºC) wine, which had the highest concentration of 0.86 ng $L^{-1}$ (Table 3.20). However, in 2021, 4-MSP concentrations were higher than in 2020. The highest concentrations were detected in the T4 wines made from WG (-4 ºC) (6.28 ng $L^{-1}$), T0 wines made from TM (-20 ºC) (4.89 ng $L^{-1}$), concentrations in the wines from the remaining treatments (1.49 to 3.894 ng $L^{-1}$) and followed by the unfrozen control with a concentration of 2.65 ng $L^{-1}$. Overall, for Producer C, the concentrations of 4-MSP varied between vintages, with concentrations in 2020 falling below the aroma perception threshold (0.8 ng $L^{-1}$) and range (0-21.9 ng $L^{-1}$) typically reported for SA Sauvignon blanc wines when compared to 2021 (Tables 2.2 and 3.20).

For Producer D, the highest concentrations of 4-MSP were detected in Chenin blanc wines made from the unfrozen control grapes (0.54 ng $L^{-1}$), T4 CJ (-4 ºC) (0.54 ng $L^{-1}$), T0 CJ (-4 ºC) (0.52 ng $L^{-1}$), T0 WG (-20 ºC) (0.52 ng $L^{-1}$) and WG (-4 ºC) (0.50 ng $L^{-1}$) (Table 3.21). The remaining treatments had concentrations ranging from the limit of detection (0.2 ng $L^{-1}$) to 0.45 ng $L^{-1}$ (Table 3.21). Furthermore, the levels of 4-MSP measured in all the Chenin blanc wines fell below the aroma perception threshold (0.8 ng $L^{-1}$) and above the global reported range (0-23 ng $L^{-1}$) and the typically reported range for SA Chenin blanc wines (n.d.) (Table 2.2). The detection of 4-MSP in this study supports its presence in SA Chenin blanc wines, despite earlier studies yielding inconsistent results likely attributed to its inherently low concentrations or the sensitivity limits of the analytical methods employed (du Plessis & Augustyn, 1981:102; Piano et al., 2015:46; Coetzee et al., 2018:182). Moreover, in cases where cryogenic freezing led to higher 4-MSP levels, the increased extraction of this compound may be attributed to the formation of ice crystals within the grape skin cells, which enhances cell wall rupture and compound release, as reported in previous research (Olejar et al., 2015:187). However, since 4-MSP concentrations varied across treatments, additional research, including data from a second vintage, is recommended to confirm these findings and better understand the impact of cryogenic freezing on varietal thiol extraction.

*3-SH*

The highest concentrations of the varietal thiol 3-SH for Producer A (2020) were detected in the T4 wines made from WG (-4 ºC) (1320.81 ng $L^{-1}$), T0 WG (-20 ºC) (859.67 ng $L^{-1}$), T4 WG (-20 ºC) (778.94 ng $L^{-1}$) followed by the T4 MG (-4 ºC) (777.02 ng $L^{-1}$) (Table 3.18). The concentration in the unfrozen control wines was 430.56 ng $L^{-1}$ and the remaining treatments ranged from 154.00 to 718.93 ng $L^{-1}$. In 2021, the highest concentrations of 3-SH were detected in wines made from the T4 TM (-4 ºC) (375.52 ng $L^{-1}$), T4 CJ (-4 ºC) (361.50 ng $L^{-1}$), followed by the T4 wines made from TM (-20 ºC) (340.79 ng $L^{-1}$). The concentrations detected in the unfrozen control wines (204.75 ng $L^{-1}$) and remaining treatments ranged from 67.45 to 283.89 ng $L^{-1}$ (Table 3.18). Overall, the levels of 3-SH were lower in 2021 compared to 2020, for most treatments and control wines, indicating that the differences observed could be vintage-related, rather than a result of the cryogenic pre-treatments. This observation differed from the findings of Olejar et al. (2015:187-188) and Chen et al. (2019:642-644), who found an increase in the varietal thiol levels of hand-picked Sauvignon blanc grapes subjected to cryogenic maceration and pre-fermentative freezing, respectively. Moreover, in the current study, the levels of 3-SH were within the aroma perception threshold (60 ng $L^{-1}$) levels and range for SA Sauvignon blanc wines (178-904 ng $L^{-1}$) as reported by Jeffery (2016:1324) and Coetzee et al. (2018:182), except for some wines which exceeded these ranges (Tables 2.2 and 3.18).

For Producer B (2020), the highest concentrations of 3-SH were detected in the wines made from hand-harvested WG (-4 ºC) (1195.10 ng $L^{-1}$), followed by the unfrozen control (899.63 ng $L^{-1}$) and the wines made from WG (-20 ºC) (252.98 ng $L^{-1}$) (Table 3.19). In the wines made from machine-harvested grapes, the highest concentrations of 3-SH were detected in wines made from MG (-20 ºC) (4137.70 ng $L^{-1}$), followed by the MG (-4 ºC) (2632.96 ng $L^{-1}$) (Table 3.19). Furthermore, high concentrations were also detected in the wines made from the CJ (-20 ºC)

(1692.30 ng $L^{-1}$), TM (-20 ºC) (1334.93 ng $L^{-1}$), CJ (-4 ºC) (1258.20 ng $L^{-1}$), TM (-4 ºC) (1067.40 ng $L^{-1}$) whilst the wines made from the unfrozen control had the lowest concentration of 549.46 ng $L^{-1}$. The current results support the research conducted by Olejar et al. (2015:187), who found that the thiol 3-SH was higher in wines made from cryogenically treated machine-harvested grapes when compared to hand-harvested treatments.

In 2021, for Producer B, all wines were made from hand-harvested grapes and the concentrations of 3-SH were found to be much lower than in 2020. The treatment with the highest concentration was the wine made from TM (-4 ºC) (524.43 ng $L^{-1}$) followed by the WG (-20 ºC) (260.43 ng $L^{-1}$), the MG (-4 ºC) (237.51 ng $L^{-1}$) and the unfrozen control (212.59 ng $L^{-1}$) (Table 3.19). This indicates that the harvesting method more so than the cryogenic treatments, had a possible effect on the levels of 3-SH in the final wines, which was similar to the findings of Kilmartin (2012:82), who found the levels of 3-SH to be higher in wines made from machine-harvested grapes compared to hand-harvested grapes. Overall, for Producer B, concentrations of 3-SH in most wines exceeded the aroma perception threshold of 60 ng $L^{-1}$. Furthermore, in 2020, the concentrations generally fell above the reported range (178-904 ng $L^{-1}$) for SA Sauvignon blanc wines, whereas in 2021, levels were lower, typically falling within or below this range (Tables 2.2 and 3.19). The highest levels of 3-SH for Producer C (2020) were detected in T0 wines made from WG (-4 ºC) (963.61 ng $L^{-1}$), the T4 wines made from MG (-20 ºC) (730.92 ng $L^{-1}$), T4 wines made from CJ (-20 ºC) (6.33.05 ng $L^{-1}$) and CJ (-4 ºC) (576.13 ng $L^{-1}$) and the unfrozen control with a concentration of 568.77 ng $L^{-1}$. The remaining treatments had concentrations ranging from 4.00 ng $L^{-1}$ to 345.46 ng $L^{-1}$ (Table 3.20).

In 2021, the highest concentrations of 3-SH were detected in wines made from T0 TM (-20 ºC) (965.47 ng $L^{-1}$), followed by MG (-20 ºC) (666.09 ng $L^{-1}$), TM (-4 ºC), (635.57 ng $L^{-1}$), MG (-4 ºC) (546.00 ng $L^{-1}$) and CJ (-4 ºC) (554.89 ng $L^{-1}$). The concentration in the unfrozen control wines was 438.34 ng $L^{-1}$ with the remaining treatments ranging from 21.91 to 370.37 ng $L^{-1}$ (Table 3.20). Once again, for this producer, the levels of 3-SH were generally higher in wines produced in 2020 compared to 2021. Moreover, in the current study, the levels in most wines were above the aroma perception threshold (60 ng $L^{-1}$) for both vintages and generally at the lower end of the reported ranges for SA Sauvignon blanc wines (178-904 ng $L^{-1}$), except for a few slightly higher wines (Tables 2.2 and 3.20).

Furthermore, the highest concentrations of 3-SH detected in Chenin blanc wines from Producer D (2021) were made from T0 MG (-20 ºC) (280.51 ng $L^{-1}$),

Table 3.20: Varietal thiols detected in Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer C from the Cape South Coast wine region) in 2020 and 2021.

| Treatment | Temp[2] (ºC) | Storage Time (Months) | Varietal Thiols[1] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 3-SHA (ng $L^{-1}$) | | 4-MSP (ng $L^{-1}$) | | 3-SH (ng $L^{-1}$) | |
| | | | 2020 | 2021 | 2020 | 2021 | 2020 | 2021 |
| **Control** | na[3] | na | 231.47±122.10fgh | 399.46±66.15def | 0.20±0.00b[4] | 2.65±0.55cdef | **568.77±195.31bc**[6] | **438.34±251.23bcde** |
| **WG**[5] | -4 | 0 | 2.10±0.00h[4] | 339.59±123.85f | 0.2± 0.00b | 4.11±1.44bc | **963.61±402.68a** | 210.11±172.54efgh |
| | -20 | 0 | **999.40±114.32ab** | 262.09±43.93f | 0.20±0.00b | 3.48±1.50bcde | 28.0±5.01e | 148.62±44.21fgh |
| | -4 | 4 | 604.41±309.62cde | 599.70±42.06bc | 0.43±0.18ab | **6.28±0.81a** | 26.84±4.91e | 45.88±18.83gh |
| | -20 | 4 | **915.63±73.55abc** | 591.69±34.64bc | 0.55±0.43ab | 3.89±0.36bcd | 36.11±7.93e | 44.77±15.66gh |
| **MG** | -4 | 0 | **1229.96±207.01a** | 673.92±5.2b | 0.25±0.10b | 2.97±1.50cdef | 34.35±6.27e | **546.00±108.03bcd** |
| | -20 | 0 | **991.76±231.13ab** | **1010.81±105.65a** | 0.31±0.18ab | 3.22±2.11cde | 42.84±11.95e | **666.09±352.95b** |
| | -4 | 4 | **757.71±173.58bcd** | 293.09±132.90f | 0.31±0.08ab | 1.96±0.08ef | 76.88±20.87e | 21.91±15.66h |
| | -20 | 4 | 381.50±113.71efg | 376.12±334.79def | 0.51±0.30ab | 1.49±0.44f | **730.92±161.34ab** | 61.92±37.28gh |
| **TM** | -4 | 0 | **858.34±137.74bc** | 523.04±84.76bcd | 0.47±0.47ab | 3.55±0.73bcde | 85.17±101.10e | **635.57±327.15bc** |
| | -20 | 0 | 476.24±160.14def | 503.22±73.78cde | 0.20±0.00b | **4.89±0.44ab** | 4.00±0.00e[4] | **965.47±32.11a** |
| | -4 | 4 | 85.94±61.76gh | **663.62±87.41b** | 0.20±0.00b | 2.98±1.01cdef | 206.15±26.55de | 213.41±168.12efgh |
| | -20 | 4 | 194.15±80.29fgh | **941.62±76.37a** | 0.20±0.00b | 2.43±0.50def | 345.46±79.18cd | 278.08±120.32defgh |
| **CJ** | -4 | 0 | **1068.88±482.67ab** | 414.52±105.12def | **0.62±0.73ab** | 3.17±1.23cde | 17.24±11.71e | 554.89±57.90bcd |
| | -20 | 0 | 611.53±183.41cde | 365.96±62.67ef | **0.86±0.57a** | 2.09±0.2fe | 21.20±4.53e | 370.37±283.15cdef |
| | -4 | 4 | 159.24±94.42fgh | **608.53±105.66bc** | **0.62±0.73ab** | 2.49±0.69cdef | **576.13±280.35bc** | 317.44±163.30defg |
| | -20 | 4 | 650.94±304.56cde | **626.92±122.46bc** | 0.20±0.00b | 2.37±0.85def | **633.05±246.14b** | 150.59±109.61fgh |

[1] Means ± standard deviation (n=3).
[2]Storage temperature
[3]Not applicable - na
[4]Limits of detection (LOD) for 3-SHA, 4-MSP and 3-SH in white wine (Mafata et al., 2018).
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[6]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

Table 3.21: Varietal thiols detected in Chenin blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer D from the Stellenbosch wine region) in 2021 only.

| Treatment | Temp[2] (ºC) | Storage time (Months) | Varietal Thiols[1] | | |
|---|---|---|---|---|---|
| | | | 3-SHA (ng $L^{-1}$) | 4-MSP (ng $L^{-1}$) | 3-SH (ng $L^{-1}$) |
| **Control** | na[3] | na | 355.70±26.94c | **0.54±0.04a**[7] | **106.62±69.0bc** |
| **WG[6]** | -4 | 0 | 229.09±93.90cd | **0.50±0.42a** | 45.04±23.7bcdef |
| | -20 | 0 | 282.92cd[4] | **0.52a** | 118.89±b |
| | -4 | 4 | 244.06±121.11cd | 0.33±0.03ab | 17.97±7.3ef |
| | -20 | 4 | 269.04±73.99cd | 0.36±0.06ab | 29.01±21.2cdef |
| **MG** | -4 | 0 | 220.70±cd | 0.20±0.00b[5] | **104.63±23.3bcd** |
| | -20 | 0 | **638.01±150.57a** | 0.27±0.13ab | **280.51±67.2a** |
| | -4 | 4 | **559.15±122.05ab** | 0.33±0.11ab | **102.37±16.4bcde** |
| | -20 | 4 | **614.61±97.09a** | 0.32±0.20ab | 89.63±26.5bcdef |
| **TM** | -4 | 0 | 255.02±57.83cd | 0.20±0.00b | 58.81±3.4bcdef |
| | -20 | 0 | 211.24cd | 0.20ab[4] | 46.70bcdef[4] |
| | -4 | 4 | 296.31±84.52cd | 0.29±0.16ab | 19.15±18.0def |
| | -20 | 4 | 400.34±68.66bc | 0.20±0.00b | **106.15±57.6bc** |
| **CJ** | -4 | 0 | 136.89d | **0.52a** | 4.00f[5] |
| | -20 | 0 | 281.65cd | - | - |
| | -4 | 4 | 111.27±5.58d | **0.54±0.09a** | 23.00±13.4cdef |
| | -20 | 4 | 139.05±32.72d | 0.45±0.04ab | 13.34±13.2f |

[1]Means ± standard deviation (n=3).
[2]Storage temperatures
[3]Not applicable - na
[4]Single values without a standard deviation (n=1) and outliers were removed.
[5]Limits of detection (LOD) for 4-MSP and 3-SH in white wine (Mafata et al., 2018).
[6]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[7]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

followed by the unfrozen control (106.62 ng $L^{-1}$), T4 TM (-20 ºC) (106.15 ng $L^{-1}$), T0 MG (-4 ºC) (104.63 ng $L^{-1}$) and the T4 MG (-4 ºC) (102.37 ng $L^{-1}$) (Table 3.21). The remaining wines had 3-SH concentrations ranging from 4.00 to 118.89 ng $L^{-1}$ (Table 3.21). Furthermore, these levels were higher than the previously reported aroma perception threshold (60 ng $L^{-1}$) for SA Chenin blanc wines and below the typically reported range (99-1124 ng $L^{-1}$) (Tables 2.2 and 3.21). Cryogenic treatments, therefore, generally did not increase the 3-SH levels sufficiently above the reported ranges for Chenin blanc, even though *Botrytis cinerea* was present and previous studies reporting significant amounts of volatile thiols, especially 3-SH, due to the concentration effect that *Botrytis* has on grape berries (Dzedze et al., 2019:265-276; Dankó et al., 2021:1-5).

In conclusion, no definite trend regarding which cryogenic temperature, stage of production and storage time would consistently yield the most favourable levels of varietal thiols in the wines produced due to the variations in the levels of these varietal thiols. However, this study found that the region from which the grapes originated, the harvesting technique and the vintage played a role in the concentrations of varietal thiols. In addition, a possible correlation was observed between the YAN levels in the grape must and the varietal thiol levels in the final wines. In 2020, YAN levels in the unfrozen control and treated grape must for most producers were generally higher than in 2021, as illustrated in Figure 3.2 and Tables 3.4-3.9. Previous research showed a correlation between higher nitrogen levels in the vine and increased cysteine precursor synthesis in Sauvignon blanc grapes (Choné et al., 2006:4-5). In the present study, the concentrations of 3-SH and 3-SHA in wines made from the unfrozen control and treatments were not only generally higher in 2020 compared to 2021 (Tables 3.18-3.20), but also exceeded the aroma perception threshold of 60 ng $L^{-1}$ and 4 ng $L^{-1}$ and generally (region dependent) within the reported ranges for SA Sauvignon blanc wines (178-904 ng $L^{-1}$) and (23-151 ng $L^{-1}$) (Table 2.2) (Coetzee et al., 2018:182).

Moreover, the concentrations of 4-MSP in wines for all producers generally fell within the ranges commonly reported for SA Sauvignon blanc (0-21.9 ng $L^{-1}$) and the aroma perception threshold, varied and in some instances exceeded the reported concentration (0.8 ng $L^{-1}$) across vintages and regions. The concentrations of 3-SHA in the Chenin blanc wines produced in this study were higher than the reported ranges (5-253 ng $L^{-1}$) for SA Chenin blanc wines and above the aroma perception threshold level (4 ng $L^{-1}$) (Tables 2.2 and 3.20). The concentrations of 3-SH were generally below the reported range (99-1124 ng $L^{-1}$) and aroma perception threshold level (60 ng $L^{-1}$) for SA Chenin blanc (Tables 2.2 and 3.20). It was further seen that the levels of 4-MSP were above the reported ranges for SA Chenin blanc (n.d.) wines but below the aroma perception threshold (0.8 ng $L^{-1}$) (Table 2.2) (Wilson, 2017:28-29; Coetzee et al., 2018:182; Wilson et al., 2018:635-638; Panzeri et al., 2020:140;142). Moreover, this research confirmed the presence of the varietal thiol 4-MSP in SA Chenin blanc wines, although previous research only hypothesized about its presence, but due to the lack of appropriate methods and equipment for the quantification of thiols in wine at the time, it was not detected (Du Plessis & Augustyn 1981:102; Piano et al., 2015:46; Coetzee et al., 2018:182). These shortcomings have subsequently been resolved with the optimisation of the detection methods, such as was used in this study (Capone et al., 2011:4652; Benkwitz et al., 2012a:6294-6300; Aleixandre-Tudo et al., 2015:373; 375; Wilson, 2017:29; Coetzee et al., 2018:180-184; Mafata et al., 2018:8-9).

#### 3.3.3.2 Methoxypyrazines

Methoxypyrazine (sbMP and ibMP) levels were not affected by the cryogenic pre-fermentative freezing, stage of production and storage time, however, the vintage appeared to have an impact. ibMP concentrations in wine usually range from 2-30 ng $L^{-1}$, whilst sbMP levels in wine are typically < 10 ng $L^{-1}$, with their respective aroma perception thresholds ranging between 2-16 ng $L^{-1}$ (Table 2.3) (Sidhu et al., 2015:494-499; Lei et al., 2018:1142). In this study, the methoxypyrazines were significantly higher in 2021 compared to 2020 (Tables 3.22-3.24), indicating that the vintage had an effect and corroborates previous research that showed methoxypyrazines in grapes and wines were primarily influenced by viticultural practices, which will not be discussed further because it was not an objective of this study (Allen & Lacey, 1993:36-37; Marais, 1994:44; Allen & Lacey, 1998:31-36; Ryona et al., 2008:10844-10845; Alberts et al., 2009:9352; Benkwitz et al., 2012:66; Sidhu et al., 2015:494-499). Moreover, in a blog, Coetzee (2019) highlighted the effect of the vinification process and how methoxypyrazines were managed through cellar practices such as sorting of grapes, destemming, skin contact, juice settling, thermo-vinification, fermentation, fining, blending, light exposure, wood ageing, and oxidation. The current research, however, explored the effect of cryogenic pre-fermentative treatments on the methoxypyrazine compounds in Sauvignon blanc wines (Tables 3.22-3.24).

*sbMP*

In 2020, it was found that the concentrations of sbMP in wines produced from control grapes and cryogenically pre-treated WG, MG, TM and CJ from Producer A ranged between 0-4.84 ng $L^{-1}$ for all treatments (Table 3.22). In 2021, the highest concentrations of sbMP were detected in the T4 wines made from WG (-4 ºC and -20 ºC) (35.22 to 42.81 ng $L^{-1}$), T0 MG (-20 ºC) (32.53 ng $L^{-1}$), WG (-4 ºC and -20 ºC) (26.52 to 31.66 ng $L^{-1}$), followed by the T4 wines made from MG (-4 ºC) (26.31 ng $L^{-1}$). Furthermore, the concentrations of sbMP in the remaining T0 and T4

MG (-4 ºC and -20 ºC) ranged from 20.59 to 20.62 ng $L^{-1}$. The T0 and T4 wines made from TM (-4 ºC and -20 ºC) had concentrations of sbMP ranging from 3.56 to 8.53 ng $L^{-1}$, with the concentrations of the T0 treatments being higher than the T4 treatments. Furthermore, the concentrations of sbMP in the control wines (12.69 ng $L^{-1}$) were generally not significantly higher than those in the wines made from cryogenically pre-treated TM (3.56 to 8.53 ng $L^{-1}$) and CJ (8.74 to 14.45 ng $L^{-1}$). However, the concentrations were lower than in most wines made from WG (26.52 to 42.81 ng $L^{-1}$) and MG (20.59 to 32.53 ng $L^{-1}$) for both vintages (Table 3.22). Furthermore, the concentrations of sbMP for this producer fell within the aroma perception threshold (2-16 ng $L^{-1}$) and above the range (< 10 ng $L^{-1}$) typically reported in Sauvignon blanc wine (Table 2.3).

For Producer B (2020), hand-harvested and machine-harvested grapes were received and subjected to cryogenic pre-fermentative freezing at T4 (Table 3.23). For the 2020 vintage, sbMP was not detected in wines made from the hand-harvested control and cryogenically pre-treated WG. For the machine-harvested grapes, sbMP was only detected in wines made from the unfrozen control MG at a concentration of 0.61 ng $L^{-1}$, which is below the aroma perception threshold typically reported in Sauvignon blanc wine (2-16 ng $L^{-1}$) (Table 2.3). In 2021, the levels of sbMP detected in wines made from the hand-harvested control and cryogenically pre-treated WG, MG, TM and CJ were within and above the aroma perception threshold (2-16 ng $L^{-1}$) and range (< 10 ng $L^{-1}$) typically reported in Sauvignon blanc wine (Table 2.3). The highest concentrations of sbMP were detected in the wine made from CJ (-4 ºC and -20 ºC) (17.43 to 20 ng $L^{-1}$), the control (16.75 ng $L^{-1}$), WG (-4 ºC and -20 ºC) (14.26 to 15.28 ng $L^{-1}$) and TM (-4 ºC) (15.13 ng $L^{-1}$). This was followed by the wines made from TM (-20 ºC) (13.54 ng $L^{-1}$), MG (-20 ºC) (13.47 ng $L^{-1}$) and MG (-4 ºC) (10.75 ng $L^{-1}$), which had the lowest concentration of sbMP, but still within the aroma perception threshold (2-16 ng $L^{-1}$) and above the typically reported range (< 10 ng $L^{-1}$) (Tables 2.3 and 3.23). Moreover, for this Producer, in general, the levels of sbMP in the control wines were not significantly different to those in the wines made from the cryogenic pre-treatments for 2020, however, it was significantly different to the levels in the wines made from MG (-4 ºC) in 2021 (Table 3.23).

The concentrations of sbMP detected in the wines from Producer C (2020) for the control and cryogenically pre-treatments ranged from 0 to 2.08 ng $L^{-1}$ for most wines, except for the T4 wine made from TM (20 ºC), which had the highest concentration of 8.26 ng $L^{-1}$ (Table 3.24). In 2021, the highest concentrations of sbMP were detected in the T0 wines made from CJ (-4 ºC) (12.09 ng $L^{-1}$), T4 CJ (-20 ºC) (11.14 ng $L^{-1}$), T0 WG (-4 ºC) (10.86 ng $L^{-1}$), MG (-20 ºC) (9.96 ng $L^{-1}$) and the T0 TM (9.75 ng $L^{-1}$). This was followed by wines made from the T0 MG (-4 ºC) (7.81 ng $L^{-1}$), unfrozen control (7.35 ng $L^{-1}$), T0 WG (-20 ºC) (6.29 ng $L^{-1}$), and T0 CJ (-20 ºC) (6.09 ng $L^{-1}$) whilst the wines from the remainder of the treatments had sbMP concentrations < 2 ng $L^{-1}$ (Table 3.24).

*ibMP*

In 2020, the concentrations of ibMP in most wines produced from the control grapes and cryogenically pre-treated WG, MG, TM and CJ from Producer A ranged between 0-10 ng $L^{-1}$ except wines from the T0 and T4 WG (-20 ºC), which had concentrations of 12.6 ng $L^{-1}$ and 11.82 ng $L^{-1}$, respectively, followed by the T0 MG (-4 ºC) (14.70 ng $L^{-1}$) and MG (-20 ºC) (14.81 ng $L^{-1}$) (Table 3.22). In 2021, the highest levels of ibMP were detected in the T4 wines made from TM (-20 ºC) (105.63 ng $L^{-1}$), WG (-20 ºC) (98.72 ng $L^{-1}$), WG (-4 ºC) (96.29 ng $L^{-1}$), and the MG (-20 ºC) (95.80 ng $L^{-1}$) (Table 3.22). This was followed by the T0 wines made from WG (-20 ºC) (87.73 ng $L^{-1}$), TM (-20 ºC) (84.96 ng $L^{-1}$) MG (-20 ºC) (84.75 ng $L^{-1}$). The ibMP levels in the wines made from the control and remaining treatments ranged between 66.94-83.12 ng $L^{-1}$ (Table 3.22), although lower concentrations were still above the aroma perception threshold (2-16 ng $L^{-1}$) and range (2-30 ng $L^{-1}$) typically reported in Sauvignon blanc wine (Table 2.3).

For Producer B (2020), the highest concentration of ibMP in wines made from the hand-harvested grapes was detected in wines made from the T4 WG (-20 ºC) (8.44 ng $L^{-1}$), the WG (-4 ºC) (7.87 ng $L^{-1}$), followed by the control grapes (4.45 ng L-$^{1}$) (Table 3.23). For the machine-harvested grapes, the highest concentrations of ibMP were detected in the T4 wines made from MG (-20 ºC) (13.47 ng $L^{-1}$), MG (-4 ºC) (12.80 ng $L^{-1}$), followed by the unfrozen control (12.03 ng $L^{-1}$) and all remaining treatments ranging from 8.10 to 8.56 ng $L^{-1}$. This increase in ibMP could be because of the increased contact between the juice and skin, which has been shown to increase the concentration of ibMP (Maggu et al., 2007:10284). In 2021, the highest concentrations of ibMP were detected in wines made from the WG (-4 ºC) (12.41 ng $L^{-1}$), MG (-20 ºC) (8.98 ng $L^{-1}$), WG (-20 ºC) (8.10 ng $L^{-1}$) and the MG (-4 ºC) (7.13 ng $L^{-1}$). The remaining wines had concentrations ranging from 4.69 to 6.10 ng $L^{-1}$ with the lowest concentration detected in the unfrozen control wines (3.28 ng $L^{-1}$) (Table 3.23). All ibMP levels detected in wines produced from the hand-harvested and machine-harvested grapes fell within the aroma perception threshold (2-16 ng $L^{-1}$) and range (2-30 ng $L^{-1}$) typically reported for Sauvignon blanc wine (Tables 2.3 and 3.23) (Sidhu et al., 2015:494-499; Lei et al., 2018:1142). For Producer C (2020), the highest concentrations of ibMP were detected in wines made from T4 CJ (-20 ºC) (21.10 ng $L^{-1}$), the T0 MG (-4 ºC) (14.88 ng $L^{-1}$) and WG (-20 ºC) (14.14 ng $L^{-1}$) (Table 3.24). Furthermore, the concentrations in the unfrozen control and remaining wines ranged from 0.33 to 6.69 ng $L^{-1}$ (Table 3.24). In 2021, for Producer C, the concentrations of ibMP were once again higher than in 2020. The highest concentrations were detected in the T4 wines made from WG (-20 ºC) (17.95 ng $L^{-1}$), MG (-4 ºC) (12.44 ng $L^{-1}$), WG (-4 ºC) (11.81 ng $L^{-1}$), T0 WG (-20 ºC) (10.70 ng $L^{-1}$) and MG (-4 ºC) (10.65 ng $L^{-1}$). The unfrozen control and remaining treatments had concentrations ranging from 5.56 to 9.00 ng $L^{-1}$, which fell within the aroma perception threshold and reported range (2-30 ng $L^{-1}$) typically reported for Sauvignon blanc wine (Tables 2.3 and 3.24).

The general trend observed in 2021, was that the methoxypyrazine levels were higher in wines made from grapes originating from all the wine regions and higher than the typically reported aroma perception threshold (2-16 ng $L^{-1}$) and ranges, i.e., ibMP (2-30 ng $L^{-1}$) and sbMP (< 10 ng $L^{-1}$) compared to the 2020 vintage (Tables 2.3 and 3.23). In contrast to the thiol levels, which were generally lower in 2021 compared to 2020, methoxypyrazine concentrations were notably higher in 2021. This increase was particularly evident in the WG and MG treatments, especially those subjected to cryogenic freezing at -20 °C at T4. Sidhu et al. (2015:498) reported that sbMP concentrations do not increase during vinification, suggesting that grape skins may inherently contain lower levels of sbMP compared to ibMP. In the present study, beyond the observed vintage variation and the influence of cryogenic treatments on methoxypyrazine levels, harvesting technique and regional origin also had a noticeable impact. These findings align with previous research by Allen & Lacey (1993:36-37; 1998:31-36); Marais (1994:44) and Ryona et al. (2008:10844-10845).

### 3.3.4 Differences in wine varietal aroma compounds between vintages and wine regions in South Africa

The SA wine-growing regions are divided by geographic units, regions and districts, mainly outlined by provincial boundaries (wards are defined by unique, terroir characteristics). The Western Cape wine region is the largest wine-producing geographical unit, comprising sub-areas/regions (see the map in Chapter 2, LiteratureReview, p 50) (Anonymous, 2021). In 2020 and 2021, for this research, Sauvignon blanc grapes were sourced from three wine producers, two in the Cape Coastal (Stellenbosch and Durbanville) and one in the Cape South Coast (Agulhas) wine districts. Additionally, in 2021, Chenin blanc grapes were sourced from a producer in the Cape Coastal (Stellenbosch) wine district. Although not one of the objectives of the study, the data from the various wine regions were compared to observe the variations in aroma composition of the wines.

The levels of varietal thiols (3-SH, 4-MSP and 3-SHA) in the wines from all the producers varied between treatments and vintages (2020 and 2021) (Tables 3.18-and 3.21). Consequently, no definite trend could be seen in terms of which cryogenic

Table 3.22: Methoxypyrazines detected in Sauvignon blanc wines produced from unfrozen control grapes and cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer A from the Stellenbosch wine region) in 2020 and 2021.

| **Treatment** | **Temp[2] (ºC)** | **Storage time (Months)** | **Methoxypyrazines[1]** | | | |
|---|---|---|---|---|---|---|
| | | | **sbMP (ng $L^{-1}$)** | | **ibMP (ng $L^{-1}$)** | |
| | | | **2020** | **2021** | **2020** | **2021** |
| **Control** | na[3] | na | 3.11±1.09bcd | 12.69±4.62de | 3.73±1.91fg | **75.51±5.54fe**[5] |
| **WG[4]** | -4 | 0 | 3.59±0.91abc | **26.52±1.87bc** | 2.97±1.40g | **78.94±3.72def** |
| | -20 | 0 | **4.84±1.21a** | **31.66±6.66abc** | **12.60±0.76ab** | **87.73±5.79bcde** |
| | -4 | 4 | 1.38±1.61efgh | **42.81±12.30a** | 8.43±0.58dc | **96.29±3.27abc** |
| | -20 | 4 | 1.78±0.85def | **35.22±1.72ab** | **11.82±2.34abc** | **98.72±12.61ab** |
| **MG** | -4 | 0 | 3.66±1.33abc | 20.59±0.04*ad | **14.70±1.75a** | **76.41±2.44ef** |
| | -20 | 0 | 3.88±0.91ab | **32.53±12.37ab** | **14.81±2.89a** | **84.75±2.18bcde***|
| | -4 | 4 | 0.67±0.58fgh | **26.31±9.66bc** | 7.39±0.94de | **78.81±16.67ef** |
| | -20 | 4 | 0.67±0.58fgh | 20.62±5.94ad | **10.24±3.01bdc** | **95.80±5.29abcd** |
| **TM** | -4 | 0 | 0.33±0.58gh | 8.37±2.99e | 4.03±2.75efg | 67.66±16.91f |
| | -20 | 0 | 2.39±0.42cde | 8.53±1.68e | 4.22±2.04efg | **84.96±3.42bdce** |
| | -4 | 4 | 0.33±0.58gh | 6.11±2.48e | 2.74±1.35g | **83.12±14.87bcdef** |
| | -20 | 4 | 2.42±0.17cde | 3.56e** | 3.19±1.74g | **105.63±3.77a** |
| **CJ** | -4 | 0 | 1.70±0.67efg | 14.45±4.45de | 7.10±5.44def | 66.94±11.16ef |
| | -20 | 0 | 1.59±0.44efg | 11.77±4.73de | 2.06±0.91g | **78.02±0.99ef** |
| | -4 | 4 | 0.67±0.58fgh | 11.16±7.96de | 2.48±0.82g | **80.79±13.34cdef** |
| | -20 | 4 | 0.00±0.00h | 8.74±3.50e | 2.79±0.63g | **76.59±9.8ef** |

[1]Means ± standard deviation (n=3) for all samples.
[2]Cryogenic temperature treatment
[3]Not applicable - na
[4]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[5]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
*Means ± standard deviation (n=2), outliers were removed, **(n=1).
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

Table 3.23: Methoxypyrazines detected in Sauvignon blanc wines produced from unfrozen control grapes and cryogenically pre-treated hand-harvested WG and machine-harvested MG, TM and CJ originating from Producer B in the Durbanville wine region in 2020 and 2021.

| **Treatment** | **Harvesting[2] technique** | **Temp[3] (ºC)** | **Storage time (Months)** | **Methoxypyrazines[1]** | | | |
|---|---|---|---|---|---|---|---|
| | | | | **sbMP (ng L$^{-1}$)** | | **ibMP (ng L$^{-1}$)** | |
| | | | | **2020** | **2021** | **2020** | **2021** |
| **Control** | H | na[4] | na | 0.00±0.00a | **16.75±0.99ab**[6] | 4.45±0.35b | 3.28±0.77d |
| **WG[5]** | H | -4 | 4 | 0.00±0.00a | **15.28±1.97abc** | 7.87±1.59ab | **12.41±2.13a***  |
| | H | -20 | 4 | 0.00±0.00a | **14.26±0.61bc** | 8.44±2.12ab | **8.10±1.11b** |
| **MG** | H | -4 | 4 | na | 10.75±1.20c | na | **7.13±1.53bc** |
| | H | -20 | 4 | na | 13.47±2.34bc | na | **8.98±3.32b** |
| **TM** | H | -4 | 4 | na | **15.13±4.57abc*** | na | 6.10±1.72bcd |
| | H | -20 | 4 | na | 13.54±4.10bc | na | 4.69±0.75cd |
| **CJ** | H | -4 | 4 | na | **17.43±3.52ab** | na | 4.89±0.62cd |
| | H | -20 | 4 | na | **20.01±4.76a** | na | 4.91±0.52cd* |
| **Control** | M | na | na | 0.61±1.06a | na | **12.03±8.93a** | na |
| **MG** | M | -4 | 4 | 0.00±0.00a | na | **12.80±1.65a** | na |
| | M | -20 | 4 | 0.00±0.00a | na | **13.47±1.12a** | na |
| **TM** | M | -4 | 4 | 0.00±0.00a | na | 8.10±0.39ab | na |
| | M | -20 | 4 | 0.00±0.00a | na | 8.24±0.40ab | na |
| **CJ** | M | -4 | 4 | 0.00±0.00a | na | 8.19±0.34ab | na |
| | M | -20 | 4 | 0.00a** | na | 8.56±.ab** | na |

[1]Means ± standard deviation (n=3).
[2]Hand-harvesting and machine-harvesting
[3]Cryogenic temperature treatment
[4]Not applicable - na
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[6]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
*Means ± standard deviation (n=2), outliers were removed, **(n=1).
Different letters within columns indicate significant differences at the p < 0.05 level between treatments.

Table 3.24: Methoxypyrazines detected in Sauvignon blanc wines produced from unfrozen control grapes and cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer C from the Cape South Coast wine region) in 2020 and 2021.

| Treatment | Temp[2] (ºC) | Storage Time (Months) | Methoxypyrazines[1] | | | |
|---|---|---|---|---|---|---|
| | | | sbMP (ng $L^{-1}$) | | ibMP (ng $L^{-1}$) | |
| | | | 2020 | 2021 | 2020 | 2021 |
| **Control** | na[3] | na | **2.08±1.09a**[6] | **7.35±5.74abc** | 2.48±1.24ef | 7.29±1.43def |
| **WG[5]** | -4 | 0 | 1.92±1.08b | **10.86±1.23ab** | 3.19±1.99def | 7.94±0.74cdef |
| | -20 | 0 | 1.17±1.26b | 6.29±4.74bc | **14.14±1.76b** | **10.70±2.05bcd** |
| | -4 | 4 | 0.00±0.00b | 1.00±0.00d[4] | 5.04±2.68de | **11.81±0.52bc** |
| | -20 | 4 | 0.00±0.00b | 1.00±0.00d | **9.56±1.02c** | **17.95±0.96a** |
| **MG** | -4 | 0 | 1.12±0.10b | **7.81±1.05abc** | **14.88±1.60ef** | **10.65±3.57bcd** |
| | -20 | 0 | 0.45±0.79b | **9.96±1.59ab** | 6.69±2.43cd | 5.56±4.64ef |
| | -4 | 4 | 0.00±0.00b | 3.94±5.09cd | 3.24±1.77def | **12.44±4.44b** |
| | -20 | 4 | 0.00±0.00b | 1.00±0.00d | 2.60±1.03b | 9.00±2.24bcde |
| **TM** | -4 | 0 | 0.00±0.00b | **7.31±5.96abc** | 2.78±3.98ef | 5.81±1.96ef |
| | -20 | 0 | 0.00±0.00b | **9.75±1.97ab** | 0.67±0.58f | 5.22±1.45ef |
| | -4 | 4 | 0.00±0.00b | 1.00±0.00d | 0.33±0.58f | 7.27±4.43def |
| | -20 | 4 | **8.26±14.30b** | 1.00±0.00d | 0.67±0.58f | 4.59±1.43d |
| **CJ** | -4 | 0 | 0.00±0.00b | **12.09±1.78a** | 1.26±1.41f | 5.83±1.76ef |
| | -20 | 0 | 0.00±0.00b | 6.09±4.61bcd | 1.44±1.71ef | 6.28±1.80ef |
| | -4 | 4 | 0.00±0.00b | 1.00±0.00d | 1.70±1.94ef | 6.56±1.90def |
| | -20 | 4 | 0.99±1.72b | **11.14±2.61ab** | **21.10±5.61a** | 8.05±1.83cdef |

[1]Means ± standard deviation (n=3) for all samples.
[2]Cryogenic temperature treatment
[3]Not applicable – na
[4]Limits of detection (LOD) for sbMP and ibMP in white wine.
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
[6]Bold script highlights values that fall above or below the typical range for that physicochemical parameter.
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

temperature (-4 ºC and -20 ºC), stage of production (WG, MG, TM, and CJ) and storage time (T0 and T4) would be most favourable in increasing the levels of varietal thiols in wines. This research found that the region from which the grapes originated, the harvesting technique and the vintage played a greater role in the concentrations of varietal thiols in the final wine. The concentrations of 3-SH and 3-SHA in the Sauvignon blanc wines made from the unfrozen control and treatments exceeded the aroma perception threshold of 60 ng $L^{-1}$ and 4 ng $L^{-1}$ for SA Sauvignon blanc wines, respectively, and fell within or above the reported ranges for SA Sauvignon blanc wines (198-904 ng $L^{-1}$) and (23-151 ng $L^{-1}$) (Table 2.2) (Coetzee et al., 2018:182). Moreover, the concentrations of 4-MSP in the Sauvignon blanc wines for all producers generally fell within the ranges commonly reported for SA Sauvignon blanc (0-21.9 ng $L^{-1}$), however, the aroma perception threshold values fluctuated and fell below the reported aroma perception threshold (0.8 ng $L^{-1}$) across vintages and regions (Table 2.2).

For the Chenin blanc wines, the concentrations of 3-SHA were within or higher than previously reported ranges (5-253 ng $L^{-1}$) for SA Chenin blanc wines and above the aroma perception threshold levels (4 ng $L^{-1}$). The concentrations of 3-SH were generally below the reported ranges (99-1124 ng $L^{-1}$) and aroma perception threshold levels (60 ng $L^{-1}$) for SA Chenin blanc. It was further seen that the levels of 4-MSP were above the reported ranges (n.d.) for SA Chenin blanc (Table 2.2) wines but below the aroma perception threshold levels (0.8 ng $L^{-1}$) (Table 2.2) (Wilson, 2017:28-29; Coetzee et al., 2018:182; Wilson et al., 2018:635-638). Chenin blanc grapes were only obtained in one vintage and from one producer in the Stellenbosch wine region, therefore, to validate the findings of this research, it is recommended to repeat this study for another vintage with grapes from another region. Furthermore, it should be noted that at the time of harvest, the Chenin blanc grapes had traces of *Botrytis cinerea*. Infected grape berries and bunches were removed as far as possible, but could still have affected the berry composition and final wine profile. Previous studies have reported significant amounts of volatile thiols, especially 3-SH, largely due to the concentration effect that *Botrytis* has on grape berries (Dzedze et al., 2019:265-276; Dankó et al., 2021:1-5).

In 2020, for Producers A, B and C, it was found that the Methoxypyrazine compound concentrations, i.e., sbMP and ibMP were lower or within the normal reported levels, < 10 ng $L^{-1}$ and 2-30 ng $L^{-1}$, respectively (Tables 2.3 and 3.22-3.24) (Sidhu et al., 2015:494-499; Lei et al., 2018:1142). However, in 2021, wines made from grapes from the same producers and subjected to the same treatments generally had higher concentrations of the methoxypyrazines. Therefore, this observation indicates that the higher concentrations of methoxypyrazines were not necessarily due to the treatments, but that vintage and region also played a role. Similar findings have been reported in previous research, indicating that viticultural factors rather than vinification techniques primarily influence this varietal aroma compound (Allen & Lacey, 1993:36-37; Allen & Lacey, 1998:31-36; Marais, 1994:44; Ryona et al., 2008:10844-10845; Jouanneau et al., 2012:335; Sidhu et al., 2015:494-499). Furthermore, for Producer B (Durbanville) in 2020, the levels of sbMP were generally 0 ng $L^{-1}$ for the hand-harvested control and hand and machine-harvested treatments, except the machine-harvested control (0.61 ng $L^{-1}$) (Table 3.23). The Stellenbosch wine region has a Mediterranean climate with warm days and cool ocean breezes, which may contribute to the variation in methoxypyrazine levels between vintages. Moreover, previous research has shown that grapes grown in cooler climate regions tend to have higher methoxypyrazine levels (Allen & Lacey, 1993:35-36; Marais, 1994:41-44; Parr et al., 2013:474). Generally, the same trends in terms of vintage effect were seen for the varietal thiol and methoxypyrazine compounds.

### 3.3.5 Influence of Harvesting Technique

#### 3.3.5.1 Machine-harvested versus hand-harvested

The choice of mechanical harvesting of wine grapes depends on many factors, including vineyard location, crop value, availability and cost of labour, efficiency of fruit removal, and transporting the harvested product (Allen et al., 2011:10642; Kilmartin, 2012:86; Coetzee, 2020a; b; Kemp et al., 2022:339). In recent years, wine producers have frequently used mechanical harvesters to increase yield and reduce time and cost (Allen et al., 2011:10641-10650; Jouanneau, 2011:239; Anonymous, 2022). Furthermore, in many instances, it was found that the grape juice and wines resulting from mechanically harvested grapes had higher levels of the varietal thiol precursors 3-S-cysteinylhexan-1-ol (Cys-3-SH) and 3-S-glutathionylhexan-1-ol (Glut-3-SH) and varietal thiols in the final wines. (Allen et al., 2011:10641-10642; Kilmartin, 2012:81-83; Jeffery, 2016:1323-1330; Coetzee, 2020a; b). However, manual or hand harvesting grapes still has several advantages over mechanical harvesting, including, specific cluster selection and elimination of poor quality and rotten grapes (Coetzee, 2020b; Kilmartin, 2012:86). Therefore, in 2020 this study, included hand and machine-harvested Sauvignon blanc grapes from Producer B (Durbanville) to determine the effect of the harvesting technique in combination with the stage of production and cryogenic pre-treatment on the chemical and varietal profiles of the wine (Fig. 3.9).

From the MFA, it was seen that the chemical and aroma compounds of the hand-harvested, machine-harvested and the WG (-20 ºC) clustered together and associated with TA and the varietal thiol 4-MSP. In 2020, the wines made from the machine-harvested unfrozen control and treatments subjected to cryogenic

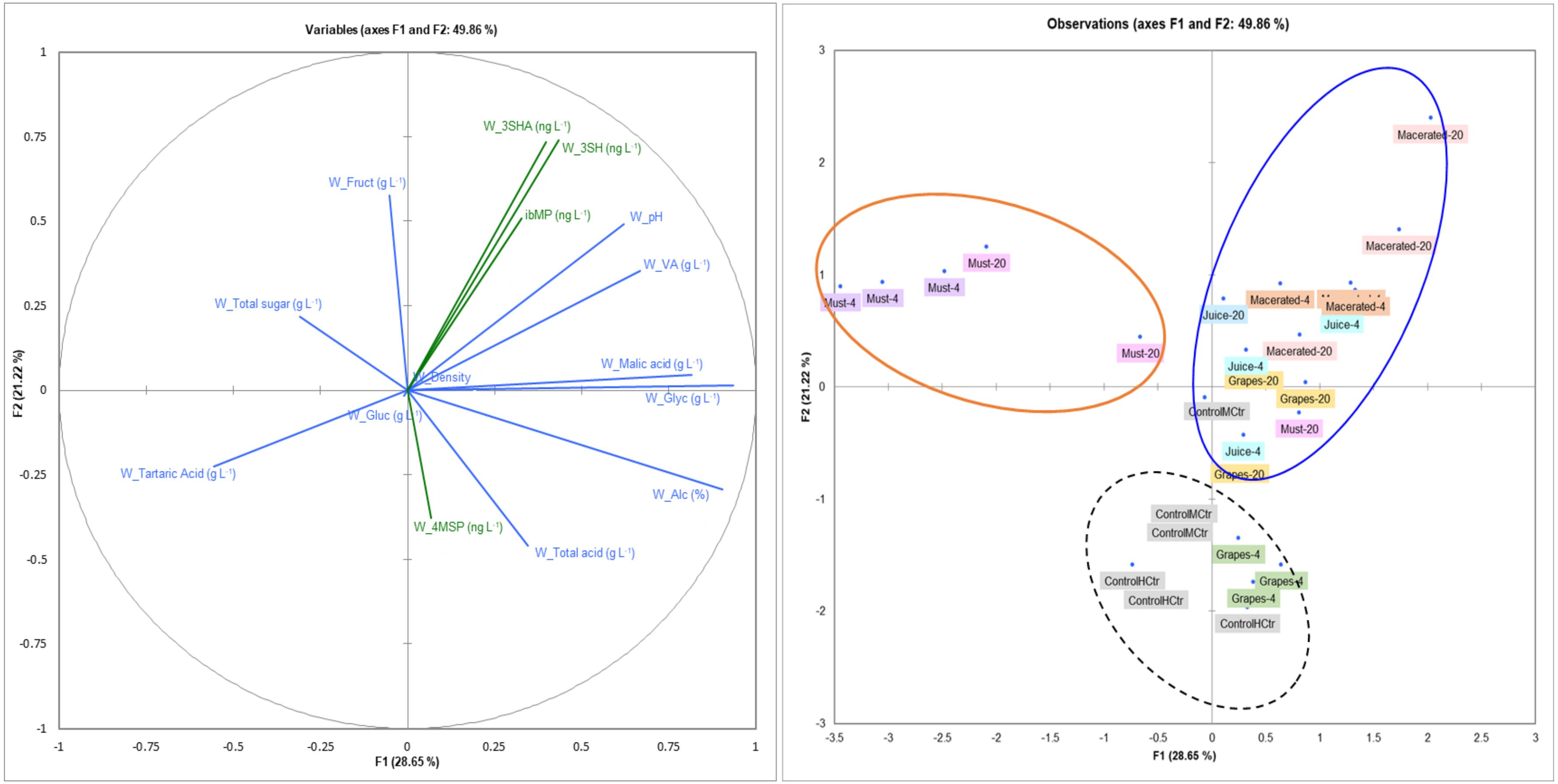



Figure 3.9. Multi-Factor Analysis (MFA) illustrating the differences (indicated by circles around groupings) observed in wine chemical parameters from Producer B (Durbanville wine region), for control wines and wines obtained from hand and machine-harvested cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-4 ºC and -20 ºC) for a T4 storage period in 2020.

pre-fermentative freezing had concentrations of 4-MSP lower than the detection limit (0.2 ng $L^{-1}$) (Table 3.19). Moreover, it appears that the cryogenic treatments did not have a significant impact on the concentrations of 4-MSP detected in the final wines, however, the harvesting technique may have had an effect. It was determined that for all wines made from machine-harvested grapes, the 4-MSP concentrations fell below the detection limit, while this compound was detected in all the wines made from hand-harvested grapes. These results contradict the findings of Kilmartin (2012:86) and Coetzee (2020b), who found that compounds such as the methoxypyrazines and 4-MSP were similar in wines made from grapes harvested by hand and machine. Moreover, the levels of 4-MSP fell below the aroma perception threshold (0.5 ng $L^{-1}$) and reported range (0-21.9 ng $L^{-1}$) for SA Sauvignon blanc wines (Table 2.2) (Jeffery, 2016:1324; Coetzee et al., 2018:182).

Furthermore, the wines resulting from all treatments associated with the varietal thiol 4-MSP, had a "greener" profile, whilst wines made from the MG, WG and some CJ clustered close to the varietal thiols 3-SH and 3-SHA, indicating that these wines were likely to have higher "tropical fruit" and/or "citrus" profiles (Fig. 3.9). Moreover, for the methoxypyrazine compounds, in 2020, sbMP was not detected in wines made from the hand-harvested control and cryogenically pre-treated WG, whilst for the machine-harvested grapes, sbMP was only detected in wines made from the unfrozen control MG, at concentrations below the aroma perception threshold reported in Sauvignon blanc wine (2-16 ng $L^{-1}$) (Fig. 3.9, Tables 2.3 and 3.23). This therefore suggests that the effect of the harvesting method and cryogenic pre-treatments on the concentrations of sbMP in the final wine was negligible. Furthermore, the wines made from the machine-harvested cryogenically pre-treated MG (-4 ºC and -20 ºC), grouped closely with the ibMP compound (Fig. 3.9). However, when looking at the actual concentrations (Table 3.23), the wines made from the machine-harvested MG control and machine-harvested MG (-4 ºC and -20 ºC) were similar, indicating that the cryogenic treatment and harvesting method did not have a notable effect, but the stage of production did. The aforementioned results were similar to previous studies that investigated the effect of the harvesting technique on varietal thiols and methoxypyrazines in wines, but without cryogenic treatments (Jouanneau et al., 2011:218-220; Kilmartin, 2012:81-82; 86 Coetzee, 2020b).

## 3.4 Conclusion

This study aimed to determine whether the stage of production, cryogenic pre-fermentative storage temperature, storage time and harvest method affected the wine chemical and volatile aroma profiles of the resultant wines. Previous research found that the levels of specific compounds in final wines were often cultivar and vintage-dependent and less affected by winemaking techniques (Gambacorta et al., 2011:1062-1065). Overall, in this study, it was found that the chemical parameters of the grape must following cryogenic pre-treatments and defrosting remained stable, with the pH and total acidity being the most affected. These observations were similar to previous research, which found that the increase in pH was due to the precipitation of potassium (K+) salts during the freezing process and the lower solubility of the acidic salts when the grapes were defrosted (Schmid & Jiranek, 2011:29; Olarte Mantilla et al., 2013:352-353; Santesteban et al., 2013:3011;3015; Zhang et al., 2015:21611, Ruiz-Rodríguez et al., 2020:5; Pedrosa-López et al., 2022:5).

Furthermore, the TA levels for the WG, MG, TM, and CJ, subjected to the T4 cryogenic treatments, were generally, but not always, lower than the T0 treatments for both vintages. Most differences seen for the measured wine chemical parameters from the WG, MG, TM, and CJ subjected to cryogenic treatment were attributed to the vintages. The physicochemical parameters of the wines made from the cryogenically treated WG, MG, TM and CJ were similar to results reported previously (Peinado et al., 2004:586; Salinas et al., 2005:1529 1530; Carillo et al., 2011:11-12; Naviglio et al., 2018:6-10; Naranjo et al., 2021:1-13).

The alcohol, VA, MA, TS, glucose and fructose levels were similar in wines made from the unfrozen control and cryogenically pre-treated WG, MG, TM and CJ for both cultivars and across vintages. The physicochemical parameters most affected were once again the pH, which was higher for wines made from the cryogenic treatments. No trend was observed regarding the impact of the stage of production, cryogenic temperature and storage period on the TA levels, which fluctuated above or below the levels found in the control wines and contradicted the findings of previous research (Darias-Martıń et al., 2000:484; Zhang et al., 2015:21610-21611; Radeka et al., 2023:6; Gu et al., 2025:3). It was also seen that the harvesting technique had an impact on the TA levels because higher levels were seen in wines from hand-harvested control grapes compared to the machine-harvested control grapes. Furthermore, the findings of this research are not in complete agreement with previous research, which showed that the type of cryomaceration treatment applied and the stage of production, i.e., WG, MG or CJ, can influence must and wine physicochemical parameters (Zhang et al., 2015:21611; 21621-21622; Naviglio et al., 2018:6-10; Ruiz-Rodríguez et al., 2020:1-13; Naranjo et al., 2021:1-13; Radeka et al., 2023:6; Gu et al., 2025:3).

The observations made in terms of the aroma compounds were that no definite trend regarding which cryogenic temperature, stage of production and storage time would consistently yield the most favourable levels of varietal thiols in the wines. However, the region from which the grapes originated, the harvesting technique and the vintage played a role in the concentrations of varietal thiols in the final wines. The levels of 3-SH and 3-SHA in wines made from the unfrozen control and cryogenic treatments exceeded both the aroma perception thresholds of 60 ng $L^{-1}$ and 4 ng $L^{-1}$ and the reported ranges for SA Sauvignon blanc wines (178-904 ng $L^{-1}$) and (23-151 ng $L^{-1}$) (Coetzee et al., 2018:182). Furthermore, the concentrations of 4-MSP in the wines for all producers generally fell within the ranges commonly reported for SA Sauvignon blanc (0-21.9 ng $L^{-1}$), however, the aroma perception threshold values fluctuated and were above the reported aroma perception threshold (0.8 ng $L^{-1}$) across vintages and regions. For the Chenin blanc wines, the concentrations of 3-SHA were higher than the reported ranges (5-253 ng $L^{-1}$) for SA Chenin blanc wines and above the aroma perception threshold levels (4 ng $L^{-1}$). The concentrations of 3-SH were generally below the reported ranges (99-1124 ng $L^{-1}$) and aroma perception threshold levels (60 ng $L^{-1}$) for SA Chenin blanc. Moreover, the levels of 4-MSP were above the reported ranges for SA Chenin blanc (n.d.) wines but below the aroma perception threshold levels (0.8 ng $L^{-1}$) (Wilson, 2017:28-29; Coetzee et al., 2018:182; Wilson et al., 2018:635-638; Wilson et al., 2019:635-640). These results were significant because they confirmed the presence of the varietal thiol 4-MSP in SA Chenin blanc wines. The effects of cryogenic pre-treatments on volatile thiols have been previously investigated, but not on methoxypyrazine compounds. This was further confirmed by an internet search using the keywords “Effect of cryogenic pre-fermentative freezing treatments on the methoxypyrazine levels in Sauvignon blanc wine” on the academic search engine, Google Scholar. The search only generated 12 results for methoxypyrazines, none relating to the effect of cryogenic pre-fermentative freezing (Anonymous, 2025b). The Google Scholar search engine suggested the use of the relatively new academic search engine, SciSpace AI, an AI-driven search engine. These search results showed the top 20 papers researching methoxypyrzines in wines, which predominantly focused on viticultural and climatic conditions and, to a lesser extent, vinification practices (Anonymous, 2025c). The only scientific paper found was the authors' own published review article, “Pre-Fermentative Cryogenic Treatments: The Effect on Aroma Compounds and Sensory Properties of Sauvignon Blanc and Chenin Blanc Wine” (van Breda et al., 2024:8-9).

In 2021, the methoxypyrazine levels were higher in wines made from grapes originating from all the wine regions and higher than the typical aroma perception threshold levels and the typical ranges found in wines, compared to the 2020 vintage. The levels of sbMP and ibMP detected in all the wines, regardless of the treatments, were similar to previous research that found the concentrations of sbMP did not increase during vinification (Coetzee, 2011:19-20; Sidhu et al., 2015:498). Furthermore, again, no definite trend as to which cryogenic temperature, stage of production and storage time would yield the most favourable levels of methoxypyrazines because the levels varied between treatments. However, as with previous research, the region, harvesting technique and the vintage played the biggest role in the differences observed (Allen & Lacey, 1993:36-37; Allen & Lacey, 1998:31-36; Marais, 1994:44; Ryona et al., 2008:10844-10845; Jouanneau, 2011:182-185).

Therefore, based on these observations, it can be concluded that applying cryogenic pre-treatments in winemaking practices will not necessarily lead to an increase in varietal compounds in the final wine. The freezing of WG and MG, when compared to the freezing of TM and CJ showed more promise, although not consistently. Furthermore, freezing in large refrigerator chambers or rooms will increase the cost and large freezer facilities will be required. Nevertheless, the time for the grapes to be frozen will allow winemakers to determine a better schedule for winemaking procedures and produce fresher white wines throughout the year. Furthermore, maceration practices are largely cultivar-dependent and vary with vineyard and vintage conditions and the desired style of wine to be produced.

## 3.5 References

Alberts, P., Stander, M.A., Paul, S.O. & de Villiers, A. 2009. Survey of 3-Alkyl-2-methoxypyrazine content of South African Sauvignon blanc wines using a novel LC−APCI-MS/MS method. *Journal of Agricultural and Food Chemistry*, 57(20):9347-9355. https://doi.org/10.1021/jf9026475.

Aleixandre-Tudo, J. L., Weightman, C., Panzeri, V., Nieuwoudt, H. H.& Du Toit, W. J. 2015. Effect of skin contact before and during alcoholic fermentation on the chemical and sensory profile of South African Chenin blanc white wines. South African Journal of Enology and Viticulture, 36(3):366-377. http://www.scielo.org.za/scielo.php?script=sci_arttextandpid=S2224-79042015000300007andlng=enandtlng=es.

Aleixandre-Tudo, J. L. & du Toit, W. 2018. Cold maceration application in red wine production and its effects on phenolic compounds: A review. *LWT-Food Science and Technology* 95:200-208. https://doi.org/10.1016/j.lwt.2018.04.096.

Allen, M.S., Lacey, M.J., Harris, R.L. & Brown, W.V. 1991. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *American Journal of Enology and Viticulture,* 42(2):109-112. https://doi.org/10.5344/ajev.1991.42.2.109.

Allen, M.S. & Lacey, M.J. 1993. Methoxypyrazine grape flavour: influence of climate, cultivar and viticulture. *Wein-Wissenschaft*, 48(3-6):211-213.

Allen, M.S. & Lacey, M.J. 1998. Methoxypyrazines of grapes and wines. In Waterhouse, A.L. & Ebeler, E.E. (eds.). *Chemistry of Wine Flavour*. Washington DC: American Chemical Society; 31-38. https:/doi.org/10.1021/bk-1998-0714.ch003.

Allen, T., Herbst-Johnstone, M., Girault, M., Butler, P., Logan, G., Jouanneau, S., Nicolau, L. & Kilmartin, P.A. 2011. Influence of grape-harvesting steps on varietal thiol aromas in Sauvignon blanc wines. *Journal of Agricultural and Food Chemistry*, 59(19):10641-10650. https://doi.org/10.1021/jf2018676.

Anonymous, 2021. Map of South African Wine Regions. [WWW document] URL http://www.sawis.co.za/cert/download/Regions - Mrt2020.pdf (02 June 2021).
Anonymous, 2022. Wine grape Harvest. [WWW document] URL https://www.thespruc eeats. com/wine–grape–harvest–3511325/. [08 March 2022].

Anonymous, 2025a. [WWW document] URL https://vinlab.com/wpcontent/uploads/20 22/09/Legal-limits-and-Sensory-thresholds-1.pdf. [26 March 2025].

Anonymous, 2025b. [WWW document] URL https://https://scholar.google.com/schola r?hl=en&as_sdt=0%2C5&q=Effect+of+cryogenic+pr-fermentative+freezing+treatment s+on+the+methoxypyrazine+levels+in+Sauvignon+blanc+wine&btnG. [21 May 2025].

Anonymous, 2025c. [WWW document] URL https://https://scispace.com/search?q=Ef fect%20of%20cryogenic%20pre-fermentative%20freezing%20treatments%20on%20t he%20methoxypyrazine%20levels%20in%20Sauvignon%20blanc%20wine&utm_sou rce=scholar.google.com&utm_content=global_search. [21 May 2025].

Antonelli, A., Arfelli, G., Masino, F. & Sartini, E. 2010. Comparison of traditional and reductive winemaking: influence on some fixed components and sensorial characteristics. *European Food Research and Technology*, 231:85-91. https://doi.org/10.1007/s00217-010-1250-6.

Aragón-García, F., Ruíz-Rodríguez, A. & Palma, M. 2021. Changes in the Aromatic Compounds Content in the Muscat Wines as a Result of the Application of Ultrasound during Pre-Fermentative Maceration. *Foods*, 10(7):1462. https://doi.org/10.3390/food s10071462.

Arora, R., 2018. Mechanism of freeze-thaw injury and recovery: a cool retrospective and warming up to new ideas. *Plant Science*, 270:301-313. https://doi.org/10.1016/j.pl antsci.2018.03.002.

Arora, R. Chen, K. 2025. Metabolites in Cellular Leachate as Biomarkers for the Nature and Severity of Injury Following a Short vs. Prolonged Freezing Stress. *Physiologia Plantarum*, 177(3):70273. https://doi.org/10.1111/ppl.70273.

Baiano, A., Terracone, C., Longobardi, F., Ventrella, A., Agostiano, A. & Del Nobile, M.A. 2012. Effects of different vinification technologies on physical and chemical characteristics of Sauvignon blanc wines. *Food Chemistry*, 135(4):2694-2701. https://doi.org/10.1016/j.foodchem.2012.07.075.

Bavčar, D., BaÅ, H., ÄŒuÅ, F., Vanzo, A., GaÅ, L. & KoÅ, T. 2011. Impact of alternative skin contact procedures on the aroma composition of white wine. *South African Journal of Enology and Viticulture*, 32(2):190-203.

Bell, S.J. & Henschke, P.A. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian journal of grape and wine research*, 11(3):242-295. https://doi.org/10.1111/j.1755-0238.2005.tb00028.x.

Benkwitz, F., Nicolau, L., Lund, C., Beresford, M., Wohlers, M. & Kilmartin, P. A. 2012a. Evaluation of key odorants in Sauvignon blanc wines using three different methodologies. *Journal of Agricultural and Food Chemistry*, 60(25):6293-6302. https://doi.org/10.1021/jf300914n.

Benkwitz, F., Tominaga, T., Kilmartin, P.A., Lund, C., Wohlers, M. & Nicolau, L. 2012b. Identifying the chemical composition related to the distinct aroma characteristics of New Zealand Sauvignon blanc wines. *American Journal of Enology and Viticulture*, 63(1):62-72. https://doi.org/10.5344/ajev.2011.10074.

Benucci, I., Cerreti, M., Liburdi, K., Nardi, T., Vagnoli, P., Ortiz-Julien, A. & Esti, M. 2018. Pre-fermentative cold maceration in presence of non-*Saccharomyces* strains: Evolution of chromatic characteristics of Sangiovese red wine elaborated by sequential inoculation. *Food Research International*, 107:257-266.https://doi.org/10.1016/j.foodr es.2018.02.029.

Bestulić, E., Rossi, S., Plavša, T., Horvat, I., Lukić, I., Bubola, M., Peršurić, A.S.I., Jeromel, A. & Radeka, S. 2022. Comparison of different maceration and non-maceration treatments for enhancement of phenolic composition, colour intensity, and taste attributes of Malvazija istarska (*Vitis vinifera* L.) white wines. *Journal of Food Composition and Analysis*, 109:104472. https://doi.org/10.1016/j.jfca.2022.104472.

Blesic, M., Zele, M., Bavcar, D., Spaho, N. & Smajic-Murtic, M. 2016. Monoterpenes in cv. Zilavka free-run musts from prefermentatively macerated pomace. *American Journal of Enology and Viticulture*, 67(1):116-119.https://doi.org/10.5344/ajev.2015.15053.

Cai, J., Zhu, B.Q., Wang, Y.H., Lu, L., Lan, Y.B., Reeves, M.J. & Duan, C.Q. 2014. Influence of pre-fermentation cold maceration treatment on aroma compounds of Cabernet Sauvignon wines fermented in different industrial scale fermenters. *Food Chemistry*, 154:217-229. https://doi.org/10.1016/j.foodchem.2014.01.003.

Capone, D.L. & Jeffery, D.W. 2011. Effects of transporting and processing Sauvignon blanc grapes on 3-mercaptohexan-1-ol precursor concentrations. *Journal of Agricultural and Food Chemistry*, 59(9):4659-4667. https://doi.org/10.1021/jf200119z.

Capone, D.L., Sefton, M.A. & Jeffery, D.W. 2011. Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. *Journal of Agricultural and Food Chemistry*, 59(9):4649-4658. https://doi.org/10.1021/jf200116q.

Carbone, K. & Fiordiponti, L. 2016. Colour evaluation, bioactive compound content, phenolic acid profiles and in vitro biological activity of Passerina del Frusinate white wines: Influence of pre-fermentative skin contact times. *Molecules*, *21*(7):960.

Carillo, M., Formato, A., Fabiani, A., Scaglione, G. & Pucillo, G. P. 2011. An inertizing and cooling process for grapes cryomaceration. *Electronic Journal of Biotechnology*, 14(6) 8-8. https://doi.org/10.2225/vol14-issue6-fulltext-10.

Casalta, E., Salmon, J.M., Picou, C. & Sablayrolles, J.M. 2019. Grape solids: Lipid composition and role during alcoholic fermentation under enological conditions. *American Journal of Enology and Viticulture*, 70(2):147-154. https://doi.org/10.5344/ajev.2018.18049.

Casalta, E., Vernhet, A., Sablayrolles, J.M., Tesniere, C. & Salmon, J.M. 2015. Characterization and role of grape solids during alcoholic fermentation under enological conditions. *American Journal of Enology and Viticulture*, 67(2):133-138. https://doi.org /10.5344/ajev.2015.15060.

Casassa, L.F. & Sari, S.E. 2015. Sensory and chemical effects of two alternatives of pre-fermentative cold soak in Malbec wines during winemaking and bottle ageing. *International Journal of Food Science & Technology*, 50(4):1044-1055. https://doi.org/ 10.1111/ijfs.12572.

Chen, K. & Li, J. 2022. In White Wine Technology. A. Morata (ed.). A glance into the aroma of white wine: Academic Press; 313 326. https://doi.org/10.1016/B978-0-12-823497-6.00018-1.

Chen, L., Capone, D.L., Nicholson, E.L. & Jeffery, D.W. 2019. Investigation of intraregional variation, grape amino acids, and pre-fermentation freezing on varietal thiols and their precursors for Vitis vinifera Sauvignon blanc. *Food Chemistry*, 295:637-645. https://doi.org/10.1016/j.foodchem.2019.05.126.

Chidi, B.S., Bauer, F.F. & Rossouw, D. 2018. Organic acid metabolism and the impact of fermentation practices on wine acidity: A review. *South African Journal of Enology and Viticulture*, 39(2):1-15. https://doi.org/10.21548/39-2-3164.

Choné, X., Lavigne-Cruège, V., Tominaga, T., van Leeuwen, C., Castagnède, C., Saucier, C. & Dubourdieu, D. 2006. Effect of vine nitrogen status on grape aromatic potential: flavor precursors (S-cysteine conjugates), glutathione and phenolic content in *Vitis vinifera* L. Cv Sauvignon blanc grape juice. *Oeno One*, 40(1):1-6. https://doi.org/10.20870/oeno-one.2006.40.1.880.

Coetzee, C. 2011. *Oxygen and sulfur dioxide additions to Sauvignon blanc: effect on must and wine composition* (Doctoral dissertation, Stellenbosch: University of Stellenbosch). http://hdl.handle.net/10019.1/6733.

Coetzee, C. 2019. *Managing green aromas in the cellar: Methoxypyrazines*. [Blog, 02 December]. https://sauvignonblanc.com/managing-green-aromas-in-the-cellar-metho xypyrazines/ [09 June 2021].

Coetzee, C. 2020a. *Effect of mechanical harvesting on Sauvignon blanc protein content*. [Blog, 21 January]. https://sauvignonblanc.com/effect-of-mechanical-harvesting-on-sauvignon-blanc-protein-content/ [8 June 2021].

Coetzee, C. 2020b. *Hand harvest vs Machine harvesting? The effect on the volatile thiols*. [Blog, 12 February]. https://sauvignonblanc.com/hand-harvest-vs-mechanical-harvesting-the-effect-on-the-volatile-thiols/ [8 June 2021].

Coetzee, C., 2022. Cooling Sauvignon blanc grapes before processing – is it worth the effort, time and energy? [Blog, 17 May] https://www.wineland.co.za/cooling-sauvignon-blanc-grapes-before-processing-is-it-worth-the-effort-time-and-energy/ [17 May 2022].

Coetzee, C. & du Toit, W.J. 2012. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. *Food Research International*, 45:287-298. https://doi.org/10.1016/j.foodres.2011.09.017.

Coetzee, C., Schulze, A., Mokwena, L., Du Toit, W. J. & Buica, A. 2018. Investigation of thiol levels in young commercial South African Sauvignon Blanc and Chenin Blanc wines using propiolate derivatization and GC-MS/MS. *South African Journal of Enology and Viticulture*, 39(2):180-184. http://dx.doi.org/10.21548/39-2-2683.

Cosme, F., Gonçalves, B., Inês, A., Jordão, A.M. & Vilela, A. 2016. Grape and wine metabolites: Biotechnological approaches to improve wine quality. *Grape and Wine Biotechnology*: 187-214. https://doi.org/10.5772/64822.

Cynkar, W.U., Cozzolino, D., Dambergs, R.G., Janik, L. & Gishen, M. 2004. The effects of homogenisation method and freezing on the determination of quality parameters in red grape berries of *Vitis vinifera*. *Australian Journal of Grape and Wine Research*, 10(3):236-242. https://doi.org/10.1111/j.1755-0238.2004.tb00027.x.

Cynkar, W., Cozzolino, D. & Dambergs, R.G. 2009. The effect of sample storage and homogenisation techniques on the chemical composition and near-infrared spectra of white grapes. *Food Research International*, 42(5-6):653-658. https://doi.org/10.1016/j. foodres.2009.02.002.

Dankó, T., Szelényi, M., Janda, T., Molnár, B. P. & Pogány, M. 2021. Distinct volatile signatures of bunch rot and noble rot. *Physiological and Molecular Plant Pathology*, 114:101626. https://doi.org/10.1016/j.pmpp.2021.101626.

Darias-Martín, J.J., Rodríguez, O., Díaz, E. & Lamuela-Raventós, R.M. 2000. Effect of skin contact on the antioxidant phenolics in white wine. *Food Chemistry*, 71(4):483-487. https://doi.org/10.1016/S0308-8146(00)00177-1.

De Santis, D. & Frangipane, M. T. 2010. Effect of prefermentative cold maceration on the aroma and phenolic profiles of a Merlot red wine. *Italian Journal of Food Science*, 22(1), 47. ISSN:1120-1770.

Du Plessis, C.S. & Augustyn, O.P.H. 1981. Initial study on the guava aroma of Chenin blanc and Colombar wines. *South African Journal of Enology and Viticulture*, 2(2):101-103. https://doi.org/10.21548/2-2-2401.

Dzedze, N., Van Breda, V., Hart, R.S. & Van Wyk, J. 2019. Wine chemical, sensory, aroma compound and protein analysis of wines produced from chemical and biological fungicide treated Chenin blanc grapes. *Food Control*, 105:265-276. https://doi.org/10.1016/j.foodcont.2019.06.007.

Fragrasso, M., Antonacci, D., Pati, S., La Gatta, B., La Gatta, M., Coletta, A. & La Notte, E. 2010, June. Pre-fermentative cold maceration for the aroma enhancement of Aglianico and Montepulciano wines. In *33rd World Congress of Vine and Wine. 8th General Assembly of the OIV* :20-25.

Gambacorta, G., Antonacci, D., Pati, S., La Gatta, M., Faccia, M., Coletta, A. & La Notte, E. 2011. Influence of winemaking technologies on phenolic composition of Italian red wines. *European Food Research and Technology*, 233:1057-1066. https://doi.org/10.1007/s00217-011-1613-7.

García, S., Santesteban, L.G., Miranda, C. & Royo, J.B. 2011. Variety and storage time affect the compositional changes that occur in grape samples after frozen storage. *Australian Journal of Grape and Wine Research*, 17(2):162-168. https://doi.org/10.1111/j.1755-0238.2011.00134.x.

Giametta, F., Catalano, F., Tanucci, G., Fioschi, G., Paradiso, V.M. & Bianchi, B. 2025. Energy and Quality Assessment in the Cooling of Crushed Bombino Nero Grapes with Indirect Heat Exchange System and Direct Heat Exchange System with $CO_2$. *Sci*, 7(2):42. https://doi.org/10.3390/sci7020042.

Gil-Muñoz, R., Moreno-Pérez, A., Vila-López, R., Fernández-Fernández, J.I., Martínez-Cutillas, A. & Gómez-Plaza, E. 2009. Influence of low temperature prefermentative techniques on chromatic and phenolic characteristics of Syrah and Cabernet Sauvignon wines. *European Food Research and Technology*, 228:777-788. https://doi.org/10.1007/s00217-008-0989-5.

Godelmann, R., Limmert, S. & Kuballa, T. 2008. Implementation of headspace solid-phase-microextraction-GC-MS/MS methodology for determination of 3-alkyl-2-methoxypyrazines in wine. *European Food Research and Technology*, 227:449-461. https://doi.org/10.1007/s00217-007-0741-6.

Gómez-Míguez, M. José, Gómez-Míguez, M., Vicario, I.M. & Heredia, F.J. 2007. Assessment of colour and aroma in white wines vinifications: effects of grape maturity and soil type. *Journal of Food Engineering*. 79:758-764. https://doi.org/10.1016/j.jfood eng.2006.02.038.

Gu, X., Liu, Y., Suo, R., Yu, Q., Xue, C., Wang, J., Wang, W., Wang, H. & Qiao, Y. 2025. Effects of different low-temperature maceration times on the chemical and sensory characteristics of Syrah wine. *Food Chemistry*, 463:141230. https://doi.org/1 0.1016/j.foodchem.2024.141230.

Guerrini, S., Galli, V., Mangani, S. & Granchi, L. 2024. Influence of Cryoextraction and Cold Pre-Fermentative Maceration on the Yeast Microbiota and the Volatile Compounds Profile of Sangiovese Wine. *Fermentation*, 10(3):148. https://doi.org/10.3 390/fermentation10030148.

Hart, R.S., Jolly, N.P. & Ndimba, B.K. 2019. Characterisation of hybrid yeasts for the production of varietal Sauvignon blanc wine–A review. *Journal of Microbiological Methods*, 165:105699. https://doi.org/10.1016/j.mimet.2019.105699.

Helwi, P., Guillaumie, S., Thibon, C., Keime, C., Habran, A., Hilbert, G., Gomes, E., Darriet, P., Delrot, S. & Van Leeuwen, C. 2016. Vine nitrogen status and volatile thiols and their precursors from plot to transcriptome level. *BMC Plant Biology*, *16*(1):173. https://doi.org/10.1186/s12870-016-0836-y.

Henschke, P.A. & Jiranek, V. 1993. Yeasts-metabolism of nitrogen compounds. In Fleet, G.H. (Editor), *Wine Microbiology and Biotechnology*. Chur: Harwood Academic Publishers, 77-83.

Heydarov, E. E., Mammadov, B. A., Fataliyev, H. K., Alekberov, A. M., Qadimova, N. S. & İmanova, K. F., 2020. Substantiation of Cryoprocessing regimes of white and red wine materials. *Vitivinícola*,35(5), 40-48. ISSN:2416-3953.

Jeffery, D.W. 2016. Spotlight on varietal thiols and precursors in grapes and wines. *Australian Journal of Chemistry*, 69:1323-1330. https://doi.org/10.1071/CH16296.

Jiang, Y.T., Yang, L.H., Ferjani, A. & Lin, W.H. 2021. Multiple functions of the vacuole in plant growth and fruit quality. *Molecular Horticulture*, 1(1):4. https://doi.org/10.1186/s43897-021-00008-7.

Jouanneau, S., 2011. Survey of aroma compounds in Marlborough Sauvignon blanc wines-Regionality and small-scale winemaking. Unpublished Doctoral dissertation, The University of Auckland, Auckland, New Zealand.

Jouanneau, S., Weaver, R.J., Nicolau, L., Herbst-Johnstone, M., Benkwitz, F. & Kilmartin, P.A. 2012. Subregional survey of aroma compounds in Marlborough Sauvignon Blanc wines. *Australian Journal of Grape and Wine Research*, 18(3):329-343. https://doi.org/10.1111/j.1755-0238.2012.00202.x.

Karabagias, I. K., Sykalia, D., Mannu, A. & Badeka, A. V. 2020. Physico-chemical parameters complemented with aroma compounds fired up the varietal discrimination of wine using statistics. *European Food Research and Technology*, 246(11):2233-2248. https://doi.org/10.1007/s00217-020-03568-y.

Kemp, B., Botezatu, A., Charnock, H., Inglis, D., Marchal, R., Pickering, G., Yang, F. & Willwerth, J. 2022. White winemaking in cold climates. In *White Wine Technology*. London: Academic Press United Kingdom, 339-354. https://doi.org/10.1016/B978-0-12-823497-6.00007-7.

Kilmartin, P. 2012. Machine harvesting versus handpicking: impacts on tropical and green characters in Sauvignon blanc wines. *The Australian and New Zealand Grapegrower and Winemaker*:81-86.

Korenika, A.M.J., Maslov, L., Jakobović, S., Palčić, I. & Jeromel, A. 2019. Comparative study of aromatic and polyphenolic profiles of Croatian white wines produced by cold maceration. *Czech Journal of Food Sciences*, 36(6):459-469. https://doi.org/10.17221/448/2017-CJFS.

Korenika, A.M.J, Prusina, T. & IviÄ, S. 2020. Influence of cold maceration treatment on aromatic and sensory properties of Vugava wine (*Vitis vinifera* L.). *Journal of Microbiology, Biotechnology and Food Sciences*, 10(1):49-53. https://doi.org/10.1541 4/jmbfs.2020.10.1.49-53. https://doi.org/10.15414/jmbfs.2020.10.1.49-53.

Kotseridis, Y., Baumes, R. & Skouroumounis, G.K. 1998. Synthesis of labelled [2H4] β-damascenone,[2H2] 2-methoxy-3-isobutylpyrazine,[2H3] α-ionone, and [2H3] β-ionone, for quantification in grapes, juices and wines. *Journal of Chromatography A*, 824(1):71-78. https://doi.org/10.1016/S0021-9673(98)00650-5.

Lei, Y., Xie, S., Guan, X., Song, C., Zhang, Z. & Meng, J. 2018. Methoxypyrazines biosynthesis and metabolism in grape: A review. *Food Chemistry*, 245:1141 1147. https://doi.org/10.1016/j.foodchem.2017.11.056.

Losada, M.M., Andrés, J., Cacho, J., Revilla, E. & López, J.F. 2011. Influence of some pre-fermentative treatments on aroma composition and sensory evaluation of white Godello wines. *Food Chemistry*, 125(3):884-891. https://doi.org/10.1016/j.foodchem.2 010.09.060.

Lukić, I., Budić-Leto, I., Bubola, M., Damijanić, K. & Staver, M. 2017. Pre-fermentative cold maceration, saignée, and various thermal treatments as options for modulating volatile aroma and phenol profiles of red wine. *Food Chemistry*, 224:251-261. https://doi.org/10.1016/j.foodchem.2016.12.077.

Mafata, M., Stander, M.A., Thomachot, B. & Buica, A. 2018. Measuring thiols in single cultivar South African red wines using 4, 4-dithiodipyridine (DTDP) derivatization and ultraperformance convergence chromatography-tandem mass spectrometry. *Foods*, 7(9):138. https://doi.org/10.3390/foods7090138.

Maggu, M., Winz, R., Kilmartin, P.A., Trought, M.C. & Nicolau, L. 2007. Effect of skin contact and pressure on the composition of Sauvignon Blanc must. *Journal of Agricultural and Food Chemistry*, 55(25):10281-10288. https://doi.org/10.1021/jf0721 92o.

Malićanin, M., Danilović, B., Stamenković Stojanović, S., Cvetković, D., Lazić, M., Karabegović, I. & Savić, D. 2022. Pre-fermentative Cold Maceration and Native Non-*Saccharomyces* yeasts as a tool to enhance aroma and sensory attributes of

Chardonnay wine. *Horticulturae*, 8(3):212. https://doi.org/10.3390/horticulturae8030212.

Marais, J. 1994. Sauvignon blanc cultivar aroma-A review. *South African Journal of Enology and Viticulture*, 15(2):41-45. https://doi.org/10.21548/15-2-2283.

Marais, J. 1998. Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. *South African Journal of Enology and Viticulture*, 19(1):10-16.

Marais, J. 2001. Effect of grape temperature and yeast strain on Sauvignon blanc wine aroma composition and quality. *South African Journal of Enology and Viticulture*, 22(1):47-50.

Miller, M. 2023. *Wine: From the inside out.* [Blog, 08 December] https://www.gencowinemakers.com/docs/Acids%20Presentation.pdf/ [08 December 2023].

Modesti, M., Shmuleviz, R., Macaluso, M., Bianchi, A., Venturi, F., Brizzolara, S., Zinnai, A. & Tonutti, P. 2021. Pre-processing cooling of harvested grapes induces changes in berry composition and metabolism and affects quality and aroma traits of the resulting wine. *Frontiers in Nutrition*, 8:728510. https://doi.org/10.3389/fnut.2021.728510.

Naranjo, A., Martínez-Lapuente, L., Ayestarán, B., Guadalupe, Z., Pérez, I., Canals, C. & Adell, E. 2021. Aromatic and sensory characterization of Maturana Blanca wines made with different technologies. *Beverages*, 7(1):10. https://doi.org/10.3390/beverages7010010.

Naviglio, D., Formato, A., Scaglione, G., Montesano, D., Pellegrino, A., Villecco, F. & Gallo, M. 2018. Study of the grape cryo-maceration process at different temperatures. *Foods*, 7(7):107. https://doi.org/10.3390/foods7070107.

Nicolini, G., Moser, S., Roman, T., Mazzi, E. & Larcher, R. 2011. Effect of juice turbidity on fermentative volatile compounds in white wines. *Vitis*, 50(3):131-135. https://doi.org/10.5073/vitis.2011.50.131-135.

Olarte Mantilla, S.M., Collins, C., Iland, P.G., Kidman, C.M., Jordans, C. & Bastian, S.E.P. 2013. Comparison of sensory attributes of fresh and frozen wine grape berries using Berry Sensory Assessment. *Australian Journal of Grape and Wine Research*, 19(3):349-357. https://doi.org/10.1111/ajgw.12041.

Olejar, K.J., Fedrizzi, B. & Kilmartin, P.A. 2015. Influence of harvesting technique and maceration process on aroma and phenolic attributes of Sauvignon blanc wine. *Food Chemistry*, 183:181-189. https://doi.org/10.1016/j.foodchem.2015.03.040.

Olejar, K.J., Fedrizzi, B. & Kilmartin, P.A. 2016. Enhancement of Chardonnay antioxidant activity and sensory perception through maceration technique. *LWT-Food Science and Technology*, 65:152-157.https://doi.org/10.1016/j.lwt.2015.08.001.

O'Kennedy, K. 2020. Freezing Sauvignon blanc grapes increases volatile thiols in wines. [Blog, 02 April]. https://sauvignonblanc.com/freezing-sauvignon-blanc-grapes-increases-volatile-thiols-in-wines/ [20 April 2020].

Panzeri, V., Ipinge, H.N. & Buica, A. 2020. Evaluation of South African chenin blanc wines made from six different trellising systems using a chemical and sensorial approach. *South African Journal of Enology and Viticulture*, 41(2):133-150. https://doi.org/10.21548/41-2-3889.

Parenti, A., Spugnoli, P., Calamai, L., Ferrari, S. & Gori, C. 2004. Effects of cold maceration on red wine quality from Tuscan Sangiovese grape. *European Food Research and Technology*, 218:360-366. https://doi.org/10.1007/s00217-003-0866-1.

Parr, W.V., Schlich, P., Theobald, J.C. & Harsch, M.J. 2013. Association of selected viniviticultural factors with sensory and chemical characteristics of New Zealand Sauvignon blanc wines. *Food Research International*, 53(1):464-475. https://doi.org/10.1016/j.foodres.2013.05.028.

Pedrosa-López, M. D. C., Aragón-García, F., Ruíz-Rodríguez, A., Piñeiro, Z., Durán-Guerrero, E. & Palma, M. 2022. Effects from the freezing of either whole or crushed grapes on the volatile compound's contents in Muscat wines. *Foods*, 11(12):1782. https://doi.org/10.3390/foods11121782.

Peinado, R.A., Moreno, J., Bueno, J.E., Moreno, J.A. & Mauricio, J.C. 2004. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chemistry*, 84(4):585-590. https://doi.org/10.1016/S0308-8146(03)00282-6.

Piano, F., Fracassetti, D., Buica, A., Stander, M., Du Toit, W.J., Borsa, D. & Tirelli, A. 2015. Development of a novel liquid/liquid extraction and ultra-performance liquid chromatography tandem mass spectrometry method for the assessment of thiols in South African Sauvignon blanc wines. *Australian Journal of Grape and Wine Research*, 21(1):40-48. https://doi.org/10.1111/ajgw.12117.

Pocock, K.F., Hayasaka, Y., McCarthy, M.G. & Waters, E.J. 2000. Thaumatin-like proteins and chitinases, the haze-forming proteins of wine, accumulate during ripening of grape (*Vitis vinifera*) berries and drought stress does not affect the final levels per berry at maturity. *Journal of Agricultural and Food Chemistry*, 48(5):1637-1643. https://doi.org/10.1021/jf9905626.

Radeka, S., Bestulić, E., Rossi, S., Orbanić, F., Bubola, M., Plavša, T., Lukić, I. & Jeromel, A. 2023. Effect of different vinification techniques on the concentration of volatile aroma compounds and sensory profile of Malvazija istarska wines. *Fermentation*, 9(7):676. https://doi.org/10.3390/fermentation9070676.

Ribereau-Gayon, P., Dubourdieu, D., Donèche, B. & Lonvaud, A., eds., 2000. *Handbook of enology: 2nd ed., The microbiology of wine and vinifications*, (Vol 1), Chichester, England: John Wiley and Sons Ltd., pp.3-49.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B. & Lonvaud, A. 2006. *Handbook of enology.* 2nd ed., *The chemistry of wine Stabilization and Treatments,* (Vol. 2), Chichester, England: John Wiley & Sons Ltd., 241-297.

Roland, A., Schneider, R., Razungles, A. & Cavelier, F. 2011a. Varietal thiols in wine: discovery, analysis and applications. *Chemical Reviews*, 111(11):7355-7376. https://doi.org/10.1021/cr100205b.

Roland, A., Schneider, R., Charrier, F., Cavelier, F., Rossignol, M. & Razungles, A. 2011b. Distribution of varietal thiol precursors in the skin and the pulp of Melon B. and Sauvignon Blanc grapes. *Food Chemistry*, 125(1):139-144. https://doi.org/10.1016/j.foodchem.2010.08.050.

Ruiz, J., Kiene, F., Belda, I., Fracassetti, D., Marquina, D., Navascués, E., Calderón, F., Benito, A., Rauhut, D., Santos, A. & Benito, S. 2019. Effects on varietal aromas during winemaking: A review of the impact of varietal aromas on the flavor of wine. *Applied Microbiology and Biotechnology*, 103(18):7425-7450. https://doi.org/10.1007/ s00253-019-10008-9.

Ruiz-Rodríguez, A., Durán-Guerrero, E., Natera, R., Palma, M. & Barroso, C. G. 2020. Influence of two different cryoextraction procedures on the quality of wine produced from muscat grapes. *Foods*, 9(11):1529. https://doi.org/10.3390/foods9111529.

Ryona, I., Pan, B.S., Intrigliolo, D.S., Lakso, A.N. & Sacks, G.L. 2008. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. cv. Cabernet Franc). *Journal of Agricultural and Food Chemistry*, 56(22):10838-10846. https://doi.org/10.1021/jf801877y.

Salinas, M.R., Garijo, J., Pardo, F., Zalacain, A. & Alonso, G.L. 2005. Influence of prefermentative maceration temperature on the colour and the phenolic and volatile composition of rosé wines. *Journal of the Science of Food and Agriculture*, 85(9):1527-1536. https://doi.org/10.1002/jsfa.2133.

Santesteban, L.G., Miranda, C. & Royo, J.B. 2013. Influence of the freezing method on the changes that occur in grape samples after frozen storage. *Journal of the Science of Food and Agriculture*, 93(12):3010-3015. https://doi.org/10.1002/jsfa.6133.

Schmid, F. & Jiranek, V. 2011. Use of fresh versus frozen or blast-frozen grapes for small-scale fermentation. *International Journal of Wine Research*. 3:25-30. https://doi.org/ 10.2147/IJWR.S23325.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika*, 52(3-4):591-611. https://doi.org/10.2307/2333709.

Sidhu, D., Lund, J., Kotseridis, Y. & Saucier, C. 2015. Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. *Critical Reviews in Food Science and Nutrition*, 55(4):485-502. https://doi.org/10.1080/10408398.2012.658587.

Steel, C.C., Blackman, J.W. & Schmidtke, L.M. 2013. Grapevine bunch rots: impacts on wine composition, quality, and potential procedures for the removal of wine faults. *Journal of Agricultural and Food Chemistry*, 61(22):5189-5206. https://doi.org/10.1021/jf400641r.

Tian, R.R., Li, G., Wan, S.B., Pan, Q.H., Zhan, J.C., Li, J.M., Zhang, Q.H. & Huang, W.D. 2009. Comparative study of 11 phenolic acids and five flavan-3-ols in cv. Vidal: impact of natural icewine making versus concentration technology. *Australian Journal of Grape and Wine Research*, 15(3):216-222. https://doi.org/10.1111/j.1755-0238.2009.00055.x.

Tomaz, I., Šeparović, M., Štambuk, P., Preiner, D., Maletić, E. & Karoglan Kontić, J. 2018. Effect of freezing and different thawing methods on the content of polyphenolic compounds of red grape skins. *Journal of Food Processing and Preservation*, 42(3):13550. https://doi.org/10.1111/jfpp.13550.

Threlfall, R., Main, G. & Morris, J. 2006. Effect of freezing grape berries and heating must samples on extraction of components and composition parameters of red wine grape varieties. *Australian Journal of Grape and Wine Research*, 12(2):161-169. https://doi.org/10.1111/j.1755-0238.2006.tb00056.x.

Van Breda, V.M., van Jaarsveld, F.P. & van Wyk, J. 2024. Pre-Fermentative Cryogenic Treatments: The Effect on Aroma Compounds and Sensory Properties of Sauvignon Blanc and Chenin Blanc Wine A Review. *Applied Sciences*, 14(4):1-14. https://doi.org/10.3390/app14041483.

Van Wyngaard, E. 2013. *Volatiles playing an important role in South African Sauvignon blanc wines* (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Vernarelli, L.A. 2018. Novel Vinification Techniques to Improve Pennsylvania Wine Quality. Unpublished Masters thesis. Pennsylvania State University, University Park. https://etda.libraries.psu.edu/catalog/15364lav7 [12 October 2024].

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I.S. & Henschke, P.A. 2007. Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. *Applied Microbiology and Biotechnology*, 77:145-157. https://doi.org/10.1007/s00253-007-1145-z.

Vilanova, M., Siebert, T.E., Varela, C., Pretorius, I.S. & Henschke, P.A. 2012. Effect of ammonium nitrogen supplementation of grape juice on wine volatiles and non-volatiles composition of the aromatic grape variety Albariño. *Food Chemistry*, 133(1):124-131. https://doi.org/10.1016/j.foodchem.2011.12.082.

Vilanova, M., Pretorius, I.S. & Henschke, P.A. 2015. Influence of diammonium phosphate addition to fermentation on wine biologicals. In *Processing and impact on active components in Food*. London: Academic Press United Kingdom, 483-491. https://doi.org/10.1016/B978-0-12-404699-3.00058-5.

Vilar-Bustillo, J., Ruiz-Rodríguez, A., Carrera, C.A., Piñeiro, Z. & Palma, M. 2023. Effects of Different Freezing Treatments during the Winemaking of a Varietal White Wine with regard to Its Phenolic Components. *Foods*, 12(10):1963. https://doi.org/10. 3390/foods12101963.

Volschenk, H., Van Vuuren, H.J. & Viljoen-Bloom, M. 2006. Malic acid in wine: Origin, function and metabolism during vinification. *South African Journal of Enology and Viticulture*, 27(2):123-136. https://doi:10.21548/27-2-1613.

Wilson, C. L. 2017. *Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines* (Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch). http://hdl.handle.net/10019.1/101250.

Wilson, C., Brand, J., du Toit, W. & Buica, A. 2018. Interaction effects of 3-mercaptohexan-1-ol (3MH), linalool and ethyl hexanoate on the aromatic profile of South African dry Chenin blanc wine by descriptive analysis (DA). *South African Journal of Enology and Viticulture*, 39(2):1-13. http://dx.doi.org/10.21548/39-2-3165.

Wilson, C., Brand, J., du Toit, W. & Buica, A. 2019. Matrix effects influencing the perception of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) in different Chenin Blanc wines by Projective Mapping (PM) with Ultra Flash profiling (UFP) intensity ratings. *Food Research International*, 121:633-640. htt ps://doi.org/10.1016/j.foodres.2018.12.032.

Zhang, S., Petersen, M.A., Liu, J. & Toldam-Andersen, T.B. 2015. Influence of pre-fermentation treatments on wine volatile and sensory profile of the new disease tolerant cultivar Solaris. *Molecules*, 20(12):21609-21625. https://doi.org/10.3390/mole cules201219791.

Zhang, Q., Sun, X., Sheng, Q., Chen, J., Huang, W. & Zhan, J. 2016. Effect of suspension freeze-concentration technology on the quality of wine. *South African Journal of Enology and Viticulture*, 37(1):39-46.

## CHAPTER FOUR: RESEARCH CHAPTER 2

## Cryogenic pre-treatment technologies: Impact on varietal aroma and sensory profiles of South African Sauvignon blanc and Chenin blanc wine


### Abstract

The distinctive aroma of Sauvignon blanc is attributed to key aroma compounds, i.e., methoxypyrazines, contributing to the "greener" aromas and the varietal thiols correlated with "tropical" and passion fruit aromas. These compounds are present in the grape skin and pulp and are released during the alcoholic fermentation process. Previous research has explored a range of viticultural practices and vinification techniques to enhance such varietal aroma compounds, specifically the varietal thiols. However, the results reported were often contradictory. Alternative technologies such as low-temperature cryogenic processing of grapes have also been explored and show promise. Therefore, this research aimed to investigate the impact of cryogenic pretreatment technologies on the levels of varietal thiols, methoxypyrazine, and the effect on the sensory profiles of South African (SA) Sauvignon blanc and Chenin blanc wines.

The concentrations of 3-SHA detected in most of the wines produced in this study exceeded both the aroma perception threshold (4 ng $L^{-1}$) and reported range for SA Sauvignon blanc wines (23-151 ng $L^{-1}$) whilst the levels of 3-SH were generally within the aroma perception threshold (60 ng $L^{-1}$) and reported range for SA Sauvignon blanc wines (178-904 ng $L^{-1}$). Moreover, the levels of 4-MSP were found to be below the aroma perception threshold (0.8 ng $L^{-1}$) and the typical range reported for SA Sauvignon blanc (0-21.9 ng $L^{-1}$). Furthermore, the levels of 3-SHA in the Chenin blanc wines produced in this study were once again higher than both the reported ranges for SA Chenin blanc wines (5-253 ng $L^{-1}$) and the aroma perception threshold (4 ng $L^{-1}$). The concentration of 3-SH was generally below the reported range (99-1124 ng $L^{-1}$) and aroma perception threshold (60 ng $L^{-1}$) values for SA Chenin blanc. It was further observed that the concentrations of 4-MSP were above the reported ranges for South African Chenin blanc wines (nd) but below the aroma perception threshold levels (0.8 ng $L^{-1}$). The methoxypyrazines, ibMP and sbMP were detected at levels above the reported aroma perception threshold levels (2-16 ng $L^{-1}$) and ranges, 2-30 ng $L^{-1}$ and < 10 g $L^{-1}$, respectively in Sauvignon blanc wines, for most cryogenic treatments compared to the control wines. Differences were also observed between the producers in different regions and between vintages (2020 and 2021) with overall thiol and methoxypyrazine concentrations higher in 2021.

Furthermore, the observations made based on the results of this study, from a sensory perspective, is that the wines produced from the WG and MG subjected to cryogenic pre-treatment technologies yielded wines with higher tropical, thiol type,

pineapple and banana aromas as well as higher body, general quality and overall intensity when compared to most wines made from the control grapes as well as the remaining cryogenic treatments. This study further highlighted that the production stage at which the cryogenic treatment was applied had the most prominent effect, whilst the effects of the cryogenic temperatures and storage times were negligible. Moreover, vintage and regional differences also played a role and had an impact on the final wine sensory profiles. Therefore, cryogenic pre-fermentation treatments applied before fermentation can enhance white wine varietal aromas and the production of wines with unique styles. It should be noted that the above-mentioned results are in context to SA Sauvignon blanc and Chenin blanc wine and might differ when these grape varieties originate from other countries.

## 4.1 Introduction

Sauvignon blanc (*Vitis vinifera*), a white grape variety cultivated globally, originates on the West Coast of France. Sauvignon blanc wines have diverse aroma profiles, influenced by region, climate, soil, and winemaking techniques. The distinctive varietal aroma of Sauvignon blanc is characterised by green or unripe notes like citrus, green capsicum, grassy, herbs, as well as tropical fruits such as passion fruit, guava, and gooseberries (Marais 1994:44; Ribéreau-Gayon et al., 2006:214; Parr et al., 2013:474). Mineral and flinty notes can also be detected (Parr et al., 2016:178). These profiles result from the differences based on the wine's country of origin, i.e., Sauvignon blanc wines from New Zealand, predominantly from Marlborough, are renowned for their distinct fruity notes of tropical and passion fruit, in comparison with French or South African wines, previously characterised by mineral, flinty, and bourbon-like aromas (Benkwitz et al., 2012:66,70-71). The typical Sauvignon blanc aroma is attributed to key chemical compounds, with methoxypyrazines contributing to the greener aromas and varietal thiols correlated with tropical fruits, including passion fruit notes (Marais, 1994:41-42; 44; Tominaga et al., 1998a:5219; 1998b:162; Benkwitz et al., 2012:66; 70-71). The interaction between methoxypyrazines and varietal thiols is not clear, however, Campo et al. (2005:5688-5689) showed that the varietal thiol 3-sulfanylhexyl acetate (3-SHA) is negatively affected by the presence of methoxypyrazines. The study showed that the higher the levels of methoxypyrazines, the lower the tropical fruit notes imparted by 3-SHA will be (Campo et al., 2005:5688-5689).

Methoxypyrazines naturally occur in grapes, particularly in the skins, as byproducts of amino acid metabolism, with higher concentrations in under-ripe fruits, diminishing during grape maturation. Factors such as sunlight exposure and warmer climates inhibit the accumulation of methoxypyrazines in grape berries during maturation (Allen & Lacey, 1993:36-37; Allen & Lacey, 1998:31-36; Marais, 1994:44;

Ryona et al., 2008:10844-10845). Consequently, grapes grown in cooler climate regions show higher methoxypyrazine levels. These compounds are stable and unaffected by oxidative conditions and include key pyrazines like 3-*iso*butyl-2-methoxypyrazine (ibMP), 2-methoxy-3-*sec*-butylpyrazine (sbMP) and 3-*iso*propyl-2-methoxypyrazine (ipMP) in Sauvignon blanc wines, crucial for the perceived green characteristics (Allen & Lacey, 1993:35-36; Parr et al., 2013:474). In wines, the levels of ibMP range from 2-30 ng $L^{-1}$, whilst the levels of ipMP and sbMP are < 10 ng $L^{-1}$ (Sidhu et al., 2015:485-487; Lei et al., 2018:1142). The methoxypyrazines mentioned above have low aroma perception thresholds ranging from 2-16 ng $L^{-1}$, emphasizing the significance of these compounds as key odourants in Sauvignon blanc wines (Benkwitz et al., 2012:66,70-71; Ribéreau-Gayon et al., 2006:214-216; Sidhu et al., 2015:485-499; van Breda et al., 2024:9) (Table 4.1).

Table 4.1: Methoxypyrazines present in Sauvignon blanc wines: aroma description, perception, and range in wine.

| Compound | Aroma description | Aroma perception in water (ng $L^{-1}$) | Aroma perception in wine (ng $L^{-1}$) | Range in wine (ng $L^{-1}$) |
|---|---|---|---|---|
| ibMP[1] | vegetative, green pepper | 1-2 | 2-16 | 2-30 |
| ipMP[2] | earthy, mushroom, cooked, or canned asparagus, green beans | 1-2 | 2-16 | < 10 |
| sbMP[3] | green (peas, bell pepper, galbanum), ivy leaves, bell pepper | 1-2 | 2-16 | < 10 |

[1]ibMP-2-methoxy-3-*iso*butylpyrazine
[2]ipMP-2-methoxy-3-*iso*propylpyrazine
[3]sbMP-2-methoxy-3-*sec*-butylpyrazine
Adapted from Sidhu et al. (2015:485-487); Lei et al. (2018:1142).

The key varietal thiols determining the aroma profile of Sauvignon blanc wines are 3-SH, its derivative ester 3-SHA, and 4-MSP. These compounds, particularly 3-SH and 3-SHA, are present in wine as a combination of R and S enantiomers, with racemic mixtures exhibiting notably low sensory perception thresholds of 60 ng $L^{-1}$ and 4 ng $L^{-1}$, respectively. However, in Sauvignon blanc and Chenin blanc wine, these varietal thiols, 3-SH, 3-SHA and 4-MSP are present in levels ranging between 350-5664 and 10-1368 ng $L^{-1}$, 0-106 and 0-100 ng $L^{-1}$ and 0-88 and 0.23 ng $L^{-1}$, respectively (Table 4.2) (Wilson, 2017:6-20; 28-38; Coetzee et al., 2018:180-184; Wilson et al., 2018:12-13; Wilson et.al., 2019:634-635). The aromatic characteristics of these varietal thiols are heavily influenced by their molecular structure, the *R* form of 3-SH imparts a grapefruit aroma, while the *S* form offers hints of passion fruit. Similarly, the *R* form of 3-SHA is less aromatic with notes of passion fruit, whereas the *S* form produces an herbaceous aroma similar to boxwood (Tominaga et al., 2006:7254-7255). Several authors, such as Lund et al. (2009:1-12); Green et al. (2011:2791-2797) and Benkwitz, et al. (2012:62-72), have explored the impact of varietal thiols on the overall aroma profile of Sauvignon blanc wines and showed that the concentrations of these compounds have strong correlations with sensory descriptors like "Tropical", "Passion fruit", "Sweet sweaty passion fruit", "Gooseberry", "Cat urine" and "Boxwood".

Table 4.2: Varietal thiols present in Sauvignon blanc and Chenin blanc wines: aroma description, perception, and range in wine.

| Cultivar | Compound | Aroma description | Aroma Perception in wine ($ng\ L^{-1}$) | Range in wine ($ng\ L^{-1}$) | Range in SA[1] wine ($ng\ L^{-1}$) |
|---|---|---|---|---|---|
| Sauvignon blanc | 4-MSP[2] | Boxwood, blackcurrant | 0.8 | 0-88 | 0-21.9 |
| Chenin blanc | | | | 0-23 | n.d.[*] |
| Sauvignon blanc | 3-SHA[3] | Passionfruit, tropical, boxwood | 4 | 0-106 | 23-151 |
| Chenin blanc | | | | 0-100 | 5-253 |
| Sauvignon blanc | 3-SH[4] | Grapefruit, tropical, passionfruit | 60 | 350-5664 | 178-904 |
| Chenin blanc | | | | 10-1368 | 99-1124 |
| Sauvignon blanc | BM[5] | Smoke, toasty, struck flint | 0.3 | 0.6-5.5 | n.d.[*] |
| Chenin Blanc | | | | 30-40 | n.d.[*] |
| Sauvignon blanc | FFT[6] | Roasted Coffee | 0.4 | 1-36 | n.d.[*] |
| Chenin blanc | | | | 14 | n.d.[*] |

[1]South Africa
[2]4-methyl-4-sulfanylpentan-2-one
[3]3-sulfanylhexyl acetate
[4]3-sulfanylhexan-1-ol
[5]benzyl mercaptan
[6]2-furfurylthiol
[*]Not detected
Adapted from Capone et al. (2015:1227); Jeffery (2016:1324); Coetzee et al. (2018:180-184).

Varietal thiols in wine are released from their precursors through three proposed biogenesis pathways as suggested by various researchers (Tominaga et al., 1998a:161-162; Ebeler & Thorngate, 2009:8101; Coetzee & du Toit, 2012:289-290; Kapaklis, 2014:22). The primary pathway involves the breakdown of cysteinylated precursors of 3-SH and 4-MSP via the *β*-lyase enzyme activity of the *S. cerevisiae* yeast (Hart et al., 2016:276-277; 2017:146-147; Williams et al., 2021:15-22). The second pathway involves the glutathionylated precursors, known for their role in cellular detoxification across various organisms like plants and animals (Peyrot des Gachons et al., 2002:4077-4078; Chen et al., 2019a:1-24; Künstler et al., 2020:5-6). Furthermore, the presence of S-cysteine conjugates alongside S-glutathione conjugates is frequently observed. The latter involves the conjugation of an endogenous or exogenous toxic compound with glutathione by the S-glutathione transferase enzyme, followed by degradation through γ-glutamyltranspeptidase and carboxypeptidase, resulting in the formation of the S-cysteine conjugate (Tominaga et al., 1998a:161-162; Peyrot des Gachons et al., 2002:4077-4078; Ebeler & Thorngate, 2009:8101). Thibon et al. (2009:714-715; 2011:1347; 2016:714-715) demonstrated the ability of *Vitis vinifera* cells to synthesise Cys-3SH from G-3SH, upon exposure to *Botrytis cinerea,* leading to a significant increase in Cys-3SH production. The third biogenesis pathway involves conjugated carbonyl compounds, such as (E)-2-hexanal and mesityl-oxide, which, through sulfur addition and yeast-mediated conversion during fermentation, result in the formation of 3-SH and 4-MSP (Schneider et al., 2006:58; Ebeler & Thorngate, 2009:8101; Roland et al., 2011:7356-7358). Regardless of extensive research, the pathway responsible for the formation of 3-SH is only partially elucidated. Therefore, the current understanding is that there is a breakdown of the conjugated precursors of glutathione or cysteine and 3-SH through the interaction with the peptide and (E)-2-hexenal, as proposed by Tominaga et al. (1998b:5218-5219), Peyrot des Gachons et al. (2002:4078) and Thibon et al. (2016:717-718). However, doubts have been raised concerning the significance of the abovementioned pathway, due to the inconsistency in terms of a direct correlation between the concentrations of these conjugates in grape juice and the levels of varietal thiols in the final wine (Pinu et al., 2012:409-411). An alternative hypothesis suggests that 3-SH is formed through the direct addition of $H_2S$, produced by yeast during the alcoholic fermentation process, to (E)-2-hexenal, resulting in an aldehyde intermediate that is subsequently converted to 3-SH through yeast metabolic processes. Originally proposed by Schneider et al. (2006:62-63) and later evaluated for its high 3-SH production potential by Harsch et al. (2013:3710-3711), the existence of this proposed pathway is challenged due to inconsistencies between the utilisation of the aldehyde and the production of $H_2S$ by the yeast, limiting the timeframe required for the reaction. Recent research has revealed that elemental sulfur reduction in grape juice can lead to $H_2S$ production, thereby expanding the window for interaction with (E)-2-hexenal. Notably, an increase in elemental sulfur residues in the juice was found to directly elevate the levels of varietal thiols in the resulting wine (Araujo et al., 2017:85).

Given their significance in sensory perception, extensive research has focused on investigating the viticultural and oenological factors that influence the development of varietal thiols and their precursors. These factors include harvesting practices, maceration techniques, pressing methods, yeast selections, and the use of antioxidants (Parenti et al., 2004:364-365; Coetzee, 2020a; b; Coetzee & du Toit, 2012:292-293; Hart et al., 2019:2; Van Breda et al., 2024:3-5). It has been demonstrated that the application of advanced oxidation techniques using grape juice significantly reduces its capacity to generate the varietal thiols 3-SH and 3-SHA (Allen et al., 2011:10648-10649; Makhotkina et al., 2013:204). This phenomenon may be attributed to reactive quinones, byproducts resulting from the oxidation of polyphenolic compounds, which eliminate the produced thiols or $H_2S$. Makhotkina et al. (2013:204-206;211-212) illustrated that the gradual addition of $SO_2$ to grapes harvested by machines led to the production of wines with elevated levels of varietal thiols, supporting the significance of safeguarding against oxidation at the initial stages of grape processing to facilitate the creation of these essential compounds. As mentioned, another factor affecting varietal thiols is the harvesting technique. Grapes can be hand-harvested or harvested using mechanical devices. Manual grape picking allows for the removal of substandard fruit and other non-grape substances. This method is also more delicate, preventing issues related to excessive oxidation and exposure to unregulated microbial growth, problems often linked to machine harvesting which can result in the production of lower-quality wines (Arfelli et al., 2010:108-112; Kilmartin, 2012:82;86). Nonetheless, advancements in harvesting technologies combined with post-harvest treatments to counteract the depletion of natural antioxidants have proven effective in preserving the quality of machine-harvested grapes (Arfelli et al., 2010:108-112). Notably, the mechanical harvesting of grapes did not result in any negative sensory effects and the quality of wine, particularly when incorporating leaves intentionally (Arfelli et al., 2010:108-112; Kilmartin, 2012:82;86; Coetzee, 2020a; b). It was also noted that machine-harvested Sauvignon blanc grapes generally displayed a greater potential for thiol formation than hand-harvested grapes (Allen et al., 2011:10644-10649). Wines made from machine-harvested grapes showed elevated levels of varietal thiols and fruity aromas, lower acidity, and enhanced perception on the palate compared to wines made from hand-harvested grapes (Parr et al., 2013:470-473). Therefore, this research was conducted to investigate whether pre-fermentative cryogenic technologies would affect the key varietal aroma compound (varietal thiol and methoxypyrazine) levels, hence influencing the sensory perception of Sauvignon blanc and Chenin blanc wines. In addition, the effect of region and harvesting method, i.e., hand-harvested grapes vs machine-harvested Sauvignon blanc grapes were also investigated.

## 4.2 Materials and methods

### 4.2.1 Wines

During the 2020 vintage, Sauvignon blanc grapes (2000 kg), the primary cultivar of interest for this study, were obtained from three producers located within the Coastal (Stellenbosch and Durbanville) and Cape South Coast (Napier) wine regions. All grapes were hand-harvested, except for 500 kg of Sauvignon blanc from the Durbanville-based producer, which was machine-harvested and then hand-harvested to reflect their harvesting methods. During the 2021 vintage, Sauvignon blanc grapes were received from the same producers, with the addition of Chenin blanc grapes from a producer in the Stellenbosch wine region. Following harvesting, the grapes were delivered to the Agricultural Research Council (ARC) Nietvoorbij Research cellar where crates were weighed and divided into batches for the cryogenic pre-treatments. Before processing, grapes were also sorted to remove any rotten or undesirable bunches. For both the 2020 and 2021 vintages, wines were made from WG, MG, TM and CJ, subjected to two cryogenic pre-treatment temperatures, i.e., -4 ºC and -20 ºC at a T0 and T4 storage period in large capacity freezer rooms. Following thawing and wine production, as described in Chapter 3, the wines were chemically analysed for the aroma compounds and also sensorially evaluated.

### 4.2.2 Chemical analyses

#### 4.2.2.1 Standard chemical analysis

All wines produced were subjected to standard chemical analysis as described in Chapter 3.

#### 4.2.2.2 Key aroma compounds

##### *4.2.2.2.1 Varietal Thiol Analysis*

All samples were prepared and analysed by the Central Analytical Facility (CAF) at Stellenbosch University according to the method used by Mafata et al. (2018:2). All solutions prepared were expressed in terms of volume percentage ($^{v}/_{v}$ %) and made up with Milli-Q water, unless otherwise specified. All reagents and standards, including 3-SH, 3-SHA, 4-MSP and 98% 4,4′-Dithiodipyridine (98% DTDP), ethylenediaminetetr aacetic acid disodium salt (EDTA-$Na_2$), methanol, 96% ethanol, sodium hydroxide (NaOH), tartaric acid, anhydrous acetaldehyde ≥ 98% and 37% hydrochloric acid (HCl) were purchased from Sigma-Aldrich (Louisville, MO, USA), and the solid phase extraction (SPE) cartridges (Supelclean ENVI-18 SPE) from Supelco (Bellefonte, PA, USA).

##### *4.2.2.2.2 Methoxypyrazine Analysis*

All samples were prepared and analysed by the Central Analytical Facility (CAF) at Stellenbosch University according to the methods described by Kotseridis et al. (1998:72) and Godelmann et al. (2008:450). Reference standards of 2-methoxy-3-iso butylpyrazine (purity 99%), 2-methoxy-3-isopropylpyrazine (purity 97%), 2-methoxy-3-sec-butylpyrazine (purity 99%) were obtained from Sigma-Aldrich, South Africa. The isotopically labelled internal standards were synthesised according to the procedures described by Kotseridis et al. (1998:72) and Godelmann et al. (2008:450). The purity was checked by full scan GC-MS. Stock solutions of each pyrazine of 2000 μg mL$^{-1}$ were prepared in analytical grade absolute ethyl alcohol and stored at 4 °C. The standard solutions were diluted as required for calibration standards (ibMP and sbMP) with the concentration ranges shown in Table 4.3. All reagents, including $H_2SO_4$-$D_2$ solution (96% $^{w}/_{w}$ in $D_2O$), deuterium oxide ($D_2O$), and ethyl alcohol absolute pro analysis (p.a.), sodium carbonate p.a., sodium chloride purissimum (puriss.) and sodium sulphate p.a., were purchased from Merck Life Sciences, South Africa.

Table 4.3. The concentration range of the sbMP and ibMP standards used for the calibration curve.

| Concentration in (ng L$^{-1}$) | | |
|---|---|---|
| **Range** | **sbMP** | **ibMP** |
| Level 1 | 1.00 | 1.00 |
| Level 2 | 2.50 | 2.50 |
| Level 3 | 5.00 | 5.00 |
| Level 4 | 10.00 | 10.00 |
| Level 5 | 25.00 | 25.00 |
| Level 6 | 50.00 | 50.00 |

### 4.2.3 Sensory evaluation

Immediately following bottling, bottled wines were subjected to sensory evaluations in three sessions, i.e., sensory profiling, training and evaluation sessions. Sensory profiling sessions were carried out on all Sauvignon blanc and Chenin blanc wines over two sessions. In the first session, six trained tasters, all members of the South African Wine and Spirit Board Sensory panels, evaluated wines and established the most prominent qualitative and quantitative aroma and taste descriptors for all wines (Control, 0 months and 4 months). Following the profiling sessions, the second session involved the training of the sensory panel members. Sensory training sessions were held at the ARC Nietvoorbij research cellar sensory facility with the panel comprising between 13 and 15 members.

During the six training sessions, panel members were presented with the preliminary descriptors established for the Sauvignon blanc wines, i.e., Overall intensity, yellow fruit (e.g., Banana, Pineapple), Tropical fruit (Thiol type aroma, e.g., Guava, Passion fruit, Melon), Citrus (e.g., Grapefruit, Lemon), Herbaceous (e.g., Fresh, vegetative), Reductive (e.g., Cooked vegetative, Rotten egg) and $SO_2$. The final aroma descriptors selected by the panel were Overall intensity, Thiol type aroma (e.g., Guava, Passion fruit), Citrus (e.g., Grapefruit, Lemon), Herbaceous (Fresh), Banana, Pineapple, Apple, Reductive (e.g., Cooked vegetative, Rotten egg) and $SO_2$. Taste attributes (Acid and Body) and general quality remained unchanged for the profiling, training and sensory evaluation sessions. The same procedure was followed for the Chenin blanc wines with final aroma descriptors selected by the panel, i.e., Overall intensity, Tree fruit (e.g., Peach, Pear, Apple), Thiol type aroma (e.g., Guava, Passionfruit, Melon), Citrus (e.g., Grapefruit, Lemon), Sweet correlated (e.g., Candy, Toffee), Stewed fruit, Vegetative (e.g., Fresh, Dried, Cooked) and reductive (e.g., Green apple skin, Overripe apple). The taste attributes (Acid and Body) and General quality once again remained unchanged.

The tasting sheets were developed based on the descriptors identified in the training sessions, using the predominant descriptors detected in the wines, formulated during discussions with the selected panel (Appendices A-E). Final wines were served at room temperature, i.e., ~20 ºC and presented in black International Organisation for Standardisation (ISO 3591:1977) wine-tasting glasses (Figure 4.1).

Figure 4.1. Black International Organization for Standardization (ISO 3591:1977) wine-tasting glasses were used during the sensory evaluation sessions.

### 4.2.4 Experimental design and Statistical analysis

This study was conducted on Sauvignon blanc grapes from three wine regions and Chenin blanc grapes from one wine region. For each region, the experimental design was completely random with nine treatment combinations replicated three times. The treatment structure comprised of four wine-making stages (WG, MG, TM and CJ), two freezing temperatures (-4 ºC and -20 ºC) and a control. Sensory evaluations and chemical analyses were conducted on T0 and T4 wines. The twenty-seven wines (nine treatment combinations with three replicates each) were presented to each of the twelve trained panel members, comprising four females and eight males (aged between 35 to 65) in a random order (according to a Williams design). The panel reliability was tested by subjecting the sensory data to an analysis of variance (ANOVA) model comparable to 5.11 in Næs et al. (2010:55) using SAS (Version 9.4; SAS Institute Inc., Cary, USA).

One ANOVA assumption is that the residuals must follow a normal distribution, however, residuals (deviation of an observation from the predicted model value) are standardised to normal (0, 1) (by subtracting the mean and dividing by the standard deviation) to be able to detect outliers. It is expected that 99% of the data should fall within three standard deviations from the mean (0) when the data is normally distributed. Therefore, values with standardised residuals > 3 are identified as outliers. In addition, standardised residuals from the model were tested for deviation from normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965:610). Observations deviating by more than three standard deviations from the model value were identified as outliers and removed. Following the confirmation of panel reliability and normality, all subsequent statistical analyses of sensory data were conducted on means over judges for the twenty-seven wines, following the experimental design.

All analyses were performed on wines from each region and storage period (T0 and T4). Univariate analysis of variance (ANOVA) was performed on sensory and chemical data using the General Linear Models (GLM) procedure of SAS software (Version 9.4; SAS Institute Inc., Cary, NC, USA). Fisher's least significant difference was calculated at the 5% level to compare treatment means. A probability level of 5% was considered significant for all significance tests. Principal component analysis (PCA) of the sensory and chemical data, using the correlation matrix, was performed to visualise and elucidate the association between the treatments and observed variables. Discriminant analysis (DA) was done to determine whether treatments can be mathematically distinguished based on the observed variables. Multivariate analyses were conducted using XLStat (Addinsoft, 2022). XLSTAT statistical and data analysis solution. New York, USA: Addinsoft. Available at: https://www.xlstat.com/en).

## 4.3 Results and Discussion

### 4.3.1 Key aroma compounds

#### 4.3.1.1 Varietal Thiols

Overall, in terms of the desired levels of specific varietal thiols (3-SH, 4-MSP, 3-SHA), there was no definite trend as to what cryogenic temperature, stage of production and storage time will yield increased levels in final wines, as in this study, a variation in the levels of thiols between these treatments were observed, as graphically illustrated (Figs. 4.2-4.5). These findings were similar to observations made in previous studies

(Peng et al., 2013:1551-1552; Chen et al., 2019b:640-642; Ruiz-Rodríguez et al., 2020:9-10). These compounds contribute greatly to the perceived varietal aroma of Sauvignon blanc wines, responsible for the “grapefruit”, “passion fruit”, “tropical aromas” and the green aromas, i.e., “broom”, “box tree” and “cat urine” (Benkwitz et al., 2012:6296-6298; Ruiz et al., 2019:7426; 7433-7435; Chen et al., 2019b:637; Chen & Li, 2022:313-315).

*4.3.1.1.1 3-SHA*

For Producer A it was observed that in 2020, the highest concentrations of 3-SHA were detected in the T0 wines made from TM (-4 ºC) (900-1000 ng $L^{-1}$), followed by the MG (-20 ºC) (900 ng $L^{-1}$) (Fig. 4.2). In 2021, the highest concentrations were found in the T4 wines made from CJ (-4 ºC and -20 ºC) (600-900 ng $L^{-1}$), followed by the TM (-4 ºC and -20 ºC) (600-700 ng $L^{-1}$). Moreover, the concentrations in the wines made from the unfrozen control was considerably lower, ranging from 0-100 ng $L^{-1}$ and 0-300 ng $L^{-1}$ for 2020 and 2021, respectively (Fig. 4.2). These concentrations were found to be higher than the aroma perception threshold (4 ng $L^{-1}$) and range (23-151 ng $L^{-1}$) typically reported in SA Sauvignon blanc wines (Table 4.2) (Benkwitz et al., 2012:66-67; Van Wyngaard, 2013:65; Piano et al., 2015:46; Coetzee et al., 2018:182).

The concentrations of 3-SHA detected in wines made from grapes originating from Producer B were generally higher than those observed for all the other producers. In 2020, the highest concentrations in the T4 wines made from hand-harvested WG (-20 ºC) ranged between 2200-2400 ng $L^{-1}$, followed by the unfrozen control (600-800 ng $L^{-1}$) and WG (-4 ºC) (< 200 ng $L^{-1}$) (Fig. 4.3). These concentrations far exceeded the typically reported aroma perception threshold (4 ng $L^{-1}$) and range (23-151 ng $L^{-1}$) in SA Sauvignon blanc wine (Table 4.2). Moreover, it was observed that when grapes from the same vineyard were harvested by machine and subjected to cryogenic pre-fermentative treatments, the concentrations of 3-SHA were even higher (Fig. 4.4). For the T4 MG (-20 ºC), the concentration of 3-SHA ranged between 3200 to 3600 ng $L^{-1}$. Furthermore, concentrations in wines made from the CJ (-4 ºC and -20 ºC), TM (-20 ºC) and MG (-4 ºC) ranged between 2400 to 2800 ng $L^{-1}$. Wines made from the TM (-4 ºC) had concentrations of 3-SHA ranging between 1200-1600 ng $L^{-1}$, whilst the control had much lower concentrations (400-800 ng $L^{-1}$) (Fig. 4.4). In 2021, only hand-harvested grapes were obtained from Producer B and the concentrations of 3-SHA were generally lower for all treatments, including the control, ranging from < 200 to 600 ng $L^{-1}$, it was still higher than the typically reported aroma perception threshold (4 ng $L^{-1}$) and range (23-151 ng $L^{-1}$) in SA Sauvignon blanc wine (Table 4.2 and Fig. 4.3).

Furthermore, the highest concentrations of 3-SHA were detected in wines produced from grapes originating from Producer C (2020), subjected to the pre-fermentative cryogenic treatments, were observed in the T0 wines made from MG (-4 ºC), ranging between 1200-1300 ng $L^{-1}$ (Fig. 4.5), whilst the concentration in the control wine ranged between 200-300 ng $L^{-1}$. In 2021, wine made from the T0 MG (-20 ºC), had the highest concentration (1000 ng $L^{-1}$) of 3-SHA, whilst the concentration in the control wine was 400 ng $L^{-1}$ (Fig. 4.5). Moreover, once again for this producer the concentrations of 3-SHA exceeded the aroma perception threshold (4 ng $L^{-1}$) and reported range (23-151 ng $L^{-1}$) for SA Sauvignon blanc wine (Table 4.2). In the case of the wines made from Chenin blanc grapes subjected to the cryogenic treatments, the highest concentrations of 3-SHA were detected in the T4 wines made from MG (-20 ºC), ranging between 600-700 ng $L^{-1}$ (Fig. 4.6), followed by the T4 MG (-4 ºC) (500-600 ng $L^{-1}$). The wines made from the control grapes ranged between 300-400 ng $L^{-1}$, similar to the concentrations of the remainder of the treatments (Fig. 4.6). The Chenin blanc wine made from the MG cryogenic treatment thus gave significantly higher concentrations of 3-SHA and exceeded the aroma perception threshold (4 ng $L^{-1}$) and typical range (5-253 ng $L^{-1}$) reported in SA Chenin blanc wine (Table 4.2 and Fig. 4.6).

##### *4.3.1.1.2 4-MSP*

For the 4-MSP varietal thiol, for Producer A (2020), the highest concentrations were detected in the T0 wines made from WG (-4 ºC) (10-12 ng $L^{-1}$) (Fig. 4.2). This was followed by the T0 wines made from MG (-20 ºC) (8-10 ng $L^{-1}$), TM (-4 ºC) (8 ng $L^{-1}$), T4 WG (-4 ºC) and T0 TM (-20 ºC) (6-8 ng $L^{-1}$) and T4 WG (-20 ºC) (4-6 ng $L^{-1}$) whilst the concentrations detected in the control wines were below the limit of detection (0.2 ng $L^{-1}$). In 2021, the concentrations detected in the wines for all treatments were below 2 ng $L^{-1}$ and lower than the limit of detection (0.2 ng $L^{-1}$) in the control wines (Fig. 4.2). Furthermore, it was noted that the aforementioned concentrations of 4-MSP were equal to and above the aroma perception threshold (0.8 ng $L^{-1}$) and within the typically reported range (0-21.9 ng $L^{-1}$) for SA Sauvignon blanc wine (Table 4.2).

The highest concentration of 4-MSP for Producer B (2020) was detected in wines made from hand-harvested WG (-4 ºC), ranging between 4-5 ng $L^{-1}$, whilst for the WG (-20 ºC) and the control, the concentrations fell below the limit of detection (0.2 ng $L^{-1}$) (Fig. 4.3). However, for the machine-harvested control and all the treatments, the concentrations of 4-MSP were lower than the limit of detection (0.2 ng $L^{-1}$) (Fig. 4.4). Moreover, in 2021, the highest concentrations of 4-MSP were detected in wines made from hand-harvested WG (-4 ºC) (6-7 ng $L^{-1}$), followed by the control (4-5 ng $L^{-1}$) and WG (-20 ºC) (3-4 ng $L^{-1}$), with concentrations in the remaining treatments ranging between 1-3 ng $L^{-1}$ (Fig. 4.3). The concentrations of 4-MSP in the hand-harvested grapes were equal to and above the aroma perception threshold (4 ng $L^{-1}$) and within the typically reported range (0-21.9 ng $L^{-1}$) for SA Sauvignon blanc wine, whilst that of the machine-harvested grapes fell below, indicating that the vintage and harvesting technique could have affected the concentrations of this varietal thiol because wines made from hand-harvested grapes in the same vintage (2020) had higher concentrations of 4-MSP (Tables 4.3 and 4.4). These results differ from the research of Allen et al. (2011:10646-10647), who showed that harvesting technique did not affect the levels of 4-MSP.

For the wines originating from Producer C (2020), concentrations of 4-MSP for the control and all treatments ranged between 0.2-1 ng $L^{-1}$ (Fig. 4.5). However, in 2021, the highest concentrations were detected in the T4 wines made from WG (-4 ºC) (6-7 ng $L^{-1}$), followed by the T0 wines (-4 ºC and -20 ºC) (3-4 ng $L^{-1}$), and the control (2-3 ng $L^{-1}$). For most of the T4 wines (-4 ºC and -20 ºC), the concentrations were lower and ranged between 1-3 ng $L^{-1}$ (Fig. 4.5). Furthermore, for Producer C, the concentrations of 4-MSP fell above the aroma perception threshold (0.8 ng $L^{-1}$) and within the typically reported range (0-21.9 ng $L^{-1}$) for SA Sauvignon blanc wine (Table 4.2). The highest concentrations of 4-MSP detected in the Chenin blanc wines ranged between 0.4-0.6 ng $L^{-1}$ for the unfrozen control and T0 WG (-4 ºC and -20 ºC) (Fig. 4.6). The same concentrations (0.4-0.6 ng $L^{-1}$) were also detected in the T0 wines made from CJ (-4 ºC), and the T4 wines made from CJ (-4 ºC and -20 ºC), with all remaining treatments having concentrations ranging from 0.2 to 0.4 ng $L^{-1}$ (Fig. 4.6). Although the concentrations of 4-MSP fell below the aroma perception threshold (0.8 ng $L^{-1}$), it was detected in concentrations above the typically reported range (n.d.) for SA Chenin blanc and within the globally reported range (0-23 ng $L^{-1}$) (Table 4.2). Moreover, these results confirmed the presence of the varietal thiol, 4-MSP in SA Chenin blanc, which was not detected until recently with the optimisation of analytical methods (du Plessis & Augustyn, 1981:102; Piano et al., 2015:46; Coetzee et al., 2018:182; Mafata et al., 2018:8-9; Panzeri et al., 2020:140; 142). The concentrations of 4-MSP were not consistent across treatments and further research is recommended to confirm these findings to better understand the impact of cryogenic freezing on varietal thiol extraction in Chenin blanc.

##### *4.3.1.1.3 3-SH*

The highest concentrations of 3-SH for Producer A (2020) were detected in the T4 wines made from WG (-4 ºC) (1300-1400 ng $L^{-1}$) followed by the T0 WG (-20 ºC) (800-900 ng $L^{-1}$) (Fig. 4.2). The T4 MG (-4 ºC and -20 ºC) and T0 MG (-20 ºC) had concentrations ranging between 700-800 ng $L^{-1}$ and the remaining treatments ranging between 100-600 ng $L^{-1}$ (Fig. 4.2). The concentrations of 3-SH in the control wines ranged between 400 and 500 ng $L^{-1}$ (Fig. 4.2). In 2021, the highest concentrations of 3-SH were detected in the TM and CJ (-4 ºC and -20 ºC), which ranged between 200 to 400 ng $L^{-1}$, whilst the concentration detected in the control was 200 ng $L^{-1}$ (Fig. 4.2).

For Producer B (2020), the highest concentrations of 3-SH detected in the hand-harvested T4 wines were made from WG (-20 ºC) (1100-1200 ng $L^{-1}$), followed by the unfrozen control (900 ng $L^{-1}$) and the WG (-4 ºC) (200-300 ng $L^{-1}$) (Fig. 4.3). Concentrations of 3-SH detected in wines made from machine-harvested grapes were considerably higher than those of the hand-harvested grapes with values above 4000 ng $L^{-1}$ by far exceeding the aroma perception threshold (60 ng $L^{-1}$) and typically reported range (178-904 ng $L^{-1}$) for SA Sauvignon blanc (Table 4.2 and Figs. 4.3-4.4). The wines with the highest concentrations were made from the MG (-20 ºC) ranging between 4000-4500 ng $L^{-1}$, followed by the MG (-4 ºC) (2500-3000 ng $L^{-1}$) (Fig. 4.4). Furthermore, high concentrations were also seen in wines made from the CJ (-20 ºC) (1500-2000 ng $L^{-1}$), TM (-4 and -20 ºC), CJ (-4 ºC) (1000-1500 ng $L^{-1}$) whilst the control wines had the lowest concentration (500 ng $L^{-1}$) (Fig. 4.4). In 2021, the concentrations of 3-SH detected in the wines were much lower for all treatments (Fig. 4.3). The treatment with the highest concentration was the wine made from the TM (-4 ºC) which ranged between 500-600 ng $L^{-1}$. The remainder of the treatments ranged from < 100 to 300 ng $L^{-1}$, while the concentration in the control wine was 200 ng $L^{-1}$ (Fig. 4.3). Although the concentrations were considerably lower, for most wines it fell above the aroma perception threshold (60 ng $L^{-1}$) and within the typically reported range (178-904 ng $L^{-1}$) for SA Sauvignon blanc (Table 4.2).

For Producer C (2020), the highest concentrations of 3-SH were detected in wines made from WG (-4 ºC) (900-1000 ng $L^{-1}$), followed by the T4 MG (-20 ºC) (700-800 ng $L^{-1}$) and CJ (-20 ºC) (600-700 ng $L^{-1}$) whilst the CJ (-4 ºC) and unfrozen control had the same range (500-600 ng $L^{-1}$) (Fig. 4.5). The concentrations in the remaining treatments ranged between 100 to 400 ng $L^{-1}$. In 2021, the highest concentrations of 3-SH were detected in the T0 wines made from TM (-20 ºC) (900-1000 ng $L^{-1}$) followed by the T0 wines made from MG (-20 ºC) and TM (-4 ºC) (600-700 ng $L^{-1}$) as well as the MG (-4 ºC) and CJ (-4 ºC) (500-600 ng $L^{-1}$) (Fig. 4.5). The concentration in the control wines ranged between 400-500 ng $L^{-1}$, with the remaining treatments ranging from < 100 to 400 ng $L^{-1}$ (Fig. 4.5). Moreover, for Producer C, the concentrations in all wines fell above the aroma perception threshold (60 ng $L^{-1}$) and within the typically reported range (178-904 ng $L^{-1}$) for SA Sauvignon blanc (Table 4.2). It was further seen that for the wines made from the Chenin blanc grapes subjected to pre-fermentative cryogenic freezing, the highest concentrations were detected in the T0 wines made from MG (-20 ºC) (250-300 ng $L^{-1}$) (Fig. 4.6).

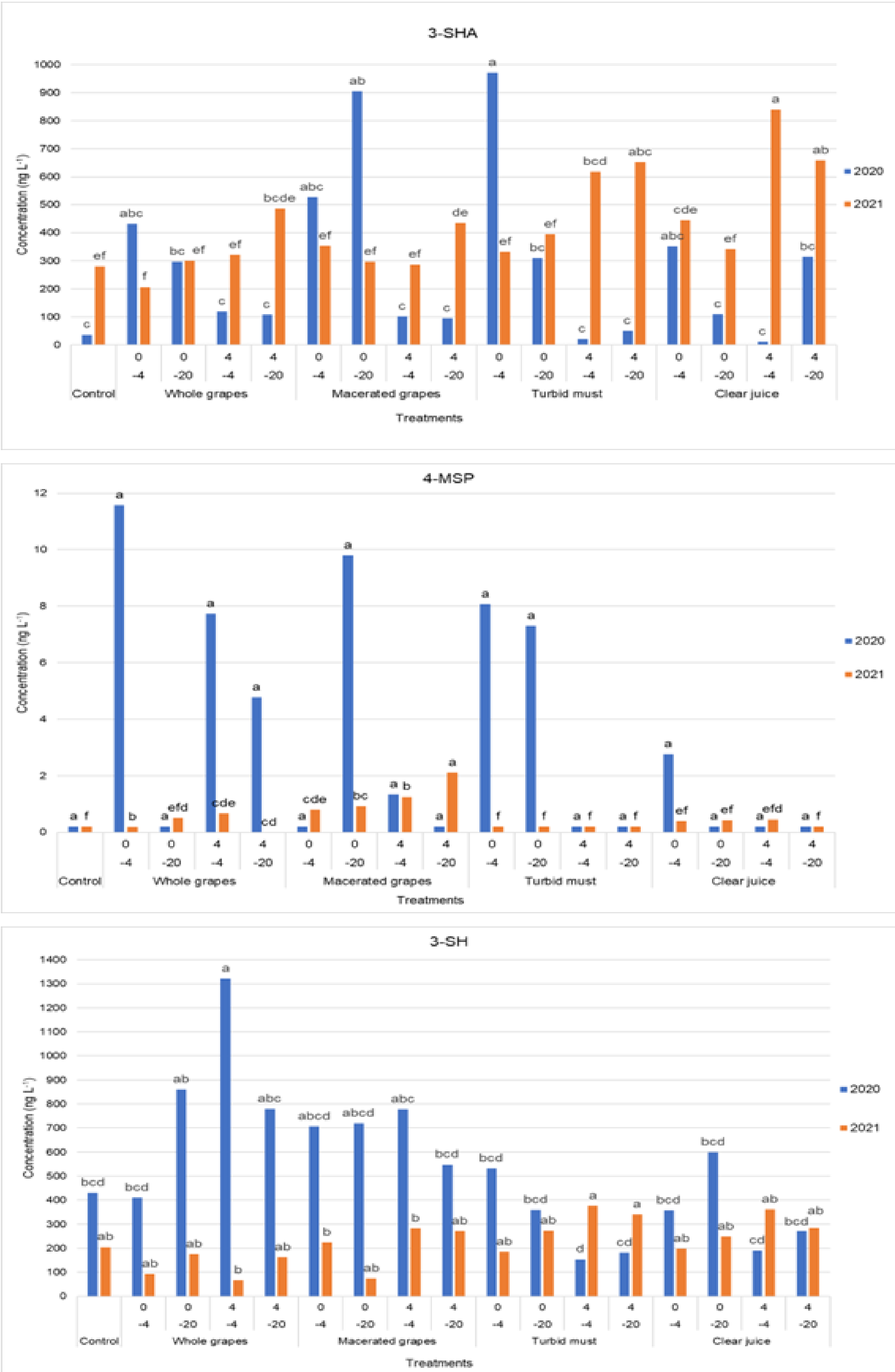



Figure 4.2. Concentrations (ng $L^{-1}$) of the varietal thiols 3-SHA, 4-MSP and 3-SH detected in Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ from Producer A (Stellenbosch wine region) in 2020 and 2021.

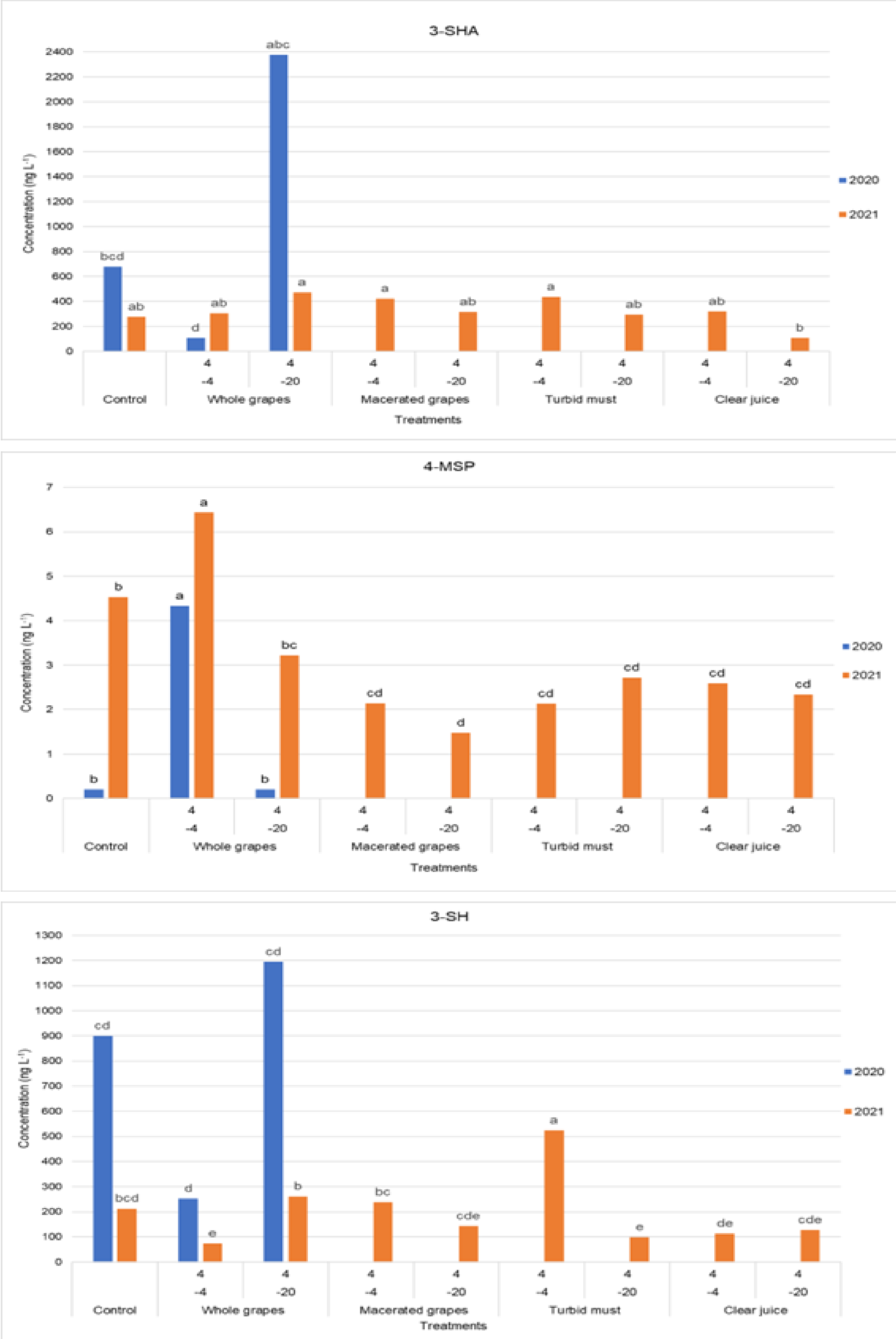



Figure 4.3. Concentrations (ng L$^{-1}$) of the varietal thiols 3-SHA, 4-MSP and 3-SH detected in Sauvignon blanc wines produced from hand-harvested cryogenically pre-treated WG, MG, TM and CJ from Producer B (Durbanville wine region) in 2020 and 2021.

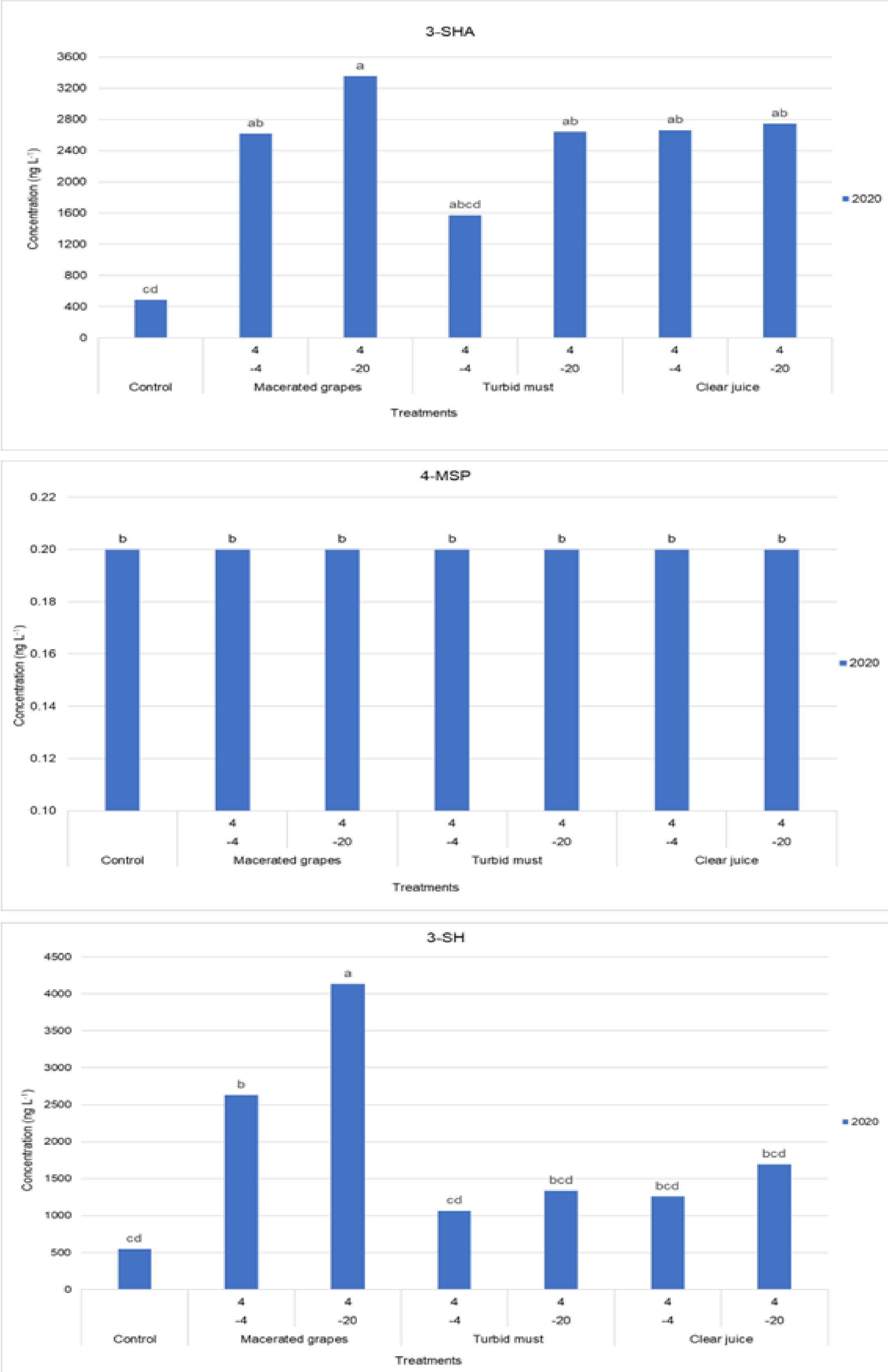



Figure 4.4. Concentrations (ng $L^{-1}$) of the varietal thiols 3-SHA, 4-MSP and 3-SH detected in Sauvignon blanc wines produced from cryogenically pre-treated machine-harvested MG, TM and CJ from Producer B (Durbanville wine region) in 2020.


Figure 4.5. Concentrations (ng $L^{-1}$) of the varietal thiols 3-SHA, 4-MSP and 3-SH detected in Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ from Producer C (Cape South Coast wine region) in 2020 and 2021.


Figure 4.6. Concentrations (ng $L^{-1}$) of the varietal thiols 3-SHA, 4-MSP and 3-SH detected in Chenin blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ from Producer D (Stellenbosch wine region) in 2021.

The T0 wines made from WG (-20 ºC), T0 and T4 MG (-4 ºC), T4 TM (-20 ºC) and the control wines had concentrations that ranged between 100-150 ng $L^{-1}$ with the remaining treatments all having concentrations < 50 ng $L^{-1}$ (Fig. 4.6). For the Chenin blanc wines, the concentrations detected were less than and above the aroma perception threshold (60 ng $L^{-1}$) and typically reported range (99-1124 ng $L^{-1}$) in SA Chenin blanc.

As mentioned, there was no definite trend as to what cryogenic temperature, stage of production and storage time would consistently yield the most favourable levels of varietal thiols in the wines produced, as the concentrations varied between these treatments. These observations were similar to the findings of Jouanneau et al. (2012:335), who conducted a subregional survey of aroma compounds in Marlborough Sauvignon blanc wines and found a large variation in the concentrations of the varietal thiol compounds (3-SHA, 4-MSP and 3-SH) between wines and no visible subregional trend across vintages. However, in the current study, there are indications that the region from which the grapes originated, the harvesting technique, and the vintage played a substantial role in the concentrations of varietal thiols.

Furthermore, the concentrations of 3-SHA detected in most of the wines produced in this study exceeded the aroma perception threshold (4 ng $L^{-1}$) and the reported range (23-151 ng $L^{-1}$) for SA Sauvignon blanc wines (Table 4.2). The concentrations of 3-SH were generally within the aroma perception threshold (60 ng $L^{-1}$) and typically reported range (178-904 ng $L^{-1}$). Moreover, the levels of 4-MSP in wines for all producers fell within the typical reported range (0-21.9 ng $L^{-1}$) for SA Sauvignon blanc wines. However, the aroma perception threshold fluctuated below or above the reported aroma perception threshold (0.8 ng $L^{-1}$) across vintages and regions (Table 4.2). The levels of 3-SHA in the Chenin blanc wines produced in this study were higher than the reported range (5-253 ng $L^{-1}$) for SA Chenin blanc wines and above the aroma perception threshold (4 ng $L^{-1}$) (Table 4.2). The concentrations of 3-SH were generally below the reported range (99-1124 ng $L^{-1}$) and aroma perception threshold (60 ng $L^{-1}$). It was further observed that 4-MSP concentrations were above the reported range (not detected) for SA Chenin blanc wines but below the aroma perception threshold (0.8 ng L-1) (Table 4.2) (Coetzee et al., 2018:180-183; Wilson, 2017:27-38; Wilson et al., 2019:635-640).

#### 4.3.1.2 Methoxypyrazines

Methoxypyrazine (sbMP and ibMP) concentrations were not affected by the cryogenic temperature, stage of production and storage time, however, the vintage appeared to have an impact. IbMP levels in wine typically range from 2 to 30 ng $L^{-1}$, whilst sbMP concentrations in wine are typically < 10 ng $L^{-1}$, with their respective aroma perception threshold ranging between 2-16 ng $L^{-1}$ (Table 4.1) (Sidhu et al., 2015:494-499; Lei et al., 2018:1142). In this study, the methoxypyrazines mentioned above were found to be much higher in 2021 when compared to 2020, as graphically illustrated (Figs. 4.7-4.10). These findings validate previous research where authors found that methoxypyrazines in grapes and wines are primarily influenced by viticultural conditions, such as the temperature during ripening, berry maturation and the fruit exposure to sunlight and a lesser extent by vinification practices such as maceration (Allen & Lacey, 1993:36-37; Allen & Lacey, 1998:31-36; Marais, 1994:44; Ryona et al., 2008:10844-10845; Sidhu et al., 2015:494-499).

In 2020 and 2021, the reported weather conditions differed significantly in the Western Cape (Vinpro, 2020:3; 2021:6-7). In 2020, the weather conditions were generally characterised as favourable, warm, and dry, resulting in the formation of smaller, more concentrated berries, increasing flavour retention in the grapes, whilst in 2021, weather conditions were notably cooler and more moderate (Vinpro, 2020:3; 2021:6-7). It was further reported that due to rainfall throughout the ripening period, a surge in disease (downy mildew, sour rot and botrytis) was experienced, which is known to affect the berry composition (Roland et al., 2011:7370; Steel et al., 2013:5193; Magyar & Soos, 2016:29-37; Avizcuri-Inac et al., 2018:15-28; Vinpro, 2020:3; Dankó et al., 2021:1-5; Santos et al., 2022:1-12). This study is the first to explore the effect of cryogenic pre-fermentative treatments on methoxypyrazine compounds in Sauvignon blanc wines.

*4.3.1.2.1 sbMP*

In 2020, it was seen that the concentrations of sbMP in wines made from the control grapes and cryogenically pre-treated WG, MG, TM and CJ from Producer A ranged between 0-5 ng $L^{-1}$ for all treatments (Fig. 4.7). In 2021, the highest concentrations of sbMP were detected in the T0 and T4 wines made from WG (-4 ºC and -20 ºC) (25-45 ng $L^{-1}$), with the T4 WG (-4 ºC) being the highest (40-45 ng $L^{-1}$) followed by the WG (-20 ºC) (35 ng $L^{-1}$), T0 and T4 wines made from MG (-4 ºC and -20 ºC) (20-35 ng $L^{-1}$) with the T0 MG (-20 ºC) having the highest concentration (30-35 ng $L^{-1}$) (Fig. 4.7). Furthermore, the concentrations of sbMP in the T0 and T4 wines made from CJ (-4 ºC and -20 ºC) ranged from < 10 to 15 ng $L^{-1}$ with the T0 wines (-4 ºC) having the highest range between 10-15 ng $L^{-1}$. The T0 and T4 wines made from TM (-4 ºC and -20 ºC) had concentrations ranging from < 5 to 10 ng $L^{-1}$, with the concentrations in the T0 wines being higher than those in the T4 wines (Fig. 4.7). The concentrations of sbMP in the control wines were similar to the wines made from CJ (10-15 ng $L^{-1}$) and fell within the aroma perception threshold (2-16 ng $L^{-1}$) and range (< 10 ng $L^{-1}$) typically reported in wine (Table 4.1). However, in 2021, the concentrations of sbMP detected in the control wine and wines made from the cryogenic treatments were higher (5-45 ng $L^{-1}$) for most treatments (Fig. 4.7) and fell above the aroma perception threshold (2-16 ng $L^{-1}$) and typically reported range (< 10 ng $L^{-1}$) in wine (Table 4.1).

For Producer B (2020), hand-harvested and machine-harvested grapes were subjected to cryogenic pre-fermentative freezing over T4 (Figs. 4.8-4.9). In 2020, no sbMP was detected in wines made from the hand-harvested control and cryogenically pre-treated WG, MG, TM and CJ (Fig. 4.8). However, in 2021, the concentrations of sbMP detected in wines made from the above-mentioned treatments fell within and above the aroma perception threshold (2-16ng $L^{-1}$) and typically reported range (< 10 ng $L^{-1}$) found in wine (Table 4.1 and Fig. 4.8). The highest concentrations of were detected in the wines made from CJ (-20 ºC) (20 ng $L^{-1}$), control wines and wines made from CJ (-4 ºC) (16-18 ng $L^{-1}$). This was followed by the wines made from the WG (-4 ºC and -20 ºC) and TM (-4 ºC), with concentrations ranging from 14-16 ng $L^{-1}$ (Fig. 4.8). The wines made from MG and TM (-20 ºC) had concentrations ranging from 12-14 ng $L^{-1}$ whilst the wine made from MG (-4 ºC) had the lowest concentrations (10-12 ng $L^{-1}$) (Fig. 4.8). For the machine-harvested grapes, sbMP was only detected in wines made from the control MG (0.6 ng $L^{-1}$) (Fig. 4.9), which fell below the aroma perception threshold typically found in wine (2-16ng $L^{-1}$) and within the typically reported range (< 10 ng $L^{-1}$) in wine (Table 4.1).

Furthermore, the concentrations of sbMP detected in wines from Producer C (2020) ranged between 0-2 ng $L^{-1}$ for most wines, except for the T4 wines made from TM (-20 ºC) which ranged between 8-10 ng $L^{-1}$ (Fig. 4.10). In 2021, the highest concentrations of sbMP were detected in the T0 wines made from CJ (-4 ºC) (12 ng $L^{-1}$), the T4 wines made from CJ (-20 ºC) and T0 wines made from WG (-4 ºC), ranging between 10-12 ng $L^{-1}$ (Fig. 4.10). This was followed by wines made from the control, T0 wines made from WG (-20 ºC), MG (-4 ºC), and TM (-4 ºC) with sbMP concentrations ranging between 6-8 ng $L^{-1}$. Furthermore, wine made from the T0 CJ (-20 ºC) had sbMP concentrations of 6 ng $L^{-1}$, followed by T4 MG (-4 ºC) (4 ng $L^{-1}$), whilst the remaining wines had concentrations < 2 ng $L^{-1}$ (Fig. 4.10). For Producer C, the concentrations of sbMP for most wines were above the aroma perception threshold (2-16 ng $L^{-1}$) and within the typically reported range (< 10 ng $L^{-1}$) in wine (Table 4.1).

##### *4.3.1.2.2 ibMP*

In 2020, for Producer A, the concentrations of ibMP in wines made from the control grapes and cryogenically pre-treated WG, MG, TM and CJ ranged between 0-10 ng $L^{-1}$ except the T0 and T4 wines made from WG (-20 ºC) and the T0 MG (-4 ºC and -20 ºC) (10-20 ng $L^{-1}$) (Fig. 4.7). In 2021, the highest concentrations of ibMP were detected in the T4 wines made from TM (-20 ºC) (100-110 ng $L^{-1}$), WG (-4 ºC and -20 ºC) and MG

(-20 ºC) (90-100 ng $L^{-1}$), followed by the T0 wines made from WG, MG and TM (-20 ºC) (80-90 ng $L^{-1}$) and the T4 wines made from TM (-4 ºC) (80-90 ng $L^{-1}$). The ibMP levels for the remaining treatments ranged between 60-80 ng $L^{-1}$ (Fig. 4.7). Furthermore, the concentrations of ibMP for most wines were above the aroma perception threshold (2-16 ng $L^{-1}$) and typically reported range (2-30 ng $L^{-1}$) in wine (Table 4.1)

For Producer B (2020), IbMP was only detected in wines made from the hand-harvested control grapes (4-6 ng $L^{-1}$) followed by the T4 wines made from WG (-4 ºC) (6-8 ng $L^{-1}$) and WG (-20 ºC) (8-10 ng $L^{-1}$) (Fig. 4.8). No ibMP was detected in wines from the remaining cryogenically pre-treated MG, TM and CJ (Fig. 4.8). In 2021, the highest concentrations of ibMP were detected in wines made from the WG (-4 ºC) (12-14 ng $L^{-1}$), MG (-20 ºC) (8-10 ng $L^{-1}$) and WG (-20 ºC) (8 ng $L^{-1}$). This was followed by wines made from MG (-4 ºC) (6-8 ng $L^{-1}$), TM (-4 ºC) (6 ng $L^{-1}$), TM (-20 ºC) (4-6 ng $L^{-1}$) and CJ (-4 ºC and -20 ºC) (4-6 ng $L^{-1}$). The lowest levels of ibMP were detected in the control wines and ranged between 2-4 ng $L^{-1}$ (Fig. 4.8). For the machine-harvested grapes, the highest concentrations of ibMP were detected in the T4 wines made from MG (-4 ºC and -20 ºC) (12-14 ng $L^{-1}$) and the control (12 ng $L^{-1}$) (Fig 4.9). The remainder of the treatments had concentrations ranging between 8-10 ng $L^{-1}$ (Fig. 4.9). All ibMP concentrations detected in wines made from the hand-harvested and machine-harvested grapes were within the aroma perception threshold (2-16 ng $L^{-1}$) and range (2-30 ng $L^{-1}$) typically reported for wine (Table 4.1) (Sidhu et al., 2015:494-499; Lei et al., 2018:1142). For Producer C, the concentrations of ibMP in 2020 were highest in the T4 wines made from CJ (-20 ºC) (20-25 ng $L^{-1}$) and the T0 wines made from WG (-20 ºC) (10-15 ng $L^{-1}$) and MG (-4 ºC) (10-15 ng $L^{-1}$). This was followed by wines made from the T4 WG (-20 ºC), T0 MG (-20 ºC) (5-10 ng $L^{-1}$) and the remaining treatments ranging between 0-5 ng $L^{-1}$ (Fig. 4.10).

Overall, during 2020, the concentrations of sbMP and ibMP in this study were found to be lower or within the normal reported ranges in wines from the Cape South Coast and Durbanville wine regions. However, methoxypyrazine levels in wines made from the Stellenbosch region were considerably higher in 2021 when compared 2020. Moreover, the general trend observed was that in 2021, the methoxypyrazine levels were higher in wines made from grapes originating from all the wine regions and higher than the typical aroma perception threshold levels in wines (2-16 ng $L^{-1}$) as well as higher than the typical range found in wines, ibMP (2-30 ng L-1) and sbMP (< 10 ng $L^{-1}$) when compared to 2020.

The higher methoxypyrazine concentrations observed in 2021 for all Producers, across regions, compared to 2020, can therefore be attributed to the cooler climatic conditions of the 2021 growing season, which is consistent with previous research (Vinpro, 2020:3; 2021:6-7).


Figure 4.7. Concentrations (ng $L^{-1}$) of the methoxypyrazines, sbMP and ibMP detected in Sauvignon blanc wines produced from control grapes and cryogenically pre-treated WG, MG, TM and CJ from Producer A (Stellenbosch wine region) in 2020 and 2021.

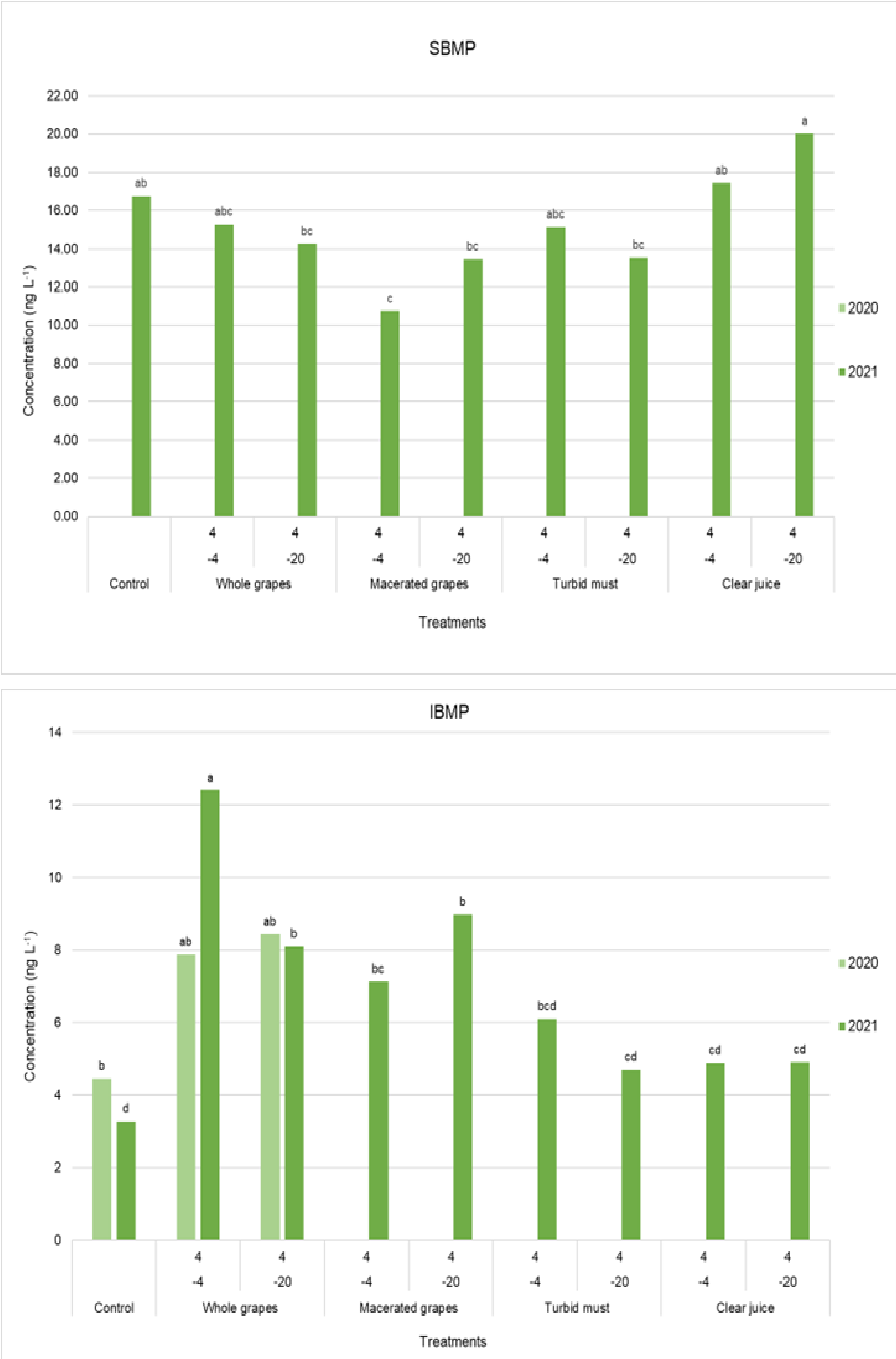
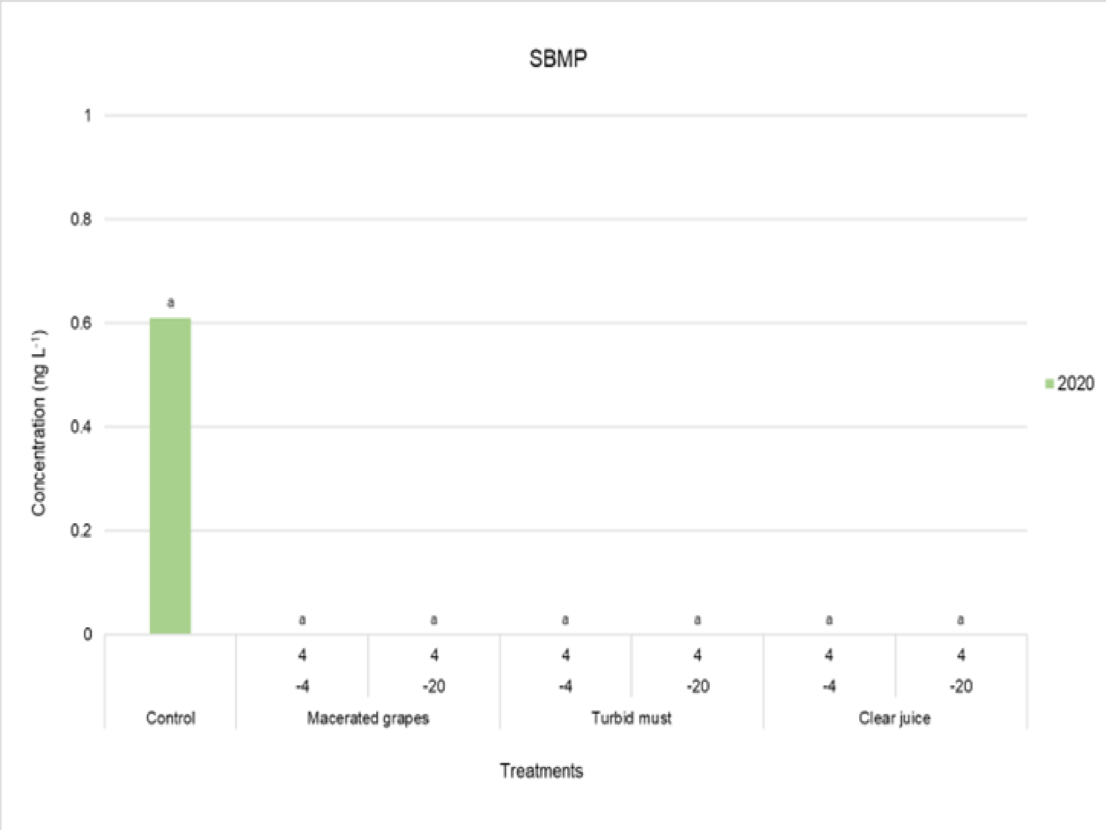



Figure 4.8. Concentrations (ng $L^{-1}$) of the methoxypyrazines sbMP and ibMP detected in Sauvignon blanc wines produced from control grapes and hand-harvested cryogenically pre-treated WG, MG, TM and CJ from Producer B (Durbanville wine region) in 2020 and 2021.



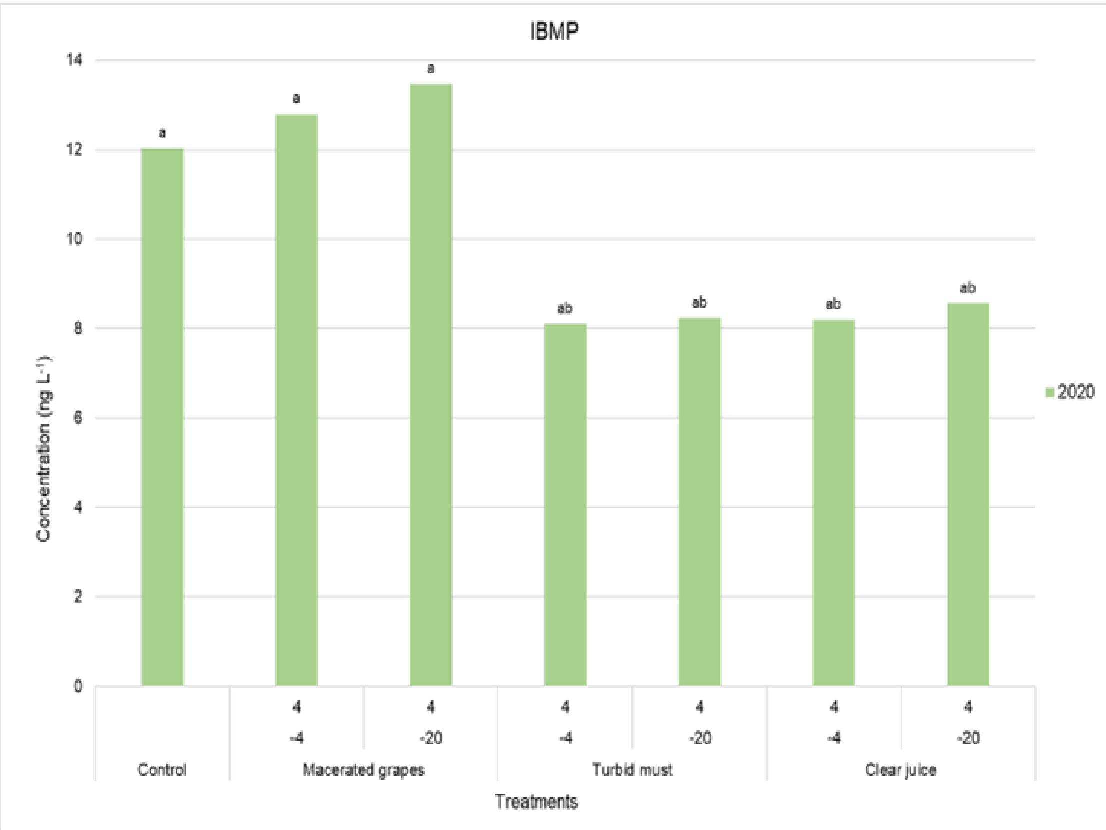
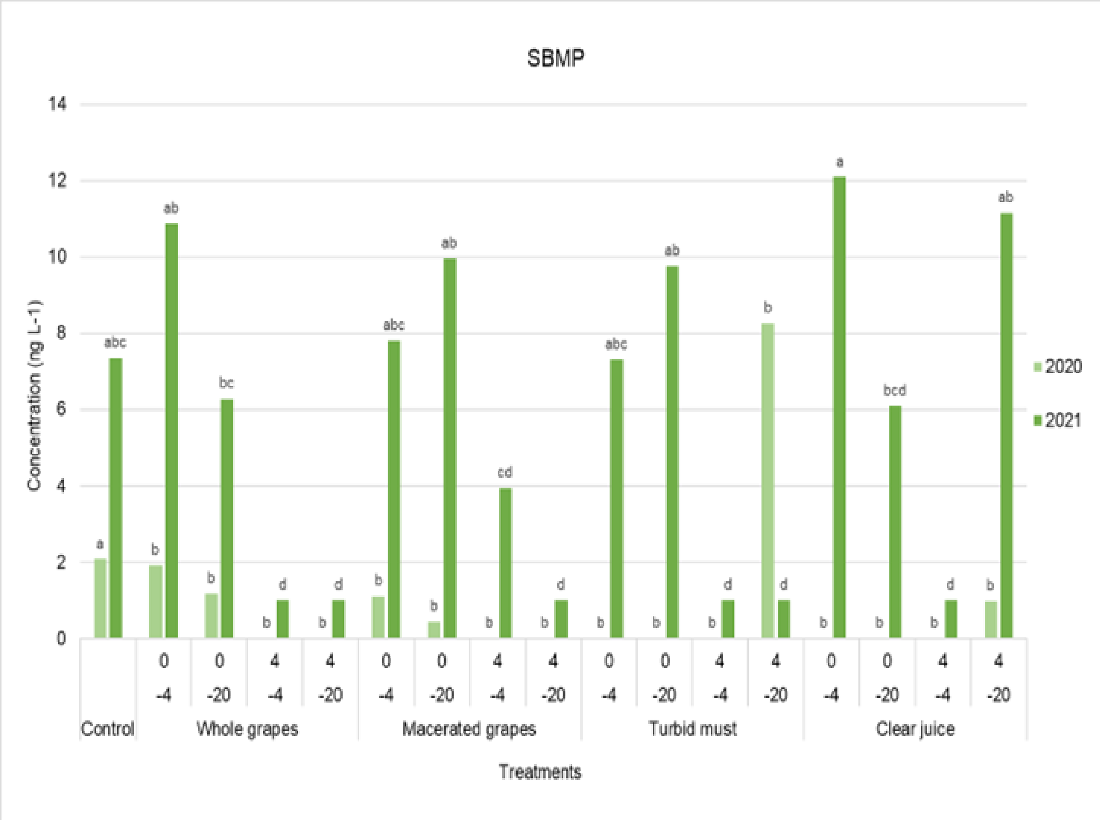




Figure 4.9. Concentrations (ng $L^{-1}$) of the methoxypyrazines sbMP and ibMP detected in Sauvignon blanc wines produced from machine-harvested cryogenically pre-treated MG, TM and CJ from Producer B (Durbanville wine region) in 2020.



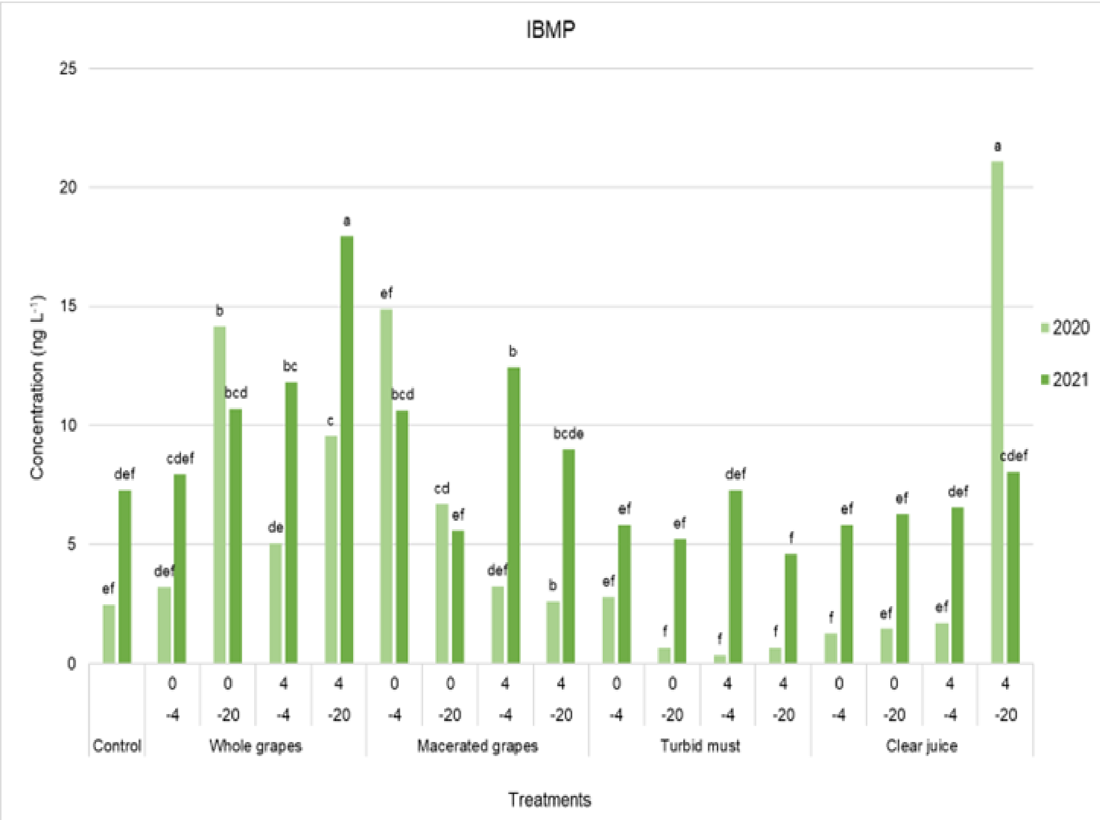




Figure 4.10. Concentrations (ng L$^{-1}$) of the methoxypyrazines sbMP and ibMP detected in Sauvignon blanc wines produced from control grapes and cryogenically pre-treated WG, MG, TM and CJ from Producer C (Cape South Coast wine region) in 2020 and 2021.

Furthermore, no definite trend as to what cryogenic temperature, stage of production and storage time would yield the most favourable concentrations of methoxypyrazines in the wines because the concentrations varied between these treatments. However, in this study, there are indications that the region from which the grapes originated, the harvesting technique and the vintage played a definitive role in the concentrations of methoxypyrazines and confirm the findings of previous research (Allen & Lacey, 1993:36-37; Allen & Lacey, 1998:31-36; Marais, 1994:44; Ryona et al., 2008:10844-10845). Overall, the observations that were seen for the varietal thiol compounds were also seen for the methoxypyrazines.

### 4.3.2 Sensory data

Target volatile compounds, i.e., varietal thiols and methoxypyrazines were selected based on their contribution to the sensory properties of Sauvignon blanc wines. Chenin blanc, which shares a genetic origin with Sauvignon blanc, both originating from Savagnin blanc or Traminer, was also shown to possess varietal thiols. Sensory analyses were performed on all wines through descriptive sensory analysis, based on descriptors identified during screening and training sessions. Descriptors identified for the Sauvignon blanc wines were divided into three main groups, i.e., "Thiol type aromas", "Citrus aromas" and "Herbaceous aromas" and for the Chenin blanc wines were "Tree fruit" (e.g. Peach, Pear, Apple), "Thiol type aroma" (e.g. Guava, Passionfruit, Melon), "Citrus" (e.g. grapefruit, lemon), "Sweet Correlated" (e.g. candy, toffee), "Stewed fruit" and "Vegetative" (e.g. Fresh, Dried, Cooked) (Tables 4.4-4.10).

The thiol-type aromas correlated with attributes such as "Melon", "Guava" and "Passion fruit". The citrus aromas were correlated with attributes such as "Grapefruit", "Lemon", and "Lime", while the herbaceous aromas were correlated with attributes such as "Fresh" and "Vegetative". These attributes are believed to be associated with aromatic compounds such as the sulfur-containing compounds like 3-SH, 3-SHA, and 4-SMP ("Grapefruit", "Guava", and "Passion fruit") and methoxypyrazines ("Fresh green") and are often used to profile young, fresh, and fruity Sauvignon blanc wines. As mentioned, varietal thiols, 3-SH and 3-SHA, are present in wine as a mixture of *R* and *S* enantiomers, from which racemic mixtures have very low sensory thresholds of 60 ng $L^{-1}$ and 4 ng $L^{-1}$, respectively. The aroma of these varietal thiols depends on their form, i.e., 3-SH in the *R* form has a grapefruit aroma and the *S* form has a passion fruit aroma, while 3-SHA in the *R* form is less aromatic and smells like passion fruit and the *S* form has an herbaceous aroma similar to boxwood (Tominaga et al., 2006:7255; Aruajo et al., 2007:10).

Furthermore, sensory attributes such as "Vegetative fresh" (Herbaceous, Green cut grass, Bell pepper), "Vegetative cooked" (Green beans, Asparagus, Green olive,

Table 4.4: ANOVA of Sensory evaluation data resulting from Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer A from the Stellenbosch wine region) in 2020.

| Treatment | Temp[2] (ºC) | Storage Time (Months) | Sensory Descriptors[1] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Overall intensity | Vegetative fresh | Vegetative cooked | Vegetative dried | 3-SH[4] aromas | 3-SHA[5] aromas | 4-MSP[6] aromas | Acidity | Other | Body | General quality |
| Control | na[3] | na | 56.67cde | 26.67bcdef | 15.19abc | 14.44abc | 11.23ef | 26.48fgh | 28.60ab | 56.90abc | 22.13ab | 50.99def | 42.87g |
| WG[7] | -4 | 0 | 61.62bcd | 32.78abcd | 16.85ab | 16.11ab | 20.39ab | 38.52cde | 17.66cd | 55.14bcd | 7.76cd | 55.86abc | 57.40bcde |
| | -20 | 0 | 55.18de | 34.63abc | 16.16ab | 13.06abcd | 18.70bc | 35.93def | 18.70bcd | 54.07bcde | 7.25cd | 54.63abcd | 56.11bcde |
| | -4 | 4 | 67.36ab | 21.94ef | 12.72bcdef | 14.12abc | 12.51ef | 50.78ab | 9.60d | 50.55ef | 12.30cd | 54.30abcd | 62.35abc |
| | -20 | 4 | 59.43bcde | 26.88bcdef | 6.91def | 18.22a | 8.23fg | 34.67defg | 15.67cd | 50.29ef | 7.64cd | 52.48bcde | 59.85abcd |
| MG | -4 | 0 | 67.22ab | 31.32abcde | 22.57a | 10.86bcd | 20.39ab | 48.61abc | 31.20a | 52.04def | 14.37bc | 56.48abc | 59.55abcd |
| | -20 | 0 | 71.67a | 38.15a | 9.93bcdef | 13.06abcd | 25.18a | 52.41a | 22.40abc | 53.88bcde | 14.79abc | 58.15a | 67.77a |
| | -4 | 4 | 65.67abc | 22.04ef | 7.53cdef | 13.58abc | 12.76ef | 46.33abcd | 15.75cd | 49.97ef | 9.78cd | 56.69ab | 63.78ab |
| | -20 | 4 | 60.24bcde | 25.82cdef | 6.59ef | 16.01ab | 12.39ef | 38.42cde | 12.87cd | 49.24f | 10.24cd | 57.17a | 57.88bcd |
| TM | -4 | 0 | 61.48bcd | 35.92ab | 16.11ab | 13.15abcd | 20.85ab | 43.89abcd | 15.31cd | 56.92abc | 12.34cd | 56.64abc | 57.59bcde |
| | -20 | 0 | 51.09e | 32.73abcd | 14.07bcde | 14.63abc | 12.78ef | 32.04efgh | 16.85cd | 60.40a | 9.26cd | 44.79gh | 45.58fg |
| | -4 | 4 | 51.30e | 20.99f | 7.87cdef | 9.61cd | 14.00cde | 23.30gh | 9.34d | 53.05cdef | 21.46ab | 42.90h | 43.92fg |
| | -20 | 4 | 56.94cde | 23.76def | 6.01f | 11.46bcd | 13.70de | 38.12cdef | 12.45cd | 51.32def | 13.18cd | 46.69fgh | 51.48def |
| CJ | -4 | 0 | 60.67bcd | 32.41abcd | 15.93ab | 13.81abc | 18.52bcd | 35.83def | 21.48abc | 57.53ab | 5.92d | 54.35abcd | 56.85bcde |
| | -20 | 0 | 55.00de | 30.93abcde | 14.63bcd | 17.59a | 19.63b | 39.82bcde | 21.76abc | 55.56bcd | 10.74cd | 56.48abc | 60.55abc |
| | -4 | 4 | 55.64de | 23.91def | 7.88cdef | 11.03bcd | 12.23ef | 36.39def | 13.24cd | 49.17f | 9.88cd | 52.12cde | 54.27cde |
| | -20 | 4 | 57.27cde | 22.51ef | 8.97bcdef | 10.30cd | 12.94ef | 37.88cdef | 9.24d | 50.21ef | 11.33cd | 53.76abcd | 57.21bcde |

[1]Means (n=3) for all samples.
[2]Cryogenic pre-treatment temperature
[3]Not applicable - na
[4]3-sulfanylhexan-1-ol (3-SH) aromas, i.e., lime, lemon, sweet grapefruit.
[5]3-sulfanylhexyl acetate (3-SHA) aromas, i.e., pineapple, passionfruit, sweet melon, banana, guava, gooseberry, kiwi.
[6]4-methyl-4-sulfanylpentan-2-one (4-MSP) aromas, i.e., Box tree (cat urine), broom, blackcurrant, and tomato leaf aromas.
[7]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

Table 4.5: ANOVA of Sensory evaluation data resulting from Sauvignon blanc wines produced from cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer A from the Stellenbosch wine region) in 2021.

| Treatment | Temp[2] (ºC) | Storage Time (Months) | Sensory Descriptors[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Overall intensity | Thiol type[4] aroma | Citrus aroma | Herbaceous aroma | Banana aroma | Pineapple aroma | Apple aroma | Reductive | $SO_2$ aroma | Acidity | Body | General quality |
| **Control** | na[3] | na | 65.15abc | 40.23b | 34.86ab | 32.60a | 18.23bc | 21.52a | 15.44b | 18.68a | 0.21a | 57.44a | 48.40ab | 47.35b |
| **WG[5]** | -4 | 0 | 66.32abc | 45.69ab | 27.42b | 33.12a | 29.65abc | 21.07a | 21.88ab | 16.88a | 0.72a | 50.02b | 48.39ab | 49.86ab |
| | -20 | 0 | 67.45abc | 53.56a | 33.27ab | 29.82a | 34.66a | 26.29a | 19.35ab | 8.38a | 0.63a | 53.28ab | 49.75ab | 55.88a |
| | -4 | 4 | 67.51abc | 47.36abc | 30.22ab | 40.95a | 27.44abcde | 25.08ab | 22.67abc | 6.70bcd | 0.52ab | 51.74bcde | 53.35abc | 56.79b |
| | -20 | 4 | 64.92abc | 46.99abc | 30.09ab | 29.45abcd | 32.37abcd | 30.94ab | 19.56abc | 5.70cd | 0.56ab | 51.64cde | 50.98abcd | 54.17bc |
| **MG** | -4 | 0 | 63.38c | 43.51ab | 31.88ab | 33.38a | 21.37abc | 20.78a | 22.18ab | 7.03a | 0.37a | 53.56ab | 48.75ab | 50.92ab |
| | -20 | 0 | 68.17ab | 54.43a | 34.53ab | 28.23a | 31.69ab | 27.87a | 25.50a | 7.43a | 0.67a | 54.04ab | 53.07a | 55.78a |
| | -4 | 4 | 68.85a | 55.14a | 32.41ab | 24.38cd | 42.08a | 35.00a | 24.24ab | 3.41d | 0.54ab | 52.23abcde | 55.51ab | 60.21a |
| | -20 | 4 | 65.73abc | 53.51ab | 32.75ab | 21.07d | 35.88ab | 29.61ab | 22.11abc | 5.39cd | 0.35b | 57.29bc | 55.65a | 57.17b |
| **TM** | -4 | 0 | 65.19abc | 45.92ab | 33.16ab | 30.90a | 19.75abc | 25.75a | 18.00ab | 16.67a | 0.16a | 55.05ab | 44.08b | 47.39b |
| | -20 | 0 | 64.79abc | 43.90ab | 29.87ab | 33.17a | 15.40c | 22.68a | 16.47b | 12.27a | 0.75a | 51.03ab | 44.64b | 45.82b |
| | -4 | 4 | 65.12abc | 42.81bc | 33.17ab | 35.69abc | 15.55e | 22.17b | 20.33abc | 27.47a | 0.48ab | 57.53a | 44.72d | 45.30d |
| | -20 | 4 | 67.98abc | 47.85abc | 35.42ab | 30.56abcd | 30.67abcd | 28.26ab | 19.65abc | 6.25cd | 0.56ab | 54.17abcde | 50.79abcd | 54.13abc |
| **CJ** | -4 | 0 | 66.50abc | 44.15ab | 37.81a | 36.28a | 23.16abc | 27.97a | 18.61ab | 6.03a | 0.74a | 55.82ab | 48.45ab | 52.97ab |
| | -20 | 0 | 63.81bc | 43.99ab | 27.86ab | 27.21a | 20.86ab | 22.94a | 16.83ab | 12.75a | 1.02a | 52.63ab | 48.24ab | 49.38ab |
| | -4 | 4 | 65.71a | 46.52abc | 35.56ab | 37.00ab | 17.61de | 25.45ab | 21.32abc | 17.13abc | 1.54a | 56.49abcd | 49.83abcd | 46.31cd |
| | -20 | 4 | 63.57a | 41.36c | 37.29a | 28.19bcd | 21.89bcde | 25.31ab | 23.24abc | 10.06bcd | 0.64ab | 53.67abcde | 49.18abcd | 51.81bcd |

[1]Means (n=3) for all samples, except for the TM -20º CT4 wine where n=2.
[2]Cryogenic pre-treatment temperature
[3]Not applicable - na
[4]Thiol type aroma, i.e., melon, tropical fruit, passion fruit.
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

Table 4.6: ANOVA of Sensory evaluation data resulting from Sauvignon blanc wines produced from hand and machine-harvested cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer B from the Durbanville wine region) in 2020.

| Treatment | Harvesting[2] technique | Temp[3] (ºC) | Storage Time (Months) | Sensory Descriptors[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Overall intensity | Yellow Fruit | Tropical Fruit | Citrus | Herbaceous | Reductive | $SO_2$ aroma | Acidity | Body | General quality |
| **Control** | H | na[4] | na | 64.39a | 32.64ab | 48.25a | 27.88abc | 30.75b | 7.86b | 17.36ab | 61.25a | 53.17ab | 55.64ab |
| **WG[5]** | H | -4 | 4 | 59.58ab | 40.14ab | 47.08a | 30.00ab | 31.25b | 7.92b | 3.44b | 52.22bc | 56.39a | 60.97a |
| | H | -20 | 4 | 60.97ab | 34.58ab | 41.11a | 30.42ab | 38.61a | 15.00b | 7.50ab | 50.83bcd | 54.87a | 56.96ab |
| **Control** | M | 15 | na | 64.39a | 32.27ab | 45.91a | 25.15abc | 21.67c | 7.57b | 18.94a | 55.45b | 51.82ab | 53.60ab |
| **MG** | M | -4 | 4 | 57.50ab | 35.69ab | 38.19a | 25.61abc | 26.67bc | 12.50b | 7.06ab | 51.52bcd | 54.70a | 55.01ab |
| | M | -20 | 4 | 56.80ab | 29.58ab | 33.88a | 25.00abc | 30.42b | 14.86b | 7.41ab | 51.58bcd | 53.75ab | 53.53ab |
| **TM** | M | -4 | 4 | 56.67ab | 27.91b | 16.25b | 14.31d | 30.83b | 44.17a | 11.39ab | 46.94d | 35.14c | 31.47c |
| | M | -20 | 4 | 55.69b | 14.1c | 35.00a | 20.97cd | 26.66bc | 18.89b | 8.97ab | 50.42cd | 46.67b | 47.08b |
| **CJ** | M | -4 | 4 | 54.58b | 32.50ab | 40.00a | 31.11a | 28.19b | 3.52b | 4.83ab | 51.39bcd | 53.47ab | 54.82ab |
| | M | -20 | 4 | 53.96b | 29.79ab | 36.25a | 22.75bc | 25.62bc | 12.71b | 5.50ab | 50.83bcd | 46.46b | 46.04b |

[1]Means (n=3) for all samples, except for the CJ -20º Ctreatments where n=2.
[2]Hand-harvested grapes (H) or Machine harvested grapes (M).
[3]Storage temperature
[4]Not applicable – na
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

Table 4.7: ANOVA of Sensory evaluation data resulting from Sauvignon blanc wines produced from hand-harvested cryogenically pre-treated WG, MG, TM and CJ (supplied by Producer B from the Durbanville wine region) in 2021.

| Treatment | Harvesting[2] technique | Temp[3] (ºC) | Storage Time (Months) | Sensory Descriptors[1] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Overall intensity | Thiol type[5] aroma | Citrus aroma | Herbaceous aroma | Banana aroma | Pineapple aroma | Apple aroma | Reductive | Acid | Body | General quality |
| Control | H | na[4] | na | 68.08cd | 37.95d | 37.29ab | 32.36ab | 18.33d | 17.68b | 15.00cd | 24.80a | 55.23ab | 56.28ab | 55.42bc |
| WG[6] | H | -4 | 4 | 67.31cd | 45.05abcd | 30.58b | 34.86a | 21.38d | 22.50ab | 13.97d | 19.97a | 51.55bcd | 57.42ab | 54.76bc |
| | H | -20 | 4 | 69.58abc | 53.03ab | 35.00ab | 31.15ab | 35.42ab | 29.41a | 19.61abcd | 6.11b | 51.25bcd | 54.77ab | 61.80a |
| MG | H | -4 | 4 | 72.60ab | 55.23a | 36.25ab | 30.19ab | 39.88a | 28.38a | 17.25bcd | 7.09b | 50.45cd | 57.83a | 63.21a |
| | H | -20 | 4 | 69.07bc | 46.12abcd | 32.70b | 30.37ab | 33.46abc | 24.83a | 22.04abc | 4.58b | 49.53d | 49.59c | 58.98ab |
| TM | H | -4 | 4 | 73.01a | 49.84abc | 35.48ab | 31.86ab | 27.08bcd | 25.83a | 19.58abcd | 5.23b | 54.35abc | 55.12ab | 59.31ab |
| | H | -20 | 4 | 66.35cd | 44.14bcd | 44.67a | 25.97b | 19.20d | 27.28a | 16.61bcd | 5.56b | 52.44bcd | 56.54ab | 62.62a |
| CJ | H | -4 | 4 | 65.48d | 41.82cd | 35.95ab | 25.78b | 23.08cd | 27.42a | 24.69a | 6.28b | 57.16a | 52.73bc | 53.85c |
| | H | -20 | 4 | 69.38bc | 41.81cd | 38.18ab | 27.88ab | 22.09cd | 24.42ab | 23.32ab | 5.30b | 53.26abcd | 58.53a | 61.39a |

[1]Means (n=3) for all samples, except for the CJ -20º C treatment where n=2.
[2]Hand-harvested grapes (H).
[3]Storage temperatures
[4]Not applicable - na
[5]Thiol type aroma, i.e., melon, tropical fruit, passion fruit
[6]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

Table 4.8: ANOVA of Sensory evaluation data resulting from Sauvignon blanc wines produced from cryogenic pre-treated WG, MG, TM and CJ originating from Producer C in the Cape South Coast wine region in 2020.

| Treatment | Temp[2] (ºC) | Storage Time (Months) | Sensory Descriptors[1] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Overall intensity | Veg Fresh | Veg Cooked | Veg Dried | 3-SH[4] aromas | 3-SHA[5] aromas | 4-MSP[6] aromas | Acidity | Other | Body | General quality |
| **Control** | na[3] | na | 69.44a | 35.37abc | 11.48bc | 13.05bc | 25.88a | 53.08a | 20.23cde | 56.48abc | 24.42a | 56.28abc | 69.28a |
| **WG[7]** | -4 | 0 | 67.14ab | 32.29abcd | 23.61a | 14.26abc | 21.48ab | 43.33bcd | 37.02a | 55.18abcd | 17.68ab | 56.27abcd | 57.48cdef |
| | -20 | 0 | 64.82abcde | 35.92ab | 27.27a | 13.54abc | 19.63abc | 39.44cde | 29.63bc | 54.07bcde | 11.67bc | 56.76ab | 65.00ab |
| | -4 | 4 | 65.88abc | 32.87abcd | 5.81cd | 15.10abc | 8.79f | 42.58cde | 13.35fgh | 50.33fg | 16.95abc | 55.25abcd | 60.79bc |
| | -20 | 4 | 62.24bcdefg | 26.33defg | 5.59cd | 16.76ab | 10.88f | 30.88fgh | 12.69gh | 50.27fg | 14.05bc | 54.48abcd | 59.42cde |
| **MG** | -4 | 0 | 66.67ab | 36.16ab | 28.98a | 12.02bc | 14.79bcdef | 40.42cde | 33.34ab | 54.07bcde | 11.47bc | 58.15a | 56.29cdefg |
| | -20 | 0 | 65.00abcd | 37.59a | 12.61b | 11.11bc | 24.98a | 51.67ab | 26.30cd | 55.56abcd | 15.69bc | 56.29abc | 65.18ab |
| | -4 | 4 | 59.30cdefgh | 27.28defg | 6.06cd | 13.56abc | 11.94def | 40.43cde | 11.44gh | 51.36efg | 9.63c | 55.09abcd | 60.00cd |
| | -20 | 4 | 64.15abcdef | 25.03efg | 5.12d | 14.34abc | 14.02bcdef | 40.15cde | 13.88fgh | 50.03g | 15.84bc | 54.52abcd | 59.96cd |
| **TM** | -4 | 0 | 59.44cdefgh | 37.59a | 13.52b | 10.46c | 25.07a | 43.70bc | 18.33efg | 55.69abcd | 13.15bc | 55.03abcd | 59.45cde |
| | -20 | 0 | 50.30i | 31.29abcde | 11.48bc | 11.92bc | 15.49bcdef | 28.43gh | 18.31efg | 56.62abc | 9.63c | 51.69def | 48.52h |
| | -4 | 4 | 54.90hi | 22.59g | 4.59a | 12.46bc | 11.12ef | 28.43gh | 7.697h | 58.13a | 16.79abc | 42.76g | 40.30i |
| | -20 | 4 | 57.18fghi | 23.49fg | 4.36d | 11.09bc | 13.08cdef | 34.64efgh | 12.48gh | 57.06ab | 17.51ab | 49.09f | 52.02gh |
| **CJ** | -4 | 0 | 57.73efgh | 28.54cdefg | 14.44a | 12.69bc | 18.56abcde | 35.18efgh | 24.79cde | 56.19abcd | 11.53bc | 53.15bcde | 54.39fg |
| | -20 | 0 | 54.79hi | 35.74ab | 12.59b | 13.54abc | 19.35abcd | 36.83cdef | 19.81def | 57.36a | 11.67bc | 55.05abcd | 55.37defg |
| | -4 | 4 | 58.45defgh | 22.89g | 4.75d | 13.85abc | 15.45bcdef | 36.94cdef | 11.79gh | 53.06defg | 12.48bc | 52.55cdef | 55.00efg |
| | -20 | 4 | 57.18ghi | 28.15defg | 5.22d | 19.24a | 12.54cdef | 26.33h | 11.68gh | 55.12abcd | 9.52c | 51.91def | 54.06fg |

[1]Means (n=3) for all samples between treatments within columns.
[2]Storage temperatures
[3]Not applicable - na
[4]3-sulfanylhexan-1-ol (3-SH) aromas, i.e., lime, lemon, sweet grapefruit.
[5]3-sulfanylhexyl acetate (3-SHA) aromas, i.e., pineapple, passionfruit, sweet melon, banana, guava, gooseberry, kiwi.
[6]4-methyl-4-sulfanylpentan-2-one (4-MSP) aromas, i.e., Box tree (cat urine), broom, blackcurrant, and tomato leaf aromas.
[7]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

Table 4.9: ANOVA of Sensory evaluation data resulting from Sauvignon blanc wines produced from cryogenic pre-treated WG, MG, TM and CJ (supplied by Producer C from the Cape South Coast wine region) in 2021.

| Treatment | Temp[2] (ºC) | Storage Time (Months) | Sensory Descriptors[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Overall intensity | Thiol type[4] aroma | Citrus aroma | Herbaceous aroma | Banana aroma | Pineapple aroma | Apple aroma | Reductive | $SO_2$ aroma | Acid | Body | General quality |
| Control | na[3] | na | 65.06a | 50.30ab | 31.27ab | 27.18de | 19.17ab | 22.98abc | 8.02ab | 13.02cdef | 2.39a | 57.54a | 54.56ab | 51.52a |
| WG[5] | -4 | 0 | 62.33a | 42.57ab | 29.17ab | 42.03ab | 17.26abc | 22.82abc | 9.05ab | 4.62ef | 3.11a | 48.06de | 52.98abcd | 53.56a |
| | -20 | 0 | 64.16a | 50.30ab | 32.00ab | 41.36abc | 19.24ab | 18.45abc | 10.58ab | 4.59ef | 2.11a | 49.96bcde | 51.70abcde | 53.61a |
| | -4 | 4 | 67.20a | 31.64ef | 26.85bcde | 43.36a | 11.36abc | 18.08abc | 6.59b | 22.76abc | 2.44a | 49.11cde | 55.58a | 52.24a |
| | -20 | 4 | 64.77a | 27.38f | 26.30cde | 38.62abcd | 10.83bc | 16.20c | 7.88ab | 20.17abc | 2.50a | 50.17bcde | 54.67ab | 48.52a |
| MG | -4 | 0 | 61.96a | 44.63ab | 29.98ab | 40.97abc | 18.70ab | 21.58abc | 7.80ab | 8.76def | 2.15a | 51.83abcde | 52.65abcde | 55.00a |
| | -20 | 0 | 63.61a | 50.18ab | 29.18ab | 31.30abcde | 16.88abc | 19.89abc | 6.67b | 5.36ef | 2.33a | 49.31bcde | 53.65ab | 52.89a |
| | -4 | 4 | 65.00a | 40.68bcde | 29.33abcde | 31.70abcde | 15.29abc | 19.91abc | 9.26ab | 8.87def | 1.50a | 49.48bcde | 51.22abcde | 48.05a |
| | -20 | 4 | 66.33a | 39.54bcde | 28.57abcde | 25.15e | 20.48a | 22.12abc | 13.70a | 13.96bcde | 2.65a | 47.29e | 49.82bcde | 47.56a |
| TM | -4 | 0 | 63.20a | 47.12ab | 30.61ab | 32.53abcde | 16.33abc | 24.37ab | 8.75ab | 2.92f | 2.61a | 52.11abcde | 54.67ab | 54.02a |
| | -20 | 0 | 61.91a | 41.15b | 34.16a | 36.12abcde | 10.42bc | 24.99a | 9.44ab | 7.57def | 3.09a | 52.64abcde | 47.73ef | 50.33a |
| | -4 | 4 | 63.25a | 31.36ef | 27.32bcde | 30.97abcde | 8.38c | 17.02bc | 9.05ab | 24.50ab | 2.52a | 53.67abcde | 48.65def | 46.59a |
| | -20 | 4 | 63.43a | 32.50def | 23.27e | 29.00cde | 10.39bc | 19.21abc | 8.40ab | 29.76a | 2.17a | 54.74abc | 47.91def | 46.94a |
| CJ | -4 | 0 | 61.55a | 45.09ab | 25.92b | 26.30de | 14.59abc | 20.46abc | 8.29ab | 5.88ef | 2.81a | 55.58ab | 51.06abcde | 51.37a |
| | -20 | 0 | 65.18a | 47.12a | 35.83a | 27.05de | 13.16abc | 19.73abc | 8.71ab | 5.81ef | 3.06a | 53.79abcd | 44.59f | 51.64a |
| | -4 | 4 | 62.14a | 38.11cdef | 28.39abcde | 32.85abcde | 13.22abc | 18.41abc | 7.56ab | 17.95bcd | 2.19a | 53.15abcde | 50.48abcde | 47.56a |
| | -20 | 4 | 63.35a | 43.61abcd | 24.17de | 29.56bcde | 12.67abc | 23.38abc | 11.06ab | 17.53bcd | 2.00a | 52.00abcde | 49.33cdef | 46.32a |

[1]Means (n=3) for all samples.
[2]Storage temperature
[3]Not applicable - na
[4]Thiol type aroma, i.e., melon, tropical fruit, passion fruit
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

Table 4.10: ANOVA of Sensory evaluation data resulting from Chenin blanc wines produced from cryogenic pre-treated WG, MG, TM and CJ (supplied by Producer D from the Stellenbosch wine region) in 2021.

| Treatment | Temp[2] (ºC) | Storage Time (Months) | Sensory Descriptors[1] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Overall intensity | Thiol type[4] aroma | Citrus aroma | Tree fruit aroma | Stewed fruit aroma | Vegetative aroma | Reductive | $SO_2$ aroma | Acid | Body | General quality |
| **Control** | na[3] | na | 62.99a | 36.97bc | 31.41abc | 50.45ab | 2.11ab | 28.60bcde | 11.52bc | 0.37ab | 51.83b | 52.74bcd | 51.69cd |
| **WG[5]** | -4 | 0 | 65.14a | 42.89abc | 27.94bc | 51.79ab | 2.81ab | 29.19bcde | 9.47cd | 0.00b | 50.22b | 56.22abcd | 58.01abcd |
| | -20 | 0 | 65.56a | 45.00abc | 43.00a | 53.75ab | 3.33ab | 37.50ab | 10.50cd | 1.25ab | 53.00ab | 56.00abcd | 59.00abc |
| | -4 | 4 | 69.58a | 45.15abc | 23.35bc | 50.87ab | 3.19ab | 27.68bcde | 6.45cd | 0.00b | 50.83b | 57.72abc | 61.74ab |
| | -20 | 4 | 63.66a | 41.31abc | 28.69bc | 50.61ab | 1.79ab | 30.63bcd | 7.76cd | 0.70ab | 54.07ab | 56.20abcd | 59.31abc |
| **MG** | -4 | 0 | 64.42a | 45.75abc | 29.15bc | 54.52ab | 2.25ab | 23.32de | 9.78cd | 0.50ab | 53.89ab | 55.04abcd | 57.34abcd |
| | -20 | 0 | 66.44a | 47.80ab | 29.91bc | 54.18ab | 1.99ab | 27.41bcde | 9.29cd | 0.48ab | 51.08b | 59.56ab | 59.04abc |
| | -4 | 4 | 68.92a | 51.83a | 31.69ab | 56.92a | 3.78ab | 29.71bcde | 6.72cd | 1.14ab | 50.92b | 58.04abc | 61.54ab |
| | -20 | 4 | 68.47a | 51.31a | 29.03bc | 59.26a | 4.93a | 26.52cde | 6.34cd | 0.67ab | 53.84ab | 60.59a | 65.37a |
| **TM** | -4 | 0 | 63.07a | 42.46abc | 29.46bc | 55.75a | 3.34ab | 23.73de | 11.95bc | 0.28ab | 54.00ab | 53.00bcd | 57.75abcd |
| | -20 | 0 | 67.50a | 45.50abc | 30.56bc | 56.00a | 0.00b | 19.55e | 10.91cd | 0.00b | 57.73a | 53.18bcd | 57.73abcd |
| | -4 | 4 | 69.42a | 34.43c | 19.99bc | 52.16ab | 1.30ab | 31.27bcd | 20.52a | 1.04ab | 52.64b | 52.65cd | 50.36d |
| | -20 | 4 | 65.76a | 46.54abc | 31.12abc | 47.26ab | 2.45ab | 30.62bcd | 10.80cd | 0.50ab | 51.92b | 56.25abcd | 58.47abcd |
| **CJ** | -4 | 0 | 62.92a | 43.00abc | 25.00bc | 50.91ab | 3.00ab | 35.91bcd | 18.33ab | 1.82a | 50.45b | 50.00d | 51.36cd |
| | -20 | 0 | 69.58a | 47.92ab | 27.92bc | 50.45ab | 2.73ab | 47.50a | 4.00d | 0.00b | 49.17b | 55.91abcd | 59.09abc |
| | -4 | 4 | 67.50a | 42.77abc | 27.59bc | 53.80ab | 2.25ab | 27.73bcde | 8.64cd | 0.00b | 50.23b | 52.73cd | 54.57bcd |
| | -20 | 4 | 67.86a | 36.93bc | 19.28c | 41.82b | 2.50ab | 21.59de | 6.00cd | 0.91ab | 52.50b | 52.00cd | 57.96abcd |

[1]Means (n=3) for all samples.
[2]Storage temperature
[3]Not applicable – na
[4]Thiol type aroma, i.e., melon, tropical fruit, passion fruit.
[5]Whole Grapes (WG), Macerated Grapes (MG), Turbid Must (TM), Clear Juice (CJ).
Different letters within columns indicate significant differences at the $p < 0.05$ level between treatments.

Artichoke), “Vegetative dried” (hay/straw, tea, tobacco), “Tree fruit” (Apricot, White peach, Green apple), “Dried fruit” (Strawberry jam, Raisin, Prune, Fig), “Floral” (Elderberry) and “Flinty” (minerality) also typically associated with Sauvignon blanc wines were also included in the sensory sheets.

In 2020, the ANOVA results showed that the T4 wines made from MG (-4 ºC) and the T0 wines made from MG (-20 ºC) originating from Producer A scored the highest for body, overall intensity, general quality and thiol aromas (3-SH and 3-SHA) when compared to the control wines and wines made from the cryogenically treated WG, TM and CJ (Fig. 4.11 and Tables 4.4-4.5). The same observations were once again made in 2021 where the wines made from the MG subjected to cryogenic treatments scored the highest for body, overall intensity, general quality, thiol-type aromas, banana, pineapple and apple aromas, when compared to the control and wines made from the cryogenically treated WG, TM and CJ (Fig. 4.11 and Tables 4.4-4.5). Additionally, the T4 wines made from MG (-4 ºC) scored the highest for banana and pineapple aromas. Furthermore, the T4 wines made from WG (-4 ºC) scored the highest for herbaceous aroma, whilst the T4 wines made from TM (-4 ºC) scored the highest for reductive aromas when compared to the rest of the wines (Fig. 4.11 and Tables 4.4-4.5).

Wines made from hand-harvested and machine-harvested control grapes obtained from Producer B (2020) had similar sensory profiles for all the sensory descriptors, except for the scoring of the herbaceous aroma, which was higher in the wines made from the hand-harvested WG (-20 ºC) (Fig. 4.12 and Tables 4.6-4.7). It was also seen that the hand-harvested and machine-harvested control wines scored the highest acidity compared to those from the cryogenic treatments. Furthermore, the wines made from hand-harvested WG (-4 ºC) scored the highest ($p > 0.05$) for general quality. Additionally, the wines made from the TM (-4 ºC) scored the highest for reductive aroma and the lowest for yellow fruit, body and general quality (Fig. 4.12 and Tables 4.6-4.7). The rest of the wines had similar aroma profiles with no notable differences between treatments, indicating that the harvesting technique did not significantly affect the sensory profiles of wines (Fig. 4.12 and Tables 4.6-4.7). In 2021 (Fig. 4.12 and Tables 4.6-4.7), the wines made from the MG (-4 ºC) and WG (-20 ºC) scored the highest for thiol-type aromas. In addition, the wines made from the MG (-4 ºC) also had the highest score for the banana aroma, followed by the wines made from the whole and MG frozen at -20 ºC (Fig. 4.12 and Tables 4.6-4.7). Overall, most wines made from cryogenically treated WG, MG, TM and CJ had the highest scores for general quality and overall intensity compared to those made from the unfrozen control grapes (Fig. 4.12 and Tables 4.6-4.7).

The control wines and T0 wines made from MG (-20 ºC) originating from Producer C (2020) had the highest scores for the 3-SH, 3-SHA aromas, general quality and overall intensity compared to the rest of the wines, therefore, cryogenic treatments did not enhance these varietal aromas for the T0 wines made from MG (-20 ºC) (Fig. 4.13 and Tables 4.8-4.9). The results also showed that the T0 wines made from WG (-20 ºC) and MG (-4 ºC) scored higher than the control wines and remaining treatments for vegetative cooked and 4-MSP aromas, indicating that these wines had more vegetative aromas compared to the other wines. In 2021, most wines grouped closely for most sensory attributes, except for the herbaceous and thiol-type aromas, where variation was seen between treatments. (Fig. 4.13 and Tables 4.8-4.9). Wines made from all the WG, for all the treatments, i.e., both freezing and storage treatments, had the highest scores for the herbaceous aroma, whilst the control wine and T0 MG (-20 ºC) had the highest thiol-type aroma. The latter was similar to that observed for 2020. Overall, scores for the body, general quality and overall intensity of all the wines were similar with no notable differences observed (Fig. 4.13 and Tables 4.8-4.9).

In 2021, for Producer D (Chenin blanc), the T4 wines made from MG (-4 ºC and -20 ºC) had the highest scores for the thiol type aroma, tree fruit aroma, body and general quality when compared to the control wines and remaining cryogenic treatments (Fig. 4.14 and Table 4.10). Furthermore, the T0 wines made from WG (-20 ºC) had the highest score for the citrus aroma. Overall, pre-fermentative cryogenic treatments produced wines with better sensory descriptors when compared to the control wines (Fig. 4.14 and Table 4.10).

### 4.3.3 Effect of machine-harvesting versus hand-harvesting on impact aroma compounds and sensory parameters of wine

Wine producers predominantly use mechanical harvesters to increase the yield of the harvest, reduce harvest time and cost. However, previous research found that the grape juice and wines resulting from mechanically harvested grapes had higher levels of varietal thiol precursors and varietal thiols in wines (Anonymous, 2022; Allen et al., 2011:10641-10650). Subsequently, numerous studies have been conducted, although the results varied greatly (Allen et al., 2011:10641-10642; Kilmartin, 2012:81-83; Jeffery, 2016:1323-1330; Coetzee, 2020a; b). In this study, Sauvignon blanc grapes from a Producer in the Durbanville wine region were harvested by hand and machine (2020) and by hand only in 2021. WG, MG, TM and CJ were subjected to pre-fermentative cryogenic freezing (-20 ºC and -4 ºC) for T4 before processing. The impact of these viticultural and pre-vinification techniques on the wine aroma compounds and sensory properties is illustrated by Multi-Factorial Analysis (MFA) (Fig. 4.15-4.16).

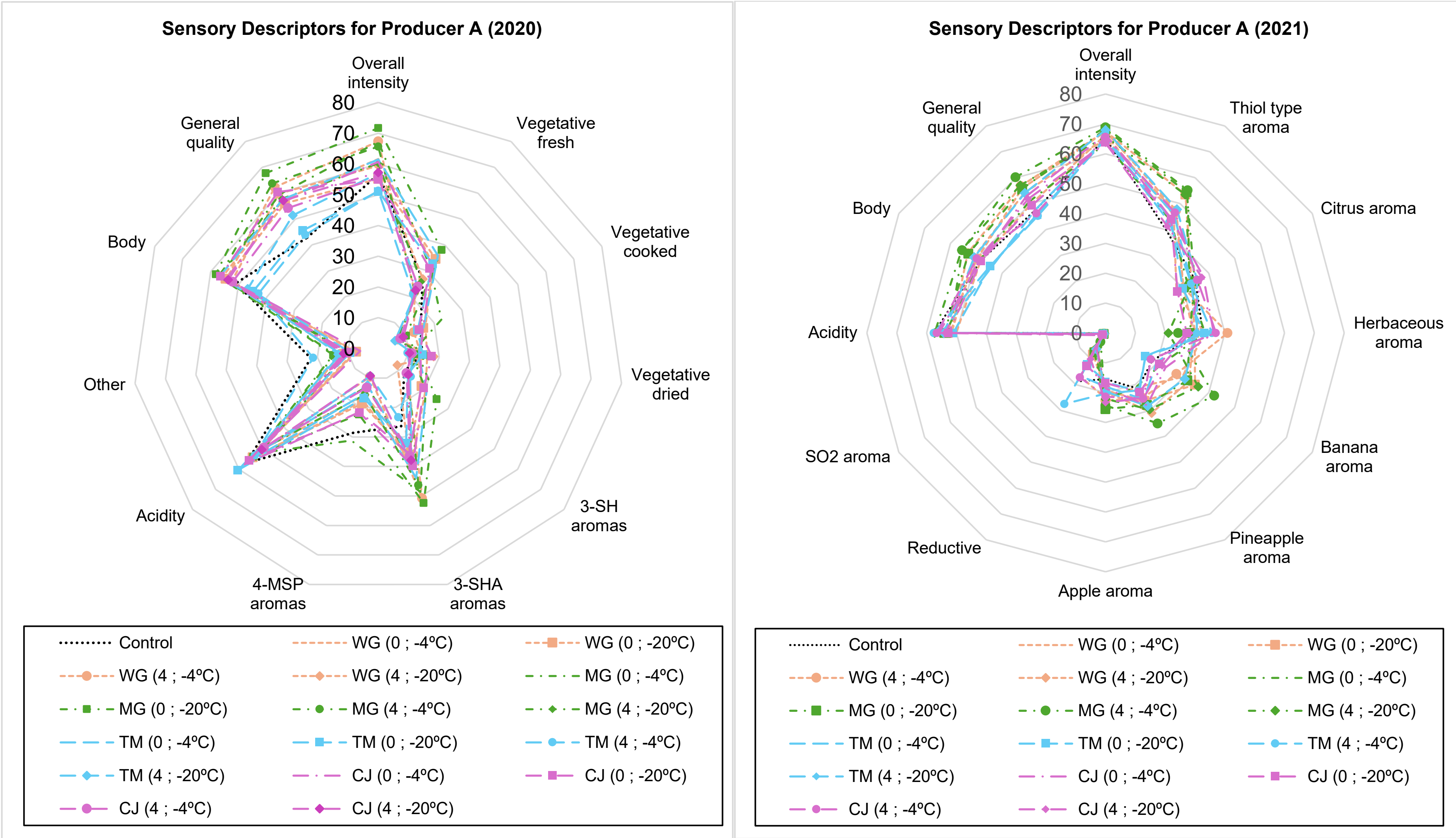



Figure 4.11. Radar plot illustrating the mean sensory scores of wines from Producer A (Stellenbosch wine region), made from control grapes and cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-20 ºC and -4 ºC) at T0 and T4 in 2020 and 2021.

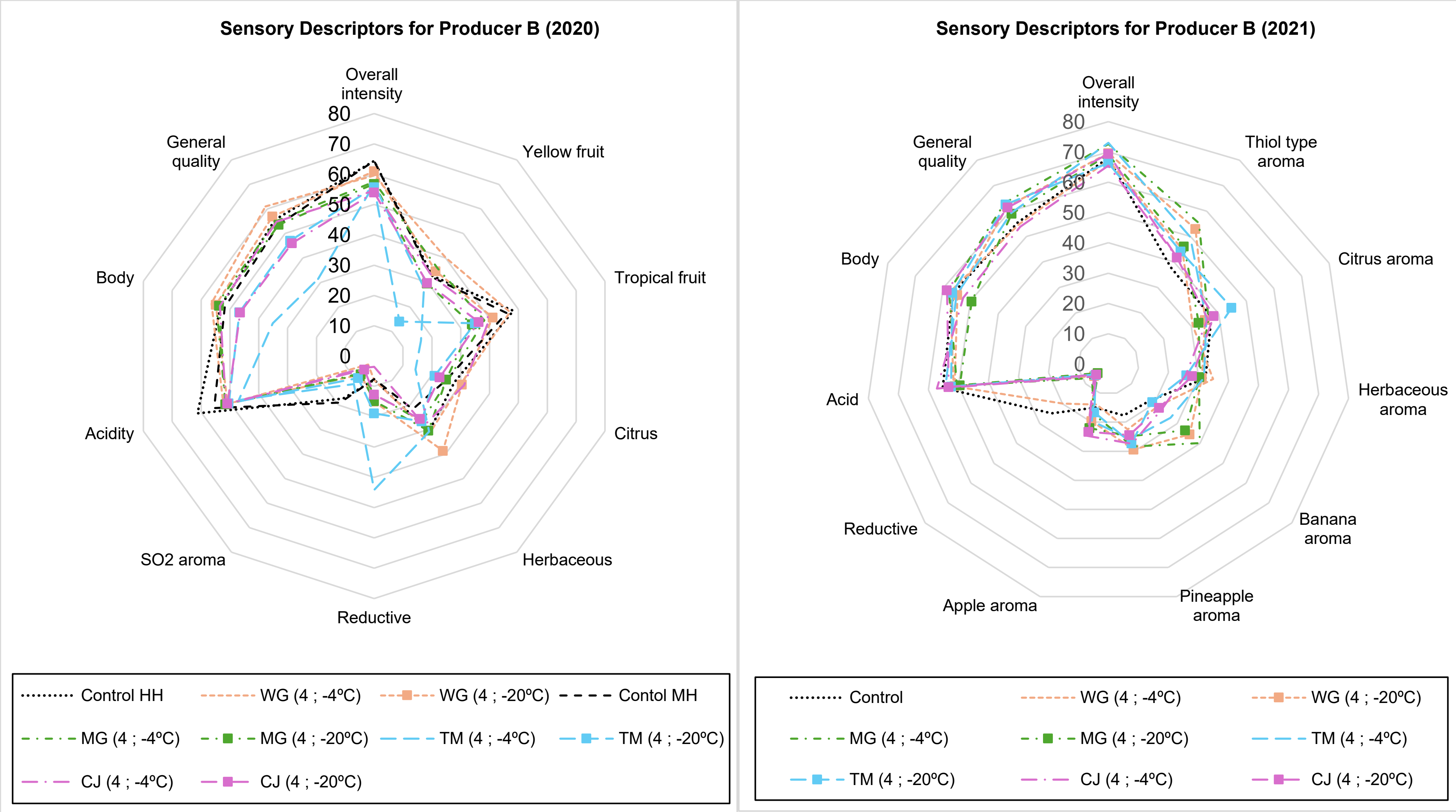



Figure 4.12. Radar plot illustrating the mean sensory scores of wines from Producer B (Durbanville wine region), made from control grapes and cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-20 °C and -4 °C) at T0 and T4 in 2020 and 2021.

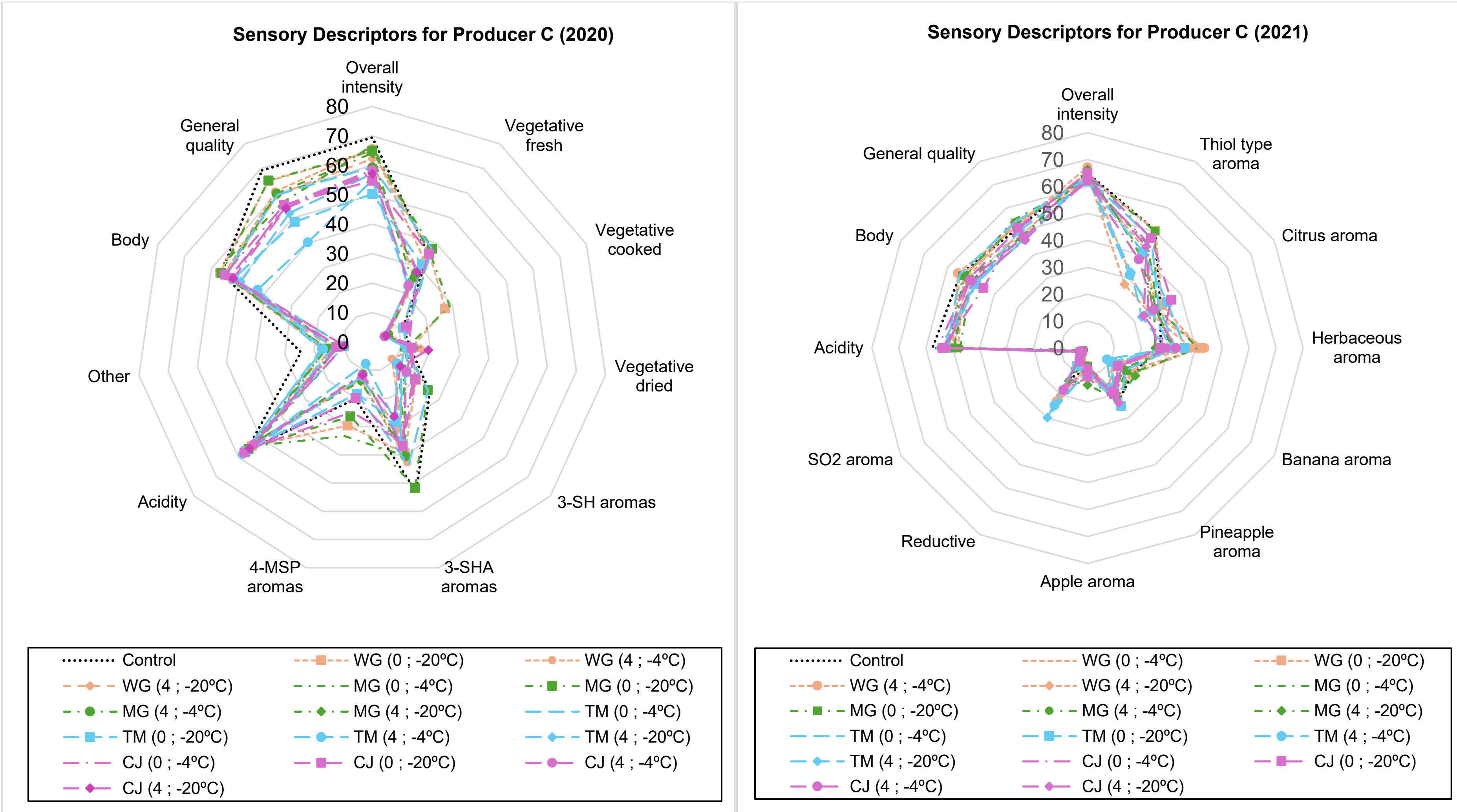



Figure 4.13. Radar plot illustrating the mean sensory scores of wines from Producer C (Cape South Coast wine region), made from control grapes and cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-20 ºC and -4 ºC) at T0 and T4 in 2020 and 2021.

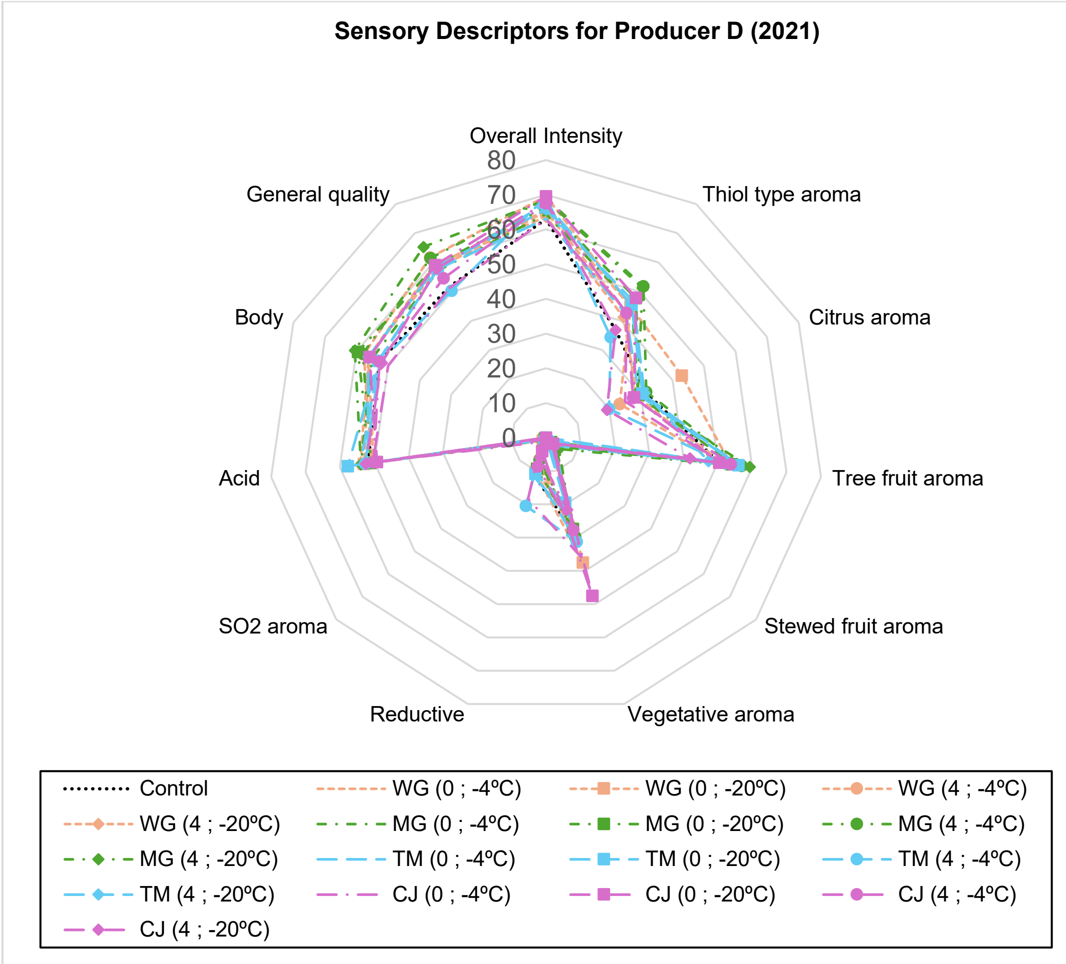



Figure 4.14. Radar plot illustrating the mean sensory scores of wines from Producer D (Stellenbosch wine region), made from control grapes and cryogenically pre-treated Chenin blanc WG, MG, TM and CJ subjected to freezing (-20 ºC and -4 ºC) at T0 and T4 in 2021.

For 2020, the control wines made from the machine and hand-harvested grapes were positioned in the right half of the MFA and clustered with the wines made from the WG (-4 ºC and -20 ºC) (Fig. 4.15). These wines are associated with the varietal thiol 4-MSP and the sensory descriptors, i.e., "Herbaceous", "Overall quality", "Acid", "Citrus", "Tropical fruit", "Yellow fruit", "General quality", "Body" and "$SO_2$". Although most control wines correlated with positive sensory attributes, it was positioned in the bottom half of the MFA, which also correlated with the less desirable reductive and $SO_2$ sensory descriptors. In terms of sensory, this is not necessarily negative, because varietal thiols exist as sulfur compounds in grapes and are released through biogenesis pathways (Tominaga et al., 1998a:161-162; Ebeler & Thorngate, 2009:8101; Coetzee & du Toit, 2012:289-290; Kapaklis, 2014:22). It is known that when varietal thiols are present in low concentrations, they impart desirable aromas and when present in high concentrations, less desirable aromas (Roland et al., 2011:7359; Coetzee & du Toit, 2012:289; Benkwitz et al., 2012:6294). These wines are also positioned on the right half of the MFA and correlate with the sensory descriptors "Overall intensity", "Acid", "Tropical fruit", "Citrus", "Yellow fruit", "General quality" and "Body", all known to be positive or desired sensory attributes (Fig. 4.15).

When looking at the groupings, it is evident that the cryogenic pre-treated MG, positioned in the top left quadrant of the MFA, correlated with the varietal thiol compounds, i.e., 3-SH and 3-SHA and the methoxypyrazine, ibMP. These wines, although in the top left quadrant and opposite to the desired sensory attributes, were still situated in the top half of the MFA and therefore correlated with the desired sensory attributes (Fig. 4.15). It was further seen that most of the wines made from the cryogenic pre-treated TM (-4 ºC and -20 ºC), were positioned in the bottom left quadrant and correlated with less desirable sensory descriptors, i.e., "Reductive" and "$SO_2$" (Fig. 4.15). It was also noted that for 2020, no sbMP was detected in the hand and machine-harvested grapes from this Producer, which is not surprising because the range in wines is usually low (< 10 ng $L^{-1}$) and similar to the findings reported in previous research (Allen & Lacey, 1998:35-37; Rajchl et al., 2009:264).

In 2021, the control wines once again clustered with wines made from WG frozen at -4 ºC (Fig. 4.16). The wines were positioned in the bottom left quadrant of the MFA and correlated with the varietal thiol compound, 4-MSP and the sensory descriptor, i.e., "Body". In addition, the wines on the left side of the MFA plot correlated with the methoxypyrazine aroma compound, sbMP and sensory descriptors, i.e., "Herbaceous", "Reductive" and "Acid". Although the latter is usually associated with less desirable sensory attributes, "Herbaceous" is a sensory descriptor that can also have a positive sensory connotation with the varietal thiol, 4-MSP and the methoxypyrazine, sbMP (Fig. 4.16).

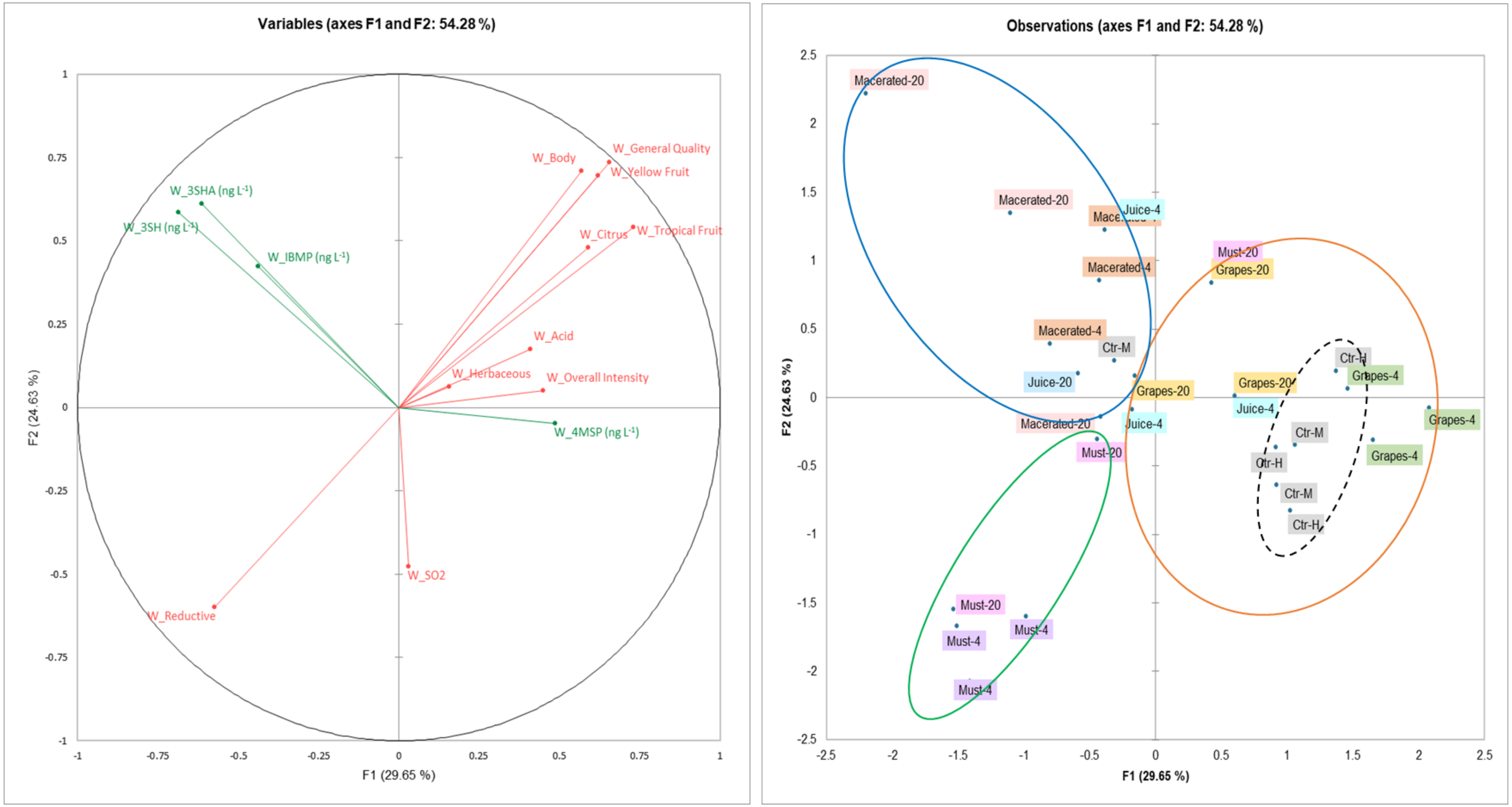



Figure 4.15. Multiple Factor Analysis (MFA) showing the correlation between (indicated by circles around groupings) the aroma compounds and sensory profiles of wine from Producer B (Durbanville wine region) made from hand and machine-harvested control and cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-20 ºC and -4 ºC) at T4 in 2020.

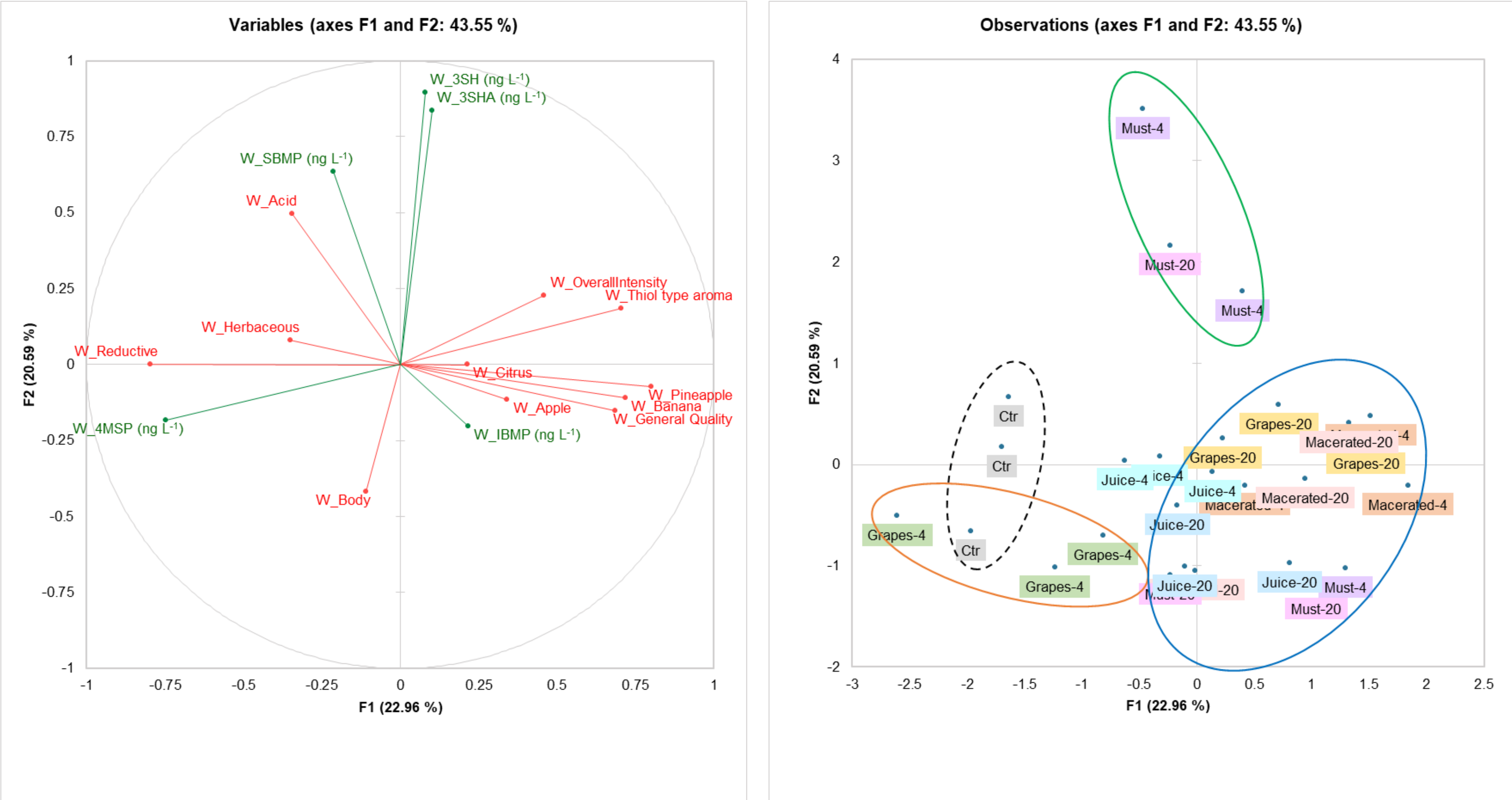



Figure 4.16. Multiple Factor Analysis (MFA) showing the correlation between the aroma compounds and sensory profiles of wines from Producer B (Durbanville wine region), made from control grapes and hand-harvested cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-20 ºC and -4 ºC) at T4 in 2021.

It was also seen that certain TM treatments, frozen at -4 ºC and -20 ºC clustered in the top half of the MFA and correlated with the varietal thiol compounds, i.e., 3-SH and 3-SHA and the sensory descriptors, i.e., “Overall intensity”, “Thiol type aroma”, “Acid”, “Herbaceous” and “Reductive”, whilst the remainder of the triplicates clustered in the bottom right half of the MFA with the WG, MG and CJ treatments. The latter correlated with the methoxypyrazine aroma compound, ibMP and the more desirable sensory descriptors, i.e., “Body”, “Citrus’, “Apple”, “Pineapple”, “Banana” and “General quality” (Fig. 4.16).

This study demonstrates that harvest method and cryogenic pre-treatment strongly influence Sauvignon blanc aroma composition and sensory style. Wines made from machine-harvested grapes (2020), particularly the MG and CJ (-4 ºC and -20 ºC), clustered and correlated with the desired varietal thiol compounds (3-SH and 3-SHA), indicating fruit-driven sensory profiles (“Tropical fruit”, “Citrus” and “Yellow fruit”) (Tominaga et al., 2006:7254-7255; Coetzee & du Toit., 2012:289; Capone et al., 2015:1227; Jeffery, 2016:1324; Coetzee et al., 2018:180-184). The wines made from hand-harvested WG (-4 ºC and -20 ºC) correlated closely with 4-MSP and “Herbaceous” sensory descriptors, indicating a greener style sensory profile (Fig. 4.15). Moreover, ibMP did not consistently align with “Herbaceous” in the MFA but instead positioned closer to the fruity sensory descriptors and sometimes contrasted with 4-MSP. This highlights that the herbaceous perception in Sauvignon blanc is not explained by a single compound but rather by a complex interaction of methoxypyrazines, thiols, and matrix effects (Fig. 4.16). These findings corroborate previous research that found wines made from machine-harvested grapes to exhibit thiol-related tropical and fruity aromas when compared to wines made from hand-harvested grapes (Allen et al., 2011:10646; Kilmartin, 2012:82-86).

For 2021, wines made from WG (-20 ºC), MG, CJ and some TM (-20 ºC and -4 ºC) clustered in the bottom right quadrant and correlated with the methoxypyrazine, ibMP, however, it correlated with the sensory descriptors, “Apple”, “Citrus”, “Tropical”, “Banana”, “Thiol type aroma” which are sensory descriptors related to “Tropical” and are typically associated with varietal thiols (Tominaga et al., 2006:7254-7255; Coetzee & du Toit, 2012:289; Capone et al., 2015:1227; Jeffery, 2016:1324; Coetzee et al., 2018:180-184). Furthermore, the varietal thiol compounds clustered in the top right quadrant (3-SH and 3-SHA), whilst the 4-MSP compound was situated in the bottom left quadrant of the MFA and away from the sensory descriptors associated with varietal thiols. The T4 wines made from the WG (-4 ºC) and hand-harvested control grapes clustered in the left half of the MFA and correlated with the varietal thiol, 4-MSP and the sensory descriptors, i.e., “Herbaceous”, “Acid”, “Reductive” and “Body”, which were similar to the results for 2020. This correlation indicates that these wines were more “Herbaceous”, which is a sensory descriptor typically used to describe the aroma of the varietal thiol 4-MSP (Tominaga et al., 2006:7254-7255; Coetzee& du Toit, 2012:289; Capone et al., 2015:1227; Jeffery, 2016:1324; Coetzee et al., 2018:180-184). Furthermore, for 2020, the wines made from TM (-4 ºC and -20 ºC), clustered in the bottom left quadrant of the MFA and indicating increased reductive notes and a reduction in positive “Fruit intensity”, “Body” and “General quality” (Fig. 4.15), whilst for 2021, the wines made from the TM (-4 ºC and -20 ºC) clustered in the top half (across quadrants) of the MFA and correlated with the desired varietal thiol (3-SH and 3-SHA) and methoxypyrazine (sbMP) compounds. This highlights the effect of the harvesting technique and is therefore evident that a combination of the stage of production, vintage and harvesting methods played a role in the aroma compound levels and sensory profiles (“Tropical” and “Herbaceous”) of the wines (Fig. 4.15-4.16). These findings suggest that winemakers can use harvest method and cryogenic pre-treatment as tools to manage the style of Sauvignon blanc wine. Machine-harvested grapes, in combination with cryogenic pre-treatment, result in tropical, thiol-driven wines, whereas hand-harvested grapes, in combination with cryogenic pre-treatment, result in greener, herbaceous-style wines (linked to 4-MSP and not only ibMP). Furthermore, when making wines with TM, there is a risk of reductive characters and lower-quality aroma expression.

### 4.3.4 Effect of time, temperature and stage of production on aroma compounds and sensory profiles of wine

Cryogenic pre-fermentative freezing (-4 ºC and -20 ºC) was applied to WG, MG, TM and CJ for T0 and T4, followed by defrosting and vinification for all Producers, except for Producer B who only had the treatments applied for T4, as illustrated in the Principal Component Analysis (PCA) biplots (Figs. 4.17-4.22). For Producer A, in 2020, two definite clusters were observed for the wines regarding the period over which the cryogenic pre-treatments were applied, as illustrated by the PCA biplot (Figs. 4.17A). The control wines clustered with the T0 wines, mostly in the top half of the PCA bi-plot and correlated with the varietal aroma compounds, i.e., 3-SH, 3-SHA and 4-MSP, the methoxypyrazine, i.e., sbMP and sensory descriptors, i.e., “Acid”, “Vegetative cooked”, “Vegetative fresh”, “Varietal aroma 2 (4-MSP)”, “Varietal aroma 3 (3-SH)”, “Vegetative dried”. A few T0 treatments clustered in the bottom right quadrant and correlated more closely with the varietal aroma compound, i.e., 3-SH, the methoxypyrazine, i.e., ibMP and sensory descriptors, i.e., “Body”, “Overall intensity”, “General quality” and the “Varietal aroma 1 (3-SHA)” contribute equally to both groupings and are not influenced by the time of the cryogenic freezing (T0 or T4). For 2021, no clustering of wines was observed in relation to the time over which the cryogenic pre-treatments were applied, as illustrated by the PCA biplot (Figs. 4.17B). Therefore, for Producer A in 2020, clear clustering was observed between T0 and T4 cryogenic treatments, indicating that storage duration influenced both aroma compounds (3-SH, 3-SHA, 4-MSP, sbMP, ibMP) and sensory attributes, whereas in 2021, no such clustering occurred, suggesting that the impact of cryogenic storage time is strongly vintage-dependent (Figs. 4.17A-4.17B).

When looking at the stage of production (WG, MG, TM and CJ) and the cryogenic temperatures (-20 ºC and -4 ºC), two distinct clusters were seen for all wines (2020 and 2021) (Figs. 4.18A-4.18B and tables 4.4-4.5). For both vintages, the WG and MG clustered together and the TM and CJ, regardless of the cryogenic temperatures. Furthermore, the wines made from the control grapes clustered with the wines made from the TM and CJ, for both vintages. This shows that the effect of the different cryogenic temperatures on the levels of aroma compounds and sensory profiles of the wines was negligible, but the stage of production had a definite impact. The sensory descriptor "Other" is significantly higher and clustered with the "Control" wines for 2020 (Fig. 4.18A and Table 4.4). Wines made from the cryogenically pre-treated WG and MG clustered predominantly in the right bottom half of the PCA bi-plot and to a greater extent correlated with the varietal aroma compound, i.e., 3-SH, the methoxypyrazine aroma compound, i.e., ibMP and the sensory descriptors, i.e., "Body", "Overall intensity", "Varietal aromas 1 (3-SHA)" and "General quality". These sensory descriptors were significantly higher in the wines subjected to pre-fermentative freezing compared to the control wines, showing that these wines had better desirable sensory attributes and varietal aroma compounds compared to the control wines (Fig. 4.18A and Table 4.4).

In 2021, once again, two clusters of wines were observed. Wines made from the TM and CJ (-4 ºC and -20 ºC) as well as the control clustered whilst wines from the WG and MG (-4 ºC and -20 ºC) clustered (Fig. 4.18B and Table 4.5). The wines made from the treated TM and CJ (-4 ºC and -20 ºC) and the control clustered predominantly on the left side of the PCA bi-plot and correlated with the varietal aroma compounds, i.e., 3-SH and 3-SHA and sensory descriptors, i.e., "Citrus", "Acid" and "$SO_2$", "Reductive" and "Herbaceous". Although reductive aromas are seen as a negative attribute, herbaceous aromas are considered favourable sensory profiles, especially for Sauvignon blanc wines (Lacey et al., 1991:105-107; Marais, 1994:41-44, Allen & Lacey, 1998:31;35) (Fig. 4.18B and Table 4.5). Furthermore, the wines made from the WG and MG (-4 ºC and -20 º) clustered mostly on the right side of the bi-plot and correlated with the varietal aroma compound, i.e., 4-MSP, methoxypyrazine compounds (ibMP and sbMP) and the positive sensory descriptors ("Overall intensity", "Pineapple", "Thiol type aroma", "Body", "General quality", "Banana" and "Apple") showing a higher to significantly higher score for these cryogenically treated wines compared to control wines (Fig. 4.18B and Table 4.5). Therefore, for Producer A, the PCA biplots revealed that the stage of production (WG, MG vs. TM, CJ) had a clear impact on the aroma compounds and sensory profiles of Sauvignon blanc wines, whilst the different cryogenic freezing temperatures (-20 ºC and -4 ºC) had negligible effects. Wines from cryogenically pre-treated WG and MG showed significantly higher desirable sensory attributes and varietal aroma compounds compared to controls, with reduced negative, reductive aromas and enhanced favourable herbaceous and fruity notes across both vintages (2020 and 2021) (Fig. 4.18B and Table 4.5).

For Producer B, the cryogenic treatments were only applied at T4, for both vintages (2020 and 2021), regardless of the harvesting method (hand or machine). The wines clustered into three groups. For the 2020 vintage, the control grapes (hand and machine) clustered with the cryogenically pre-treated WG (-4 ºC) and correlated with the varietal thiol compound, i.e., 4-MSP and the sensory descriptors, i.e., “Overall intensity”, “Acid” and “Tropical fruit” (Fig. 4.19A and Table 4.6). Wines made from the machine-harvested TM (-4 ºC) clustered separately on the left side of the PCA biplot ($p < 0.05$) with higher “Reductive” (a descriptor with a negative sensory association), and less of the positive sensory descriptors (“Yellow and Tropical Fruit”, and “Citrus”) and higher “Acidity”, “Body” and “General Quality” (Fig. 4.19A and Table 4.6). Furthermore, wines made from the WG (-20 ºC), MG, some TM (-20 ºC) and all the CJ clustered in the top half of the PCA bi-plot and correlated with the varietal thiol compounds, i.e., 3-SH and 3-SHA, methoxypyrazine compound, i.e., ibMP, and the sensory descriptors, i.e., “Yellow fruit”, “General quality”, “Citrus”, “Herbaceous” and “Body” which were generally higher ($P > 0.05$) for wines made from the pre-fermentative cryogenic treatments than the control wines (Fig. 4.19A and Table 4.6).

In 2021, the wines made from the control grapes (hand-harvested only), the WG (-4 ºC), TM (-4 ºC and -20 ºC) and CJ (-4 ºC and -20 ºC) clustered on the left side of the PCA bi-plot and correlated with the varietal thiol compound, i.e., 4-MSP, the methoxypyrazine compound, i.e., sbMP and the sensory descriptors, i.e., “Herbaceous”, “Reductive”, “Body” and acid (Fig. 4.19B, Table 4.7). Therefore, cryogenic treatments significantly ($p < 0.05$) reduced the reductive character of the control wines (Table 4.7). The wines made from the TM (-4 ºC and -20 ºC), CJ (-20 ºC) and MG (-4 ºC and -20 ºC) clustered to the right of the control wines in the PCA bi-plot and correlated with the varietal thiol compounds (3-SH and 3-SHA), the methoxypyrazine compound (ibMP) and the sensory descriptors, i.e., “Acid”, “Citrus”, “Overall intensity”, “Thiol type”, “Pineapple”, “Body”, “Banana”, “Apple” and “General quality” (Fig. 4.19B and Table 4.7).

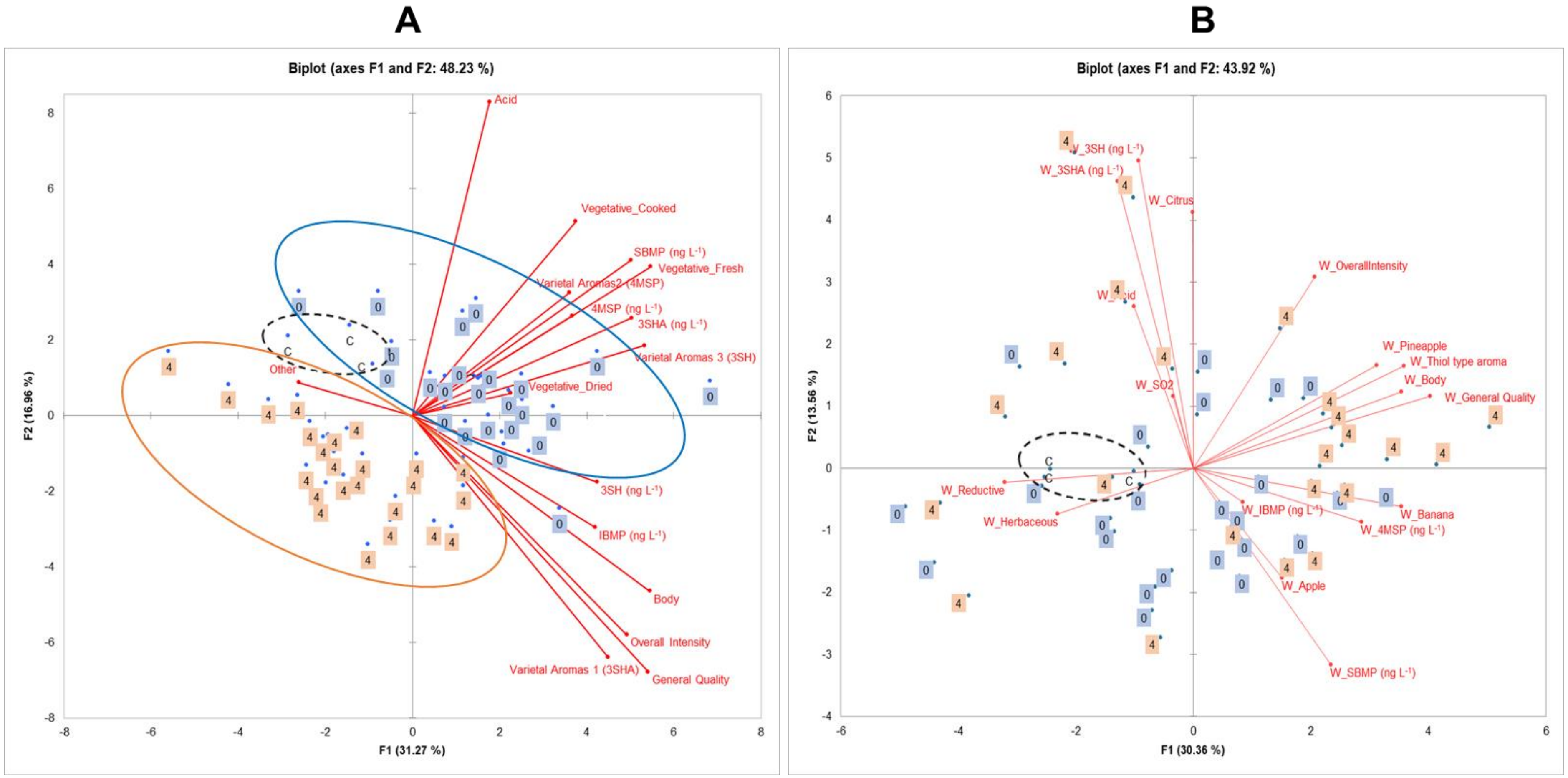



Figure 4.17. PCA bi-plots showing the correlations (indicated by circles around groupings) between the aroma compounds and sensory profiles of wines from Producer A (Stellenbosch wine region), made from control grapes and cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-20º C and -4 C), illustrating the effect of storage time (T0 and T4) as the variable in 2020(A) and 2021(B).

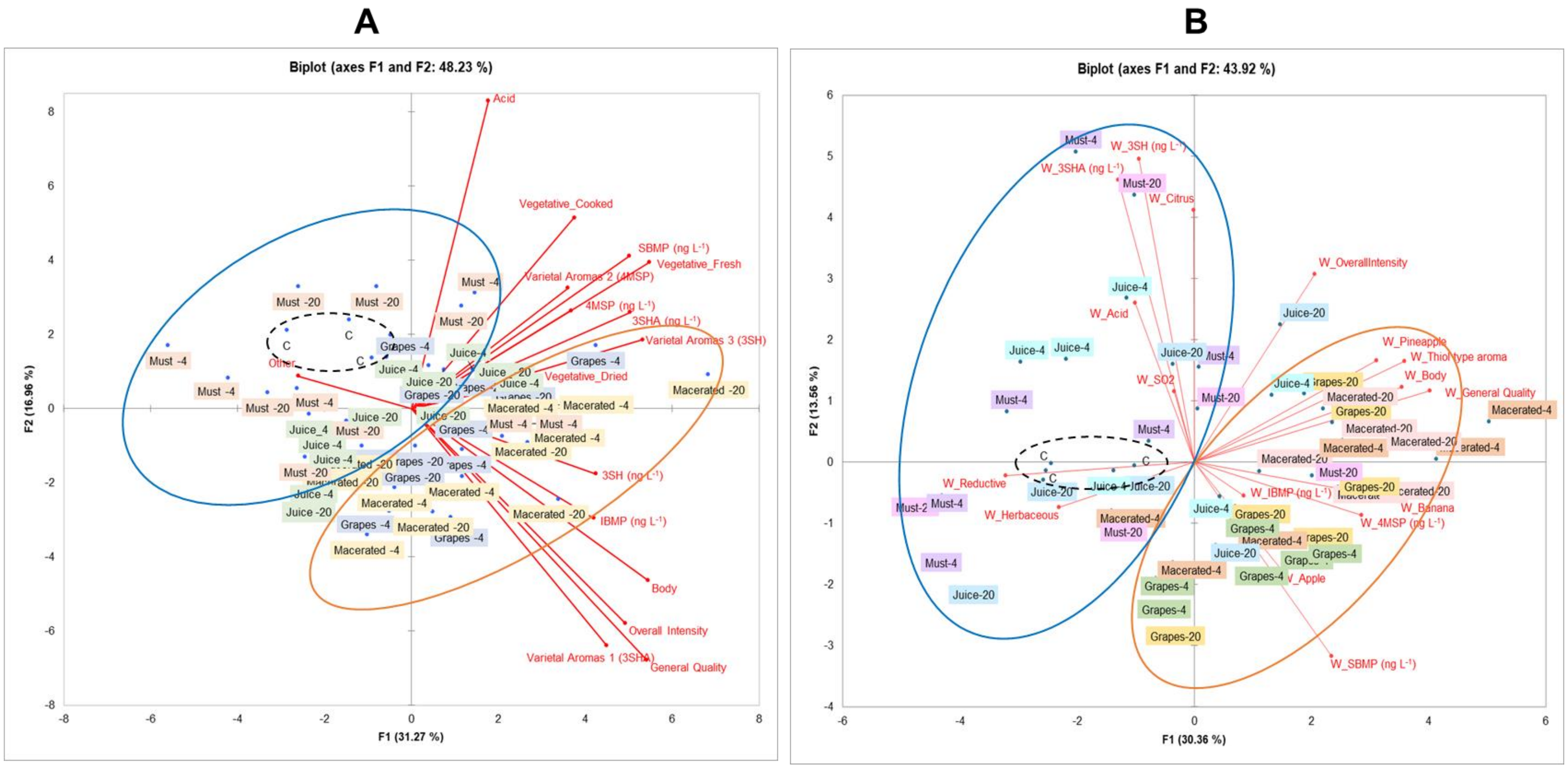



Figure 4.18. PCA bi-plots showing the correlations (indicated by circles around groupings) between the aroma compounds and sensory profiles of wines from Producer A (Stellenbosch wine region), made from control grapes and cryogenically pre-treated Sauvignon blanc subjected to freezing (-20 ºC and -4 ºC), illustrating the effect of production stage (WG, MG, TM and CJ) as the variable in 2020(A) and 2021(B).

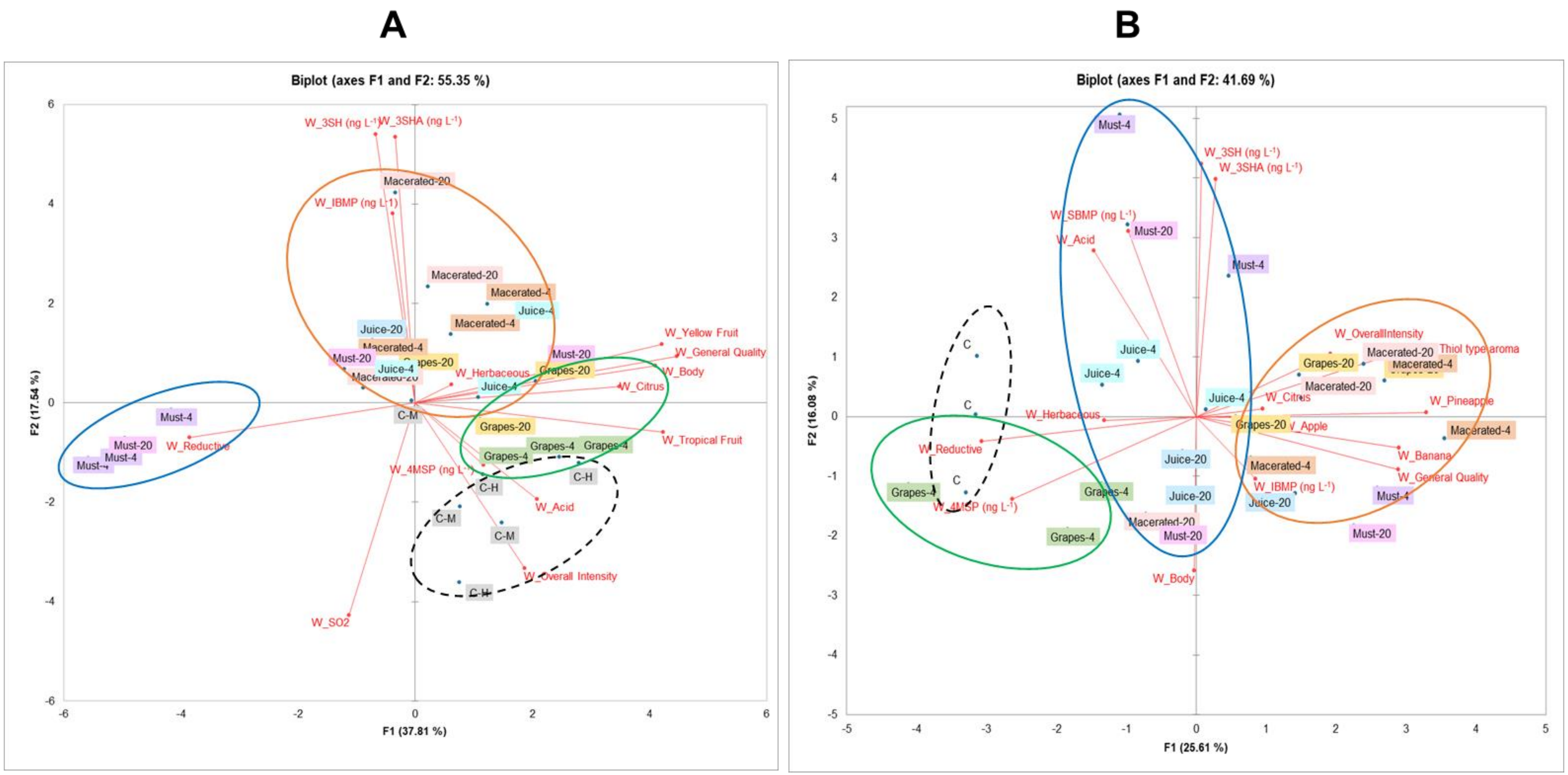



Figure 4.19. PCA bi-plots showing the correlations (indicated by circles around groupings) between the aroma compounds and sensory profiles of wines from Producer B (Durbanville wine region) made from control grapes and from hand-harvested and machine-harvested Sauvignon blanc, illustrating the effect of production stage (WG and MG, TM and CJ), cryogenic temperature (-20 ºC and -4 ºC) and storage time (T4) in 2020(A) and 2021(B).

These results show that the wines made from pre-fermentative cryogenic treatments, when compared to the control, had significantly increased positive sensory descriptors (Fig. 4.19B and Table 4.7). Although similar groupings were observed for the production stages of the grapes, the correlations with the sensory descriptors and aroma compounds differed for 2020 and 2021 (Figs. 4.19A and 4.19B).

Wines made from cryogenically pre-treated grapes from Producer C clustered into two distinct groupings for both vintages (2020 and 2021) when comparing the time period (T0 and T4) over which the cryogenic treatments were applied (Figs 4.20A and 4.20B). Additionally, for 2020 the control wines clustered on their own, whilst for 2021, the control wines clustered with the T0 wines. The control wines for both vintages (2020 and 2021) clustered on the right half of the PCA bi-plot and correlated with the desirable sensory descriptors, i.e., “Varietal thiol aromas 1 (4-MSP)”, “Overall intensity”, “Varietal thiol aromas 3 (3-SH)”, “General quality”, “Other”, “Herbaceous”, “Body”, “Pineapple”, “Citrus”, “Banana”, “Thiol type aroma” and “Apple” (Figs 4.20A and 4.20B, Table 4.9-4.10). The abovementioned wines also correlated with the varietal thiol compound, i.e., 3-SH and the methoxypyrazine compound, i.e., sbMP (Fig. 4.20A). For 2021, the control wines predominantly clustered with the T0 wines and correlated with the varietal thiol compound, i.e., 3-SH, methoxypyrazine compound, i.e., sbMP and sensory descriptors, i.e., “Citrus”, “Pineapple”, “Banana”, “Thiol type aroma”, “Apple” and “Acid” (Fig. 4.20B).

Furthermore, for 2020, the T0 wines predominantly clustered in the right half of the PCA bi-plot and correlated with the varietal thiol compound, i.e., 4-MSP, the methoxypyrazine compound, i.e., ibMP and the sensory descriptors, i.e., “Varietal aromas 2 (3-SHA)”, “Body”, “Vegetative fresh”, “Vegetative cooked” (Fig. 4.20A and Table 4.8). The increased vegetative and 4-MSP aromas associated with immediate cryogenic freezing reflect increased "Green" sensory profiles, typical of cool climate Sauvignon blanc (Allen & Lacey, 1993:35-36; Parr et al., 2013:474). A similar observation was made for 2021, where the control and T0 wines predominantly clustered in the right half of the PCA bi-plot and correlated with the varietal thiol compound, i.e., 4-MSP, methoxypyrazine compound, i.e., sbMP and tropical and sensory descriptors, i.e., “Citrus”, “Pineapple”, “Banana”, “Thiol type aroma”, “Acid” and “Apple” (Fig. 4.20B).

Moreover, for 2020, the T4 wines (-4 ºC and -20 ºC), clustered in the left half of the PCA bi-plot (Fig. 4.20A) and correlated with the varietal thiol compound, i.e., 3-SH, methoxypyrazine compound, i.e., sbMP and the sensory descriptors, i.e., “Acid” and “Vegetative dried”. For 2021, wines made from the abovementioned treatment once again predominantly clustered in the left half of the PCA bi-plot and correlated with the varietal thiol compound, i.e., 3-SHA and sensory descriptors, “Reductive” and “$SO_2$”

(Fig. 4.20B). Although these wines correlated with negative sensory descriptors, they also correlated with a varietal thiol (3-SHA), associated with desirable aroma descriptors when present at acceptable levels (Tables 4.1 & 4.2). Furthermore, in 2020, the T4 wines correlated with desirable sensory descriptors, however, for 2021, these wines correlated with negative sensory descriptors (Figs. 4.20A and 4.20B).

When considering the stage of production for Producer C, (WG, MG, TM and CJ) and the cryogenic temperatures (-20 ºC and -4 ºC), two distinct clusters were seen for the wines for both vintages (2020 and 2021) (Figs. 4.21A-4.21B, Tables 4.8-4.9). In 2020, the control wines clustered on their own, in the top right quadrant of the PCA bi-plot and correlated with the sensory descriptors, i.e., “Other”, “Varietal aromas 1 (4-MSP)”, “Overall intensity”, “Varietal aromas 3 (3-SH)” and “General quality” (Fig. 4.21A). Once again, for Producer C the cryogenic pre-treated WG clustered with the MG and the TM clustered with the CJ. The cryogenically pre-treated WG and MG clustered in the right half of the PCA bi-plot and correlated with the varietal thiol compound, i.e., 3-SHA, methoxypyrazine compound, i.e., ibMP and sensory descriptors, i.e., “Other”, “Varietal aromas 1 (4-MSP)”, “Overall intensity”, “Varietal aromas 3 (3-SH)”, “General quality”, “Varietal aromas 2 (3-SHA)”, “Body”, “Vegetative fresh” and “Vegetative cooked” (Fig. 4.21A).

This indicates that for 2020, wines made from cryogenically pre-treated WG and MG had a higher correlation with the varietal thiol compounds 3-SH and 3-SHA and the sensory descriptors associated with varietal aromas and some vegetative aromas, regardless of the cryogenic temperature and period that it is stored for. In 2021, the cryogenically pre-treated WG clustered with the MG in the top half of the PCA biplot and correlated with the varietal thiol compounds, i.e., 3-SHA and 4-MSP, the methoxypyrazine compound, i.e., ibMP and sensory descriptors, i.e., “Reductive”, “Herbaceous”, “Body”, “Overall intensity”, “General quality” and “Citrus” (Fig. 4.21B). The varietal thiol compounds 4-MSP and methoxypyrazine impart green, herbaceous notes. The control wine, clustered in the bottom half of the PCA bi-plot with the TM and CJ (-4 ºC and -20 ºC) wines and correlated with the varietal thiol compound, i.e., 3-SH, methoxypyrazine compound, i.e., sbMP and sensory descriptors, i.e., “Pineapple”, “Banana”, “Thiol type aroma” and “Apple” therefore, pre-fermentative cryogenic treatments did not improve the tropical aromas over and above the Control wines (Fig. 4.21B). Moreover, for Producer C, the PCA biplots revealed that the region (Cape South Coast) from where the grapes originated and the vintage had the biggest effect on the aromas in the resultant wines, rather than the stage of production and cryogenic treatments (Figs. 4.20A and 4.20B).

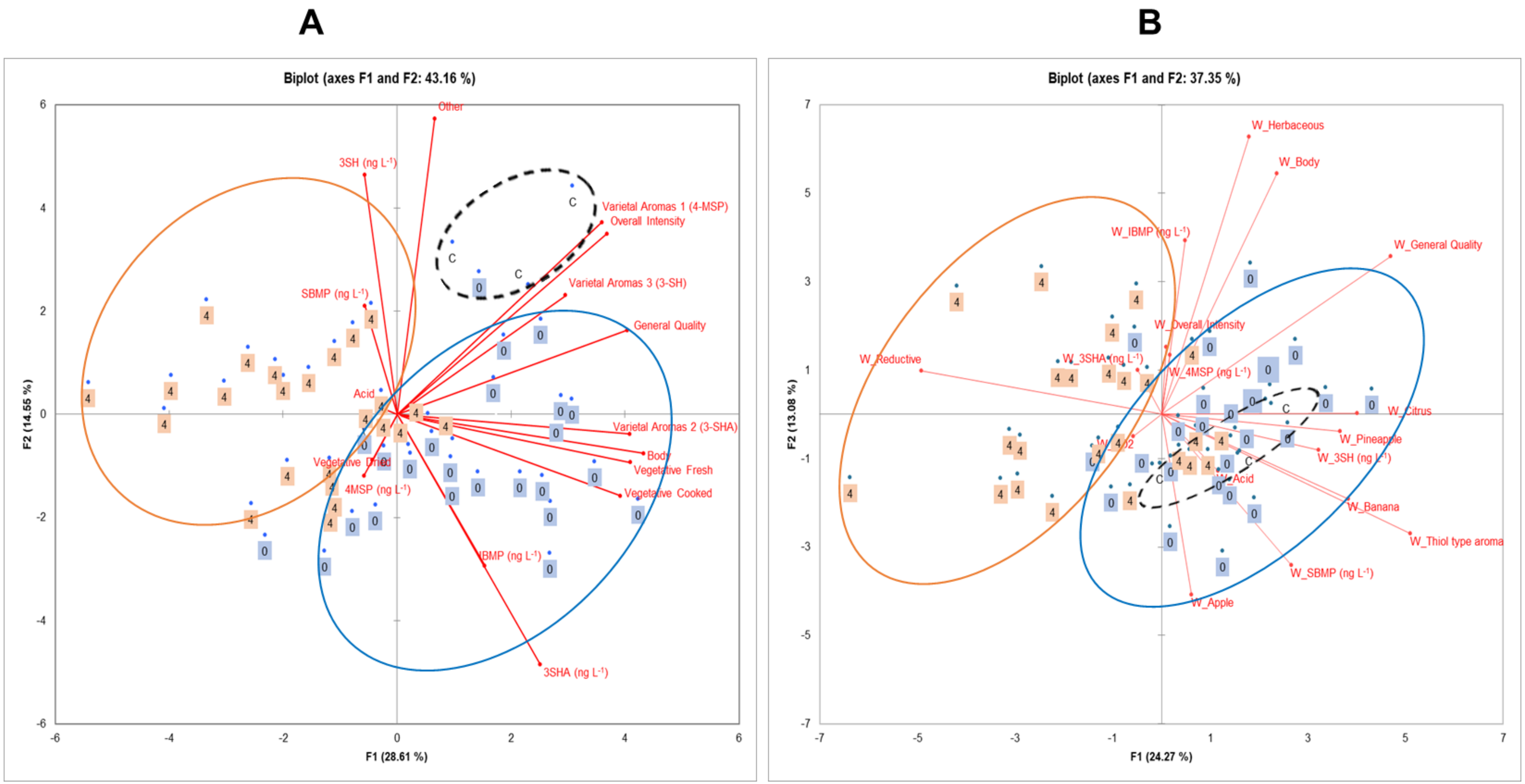



Figure 4.20. PCA bi-plots showing the correlations (indicated by circles around groupings) between the aroma compounds and sensory profiles of wines from Producer C (Cape South Coast), made from control grapes and cryogenically pre-treated Sauvignon blanc WG, MG, TM and CJ subjected to freezing (-20º C and -4º C), illustrating the effect of storage time (T0 and T4) as the variable in 2020(A) and 2021(B).

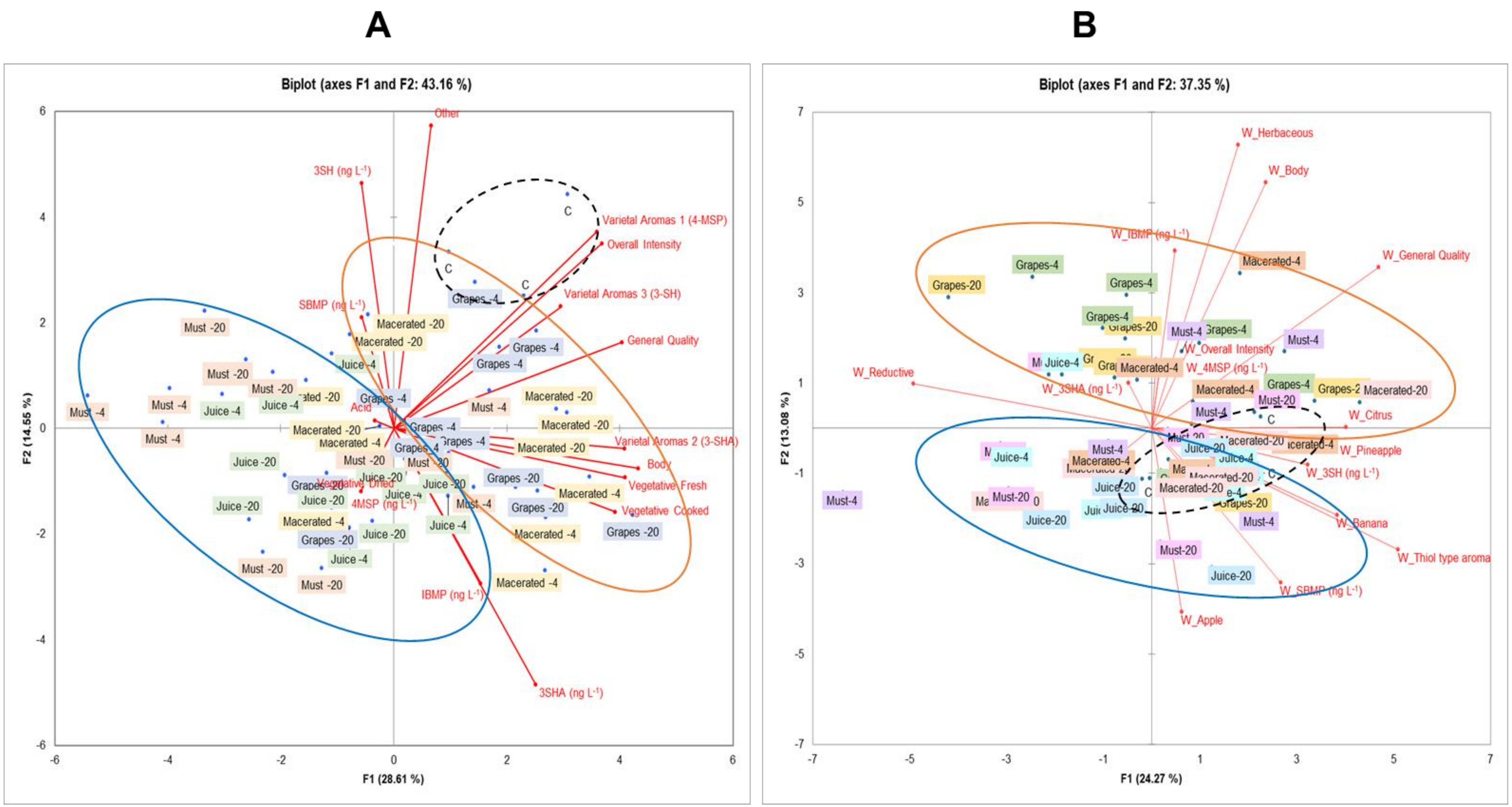
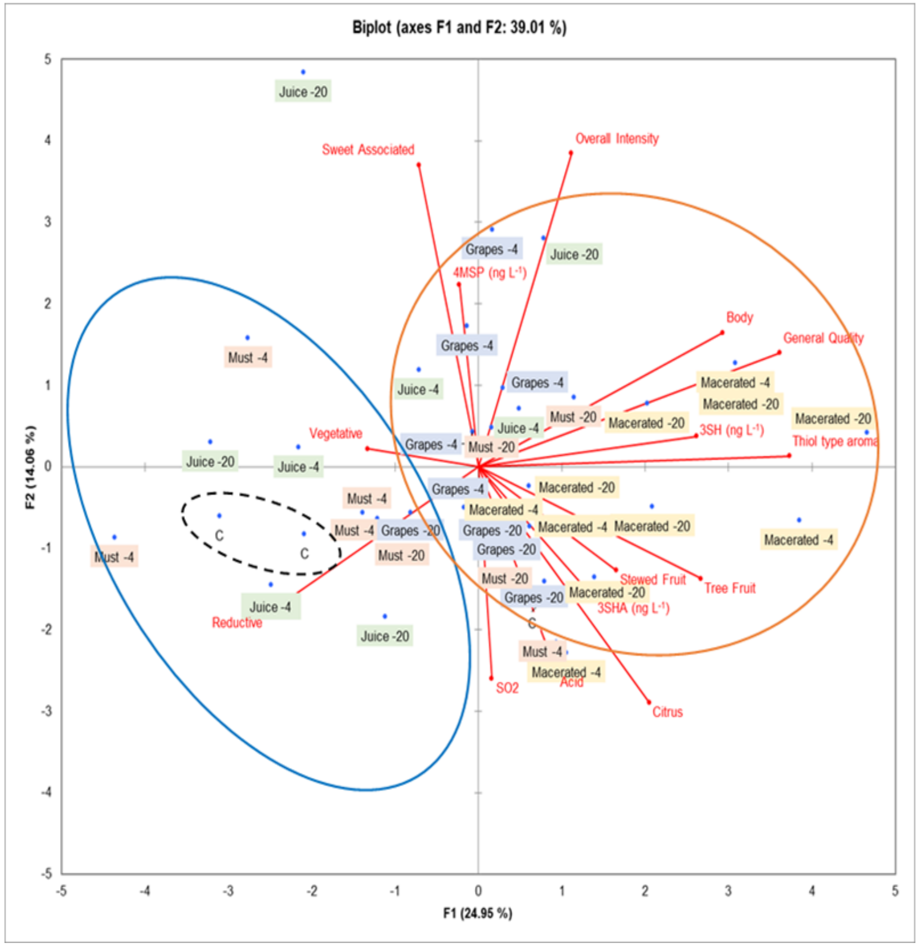



Figure 4.21. PCA bi-plots showing the correlations (indicated by circles around groupings) between the aroma compounds and sensory profiles of wines from Producer C (Cape South Coast wine region), made from control grapes and cryogenically pre-treated Sauvignon blanc subjected to freezing (-20 ºC and -4 ºC), illustrating the effect of production stage (WG, MG, TM and CJ) as the variable in 2020(A) and 2021(B).

A                                                                B



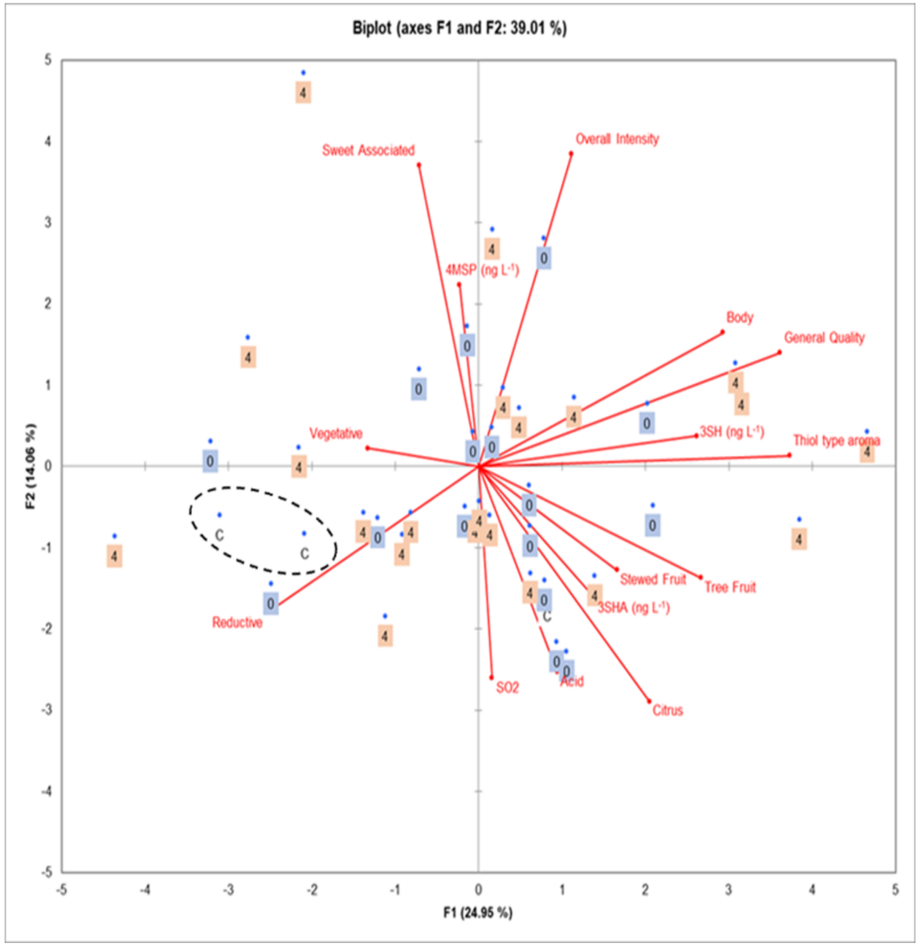




Figure 4.22. PCA bi-plots showing the correlations (indicated by circles around groupings) between the aroma compounds and sensory profiles of wines from Producer D (Stellenbosch wine region), made from control grapes and cryogenically pre-treated Chenin blanc WG, MG, TM and CJ (A) subjected to freezing (-20 ºC and -4 ºC), illustrating the effect of production stage (A) and storage time (B) as the variables in 2021.

In 2021, Chenin blanc grapes from Producer D (Stellenbosch wine region) were divided into the same production stages, storage period (T0 and T4) and subjected to the same cryogenic treatments (-4 ºC and -20 ºC) (Figs. 4.22A and 4.22B). It was observed that the wines were grouped into two distinct clusters when looking at the production stage (Fig. 4.22A). The cryogenically pre-treated WG clustered with the MG and the TM with the CJ. This observation was also seen for wines made from cryogenically pre-treated Sauvignon blanc grapes from other Producers (Figs. 4.18-4.19 and 4.21). When comparing the T0 wines to the T4 wines, no clusters were observed (Fig. 4.22B). This observation was similar to that observed for Sauvignon blanc wines produced for 2021 from Producer A (Stellenbosch wine region). Furthermore, the control wines clustered with the TM and CJ (-4 ºC and -20 ºC) wines in the left half of the PCA bi-plot and correlated predominantly with the varietal thiol compound, i.e., 4-MSP and sensory descriptors, i.e., “Sweet associated”, “Vegetative” and “Reductive” (Fig. 4.22A).

Wines made from the cryogenically pre-treated WG and MG predominantly clustered in the right half of the PCA bi-plot and correlated with the varietal thiol compounds, i.e., 3-SH and 3-SHA and the sensory descriptors, i.e., “Overall intensity”, “Body”, “General quality”, “Thiol type aroma”, “Stewed fruit”, “Tree fruit”, “Citrus”, “Acid” and “$SO_2$” (Fig. 4.22A). These wines displayed sensory descriptors generally associated with Chenin blanc wines (“Stewed fruit”, “Tree fruit”), as well as descriptors generally associated with varietal thiols (“Thiol type aroma” and “Citrus”). The present study confirmed previous research where varietal thiols were detected in Chenin blanc wines, at concentrations above the aroma perception threshold and typically reported range in wine (Wilson, 2017:6-20; 28-38; Coetzee et al., 2018:180-184; Wilson et al., 2018:12-13; Wilson et.al., 2019:634-635) (Table 4.2). Therefore, this indicates that when applying cryogenic pre-treatments to Chenin blanc WG and MG, regardless of the cryogenic temperature and storage period, the wines will have “Tropical” and “Chenin blanc associated” sensory profiles as opposed to the cryogenic pre-treated TM and CJ. The results from this research support those of previous studies where varietal thiols were detected in Chenin blanc wines at levels higher than expected (Wilson, 2017:6-20; Coetzee et al., 2018:180-184).

## 4.4 Conclusion

The diversity of the viticultural regions in South Africa allows for the production of Sauvignon blanc wines with a range of aromatic profiles, including “Vegetative/Grassy”, “Citrus/Grapefruit”, “Tropical”, or “Green apple skin”. Sauvignon blanc grapes cultivated in warmer climatic regions (Stellenbosch, Robertson, and Paarl) tend to produce wines characterized by flavours of “Tropical” and “Yellow fruits”, whereas those grown in cooler climatic regions (Elgin and Cape South Coast) typically result in wines that possess more pronounced spicy and herbaceous aromas, such as "Grass", "Asparagus" and "Green figs" (Marais, 1994:41-44; Coetzee & du Toit, 2012:288-295; Ruiz et al., 2019:7425-7450; Anonymous, 2021).

The observations made from this study, from a sensory perspective, are that in most cases the wines produced from the WG and MG subjected to cryogenic pre-treatment technologies yielded wines with higher tropical, thiol-type, pineapple and banana aromas as well as higher body, general quality and overall intensity when compared to most wines made from the control grapes as well as the remaining cryogenically treated TM and CJ. In terms of the effect of cryogenic treatments on the levels of impact aroma compounds, i.e., varietal thiols and methoxypyrazines, no definitive trend was observed in terms of how each specific cryogenic treatment (temperature and storage time) affected the levels of specific aroma compounds. However, there were vintage effects because in 2020, the varietal thiol levels in most wines were higher compared to 2021, where the methoxypyrazine levels were higher. The aforementioned results support previous research which showed that due to the complex nature of wine, there are major interactions between compounds, leading to suppression or masking of aromas and flavour, i.e., when thiols are present in high enough concentrations, they can suppress the sensory effect of methoxypyrazine compounds and the other way around (King et al., 2011:179).

The production stage at which the cryogenic treatment was applied had the most notable effect, whilst the cryogenic treatment (temperature and storage time) had no consistent effect. For the machine-harvested grapes for 2020, the cryogenically pre-treated MG and CJ, regardless of the temperature, clustered and correlated with the desired varietal thiol compounds, i.e., 3-SH and 3-SHA and the methoxypyrazine (ibMP), indicating that the levels of these compounds in the wines were higher for these treatments. The wines correlated with the sensory descriptors, i.e., "Thiol type aroma", "Yellow fruit" and "Citrus", which are all descriptors known to be associated with varietal thiols (Tominaga et al., 2006:7254-7255; Coetzee et al., 2012:289; Capone et al., 2015:1227; Jeffery, 2016:1324; Coetzee et al., 2018:180-184). The correlations can be attributed to the preservation of these descriptors in the wines from these treatments and the negative performance of the TM treatments. The wines also correlated with "General quality" and "Body", which are favourable sensory descriptors. The hand-harvested WG (-20 ºC and -4 ºC) clustered with the control and correlated with the varietal thiol, i.e., 4-MSP and the sensory descriptors, i.e., "Herbaceous", "Overall intensity" and "Acid". Therefore, this indicates that the aforementioned wines have a "Herbaceous" sensory profile, whilst the wines made from machine-harvested grapes had a "Tropical" sensory profile. These findings corroborate previous research where it was found that wines made from machine-harvested grapes exhibit thiol-related tropical and fruity aromas when compared to wines made from hand-harvested grapes (Allen et al., 2011:10646; Kilmartin, 2012:82-86).

Moreover, again, the vintage played a role because the differences in the sensory profiles were more pronounced for 2020, whilst for 2021, the wines clustered and showed more overlap, with regional differences and the harvesting methods used had less of an impact on the final wine sensory profiles. Enhanced tropical and thiol aromas, greater body and quality, and reduced negative/reductive notes were observed in the sensory profiles of both Sauvignon blanc and Chenin blanc wines when made from cryogenically pre-treated WG and MG. Therefore, within the range of parameters applied in this study, the pre-fermentative cryogenic treatments can be applied to both white wine cultivars with similar outcomes.

It can, therefore, be concluded that cryogenic pre-fermentation treatments applied at the correct stage, before the fermentation process and wine production, have the potential to enhance white wine varietal aromas and the production of unique wines. It is recommended that winemakers apply these treatments to WG or MG, using any of the cryogenic temperatures used in practice (-4 ºC, which is more energy efficient or -20 ºC), subjected to T0 (more economical).

## 4.5 References

Allen, M.S. & Lacey, M.J. 1993. Methoxypyrazine grape flavour: influence of climate, cultivar and viticulture. *Wein-Wissenschaft*, 48(3-6):211-213.

Allen, M.S. & Lacey, M.J. 1998. Methoxypyrazines of grapes and wines. In Waterhouse, A.L. & Ebeler, E.E. (eds.). *Chemistry of Wine Flavour*. Washington DC: American Chemical Society; 31-38.

Allen, T., Herbst-Johnstone, M., Girault, M., Butler, P., Logan, G., Jouanneau, S., Nicolau, L. & Kilmartin, P.A. 2011. Influence of grape-harvesting steps on varietal thiol aromas in Sauvignon blanc wines. *Journal of Agricultural and Food Chemistry*, 59(19):10641-10650. https://doi.org/10.1021/jf2018676.

Anonymous, 2021. White wine varieties grown in South Africa (Wine of South Africa). http://www.wosa.co.za/The–Industry/Varieties–and–Styles/White Wine-Varieties/. [31 March 2021].

Anonymous, 2022. Wine grape Harvest. Retrieved from https://www.thespruceeats.com/wine–grape–harvest–3511325/. [08 March 2022].

Araujo, L.D., Vannevel, S., Buica, A., Callerot, S., Fedrizzi, B., Kilmartin, P.A. & du Toit, W.J. 2017. Indications of the prominent role of elemental sulfur in the formation of the varietal thiol 3-mercaptohexanol in Sauvignon blanc wine. *Food Research International*, 98:79-86. https://doi.org/10.1016/j.foodres.2016.12.023.

Arfelli, G., Sartini, E., Bordini, F., Caprara, C. & Pezzi, F. 2010. Mechanical harvesting optimization and postharvest treatments to improve wine quality. *OENO One*, 44(2):101-115. https://doi.org/10.20870/oeno-one.2010.44.2.1461.

Avizcuri-Inac, J. M., González-Hernández, M., Rosáenz-Oroz, D., Martínez-Ruiz, R. & Vaquero-Fernández, L. 2018. Chemical and sensory characterisation of sweet wines obtained by different techniques. *Ciência e Técnica Vitivinícola*, 33(1):15-30. https://doi.org/10.1051/ctv/20183301015.

Benkwitz, F., Nicolau, L., Lund, C., Beresford, M., Wohlers, M. & Kilmartin, P. A. 2012. Evaluation of key odorants in Sauvignon blanc wines using three different methodologies. *Journal of Agricultural and Food Chemistry*, 60(25):6293 6302. https://doi.org/10.1021/jf300914n.

Campo, E., Ferreira, V., Escudero, A. & Cacho, J. 2005. Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography−olfactometry data. *Journal of Agricultural and Food Chemistry*, 53(14):5682-5690. https://doi.org/10.1021/jf047870a.

Capone, D. L., Ristic, R., Pardon, K. H. & Jeffery, D. W. 2015. Simple quantitative determination of potent thiols at ultra-trace levels in wine by derivatization and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. *Analytical chemistry*, 87(2):1226-1231. https://doi.org/10.1021/ac503883s

Chen, K. & Li, J. 2022. In White Wine Technology. A. Morata (ed.). A glance into the aroma of white wine: Academic Press; 313-326. https://doi.org/10.1016/B978-0-12-823497-6.00018-1.

Chen, L., Capone, D.L. & Jeffery, D.W. 2019a. Analysis of potent odour-active volatile thiols in foods and beverages with a focus on wine. *Molecules*, 24(13):2472. https://doi.org/10.3390/molecules24132472.

Chen, L., Capone, D.L., Nicholson, E.L. & Jeffery, D.W. 2019b. Investigation of intraregional variation, grape amino acids, and pre-fermentation freezing on varietal thiols and their precursors for Vitis vinifera Sauvignon blanc. *Food Chemistry*, 295:637-645. https://doi.org/10.1016/j.foodchem.2019.05.126.

Coetzee, C., Schulze, A., Mokwena, L., Du Toit, W. J. & Buica, A. 2018. Investigation of thiol levels in young commercial South African Sauvignon Blanc and Chenin Blanc wines using propiolate derivatization and GC-MS/MS. *South African Journal of Enology and Viticulture*, 39(2):180-184. http://dx.doi.org/10.21548/39-2-2683.

Coetzee, C. 2020a. *Effect of mechanical harvesting on Sauvignon blanc protein content*. [Blog, 21 January]. https://sauvignonblanc.com/effect-of-mechanical-harvesting-on-sauvignon-blanc-protein-content/ [8 June 2021].

Coetzee, C. 2020b. *Hand harvest vs Machine harvesting? The effect on the volatile thiols*. [Blog, 12 February]. https://sauvignonblanc.com/hand-harvest-vs-mechanical-harvesting-the-effect-on-the-volatile-thiols/ [8 June 2021].

Coetzee, C. & du Toit, W.J. 2012. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. *Food Research International*, 45:287-298. https://doi.org/10.1016/j.foodres.2011.09.017.

Coetzee, C., Schulze, A., Mokwena, L., Du Toit, W. J. & Buica, A. 2018. Investigation of thiol levels in young commercial South African Sauvignon Blanc and Chenin Blanc wines using propiolate derivatization and GC-MS/MS. *South African Journal of Enology and Viticulture*, 39(2):180-184. http://dx.doi.org/10.21548/39-2-2683.

Dankó, T., Szelényi, M., Janda, T., Molnár, B. P. & Pogány, M. 2021. Distinct volatile signatures of bunch rot and noble rot. *Physiological and Molecular Plant Pathology*, 114:101626. https://doi.org/10.1016/j.pmpp.2021.101626.

Du Plessis, C.S. & Augustyn, O.P.H. 1981. Initial study on the guava aroma of Chenin blanc and Colombar wines. *South African Journal of Enology and Viticulture*, 2(2):101-103. https://doi.org/10.21548/2-2-2401.

Ebeler, S.E. & Thorngate, J.H. 2009. Wine chemistry and flavor: looking into the crystal glass. *Journal of Agricultural and Food Chemistry*, 57(18):8098-8108. https://doi.org/10.1021/jf9000555.

Godelmann, R., Limmert, S. & Kuballa, T. 2008. Implementation of headspace solid-phase-microextraction-GC-MS/MS methodology for determination of 3-alkyl-2-methoxypyrazines in wine. *European Food Research and Technology*, 227:449-461. https://doi.org/10.1007/s00217-007-0741-6.

Green, J.A., Parr, W.V., Breitmeyer, J., Valentin, D. & Sherlock, R. 2011. Sensory and chemical characterisation of Sauvignon blanc wine: Influence of source of origin. *Food Research International*, 44(9):2788-2797. https://doi.org/10.1016/j.foodres.2011.06.005.

Harsch, M.J., Benkwitz, F., Frost, A., Colonna-Ceccaldi, B., Gardner, R.C. & Salmon, J.M. 2013. New precursor of 3-mercaptohexan-1-ol in grape juice: thiol-forming potential and kinetics during early stages of must fermentation. *Journal of Agricultural and Food Chemistry*, 61(15):3703-3713. https://doi.org/10.1021/jf3048753.

Hart, R.S., Jolly, N.P. & Ndimba, B.K. 2019. Characterisation of hybrid yeasts for the production of varietal Sauvignon blanc wine–A review. *Journal of Microbiological Methods*, 165:105699. https://doi.org/10.1016/j.mimet.2019.105699.

Hart, R. S., Ndimba, B. K., & Jolly, N. P. 2017. Characterisation of thiol-releasing and lower volatile acidity-forming intra-genus hybrid yeast strains for Sauvignon blanc wine. *South African Journal of Enology and Viticulture*, 38(2):44-155. https://doi.org/10.21548/38-2-1322.

Hart, R.S., Jolly, N.P. Mohamed, G. Booyse, M. & Ndimba, B.K. 2016. Characterisation of Saccharomyces cerevisiae hybrids selected for low volatile acidity formation and the production of aromatic Sauvignon blanc wine. *African Journal of Biotechnology*, 15(38):2068-2081. https://doi.org/10.5897/AJB2016.15388.

Jeffery, D.W. 2016. Spotlight on varietal thiols and precursors in grapes and wines. *Australian Journal of Chemistry*, 69:1323-1330. https://doi.org/10.1071/CH16296.

Jouanneau, S., Weaver, R.J., Nicolau, L., Herbst-Johnstone, M., Benkwitz, F. & Kilmartin, P.A. 2012. Subregional survey of aroma compounds in Marlborough Sauvignon Blanc wines. *Australian Journal of Grape and Wine Research*, 18(3):329-343. https://doi.org/10.1111/j.1755-0238.2012.00202.x.

Kapaklis, A. 2014. Impact of specific volatile thiols on varietal aroma of wines produced from Greek and some international grape varieties. Unpublished Doctoral dissertation, Institute for Phytopathology, Justus Liebig University of for Phytopathology, Justus Liebig University of Giessen.

Kilmartin, P. A. 2012. Machine harvesting versus handpicking: impacts on tropical and green characters in Sauvignon Blanc wines. *Grapegrower & Winemaker:*81-86.

King, E.S., Osidacz, P., Curtin, C., Bastian, S.E.P. & Francis, I.L. 2011. Assessing desirable levels of sensory properties in Sauvignon blanc wines–consumer preferences and contribution of key aroma compounds. *Australian Journal of Grape and Wine Research*, 17(2):169-180. https://doi.org/10.1111/j.1755-0238.2011.00133.x.

Kotseridis, Y., Baumes, R. & Skouroumounis, G.K. 1998. Synthesis of labelled [2H4] *β*-damascenone,[2H2] 2-methoxy-3-isobutylpyrazine, [2H3] α-ionone, and [2H3] *β*-ionone, for quantification in grapes, juices and wines. *Journal of Chromatography A*, 824(1):71-78. https://doi.org/10.1016/S0021-9673(98)00650-5.

Künstler, A., Gullner, G., Ádám, A.L., Kolozsváriné Nagy, J. & Király, L. 2020. The versatile roles of sulfur-containing biomolecules in plant defense-A road to disease resistance. *Plants*, 9(12):1705. https://doi.org/10.3390/plants9121705.

Lacey, M.J., Allen, M.S., Harris, R.L. & Brown, W.V. 1991. Methoxypyrazines in Sauvignon blanc grapes and wines. *American Journal of Enology and Viticulture*, *42*(2):103-108. https://doi.10.5344/ajev.1991.42.2.103.

Lei, Y., Xie, S., Guan, X., Song, C., Zhang, Z. & Meng, J. 2018. Methoxypyrazines biosynthesis and metabolism in grape: A review. *Food Chemistry*, 245:1141-1147. https://doi.org/10.1016/j.foodchem.2017.11.056.

Lund, C.M., Thompson, M.K., Benkwitz, F., Wohler, M.W., Triggs, C.M., Gardner, R., Heymann, H. & Nicolau, L. 2009. New Zealand Sauvignon blanc distinct flavor characteristics: Sensory, chemical, and consumer aspects. *American Journal of Enology and Viticulture*, 60(1):1-12. https://doi.org/10.5344/ajev.2009.60.1.1.

Mafata, M., Stander, M.A., Thomachot, B. & Buica, A. 2018. Measuring thiols in single cultivar South African red wines using 4, 4-dithiodipyridine (DTDP) derivatization and ultraperformance convergence chromatography-tandem mass spectrometry. *Foods*, 7 (9):138. https://doi.org/10.3390/foods7090138.

Magyar, I. & Soos, J. 2016. Botrytized wines–current perspectives. *International Journal of Wine Research*,8*:*29-39. https://doi.org/10.2147/IJWR.S100653.

Makhotkina, O., Herbst-Johnstone, M., Logan, G., du Toit, W. & Kilmartin, P.A. 2013. Influence of sulfur dioxide additions at harvest on polyphenols, C6-compounds, and varietal thiols in Sauvignon blanc. *American Journal of Enology and Viticulture*, 64(2):203-213. https://doi.org/10.5344/ajev.2012.12094.

Marais, J. 1994. Sauvignon blanc cultivar aroma-A review. *South African Journal of Enology and Viticulture*, 15(2):41-45. https://doi.org/10.21548/15-2-2283.

Næs, T., Brockhoff, P.B. & Tomic, O. 2010. Statistics for sensory and consumer science. United Kingdom: John Wiley & Sons, 47-66.

Panzeri, V., Ipinge, H.N. & Buica, A. 2020. Evaluation of South African chenin blanc wines made from six different trellising systems using a chemical and sensorial approach. *South African Journal of Enology and Viticulture*, 41(2):133-150. https://doi.org/10.21548/41-2-3889.

Parenti, A., Spugnoli, P., Calamai, L., Ferrari, S. & Gori, C. 2004. Effects of cold maceration on red wine quality from Tuscan Sangiovese grape. *European Food Research and Technology*, 218:360-366. https://doi.org/10.1007/s00217-003-0866-1.

Parr, W.V., Schlich, P., Theobald, J.C. & Harsch, M.J. 2013. Association of selected viniviticultural factors with sensory and chemical characteristics of New Zealand Sauvignon blanc wines. *Food Research International*, 53(1):464-475. https://doi.org/10.1016/j.foodres.2013.05.028.

Parr, W.V., Valentin, D., Breitmeyer, J., Peyron, D., Darriet, P., Sherlock, R., Robinson, B., Grose, C. & Ballester, J. 2016. Perceived minerality in Sauvignon blanc wine: Chemical reality or cultural construct? *Food Research International*, 87:168-179. https://doi.org/10.1016/j.foodres.2016.06.026.

Peng, C.T., Wen, Y., Tao, Y.S. & Lan, Y.Y. 2013. Modulating the formation of Meili wine aroma by pre-fermentative freezing process. *Journal of Agricultural and Food Chemistry*,61(7):1542-1553. https://doi.org/10.1021/jf3043874.

Peyrot des Gachons, C., Tominaga, T. & Dubourdieu, D. 2002. Sulfur aroma precursor present in S-glutathione conjugate form: identification of S-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon blanc. *Journal of Agricultural and Food Chemistry*, 50(14):4076-4079. https://doi.org/10.1021/jf020002y.

Piano, F., Fracassetti, D., Buica, A., Stander, M., Du Toit, W.J., Borsa, D. & Tirelli, A. 2015. Development of a novel liquid/liquid extraction and ultra-performance liquid chromatography tandem mass spectrometry method for the assessment of thiols in South African Sauvignon blanc wines. *Australian Journal of Grape and Wine Research*, 21(1):40-48. https://doi.org/10.1111/ajgw.12117.

Pinu, F.R., Jouanneau, S., Nicolau, L., Gardner, R.C. & Villas-Boas, S.G. 2012. Concentrations of the volatile thiol 3-mercaptohexanol in Sauvignon blanc wines: no correlation with juice precursors. *American Journal of Enology and Viticulture*, 63(3):407-412. https://doi.org/10.5344/ajev.2012.11126.

Rajchl, A., Čížková, H., Voldřich, M., Lukešová, D. & Panovska, Z. 2009. Methoxypyrazines in Sauvignon blanc wines, detection of addition of artificial aroma. *Czech Journal of Food Sciences*, 27(4):259-266. https://DOI: 10.17221/4/2009-CJFS.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B. & Lonvaud, A. (eds.) 2006. *Handbook of enology: 2nd ed., The Chemistry of Wine Stabilization and Treatments*, (Vol. 2), Chichester, England: John Wiley & Sons Ltd., pp. 241-297.

Roland, A., Schneider, R., Razungles, A. & Cavelier, F. 2011. Varietal thiols in wine: discovery, analysis and applications. *Chemical Reviews*, 111(11):7355-7376. https://doi.org/10.1021/cr100205b.

Ruiz, J., Kiene, F., Belda, I., Fracassetti, D., Marquina, D., Navascués, E., Calderón, F., Benito, A., Rauhut, D., Santos, A. & Benito, S. 2019. Effects on varietal aromas during winemaking: A review of the impact of varietal aromas on the flavor of wine. Applied Microbiology and Biotechnology, 103(18):7425-7450. https://doi.10.1007/s00253-019-10008-9.

Ruiz-Rodríguez, A., Durán-Guerrero, E., Natera, R., Palma, M. & Barroso, C. G. 2020. Influence of two different cryoextraction procedures on the quality of wine produced from muscat grapes. Foods, 9(11):1529. https://doi.org/10.3390/foods9111529.

Ryona, I., Pan, B.S., Intrigliolo, D.S., Lakso, A.N. & Sacks, G.L. 2008. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. cv. Cabernet Franc). *Journal of Agricultural and Food Chemistry*, 56(22):10838-10846. https://doi.org/10.1021/jf801877y.

Santos, H., Augusto, C., Reis, P., Rego, C., Figueiredo, A. C. & Fortes, A. M. 2022. Volatile metabolism of wine grape Trincadeira: impact of infection with *Botrytis cinerea*. *Plants*, 11(1):141. https://doi.org/10.3390/plants11010141.

Schneider, R., Charrier, F., Razungles, A. & Baumes, R. 2006. Evidence for an alternative biogenetic pathway leading to 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one in wines. *Analytica Chimica Acta*, 563(1-2):58-64. https://doi.org/10.1016/j.aca.2006.01.057.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika*, 52(3-4):591-611. https://doi.org/10.2307/2333709.

Sidhu, D., Lund, J., Kotseridis, Y. & Saucier, C. 2015. Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. *Critical Reviews in Food Science and Nutrition*, 55(4):485-502. https://doi.org/10.1080/10408398.2012.658587.

Steel, C.C., Blackman, J.W. & Schmidtke, L.M. 2013. Grapevine bunch rots: impacts on wine composition, quality, and potential procedures for the removal of wine faults. *Journal of Agricultural and Food Chemistry*, 61(22):5189-5206. https://doi.org/10.1021/jf400641r.

Thibon, C., Dubourdieu, D., Darriet, P. & Tominaga, T. 2009. Impact of noble rot on the aroma precursor of 3-sulfanyl hexanol content in *Vitis vinifera* L. cv Sauvignon blanc and Semillon grape juice. *Food Chemistry*, 114(4):1359-1364. https://doi.org/10.1016/j.foodchem.2008.11.016.

Thibon, C., Cluzeet, S., Mérillon, J. M., Darriet, P., & Dubourdieu, D. 2011. 3-Sulfanylhexanol precursor biogenesis in grapevine cells: The stimulating effect of

*Botrytis cinerea*. *Journal of Agricultural and Food Chemistry*, 59:1344-1351. https://doi.org/10.1021/jf103915y.

Thibon, C., Böcker, C., Shinkaruk, S., Moine, V., Darriet, P. & Dubourdieu, D. 2016. Identification of S-3-(hexanal)-glutathione and its bisulfite adduct in grape juice from *Vitis vinifera* L. cv. Sauvignon blanc as new potential precursors of 3SH. *Food Chemistry*, 199:711-719. https://doi.org/10.1016/j.foodchem.2015.12.069.

Tominaga, T., Peyrot des Gachons, C. & Dubourdieu, D. 1998a. A new type of flavor precursors in Vitis vinifera L. cv. Sauvignon blanc: S-cysteine conjugates. *Journal of Agricultural and Food Chemistry*, 46(12):5215-5219. https://doi.org/10.1021/jf980481u.

Tominaga, T., Furrer, A., Henry, R. & Dubourdieu, D. 1998b. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour and Fragrance Journal*, 13(3):159-162. https://doi.org/10.1002/(SICI)1099-1026(199805/06)13:3<159:AID-FFJ709>3.0.CO;2-7.

Tominaga, T., Niclass, Y., Frérot, E. & Dubourdieu, D. 2006. Stereoisomeric distribution of 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate in dry and sweet white wines made from *Vitis vinifera* (Var. Sauvignon Blanc and Semillon). *Journal of Agricultural and Food Chemistry*, 54(19):7251-7255. https://doi.org/10.1021/jf061566v.

Van Breda, V.M., van Jaarsveld, F.P. & van Wyk, J. 2024. Pre-Fermentative Cryogenic Treatments: The Effect on Aroma Compounds and Sensory Properties of Sauvignon Blanc and Chenin Blanc Wine-A Review. *Applied Sciences*, 14(4):1-14. https://doi.org/10.3390/app14041483.

Van Wyngaard, E. 2013. Volatiles playing an important role in South African Sauvignon blanc wines (Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch). http://hdl.handle.net/10019.1/80274.

Vinpro. 2020. *South African Wine Harvest report.* https://chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://vinpro.co.za/wp-content/uploads/2020/05/SA-Wine-Harvest-Report-2020_5May2020.pdf [02 September 2025].

Vinpro. 2021. *South African Wine Harvest report*. https://hrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://vinpro.co.za/wp-content/uploads/2021/05/South-African-Wine-Harvest-Report-2021_full.pdf [02 September 2025].

Williams, M., Khan, W., Ntushelo, N. & Hart, R. 2021. An indigenous *Saccharomyces cerevisiae* yeast strain isolated from Paarl regional Shiraz grapes to enhance Shiraz wine typicity. *OENO One*, 55(2):209-225. https://doi.org/10.20870/oeno-one.2021.55.2.4552.

Wilson, C. L. 2017. Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines (Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch). http://hdl.handle.net/10019.1/101250.

Wilson, C., Brand, J., du Toit, W. & Buica, A. 2018. Interaction effects of 3-mercaptohexan-1-ol (3MH), linalool and ethyl hexanoate on the aromatic profile of South African dry Chenin blanc wine by descriptive analysis (DA). *South African Journal of Enology and Viticulture*, 39(2):1-13. http://dx.doi.org/10.21548/39-2-3165.

Wilson, C., Brand, J., du Toit, W. & Buica, A. 2019. Matrix effects influencing the perception of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) in different Chenin Blanc wines by Projective Mapping (PM) with Ultra Flash profiling (UFP) intensity ratings. *Food Research International*, 121:633-640. https://doi.org/10.1016/j.foodres.2018.12.032.

## APPENDICES:

## APPENDIX A: Consent form

Cape Peninsula University of Technology

**Consent form**

RESEARCH PARTICIPANT CONSENT FORM
Wine Sensory Evaluation
Ms V. van Breda
(Department of Food Science & Technology, Cape Peninsula University of Technology)

Purpose of Research: To evaluate the sensory profile of wines produced from WG, MG, TM and CJ, subjected to freezing (-20ºC) and chilling (-4ºC), immediately and after a T4 storage period prior to winemaking as well as wines produced by standard (Control) winemaking practices.

Specific Procedures to be Used: Sensory evaluation of wines.

Duration of Participation: The sensory evaluation sessions will be divided into three sessions over a period of two to three days, once a year. This will involve a sensory evaluation session of about 1 hour per day in the ARC Nietvoorbij Research cellar or alternative venue as stipulated by the Researcher.

Benefits to the Individual: Participants will have the satisfaction in knowing they have assisted with this research project, the wine industry and economy as a whole, investigating delayed fermentation strategies for fresher wines with improved flavour complexity and superior quality.

Risks to the Individual: The risk in tasting these wine samples will be no greater than tasting wine purchased in the retail market. The wine samples contain alcohol and sulfur (preservative).

Medical Liability: I understand that no financial compensation will be paid to me in connection with any physical injury or illness in the unlikely event of physical injury or illness as a direct or indirect result of my participation in this sensory project.

Confidentiality: Participants are not required to divulge any confidential data.

Voluntary Nature of Participation: You do not have to participate in this research project. If you do agree to participate you can withdraw your participation at any time without penalty.

Human Subject Statement:
If you have any questions about this research project, contact **Ms V. van Breda, Tel: 021-8093039**

______________________________
Researcher's Signature

I HAVE HAD THE OPPORTUNITY TO READ THIS CONSENT FORM, AND ASK QUESTIONS ABOUT THE RESEARCH PROJECT AND AM PREPARED TO PARTICIPATE IN THIS PROJECT.

______________________________ ________________________
Participant's Signature Date

______________________________
Participant's Name (print clearly)

## APPENDIX B: 2020 Sensory evaluation sheet

**Sb9**

## SCORECARD

Judge: ______________________________ Date:_________________

Cultivar: **Sauvignon blanc** Wine no: ______________

Judge the wine on the line scale.

| AROMA (NOSE INTENSITY) | | |
|---|---|---|
| | Undetectable | Prominent |
| **Overall intensity** | ____________________________________________ | |
| **Vegetative - fresh** | ____________________________________________ | |
| **Vegetative - cooked** | ____________________________________________ | |
| **Vegetative - dried** | ____________________________________________ | |
| **Tropical fruit (3SHA)**[1] | ____________________________________________ | |
| **Tropical fruit (4MSP)**[2] | ____________________________________________ | |
| **Citrus** | ____________________________________________ | |
| **Other** .....................** | ____________________________________________ | |

| TASTE (INTENSITY) | | | |
|---|---|---|---|
| **Acid** | Low | ______________________________ | High |
| **Body _** | Thin | ______________________________ | Full |

| GENERAL QUALITY | | | |
|---|---|---|---|
| **General quality** | Poor | ______________________________ | Excellent |

**Comments:** ____________________________________________

**Descriptive term:**

Vegetative (Fresh) - Herbaceous, green cut grass, green bell pepper

(Cooked) - Green beans, asparagus, green olive, artichoke

(Dried) - Hay/straw, tea, tobacco

[1]Tropical fruit (3SHA) -Pineapple, passionfruit, sweet melon, banana, guava, gooseberry, kiwi

[2]Tropical fruit (4MSP) -Box tree (cat urine), broom, blackcurrant, tomato leaf aromas

Citrus - Lime, lemon, sweet grapefruit

Other - Tree fruit (apricot, white peach, green apple)

Dried fruit (strawberry jam, raisin, prune, fig)

Floral (Elderberry)

Flinty, minerality

****Please note, if no "Other" aromas are indicated, mark "0" on the descriptor line**

**APPENDIX C:2020 & 2021 Sauvignon blanc sensory evaluation sheet – modified**

**Sb10**

## SCORECARD

Judge: ________________________ Date:_________

Cultivar: **Sauvignon blanc** Wine no: ___________

Judge the wine on the line scale.

**AROMA (NOSE INTENSITY)**

Undetectable Prominent

**Overall intensity** ______________________________

**Yellow fruit** ______________________________
(e.g. banana, pineapple)

**Tropical fruit** ______________________________
(Thiol type aroma)
(e.g. guava, passionfruit, melon)

**Citrus** ______________________________
(e.g. grapefruit, lemon)

**Herbaceous** ______________________________
(e.g. fresh, vegetative)

**Reductive** ______________________________
(e.g. cooked veg, rotten egg)

**$SO_2$** ______________________________

**TASTE (INTENSITY)**

**Acid** Low ______________________________ High

**Body** Thin ______________________________ Full

**GENERAL QUALITY**

**General quality** Poor ______________________________ Excellent

**Comments:** ______________________________

______________________________

______________________________

**APPENDIX D: 2021 Sensory profiling sheet**

**Sensory profiling of White wines**

**Judge:____________________________________**

Instructions:

1. Evaluate each wine. Please make notes in the comments section below.

2. A brief discussion will follow the screening of each flight of **9** wines.

| WINE NO | COMMENTS |
|---|---|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |
| 9 | |

**APPENDIX E: 2021 Chenin blanc sensory evaluation sheet**

## SCORECARD

**CB**

**WINE #**

Judge: ______________________________ Date:

Cultivar: **Chenin blanc**

Judge the wine on the line scale.

**AROMA (NOSE INTENSITY)**

Undetectable Prominent

**Overall intensity** ______________________________

**Tree fruit** ______________________________
(e.g. Peach, pear, apple)

**Thiol type aroma** ______________________________
(e.g. guava, passionfruit, melon)

**Citrus** ______________________________
(e.g. grapefruit, lemon)

**Sweet Correlated** ______________________________
(e.g. candy, toffee)

**Stewed fruit** ______________________________

**Vegetative** ______________________________
(e.g. fresh, dried, cooked)

**Reductive** ______________________________
(e.g. green apple skin, overripe apple)

**$SO_2$** ______________________________

**TASTE (INTENSITY)**

**Acid** Low ______________________________ High

**Body** Thin ______________________________ Full

**GENERAL QUALITY**

**General quality** Poor ______________________________ Excellent

**Comments:** ______________________________

**CHAPTER FIVE**
**GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS**

## 5.1 General Summary

The aroma profile of wine is an important attribute associated with its quality, often reflecting the style and character of the wine. These aroma profiles are a combination of various volatile compounds, which interact with one another and influenced by the grape variety, growing region, climatic conditions, agricultural practices, winemaking technology, and ageing processes (Swiegers et al., 2006:35; Peng et al., 2013:1542; Wilson et al., 2018:1; He et al., 2023:14). Therefore, this study aimed to determine the effect of pre-fermentative cryogenic treatments of Sauvignon blanc and Chenin blanc WG, MG, TM and CJ on the varietal thiol and methoxypyrazine compound levels in wines made from grapes originating from different regions, stored at two-time intervals and harvested with various techniques (machine-harvested versus hand-harvested).

The general observations made from this study were that the actual pre-fermentative cryogenic temperatures (-4 ºC and -20 ºC) and the storage times (T0 and T4) over which the treatments were applied to WG, MG, TM and CJ did not have a notable effect on the wine chemical and sensory profiles. Cryogenic treatments can be effectively applied to both Sauvignon blanc and Chenin blanc grape cultivars, as similar effects were observed in the chemical and sensory profiles of wines produced from these two white grape varieties. However, differences in terms of varietal thiol concentrations were observed for the Sauvignon blanc wine produced from the different wine regions and between the two vintages (2020 and 2021). The cryogenic treatments that had the most significant impact on the sensory and chemical profiles and the quality of the final wines were those applied to WG and MG.

The observations made regarding the effect of the cryogenic pre-treatment freezing on the physicochemical parameters of the final wines were similar to results from previous research (Peinado et al., 2004:586; Salinas et al., 2005:1529 1530; Carillo et al., 2011:11-12; Naviglio et al., 2018:6-10; Naranjo et al., 2021:1-13). The alcohol, VA, MA, TS, glucose and fructose levels were generally similar in wines made from the unfrozen control and cryogenically pre-treated WG, MG, TM and CJ for both cultivars and across vintages. The physicochemical parameters most affected were the pH, which was higher for wines made from the cryogenic treatments. No trend regarding the impact of the stage of production, cryogenic temperature and storage period on the TA levels was observed, which fluctuated above or below the levels found in the control wines. These results contradicted previous results (Darias-Martıń et al., 2000:484; Zhang et al., 2015:21610-21611; Radeka et al., 2023:6; Gu et al., 2025:3). It was also seen that the harvesting technique had an impact on the TA levels because higher levels were seen in wines from hand-harvested control grapes compared to the machine-harvested control grapes. Furthermore, it should be noted that the type of cryomaceration treatment applied and the stage of production (WG, MG or CJ) used also influence must and wine physicochemical parameters (Zhang et al., 2015:21611; 21621-21622; Naviglio et al., 2018:6-10; Ruiz-Rodríguez et al., 2020:1-13; Naranjo et al., 2021:1-13; Radeka et al., 2023:6; Gu et al., 2025:3).

Overall, in terms of the desired levels of varietal thiols, there was no definite trend regarding which cryogenic temperature, stage of production and storage time would yield the most favourable levels in final wines because in this study, the concentrations differed between these treatments. Furthermore, there are indications that the region from which the grapes originated, the harvesting technique and the vintage played a role in the concentrations of varietal thiols detected in the final wines. It was noted that the concentrations of 3-SHA, 4-MSP and 3-SH detected in the wines exceeded the aroma perception threshold (3-SHA: 4 ng $L^{-1}$; 3-SH: 60 ng $L^{-1}$ and 4-MSP: 0.8 ng $L^{-1}$) for most wines and exceeded the range typically reported in South African Sauvignon blanc, i.e., 3-SHA (23-151 ng $L^{-1}$); 3-SH (178-904 ng $L^{-1}$) and 4-MSP (0-21.9 ng $L^{-1}$) and Chenin blanc, i.e., 3-SHA (5-253 ng $L^{-1}$); 3-SH (99-1124 ng $L^{-1}$) and 4-MSP (n.d.) wines. This result was noteworthy because previous studies did not detect 4-MSP in South African Chenin blanc wines (Coetzee, 2018; Coetzee et al., 2018:180-183; Wilson, 2017:27-38; Wilson et al., 2019:635-640). Cryogenic pre-treatment technologies applied to this grape cultivar, therefore, enhanced the formation of this varietal thiol. The higher thiol levels detected in the Sauvignon blanc wines during the 2020 vintage could be because of the observed higher haze and YAN levels, which previous research showed could influence varietal thiol compound levels. The research showed that the concentration of nitrogen in the grape must influences the formation of volatile and non-volatile varietal compounds during alcoholic fermentation (Bell & Heschke, 2005:255; Vilanova et al., 2007:147;149-151; 2012:127; 2015:5-6).

The concentrations of methoxypyrazines (sbMP and ibMP) were not affected by the cryogenic temperature, stage of production and storage time, however, the vintage appeared to have an impact, which has been highlighted in previous research (Allen & Lacey, 1993:36-37; Allen & Lacey, 1998:31-36; Marais, 1994:44; Ryona et al., 2008:10844-10845; Sidhu et al., 2015:494-499). IbMP concentrations in wine usually range from 2 to 30 ng $L^{-1}$, whilst sbMP concentrations in wine are typically < 10 ng $L^{-1}$, and their respective aroma perception thresholds range between 2-16 ng $L^{-1}$ (Sidhu et al., 2015:494-499; Lei et al., 2018:1142). In this study, the methoxypyrazines were found to be much higher during the 2021 vintage when compared to the 2020 vintage. These findings validate previous research where authors found that methoxypyrazines in grapes and wines are primarily influenced by viticultural conditions, such as the temperature during ripening, berry maturation and the fruit exposure to sunlight and a lesser extent by vinification practices such as maceration (Allen & Lacey, 1993:36-37; Allen & Lacey, 1998:31-36; Marais, 1994:44; Ryona et al., 2008:10844-10845; Sidhu et al., 2015:494-499). Furthermore, the concentrations of methoxypyrazines detected in the wines were directly influenced by the weather conditions as reported in the Vinpro harvest reports for 2020 (warm and dry conditions) and 2021 (cool, wet, and late harvest) (Vinpro, 2020:3; 2021:6-7). Moreover, with impact aroma compounds such as varietal thiols and methoxypyrazines, the ability of one aroma compound to suppress or mask the aroma and flavour of another should also be considered, i.e., high thiols suppressing the sensory effect of methoxypyrazine compounds and the other way around (King et al., 2011:179).

For the 2020 vintage, cryogenically pre-treated machine-harvested MG and CJ, regardless of the temperature, clustered and correlated with the desired varietal thiol compounds, i.e., 3-SH and 3-SHA and the methoxypyrazine compound, i.e., ibMP, demonstrating that the levels of these compounds in the wines were higher for these treatments. The wines mentioned above correlated with the sensory descriptors, i.e., “Thiol type aroma”, “Yellow fruit” and “Citrus”, which are all descriptors known to be associated with varietal thiols (Tominaga et al., 2006:7254-7255; Coetzee & du Toit., 2012:289; Capone et al., 2015:1227; Jeffery, 2016:1324; Coetzee et al., 2018:180-184). The wines also correlated with “General quality” and “Body”, which are favourable sensory descriptors. The hand-harvested WG (-20 ºC and -4 ºC) clustered with the control and correlated with the varietal thiol, i.e., 4-MSP and the sensory descriptors, i.e., “Herbaceous”, “Overall intensity” and “Acid”. Therefore, this indicates that the wines mentioned above have an “Herbaceous” sensory profile, whilst the wines made from machine-harvested grapes had a “Tropical” sensory profile. These findings corroborate previous research, which found that wines made from machine-harvested grapes exhibit thiol-related tropical and fruity aromas when compared to wines made from hand-harvested grapes (Allen et al., 2011:10646; Kilmartin, 2012:82-86).

Furthermore, from a sensory perception, the wines produced from the WG and MG subjected to cryogenic pre-treatment technologies yielded wines with higher tropical, thiol type, pineapple and banana aromas as well as higher body, general quality and overall intensity when compared to most wines made from their unfrozen control grapes and the remaining cryogenically treated turbid must and clear juice. These results were similar to the findings of Giametta et al. (2025:10), who showed that wines made from white grapes cooled with $CO_2$ had an enhanced aroma and improved organoleptic quality when compared to wines made traditionally. The production stage at which the cryogenic treatment was applied had the most notable effect, whilst the temperature and storage time had a negligible effect. Moreover, the vintage also played a role because in 2020, the differences in the sensory profiles were more pronounced, whilst during 2021, the wines overlapped more. Moreover, regional differences and the harvesting methods did not impact the final wine sensory profiles. It can, therefore, be concluded that cryogenic pre-fermentation treatments applied at the correct stage, before the fermentation process and wine production, have the potential for enhancing white wine varietal aromas and the production of wines with unique styles.

## 5.2 Conclusion

In conclusion, indications are that the differences observed in the levels of aroma compounds, i.e., Varietal thiols and Methoxypyrazines, were largely a result of vintage differences and stage of production rather than the cryogenic treatment. Furthermore, freezing as a delayed winemaking strategy at the appropriate stage during winemaking can produce new styles of Sauvignon blanc and Chenin blanc with more intense aromas.

Results from the two vintages for different regions show that the production stage (WG and MG), had the most pronounced effect on the final wine chemical parameters and sensory profiles. There were no clear differences in the final wine chemical parameters and sensory profiles between treatments (T0 and T4) and therefore the application of different cryogenic temperatures for different periods was inconclusive. There were no clear differences in the wine's final chemical parameters and sensory profiles between wines made from the different cryogenic temperatures (-4 ºC and -20 ºC), therefore, the effect of the different cryogenic temperatures was negligible. Furthermore, there was no observed trend as to which of the pre-fermentative cryogenic treatment combinations will yield the most favourable levels of a specific thiol, however, there are indications that the region from which the grapes originated, the harvesting technique, and the vintage played a role in the concentrations of varietal thiols detected in final wines. Compared to the control (no cryogenic treatment), cryogenic pre-fermentative treatments generally enhanced varietal thiol concentrations and wine sensory properties.

## 5.3 Recommendations

Winemakers could use cryogenic pre-fermentative treatments applied to WG or MG, in combination with cryogenic temperatures, i.e., -4 ºC (more energy efficient) or -20 ºC, subjected to immediate freezing (more economical), taking into consideration the influence of region and vintage to achieve the desired outcome. Moreover, because cryogenic treatments have the potential to enhance white wine varietal aromas to produce unique wine styles, it is recommended to also apply these treatments to other white wine cultivars and red wine cultivars to enhance the varietal profiles of the resultant wines. When considering the positive outcomes of cryogenic pre-treatments, the wine industry should also consider modifying equipment to optimise cost reduction, specifically from an energy perspective, for future innovations.

## 5.4 References


Allen, M.S. & Lacey, M.J. 1993. Methoxypyrazine grape flavour: influence of climate, cultivar and viticulture. *Wein-Wissenschaft*, 48(3-6):211-213.

Allen, M.S. & Lacey, M.J. 1998. Methoxypyrazines of grapes and wines. In Waterhouse, A.L. & Ebeler, E.E. (eds.). *Chemistry of Wine Flavour*. Washington DC: American Chemical Society; 31-38. https://doi:10.1021/bk-1998-0714.ch003.

Allen, T., Herbst-Johnstone, M., Girault, M., Butler, P., Logan, G., Jouanneau, S., Nicolau, L. & Kilmartin, P.A. 2011. Influence of grape-harvesting steps on varietal thiol aromas in Sauvignon blanc wines. *Journal of Agricultural and Food Chemistry*, 59(19):10641-10650. https://doi.org/10.1021/jf2018676.

Bell, S.J. & Henschke, P.A. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian journal of grape and wine research*, 11(3):242-295.

Capone, D. L., Ristic, R., Pardon, K. H. & Jeffery, D. W. 2015. Simple quantitative determination of potent thiols at ultra-trace levels in wine by derivatization and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. *Analytical chemistry*, 87(2):1226-1231. https://doi.org/10.1021/ac503883s.

Carillo, M., Formato, A., Fabiani, A., Scaglione, G. & Pucillo, G. P. 2011. An inertizing and cooling process for grapes cryomaceration. *Electronic Journal of Biotechnology*, 14(6) 8-8. https://doi.org/10.2225/vol14-issue6-fulltext-10.

Coetzee, C. 2018. Grape-derived fruity volatile thiols - Adjusting Sauvignon blanc aroma and flavor complexity. *Wines and Vines*:1-11.[Blog, April 2018] https://www.winebusinessanalytics.com/sections/printout_article.cfm?content=197002&article=feature [18 May 2020].

Coetzee, C. & du Toit, W.J. 2012. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. *Food Research International*, 45:287-298. https://doi.org/10.1016/j.foodres.2011.09.017.

Coetzee, C., Schulze, A., Mokwena, L., Du Toit, W. J. & Buica, A. 2018. Investigation of thiol levels in young commercial South African Sauvignon Blanc and Chenin Blanc wines using propiolate derivatization and GC-MS/MS. *South African Journal of Enology and Viticulture*, 39(2):180-184. http://dx.doi.org/10.21548/39-2-2683.

Darias-Martín, J.J., Rodríguez, O., Díaz, E. & Lamuela-Raventós, R.M. 2000. Effect of skin contact on the antioxidant phenolics in white wine. *Food Chemistry*, 71(4):483 487. https://doi.org/10.1016/S0308-8146(00)00177-1.

Giametta, F., Catalano, F., Tanucci, G., Fioschi, G., Paradiso, V.M. & Bianchi, B. 2025. Energy and Quality Assessment in the Cooling of Crushed Bombino Nero Grapes with Indirect Heat Exchange System and Direct Heat Exchange System with $CO_2$. *Sci*, 7(2):42. https://doi.org/10.3390/sci7020042.

Gu, X., Liu, Y., Suo, R., Yu, Q., Xue, C., Wang, J., Wang, W., Wang, H. & Qiao, Y. 2025. Effects of different low-temperature maceration times on the chemical and sensory characteristics of Syrah wine. *Food Chemistry*, 463:141230. https://doi.org/10.1016/j.foodchem.2024.141230.

He, Y., Wang, X., Li, P., Lv, Y., Nan, H., Wen, L. & Wang, Z. 2023. Research progress of wine aroma components: A critical review. *Food Chemistry*, 402:1-17. https://doi.org/10.1016/j.foodchem.2022.134491.

Jeffery, D.W. 2016. Spotlight on varietal thiols and precursors in grapes and wines. *Australian Journal of Chemistry*, 69:1323-1330. https://doi.org/10.1071/CH16296.

Kilmartin, P. A. 2012. Machine harvesting versus handpicking: impacts on tropical and green characters in Sauvignon Blanc wines. *Grapegrower & Winemaker:*81-86.

King, E.S., Osidacz, P., Curtin, C., Bastian, S.E.P. & Francis, I.L. 2011. Assessing desirable levels of sensory properties in Sauvignon blanc wines–consumer preferences and contribution of key aroma compounds. *Australian Journal of Grape and Wine Research*, 17(2):169-180. https://doi.org/10.1111/j.1755-0238.2011.00133.x.

Lei, Y., Xie, S., Guan, X., Song, C., Zhang, Z. & Meng, J. 2018. Methoxypyrazines biosynthesis and metabolism in grape: A review. *Food Chemistry*, 245:1141-1147. https://doi.org/10.1016/j.foodchem.2017.11.056.

Marais, J. 1994. Sauvignon blanc cultivar aroma-A review. *South African Journal of Enology and Viticulture*, 15(2):41-45. https://doi.org/10.21548/15-2-2283.

Naranjo, A., Martínez-Lapuente, L., Ayestarán, B., Guadalupe, Z., Pérez, I., Canals, C. & Adell, E. 2021. Aromatic and sensory characterization of Maturana Blanca wines made with different technologies. *Beverages*, 7(1):10. https://doi.org/10.3390/beverages7010010.

Naviglio, D., Formato, A., Scaglione, G., Montesano, D., Pellegrino, A., Villecco, F. & Gallo, M. 2018. Study of the grape cryo-maceration process at different temperatures. *Foods*, 7(7):107. https://doi.org/10.3390/foods7070107.

Peinado, R.A., Moreno, J., Bueno, J.E., Moreno, J.A. & Mauricio, J.C. 2004. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chemistry*, 84(4):585-590. https://doi.org/10.1016/S0308-8146(03)00282-6.

Peng, C.T., Wen, Y., Tao, Y.S. & Lan, Y.Y. 2013. Modulating the formation of Meili wine aroma by pre-fermentative freezing process. *Journal of Agricultural and Food Chemistry*,61(7):1542-1553. https://doi.org/10.1021/jf3043874.

Radeka, S., Bestulić, E., Rossi, S., Orbanić, F., Bubola, M., Plavša, T., Lukić, I. & Jeromel, A. 2023. Effect of different vinification techniques on the concentration of volatile aroma compounds and sensory profile of Malvazija istarska wines. *Fermentation*, 9(7):676. https://doi.org/10.3390/fermentation9070676.

Ryona, I., Pan, B.S., Intrigliolo, D.S., Lakso, A.N. & Sacks, G.L. 2008. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. cv. Cabernet Franc). *Journal of Agricultural and Food Chemistry*, 56(22):10838-10846. https://doi.org/10.1021/jf801877y.

Ruiz-Rodríguez, A., Durán-Guerrero, E., Natera, R., Palma, M. & Barroso, C. G. 2020. Influence of two different cryoextraction procedures on the quality of wine produced from muscat grapes. *Foods*, 9(11):1529. https://doi.org/10.3390/foods9111529.

Salinas, M.R., Garijo, J., Pardo, F., Zalacain, A. & Alonso, G.L. 2005. Influence of prefermentative maceration temperature on the colour and the phenolic and volatile composition of rosé wines. *Journal of the Science of Food and Agriculture*, 85(9):1527-1536. https://doi.org/10.1002/jsfa.2133.

Sidhu, D., Lund, J., Kotseridis, Y. & Saucier, C. 2015. Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. *Critical Reviews in Food Science and Nutrition*, 55(4):485-502. https://doi.org/10.1080/10408398.2012.658587.

Swiegers, J.H., Francis, I.L., Herderich, M.J. & Pretorius, I.S. 2006. Meeting consumer expectations through management in vineyard and winery. *Wine Industry Journal*, 21(1):34-43.

Tominaga, T., Niclass, Y., Frérot, E. & Dubourdieu, D. 2006. Stereoisomeric distribution of 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate in dry and sweet white wines made from *Vitis vinifera* (Var. Sauvignon Blanc and Semillon). *Journal of Agricultural and Food Chemistry*, 54(19):7251-7255. https://doi.org/10.1021/jf061566v.

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I.S. & Henschke, P.A. 2007. Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. *Applied Microbiology and Biotechnology*, 77:145-157. https://doi.org/10.1007/s00253-007-1145-z.

Vilanova, M., Siebert, T.E., Varela, C., Pretorius, I.S. & Henschke, P.A. 2012. Effect of ammonium nitrogen supplementation of grape juice on wine volatiles and non-volatiles composition of the aromatic grape variety Albariño. *Food Chemistry*, 133(1):124-131. https://doi.org/10.1016/j.foodchem.2011.12.082.

Vilanova, M., Pretorius, I.S. & Henschke, P.A. 2015. Influence of diammonium phosphate addition to fermentation on wine biologicals. In *Processing and impact on active components in Food*. London: Academic Press United Kingdom, 483-491. https://doi.org/10.1016/B978-0-12-404699-3.00058-5.

Vinpro. 2020. *South African Wine Harvest report.* https://chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://vinpro.co.za/wp-content/uploads/2020/05/SA-Wine-Harvest-Report-2020_5May2020.pdf [02 September 2025].

Vinpro. 2021. *South African Wine Harvest report*. https://hrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://vinpro.co.za/wp-content/uploads/2021/05/South-African-Wine-Harvest-Report-2021_full.pdf [02 September 2025].

Wilson, C. L. 2017. *Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines* (Unpublished Doctoral dissertation, Stellenbosch University, Stellenbosch). http://hdl.handle.net/10019.1/101250.

Wilson, C., Brand, J., du Toit, W. & Buica, A. 2018. Interaction effects of 3-mercaptohexan-1-ol (3MH), linalool and ethyl hexanoate on the aromatic profile of South African dry Chenin blanc wine by descriptive analysis (DA). *South African Journal of Enology and Viticulture*, 39(2):1-13. http://dx.doi.org/10.21548/39-2-3165.

Wilson, C., Brand, J., du Toit, W. & Buica, A. 2019. Matrix effects influencing the perception of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) in different Chenin Blanc wines by Projective Mapping (PM) with Ultra Flash profiling (UFP) intensity ratings. *Food Research International*, 121:633-640. https://doi.org/10.1016/j.foodres.2018.12.032.

Zhang, S., Petersen, M.A., Liu, J. & Toldam-Andersen, T.B. 2015. Influence of pre-fermentation treatments on wine volatile and sensory profile of the new disease tolerant cultivar Solaris. Molecules, 20(12):216-21625. https://doi.org/10.3390/molecules201219791.