Pinking of wine – influence of different winemaking processes, causative agents and pinking treatments

by

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Declaration

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Date: 1 March 2021

Summary

Pinking is a slight salmon-red discolouration in certain white wines after some oxygen ingress. This discolouration can lead to some economical loss to the winemaker. This phenomenon was first described in the late 1960s to the early 1970s. In the 50 odd years that this phenomenon is known, only about 4 peer-reviewed scientific articles were ever written with a handful of popular articles. In the first article on pinking, the researcher looked at how to test for pinking susceptibility (PS) as well as the influence of SO_2 and pH on a pinked wine. This article established the method that is now used all over wine-producing countries. The influence of SO_2 was established and the outcome was that the wine needs at least 40 mg/L of SO_2 to protect the wine against PS. It was also established that the change in pH does not influence the pink colour showing that pinking is not associated with anthocyanins. In another article, preventative agents were researched. It was found that PVPP can bind certain compounds, presumably phenolic compounds, and settle them out, decreasing the PS. This research led to a phenomenal increase in the sale of PVPP. Literature indicated the possibility of anthocyanins in white wine and found malvidin-3-O-glucoside to play a role in PS of Siria grapes.

In this study, the influence of temperature, skin contact time and pressing methods were researched on PS. Whole bunch press was tested against crushed & destemmed grapes. Dissolved oxygen (DO) was also measured to deduce the influence of oxygen on PS. The DO of the whole bunch pressed grapes was significantly higher than the crushed & destemmed grapes. This was due to more air space between the berries. Micro oxygenation in the initial stages of the winemaking practices led to a higher PS in the whole bunch pressed wines. The temperature had the highest influence on PS. Cooler (4ºC) grapes also had a significantly higher DO than grapes at ambient (20ºC) temperature. This finding was confirmed by different winemakers which had problems with pinking. All of them picked their Sauvignon blanc grapes at the coolest temperature, keeping them as cool as possible before processing. This and the higher DO in the juice help with the oxidation of the pinking precursors leading to a wine with a high PS. Longer skin contact time leads to a higher potential to pink, but this PS was not significant. When long skin contact time was combined with a cool temperature, the PS increases significantly.

In two articles by Portuguese researchers, the presence of malvidin-3-O-glucoside was established. The theory that all cultivars have the genes to produce anthocyanins, but that the genes are repressed in white wines grapes, could hold some truth. In UV-visible spectrophotometric analysis, the spectra of pinked wines and control wines were analysed. The ranges where the differences in spectra were visible was not associated with any phenolic or anthocyanin compounds. During LC-MS analysis of the peaks showed that the compounds causing PS does not fit any of the known wine phenols. During the LC-MS analysis, the mass

and retention times were given. The masses were compared to the library of the LC-MS and no fits to the mass data were established, thus making the identification of the compounds near impossible.

A sensory analysis was done on pinked wines which are novel work and was never done before. The anecdotal evidence shows that pinked wine does not influence the aroma and taste. This was tested against a trained panel. It was found that the trained panel could not pick up the difference between a pinked wine and a control wine, although some of the tasters could pick up oxidation characters on the aroma. The sensory study showed that the panelists could pick up differences by smell but not on the taste of the wines. This study proved the anecdotal evidence and could lead to further sensory studies in the sensory science of pinked white wines. This study paved the way for further sensory research on pinked wines. The data also showed that panels can now be trained in identifying pink wine as a wine fault or to contribute a new style of wine to the public.

Opsomming

Pienkwording[1] in sommige wit wyne het 'n effense salmrooi verkleuring na aanraking met suurstof tydens sekere wynmaakprosesse. Hierdie verkleuring kan tot 'n ekonomiese verlies vir die wynmaker lei. Hierdie verskynsel is die eerste keer in die laat 1960's tot vroeë 1970's beskryf. In die om en by 50 jare wat hierdie verskynsel bekend is, is daar slegs ongeveer 4 wetenskaplike artikels en 'n handjievol populêre artikels geskryf. Die eerste artikel oor pienkwording het die navorser ondersoek ingestel na die toets metode van pienkwording, asook die invloed van SO_2 en pH op 'n pienk wyn. In hierdie artikel word die metode bepaal wat nou algemeen in die wynproduserende lande gebruik word. Die invloed van SO_2 is vasgestel en die resultaat wys dat die wyn minstens 40 mg/L SO_2 benodig om die wyn teen pienkwording te beskerm. Daar is ook vasgestel dat die verandering in pH nie die pienk kleur beïnvloed nie, wat wys dat pienk nie met antosianiene geassosieer word nie. In 'n volgende artikel is die voorkomende middels teen pienkwording ondersoek. Daar is gevind dat Polivinielpolipirrolidoon (PVPP) die vermoë het om sekere verbindings, wat vermoedelik fenoliese verbindings kan wees, te bind en uit te skei. Hierdie navorsing het gelei tot 'n fenomenale toename in die verkoop van PVPP. Die laaste artikels het die moontlikheid van antosianiene in witwyn ondersoek. Die navorsers het malvidin-3-O-glukosied in Siria-druiwe gevind, 'n kultivar wat in Portugal afkomstig is.

In hierdie navorsing is die invloed van temperatuur, dopkontak en persmetodes ondersoek. Heeltrospers is getoets teen ontstingelde en afgemaalde druiwe. Opgeloste suurstof (DO) is ook gemeet om die invloed van suurstof op pienkwording te meet. Die DO van die heeltrospers was aansienlik hoër as die ontstingelde en afgemaalde druiwe. Dit was as gevolg van meer lugruimte tussen die korrels. Suurstofopname in die beginfase van die wynmaakpraktyke, het gelei tot 'n hoër pienkwording in die heeltrosgepersde wyne. Temperatuur het die grootste invloed op pienkwording gehad. Koeler (4°C) geparste druiwe het ook 'n aansienlik hoër DO gehad as die omgewingstemperatuur (20°C). Hierdie bevinding was bevestig deur verskillende wynmakers wat pienkwordingsprobleme gehad het nadat Sauvignon blanc teen koeler temperature gepars was. Koel temperature en hoër DO in die sap het gehelp met die oksidasie van die pienkwordingsvoorgangers wat gelei het tot 'n wyn met 'n hoë pienkwordingspotensiaal. Dopkontak tyd het gelei tot 'n hoër pienkwordingspotensiaal, maar hierdie pienkwordingspotensiaal was nie betekenisvol nie. Wanneer lang dopkontak gekombineer word met koel temperature, neem die pienkwording aansienlik toe.

[1] Translation of winemaking terms and processes taken from *The South African Trilingual Wine Industry Dictionary*, An initiative of Winetech and SAWIS, 2008.

In twee artikels deur Portugese navorsers was die teenwoordigheid van malvidin-3-O-glukosied vasgestel. Die teorie dat alle kultivars die gene het om antosianiene te produseer, maar dat die gene in witwyndruiwe onderdruk word, kan 'n mate van waarheid wees. In UV-sigbare spektrofotometriese analise is die spektra van pienkwyne en kontrolewyne geanaliseer. Die variasies wat deur die verskille in spektra sigbaar was, het nie verband gehou met enige fenoliese of antosianienverbindings in die wyn nie. Tydens vloeistofchromatografie-massaspektrometrie analiese was die pieke ontleed, maar die aangeduide pieke het nie by enige van die bekende wynfenole gepas het nie.

'n Sensoriese ontleding was op pienk wyne gedoen, wat nuwe werk was en nog nooit tevore gedoen is nie. Die anekdotiese bewyse toon dat pienk wyn nie die aroma en smaak beïnvloed nie. Dit is getoets deur 'n opgeleide proepaneel. Daar is gevind dat die opgeleide proepaneel nie 3die verskil tussen 'n pienkwyn en 'n kontrolewyn op smaak kon opspoor nie, maar dat sommige van die proeërs die pienkwyn op die aroma kon optel. Die opmerkings was dat hulle 'n bietjie oksidasie op die neus kon optel.

This dissertation is dedicated to:
My parents, Joppie and Jeanette Nel.
My wife, Helma, and my two kids, Pieter and Hesmarie, who supported me throughout the difficult years and tolerate a dad which was not always there for them.

Biographical sketch

Anton Pieter Nel was born in Windhoek, Namibia (the old South West Africa) in 1969. He matriculated at Adamantia High School, Kimberley in 1987. After matric, he completed two years of military service in 1 Parachute Battalion. Anton chose a career in the wine industry due to his love for science and nature. During his 14 years in the wine industry, he worked for KWV (1990), was an assistant winemaker at Uitvlucht Wine Cellar, Montagu (1994) and Louwshoek-Voorsorg (1996) before becoming a winemaker at Kango Wine Cellar (2001) in Oudtshoorn.

He obtained a BScAgric-degree at Stellenbosch University in 2007, majoring in Oenology and Viticulture. He enrolled for the MScAgric-degree in Oenology during 2008 at the same institution and obtained his degree in 2010. At that time, he was working for Ernita Nursery in Wellington and became a lecturer in Viticulture at Elsenburg. He is currently working for Cape Peninsula University of Technology (CPUT) as a lecturer in Oenology and Crop Protection. Anton is married to Helma Nel and is the father of two children, Pieter and Hesmarie Nel.

His philosophy in life is "*in vino veritas*" (in wine there is truth) as well as "*Carpe Diem*" (seize the day) as one does not know what will come next.

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I wish to express my sincere gratitude and appreciation to many people who made valuable contributions to the completion of this project. While it would be difficult to mention each person by name, I would like to single out the following people:

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- **Winetech, the National Research Foundation (NRF)** of South Africa, and **THRIP** for funding

Preface

This dissertation is presented as a compilation of 6 chapters. Each chapter is introduced separately and is written according to the style of the South Africa Journal of Enology and Viticulture to which Chapter 2 was submitted for publication.

Chapter 1 **General Introduction and project aims**

Chapter 2 **Literature review**

Pinking in white wines: A review

Chapter 3 **Research results**

Different winemaking processes influencing the pinking potential of Sauvignon blanc

Chapter 4 **Research results**

Causative compound(s) causing pinking in white wines

Chapter 5 **Research results**

Sensory evaluation of pinked Sauvignon blanc

Chapter 6 **General discussion and conclusions**

List of Outputs

The work presented in this dissertation was submitted for publication to peer-reviewed scientific journals, presented at scientific conferences and communicated through the publication of popular articles.

Scientific articles

Nel, A.P., du Toit, W.J. and van Jaarsveld, F.P., 2020. Pinking in white wines – A review. *S. Afr. J. Enol. Vitic.* 41 (2), 151-157. (Chapter 2)

Nel, A.P., du Toit, W.J. and van Jaarsveld, F.P., 2021. Aim to the identification of causal constituents of pink discolouration in white wines. *S. Afr. J. Enol. Vitic.* Currently under review and preliminary approved (Chapter 4)

Nel, A.P., du Toit, W.J. and van Jaarsveld, F.P., 2021. Sensory evaluation of pinked Sauvignon blanc. *S. Afr. J. Enol. Vitic.* Currently under review and preliminary approved (Chapter 5)

Conference participation

Oral presentations

Nel, A.P., du Toit, W.J. and van Jaarsveld, F.P., 2016. Pinking potential of South African Sauvignon blanc. *SASEV International Conference*, 23-25 August 2016, Lord Charles Hotel, Somerset West

Nel, A.P., du Toit, W.J. and van Jaarsveld, F.P., 2017. The mystery of the pinking wine. Skype workshop, Italian workshop on pinking.

Nel, A.P., du Toit, W.J. and van Jaarsveld, F.P., 2020. Oenological practices that may lead to pinking in Sauvignon blanc. *42nd SASEV Virtual Conference*. 3-5 November 2020, South Africa

Popular articles

Nel, A.P. How to reduce the pinking potential of white wines. *Wineland Technical,* February 2017, 81-82.

Nel, A.P. & van Jaarsveld, F., 2018. Die pienking potensiaal van witwyne. *Wineland Technical*, March 2018, 70–71.

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Chapter 1

General introduction and project aims

1. General introduction and project aims

1.1 PROBLEM STATEMENT

The term pinking describes the infrequent but troublesome, subtle discolouration or undesirable pinkish blush that sometimes develops during the production or storage of white wine. White wine turns pink due to the ingress of oxygen. The pinking phenomenon can even occur in young white wines carefully protected from oxidation, therefore the introduction of protective procedures in the white winemaking process does not guarantee a wine with no pinking susceptibility. Pinking can be slight or intense; in both cases, the wine will be commercially unacceptable. Cultivars that were reported to pink are Sauvignon blanc, Thompson Seedless, Semillon and Chenin blanc (Simpon *et al.*, 1982; Tobe, 1983). While the problem is not new (it was first mentioned by Singleton & Esau in 1969), it is not easy to find reports describing curative treatments. Additionally, wines identified as having a potential for pinking can be pre-treated with polyvinylpolypyrrolidone (PVPP) as the current method of choice to prevent pinking (Lamuela-Raventós *et al.*, 2001), however, PVPP is costly and therefore not a routine addition to white winemaking to manage the problem.

Although the cause of pinking is still speculative, the physico-chemical properties of the pink deposited material indicate that these materials are unstable flavonoid phenols (Simpson *et al.*, 1982). The means for reducing the likelihood of pink colour development will include treatments to reduce the quantities of components capable of producing pink colour. Therefore, the identification of the compounds responsible for pinking will also be attempted. Although Sauvignon blanc is not the only cultivar that pink, it is the most widely reported on in this regard and will be the cultivar focussed on in this study.

1.2 RATIONALE

The pinking phenomenon can appear even with the introduction of protective procedures in the white winemaking process, such as the use of sulphites at crush, low-temperature fermentation, and the use of inert gas (argon, nitrogen or carbon dioxide). Some reports suggest that aerobic juice handling can reduce the pinking of finished wines, however, induced changes in flavour generally are not desirable.

Browning and pinking are two different phenomena. Both occur as a result of contact with or exposure to air or atmospheric oxygen, however, browning may occur in a wine in the absence of pinking. In some white wines, where both phenomena are observed, pinking is observed after slight exposure to oxygen, with browning occurring after further oxidation (Simpson, 1980). Therefore, the precursor(s) of the pink chromophore is the first component(s) to oxidise and does so before browning. In contrast to browning, which is associated with more

gross oxidation and undesirable changes in odour and taste, pinking does not seem to influence odour/flavour sensory characteristics (Simpson, 1977; Simpson *et al.*, 1982).

The cause of pinking is still speculative, attributed to several possible chromophores, implicating unstable flavonoid phenols, including astilbin and engeletin (Trousdale & Singleton, 1983), and the chemical degradation of small quantities of procyanidins in the presence of oxygen to form anthocyanidols and the appearance of a pink colour (Trousdale & Singleton, 1983). Oxidative enzymes, for example, polyphenol oxidase (PPO), could be linked to pinking (Vaimakis & Roussis, 1993). 2-S-glutathionyl-caftaric acid derivatives may be linked to pink chromophores (van Wyk et al., 1976).

Reported pre- or post-pinking treatments for the removal of pink materials and their precursors in must/wine utilise fining agents (such as nylon, casein, bentonite, kieselsol, patatin and PVPP), antioxidants (such as ascorbic acid), or chelating agents (such as diethyldithiocarbamate), as well as mannoproteins, and dehydrated yeast cells (Gambuti *et al.*, 2016; Lamuela-Raventós *et al.*, 2001). PVPP, although not necessarily always the best, remains the method of choice if the pros and cons of each method is considered. PVPP, however, is costly, therefore it is important to screen wines for pinking potential and treat only these wines. The concentration of PVPP to be used needs to be optimised depending on the grape variety and the PS of the wine.

Factors affecting pinking susceptibility could include light, oxidising agents (i.e. hydrogen peroxide (H_2O_2)), time of storage (linear increases over 24h, and decreasing after maximum pinking at three days upon induced pinking), temperature, SO_2 concentration, grape variety (some varieties demonstrate greater susceptibility to oxygen and are more inclined to pinking (Day *et al.*, 2010)), vintage, region (considerable regional variation to the susceptibility for pinking was observed), fungal enzymes and oenological conditions. Oenological conditions affecting pinking: Treatments to protect the wine against oxidation such as cold fermentation and inert gas could induce pinking; reductive musts are more sensitive to pinking than oxidised musts (Day *et al.*, 2010); oxygenation during wine preparation and storage (Simpson, 1977); Macerated Grape skin contact time with varietal differences to pinking susceptibility have been reported (Simpson, 1977); and others, including glucose-oxidase treatment of juice (Pickering *et al.*, 1999).

This project formed part of a collaboration between the Wine Chemistry groups at ARC Infruitec-Nietvoorbij and Stellenbosch University (Departments of Viticulture and Oenology, and Chemistry) and formed part of a Doctoral study for which industry and THRIP funding has been allocated.

1.3 AIMS AND OBJECTIVES

The aims of this study is to find the winemaking and viticultural pactices that may influence pinking susceptibility in white wines. The identification of compound(s) that may influence pinking susceptibility will also be attempted. This study also aim to test the hypothesis that panelists cannot identify a pink wine on taste and aroma alone, while, also testing the panelists ability to identify pink wine as a wine fault.

This study have several objectives:

(i) Determine the effectiveness of different fining agents and related products, added during different winemaking stages, on the prevention of pinking,

(ii) Determine the effectiveness of antioxidants such as ascorbic acid in conjunction with SO_2 as curative agents for the prevention of pinking.

(iii) Investigate winemaking processes that affect the pinking susceptibility of white wine.

(iv) Provide practical guidelines for the prevention of pinking to enable winemakers to establish pinking risk practices;

(v) Identify the compounds responsible for pinking.

(vi) Assess panelists ability to identify the pink wine on aroma and taste, as well as their ability to identify pinked and oxidised wines as wine faults.

1.4 METHODOLOGY

The study will be approached as follows:

(i) Different winemaking pactices will be investigated by making wine form grapes that show high pinking susceptibility. Pinking susceptibility, metal and phenol analyses will be performed to find correlations to pinking.

(ii) Different fining agents and related products used in winemaking and their possible influence on pinking will be investigated (29 different fining agents that are commercially available in the wine industry are going to be tested at different winemaking stages, i.e. settling, fermentation and fining).

(iii) Antioxidants used in winemaking, such as ascorbic acid and SO_2, and their effectiveness as pinking preventative treatments, and at different ratios, under standard, oxidative and reductive winemaking conditions, will be investigated to prevent or avoid unwanted oxidation reactions and pinking.

(iv) Attempting the isolation of the pinking compounds in South African wines by solid-phase extraction and attempting to characterise these compounds by LC-MS, GC

and ICP-OES. Wines that show pinking potential will be divided into 3-4 groups. One wine will be kept as a control (C), one will be pinking induced (PI), one wine will be pinked naturally (NP) and one will be pinked and exposed to sunlight to remove the pink colour of the wine. These samples will then undergo the abovementioned analyses.

(v) If the compounds have been identified with LC-MS, develop a routine LC-UV method for identifying pinking compounds in wine/must samples in future.

Laboratory-scale trials will be performed on young Sauvignon blanc commercial wines, and juice samples where applicable. Cellar trials will also be performed where oenological treatments are involved that cannot be performed at a laboratory scale.

During the course of this study, the following will be dedicated to a continued literature survey, optimisation of assays for pinking potential, commissioning of analytical instrumentation, the investigation of different fining agents and related products used in winemaking and their possible influence on pinking, and antioxidants used in winemaking and their effectiveness as pre-pinking treatments. To investigate winemaking processes that could induce pinking in white wine. Commercial-scale trials of selected treatments, and to provide practical guidelines for the prevention of pinking that can be used by cellars to establish pinking risk practices, and to attempt to develop a routine LC-UV method for identifying pinking compounds in the wine/must samples (if the pinking chromophore is a phenol).

All experiments will be done in triplicate where possible. Standard small-scale white wine vinification techniques refer to are those at Nietvoorbij. Wherever reductive winemaking processes are used, the available techniques will be adapted to work as reductive as possible. Wines will be analysed for their ability to pink according to the SASEV Laboratory Manual (2002), and chemical analyses will also be performed at Central Analytical Facilities (CAF) (Stellenbosch University, Stellenbosch, Western Cape, South Africa) to keep consistency and accuracy of the highest order. These analyses will include standard wine analyses (WineScan), LC-MS, GC-MS, and ICP-OES. Although Sauvignon blanc is not the only cultivar that pink, it is the most widely reported on in this regard and will therefore be the cultivar focussed on in this study.

1.5 LITERATURE CITED

Day, M.P., Aagaard, O. and Waters, E.J., 2010. Dissolved oxygen measurements during processing in the winery. Blair, R.J., Lee, T.H. and Pretorius, I.S. (Eds.). Proceedings of the 14th Australian wine industry technical conference; 3–8 July 2010; Adelaide, SA, Australia (The Australian Wine Industry Technical Conference: Urrbrae, SA, Australia), 379–380.

Gambuti, A., Rinaldi, A., Romano, R., Manzo, N. And Moio, L., 2016. Performance of a protein extracted from potatoes for fining of white musts. Food Chem. 190, 237–243.

Lamuela-Raventós, R.M., Huix-Blanquera, M. and Waterhouse, A.L., 2001. Treatments for Pinking Alteration in White Wines. Am. J. Enol. Vitic. 52 (2), 156–158.

Pickering, G.J., Heatherbell, D.A. and Barnes, M.F., 1999. The Production of Reduced-Alcohol Wine Using Glucose Oxidase Treated Juice. Part I. Composition. Am. J. Enol. Vitic. 50 (3), 291–298.

SASEV, 2002. Methods of analysis for wine laboratories. South African Wine Laboratory Association, Stellenbosch

Simpson, R.F., 1977. Pinking in Australian white table wines. Australian wine, brewing and spirit review. 97 (1), 56–58.

Simpson, R.F., January 1980. Some aspects of oxidation and oxidative browning in white table wines. The Australian Grapegrower & Winemaker. 20–21.

Simpson, R.F., Miller, G.C. and Orr, G.L., 1982. Oxidative pinking of white wines: recent observations. Food Techn. Austr. 34 (1), 44–47.

Singleton, V.L. and Esau, P., 1969. Phenolic substances in grapes and wine and their significance. Adv. Food Res. Suppl. 1, 1–282.

Tobe, S.J., 1983. Pinking of table wines from white grapes. Unpublished Master's thesis. University of California, Davis.

Trousdale, E.K. and Singleton, V.L., 1983. Astilbin and engeletin in grapes and wine. Phytochem. 22 (2), 619-620.

Vaimakis, V. and Roussis, L., 1993. Must oxygenation and polyphenoloxidase inhibition and the oxidation of white wine. Lebensm. Wiss. Technol. 26(2), 133–137.

Van Wyk, C., Louw, A. and Rabie, M., 1976. The effect of reductive winemaking conditions on wine quality and composition. In Proceedings of the 11th International Oenological symposium, June 3-5, Sopron, Budapest, Hungary. 180–200.

Chapter 2

Pinking in white wines: A review

2. Pinking in white wines: A review

2.1 INTRODUCTION

The first incidence of pinking in white wines was reported by Singleton and Esau in 1969. This led to a series of research articles on pinking ranging from 1977 to 1983 by an Australian researcher, Dr Bob Simpson (1977a, b; 1980a, b; Simpson *et al.*, 1982, 1983). This was followed by a research article on the use of polyvinyl polypyrrolidone (PVPP) by Lamuela-Raventós *et al.* (2001) and two articles on the presence of anthocyanins by Andrea-Silva *et al.* (2014) & Cosme *et al.* (2019). This literature review reports on the findings of Simpson and other researchers that investigated pinking susceptibility in white wines.

Simpson (1977a) defines pinking as “the troublesome discolouration” that develops during the storage of white wines. He later adds that it develops over several days, but most likely after vinification or when the wine is no longer protected by a CO_2 blanket. This led to the discovery that pinking occurs after contact with air (Simpson, 1980a). In 1982, Simpson stated that “white wines develop a pink colouration on exposure to air”. Andrea-Silva *et al.* (2014) defined pinking as “the appearance of a salmon-red blush in white bottled wines produced exclusively from white varieties”. Therefore, a comprehensive definition of pinking could be established, as follows:

Pinking, or oxidative pinking, is the slight discolouration of white wines from a pinkish to a salmon-red blush colour, affected by certain oenological processes before and after the fermentation until storage during which the wine could come into contact with air.

Different cultivars have been reported to show some degrees of pinking susceptibility. In America, the white cultivars reported are Thompson Seedless, Semillon, Sauvignon blanc, Chardonnay and Chenin blanc (Tobe, 1983; Jones, 1989). In Australia, the cultivars reported to be prone to pinking are Muscat Gordo Blanco, Sultana, Palomino, Riesling, Doradillo and Crouchen (Simpson, 1977a). In Spain, the cultivars Sauvignon blanc, White Riesling, Chardonnay, Albariño, Macabeo, Xarel·lo, Parellada, Garnatxa Blanca (Grenache) and Verdejo were reported to have the potential to pink (Lamuela-Raventós *et al.*, 2001). In Portugal, the cultivars reported was Síria (Andrea-Silva *et al.*, 2014), and in the Czech Republic, it was Pinot blanc, Pálava, Pinot Gris, Sauvignon blanc, Grüner Veltliner and Chardonnay (unpublished data). This is an extensive list of white cultivars. According to Simpson (1977a, b), Tobe (1983), Jones (1989), Lamuela-Raventós *et al.* (2001) and Andrea-Silva *et al.* (2014), the predominant cultivar that shows susceptibility to pinking is Sauvignon blanc. Winemakers must take note of this when producing Sauvignon blanc. Although these cultivars showed a tendency to pink, regional variations and yearly differences also influence the potential of these wines to pink (Simpson, 1977a; Andrea-Silva *et al.*, 2014).

Wines made by winemaking practices such as cooling of the must, cold fermentation and the use of inert gasses (Ar, N_2 and CO_2) show higher susceptibility to pinking (Singleton & Esau, 1969; Simpson *et al.*, 1982). This led to the assumption that air contact or O_2 increases pinking susceptibility (Simpson, 1980b). Other factors such as storage temperatures, the presence of light, free SO_2 content and the pH of the wine also play a role in pinking susceptibility (Simpson, 1977a; Simpson *et al.*, 1982). With the influence of light came the suggestion wine must not be bottled in clear glass bottles, but rather in green or dark green bottles (Lamuela-Raventós *et al.*, 2001). Anecdotal evidence also suggests that pinking does not affect the aroma or taste of white wines (Simpson, 1980b & Lamuela-Raventós *et al.*, 2001), but this was never proven scientifically.

Simpson (1980b) states that there is “good evidence” that the compounds causing pinking have their origin in phenolics. This led to a worldwide belief that phenols cause pinking in white wines (Jacobson, 2006; Jackson, 2014, 2016), and the use of polyvinylpolypyrrolidone (PVPP) should be used for their removal (Lamuela-Raventos *et al.*, 2001).

2.2 MEASUREMENT OF PINK SUSCEPTIBILITY

2.2.1 Development of an assay

Simpson (1977a, 1980b) did extensive studies on the pinking susceptibility of white wines and the analysis thereof. Spectrophotometric studies on normal white wines and wines with a visible pinking showed a distinctive bump over the 500 nm absorbency range (Figures 2.1 & 2.2). Therefore, because the greatest differences occurred at an optical density of 500 nm, this wavelength was chosen as a suitable wavelength for testing for pinking susceptibility.

The absorbance of normal white wine, therefore, will have a smooth curve at 500 nm, but a white wine with a visible pink colouration will show an absorbency at 500 nm. Thus, when white wine is tested for pinking susceptibility, two samples of the wine are taken. One will be the control and the other the treatment. The control sample is determined first at 500 nm, followed by the pink induced sample. The difference between the two samples will show the pinking susceptibility of the wine. Simpson found that light coloured wines will show a pinking susceptibility of 5 (0.005 AU x 10^3), and darker coloured white wines will have a pinking susceptibility above 10 (0.01 AU x 10^3). With darker coloured white wines, Simpson meant wines that border on a more yellowish colour.

Wines that show a tendency to browning rather than pinking will show a greater absorbency at 420 nm. At the wavelength of 420 nm, there will be no interference from the pink colouration. Both pinking and browning therefore can be measured in white wines.

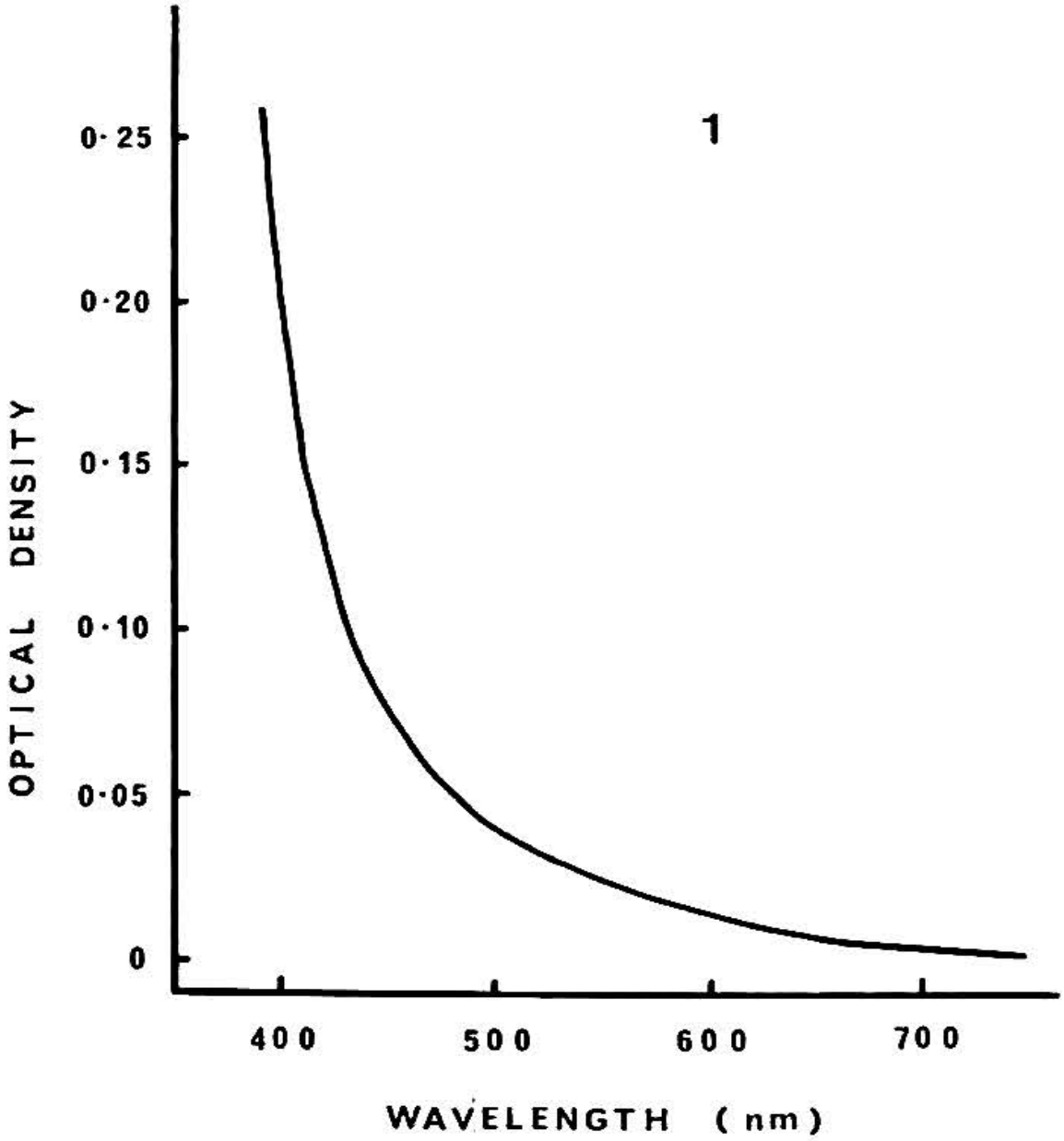

Fig. 2.1: Spectrum of a wine showing no pinking (adopted from Simpson, 1977a).

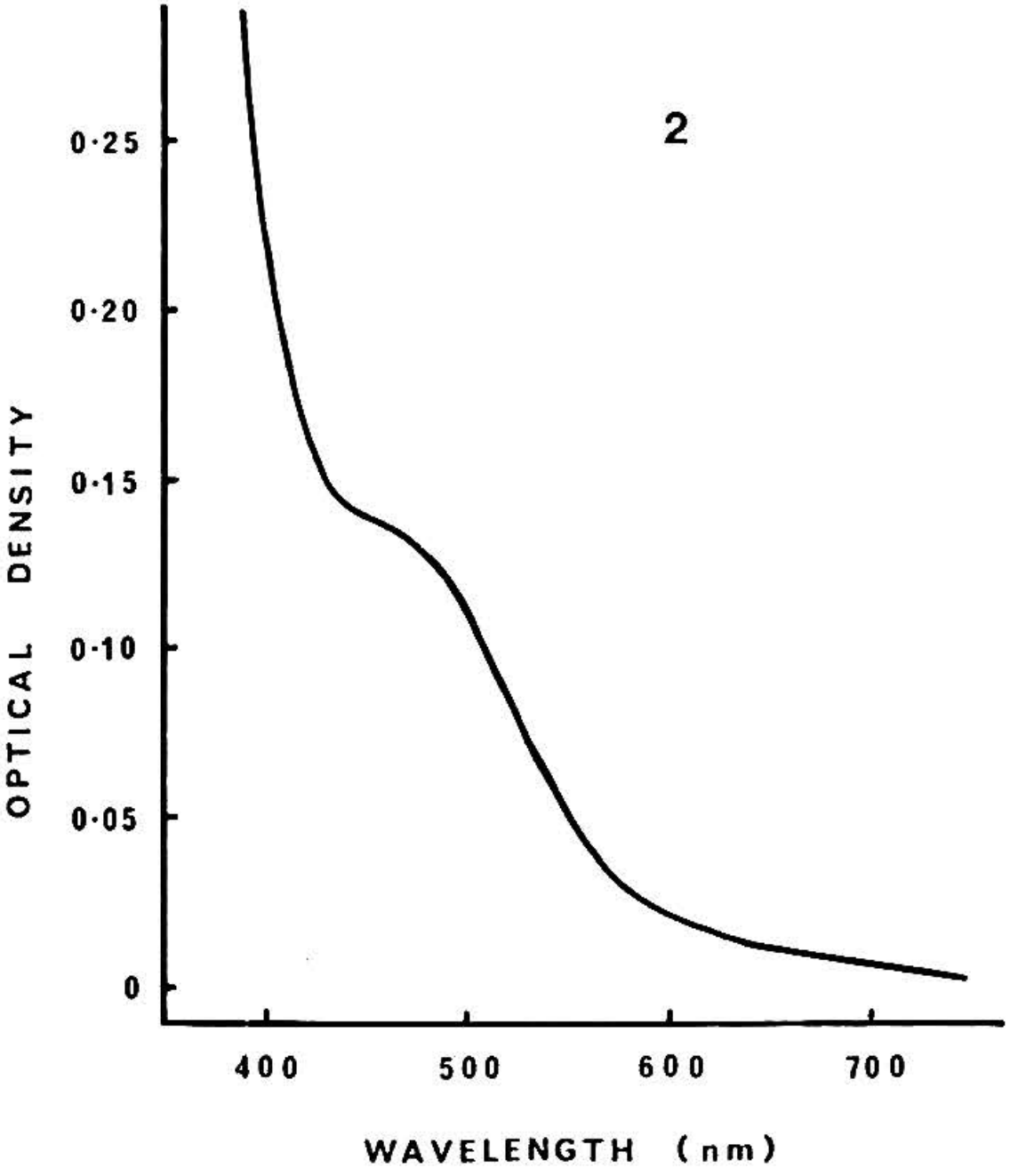

Fig. 2.2: Spectrum of a wine showing pinking (adopted from Simpson, 1977a).

Simpson (1977a) prepared a 0.3% (w/v) solution (1 mL in 100 mL distilled water) of 30% hydrogen peroxide (H_2O_2). He used increments of 0.05 mL, starting from 0.05 mL in a 10 mL wine sample, and ending with 0.40 mL from this 0.3% (w/v) H_2O_2 solution to end up with concentrations ranging from 15 mg/L to 120 mg/L H_2O_2. For each wine tested, there were two samples, one of which was the control and the other one that received the H_2O_2 addition. These two samples of each wine were then kept in the dark for 24 hours before being analysed spectrophotometrically.

Simpson (1977a) found that the increase of pinking was linear up to 24 hours, reaching a peak at 3 days and then decreasing to 14 days. However, the reason for the shorter than 3-day assay periods for pinking susceptibility used in practice today could be that winemakers need to make a decision as quickly as possible and waiting three days for results is too long.

Simpson (1977a, 1980b) reports that, at a concentration of 75 mg/L (0.25 mL) H_2O_2 shows the most consistent results. At 75 mg/L (0.25 mL) H_2O_2, Simpson also found that SO_2 did not influence the outcome, but at a concentration of 45 mg/L (0.15 mL) H_2O_2, the lack of SO_2 or low concentrations of SO_2 could influence the values and therefore give a false negative to the winemaker.

2.2.2 Assays used in Australia, South Africa, America and Europe

The assays for pinking and various approaches used in different parts of the world are described below. Although laboratories in the winemaking countries use the assay as established by Simpson (1977a), there are variations in different countries (Table 1.1) adapted to best suit their final objectives.

Australia

A 100 mL clear glass screw cap bottle is labelled as ‘control’ and another as ‘test’. The ‘control’ bottle is filled with wine. Forty mL of the same wine is measured into the ‘test’ bottle, to which 0.5 mL of 0.3% (w/v) hydrogen peroxide is added and mixed well. The ‘test’ sample is then placed in a dark cupboard at approximately 25°C overnight (about 12 hours). The degree of pinking of the ‘test’ wine is compared to that of the ‘control’. In addition to visual assessment, spectral measures of the ‘test’ and ‘control’ wine can be performed at 520 nm, which gives a quantitative comparison. In this case, the wines are filtered through a 0.45 µm filter for assessment. A change greater than 0.050 at 500 nanometres (nm) between the control and treated sample indicates significant susceptibility to pinking (https://www.awri.com.au/industry_support/winemaking_resources/frequently_asked_questions/pinking/).

South Africa

According to the SASEV Methods of Analysis for Wine Laboratories (2002), a 0.072% (w/v) H_2O_2 solution (1.2 mL of 30% (w/v) H_2O_2 in 500 mL volumetric flask with distilled water) is used. A set of 5 x 25 mL sample bottles are filled with a wine and additions of 0, 0.5, 0.75, 1.00 and 1.25 mL of the 0.072% (w/v) solution are done. The sample bottles are gently mixed and left for at least 8 hours (the temperature and whether in a dark place or not are specified in the method). After 8 hours the samples are measured on a spectrophotometer at 500 nm, zeroed with the control sample (0 mL of H_2O_2 added) and, if the optical density (OD) is above 0.05, the wine is susceptible to pinking.

America and Europe

In both America and Europe, the method described by Simpson (1977a) is roughly followed (personnel communications). In America, 250 µL of a 0.3% (w/v) H_2O_2 solution is added to a 10 mL wine sample, while in France 125 µL of the 30% (w/v) H_2O_2 is used. In both countries, the samples are kept in a dark cupboard for 24 hours. The specific method is not revealed by the laboratories and the personnel were not willing to reveal all aspects of the methods. The spectrophotometer is zeroed with distilled water and both the control and treated sample are measured at 500 nm. The difference between these two is given as AU and, when the value is ≥ 0.05, the wine is seen as having a pinking susceptibility.

Table 1.1: The table depicts the differences in the assay used in South Africa, France, America and Australia.

	H_2O_2 from 30% stock solution	***Sample volume (mL)***	***H_2O_2 added (µL)***	***Waiting period (hours)***
South Africa	0.072%	25	1000	8
Australia	0.3%	40	500	12
United States of America	0.3%	10	250	24
France	30%	10	125	24

Concluding remarks

Simpson (1977a) reports that if the AU is above 5, the wine shows potential for pinking susceptibility. In his research work, he multiplied the absorbance unit (AU) by 1000 (x 10^3) to get to a whole number. In all the methods, an AU of 0.05 (10 times higher than 0.005 Simpson used in his original work) is used. It is not sure when this decision was made and for what reason and this discrepancy has never been questioned.

The use of a 0.3% (w/v) H_2O_2 solution in Australia and America have been reported, while in South Africa, it is 0.072% (w/v). In France, a 30% (w/v) undiluted solution is added to the wine sample (Table 1.1). Different volumes of the H_2O_2 concentrations are used by the different countries (500 µL in 40 mL of wine sample, 250 µL in 10 mL of wine sample, 1000µL in 25 mL of wine sample and 125 µL in 10 mL of wine sample for Australia, USA, SA and France, respectively). The final concentrations of H_2O_2 in the treatment sample differ but could easily be worked out with the formula $C_1V_1 = C_2V_2$. This will lead to different sensitivity measurements

and different absorbency units, and possibly different conclusions on whether a wine shows pinking susceptibility or not.

Another difference between the countries is that Australia uses a wavelength of 520 nm, while South Africa and France use 500 nm, as stated by Simpson (1977a). The reason for this could be that some countries scan through a wide range of spectra, i.e. 400 to 650 nm, to determine the wavelength of maximum absorbance.

Simpson (1977a) originally used a 10 mL sample bottle for his experiments. This is also the case in America and France, while in Australia a 40 mL sample bottle is used (Iland *et al.*, 2012). In South Africa, a 25 mL sample bottle is used (SASEV, 2002). The question arises if the addition of H_2O_2 from the different stock solutions will have an impact on the values for pinking susceptibility and regarding the pinking sensitivity of the wines. Would it then not be better to standardise to the original concentrations set by Simpson (1977a)?

The above shows that there is no real standard for the testing of pinking susceptibility. In some cases, the waiting period is up to 24 hours, whereas in countries like South Africa it is reduced to 8 hours of waiting. It is evident that there is a need to develop a standardised, shorter and more reliable method for the testing of pinking susceptibility. There is also no protocol prescribed by the International Organisation of Vine and Wine (OIV) on an assay for pinking.

2.3 FACTORS INFLUENCING PINKING IN WHITE WINES

In a series of articles published from 1980 to 1983, Simpson and co-workers discussed different factors that could contribute to the pinking susceptibility of white wines. The factors influencing pinking in white wine are discussed based on these abovementioned articles and supplemented by other authors.

2.3.1 The role of phenols

Singleton and Esau (1969) discussed the possibility of colourless plant phenols turning pink in an acidic medium, like wine, when the colourless anthocyanogens turn into anthocyanidins. In 1977, Simpson also stated that the spectral and chemical properties of the pink wines tested indicated that the precursors could be phenolic in origin. This started the reasoning that the oxidation of phenols could lead to pinking.

Phenolics in wine are divided into two groups. These are the flavonoids, of which the flavan-3-ols are part, and the non-flavonoids, of which the hydroxycinnamic acids and hydroxybenzoic acids are part. The flavan-3-ols consist of catechin, epicatechin, epigallocatechin and epicatechin-gallate and are found mainly in the skins and pips of grapes (Monagas *et al.*, 2005; Aron & Kennedy, 2008; Piñeiro *et al.*, 2012). The hydroxycinnamic and

hydroxybenzoic acids are normally found in the fleshy parts of the grapes (Garrido & Borges, 2013; Nel, 2018).

Compounds that have an ortho-diphenol grouping are highly reactive with dissolved oxygen (Garrido & Borges, 2013) to form an ortho-quinone.

$$\textit{Ortho-diphenol} + O_2 \rightarrow \textit{ortho-quinone} + H_2O_2$$

These ortho-quinones are very unstable because of their highly electrophilic nature and can react in a further three ways. Firstly, the ortho-quinone can form dimers or polymers if reacting with the nucleophilic parent. Secondly, the ortho-quinone can undergo further nucleophilic additions with other nucleophiles (amino acids, glutathione, and other phenols). Thirdly, the ortho-quinone can be reduced by other reducing species, like ascorbate and other phenols, to form ortho-diphenols (Fulcrand *et al.*, 2006). All these non-enzymatic reactions are catalysed by Fe^{3+}/Fe^{2+} or Cu^{2+}/Cu^{+} (Oliveira *et al.*, 2011). The oxidation of these phenols leads to the browning of white wines (Fulcrand *et al.*, 2006; Garrido & Borges, 2013; Rustioni, 2017). The cause of pinking is still speculative when it comes to phenols as causative agents. The attribution of several possible phenols (protocatechuic acid, catechin, epicatechin, caffeic acid, gallic acid, ethyl gallate, p-hydroxybenzoic acid, quercetin-3-rutinoside, quercitin-3-glucoside, quercetin-3-L-rhamnoside, quercetin-3-D-galactoside, cis-coutaric acid, trans-coutaric acid, m-coutaric acid, p-coutaric acid, caftaric acid, ferulic acid, fertaric acid and coumaric acid), the implicating unstable flavonoid phenols (astilbin and engeletin) and the chemical degradation of some of these procyanidins in the presence of oxygen to form anthocyanogens may lead to the appearance of a pink colour (Tobe, 1983).

2.3.2 The role of temperature

Simpson (1977b) states that the development of the pink colour can generally be linked to the ingress of oxygen during winemaking processes. These processes are normally critical points in which oxygen uptake plays a role, such as pump-overs, filtration, bottling, etc. The solubility of oxygen increases with a decrease in temperature (Simpson, 1980b). Oxygen solubility in wine at room temperature and atmospheric pressure is about 6.0 mL/L (8.6 mg/L) (Castellari *et al.*, 2004; Waterhouse & Laurie, 2006). The oxygen solubility increases by about 10% with a decrease in temperature (Waterhouse & Laurie, 2006). Winemakers therefore should be careful about practices in which wine temperature is kept low and the chances for oxygen uptake are high, like crushing and destemming, pressing and pumping of wine at low temperatures.

2.3.3 Light

Simpson (1980a) suggests that when a wine pinks in the bottle it can be exposed to direct sun or UV light for about 10 minutes to reduce the pink discolouration. UV exposure might lead to reduced pinking but can have other negative effects. To explain the chemistry behind UV

exposure, Clark *et al.* (2011) showed that Fe^{3+} and light have the potential to degrade wine compounds, like tartaric acid, 3-mercaptohexanol (3MH) and 3-mercaptohexyl acetate (3MHA). Different coloured glass bottles have different degrading properties and protection against UV light and degradation compounds. The different coloured glass bottles, with their protective abilities in increasing order, are Flint < Arctic Blue < French Green < Antique Green glass (Dias *et al.*, 2012). Light can also catalyse free radical reactions that are involved in the peroxidation step of autoxidation (Simpson, 1980b). UV light furthermore promotes the browning of phenols in wine (Clark *et al.,* 2011; Parish-Virtue *et al.*, 2019). UV light has the ability to excite singlet oxygen, which is then able to diffuse over a large distance of 270 nm (2.7×10^{-4} mm). The singlet oxygen molecule is electrophilic, as it has a completely vacant $2p\pi$ orbital. Therefore, the singlet oxygen molecule can react with high e-density double bonds via a 6-membered ring. This results in the formation of hydroperoxide radicals ($HOO^{\cdot}$) that again assists in autoxidation (Choe & Min, 2009).

2.3.4 Trace metals

During non-enzymatic oxidation or chemical oxidation, H^+ ions are transferred from a diphenol to an O_2 to form H_2O_2, but these reactions can only take place in the presence of metal ion catalyses like Fe^{3+} and Cu^{2+}. This process is mediated by the redox cycle, in which Fe^{3+}/Fe^{2+} and Cu^{2+}/Cu^{+} reduce oxygen to hydrogen peroxide (Oliveira *et al.*, 2011). For this reason, knowledge of the iron and copper concentration of the wine is of utmost importance, as it can have a significant impact on the autoxidation of the wine.

2.3.5 SO_2 concentration of the wine

During a study done by Simpson (1977a) on the effect of SO_2 on pinking susceptibility, he reduced the pH of a range of wines to pH 1. Sparging of the wine with nitrogen expelled the SO_2 from the wine. Simpson then adjusted the pH of the wines back to their normal states and added SO_2 in potassium metabisulphite form up to a free SO_2 of 60 mg/L. Two concentrations of H_2O_2, viz. 15 mL/L and 75 mL/L, were added to the wines and the pinking susceptibility was tested. With the lower concentration (15 mL/L) of H_2O_2, the pinking susceptibility was reduced proportionally with the increase of free SO_2. With the higher concentration of H_2O_2 (75 mL/L), there was a reduction in pinking at the free SO_2 of 40 mg/L. The amount of free SO_2 (concentration of about 40 mg/L) in the wine was sufficient to react with H_2O_2 to form an HSO_3^- anion. This will also be sufficient to prevent pinking in white wines (Simpson, 1977a).

2.3.6 Ascorbic acid addition as an antioxidant

Ascorbic acid is a very strong antioxidant as it reacts effectively with O_2 in the wine (Simpson, 1980a, b). The H_2O_2, formed from transferring an H^+ ion to an O_2 from ascorbic acid, is also a very strong oxidising agent (Bradshaw *et al.,* 2004, 2011; Barril *et al.*, 2016) and therefore the

concentration of free SO_2 in the wine needs to be at least 40 mg/L (Simpson 1977a). Ascorbic acid is one of the agents that work very well in preventing pinking in white wines. The addition of ascorbic acid prior to bottling may also keep the wine safe against oxidative browning in the bottle (Gibson, 2006). But there could also be a downside to the addition of ascorbic acid as it may also decrease the shelf life of the wine, with the risk of oxidative browning and even pinking (Bradshaw *et al.*, 2011; Barril *et al.*, 2016). Bradshaw *et al.* (2004) found that the molar ratio of ascorbic acid to SO_2 must be 1:1.7 to prevent oxidative browning and pinking in wines.

2.3.7 Wine pH

The equilibrium of molecular SO_2, bisulphite and sulphite ions in wine is pH dependant. A sulphite anion attached to the C-4 position of the anthocyanin transforms it into a colourless form. This means that, at a lower pH, more molecular SO_2 is available for the protection of the wine against oxidation (Simpson, 1980b; Abramovič *et al.*, 2015). Simpson (1977a) tested the influence of pH on pinking. Wine with a known pinking susceptibility was used to provide a pH range from 2.75 to 4.00. Pinking values were then obtained 4 hours after the addition of 75 mg/L H_2O_2. In a second test, samples were acidified to a pH 1 and assayed for pinking. No significant differences were obtained in the pH range, as well as for the acidification test. This led Simpson to believe that the compound causing pinking is not a flavylium salt or its glucosides (anthocyanins). This was confirmed by Tobe (1983), who used seven cultivars made from grapes in an experimental wine cellar in 1981, and Jones (1989), who made wine in three consecutive years (1985 to 1987). The wines that were made were treated specially for the experiments planned. Although true for monomeric anthocyanins, polymeric anthocyanins are more resistant to SO_2 bleaching and pH changes (Somers, 1971; Andrea-Silva *et al.*, 2014). During ageing and/or maturation, a polymerisation of anthocyanins takes place at the C-8 and C-6 positions, forming anthocyanin-tannin condensation reactions (Monagas *et al.*, 2005). These reactions lead to a stable polymeric anthocyanin, which therefore is resistant to decolouration by SO_2 and to pH changes (Somers, 1971).

2.4 TREATMENT OF PINKING SUSCEPTIBILITY IN WHITE WINES

Lamuela-Raventós *et al.* (2001) did a series of experiments to find the best product to remove the precursors for pinking susceptibility in white wines. Wines were divided into four lots, control wine; wine with 1 g/L PVPP, wine with 1 g/L PVPP + 0.5 g/L bentonite and wine with 1 g/L PVPP + 15 mg/L ascorbic acid. Wine treated with 1 g/L PVPP reduced pinking by 74%, the wine with 1 g/L PVPP + 0.5 g/L bentonite reduced pinking by 90%, and the wine with 1 g/L PVPP + 15 mg/L ascorbic acid reduced pinking by 98%. However, after 20 days the capacity of ascorbic acid + PVPP to reduce pinking decreased to the same levels as that of PVPP + bentonite. Tobe

(1980) investigated the removal of precursors by bentonite and PVPP. He initially observed decreases in total phenols by bentonite fining, to be ineffective after applying the Freundlich equation. The Freundlich equation is an empirical formula to describe adsorption systems in which it does not appear to have infinite adsorption sites (Blade & Boulton, 1988). PVPP was more favourable in removing the total phenols. Lamuela-Raventós *et al.* (2001) added various concentrations of ascorbic acid to a wine, i.e. 0, 15, 30, 45 and 100 mg/L. At 30 mg/L pinking was reduced, however, at 45 mg/L pinking was completely prevented. This showed that ascorbic acid is a good agent to prevent pinking susceptibility in white wine, but it could lead to oxidative browning after an extended period (Lamuela-Raventós *et al.*, 2001).

2.5 ALTERNATIVE EXPLANATIONS TO PINKING

Andrea-Silva *et al.* (2014) reported that the compound that causes pinking susceptibility in Siria wines was malvidin-3-O-glucoside, which was the most abundant anthocyanin tested. Siria is a Portuguese cultivar, a widely planted Iberian variety also known as Roupeiro, Doña Blanca and Cigüente. The wine is aromatic but oxidises easily (Robinson *et al.*, 2012).

Andrea-Silva *et al.* (2014) mention that the wine used for their experiments pinked naturally. After pinking, 0.8 g of PVPP was added. The suspension was then filtered through a cheesecloth and washed with 100 mL of water and 100 mL of ethanol (95%). Thereafter, the PVPP was loaded into an empty SPE cartridge and eluted with acetonitrile, acetone, an aqueous solution of 1% HCl, ethanol and 0.1 M NH_3 in ethanol. Each fraction was kept separate. After evaporation and reconstitution with 0.2 mL of methanol and water, the samples were loaded onto an LC-MS. The main compound found was malvidin-3-O-glucoside. Andrea-Silva *et al.* (2014) also established that the minimum amount of total anthocyanin must be 0.3 mg/L (300 µg/L) to turn the wine a visible pink colour. This experiment was repeated in 2019 (Cosme *et al.*, 2019). Arapitsas *et al.* (2015) analysed grapes of Sauvignon blanc, Chardonnay and Riesling using a UPLC-MS/MS. They found measurable amounts of malvidin-3-O-glucoside, as well as carboxypyranomalvidin-3-O-glucoside (A-type vitisin) and pyranomalvidin-3-O-glucoside (B-type vitisin). The amounts were 55.44 µg/kg, 37.05 µg/kg and 38.99 µg/kg, respectively, for Sauvignon blanc, Chardonnay and Riesling (Arapitsas *et al.*, 2015).

In genetic analyses for anthocyanins in red and white grapes, six genes were determined in the flavonoid biosynthetic pathway. Some genes were expressed in all grapes, even where little or no anthocyanins accumulated, but an expression of the gene encoding a UDP glucose-flavonoid 3-O-glucosyl transferase (UFGT) was only detected in red grapes that synthesised anthocyanins. Analysis of the white grapes indicated that the UFGT gene was present but was not expressed (Boss *et al.*, 1996). External environmental conditions and

vineyard practices therefore can switch on these genes to start the anthocyanin metabolic pathways (Boss *et al.*, 1996).

The original researcher on pinking, Dr Bob Simpson, reported that phenols (flavonoid and non-flavonoids) and not anthocyanins are the causative compound. Research on pinking in wine is needed to identify the colour-forming compounds.

2.6 LITERATURE CITED

Abramovič, H., Košmerl, T., Ulrih, N.P. and Cigic, B., 2015. Contribution of SO_2 to antioxidant potential of white wine. Food Chem. 174, 147–153.

Andrea-Silva, J., Cosme, F., Ribeiro, L.F., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Domingues, M.R.M. and Nunes, F.M., 2014. Origin of the pinking phenomenon of white wines. J. Agric. Food Chem. 62, 5651–5659.

Arapitsas, P., Oliveira, J. and Mattivi, F., 2015. Do white grapes really exist? Food Res. Int. 69, 21–25.

Aron, P.M. and Kennedy, J.A., 2008. Flavan-3-ols: Nature, occurrence and biological activity. Mol. Nutr. Food Res. 52, 79–104.

Australian Wine Research Institute (AWRI). https://www.awri.com.au/industry_support/winemaking_resources/frequently_asked_questions/pinking/ (accessed in October 2019)

Barril, C., Rutledge, D.N., Scollary, G.R. and Clark, A.C., 2016. Ascorbic acid and white wine production: a review of beneficial versus detrimental impacts. Aust. J. Grape Wine Res. 22, 169–181.

Blade, W.H. and Boulton, R., 1988. Adsorption of protein by bentonite in a model wine solution. Am. J. Enol. Vitic., 39 (3), 193-199.

Boss, P.K., Davies, C. and Robinson, S.P., 1996. Anthocyanin composition and anthocyanin pathway gene expression in grapevine sports differing in berry skin colour. Aust. J. Grape Wine Res. 2, 163–170.

Bradshaw, M.P., Scollary, G.R. and Prenzler, P.D., 2004. Examination of the sulfur dioxide-ascorbic acid anti-oxidant system in a model white wine matrix. J Sci Food Agric. 84, 318–324.

Bradshaw, M.P., Barril, C., Clark, A.C., Prenzler, P.D. and Scollary, G.R., 2011. Ascorbic acid: A review of its chemistry and reactivity in relation to a wine environment. Crit. Rev. Food Sci. 51, 479–498.

Castellari, M., Simonato, B., Tornielli, G.B., Spinelli, P. and Ferrarini, R., 2004. Effects of different enological treatments on dissolved oxygen in wines. Ital. J. Food Sci. 3 (16), 387–397.

Choe, E. and Min, D.B., 2009. Mechanisms of antioxidants in the oxidation of foods. Compr. Rev. Food Sci. F. 8, 345–358.

Clark, A.C., Dias, D.A., Smith, T.A., Ghiggino, K/P/, and Scollary, G.R., 2011. Iron(III) tartrate as a potential precursor of light-induced oxidative degradation of white wine: Studies in a model wine system. J. Agric. Food Chem. 59, 3575–3581.

Cosme, F., Andrea-Silva, J., Filipe-Ribeiro, L., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Domingues, M.R.M. and Nunes, F.M., 2019. The origin of pinking phenomena in white wines: An update. 41st World Congress of Vine and Wine. BIO Web of Conferences 12, 1–5.

Dias, D.A., Smith, T.A., Ghiggino, K.P. and Scollary, G.R., 2012. The role of light, temperature, and wine bottle colour on pigment enhancement in white wine. Food Chem. 135, 2934–2941.

Fulcrand, H., Dueñas, M., Salas, E. and Cheynier, V., 2006. Phenolic reactions during winemaking and aging. Am. J. Enol. Vitic. 57 (3), 289–297.

Garrido, J. and Borges, F., 2013. Wine and grape polyphenols — A chemical perspective. Food Res. Int. 54, 1844–1858.

Gibson, R., 2006. Ascorbic acid – friend or foe? 35th Annual New York Wine Industry Workshop. New York.

Iland, P., Bruer, N., Edwards, G., Caloghiris, S. and Wilkes, E., 2012. Chemical analysis of grapes and wine: Techniques and concepts (2nd Ed.). Patrick Iland Wine Promotions Pty Ltd. Adelaide. Australia.

Jackson, R.S., 2014. Post-fermentation treatments and related topics. In: Wine Science: Principles and applications. Academic Press. Elsevier. 418–521.

Jackson, R.S., 2016. Wine Tasting: A Professional Handbook. Academic Press; 3rd edition. Ontario. Canada.

Jacobson, J.L., 2006. Introduction to wine laboratory practices and procedures. Springer, United States of America.

Jones, T., 1989. Pinking of white table wines: Further studies. Unpublished Master of Science thesis, University of California, Davis, CA, USA.

Lamuela-Raventós, R.M., Huix-Blanquera, M. and Waterhouse, A.L., 2001. Treatments for pinking alteration in white wines. Am. J. Enol. Vitic. 52 (2), 156–158.

Monagas, M., Bartolomé, B. and Gómez-Cordovés, C., 2005. Updated knowledge about the presence of phenolic compounds in wine. Crit. Rev. Food Sci. 45, 85–118.

Nel, A.P., 2018. Tannins and Anthocyanins: From Their Origin to Wine Analysis – A Review. S. Afr. J. Enol. Vitic. 39 (1), 1–19.

Oliveira, C.M., César, A.C., Ferreira, S., De Freitas, V. and Silva, A.M.S., 2011. Oxidation mechanisms occurring in wines. Food Res. Int. 44 (5), 1115-1126.

Parish-Virtue, K., Herbst-Johnstone, M., Bouda, F. and Fedrizzi, B., 2019. The impact of postharvest ultra-violet light irradiation on the thiol content of Sauvignon blanc grapes. Food Chem. 271, 747–752.

Piñeiro, Z., Canepa, D., Palma, M. and Barroso, C.G., 2012. Evolution of grape seed flavan-3-ols during ripening of different grape cultivars. Int. J. Food Sci. Tech. 47, 40–46.

Robinson, J., Harding, J. and Vouillamoz, J., 2012. Wine grapes: a complete guide to 1, 368 vine varieties, including their origins and flavours. London: Harper Collins.

Rustioni, L., 2017. Oxidized polymeric phenolics: Could they be considered photoprotectors? J. Agric. Food Chem. 65, 7843−7846.

SASEV, 2002. Methods of analysis for wine laboratories. South African Wine Laboratory Association, Stellenbosch.

Simpson, R.F., 1977a. Oxidative pinking in white wines. Vitis. 16, 286–294.

Simpson, R.F., 1977b. Pinking in Australian white table wines. Austr. Wine Brew. Spirit Rev. November, 56–58.

Simpson, R.F., 1980a. Some aspects of oxidation and oxidative browning in white table wines. Austr. Grapegrower & Winemaker. January, 20-21.

Simpson, R.F., 1980b. Some aspects of oxidative pinking of white wines. Austr. Grapegrower & Winemaker. December, 12-13.

Simpson, R.F., Miller, G.C. and Orr, G.L., 1982. Oxidative pinking of white wines: recent observations. Food Tech. Austr. 34 (1), 44–47.

Simpson, R.F., Bennett, S.B. and Miller, G.C., 1983. Oxidative pinking of white wines: a note on the influence of sulphur dioxide and ascorbic acid. Food Tech. Austr. 35 (1), 34–36.

Singleton, V.L. and Esau, P., 1969. Phenolic substances in grapes and wine and their significance. Adv. Food Res. Suppl. 1, 1–282.

Somers, T.C., 1971. The polymeric nature of wine pigments. Phytochem. 10(9), 2175-2186.

Tobe, S.J., 1983. Pinking in table wines from white grapes. Unpublished MSc. thesis,

University of California, Davis.

Waterhouse, A.L. and Laurie, V.F., 2006. Oxidation of wine phenolics: A critical evaluation and hypotheses. Am. J. Enol. Vitic. 57 (3), 306–313.

Chapter 3

Influence of different winemaking processes on the pinking susceptibility of Sauvignon blanc

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3.1 INTRODUCTION

The phenomena of pinking were first described by Singleton & Esau in 1969. In the 50 years that this phenomenon is known, only 10 articles and 2 dissertations have been written. These are as follows:

1. *Oxidative pinking in white wines (Simpson, 1977a)*.
2. *Pinking in Australian white table wines (Simpson, 1977b)*.
3. *Some aspects of oxidative pinking of white wines (Simpson 1980).*
4. *Oxidative pinking of white wines: Recent observations (Simpson et al., 1982)*.
5. *Oxidative pinking of white wines: A note on the influence of sulphur dioxide and ascorbic acid (Simpson et al., 1983)*.
6. *Treatments for pinking alteration in white wines (Lamuela-Raventós et al., 2001).*
7. *Origin of the pinking phenomenon of white wines (Andrea-Silva et al., 2014).*
8. *The origin of pinking phenomena in white wines: An update (Cosme et al., 2019).*
9. *Effect of certain treatments to prevent or partially reverse the pinking phenomenon in susceptible white wines (Cojocaru & Antoce, 2019).*
10. *Pinking in White Wines – A Review (Nel et al., 2020).*
11. *Alternative methods for measuring the susceptibility of white wines to pinking alteration: Derivative spectroscopy and CIEL*a*b* colour analysis (Minute et al., 2021)*

The two dissertations being

1. *Pinking in table wines from white grapes (Tobe, 1983).*
2. *Pinking of white table wines: Further studies (Jones, 1989).*

The first research on pinking was published by Simpson in 1977a. In this phenomenal work, Simpson developed the pinking assay and it is still used today throughout the wine-producing countries' laboratories. Simpson discovered that there was a peak formed at 500 nm on the chromatograph after analysing non-pinked wines and pinked wines at different spectra ranging from 380 to 780 nm. Simpson (1977a) determined that when the samples are measured on a spectrophotometer at 500 nm, the absorbency units (AU) above 0.05 AU showed that the wine has a pinking potential, and wine that is below the value of 0.05 AU is fairly safe from pinking.

In the same study, Simpson looked at the influence of SO_2 and pH on pinking. He established that SO_2 does not increase the pinking potential provided that the free SO_2 of the wine is above 40 mg/L. The effect of pH on the colour formation in red wine is also well known and at pH1 all anthocyanins are in their red form. Between a pH 2 and 5, the equilibrium shifts to quinoidal species in a blue form, while at pH 5 and 6, the species are in a colourless state, while at a neutral pH (pH 7) and higher the degradation depends on the substituent groups of the flavonoid (Castañeda-Ovando *et al.*, 2009). Simpson (1977a) found that pH does not influence the pinking of the wine, establishing that anthocyanins are not the causing agent of pinking.

In 1983 (Tobe) and 1989 (Jones) tried to identify the compound(s) causing pinking in white wines. Tobe (1983) discovered that leucoanthocyanidins are not the compounds involved, but that there could be several compounds (quercitrin, rutin, astilbin & engeletin) that could cause pinking. Jones (1989) suggested that at least 10 compounds can cause pinking. He indicated that amino acids, Grape Reaction Product (GRP) and polyphenol oxidase (PPO) could cause pinking in white wines. In their pioneering work, Andrea-Silva *et al.* (2014) discovered that malvidin-3-O-glucoside causes pinking in Siria wines. This was confirmed by Cosme *et al.* (2019) in an updated study. Extensive literature searches on studies performed over the last 50 years, related to oxidative colour changes in wine, showed that the majority of research focused on browning in white wines, with only a few of these articles referring to pinking, and even fewer to the influence of viticultural or oenological aspects on the increase or reduction of pinking in wine. Singleton *et al.* (1979) made the association that the oxidation of a colourless precursor turns into an anthocyanin. Procyanidins, which is a group of flavan-3-ols are found naturally in *Vitis vinifera* cultivars and can change into cyanidin giving white wine a pinkish colour (Porter *et al.*, 1986).

During the early stages of berry ripening, phenolic content is high and decreases as the berry ripens. Most of the phenols are situated in the skin and pips of the berry. At the destemming and crushing stage, the berries are beaten off the stems and are crushed without the mashing up of the pips. These mechanical actions can be quite harsh on the grape berry, leading to more phenolics being leached out into the juice depending on the system used. Adding stems to the winemaking process can be an extra source to increase phenolic concentrations, especially the flavan-3-ols. Pressing whole bunches may also lead to an increase in the phenolic concentration of juice and wine (Pascual *et al.*, 2016). If the cause of pinking is phenolic of sort as anecdotal evidence of some studies suggests, then the temperature may well play an intrinsic role in the pinking potential of Sauvignon blanc wines. It is a well-known fact that temperature, especially night-time temperatures, plays a role in phenolic synthesis (Jackson and Lombard, 1993; Dokoozlian & Kliewer, 1996). Mafata *et al.*

(2018) also found that warmer pressing temperatures (25ºC and 30ºC) increased phenolic concentrations. Therefore, it is important to investigate the effect that grape temperature plays in the pinking potential of Sauvignon blanc wines. According to Tobe (1983), skin contact and fermenting on skins increase the chances of pinking. Furthermore, according to a questionnaire sent out to South African Sauvignon blanc wine producers (unpublished data, 2015), the skin contact time for Sauvignon blanc can range from 0 hours to 12 hours.

Simpson (1980) also established that oxygen, especially after bottling, led to pinking. In the 1980 study, Simpson linked the use of inert gas and cool fermentation to pinking and started giving preventative methods to reduce pinking susceptibility, recommending the use of SO_2 and PVPP (polyvinyl polypyrrolidone). Simpson et al. (1982) also looked at the influence of erythorbate on pinking and discovered that the use of erythorbate can reduce the pinking susceptibility significantly. Simpson *et al.* (1983) took it a step further when they concluded that the use of SO_2 and ascorbic acid would reduce pinking significantly. Research has found that the addition of ascorbic acid prevents oxidative browning and pinking (de Beer *et al.*, 2003; Skouroumounis *et al.*, 2005; Barril *et al.*, 2016), although the addition of ascorbic acid can also enhance browning under certain circumstances (du Toit *et al.*, 2006). The enhancement of browning of white wines was confirmed by Jones (1989) and Clark *et al.* (2010). Simpson *et al.* (1982) looked at the influence of PVPP on the reduction of pinking. Lamuela-Raventós *et al.* (2001) reported that the use of PVPP, with bentonite and ascorbic acid, reduced the pinking susceptibility significantly. The phenomena of white wines that turn pink during the winemaking process have led to the manufacturing of PVPP (Lamuela-Raventós *et al.*, 2001). The use of PVPP and its effect on pinking precursors was investigated by Main and Morris (1994) and it was discovered that PVPP with bentonite and ascorbic acid can reduce pinking in white wines (Main & Morris, 1995; Lamuela-Raventós *et al.*, 2001, du Toit *et al.*, 2006; Ferreira *et al.*, 2018). This discovery led to an increase in the use of PVPP.

Research in the line of viticultural practices and oenological processes are very rare or non-existing about pinking. Although a lot of studies have been done on browning or oxidative browning (de Villiers, 1961; Caputi & Petersen, 1965; Rossi & Singleton, 1966; Singleton & Kramling, 1976; Simpson, 1980; Simpson, 1982; Sapis *et al.*, 1983; Lee & Jaworski, 1988; Cilliers & Singleton, 1989; Cheynier *et al.*, 1990; Gómez *et al.*, 1995; Bradshaw *et al.*, 2001; Razmkhab *et al.*, 2002; Galuska *et al.*, 2010; Serra-Cayuela *et al.*, 2013; Serra-Cayuela *et al.*, 2014; Nenadis & Paraskevopoulou, 2016; Grant-Preece *et al.*, 2018; Sartor *et al.*, 2019), very few of these studies touched on pinking as a problem or phenomena. The influences of viticultural aspects like ripening stages and harvesting temperatures on the pinking potential of white wine were never researched until now, which forms the main part of this study.

Sauvignon blanc is the 3rd most planted white variety in South Africa which makes out 10.3% of the total planted area (SAWIS, 2018). Many white cultivars, including Sauvignon blanc, are oxygen-sensitive (Day *et al.*, 2015), and are more prone to pinking (Simpson, 1977a; Andrea-Silva *et al.*, 2014). With a large percentage of all white cultivar wine sold locally being Sauvignon blanc while it also dominates packaged exports, and being one of the oxygen- and pinking prone white wine cultivars, Sauvignon blanc was the cultivar of choice used in this study. The overall aims of this study thus included examining the effects that different winemaking practices might have on the pinking susceptibility of South African Sauvignon blanc wines. In this study, the influence of different skin contact times on the occurrence of pinking was investigated. The influence of normal oxidative and reductive winemaking conditions was investigated to establish a correlation between juice oxidative state, skin contact time, and pinking potential. The study will include the influence of a whole bunch pressing versus bag press, the influence of different temperatures, ripeness levels and skin contact time at different maceration temperatures on the pinking susceptibility of Sauvignon blanc wines. The efficiency to prohibit pinking of different fining agents will also be investigated at different addition stages during the winemaking process.

3.2 MATERIALS AND METHODS

3.2.1 Grapes vineyards and locations

Sauvignon blanc grapes were used in all the experiments as it was the focus of this study. Grapes for the 2015 harvest season were sourced from two farms in the Durbanville ward, Tygerberg District, Coastal region, Western Cape, South Africa, that reported pinking (producer 1) and no pinking susceptibility (producer 2) of their wines, respectively. The Sauvignon blanc grapes came from a block with a northwest/southeast row direction, the soil is decomposed granite with high clay content. The planting width is 120 cm x 210 cm and the vines were planted in 1997 on a four-wire Perold training system and are dryland. The Sauvignon blanc cultivar is grafted on R99 rootstock for both producers. The grapes were used for two experiments, different additions of ascorbic acid and hyper-oxidation of juice, as well as the skin contact time with oxidative and reductive winemaking.

Grapes for the 2016 to 2018 harvest seasons were sourced from a farm in the Robertson District, Breede River Valley region, Western Cape, South Africa, which was known to have a pinking susceptibility. The Sauvignon blanc grapes came from a block with an east-west row direction, the soil is alluvial sand, with a planting width of 120 cm x 210 cm. The vines were planted in 2001 on a three-wire training system and are irrigated by drip irrigation. The rootstock is Mgt 101/14, but the top cultivar clone is unknown. Whole bunch versus crushed/destemmed bag pressed, ambient temperature (20°C) versus cool temperature (4°C),

different ripening stages, skin contact time versus different temperatures, and the fining agent experiment was performed using the Robertson grapes to investigate the influence on pinking susceptibility.

All the harvested Sauvignon blanc grapes were kept at 0ºC overnight to get all the grapes at the same temperature before experimentation commenced. The grapes were destemmed, crushed, and pressed the next day. The batches of grapes were pooled and divided to exclude vineyard heterogeneity. Every treatment was done in triplicate.

3.2.2 Winemaking practices

3.2.2.1 Standard winemaking

The standard Nietvoorbij procedures for small-scale, white wine production were followed. Twenty kilograms of grapes were used per treatment and 6.3 L of juice were pressed off. After crushing, destemming, and pressing, 50 mg/L SO_2 and 0.5 g/L pectolytic enzyme (Rapidase Clear, Anchor Oenobrands, South Africa) were added to the 5 L of pressed juice in 5 L glass/PET bottles. The pressing was done in a 20 kg stainless steel bag press. The juice was settled overnight at 14ºC in 20 L canisters or 5 L glass/PET bottles depending on the experiment. Grapes at ambient temperature (20°C) were left outside until the temperature was at 20°C as measured with a thermometer before being processed. The juice was racked from its lees the following day and active dried *Saccharomyces cerevisiae* yeast from Anchor Oenobrands (VIN13) was added at a dose of 30 g/hL. Fifty g/L diammonium phosphate and 10 mL/L of a 7.5% bentonite solution (75 g/hL) was added after three days of fermentation. After completion of fermentation, wines were analysed for residual sugar (RS), the dry wines were racked to clean canisters/glass/PET bottles, and cold stabilised (tartrate stabilisation) at 0ºC for 14 days. The cold stabilisation also helps to further clarify the wines before bottling. After cold stabilisation, the wine was filtered into 750 mL glass bottles and closed with Roté (roll-on tamper evident) capsules and stored until further analyses. All wines were analysed one month after bottling.

3.2.2.2 Reductive winemaking

The reductive winemaking process followed the same protocol as that of the standard Nietvoorbij procedures for small-scale. Extra steps were taken to minimise oxygen contact by using inert gases (CO_2 or N_2). CO_2 cylinders were used to blow gas at the destemming and crushing stages while a second cylinder was used to blow gas in the receiving bucket. These buckets were closed immediately with a lid to prevent O_2 from entering and to keep a blanket of gas on top of the marc. An N_2 cylinder was connected to the bag press to press the grapes reductively. CO_2 was blown into all canisters, glass bottles etc. before and during filling with pressed juice. A specially constructed gas manifold, connected to an N_2 gas bottle, kept a

reductive environment inside the 20 L maceration buckets. All wine transfers were performed by gas. During sampling, a custom-made laboratory filter was used to filter wine for sampling purposes as well as a special unit to transfer wine from one bottle to another under CO_2 gas pressure. After completion of fermentation, wines were analysed for residual sugar (RS), the dry wines were transferred to clean canisters/glass/PET bottles, under inert gas, and cold stabilised (tartrate stabilisation) at 0ºC for 14 days. The cold stabilisation also helps to further clarify the wines before bottling. After cold stabilisation, the wine was filtered into 750 mL glass bottles, which was filled with inert gas, and closed with Roté (roll-on tamper evident) capsules and stored until further analyses. All wines were analysed one month after bottling.

3.2.3 Experiments testing the influence on pinking susceptibility of Sauvignon blanc wines

3.2.3.1 The effect of different additions of ascorbic acid to juice on pinking susceptibility of the final wine

Treatment 1 (control wines) were prepared according to the standard Nietvoorbij procedures for small-scale, white wine production (see 3.2.2.1). For Treatment 2, the juice was hyper-oxidised at settling by bubbling air through the juice for 10 minutes until the juice had a brown colour with no SO_2 added, followed by vinification according to the Nietvoorbij standard white winemaking protocol. Treatments 3-5 entailed the addition of ascorbic acid to SO_2 ratios of 0.5:1, 1:1 and 2:1 (20 mg/L ascorbic acid:40 mg/L SO_2, 40 mg/L ascorbic acid:40 mg/L SO_2 and 80 mg/L ascorbic acid:40 mg/L SO_2), weighed before-hand respectively, and added at the destemming and crushing stage. This method was chosen as this is the practical way winemakers add ascorbic acid with sulphur to their grapes or juice. The physical ratios, as mentioned above, corresponds to the molar ratios of 3.17:1, 1.59:1 and 0.79:1 respectively. Calculation of the amounts of ascorbic acid and SO_2 to be added did not take into consideration pre-existing inherent concentrations in the grapes or juice. The reductive juices in treatments 3-5 were made under blankets of CO_2 gas during destemming and crushing.

3.2.3.2 The effect of skin contact time on the pinking susceptibility of Sauvignon blanc wines

Grapes were obtained from two different producers. The producers were chosen for the following reasons: Producer 1 claimed to have little or no pinking susceptibility in his wine, while producer 2 claimed to have regular pinking susceptibility in their wines. Each producer's grapes were first pooled and then divided to exclude vineyard heterogeneity. Grapes from each producer were divided into two batches (216 kg each group), one batch for standard winemaking and one for reductive winemaking. Each of these batches was further divided into treatments (54 kg per treatment), including control (no skin contact) and 3, 6, and 12 hours of skin contact and kept in a cold storage room at 10ºC during maceration. With the aid of a

custom-made manifold (Figure 3.1) the batch and treatments destined for reductive winemaking were kept under reductive conditions (N_2 gas blanket) during skin contact times. After each skin contact time, the macerated grape was pressed and the normal white winemaking protocol was used except for reductive winemaking.

Fig. 3.1: Custom made manifold to keep macerated grape under constant CO_2 blanket for 3, 6, and 12 hours.

3.2.3.3 The effect of grape temperature on pinking susceptibility

The grapes were sourced from a farm in the Robertson District which was known to have a pinking potential. The batches of grapes were first pooled and then divided to exclude vineyard heterogeneity. Half the grapes (one batch, 105 kg) were cooled to 4ºC, and the other half/batch (107 kg) was exposed to ambient temperature (20ºC) before winemaking commenced. For both batches, the standard Nietvoorbij procedures for small-scale, white wine production were followed. Every treatment was done in triplicate.

3.2.3.4 The effect of whole bunch versus crushed and destemmed bag press

One batch of Sauvignon blanc grapes (109 kg) was crushed and destemmed before pressing in a 20 kg stainless steel bag press that uses compressed air to blow up the bag for pressing. The

second batch (104 kg) of Sauvignon blanc grapes were whole bunch pressed (without the destemming and crushing stage) in the same 20 kg stainless steel bag press. For both batches, the standard Nietvoorbij procedures for small-scale, white wine production were followed. Every treatment was done in triplicate.

3.2.3.5 The effect of different ripening stages

Sauvignon blanc grapes were harvested at three different ripening stages from the same vineyard from a farm in the Robertson District known to have a pinking potential. Grapes from the same harvesting stage were pooled and divided to exclude vineyard heterogeneity. The first batch of grapes was harvested on (8/2/2017) at 18°B. The second batch of grapes was harvested on 7/3/2017 at 22°B, while the last batch was harvested on 20/3/2017 at 25°B. Grapes were processed and vinified according to the standard Nietvoorbij procedure for small-scale, white wine production. Every treatment was done in triplicate.

3.2.3.6 The effect of skin contact time at different temperatures

Sauvignon blanc grapes (523 kg) were used for this experiment. The grapes were pooled and divided to exclude vineyard heterogeneity. For the ambient temperature (20ºC), 312 kg of grapes were used of which 101 kg was for the control wine, (destemmed and immediately pressed 0 hours' skin contact), 102.5 kg was destemmed and left on the skins for 3 hours, and 108.5 kg grapes were destemmed and left for 6 hours on the skins and juice. The rest of the grapes were cooled to 10ºC. After 10ºC was reached, 109 kg of grapes were destemmed and left for 3 hours on the skins, and 102 kg were destemmed and left for 6 hours on the skins in a 10ºC cool storage room. Grapes were further processed and vinified according to the standard Nietvoorbij procedure for small-scale, white wine production. Every treatment was done in triplicate.

3.2.3.7 The efficacy of different fining agents to reduce pinking susceptibility

Twenty-nine different fining agents were sourced from different suppliers (Table 3.1). The fining agents were added at the maximum dose prescribed by these suppliers and tested at three different winemaking stages, settling, fermentation, and fining. At every stage, the dosages were adjusted for the loss of wine after racking. The juice was mixed thoroughly before 264 x 5 L PET bottles (261 treatments plus 3 for control) were filled up to the 4 L mark. Twenty-nine different chemicals were weighed for 4 L volumes and added to 87 of these 5 L PET bottles. The 87 bottles and the rest of the 177 bottles (with no fining agents added) were left overnight at 14ºC. The next morning the juice was racked off the lees to clean 5 L PET bottles and active dried yeast from Anchor Oenobrands (VIN13) was added at a dose of 30 g/hL. DAP was also added, and 50 g/hL bentonite solution was added 3 days later. The next 29 fining agents were added to the second group of 87 bottles for the fermentation stage. After the completion of fermentation, the wine was tested for residual sugar concentrations to ensure fermentation to

dryness and racked to clean 5 L PET bottles. The last 29 fining agents were added to the last group of 87 bottles, and all wines were cold stabilised at 0ºC for 14 days (Figure 3.2). The three control wines, therefore, received no fining agents and were processed and vinified according to the standard Nietvoorbij procedure for small-scale, white wine production. After the acquired period for cold stabilisation, the wines were filtered under reductive conditions into 750 mL glass bottles and closed with Roté capsules and stored until further analysis.

Table 3.1: The fining agents, with their active ingredients in brackets, used for the fining agent experiment

Number	**Fining agent**	**Dosage added**
1	Product 1 (*Fish-based fining agent*)	2 g/hL
2	Product 2 (P*ea protein + PVPP*)	80 g/hL
3	Product 3 (*PVPP + micro-pulverised cellulose*)	50 g/hL
4	Product 4 (F*ish-based fining agent*)	1.5 g/hL
5	Product 5 (*Liquid gelatine from porcine origin*)	100 mL/hL
6	Product 6 (*Vegetable protein – patatin*)	20 g/hL
7	Product 7 (*Gum Arabic*)	300 mL/hL
8	Product 8 (*30% liquid silicon dioxide colloidal solution*)	100 mL/hL
9	Product 9 (*Liquid gelatine from porcine origin*)	100 mL/hL
10	Product 10 (*PVPP + potassium caseinate*)	100 g/hL
11	Product 11 (*Hydrolysed and condensed tannins*)	15 g/hL
12	Product 12 (*Gelatine*)	10 g/hL
13	Product 13 (*Selected silica + bentonite*)	Must: 50 g/hL Wine: 40 g/hL
14	Product 14 (*Fish-based fining agent*)	2 g/hL
15	Product 15 (*Selected yeast hulls, selected silica*)	20 g/hl
16	Product 16 (*Potassium metabisulfite, ascorbic acid, citric acid + hydrolysed tannins*)	20 g/hL
17	Product 17 (S*elected yeast cell walls, PVPP + selected silica*)	25 g/hL
18	Product 18 (*Polyvinylpolypyrrolidone*)	20 g/hL
19	Product 19 (*Natural gum from Acacia trees*)	100 g/hL
20	Product 20 (*Solution of silica dioxide*)	Must: 100 g/hL Wine: 70 g/hL
21	Product 21 (*Natural gum from Acacia trees*)	100 g/hL
22	Product 22 (*Polyvinylpolypyrrolidone*)	10 g/hL
23	Product 23	200 mg/L
24	Product 24 (*Ascorbic acid*)	100 mg/L
25	Product 25	20 g/hL
26	Product 26 (*Ascophyllum nodosum*)	10 mg/L
27	Product 27 (*Gelatine*)	Must 10 g/hL Wine 4 g/hL
28	Product 28 (*Yeast hulls*)	Must: 20 g/hL Wine: 4 g/hL
29	Product 29 (*Specific inactivated yeast cells*)	40 g/hL

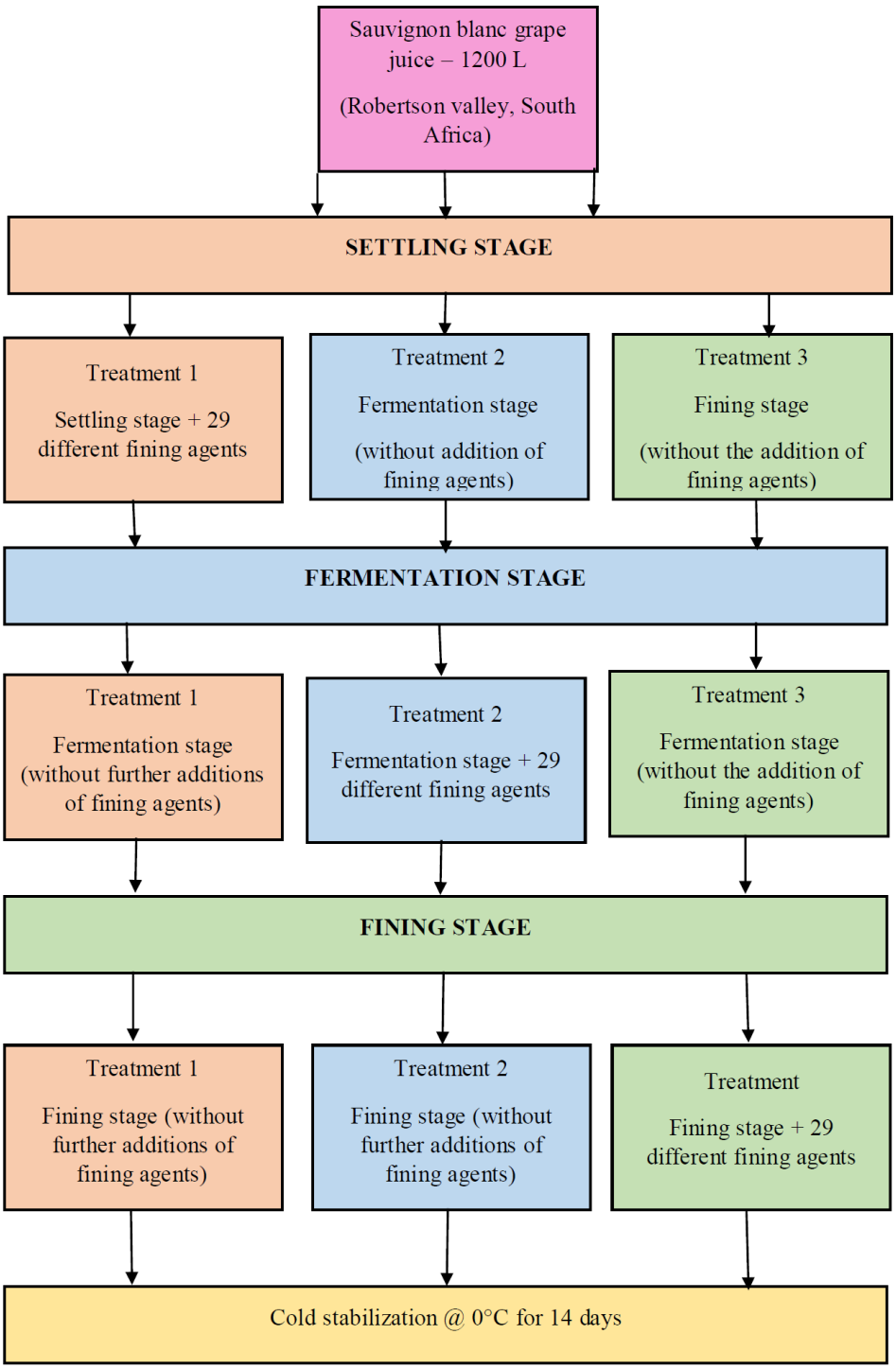

Fig. 3.2: Diagrammatic representation of the experimental layout of the fining agent experiment

3.2.3.8 The effect of sunlight on pinked wines

The effect of sunlight irradiation on the colour of pink wine has not been researched. An experiment was devised to test the effect of sunlight irradiation on the pink colour and phenolic concentration of a Sauvignon blanc wine after 15, 30, 60, and 120 minutes in direct sunlight. Sauvignon blanc wine (2017 vintage from the Robertson area) with high pinking potential (0.123 AU measured at 500 nm) was used. The wine pinked naturally (i.e. without the addition of H_2O_2, and letting the wine stand open for three days). The pinked wine was decanted, using CO_2 gas to prevent further oxidation, in 100 mL transparent Schott bottles and left in the sun for 0, 15, 30, 60, and 120 minutes. After these designated times in direct sunlight, the wine samples were analysed spectrophotometrically at 500 nm to access the colour against the original pinking values. Phenolic compounds were assessed by HPLC. Compounds analysed include gallic acid, epicatechin-3-O-gallate, caftaric acid, epigallocatechin-3-O-gallate, caffeic acid, p-coumaric acid, syringic acid, vanillic acid, chlorogenic acid, delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside.

3.2.3.9 Investigate the interactive influence of grape maturity, grape temperature, different methods of crushing and pressing, and time of skin contact on the pinking susceptibility of Sauvignon blanc wine

Grapes (harvest 2018, total of 720 kg) from a farm in the Robertson District known to have a pinking potential, were harvested at three different stages of maturity, i.e. 18°B (240 kg), 20°B (240 kg), and 25°B (240 kg) at each stage of maturity, half the grapes were cooled to 4ºC (120 kg), and the other half/batch exposed to ambient temperature (20ºC, 120 kg) before processing. For each temperature treatment, one batch was processed and vinified according to the standard Nietvoorbij procedure for small-scale white wine production (control wine, destemmed and immediately pressed 0 hours' skin contact, 60 kg (CDK)), a second batch destemmed and left for 18 hours on the skins (CK18H), and a third whole bunch pressed (without the destemming and crushing stage, 60 kg (WB)). Every treatment was done in triplicates. Dissolved oxygen (DO) were tested as described in section 3.2.4.5. Samples for juice analyses were taken after crushing and for wine analyses, after bottling.

3.2.4 Analyses

3.2.4.1 Pinking test

Pinking susceptibility was analysed as described in the SASEV laboratory manual (2002). A hydrogen peroxide solution of 0.072% was prepared by adding 120 µL of a 30% H_2O_2 solution to 50 mL of distilled water. One mL of this H_2O_2 solution was added to 25 mL of wine. No H_2O_2 is added to the control wine sample. The samples are kept in the dark for at least 8 hours. Samples were done in triplicate. All samples were analysed 1 month after bottling. The pinking

potential was measured against the control and was read at 500 nm on a spectrophotometer (Thermospectronic Heλios Gamma) with a 10 mm path length using 2 mL plastic cuvettes.

3.2.4.2 FTIR analysis

Wines were analysed for alcohol, titratable acidity (TA), volatile acidity (VA), pH, malic acid, lactic acid, glucose, fructose, and glycerol. The work was performed on a FOSS WineScan FT120 FT-MIR spectrometer equipped with a DTGS pyroelectric detector and in-house calibrations of the chemical-analytical laboratory of the South African Grape and Wine Research Institute. Measurements were carried out using a liquid flow-through cell equipped with two CaF_2 windows. Each window has a 10 mm diameter circle with 2 mm thickness, providing an optical aperture of 88.54 mm^2 and a cell volume of 3.276 µL. A polyethylene terephthalate spacer provides a 37 µm optical path length. To minimize the problem of internal reflections (interference fringe) the CaF_2 windows are mounted slightly non-parallel. The interferogram of the sample in the cell is recorded using an optical resolution of 14 cm^{-1} × 2000 cm^{-1}, and averages based on 10 scans. The instrument is equipped with a He/Ne laser (632 nm) and a silicium detector to track the position of the moving mirror in the interferometer.

3.2.4.3 Metal analysis

Trace metals were analysed on an Agilent 7900 ICP-MS, using the standard configuration of quartz spray chamber and torch, and Ni-plated sampling and skimmer cones. A 0.4 mL/min micro-mist nebulizer was used to aspirate the sample. The instrument was optimised for sensitivity and oxide formation before calibration. Instrument parameters were set as follows: USEPA Methods 6020A and 200.8 guidelines are followed for instrument calibration and data verification protocols. The instrument was calibrated using NIST traceable standards purchased from Inorganic Ventures, and the accuracy of the calibration was validated by a separate standard from Merck. A drift monitor standard was analysed after every 12 samples, with internal standard elements added online to correct for drift and matrix differences between samples and standards. The metal analysis was only done where pinking was significant.

3.2.4.4 HPLC

Samples were taken from all the experiments filtered through a 0.45 µm filter before being injected into a Waters Synapt G2 quadrupole time-of-flight mass spectrometer. It was fitted with a Waters Ultra pressure liquid chromatograph and photodiode array detection. The separation was achieved on a Waters HSS T3 column, 2.1 x 100 mm with 1.7 µm particles. A gradient was applied using 0.1% formic acid (solvent A) and acetonitrile (solvent B). The gradient started at 100% solvent A for 1 minute and changed to 50% B over 22 minutes in a linear way. It then went to 100% B after 23 minutes where it was held until 24.5 minutes, followed by re-equilibration to initial conditions for 4 minutes. The flow rate was 0.25 mL/min and the column

were kept at 60ºC. The injection volume was 3 µL. Data were acquired in MSE mode which consisted of a low collision energy scan (6V) from m/z 150 to 1500 and a high collision energy scan from m/z 40 to 1500. The high collision energy scan was done using a collision energy ramp of 30-60V. The photodiode array detector was set to scan from 220 - 600 nm. The mass spectrometer was optimized for best sensitivity, a cone voltage of 15V, desolvation gas was nitrogen at 650 L/hr and desolvation temperature 275ºC. The instrument was operated with an electrospray ionization probe in the negative mode. Sodium formate was used for calibration and leucine encephalin was infused in the background as lock mass for accurate mass determinations. The following phenols were measured on HPLC: Gallic acid, catechin, epicatechin, caffeic acid, p-coumaric acid, chlorogenic acid, ferulic acid as well as the following anthocyanins: Delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside. Phenols analysis was done where pinking differences between treatments were significant.

3.2.4.5 Dissolved oxygen measurements

The dissolved oxygen of the 2018 vintage wine was measured with a NomaSense O_2 P300 handheld meter. Dissolved oxygen was measured for the investigation of the interactive influence of grape maturity, grape temperature, different methods of crushing and pressing, and time of skin contact on the pinking potential of white wine. This meter has a sensor and a thermometer that is placed in the wine. The dissolved oxygen concentrations are measured and calibrated with the wine's temperature at that time. Dissolved oxygen was measured after crushing and destemming (O2_Bucket), transferring to 4.5 L glass bottles (O2_PET), after 1 hour (O2_17.5ºC) after settling (O2_14ºC) and after alcoholic fermentation (O1_AF). Every measurement was done for all the treatments and triplicates.

3.2.5 Statistical analysis

Analysis of variance (ANOVA) was conducted according to the experimental design using SAS statistical software (Version 9.4, SAS Institute Inc., Cary, NC, USA). Shapiro-Wilk test was performed on the standardized residuals from the model to test for deviation from normality (Shapiro & Wilks, 1965). 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 tests. Multivariate analyses were performed using XLSTAT, version 2016 (Addinsoft, Paris, France).

3.3 RESULTS AND DISCUSSIONS

3.3.1 The effect of different addition of ascorbic acid to juice on pinking susceptibility of the Sauvignon blanc wine

Grapes for the 2015 harvest season were sourced from two farms in the Durbanville area. Producer 1 claimed to have little or no pinking susceptibility in his wine, while producer 2 claimed to have regular pinking susceptibility in his wines. Juice from both producers were analysed for standard or routine parameters, i.e. degrees balling (°B), pH, and titratable acidity (TA). No significant differences were noticed between the different treatments and the control juice, except for producer 1 of which the control sample was not analysed and the significant differences could not be established. Standard wine parameters, pH, volatile acidity, alcohol, titratable acidity, malic acid, lactic acid, glucose, fructose, and glycerol were also tested by FTIR analyses (Table 3.2). Although there are slight differences between the ratios, the biggest differences that stand out are the fructose for producer 2, which is between 2 to 3 times higher in the treatments than in the control. Adding ascorbic acid to wine, it could be expected that the pH will decrease and that the TA will increase slightly. It is difficult making a conclusion of Producer 1 as the control samples were not measured. In producer 2 it is evident that there is a decrease in TA and an increase in pH in the different treatments (Table 3.2). The study done by Skouroumounis *et al.* (2005) showed that there was no significant increase in TA and decrease in pH of Riesling and Chardonnay wines with added ascorbic acid. Normally stronger acids like tartaric acid and citric acids are used to increase the acidity of the wine, but weaker acids like ascorbic acid (adjuvant to SO_2), succinic acid (developed during fermentation) etc. are not used for wine acidity increases (Ribéreau-Gayon *et al.*, 2006). Relative to the control, there was a slight decrease in TA and an increase in pH, however, there were no significant differences between the ascorbic acid ratios of the treatments. After studying the degradation products of ascorbic acid in oranges and grapefruit, Tatum *et al.* (1969) found that fructose was formed after the degradation of ascorbic acid. This theory could explain the significantly higher concentrations of fructose in the wine after the addition of 2 parts ascorbic acid for producer 2.

Table 3.2: FTIR analyses results of wines after bottling

Treatment	pH	VA (g/L)	TA (g/L)	MA (g/L)	LA (g/L)	Glc (g/L)	Fru (g/L)	Alc (% v/v)	Gly (g/L)
				PRODUCER 1					
Control	-	-	-	-	-	-	-	-	-
Hyper-oxidised	3.06^{a}	0.34^{a}	6.78^{ab}	2.94^{ab}	<0.2^{a}	0.41^{a}	2.46^{a}	13.7^{a}	6.46^{ab}
Ascorbic:SO_2 (0.5:1)	2.96^{b}	0.30^{a}	7.27^{a}	3.47^{a}	<0.2^{a}	0.36^{a}	2.14^{a}	13.3^{a}	6.62^{ab}
Ascorbic:SO_2 (1:1)	2.97^{b}	0.32^{a}	6.93^{ab}	2.96^{ab}	<0.2^{a}	0.52^{a}	5.44^{a}	13.8^{a}	6.32^{b}
Ascorbic:SO_2	3.01^{ab}	0.33^{a}	6.69^{b}	2.74^{b}	<0.2	0.51	5.61^{a}	13.8^{a}	6.84^{a}

(2:1)					a	a			
				PRODUCER 2					
Control	3.00^{b}	0.30^{b}	6.81^{a}	2.67^{ab}	<0.2 a	0.53 a	2.00^{c}	13.6^{b}	7.28^{a}
Hyper-oxidised	2.99^{b}	0.35^{a}	6.91^{a}	2.65^{ab}	<0.2 a	0.40 a	3.71^{cb}	13.7^{ab}	6.55^{b}
Ascorbic:SO_2 (0.5:1)	3.05^{a}	0.33^{ab}	6.58^{b}	2.84^{a}	<0.2 a	0.63 a	4.26^{b}	13.8^{ab}	6.95^{ab}
Ascorbic:SO_2 (1:1)	3.09^{a}	0.36^{a}	6.47^{b}	2.69^{ab}	<0.2 a	0.54 a	4.40^{b}	14.2^{a}	7.03^{a}
Ascorbic:SO_2 (2:1)	3.06^{a}	0.36^{a}	6.41^{b}	2.46^{b}	<0.2 a	0.45 a	7.07^{a}	13.7^{ab}	6.82^{ab}

VA – Volatile Acidity, TA – Titratable Acidity, MA – Malic Acid, LA – Lactic Acid, Glc – Glucose, Fru – Fructose, Alc – Alcohol & Gly – Glycerol. Ascorbic acid: SO_2 ratios of 0.5:1, 1:1 & 2:1 indicate the ratios added at crushing/destemming, with no further adjustments/additions. Statistics were done separately for each producer. Different letters depict significant differences ($p \leq 0.05$) between treatments. The control sample of Producer 1 was accidentally discarded by the lab.

After bottling, all the wine samples were tested for pinking potential. Wines did not show pinking potential during this study. Even though none of the samples, for both the two producers, showed any pinking potential, the control samples, for both the producers, showed a higher tendency for an increased absorbance at 500 nm than the ascorbic acid treatments and hyper-oxidised wines, decreasing from control to hyper-oxidised and with increased ascorbic acid: SO_2 ratio treatments (Figure 3.3). Producer 1's control showed no significant differences between the control and the hyper-oxidised wines. The 0.5:1 ascorbic acid: SO_2 showed a significant difference of a lower 500 nm value-form both the control and the hyper-oxidised wines. The ascorbic acid: SO_2 addition treatments (1:1 & 2:1) showed no significant differences between them. Producer 2 showed no significant differences between the treatments.

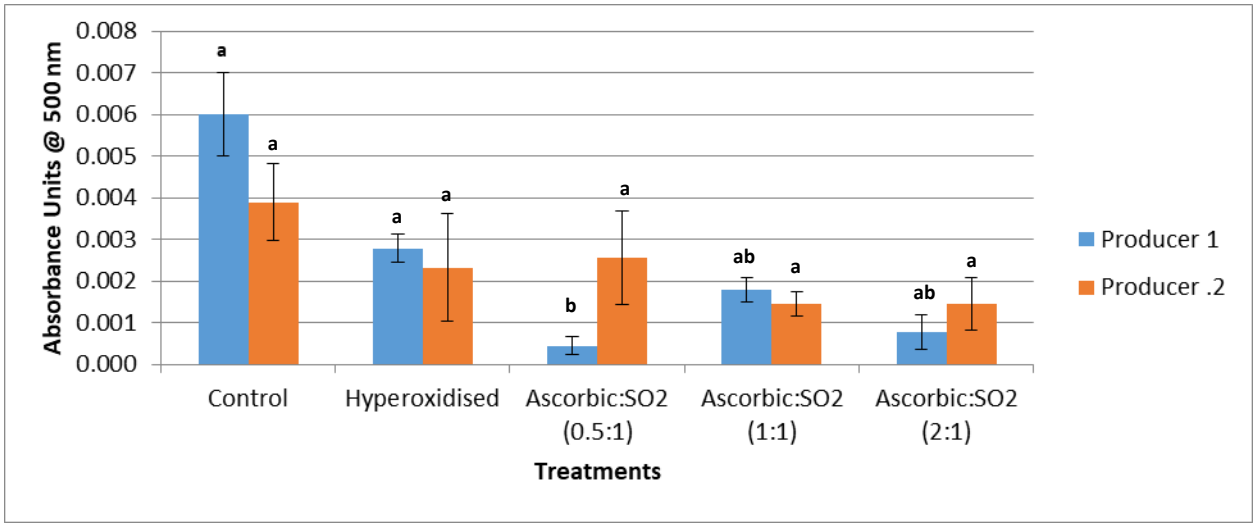

Fig. 3.3: Graph depicting absorbance at 500 nm as a function of different treatments. Ascorbic acid: SO_2 ratios of 0.5:1 (molar ratio 3.17:1), 1:1 (molar ratio 1.59:1) & 2:1 (molar ratio 0.79:1) indicate the ratios added at crushing/destemming, with no further adjustments/additions. Statistics were done separately for each producer. Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments. Error bars represent the standard error of the mean.

In both cases, for producer 1 and 2, the hyper-oxidised wine showed a lower value at 500 nm than the control wine, although not significantly. After hyper-oxidation, the juice was left to settle and was racked from its lees before fermentation the next day. This action oxidised the phenols in the wine into their colour form after which they settled out, preserving the wine against the potential for oxidative browning at later stages in the winemaking process (Ricardo-da-Silva *et al.*, 1993; Schneider, 1998). It seems that during this hyper-oxidation process some precursors to pinking are also oxidised and settled out as both producers showed a 50% decrease in pinking susceptibility. The use of ascorbic acid had a further decreasing effect on the potential to pink (De Beer *et al.*, 2003; Barril *et al.*, 2016). Ascorbic acid has antioxidative properties and is capable to protect phenols and other components against oxidation (Simpson *et al.*, 1983; Skouroumounis *et al.*, 2005). Normally, winemakers will add ascorbic acid at crushing (Day *et al.*, 2015) or just before bottling at a concentration of 40 - 100 mg/L (Barril *et al.*, 2016). The H_2O_2 formed with ascorbic acid addition could lead to an induced pinking formation as well as browning and shorter shelf life (Bradshaw *et al.*, 2001; Barril *et al.*, 2016). The importance of the use of SO_2 and the ratio of ascorbic acid to SO_2 must, however, not be underestimated. Figure 3.3 showed that for producer 2 the molar ratio of ascorbic acid:SO_2 (0.79:1) did decrease pinking susceptibility, although it was non-significant. For producer 1 the molar ratio of ascorbic acid:SO_2 (3.17:1) did reduce the pinking susceptibility significantly. Ascorbic acid reacts with SO_2 at a ratio of 1:1.7 and not 1:1 as generally accepted (Barril *et al.*, 2012; Danilewicz & Standing, 2018). Although there was no significant differences between the treatments, it still shows that ascorbic acid is an excellent antioxidant, more so when used in conjunction with SO_2. When ascorbic acid is added to wine it dissociates into dehydro-ascorbic acid and two H^+ ions. These two H^+ ions will immediately seek out O_2 anions and bind with them forming H_2O_2. For ascorbic acid to be an effective antioxidant (Barril *et al.,* 2012), it needs to be used in conjunction with SO_2 so that the SO_2 can bind to H_2O_2 preventing further complications in the wine (Simpson *et al.*, 1983; Barril *et al.*, 2012). In a study done by Simpson *et al.* (1983) three groups of wines with different concentrations of free SO_2 (FSO_2) were used, and with each level, three different concentrations of ascorbic acid were added. With the first group of 0 mg/L FSO_2, the three groups of ascorbic acid additions were 0, 50, and 100 mg/L ascorbic acid. Only with the combination of 0 mg/L FSO_2 and 0 & 50 mg/L ascorbic acid was there a pinking susceptibility noticed. With the 100 mg/L ascorbic acid added the pinking susceptibility dropped to a value of 0.06. With a low FSO_2 (ca. 15 mg/L) and high FSO_2 (ca. 34 mg/L) and the added ascorbic acid additions of 0, 50, and 100 mg/L no pinking susceptibility was noticed. It is therefore important first to determine the existing concentration of ascorbic acid in the wine before making any ascorbic acid additions or adjustments. It is always important to keep the FSO_2 concentration of the wine as close as possible to 35 – 45 mg/L when using ascorbic acid

(Tobe, 1983). As soon as dry ice and inert gas (reductive winemaking conditions) are involved, it is important to keep the SO_2 of the wine as close as possible to the above-mentioned values.

3.3.2 The effect of skin contact time on the pinking susceptibility of Sauvignon blanc wines

Standard parameters were analysed for juice: degrees balling (°B), pH, titratable acidity (TA), and free and total SO_2, and for wine: pH, volatile acidity, alcohol, titratable acidity, malic acid, lactic acid, glucose, fructose, and glycerol by FTIR analyses. No significant treatment differences were noticed for the standard parameters (results not shown). Oxidative and reductive winemaking processes were compared in their ability to promote pinking during skin contact time. The pinking susceptibility of the wines was determined after bottling. Skin contact time had very little ($p \leq 0.05$) influence on the pinking potential in wines prepared under standard winemaking conditions (Figure 3.4 A) and reductive winemaking conditions (Figure 3.4 B). The pinking susceptibility increased but insignificantly with skin contact under reductive winemaking conditions, with 12 hours of skin contact showing the highest value at 500 nm (Figure 3.4 B).

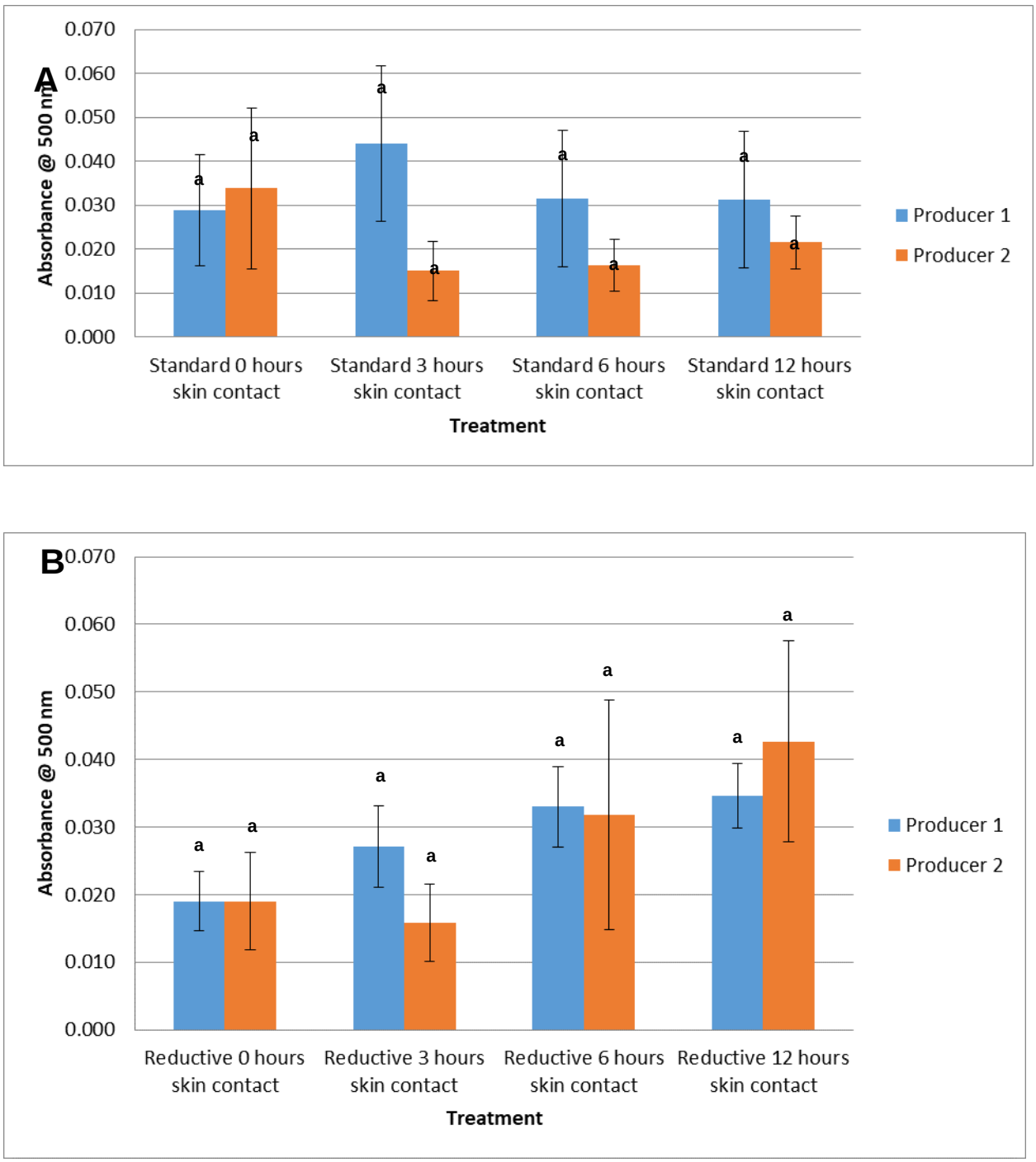

Fig. 3.4: The influence of skin contact time, under standard winemaking conditions, on the pinking potential of Sauvignon blanc wines. (A) Blue - Standard winemaking (oxidative), (B) Red – Reductive winemaking. 0, 3, 6, and 12h – depicts the different skin contact time in hours. Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments. Error bars represent the standard error of the mean. Statistics were done separately for each producer.

According to Simpson (1977a), phenols may contribute to pinking. Phenols are a broad concept and the effect on white wines are well researched. The effect of phenols on pinking is not known. Different phenols were analysed to establish the possibility of phenolic contribution to pinking susceptibility. Results of qualitative and quantitative HPLC analysis of phenols of wines produced from grapes with and without pinking potential from two producers, subjected to either standard or reductive winemaking, with and without skin contact, is shown in Table 3.3. For the standard winemaking for producer 1, the gallic acid starts with a low concentration at 0 hours' skin contact, increase with 3- & 6-hours' skin contact and then decrease to 12-hours' skin contact. This is an indication of polymerisation that is taking place during the long skin contact time. While this is evident at the standard winemaking process, the same cannot be said for the reductive winemaking process, where there are no significant differences during the skin contact time. The extraction for epicatechin follows the same tendency for both the standard and the reductive winemaking process. In both cases, the extraction is low at 0 hours' skin contact and increases with 12-hours' skin contact. There are no significant differences between skin contact time for the rest of the phenols. Catechin concentrations were only detectable at 6 hours' skin contact time. p-Coumaric acid had low concentration at 0- and 3-hours' skin contact time (6.70 mg/L and 6.97 mg/L respectively), increasing with 6- and 12-hours' skin contact time.

Gallic acid extraction for producer 2 followed the same tendency for both the standard and the reductive winemaking processes. The extraction increases with longer skin contact time. Catechin was more extracted with increased skin contact during the reductive winemaking process than with the standard winemaking process. Caffeic acid, p-coumaric acid and chlorogenic acid were more extracted from 6- and 12-hours' skin contact at the reductive winemaking process. Epicatechin concentration peaked at 6- to 12-hours' skin contact time during standard and reductive winemaking conditions.

Table 3.3: Phenolic concentrations of Sauvignon blanc wines made from two different producers.

	Gallic acid (mg/L)		Catechin (mg/L)		Epicatechin (mg/L)		Caffeic acid (mg/L)		p-Coumaric acid (mg/L)		Chlorogenic acid (mg/L)		Ferulic acid (mg/L)	
Treatment	**Producer 1**		**Producer 1**		**Producer 1**		**Producer 1**		**Producer 1**		**Producer 1**		**Producer 1**	
	Std	**Red**	**Std**	**Red**	**Std**	**Red**	**Std**	**Red**	**Std**	**Red**	**Std**	**Red**	**Std**	**Red**
O hours	2.12^{c}	10.95^{a}	nd	1.45^{a}	7.52^{b}	9.42^{b}	9.36^{a}	7.79^{b}	6.70^{b}	5.38^{b}	97.86^{a}	80.55^{b}	3.34^{a}	3.46^{a}
3 hours	11.11^{a}	11.16^{a}	nd	nd	9.43^{a}	9.05^{b}	7.85^{b}	8.10^{b}	6.97^{b}	6.55^{b}	58.60^{b}	101.70^{a}	3.36^{a}	3.48^{a}
6 hours	11.69^{a}	10.82^{a}	1.66^{a}	1.57^{a}	8.73^{a}	9.29^{b}	10.52^{a}	10.33^{a}	12.66^{a}	13.59^{a}	80.93^{a}	83.34^{b}	3.33^{a}	3.73^{a}
12 hours	9.60^{b}	11.12^{a}	nd	1.47^{a}	9.10^{a}	10.84^{a}	9.53^{a}	8.19^{b}	10.08^{a}	7.87^{b}	75.99^{a}	70.33^{c}	3.20^{a}	3.45^{a}
Treatment	**Gallic acid (mg/L) Producer 2**		**Catechin (mg/L) Producer 2**		**Epicatechin (mg/L) Producer 2**		**Caffeic acid (mg/L) Producer 2**		**p-Coumaric acid (mg/L) Producer 2**		**Chlorogenic acid (mg/L) Producer 2**		**Ferulic acid (mg/L) Producer 2**	
	Std	**Red**	**Std**	**Red**	**Std**	**Red**	**Std**	**Red**	**Std**	**Red**	**Std**	**Red**	**Std**	**Red**
O hours	7.42^{b}	7.06^{b}	nd	nd	7.57^{b}	7.34^{b}	10.27^{a}	8.97^{b}	10.02^{b}	10.76^{b}	35.69^{a}	35.74^{b}	3.82^{a}	3.71^{a}
3 hours	7.96^{a}	7.54^{b}	2.56^{a}	2.18^{b}	7.84^{a}	5.59^{c}	8.87^{b}	10.60^{b}	10.74^{b}	10.46^{b}	39.99^{a}	27.51^{b}	4.00^{a}	3.83^{a}
6 hours	8.02^{a}	7.74^{a}	2.17^{a}	2.23^{b}	8.04^{a}	8.61^{a}	11.29^{a}	14.64^{a}	12.19^{a}	15.24^{a}	37.02^{a}	57.01^{a}	3.87^{a}	3.93^{a}
12 hours	8.07^{a}	8.48^{a}	2.38^{a}	2.84^{a}	8.31^{a}	7.59^{b}	11.32^{a}	13.72^{a}	10.74^{b}	15.89^{a}	30.88^{b}	52.37^{a}	3.74^{a}	3.79^{a}

All values are in mg/L. Different letters depict significant differences ($p \leq 0.05$) between treatments. Table values represent the average of triplicate treatments. Statistics were done separately for each producer.

Only the five main anthocyanins were tested and not their glycoside species. The concentrations of anthocyanins were non-detectable (ND), which means that there may be traces of anthocyanins in the wines, but the concentrations were too low to quantify or there were no anthocyanins present. Normally anthocyanins are found in the skins of red grapes and one would not expect any traces of anthocyanins in white grapes. However, in experiments done by Arapitsas *et al.* (2015), the authors found trace amounts of delphinidin-3-O-glucoside (1.08 µg/L), cyanidin-3-O-glucoside (7.91 µg/L), petunidin-3-O- glucoside (2.51 µg/L), peonidin-3-O-glucoside (8.95 µg/L) and malvidin-3-O- glucoside (34.96 µg/L) in Sauvignon blanc grapes.

During cold maceration and skin contact phenols like protocatechuic acid, catechin, epicatechin, caffeic acid, gallic acid, ethyl gallate, *p*-hydroxybenzoic acid, quercetin-3-rutinoside, quercitin-3-glucoside, quercetin-3-L-rhamnoside, quercetin-3-D-galactoside, *cis*-coutaric acid, *trans*-coutaric acid, *m*-coutaric acid, *p*-coutaric acid, caftaric acid, ferulic acid, fertaric acid, and coumaric acid, are extracted from the skins and seeds (Hernanz *et al.*, 2007; Gómez-Míguez *et al.*, 2007; Maggu *et al.*, 2007; Ružić *et al.*, 2011). The extracted phenols can influence the colour of white wines under different conditions including exposure to polyphenol oxidase enzymes (Simpson, 1982; Lee & Jaworski, 1988; Muñoz-Pina *et al.*, 2018), storage, when exposed to light (Grant-Preece *et al.*, 2018), temperature (Scrimgeour *et al.*, 2015) and oxygen ingress (Serra-Cayuela *et al.*, 2013; Nenadis &·Paraskevopoulou, 2016). The correlation between phenols and browning is well documented (De Villiers, 1961; Peterson & Caputi, 1967; Lee & Jaworski, 1988; Cheynier *et al.*, 1990; Bradshaw *et al.*, 2001; Razmkhab *et al.*, 2002; Lopez-Toledano *et al.*, 2006; Li *et al.*, 2008; Nenadis & Paraskevopoulou, 2016). These extracted phenols could also contribute to the pinking phenomena (Simpson, 1977a; Simpson, 1980; Andrea-Silva *et al.*, 2014).

A Principal component analysis (PCA) scores and loading plot, explaining 73.62% of the variation, depicting two separate and distinct areas of association (Figure 3.5). Both the 6- and 12-hours' skin contact, reductive and standard winemaking procedures, are associated with phenols, i.e. chlorogenic acid, epicatechin, gallic acid, catechin, p-coumaric acid and caffeic acid.

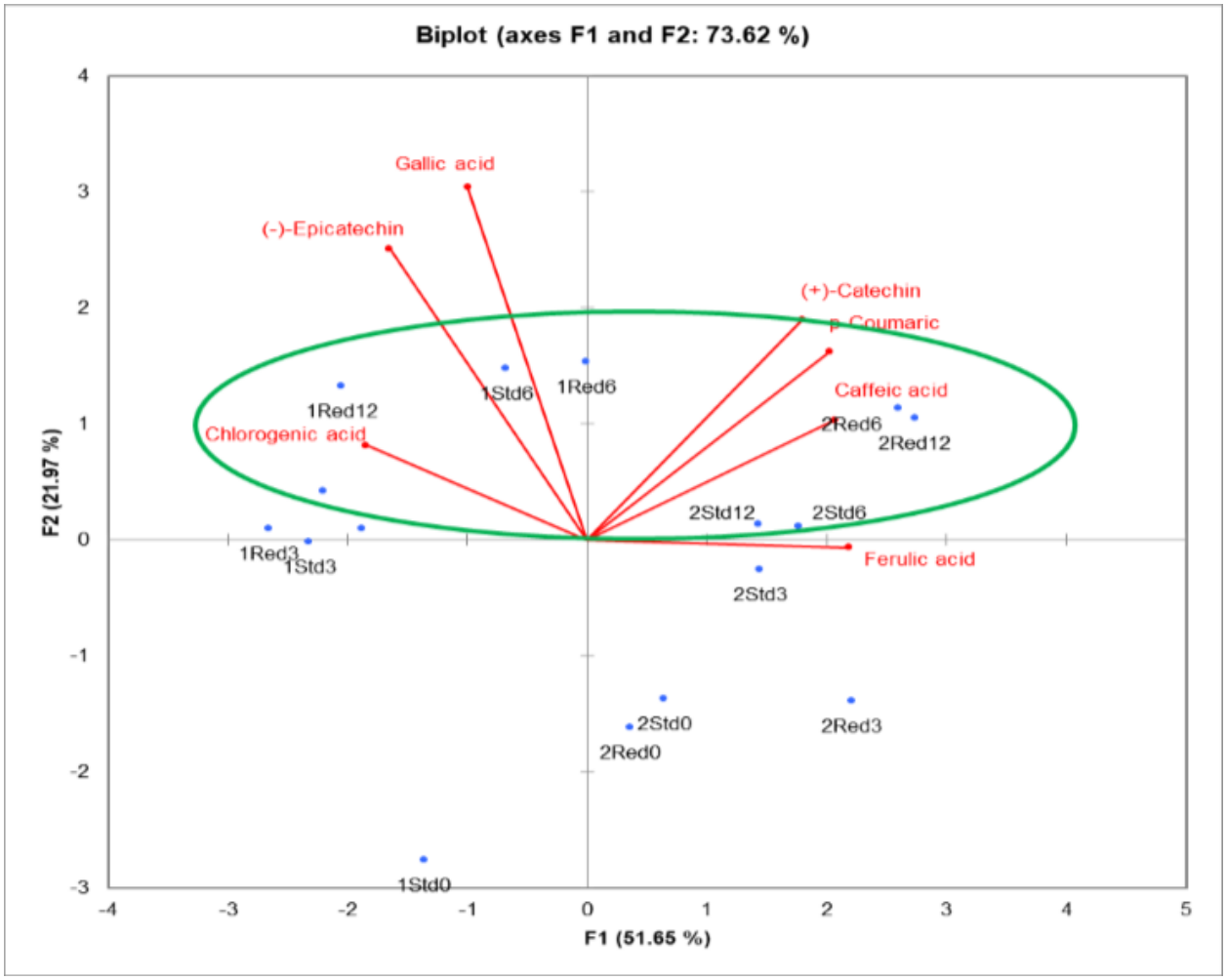

Fig. 3.5: Influence of skin contact time under reductive and standard winemaking conditions on phenols in Sauvignon blanc wines produced from grapes from two producers. Abbreviations: “Red”, reductive winemaking methods; “Std”, indicate wine prepared according to the standard Nietvoorbij white winemaking protocol; 0, 3, 6, 12 indicate the skin contact times in hours; and prefix 1 and 2 indicated producers 1 and 2.

In a study done by Amendola *et al.* (2010), they found that the phenol extraction increased until about 3 hours. Then observed flattening in phenolic extraction after 3-hours was due to either the maximum extraction or polymerization that took place. Hernanz *et al.* (2007) also reported that phenol extraction was at its peak at 12-hours of skin contact. The imperceptible lower phenolic concentration in standard wines compared to reductive wines could be the result of oxidation of phenols during standard winemaking processes compared to reductive winemaking where the use of inert gas prevents phenolic oxidation (Cáceres-Mella *et al.,* 2013). George *et al.* (2006) discovered that when caffeic acid and (+)-catechin are oxidised the result is a reddish colouration. As can be seen in Table 3.3, the highest concentration for caffeic acid and catechin is at 6- and 12-hours' skin contact for reductive winemaking (producer 2). Although non-significant, the highest values at 500 nm were consistent with 6- and 12-hours' skin contact time. This could indicate that caffeic acid, in conjunction with (+)-catechin may lead to a pinkish discolouration in white wines.

3.3.3 The effect of grape temperature on pinking susceptibility

Grapes from the Robertson valley were harvested early in the morning and brought to the experimental cellar. A batch (done in triplicate) was cooled down to 0°C, while another batch (done in triplicate) was kept outside to warm up to 20°C. Grape juice was analysed for standard parameters, i.e. degrees balling (°B), pH, and titratable acidity (TA). No significant differences were noticed for standard parameters between treatments. Standard wine parameters, pH, volatile acidity (VA), alcohol (% Alc), titratable acidity (TA), malic acid (MA), lactic acid (LA), glucose (Glc), fructose (Fru), and glycerol (Gly) were also analysed by FTIR analyses. No significant differences were noticed (data not shown). Pinking susceptibility, metal and phenolic analyses were performed a month after bottling.

As observed in figure 3.6 the pinking potential of wines made from grapes at 4ºC was about 0.05 AU, the protocol limit for pinking susceptibility. The AU for wines from grapes pressed at ambient temperature was 0.02 AU. The overall temperature impact across all interactions in experiment 3.3.10 shows synergistically higher dissolved oxygen concentrations for lower temperatures, i.e. 4°C as compared to 20°C. Cool grapes/juice, therefore, can hold much more dissolved oxygen than warm grapes/juice, thus explaining the significantly higher pinking potential of wines prepared from cold grapes as the solubility of gases increases as the temperature decreases (Castellari *et al.*, 2004; Laurie *et al.*, 2008).

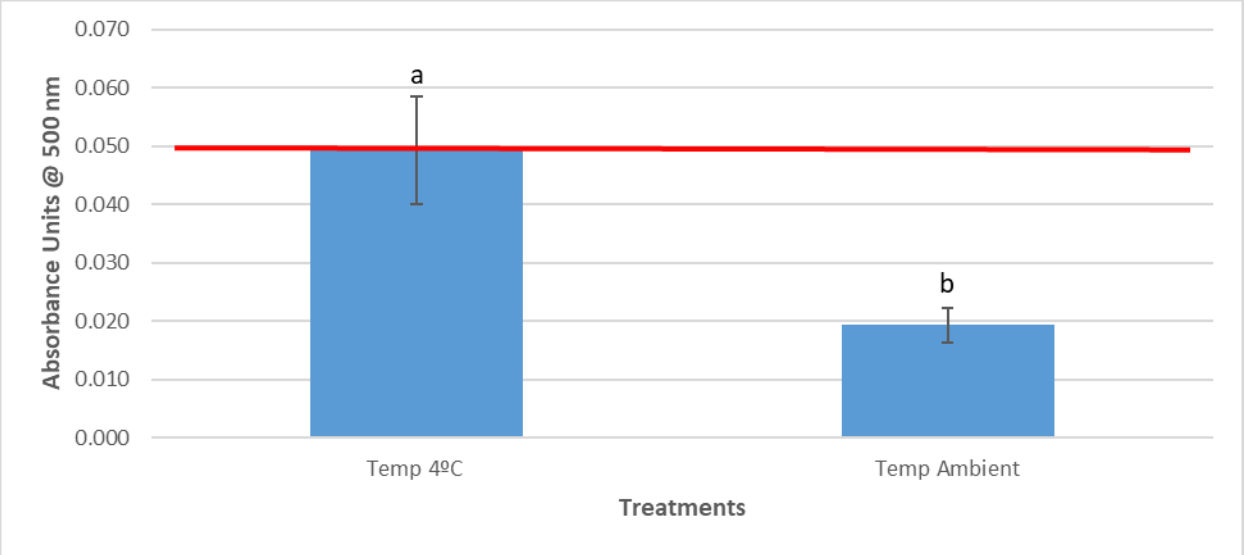

Fig. 3.6: Pinking potential measured at 500 nm for Sauvignon blanc wines made from grapes kept at 4°C and 20°C. The red line indicates the pinking potential limit as defined in the method described in the SASEV laboratory manual (2002). Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments. Error bars represent the standard error of the mean.

Juice from the 4°C and 20°C grapes were pressed and vinified, and the resulting wines were analysed for their metal contents. Wines from 4°C grapes that showed the highest potential for pinking also had higher concentrations of chromium (Cr), copper (Cu) and iron (Fe), and dissolved oxygen at the initial stages of winemaking, than wines made from 20°C grapes (Table 3.4). The two main variables that showed a definitive tendency to influence pinking are the temperature (4°C vs. 20°C) and the ripeness level (see section 3.3.5). In these two investigations, it was found that lower temperatures (4°C) and a higher ripeness level (25°B) are more prone to pinking susceptibility. A statistical analysis of metals of both these investigations showed three metals that are significantly higher in the pinking prone experiments than in the other (Tables 3.4). These metals are chromium (Cr), iron (Fe), and copper (Cu). Chromium is a heavy metal pollutant that has its origin from pesticides and equipment in the wine cellar (Pohl, 2007; Ibanez *et al.*, 2008; Čepo *et al.*, 2018). The Cr concentrations of the two wines fall well in EU of accepted concentrations of 6.5 – 31 µg/L (Ibanez *et al.*, 2008; Čepo *et al.*, 2018). The other two metals (Cu & Fe) are well linked to oxidative browning in white wines (Ibanez *et al.*, 2008; Kreitman *et al.*, 2016a, b) and could also play a role in pinking or more sensitive white cultivars.

Table 3.4: Metal concentrations of Sauvignon blanc wines produced from 4°C vs. 20°C grapes.

Treatment	Cr (µg/L)	Cu (µg/L)	Fe (µg/L)
Temp 4ºC	10.6[a]	70.1[a]	1394.1[a]
Temp Ambient	6.4[b]	39.2[b]	955.4[a]

Different letters depict significant differences ($p \leq 0.05$) between treatments. All samples were done in triplicate.

p-Coumaric acid at ambient temperature (1.95 mg/L) was the only phenol that was significantly different compared to 4ºC (1.23 mg/L). It is well known that these phenols contribute to a large extent to the protection of the vines against UV damage (Blancquaert *et al.*, 2019) and that p-coumaric acid is a very stable acid at ambient temperature and only starts to degenerate at high temperatures (Salameh *et al.*, 2008). The reason for the lower concentration of p-coumaric acid at 4ºC could be explained that less of this phenol is extracted at cooler temperatures. The hydroxycinnamic acids (of which p-coumaric acid is one) are situated in the vacuoles of the grape cells. The enzymes liberating them are inhibited by cooler temperatures, hence the lower concentration (Mafata *et al.*, 2018). As the lower temperature (4°C) showed a higher tendency for pinking susceptibility, it seems that these phenols are not linked to pinking.

3.3.4 The effect of whole bunch versus crushed and destemmed bag press

Whole bunch and crushed grape juices were analysed for degrees balling, pH, and titratable acidity. Standard wine parameters, pH, volatile acidity, alcohol, titratable acidity, malic acid, lactic acid, glucose, fructose, and glycerol were also analysed by FTIR analyses. No significant differences were noticed except for titratable acidity that was significantly ($P \leq 0.05$) lower in the wine made from a whole bunch pressed grapes (data not shown). Although the grapes were all from the same batch, harvested at the same time, there was a significant difference in the dissolved solids (°B). This could be explained that the whole bunch pressing prevents too much pressure exuded on the grapes, as the stems help to buffer the grapes. Therefore, much less dissolved solids are extracted from whole bunch pressings than for crushed and destemmed grapes. Gawel *et al.* (2016) reported a 15% higher dissolved solid in destemmed, crushed, and pressed juice compared to whole bunch pressed juice. Significant differences in pinking potential observed between treatments, dissolved oxygen, metals, and phenols as primary substrates of oxidation, are also discussed.

Most hydroxycinnamates are located in the pulp of grapes and the flavonoids in the skin and pips. Therefore, in free-run juice and lightly pressed juice, the hydroxycinnamates will be

higher in concentrations than the flavonoids (Smith & Waters, 2012). The influence of pressing on phenolics have been reported by authors Smith & Waters (2012) and Kelly *et al.* (2014). These authors reported the phenolic content to be a little bit higher in crushed and pressed juice than whole bunch pressed juice. These differences can be explained by the pressing methods used in different processes. The crushing/destemming equipment used, differ and so will the hardness/pressure of the pressing which will influence the phenolic concentrations detected.

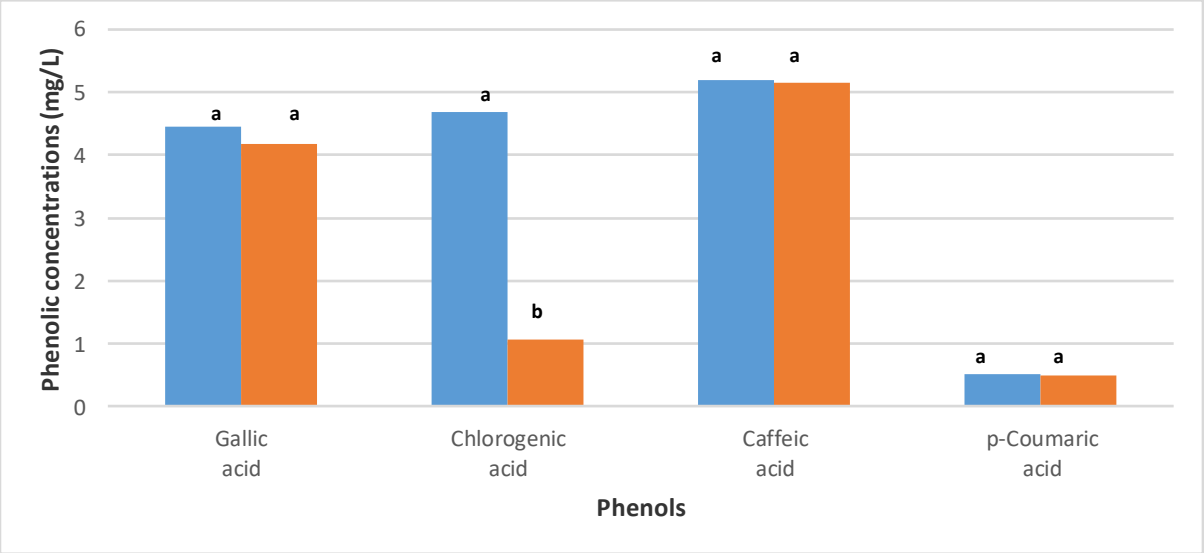

Fig. 3.7: A graph depicting phenolic concentrations of wine from bag pressed (blue bar) versus whole bunch pressed (orange bar) grapes. Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments.

Different phenols (gallic acid, chlorogenic acid, caffeic acid, and p-coumaric acid) were detected using an HPLC method (Figure 3.7). Gallic acid, caffeic acid, and p-coumaric acid showed no significant difference between bag press and whole bunch press wines, but there was a significant difference in chlorogenic acid (a 77.5% lower concentration in wine from whole bunch pressed grapes). Wine from whole bunch pressed grapes had significantly higher pinking potential than wines made from bag pressed grapes (Figure 3.8). Chlorogenic acids are formed by the esterification of quinic acid and caffeic acid (Narita & Inouye, 2013). Whole bunch pressing leads to a lower phenolic content than bag pressed wines (Hornsey, 2007). The significant lower concentrations of chlorogenic acid and the non-significant differences between the other tested phenols does not explain the higher pinking susceptibility of bag pressed wines. The causing agent for pinking susceptibility, in this case, was not tested for and could be from other phenols not tested or from another source.

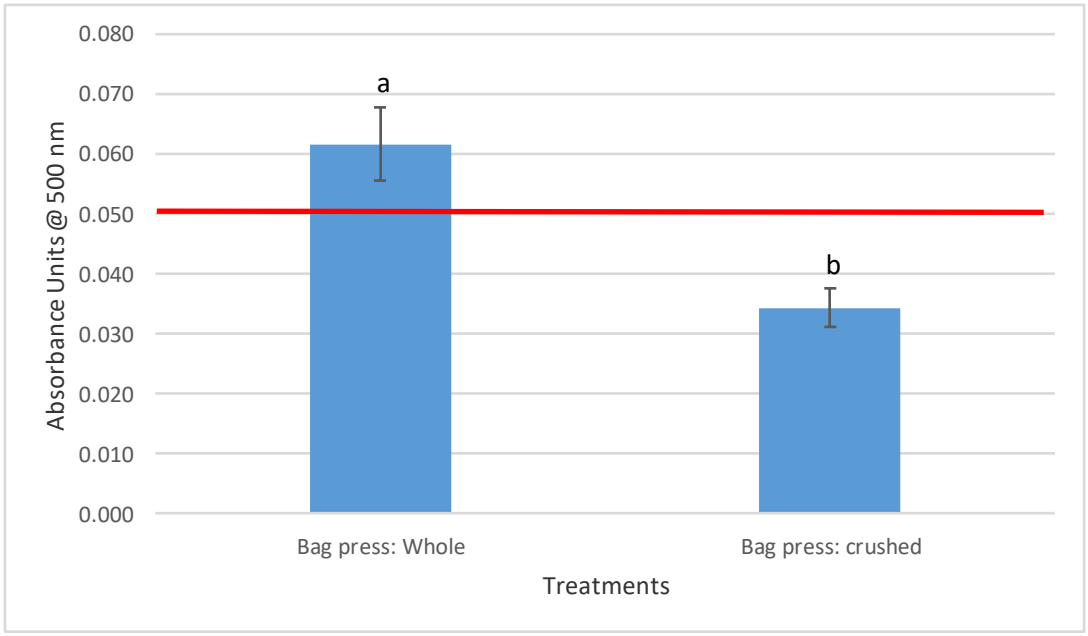

Fig. 3.8: Pinking potential of Sauvignon blanc made from whole bunch pressed and destemmed/crushed and bag pressed grapes. The red line indicates the pinking potential limit as defined in the method described in the SASEV laboratory manual (2002). Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments. Error bars represent the standard error of the mean.

As rule, whole bunch pressed grapes are used for sparkling wines and wines that are made to be fruitier. The crushed and destemmed wine had a higher concentration of phenols than the whole bunch pressed wines, which suggested that phenols or the phenols tested did not affect pinking susceptibility.

3.3.5 The effect of different ripening stages

All the standard wine analysis was done by FTIR analyses (Table 3.5). Significant differences ($p \leq 0.05$) exist between the different ripeness levels for the different parameters (except for lactic acid), with VA, glucose, fructose, ethanol, and glycerol increasing, and TA and malic acid decreasing with increasing ripeness.

Table 3.5: FTIR analyses analysis of Sauvignon blanc produced from the same vineyard block at different ripening stages.

	pH	VA (g/L)	TA (g/L)	MA (g/L)	LA (g/L)	Glu (g/L)	Fru (g/L)	EtOH (% v/v)	Gly (g/L)
Ripening stage: 18ºB	2.99^{c}	0.20^{c}	6.18^{a}	2.96^{a}	<0.2^{a}	<0.2^{c}	0.67^{b}	10.32^{c}	6.97^{c}
Ripening stage: 22ºB	3.41^{b}	0.30^{b}	5.06^{b}	1.45^{b}	<0.2^{a}	0.83^{b}	1.52^{b}	13.58^{b}	7.24^{b}
Ripening stage: 25ºB	3.60^{a}	0.48^{a}	5.10^{b}	1.27^{c}	<0.2^{a}	1.95^{a}	9.24^{a}	17.15^{a}	9.99^{a}

VA – Volatile acidity, TA – Titratable acidity, MA – Malic acid, LA – Lactic acid, Glu – Glucose, Fru – Fructose, EtOH – Ethanol, and Gly- Glycerol. Small letters depict significant differences ($p \leq 0.05$)

Anecdotal evidence suggests that ripening stages will affect the pinking potential of Sauvignon blanc wines. This was tested by harvesting Sauvignon blanc at three different ripening stages (i.e. 18°B, 22°B, and 25°B). After testing the pinking potential of the wine, it was evident that ripening levels do increase the pinking potential of the wine (Figure 3.9). An increase in ripeness increases the extraction of phenols into the wine, possibly explaining the wines' increased potential to pink (Singleton *et al.*, 1979; Singleton *et al.*, 1980; Delteil, 2004). Authors Simpson (1977a, b; 1980) and Andrea-Silva *et al.* (2014) reported the possible contribution of phenols to the pinking phenomena.

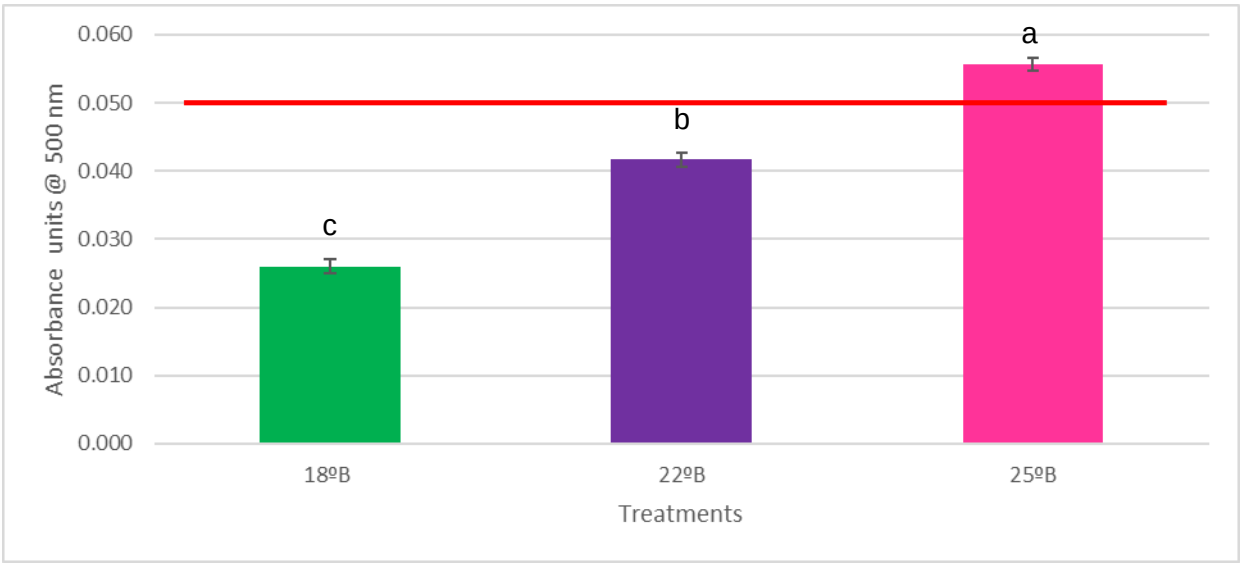

Fig. 3.9: Pinking potential of Sauvignon blanc at different ripening stages. The red line indicates the pinking potential limit as defined in the method described in the SASEV laboratory manual (2002). Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments. Error bars represent the standard error of the mean.

Significant differences in pinking potential observed between grape ripening stages, dissolved oxygen, metals, and phenols as primary substrates of oxidation, are also reported.

During the early stages of grape berry development, the berry accumulates sugars and metabolises the acids. Phenols and thiols are being synthesised at this stage and translocated to the berry. As the berry ripens, the sugars increase and acids decrease while the berry starts to develop the prominent aroma of Sauvignon blanc. As the ripening stages increased the gallic acid concentration also increased significantly from 2.55 mg/L at 18ºB to 6.73 mg/L at 25ºB. Caffeic acid was relatively constant for 18ºB (4.65 mg/L) and 22ºB (4.91 mg/L) ripening stages, increasing significantly at 25ºB (5.21 mg/L). p-Coumaric acid was the lowest of all the phenols. At ripening stages of 18ºB and 22ºB, the concentration stayed constant at 0.91 mg/L and increased to 1.26 mg/L at 25ºB. Syringic acid also increased significantly with ripeness from a non-detected to a concentration of 5.68 mg/L at 25ºB. Caftaric acid was the only phenol that decreased with ripening. It started with a high 6.11 mg/L at 18ºB and decreased to 4.60 mg/L at 25ºB. The following phenolic compounds were tested but showed as non-detectable (ND): epicatechin 3-O-gallate, epigallocatechin 3-*O*-gallate, vanillic acid, chlorogenic acid, delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside and malvidin-3-O-glucoside. This was confirmed by studies by Hernanz *et al. (*2007), Gómez-Míguez *et al. (*2007), Maggu *et al. (*2007), and Ružić *et al. (*2011) where the grapes were harvested between 17.7ºB to 22ºB. If the theory that phenols contribute to pinking is accepted, then the higher concentrations of gallic-, caffeic-, syringic- and p-coumaric acid concentrations may be contributing to the increase in pinking from 18ºB to 22ºB to 25ºB.

Juice/must from the different ripeness levels were pressed and vinified, and the resulting wines were analysed for their metal contents. A statistical analysis of metals showed basic three metals that are significantly higher in the pinking prone experiments than in the other (Tables 3.6). These metals are chromium (Cr), iron (Fe), and copper (Cu). Wines from 18°B grapes that showed the lowest potential for pinking also had the lowest concentrations of chromium (Cr), copper (Cu) but the highest concentration of iron (Fe), than wines made from 25°B grapes (Table 3.6). In these investigations, it was found that higher ripeness level (25°B) are more prone to pinking susceptibility than lower ripeness levels (18°B). Chromium is a heavy metal pollutant that has its origin from pesticides and equipment in the wine cellar (Pohl, 2007; Ibanez *et al.*, 2008*;* Čepo *et al.*, 2018). The Cr concentrations of the two wines fall well in the accepted concentration of 6.5 – 31 µg/L (Ibanez *et al.*, 2008*;* Čepo *et al.*, 2018). The other two metals (Cu & Fe) are well linked to oxidative browning in white wines (Ibanez *et al.*, 2008*;* Clark *et al.*, 2015; Kreitman *et al.*, 2016a, b) and, in this case, a combination of Cu and Fe could also play a role in pinking or more sensitive white cultivars.

Table 3.6: Metal concentrations of Sauvignon blanc produced from different ripening stages

Treatment	Cr (µg/L)	Cu (µg/L)	Fe (µg/L)
Ripening stage: 18ºB	8.4[b]	15.1[b]	2041.5[a]
Ripening stage: 22ºB	13.1[a]	70.5[a]	1125.3[b]
Ripening stage: 25ºB	11.5[ab]	55.5[a]	1138.1[b]

Different letters depict significant differences ($p \leq 0.05$) between treatments. Values represent the average of triplicate treatments.

This experiment concluded that with higher ripeness levels significantly more Cu cations and phenols are extracted, increasing the pinking susceptibility of the wine

3.3.6 The effect of skin contact time at different temperatures

Standard wine parameters, degrees balling, pH, and titratable acidity, were tested. Standard wine parameters, pH, volatile acidity, alcohol, titratable acidity, malic acid, lactic acid, glucose, fructose, and glycerol were also tested by FTIR analyses. No significant differences were noticed (data not shown).

The impact of macerated grape temperature on pinking potential was not significant, whereas the impact of grape temperature before processing was significant (see section 3.3.3). Increasing susceptibility or tendency to pink (or colour increase at 500 nm) was observed with longer skin contact times up to 6 hours at low temperature under standard winemaking conditions (Figure 3.10) ($p > 0.05$), up to 12 hours under reductive winemaking conditions (see section 3.3.2) ($p \leq 0.05$), and up to 18 hours with significance depending on temperature and ripeness level (see section 3.3.10). Increased skin contact and lower temperature favour the pinking potential of Sauvignon blanc as these juices absorb more dissolved oxygen (DO) from the surrounding air.

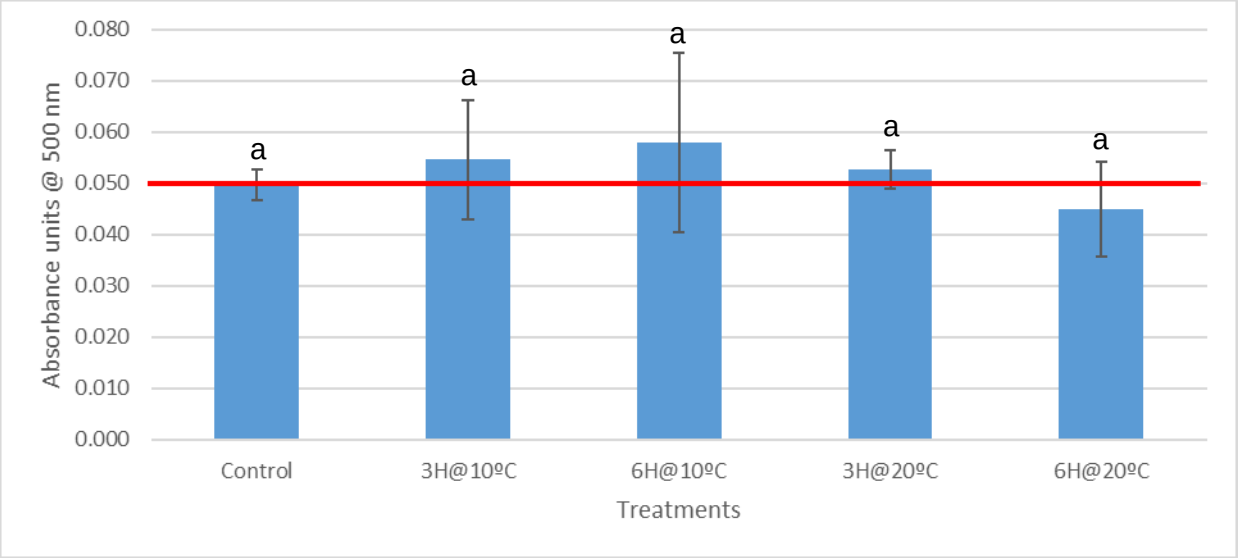

Fig. 3.10: Pinking potential of Sauvignon blanc produced from grape pomace subject to different skin contact times at different temperature treatments. The red line indicates the pinking potential limit as defined in the method described in the SASEV laboratory manual (2002). Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments. Error bars represent the standard error of the mean.

With no significant differences in pinking potential observed between treatments, dissolved oxygen, metals, and phenols as primary substrates of oxidation, were not determined.

3.3.7 The efficacy of different fining agents to reduce pinking susceptibility

Polyvinylpolypyrrolidone is the most well-known fining agent used to prevent pinking in Sauvignon blanc wines. The question that arises, is whether PVPP is the only fining agent that can be used. In this experiment, PVPP was compared to other fining agents at different winemaking stages. After contacting chemical companies to get as much info on the effect of fining agents that may influence pinking potential, 29 different products were selected and tested. The fining agents were added at three different winemaking stages, i.e. settling, fermentation, and fining (Figure 3.2). These stages were selected as these are the most popular stages where winemakers do additions to juice or wine. The fining agents at three different winemaking stages most effective in reducing the ability to pink are shown in Table 3.7.

Table 3.7: The different fining agents and the percentage of reduction in pinking each have against the control

Treatments	Settling	Fermentation	Fining
Control	100	100	100
Product 1 (*Fish-based fining agent*)	-30	-95	-43
Product 2 (*Pea protein + PVPP*)	-25	-35	-18
Product 3 (*PVPP + micro-pulverised cellulose*)	-34	0	0
Product 4 (*Fish-based fining agent*)	-26	-30	-15
Product 5 (*Liquid gelatine from porcine origin*)	-58	-36	-6
Product 6 (*Vegetable protein – patatin*)	-74	-71	-59
Product 7 (*Gum Arabic*)	-37	0	-45
Product 8 (*30% liquid silicon dioxide colloidal solution*)	-8	-34	0
Product 9 (*Liquid gelatine from porcine origin*)	-12	-64	-19
Product 10 (*PVPP + potassium caseinate*)	-31	-75	-58
Product 11 (*Hydrolysed and condensed tannins*)	-41	-5	0
Product 12 (*Gelatine*)	0	-72	0
Product 13 (*Selected silica + bentonite*)	-82	-63	0
Product 14 (*Fish-based fining agent*)	-59	-42	-50
Product 15 (*Selected yeast hulls, selected silica*)	-81	0	0
Product 16 (*Potassium metabisulfite, ascorbic acid, citric acid + hydrolysed tannins*)	-24	-94	-90
Product 17 (*Selected yeast cell walls, PVPP + selected silica*)	-14	-15	-32
Product 18 (*Polyvinylpolypyrrolidone*)	-46	-85	-43
Product 19 (*Natural gum from Acacia trees*)	-59	0	-30
Product 20 (*Solution of silica dioxide*)	-71	-25	-3
Product 21 (*Natural gum from Acacia trees*)	-93	0	-17
Product 22 (*Polyvinylpolypyrrolidone*)	-23	-63	-23
Product 23	-26	-66	-61
Product 24 (*Ascorbic acid*)	-99	-84	-100
Product 25	0	0	-43
Product 26 (*Ascophyllum nodosum*)	0	-67	0
Product 27 (*Gelatine*)	-68	-85	-44
Product 28 (*Yeast hulls*)	-84	-15	-23
Product 29 (*Specific inactivated yeast cells*)	-52	-24	0

Control wines received no fining agents. The negative sign in front of the percentage means there was a reduction in pinking susceptibility. Numbers in red indicate fining agents with no contribution to pinking reduction. The numbers in orange showed a decrease of ≤ 50%. Numbers in green showed a decrease greater than 50% in pinking. Values show the % reduction in pinking compared to the control for each product per stage of addition. Values represent the average of triplicate treatments.

Two fining agents had a significant effect in reducing pinking potential when added to any of the winemaking stages. These fining agents were product 6 and product 24, which contained ascorbic acid and patatin as active ingredients. Product 13 and product 27 work well at the settling and fermentation stages, while product 9, product 10, product 16, and product 23 are most efficient at the fermentation and fining stages. These products have ascorbic acid, PVPP, caseinate, silica and bentonite in combinations as active ingredients. PVPP, which are the fining agents that are used the most by winemakers, only work at their best when added at the fermentation stage (Table 3.7). PVPP is produced by polymerization of 1-ethyl-2-pyrrolidinone (EP), which could absorb polyphenols by an H^+-bonding. These molecules become heavy and settled down, therefore, taking polyphenols out of the wine (Laborde *et al.*,

2006). Product 13 is prepared from selected yeast hulls and silica. Product 24 (active ingredient is ascorbic acid) was very efficient, decreasing pinking potential to a near zero, but studies have shown that wines treated with ascorbic acid age faster and turn brown very quickly after bottling (Peng *et al.*, 1998; Comuzzo *et al.*, 2015). When ascorbic acid is added to wine, it breaks down to dehydro-ascorbic acid and $2H^+$ ions. These $2H^+$ ions bind with O_2 to form hydrogen peroxide (H_2O_2) which in turn is a very strong oxidising agent. Product 6 is a product derived from potato protein (patatin) that assist in the fining of white wines. Product 27 (active ingredient is gelatine) is widely used in the wine industry to bind phenols and remove them from wines. Product 16 is a mixture of potassium pyrosulfite, L-ascorbic acid, and hydrolysable tannins. Product 10 is a PVPP and potassium caseinate on cellulose support that efficiently contributes to the adsorption and removal of oxidised and oxidisable phenolic compounds. The fining agents, therefore, most efficient in reducing pinking potential should contain gelatine, PVPP, or ascorbic acid and/or potato protein as part of the product's composition as these active ingredients work the best in absorbing polyphenols.

Table 3.8: The fining agents (active ingredients) that worked the best at the three different winemaking stages

Settling	Fermentation	Fining
Patatin	Patatin	Patatin
Ascorbic acid	Ascorbic acid	Ascorbic acid
-	Potassium disulphite & ascorbic acid	Potassium disulphite & ascorbic acid
-	Gelatine	Gelatine
-	PVPP & potassium caseinate	PVPP & potassium caseinate
Silica & bentonite	Silica & bentonite	-
Gelatine	Gelatine	-
-	Potassium pyrosulfite, ascorbic acid & hydrolysable tannins	Potassium pyrosulfite, ascorbic acid & hydrolysable tannins

3.3.8 The effect of sunlight on pinked wine

Based on anecdotal evidence some winemakers will leave a pinked wine in direct sunlight for 15 minutes for the pink colour to disappear. This experiment shows that, although there was a significant ($p \leq 0.05$) reduction of 43% in absorbency after 15 minutes (Figure 3.11), longer exposure times to sunlight might be needed to bring the absorbency below the accepted level of 0.05 AU. Although spectrophotometrically a reduction in absorbency was seen, visually there was no difference in the visible colour as the wine did not change back to the natural Sauvignon blanc colour expected for this white wine (Figure 3.11). Cojocaru and Antoce (2019), found that exposing pinked wine to UV light for a month decreased the pinking potential by about 25%. They stated that the treatment helps, but that it was not enough to sufficiently decreased the pink colour.

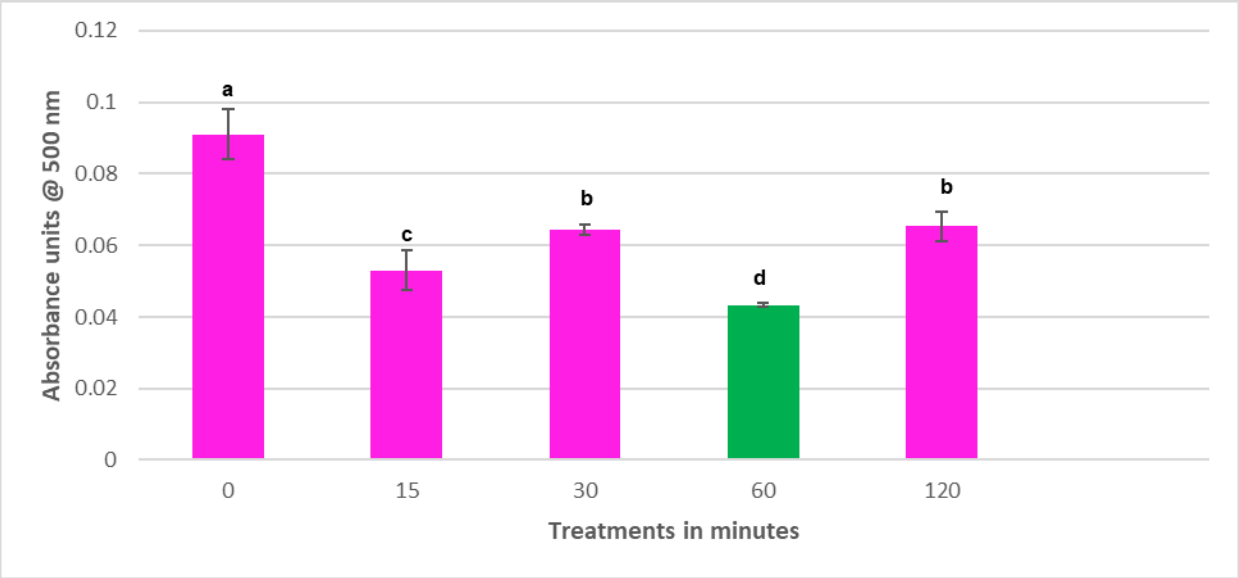

Fig. 3.11: Absorbance of Sauvignon blanc at 500 nm before and after exposure to sunlight. Absorbance values were corrected for control. Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments. Error bars represent the standard error of the mean.

There were no significant differences in standard wine parameters (pH, volatile acidity, total acidity, malic acid, lactic acid, glucose, fructose, ethanol, and glycerol) as measured by FTIR analyses between treatments. Analysing for anthocyanins (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside) showed that none was detected, or were present at concentrations lower than 10 mg/L. Simpson (1982) suggested that the pink colour of a pinked wine will disappear in direct sunlight after 10 minutes. The theory was tested in this study and the results showed that 60 minutes in direct sunlight had the most efficient effect in reducing the pink colour. Different phenols were also analysed by HPLC, and it was found that vanillic acid and chlorogenic acid were not detected. Gallic acid, epicatechin-3-O-gallate, caftaric acid, epigallocatechin-3-O-gallate, caffeic acid, p-coumaric acid, and syringic acid were the only phenols detected (Figure 3.12). Only gallic acid and caftaric acid showed significant differences between the 15 minutes and longer sunlight exposure. Gallic acid concentrations were the highest just after 15 minutes in direct sunlight where it reduced significantly to the lowest concentration after 120 minutes in direct sunlight. Caftaric acid had also the highest concentration at 15 minutes in direct sunlight and was reduced significantly after 120 minutes in direct sunlight. Although a study done by Grant-Preece *et al.* (2018) showed that an increase in these phenols increased the browning potential of a white wine, no studies so far, apart from this one, identified the phenols that change significantly in concentration upon exposure to direct sunlight for various time periods. Cojocaru and Antoce (2019), found that co-pigmented anthocyanins, polymeric anthocyanins and total pigments increased in pinked Sauvignon blanc and Chardonnay wines, which decreased slightly on the exposure to UV light, but that there were no free anthocyanins detected in these wines.

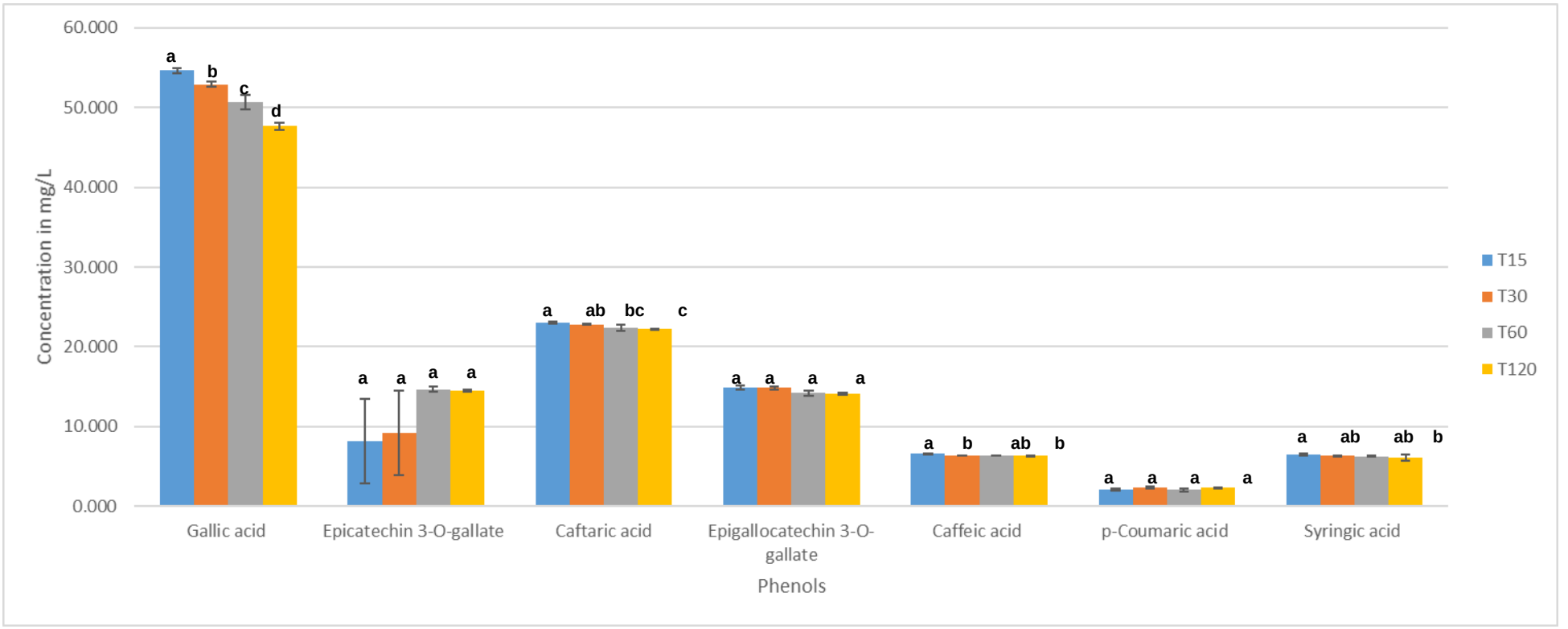

Fig. 3.12: HPLC analyses of phenolic compounds and anthocyanins of the different treatments. Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments. Error bars represent the standard error of the mean.

Treating pinked wines in direct sunlight can reduce the pink colour, but the wine colour never returns to its original colour. This is only a short-term solution as the UV spectrum of direct sunlight and the increase in wine temperature will have a negative effect on the wines.

3.3.9. Investigate the interactive influence of grape maturity, grape temperature, different methods of crushing and pressing, and time of skin contact on the pinking susceptibility of Sauvignon blanc wine

This was a three-way interactive study where grapes were harvested at three different ripeness levels (18B, 20B & 25B). In the second of the three-way interactive study, each ripeness level group was divided into two equal temperature groups (4C & 20C). In a third of the three-way interactive group, each of the temperature groups were further subdivided into three groups, a control (CDK), a whole bunch press (WB) and an 18-hour skin contact (CD18H) group. In the first of the three-way interactive study, there is a definitive distinction of pinking susceptibility between ripeness levels, where higher ripeness levels (20°B and 25°B) have higher pinking potential than lower ripeness levels (18°B). In the second of the three-way study, there are no real differences between temperature (4°C vs 20°C). In the third of the three-way study, the crushed/press grapes showed a higher tendency to pink than the whole bunch pressed grapes. The higher ripeness levels coupled with longer skin contact time and harvested at ambient temperature showed the highest pinking susceptibility. Although long skin contact time (CD18H) co-affected pinking, especially at 20°B and 25°B, this was not the case at lower ripeness levels. Long skin contact time with low ripeness levels did not affect pinking susceptibility as much. Most of the higher ripeness levels (25°B), coupled with crushed/pressed grapes indicates a high pinking susceptibility. The only whole bunch pressed treatment that showed a high pinking susceptibility was at 25°B and 4°C (Figure 3.13). Although the stats showed no significant differences between the effect of temperature, most of the 4°C showed a higher pinking susceptibility than the 20°C.

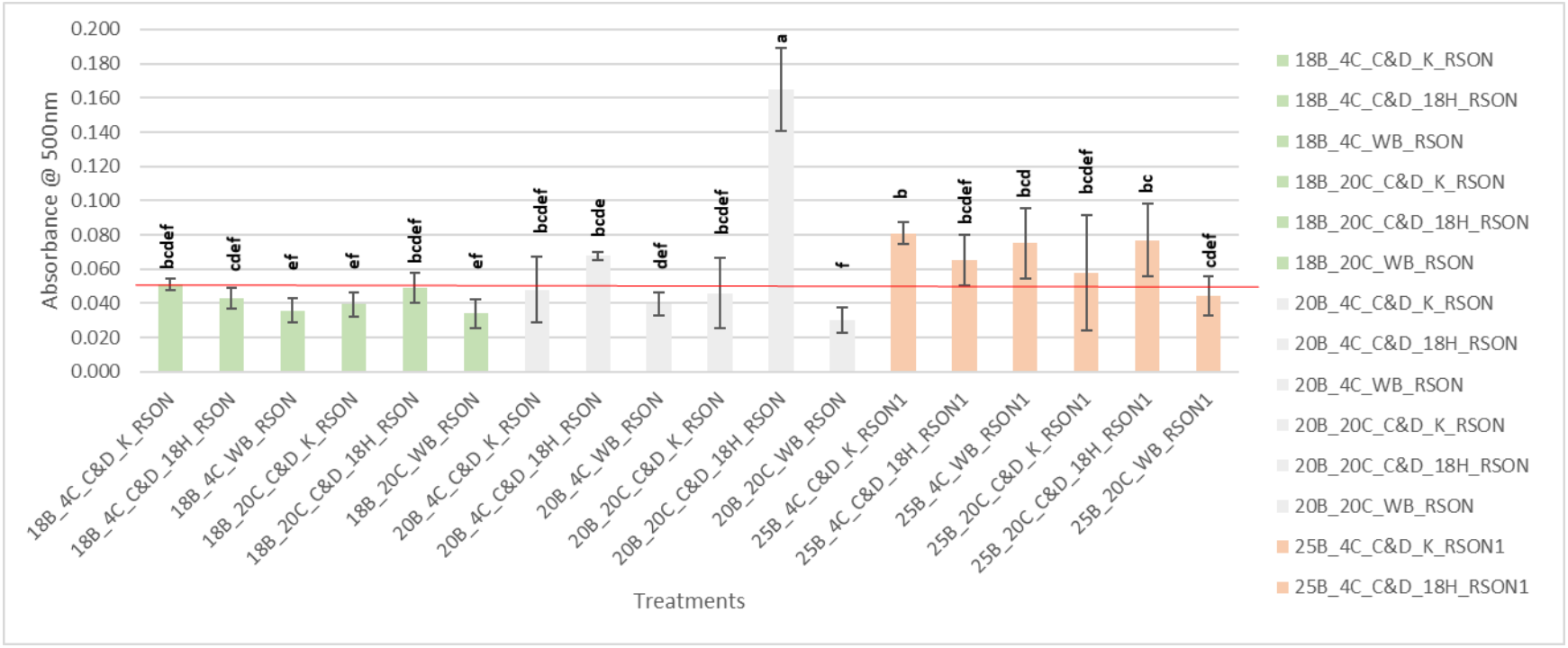

Fig. 3.13: A graph depicting pinking susceptibility of grapes harvested at three different ripeness levels, two different temperatures and different skin contact times. The number before the B is the ripeness level at which these grapes were harvested (18ºB, 20ºB & 25ºB); C – temperature of grapes at pressing in degrees Celsius; WB – whole bunch pressing; C&D – Crushed and pressed grapes; K – control wine; 18H – 18 hours' skin contact time. The red line depicts the 0.05 AU as the indication of pinking susceptibility. Different letters depict significant differences ($p \leq 0.05$) between treatments. Graph values represent the average of triplicate treatments. Error bars represent the standard error of the mean.

It is well researched that copper and iron influence the non-enzymatic oxidative browning of white wines (Bradshaw *et al.*, 2001; Danilewicz, 2003). It was for that reason that metals were analysed to establish their influence on the pinking susceptibility of the Sauvignon blanc wines. The PCA plot (Figure 3.14) explains 65.94% of the association between the different treatments and the metal concentrations. Three distinct groupings can be seen on the PCA plot (Figure 3.14). The metals associated with the lower ripeness levels (blue circle) are cadmium, zinc, selenium, cobalt and boron. These metals are normally taken up by fertilizers or from fungicides (Zeeman & du Plessis, 1980; Chen *et al.*, 1994) and are therefore more prominent at lower ripeness levels. The ripeness levels (25°B), are more associated with minerals more associated in the mediation of oxidation in white wines (copper and iron) (Bradshaw *et al.*, 2001; Danilewicz, 2003). No studies were ever done on the influence of minerals on pinking susceptibility, but the influence on non-enzymatic browning is well-known.

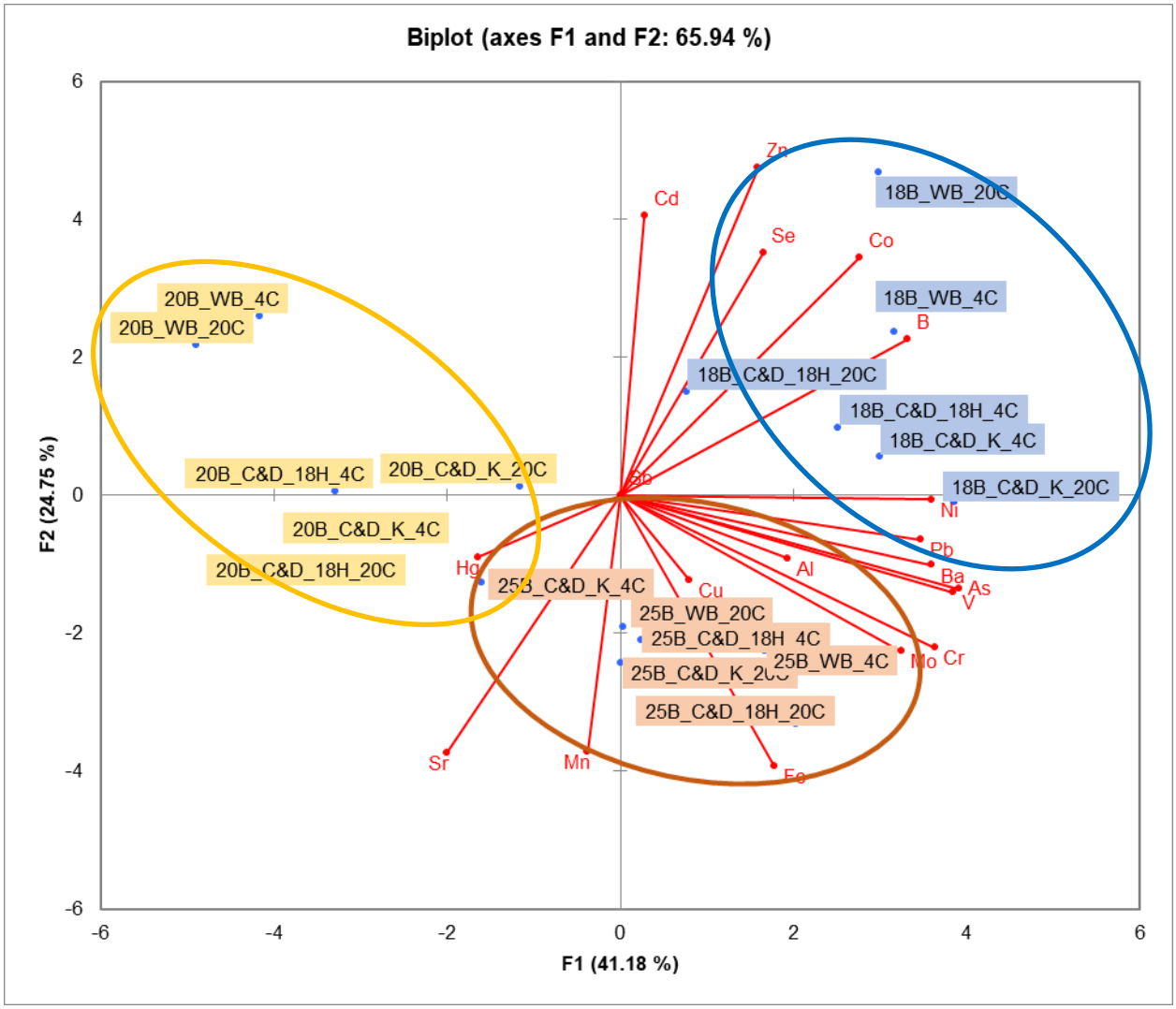

Fig 3.14: PCA graph depicting the interactive influence of minerals on grape maturity, grape temperature, different methods of crushing and pressing, and time of skin contact on the different ripening stages of

18ºB, 20ºB, and 22ºB; WB – whole bunch pressed; C&D – crushed, destemmed, and pressed; K – control wine; 18h – 18 hours of skin contact; 4C & 20C – depicts the grape temperature at pressing.

Statistical analysis of the ripeness level groups showed that the highest dissolved oxygen (DO) was measured at the highest ripeness level (22°B) just after crushing and pressing. The lowest DO was measured at the lowest ripeness levels (18°B). Statistical analysis of the temperature groups showed that the highest DO was measured at 4°C. As soon as fermentation started the DO was used up by yeast and there were no significant differences in DO concentration between the treatments (Figure 3.15). The higher concentrations of DO at lower temperature keeps the DO in the system longer provide the means for slow oxidation which may cause pinking susceptibility in white wines.

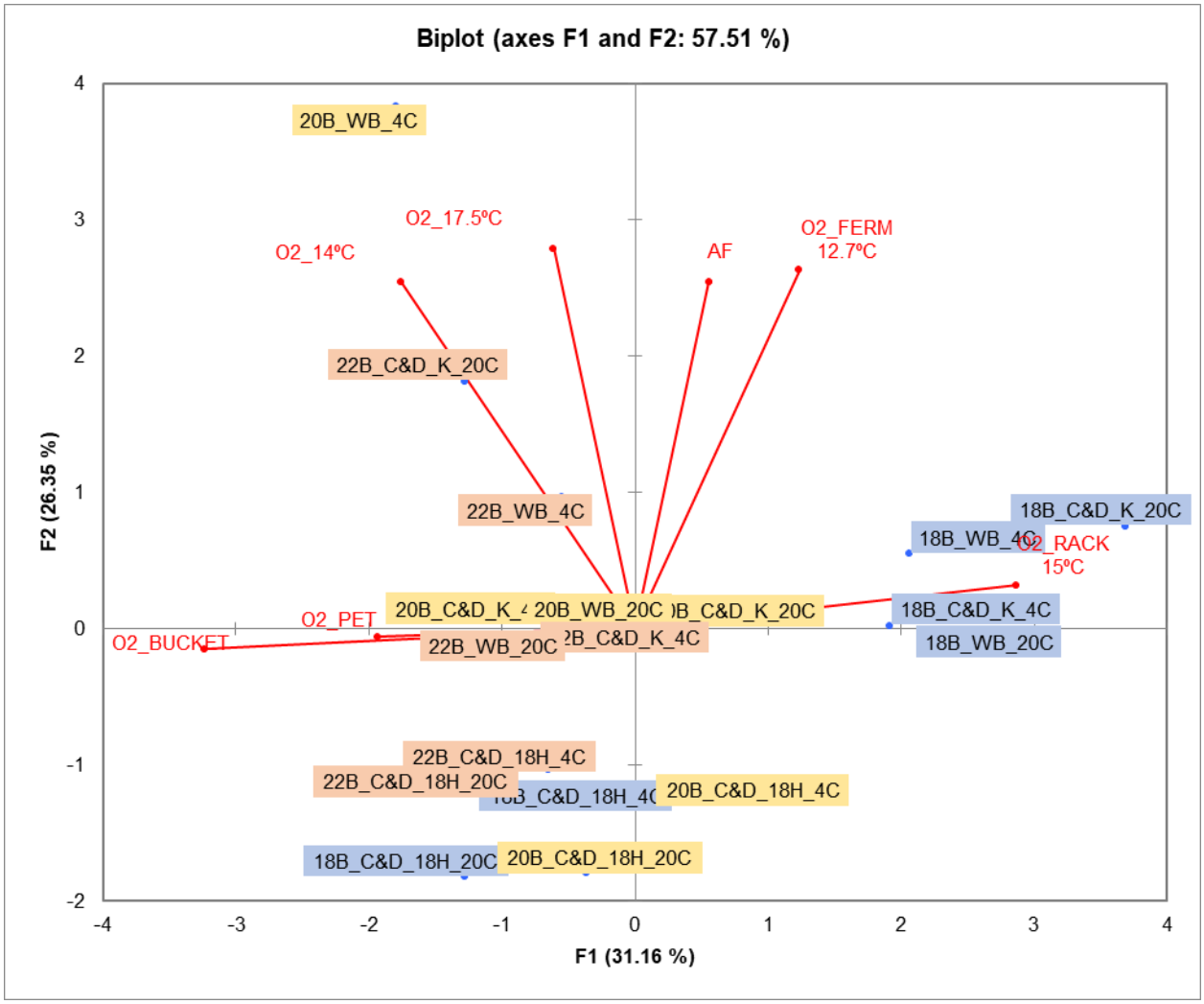

Fig. 3.15: PCA graph depicting the dissolved oxygen (DO) concentrations during winemaking for the different treatments. 18B, 20B, 22B depicts the different ripening stages of 18ºB (blue), 20ºB (yellow/orange), and 22ºB (pinkish-red); WB – whole bunch pressed; C&D – crushed, destemmed, and pressed; K – control wine; 18h – 18 hours of skin contact; 4C & 20C – depicts the grape temperature. Dissolved oxygen was measured after crushing and destemming (O2_Bucket), transferring to 4.5 L glass bottles (O2_PET), after 1 hour (O2_17.5ºC) of transfer, after settling (O2_14ºC), after alcoholic fermentation (O2_AF), and after racking (O2_RACK_15ºC).

The three-way study concludes that the pinking susceptibility of Sauvignon blanc wines will increase with ripeness levels. Although the temperature at harvest time did not affect pinking susceptibility, the crushed and destemmed wines coupled with 18 hours of skin contact did increase the pinking susceptibility. Overall, the whole bunch pressed wines did not increase the pinking susceptibility and the least affected was low ripeness levels with whole bunch pressed wines. This may be something to consider by winemakers in the future.

3.4 CONCLUSION

This study showed that certain viticultural and winemaking practices do influence the pinking potential of Sauvignon blanc wines. Viniviticultural practices that cellars, winemakers and researchers in the field should take into consideration that affect the pinking potential of final wines are grape ripeness levels, temperature of the grapes at processing, skin contact times, pressing method, and the type of fining agent and winemaking stage of addition thereof. To prevent the potential for wines to pink, grapes should be harvested at lower ripeness levels, processed at an ambient grape temperature, with short to no skin contact time, pressed after removal of the stems (destemming). Although PVPP is the most commonly used fining agent, agents like ascorbic acid and patatin are even more effective, and are recommended for use in the prevention of pinking at all winemaking stages. Literature on the influence of viniviticultural factors on pinking potential is not available, making this investigation a novel study.

Pinking is an oxidative event, and factors associated with oxidation such as metals (Fe and Cu) and oxygen did associate with higher pinking potential and related viniviticultural treatments. No particular phenol associated throughout with pinking potential or practices shown to increase the potential to pink.

This study show that practices used by winemaking such as exposing pinked wines to sunlight, has limited success, since measured colour reductions not always corresponded to the visual disappearance of pinking, questioning the effectiveness of sunlight (UV) as a post-pinking treatment. Winemakers should, therefore, rather avoid pinking with fining agents proved in this study to be effective against the potential to pink. Early detection of pinking would enable preventative measures and earlier action to be taken against pinking, however, the pinking assay used for wine, is not effective for juice as also shown in this study. Further work need be done to find an assay or compound that can be used in the development of a method for the earlier (i.e. in juice) detection of pinking potential.

3.5 LITERATURE CITED

Amendola, D., De Faveri, D.M and Spigno, G., 2010. Grape marc phenolics: Extraction kinetics, quality and stability of extracts. J. Food Engin. 97, 384–392.

Anderson, M.E., 1998. Glutathione: an overview of biosynthesis and modulation. Chem-Biol Interact. 111–112, 1–14.

Andrea-Silva, J., Cosme, F., Ribeiro, L.F., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Domingues, M.R.M. and Nunes, F.M., 2014. Origin of the pinking phenomenon of white wines. J. Agric. Food Chem. 62, 5651–5659.

Arapitsas, P., Oliveira, J. and Mattivi, F., 2015. Do white grapes really exist? Food Res. Int. 69, 21–25.

Barril, C., Clark, A.C. and Scollary, G.R., 2012. Chemistry of ascorbic acid and sulfur dioxide as an antioxidant system relevant to white wine. Anal. Chim. Acta. 732, 186–193.

Barril, C., Rutledge, D.N., Scollary, G.R. and Clark, A.C., 2016. Ascorbic acid and white wine production: a review of beneficial versus detrimental impacts. Austr. J. Grape Wine Res. 22, 169–181.

Blancquaert, E.H., Oberholster, A., Ricardo-da-Silva, J.M. and Deloire, A.J., 2019. Effects of abiotic factors on phenolic compounds in the grape berry – A review. S. Afr. J. Enol. Vitic., Vol. 40 (1), 92–105.

Bradshaw, M.P, Prenzler, P.D. and Scollary, G.R., 2001. Ascorbic acid-induced browning of (+)-catechin in a model wine system. J. Agric. Food Chem. 49 (2), 934–939.

Cáceres-Mella, A., Peña-Neira, A.I., Parraguez, J., López-Solís, R., Laurie, V.P. and Canals, J.M., 2013. Effect of inert gas and prefermentative treatment with polyvinylpolypyrrolidone on the phenolic composition of Chilean Sauvignon blanc wines. J Sci Food Agric 93, 1928–1934.

Caputi, A. and Peterson, R.G., 1965. The Browning Problem in Wines. Am. J. Enol. Vitic. 16 (1), 9-13.

Castañeda-Ovando, A., Pacheco-Hernández, d-L., Páez-Hernández, E., Rodríguez, J.A. and Galán-Vidal, C.A., 2009. Chemical studies of anthocyanins: A review. Food Chem. 113, 859–871.

Castellari, M., Simonato, B., Tornielli, G.B., Spinelli, P. and Ferrarini, R., 2004. Effects of different enological treatments on dissolved oxygen in wines. Ital. J. Food Sci. 3 (16), 387–398.

Čepo, D.V., Pelajić, M., Vrček, I.V., Krivohlavek, A., Žuntar, I. and Karoglan, M., 2018. Differences in the levels of pesticides, metals, sulphites and ochratoxin A between organically and conventionally produced wines. Food Chem. 246, 394–403.

Chen, G.N., Scollary, G.R. and Vicente-Beckett, V.A., 1994. Potentiometric stripping determination of lead, cadmium, and zinc in wine. Am. J. Enol. Vitic. 45 (3), 305–311.

Cheynier, V.F and Van Hulst, M.W.J., 1988. Oxidation of trans -caftaric acid and 2-S-Glutathionylcaftaric acid in model solutions. J. Agric. Food Chem. 36 (1), 10-15.

Cheynier, V., Rigaud, J., Souquet, J-M., Duprat, F. and Moutounet, M., 1990. Must browning in relation to the behavior of phenolic compounds during oxidation. Am. J. Enol. Vitic. 41 (4), 346–349.

Cilliers, J.J.L. and Singleton, V.L., 1989. Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. J. Agric. Food Chem. 37 (4), 891–897.

Clark, A.C., Vestner, J., Barril, C., Maury, C., Prenzler, P.D. and Scollary, G.R., 2010. The influence of stereochemistry of antioxidants and flavanols on oxidation processes in a model wine system: Ascorbic acid, erythorbic acid, (+)-catechin and (-)-epicatechin. J. Agric. Food Chem. 58 (2), 1004–1011.

Clark, A.C., Wilkes, E.N. and Scollary, G.R., 2015. Chemistry of copper in white wine: a review. Austr. J. Grape Wine Res. 21, 339–350.

Cojocaru, G.A. and Antoce, A.O., 2019. Effect of certain treatments to prevent or partially reverse the pinking phenomenon in susceptible white wines. BIO Web of Conferences 15. 42nd World Congress of Vine and Wine. https://doi.org/10.1051/bioconf/20191502003

Comuzzo, P., Battistutta, F., Vendrame, M., Páez, M.S., Luisi, G. and Zironi, R., 2015. Antioxidant properties of different products and additives in white wine. Food Chem. 168, 107–114.

Cosme, F., Andrea-Silva, J., Filipe-Ribeiro, L., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Domingues, M.R.M. and Nunes, F.M., 2019. The origin of pinking phenomena in white wines: An update. BIO Web of Conferences 12, 02013. 41st World Congress of Vine and Wine. https://doi.org/10.1051/bioconf/20191202013

Danilewicz, J.C., 2003. Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. Am. J. Enol. Vitic. 54 (2), 73–85.

Danilewicz, J. C., & Standing, M. J., 2018. Reaction mechanisms of oxygen and sulfite in red wine. Am. J. Enol. Vitic. 69 (3), 189–195.

Day, M.P, Schmidt, S.A, Smith, P.A. and Wilkes, E.N., 2015. Use and impact of oxygen during winemaking. Austr. J. Grape Wine Res. 21, 693–704.

De Beer, D., Joubert, E., Gelderblom, W.C.A and Manley, M., 2003. Antioxidant activity of South African red and white cultivar wines: Free radical scavenging. J. Agric. Food Chem. 51 (4), 902−909.

Delteil, D., 2004. The diverse functions of oxygen – 2nd part. Wine Intern. Tech. J. 4, 1–5.

De Villiers, J.P., 1961. The control of browning of white table wines. Am. J. Enol. Vitic. 12 (1), 25–30.

Dokoozlian, N. K. and Kliewer, W.M., 1996. Influence of light on grape berry growth and composition varies during fruit development. J. Am. Soc. Hort. Sci. 121(5), 869-874.

Du Toit, W.J., Marais, J., Pretorius, I.S and du Toit, M., 2006. Oxygen in must and wine: A review. S. Afr. J. Enol. Vitic. 27 (1), 76–94.

Ferreira, S.S., Alves, A.J., Filipe-Ribeiro, L., Cosme, F. and Nunes, F.M., 2018. Holistic and sustainable approach for recycling and valorization of polyvinylpolypyrrolidone used in wine fining. Sustainable Chem. Eng. 6, 14599−14606.

Galuska, S., Makris, D.P. and Kefalas, P., 2010. Browning development in wine-like liquid model matrices: Dependence on phenolic, Fe(III) and SO_2 concentrations. J. Food Process Eng. 33, 934–945.

Gawel, R., Smith, P.A. & Waters, E.J., 2016. Influence of polysaccharides on the taste and mouthfeel of white wine. Austr. J. Grape Wine Res. 22, 350–357.

George, N., Clark, A.C., Prenzler, P.D.and Scollary, G.R., 2006. Factors influencing the production and stability of xanthylium cation pigments in a model white wine system. Austr. J. Grape Wine Res. 12, 57–68.

Gómez, E., Martinez, A. and Laencina, J., 1995. Prevention of oxidative browning during wine storage. Food Res. Inter. 28 (3), 213-217.

Gómez-Míguez, M.J., González-Miret, M.L., Hernanz, d., Fernández, M.A., Vicario, I.M. and Heredia, F.J., 2007. Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. J. Food Eng. 78, 238–245.

Grant-Preece, P., Barril, C., Schmidtke, L.M. and Clark, A.C., 2018. Impact of fluorescent lighting on the browning potential of model wine solutions containing organic acids and iron. Food Chem. 243, 239–248.

Hernanz, D., Recamales, A.F., González-Miret, M.L., Gómez-Míguez, M.J., Vicario, I.M. and Heredia, F.J., 2007. Phenolic composition of white wines with a prefermentative maceration at experimental and industrial scale. J. Food Eng. 80, 327–335.

Hornsey, I., 2007. Winemaking processes. In: Hornsey, I. (ed). The chemistry and biology of winemaking. The Royal Society of Chemistry. Cambridge. UK. 173-174.

Ibanez, J.G., Carreon-Alvarez, A., Barcena-Soto, M. and Casillas, N., 2008. Metals in alcoholic beverages: A review of sources, effects, concentrations, removal, speciation, and analysis. J. Food Comp. Anal. 21, 672–683.

Jackson, D.I. and Lombard, P.B., 1993. Environmental and management practices affecting grape composition and wine quality: a review. Am. J. Enol. Vitic. 44(4), 409-430.

Jones, T., 1989. Pinking of white table wines: Further studies. Unpublished Master of Science thesis, University of California, Davis, CA, USA.

Kelly, D., Zerihun, A., Hayasaka, Y. & Gibberd, M., 2014. Winemaking practice affects the extraction of smoke-borne phenols from grapes into wines. Austr. J. Grape Wine Res. 20, 386–393.

Kreitman, G.Y., Danilewicz, J.C., Jeffery, D.W. and Elias, R.J., 2016a. Reaction mechanisms of metals with hydrogen sulfide and thiols in model wine. Part 1: Copper-catalyzed oxidation. J. Agric. Food Chem. 64, 4095−4104.

Kreitman, G.Y., Danilewicz, J.C., Jeffery, D.W. and Elias, R.J., 2016b. Reaction mechanisms of metals with hydrogen sulfide and thiols in model wine. Part 1: Iron- and copper-catalyzed oxidation. J. Agric. Food Chem. 64, 4105−4113.

Kritzinger, E.C., Bauer, F.F. and du Toit, W.J., 2013. Role of glutathione in winemaking: A review. J. Agric. Food Chem. 61, 269–277.

Laborde, B., Moine-Ledoux, V., Richard, T., Saucier, C., Dubourdieu, D. and Monti, J-P., 2006. PVPP-polyphenol complexes: A molecular approach. J. Agric. Food Chem. 54 (12), 4383-4389.

Lamuela-Raventós, R.M., Huix-Blanquera, M. and Waterhouse, A.L., 2001. Treatments for pinking alteration in white wines. Am. J. Enol. Vitic. 52 (2), 156–158.

Laurie, V.F., Law, R., Joslin, W.S. and Waterhouse, A.L., 2008. In situ measurements of dissolved oxygen during low-level oxygenation in red wines. Am. J. Enol. Vitic. 59 (2), 215–219.

Lee, C.Y. and Jaworski, A.W., 1988. Phenolics and browning potential of white grapes grown in New York. Am. J. Enol. Vitic. 39 (4), 337–340.

Li, H., Guo, A. and Wang, H., 2008. Mechanisms of oxidative browning of wine. Food Chem. 108, 1–13.

Lopez-Toledano, A., Mayen, M., Merida, J. and Medina, M., 2006. Yeasts used to delay browning in white wines. Food Chem. 97, 498–504.

Mafata, M., Buica, A., du Toit, W.J. and van Jaarsveld, F.P., 2018. The Effect of grape temperature at pressing on phenolic extraction and evolution in Méthode Cap Classique wines throughout winemaking. S. Afr. J. Enol. Vitic., Vol. 39 (1), 141–148.

Maggu, M., Winz, R., Kilmartin, P.A., Trought, M.C.T and Nicolau, L., 2007. Effect of skin contact and pressure on the composition of Sauvignon blanc Must. J. Agric. Food Chem. 55, 10281–10288.

Main, G.L. and Morris, J.R., 1994. Color of Seyval blanc juice and wine as affected by juice fining and bentonite fining during fermentation. Am. J. Enol. Vitic. 45 (4), 417–422.

Minute, F., Giotto, F., Filipe-Ribeiro, L., Cosme, F. and Nunes, F.M., 2021. Alternative methods for measuring the susceptibility of white wines to pinking alteration: Derivative spectroscopy and CIEL*a*b* colour analysis. Foods. 10, 1 – 15.

Muñoz-Pina, S., Ros-Lis, J.V., Argüelles, A., Coll, C., Martínez-Máñez, R., Andrés, A., 2018. Full inhibition of enzymatic browning in the presence of thiol-functionalised silica nanomaterial. Food Chem. 241, 199–205.

Narita, Y. and Inouye, K., 2013. Degradation kinetics of chlorogenic acid at various pH values and effects of ascorbic acid and epigallocatechin gallate on its stability under alkaline conditions. J. Agric. Food Chem. 61, 966−972.

Nel, A.P., du Toit, W.J. and van Jaarsveld, F.P., 2020. Pinking in White Wines – A Review. S. Afr. J. Enol. Vitic. 41 (2), 151–157.

Nenadis, N. and Paraskevopoulou, A., 2016. Browning susceptibility of commercial monovarietal white wines under accelerated oxidation conditions: correlation with compositional data and effect on total phenol content and radical scavenging activity. Eur Food Res Technol. 242, 1821–1828.

Pascual, O., González-Royo, E., Gil, M., Gómez-Alonso, S., García-Romero, E., Canals, J.M., Hermosín-Gutíerrez, I. and Zamora, F., 2016. Influence of grape seeds and stems on wine composition and astringency. J. Agric. Food Chem. 64, 6555−6566.

Peng, Z., Duncan, B., Pocock, K.F. & Sefton, M.A., 1998. The effect of ascorbic acid on oxidative browning of white wines and model wines. Austr. J. Grape Wine Res. 4, 127–135.

Peterson, R.G. and Caputi jr, A., 1967. The browning problem in wines II. Ion exchange effects. Am. J. Enol. Vitic. 18 (3), 105–112.

Pohl, P., 2007. What do metals tell us about wine? Trends Anal. Chem. 26 (9), 941–949.

Porter, L.J., Hrstich, L.N. and Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochem. 25 (1), 223-230.

Razmkhab, S., Lopez-Toledano, A., Ortega, J.M., Mayen, M., Merida, J. and Medina, M., 2002. Adsorption of phenolic compounds and browning products in white wines by yeasts and their cell walls. J. Agric. Food Chem. 50 (25), 7432–7437.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D., 2006. Handbook of Enology Volume 2. The chemistry of wine stabilization and treatments (2nd Ed.). John Wiley & Sons Ltd.

Ricardo-da-Silva, J.M., Cheynier, V., Samsom, A. and Bourzeix, M., 1993. Effect of pomace contact, carbonic maceration, and hyperoxidation on the procyanidin composition of Grenache blanc wines. Am. J. Enol. Vitic. 44(2), 168–172.

Rossi, J.A and Singleton, V.L., 1966. Contributions of Grape Phenols to Oxygen Absorption and Browning of Wines. Am. J. Enol. Vitic. 17 (4), 231-239.

Ružić, I., Škerget, M., Knez, Z. and Runje, M., 2011. Phenolic content and antioxidant potential of macerated white wines. Eur Food Res Technol. 233, 465–472.

Salameh, D., Brandam, C., Medawar, W., Lteif, R. and Strehaiano, P., 2008. Highlight on the problems generated by p-coumaric acid analysis in wine fermentations. Food Chem. 107, 1661–1667.

Sapis, J.C., Macheix, J.J. and Cordonnier, R.E., 1983. The browning capacity of grapes. II. Browning potential and polyphenol oxidase activities in different mature grape varieties. Am. J. Enol. Vitic., Vol. 34 (3), 157–162.

Sartor, S., Burin, V.M., Ferreira-Lima, N.E., Caliari, V. and Bordignon-Luiz, M.T., 2019. Polyphenolic profiling, browning, and glutathione content of sparkling wines produced with nontraditional grape varieties: Indicator of quality during the biological aging. J. Food Sci. 84 (12), 3546–3554.

SASEV. 2002. Methods of Analysis for wine laboratories. Compiled by the South African Wine Laboratory Association.

Schneider, V., 1998. Must hyperoxidation: A review. Am. J. Enol. Vitic. 49 (1), 65–73.

Scrimgeour, N., Nordestgaard, S., Lloyd, N.D.R and Wilkes, E.N., 2015. Exploring the effect of elevated storage temperature on wine composition. Austr. J. Grape Wine Res. 21, 713–722.

Serra-Cayuela, A., Aguilera-Curiel, M.A., Riu-Aumatell, M., Buxaderas, S. and López-Tamames, E., 2013. Browning during biological aging and commercial storage of Cava sparkling wine and the use of 5-HMF as a quality marker. Food Res. Int. 53, 226–231.

Serra-Cayuela, A., Jourdes, M., Riu-Aumatell, M., Buxaderas, S., Teissedre, P.-L. and López-Tamames, E., 2014. Kinetics of browning, phenolics, and 5-hydroxymethylfurfural in commercial sparkling wines. | J. Agric. Food Chem. 62, 1159−1166.

Shapiro, S.S. and Wilk, M.B., 1965. An Analysis of Variance Test for Normality (Complete Samples). Biometrika. 52 (3/4), 591-611.

Simpson, R.F., 1977a. Oxidative pinking in white wines. Vitis. 16, 286–294.

Simpson, R.F., 1977b. Pinking in Australian white table wines. Austr. Wine Brew. Spirit Rev. 97 (1), 56–58.

Simpson, R.F., January 1980. Some aspects of oxidation and oxidative browning in white table wines. The Australian Grapegrower & Winemaker. 20–21.

Simpson, R.F., Miller, G.C. and Orr, G.L., 1982. Oxidative pinking of white wines: recent observations. Food Techn. Austr. 34 (1), 44–47.

Simpson, R.F., Bennett, S.B. and Miller, G.C., 1983. Oxidative pinking of white wines: a note on the influence of sulphur dioxide and ascorbic acid. Food Tech. Austr. 35 (1), 34–36.

Singleton, V.L. & Esau, P., 1969. Phenolic substances in grapes and wine and their significance. Adv. Food Res. Suppl. 1, 1-282.

Singleton, V.L. and Kramling, T.E., 1976. Browning of white wines and an accelerated test for browning capacity. Am. J. Enol. Vitic. 27 (4), 157–160.

Singleton, V.L., Salgeus, M., Zaya, J. and Trousdale, E., 1985. Caftaric Acid Disappearance and Conversion to Products of Enzymic Oxidation in Grape Must and Wine. Am. J. Enol. Vitic. 36 (1), 50–56.

Singleton, V.L., Trousdale, E. and Zaya, J., 1979. Oxidation of wines I. Young white wines periodically exposed to air. Am. J. Enol. Vitic. 30 (1), 49–54.

Singleton, V.L., Zaya, J. and Trousdale, E., 1980. White table wine quality and polyphenol composition as affected by must SO_2 content and pomace contact time. Am. J. Enol. Vitic. 31 (1), 14–20.

Singleton, V.L., Zaya, J. and Trousdale, E., 1986. Compositional changes in ripening grapes: caftaric and coutaric acid. Vitis. 25, 107–117.

Singleton, V.L., Zaya, J. Trousdale, E. and Salgeus, M, 1984. Caftaric acid in grapes and conversion to a reaction product during processing. Vitis. 23, 113–120.

Skouroumounis, G.K., Kwaitkowski, M.J., Francis, I.L., Oakey, H., Capone, D.L., Peng, Z., Duncan, B., Sefton, M.A. and Waters, E.J., 2005. The influence of ascorbic acid on the composition, colour and flavour properties of a Riesling and a wooded Chardonnay wine during five years' storage. Austr. J. Grape Wine Res. 11, 355–368.

Smith, P.A. & Waters, E., 2012. Identification of the major drivers of 'phenolic' taste in white wines. AWRI Final report, AWRI 0901.

South African Wine Industry Statistics (SAWIS). 2018. Statistics nr 43. Paarl. www.sawis.co.za

Tatum, J.H., Shaw, P.E. and Berry, R.E., 1969. Degradation Products from Ascorbic Acid. J. Agr. Food Chem. 17 (1), 38–40.

Tobe, S.J., 1983. Pinking in table wines from white grapes. Unpublished thesis. The University of California Davis.

Zeeman, W. and du Plessi, C.S. 1980. Cadmium Content of South African Table Wines. S. Afr. J. Enol. Vitic. 1 (2), 123–124.

Chapter 4

Attempted identification of causal constituents of pink discolouration in white wines

4. Attempted identification of causal constituents of pink discolouration in white wines

4.1 INTRODUCTION

Pinking in wine is a phenomenon that was first described by Singleton and Esau in 1969. Andrea-Silva *et al.* (2014) found traces of malvidin-3-O-glucoside after cleaning a pinked Siria wine with PVPP (Polyclar V) and analysing it with electrospray ionization mass spectrometry (ESI-MS). At a workshop held in Italy, Andrea-Silva reported lenticel-type structures that formed on Siria grapes and presumed these patches of anthocyanin forming on the skin of the grape to be responsible for the pink colour (data presented at an Italian workshop on pinking, 2018). Siria is not the only cultivar that is prone to pinking phenomena. Other cultivars such as Sauvignon blanc, Thompson Seedless, Chardonnay, Chenin blanc, Crouchen, Muscat Gordo Blanco, Palomino, Riesling, Semillon and Sultana are also prone to this pinking phenomenon (Simpson, 1977a; Andrea-Silva *et al.,* 2014).

White wine can turn pinkish for different reasons. White grapes could be contaminated with red grapes or a small amount of red wine could come in contact with white wine (Tobe, 1983). The result in both cases would be white wine with a pinkish tint. White wines can also turn pink because of an oxidation reaction where sensitive cultivars (like Sauvignon blanc) turns pink during certain winemaking processes (Tobe, 1983). Another possibility is that the genes for anthocyanin production in the vine are turned on because of external environmental conditions (Boss *et al.*, 1996). Whatever the reason might be, the result presents a challenge and unnecessary expense for the winemaker to rectify.

The pinking phenomenon has been known since the 1960s, as described by Singleton and Esau (1969), but about 50 years later the compounds causing pinking are still unknown. It is known that the precursor to pinking appears to be present at low concentrations (Singleton *et al.*, 1979), is thermally unstable (Simpson *et al.*, 1982) and is slightly affected by SO_2 additions or changes in wine pH (Simpson *et al.*, 1982). Pinking is postulated to be the result of several reasons, namely 1) Oxidative enzymes, for example, polyphenol oxidase (PPO), could be linked to pinking (Vaimakis & Roussis, 1993). 2) The rapid conversion of accumulated flavones may lead to red flavylium salts and a pinkish colour (Zoecklein *et al.,* 1995). 3) 2-S-glutathionyl-caftaric acid (Grape Reaction Product - GRP) derivatives may be linked to pink chromophores (van Wyk *et al.*, 1976). 4) Jones (1989:61) reported that at least 10 different compounds and polymeric compounds, forming a heterogeneous group of varying composition and polarity, that

could be linked to pinking, while Tobe (1983) narrowed it down to about four compounds (astilbin, quercitrin, engeletin and rutin). 5) Certain amino acids (e.g. cysteine) form compounds with thiosulfinates to form pink chromophores (Kubec *et al.*, 2004b; Imai *et al.*, 2006; Kubec & Velíšek, 2007; Lee *et al.*, 2010; Kučerová *et al.*, 2011 & Kubec *et al.*, 2015). The first attempt to identify the compound causing pinking was done in 2014 with a follow-up confirmation of malvidin-3-O-glucoside. This was done on a Portuguese cultivar, Siria. This study will attempt to confirm the said study (null hypothesis) or will try to point to another potential causative compound(s) that can cause pinking in Sauvignon blanc wines.

4.2 MATERIALS AND METHODS

4.2.1 Grape and wine samples

White grapes and white wines come from *Vitis vinifera* L. cv. Sauvignon blanc. The grapes which are used in this study, were from the vintage 2019 and 2020, harvested in the ward of Constantia, Coastal region, Western Cape, South Africa. This ward and region are characterized by an altitude ranging between 50 and 420 m above sea level. The soils consist of decomposed granite with high clay content. Grapes were manually harvested at optimum maturity (± 24°Brix) and transported to the winery. They were immediately destemmed and crushed, and the grape juice was treated with sulphur dioxide (40 mg/L) to avoid must oxidation. The crushed grapes were then pressed at a maximum pressure of 1.5 bar in a pneumatic press machine. Grape juice was clarified by pumping to 4.5 L glass fermentation bottles and left for 24 h at 10°C before racking to 4.5 L fermentation bottles. The alcoholic fermentation was started using active dry yeasts (Vin 13, Anchor Yeast, South Africa), carried out at controlled temperature (14°C), and finished when residual sugars were <2 g/L. After alcoholic fermentation, the wine was racked, and the free sulphur dioxide was adjusted (~40 mg/L). All conventional analytical methods were performed according to OIV methods and the South African Wine Laboratory Association Manual (SAWLA, 2002; OIV, 2012). The described wines were used for the LC-MS and Fourier Transform Infrared analyses.

Wine samples selected had high pinking potential. Control samples (4 L) were kept refrigerated in closed bottles with no airspace. In addition to the control samples, two treatments were applied to the remaining wine. The treatments were as follows: Four litres of wine were left with airspace in the open for three days until the sample pinked naturally (showing a visible pink colour - NP). Hydrogen peroxide (H_2O_2) was added to another batch of 4 L of wine and left overnight until the wine turned pink (pink induced - PI).

4.2.2 Visible absorption spectra and wine chromatic characteristics

The visible absorption spectra were acquired in a 4 cm path length glass cell from 380 to 780 nm. The spectra were converted into a 1 cm path length, and the chromatic characteristics of the wines according to CIELab were calculated by using the Method OIV-MA-AS2-11 of the OIV compendium (OIV, 2012). All analyses were performed in triplicate.

4.2.3 Isolation of the pinking compounds from Sauvignon blanc wines by PVPP column chromatography

The method described by Andrea-Silva *et al.* (2014) were followed. To a white wine (1 L) presenting natural pinking (NP), pinking induced (PI) and a control (C) 0.8 g of PVPP (Polyclar V, Laffort, South Africa) was added, and the suspension was filtered through a cheesecloth and washed with 100 mL of water followed by 100 mL of ethanol 95%. After this washing, the PVPP, presenting a pink colour, was loaded on an empty SPE cartridge (without the silica-based sorbents) (Waters Corporation, South Africa) with frits and eluted sequentially with 3 mL of (1) acetonitrile, (2) acetone, (3) aqueous solution of 1% HCl, (4) ethanol, and (5) 0.1 M NH_3 in ethanol (all chemicals from Sigma-Aldrich, South Africa). Each eluent was separately collected, the solvent was removed by centrifugal evaporation and the solution was reconstituted with 0.2 mL of a methanol/water (1:1 v/v) solution before analysis by LC-MS. All analyses were performed in triplicate.

4.2.4 Determination of total monomeric anthocyanins.

Total monomeric anthocyanins were determined using the pH differential method AOAC Official Method with a minor modification (Lee, 2005). Due to the low concentration of anthocyanins present in pinking white wines, for the anthocyanin determination by the pH differential method the absorbance was measured at 520 and 700 nm using a 1 cm path length glass cell. Total monomeric anthocyanins were expressed as milligrams of malvidin-3-O-glucoside per litre (molar extinction coefficient of 28000 L/cm/mol and molecular weight of 493.43 g/mol). All analyses were performed in triplicate.

4.2.5 Isolation of pinking compounds by reversed-phase solid-phase extraction

The method described by Andrea-Silva *et al.* (2014) were followed. The pinking compounds present in the samples were purified and concentrated by reversed-phase (C-18) solid-phase

extraction (Oasis HLB SPE cartridge with 6 mL volume). Briefly, 1 g of C-18 SPE column was conditioned by applying four times 5 mL of methanol and 4 times 5 mL of a 0.1 M HCl solution. Then, the samples were applied: 100 mL of white wines from Sauvignon blanc grape variety as well as a pinked sample, adjusted to pH 1 with 3 M HCl. After application of the sample, the column was washed four times with 5 mL of the 0.1 M HCl solution, and the compounds retained were eluted four times with 5 mL of methanol. The fractions were pooled, and the methanol was removed by rotary evaporation at 35°C. The dried residue was dissolved in 2 mL of a methanol/water (1:1 v/v) solution and analysed by LC-MS. All analyses were performed in triplicate.

4.2.6 High-performance liquid chromatography

For this experiment, Sauvignon blanc wine with high pinking potential was taken. Half of the wine was kept under a blanket of CO_2 gas to exclude oxygen from the wine and the other half was left open for a couple of days to pink naturally. Solid-phase extraction (SPE) was used with a 24-position SPE vacuum manifold from Supelco. The SPE procedure used was similar for each kind of cartridge. The cartridges were activated with 5 mL methanol and conditioned with 5 mL 5% methanol solution. Two hundred and fifty millilitres of samples were loaded into the cartridges and, before elution, sorbents were washed with 5 mL ultrapure water and eluted with 15 mL methanol. The eluted samples were concentrated with N_2 gas up to 0.5 mL Concentrated samples were kept in a 1 mL Eppendorf tube and directly taken for LC-MS analysis.

4.2.6.1 Anthocyanins

A Waters Synapt G2 quadrupole time-of-flight (TOF) mass spectrometer (Waters Corporation, Milford, MA, USA), fitted with a Waters Acquity UPLC and photodiode array detector (PDA), was used for LC-MS analyses. The separation was achieved on a Waters BEH Amide UPLC column (2.1 × 100 mm, 1.7 µm) at 35°C. Solvent A consisted of 10 mM ammonium acetate in water; solvent B consisted of 10 mM ammonium acetate in 95% acetonitrile. The gradient consisted of a flow rate of 0.25 mL/min, starting with 95% B to 40% B over 9 min, applying gradient curve 7, followed by re-equilibration to initial conditions over 5 min. Electrospray ionisation was applied in the negative mode, using a capillary voltage of 2.5 kV, a cone voltage of 15 V, desolvation temperature of 250°C and desolvation gas (N_2) flow of 650 L/hr. The rest of the MS settings were optimised for best sensitivity. Data were acquired in MSE mode, consisting of a scan using low collision energy and a scan using a collision energy ramp from 25 to 60 V, which has the added advantage of acquiring low energy molecular ion data as well as fragmentation data for all analytes all the time. Data were scanned using a scan rate of 0.2 s over the range m/z 100–

1000. Leucine enkephalin was used as lock mass for accurate mass determination on the fly using a lock mass flow rate of 0.002 mL/min, acquiring lock mass data every 20 s. Sodium formate was used to calibrate the instrument. The PDA detector was set to scan over the range of 220–450 nm.

4.2.6.2 Phenols

A Waters Synapt G2 quadrupole time-of-flight (TOF) mass spectrometer was used for LC-MS analysis, fitted with a Waters Ultra pressure liquid chromatography and photodiode array detection. The separation was achieved on a Waters HSS T3 column (2.1 x 100 mm with 1.7 µm particles). A gradient was applied using 0.1% formic acid (solvent A) and acetonitrile (solvent B). The gradient started at 100% solvent A for 1 minute and changed to 50% B over 22 minutes in a linear way. It then went to 100% B after 23 minutes where it was held until 24.5 minutes, followed by re-equilibration to initial conditions for 4 minutes. The flow rate was 0.25 mL/min and the column were kept at 60ºC. The injection volume was 3 µL. Data were acquired in MSE mode which consisted of a low collision energy scan (6 V) from m/z 150 to 1500 and a high collision energy scan from m/z 40 to 1500. The high collision energy scan was done using a collision energy ramp of 30-60 V. The photodiode array detector was set to scan from 220-600 nm. The mass spectrometer was optimized for best sensitivity, a cone voltage of 15 V, desolvation gas (N_2) at 650 L/hr and desolvation temperature 275ºC. The instrument was operated with an electrospray ionization probe in the negative mode. Sodium formate was used for calibration and leucine encephalin was infused in the background as lock mass for accurate mass determinations.

4.2.7 Fourier Transform Infrared analysis

Using Fourier Transform Infrared technology (FT2 WineScan™, Foss electric, Hille-roed, DK), the spectra were recorded in a range from 929 to 4992 cm^{-1} with a spectral resolution of 14 cm^{-1}. Spectra were recorded at a sample temperature of 40°C. Measurements were carried out in transmission mode at a defined optical path length of 37 µm using a CaF_2 cuvette. Sampling was conducted with an auto-sampler, using about 30 mL of sample for a double measurement, including pre-flushing of the system. The sample transmittance spectrum is then divided by the background transmittance spectrum obtained in the standard measurement. Background measurements were taken against double-distilled water. Background measurements were taken every 20 min and the system was flushed automatically every 20 min or after 15 samples. In this instrument, the interferometer system is encapsulated to minimize the disturbance by water vapour and other gases like CO_2 in the optical pathway. The collection of data from the

entire spectrum allows the analysis of many parameters in a short period. The whole spectral range (929–4992 cm^{-1}) is stored for each sample. There are certain ranges of frequencies, which are eliminated to prevent noise from being included in the calculations. The following spectral ranges: 965–1582, 1698–2006 and 2701–2971 cm^{-1} are advised to be used. Two regions contain water absorption bands which also taken out of the spectral range, i.e. 1582–1698 and 2971–3627 cm^{-1} (Patz *et al.,* 2004). Therefore, for routine wine analysis, only about 30 data points are used to quantify the following parameters: pH, volatile acidity, titratable acidity, malic acid, lactic acid, glucose, fructose, glycerol and alcohol. There is thus a whole range of data points that are not used, and which could be used to identify unknown compounds (Palma & Barroso, 2002; Patz *et al.*, 2004; Bevin *et al.*, 2006).

4.2.8 Statistical analysis

Analysis of variance (ANOVA) was conducted according to the experimental design using SAS statistical software (Version 9.4, SAS Institute Inc., Cary, NC, USA). Shapiro-Wilk test was performed on the standardized residuals from the model to test for deviation from normality (Shapiro & Wilk, 1965). This was done for the visible absorption spectra, isolation of pinking and determination of monomeric anthocyanins. 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. Multivariate analyses were performed using XLSTAT, version 2016 (Addinsoft, Paris, France). AUC (Area Under the Curve) and ROC (Receiver Operating Characteristics) curve or as it is also written, AUROC (Area Under the Receiver Operating Characteristics) were used to detect the spectral differences between the control and pinked samples (Fawcett, 2006). This was done for the LC-MS and FTIR data. The t-test was used to determine the means of the two sets of data showing the significant difference from each other (Pfanzagl & Sheynin, 1996).

4.3 RESULTS AND DISCUSSIONS

4.3.1 Visible absorption spectra and wine chromatic characteristics

Sauvignon blanc, from Constantia ward, with a potential to pink was used. The wine was divided into two batches, of which each batch was further divided into a control (K), naturally pinked (NP), and a pink induced (PI) wine. The pH of the wine (K, NP & PI) was 3.3. To test the findings of Simpson (1977a) that pH does not influence pinked wines, the second batch (K, NP & PI) were acidified to a pH 1.0 before testing it chromatographically. Figure 4.1 presents the visible absorption spectra of the Sauvignon blanc's control wine (blue line at pH 1.0 and blue

dotted line at pH 3.3), the same white wine where the wine pinked naturally (the orange line at pH 1.0 and orange dotted line at pH 3.3) and the same wine that was pink induced by hydrogen peroxide (green line at pH 1.0 and green dotted line at pH 3.3). The control wine showed no visible peak at 500 nm at either pH 3.3 or pH 1.0 as the wine had a normal white wine colour. There are definitive differences between natural pinked wine and pinked induced wines. The pinkish discolouration was more evident at the pink induced wines, while the natural pinked wines had a light pinkish colour, which showed the oxidation effect of H_2O_2 on the wine components.

The difference between NP and PI wine is also shown in Figure 4.1. The PI wine has a much higher peak at 500 nm than the NP wines. This could be explained by the H_2O_2 mode of action which was more aggressive and therefore the colour change in the wine was more prominent (Giguere, 1983). Although the PI wine showed a higher absorbance peak than the NP wine, the difference between $PI_{1.0}$ and $PI_{3.3}$ is non-significant. The pH does not influence the pink discolouration of PI wines as much as it does the NP wines as there was only a slight increase in absorbance at 500 nm between $NP_{1.0}$ and $NP_{3.3}$.

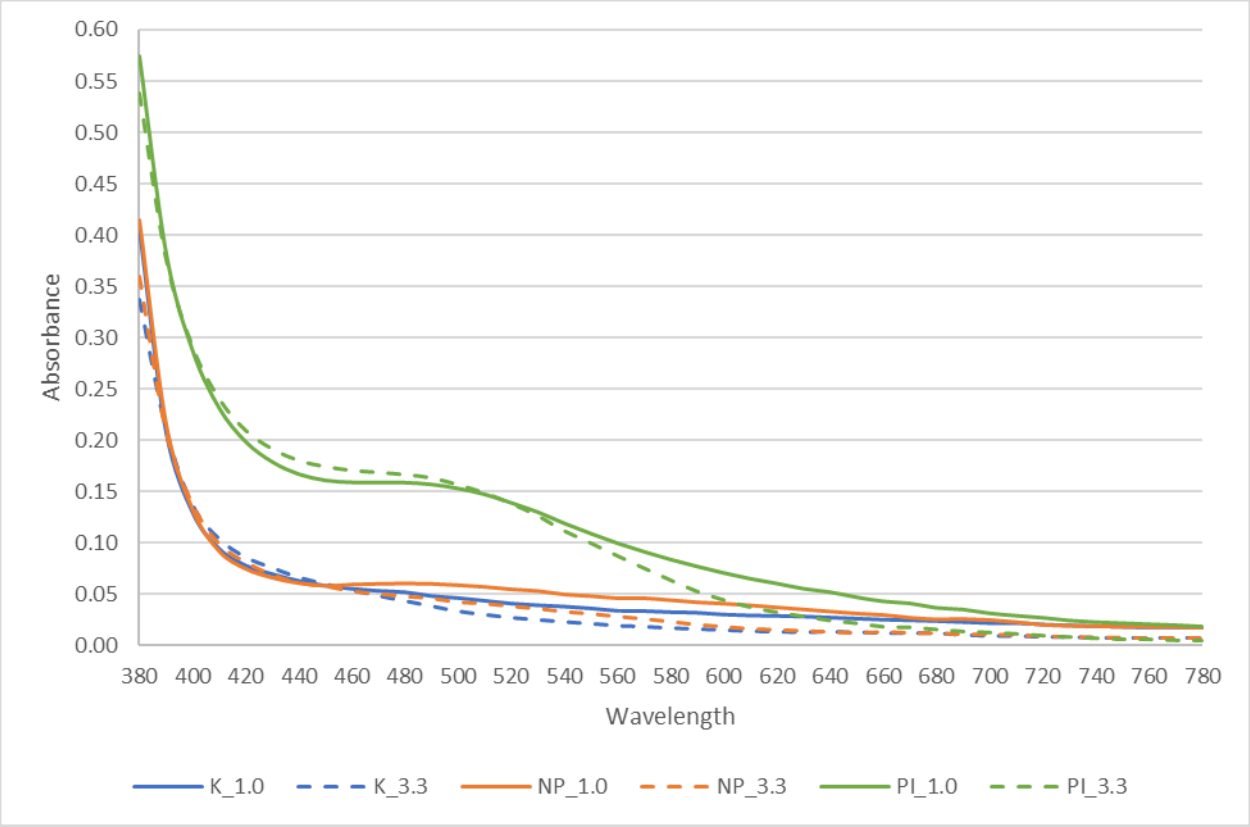

Fig. 4.1: The visible absorption spectra of a Sauvignon blanc (blue line K_1.0–control wine at pH 1.0, dotted blue line K_3.3–control wine at pH3.3, orange line NP_1.0–naturally pinked wine at pH1.0, dotted orange line NP_3.3–naturally pinked wine at pH 3.3, green line PI_1.0–pink induced wine at pH1.0 and dotted green line PI_3.3–pink induced wine at pH3.3).

The L* a• b* values describe a three-dimensional colour space. The vertical axis L* is a measure of lightness, from completely opaque (0) to completely transparent (100), while on the hue-circle a• is a measure of redness (or -a• of greenness), and b* of yellowness (or -b* of blueness) (Bakker *et al.*, 1986). The Sauvignon blanc was divided in two pH groups (pH 3.3 and pH 1.0). Each of these two pH groups were further divided in a control (K), natural pink (NP) and pink induced (PI) wine. The control wine of pH3.3 (L* = 97.9; a* = -0.6564; b* = 5.4816) and pH1.0 (L* = 96.6; a* = -0.0438; b* = 3.4173) had a pale yellow colour. The natural pink wine at pH3.3 (L* = 97.3; a* = 0.6380; b* = 4.2334) showed a slight pinkish colour, while the NP wine at pH1.0 (L* = 95.8; a* = 0.9385; b* = 2.0723) was not much darker according to visual perception. The large colour difference was perceived with the PI wine. Between the two pH levels of the PI samples, there was a slight visual difference. The pH3.3 (L* = 91.7; a* = 6.1628; b* = 11.3448) and pH1.0 (L* = 90.8; a* = 4.4952; b* = 8.2376) changed to a deeper salmon pink after the addition of H_2O_2.

The absorbance at 500 nm showed that the NP wine was just over the limit of 0.05 AU, which is regarded as the cut-off point for pinking susceptibility (Simpson, 1977a; SAWLA, 2002). Comparing the NP wine at the two pH levels at 500 nm, showed that there is a slight increase in absorbance when the pH level was lowered to pH 1.0. The absorbance of the PI wine was about three times higher than the NP wine. The addition of H_2O_2, which form part of the reactive oxygen species (ROS), reacts more aggressively with hydroxyl substrates leading to a darker hue of pink (Oliveira *et al.*, 2011). In his epic study, Simpson (1977a) proved that there is no colour difference by reducing the pH of pink wine. He concluded that, if there is no colour difference after reducing the pH, anthocyanins were not the cause of pinking.

When a white wine pinked in a bottle, a definite pinkish or salmon-red discolouration can be observed. This study showed that there were no significant differences between treatment at wine pH and its corresponding pH 1.0. This can lead to a conclusion that a decrease in pH does not influence the colour of a pinked wine, which support the findings of Simpson (1977a). The darker discolouration observed with the addition of H_2O_2 showed a strong oxidising effect on oxidisable wine components.

4.3.2 Isolation of the pinking compounds from Sauvignon blanc wines by PVPP column chromatography

In rare cases, pinking is observed after bottling. A small percentage are perceived by members of the Wine and Spirit Board tasting and are then rejected (unpublished SAWIS data). The strategy of most winemakers is to work preventatively by adding polyvinylpolypyrrolidone

(PVPP) to their wine during the blending and/or fining stage. For this reason, PVPP was used for the following experiment. To 1 L of wine, 0.8 g/L PVPP was added to the control, NP and PI wine samples. The PVPP was filtered out and loaded to empty SPE cartridges before eluted with 3 mL of acetonitrile, acetone, an aqueous solution of 1% HCl, ethanol and 0.1 M NH_3 in ethanol sequentially. All the eluted samples had a clear liquid except for the PI samples that were eluted with 0.1 M NH_3, which had a darker pink/red colour. A low-intensity peak was observed at 479.083 g/mol ([m+H]) for the PI samples of all 0.1 M NH_3 eluates. There were 62 compounds listed on Metfrag and corresponds to the molar mass of which petunidin-3-O-glucoside, petunidin-3-O-galactoside and 4-O-methyldelphinidin-3-glucoside best-fit anthocyanins that could contribute to a pinkish colour in the wine. Only the petunidin-3-O-glucoside corresponds to anthocyanins found by Andrea-Silva *et al.* (2014) and Cosme *et al.* (2019). Flavonoid concentrations in the NP and K samples were too low to be detected. There was nothing to detect in the other eluents.

It can be deduced that petunidin could be the anthocyanin that causes pinking in Sauvignon blanc, while malvidin is the anthocyanin causing pinking in Siria. It just shows that the anthocyanin fingerprint of Siria differs from Sauvignon blanc grapes.

4.3.3 Determination of total monomeric anthocyanins

Monomeric anthocyanins were measured in the control (K), NP and PI wines according to the AOAC method (Lee, 2005). The control and NP wines had very low quantities of monomeric anthocyanins (40 and 10 µg/L, respectively), while the PI wines had more than twice the concentration of monomeric anthocyanins (90 µg/L). This confirms the study done by Arapitsas *et al.* (2015), that there are trace amounts of monomeric anthocyanins in white cultivars. The concentrations measured are well below the limit determined by Andrea-Silva *et al.* (2014) of 300 µg/L for pinking to become detectable in Siria wine. The research of Andrea-Silva *et al.* (2014) and Arapitsas *et al.* (2015) showed the possibilities of anthocyanin formation in white cultivars and the different concentration that can be found in different white cultivars. Arapitsas *et al.* (2015) showed the concentrations for the 2011 harvest year for Sauvignon blanc (55.44 µg/kg), Riesling (138.99 µg/kg), Gewurztraminer (1094.90 µg/kg), Chardonnay (37.05 µg/kg) and Moscateller (922.00 µg/kg). Although this study confirms the possibility of anthocyanins present in white grape cultivars, it also is shown that the quantities are below the concentration to induce pinking in, especially, Sauvignon blanc. Several studies have shown that white grape cultivars possess the VvmybA1 gene for anthocyanin expression, but that these genes are recessive (Jeong *et al.*, 2006; Azuma *et al.*, 2007; This *et al.*, 2007; Mitani *et al.*, 2009; Terrier *et al.*, 2009; Fournier-Level *et al.*, 2010).

4.3.4 High Performance Liquid chromatography

4.3.4.1 Anthocyanins

Anthocyanins have a very distinct fingerprint in red wines and these fingerprints can be used to distinguish between different cultivars (García-Beneytez *et al.*, 2003). Although that may be the case for red cultivars, finding anthocyanins in white wine even if the wine has pinked, can be problematic. Analysis of anthocyanins by LC-MS throughout this study always showed anthocyanins to be non-detectable, reflecting either concentrations less than the detection limit of 1 mg/L or the absence of anthocyanins in the samples. LC-MS results confirmed FT-MIR results, showing no or non-detectable levels of anthocyanins. Although anthocyanin is known to only be present in coloured grape berries, a study done by Arapitsas *et al.* (2015) showed trace amounts of delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside, petunidin-3-O-glucoside and malvidin-3-O-glucoside (µg/kg) in white grape cultivars Chardonnay, Riesling, Gewurztraminer, Moscateller and Sauvignon blanc after analyses with UPLC-MS-MS. In analysing our pinked samples and their corresponding control samples (non-pinked Sauvignon blanc wines) with LC-MS, no differentiation was found between the samples analysed. This also led to the conclusion that anthocyanins were not detected, and probably, therefore, had no influence on pinking in our wine.

4.3.4.2 Phenols

An untargeted LC-MS analysis of the control and pinked sample showed peaks corresponding to phenolic compounds normally found in white wines. The difficulty with using the LC-MS became evident when peaks could be seen, but the compounds corresponding to the given mass could not be identified. As the internal library of the LC-MS was more calibrated for pharmaceuticals, wine compounds are an unknown factor for the LC-MS. A different approach was necessary to evaluate the impact of the different masses found and S-plots were constructed from the LC-MS raw data (Figure 4.2). The S-plots are a screening tool that can be used to identify possible masses contributing to the difference between the control and pinked wines. The potential distinctive time_mass numbers are distributed on the S-plot (upper right and lower left of the S-plot). A list of time_mass, which may influence the pink wine, was generated. In Table 4.1 the mass of compounds with the most influences, to either the control or pinked samples, with their retention times in brackets, are depicted.

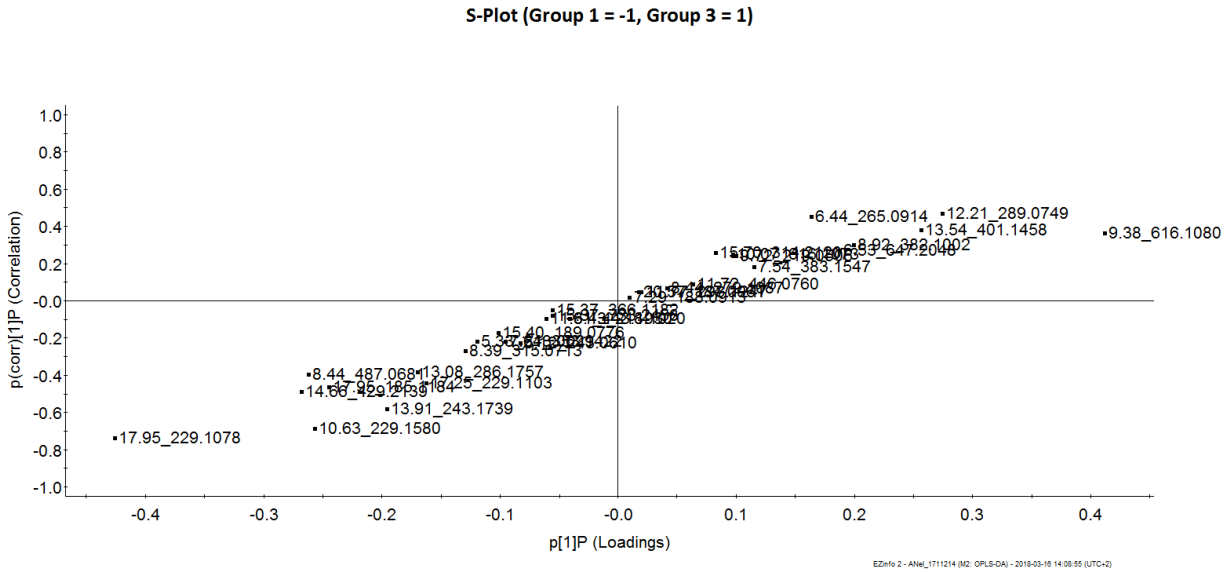

Fig. 4.2: An S-plot drawn from the chromatographic data obtained from pinked and control (Sauvignon blanc) samples. The first number in a sequence depicts the retention time, while the second set of numbers depicts the mass of a compound (e.g. 17.95_229.1078).

From this S-plot (Figure 4.2) the time_mass compounds considered to be the main contributors, but not the only ones, driving the differences between the matrices were 429 (14.66), 229 (17.95), 383 (7.54) and 487 (8.41) on the left side of the S-plot and 287 (12.21), 401 (8.54), 383 (7.54) and 616 (10.07) on the top of the S-plot. The only compound which could be positively identified, with regards to the LC-MS in Table 4.2, is GRP which correlates with a mass of 616.1073. The closest phenolic compounds that fit the masses in Table 4.2 are Daidzein 4'-O-glucuronide (429.2139 - not found in wine), dihydro-resveratrol (229.1078), 5-(3',5'-dihydroxyphenyl)-valerolactone-3-O-glucuronide (383.1547 - not found in wine), 6''-O-acetylglycitin (487.0681 - not found in wine), phlorin (287.0749 - not found in wine), 5-heneicosenylresorcinol (401.1458 - not found in wine). These compounds are plant metabolites and are associated with water stress (De Sanctis *et al.*, 2012) or the response of plants against fungal attack.

Table 4.1: Mass with retention times in brackets noted from S-Plots of LC-MS data

Mass [M-H] (Rt) - Right	Mass [M-H] (Rt) - Left
429.2139 (14.66)	287.0749 (12.21)
229.1078 (17.95)	401.1458 (8.54)
383.1547 (7.54)	383.1547 (7.54)
487.0681 (8.41)	616.1073 (10.07)

M-H–An H^+ is subtracted during the LC-MS analysis. Rt–Retention time in brackets

In all the analyses that were done on pinked wine in this study, the data generated indicates or low levels (non-detected) or any signs of the presence of phenolic substances that could cause pinking in white wines. A new theory was hypothesised that phenols may not lead to pinking but that another type of compound plays a role. This new theory took an interesting turn in the discovery of a series of articles written on pinking in onions. In these articles, the authors showed that certain amino acids (the most common one is L-cysteine) which react with thiosulfinates to form compounds that turn onions and garlic pink in the presence of oxygen (Shannon *et al.*, 1967; Kubec *et al.*, 2004a, 2004b; Dong *et al.*, 2010; Kubec & Velíšek, 2007; Kučerová *et al.*, 2011; Kubec *et al.*, 2015). This opens a new path in further research for the pinking compound.

4.3.5 Fourier Transform Infrared analysis

A Sauvignon blanc wine was naturally pinked by standing outside for three days and a control (K) sample was kept in a full bottle with no airspace. After pinking naturally (NP), the pinked and control sample was sent for FTIR analysis. The FTIR scans in the mid-infrared (MIR) spectrum from about 929–4880 cm^{-1}. MIR forms part of the infrared spectrum which can be divided into near-infrared (NIR), mid-infrared (MIR) and far-infrared (FIR). In between the ultraviolet (UV) spectrum and IR is the visible light (Vis) spectrum that is mostly used by spectrophotometers. Therefore, the ranges that are available for researchers are 280–400 nm (UV), 400–700 nm (Vis), 700–2 500 nm (NIR), 2 500–5 000 nm (MIR) and 5 000–1 000 000 nm (FIR). The initial research done by Kaffka and Norris (1976) on the use of infrared spectra to analyse wine components, opened up a whole new field of research and studies was done to correlate total soluble solids (Cao *et al.*, 2010), alcohols (Baumgarten, 1987; Yano *et al.*, 1997; Li *et al.*, 1999), volatile acids (Yano *et al.*, 1997), esters (Ribéreau-Gayon *et al.*, 2001), aroma compounds

(Vianna & Ebeler, 2001; Díaz-Maroto *et al.*, 2005; Lorenzo *et al.*, 2009), anthocyanins (Janik *et al.*, 2007; Hernández-Hierro *et al.*, 2013) and phenolic compounds (Cozzolino *et al.*, 2004; Cozzolino, *et al.*, 2008) with conventional analytical methods. Using the whole range of MIR spectra, it is possible to see at what ranges significant differences can be observed between a control wine and a pinked wine. Two regions containing strong water absorption, 1582–1698 and 2971–3627 cm^{-1} and the region from 3627 cm^{-1} onwards is eliminated because it contains very little useful information (Nieuwoudt *et al.*, 2004; Patz *et al.*, 2004).

Area Under the Curve (AUC) and Receiver Operating Characteristics (ROC) were used to look at each wavenumber. For each wavenumber, an optimal cut-off value was calculated. On each of these wavenumbers, a student t-test was done to discriminate the significant difference between the control and pink samples. The principal component analysis (PCA) graph explained 87.72% of the variation of the spectra between the control and the pinked samples (Figure 4.3) indicating distinct groupings of the control and pink samples.

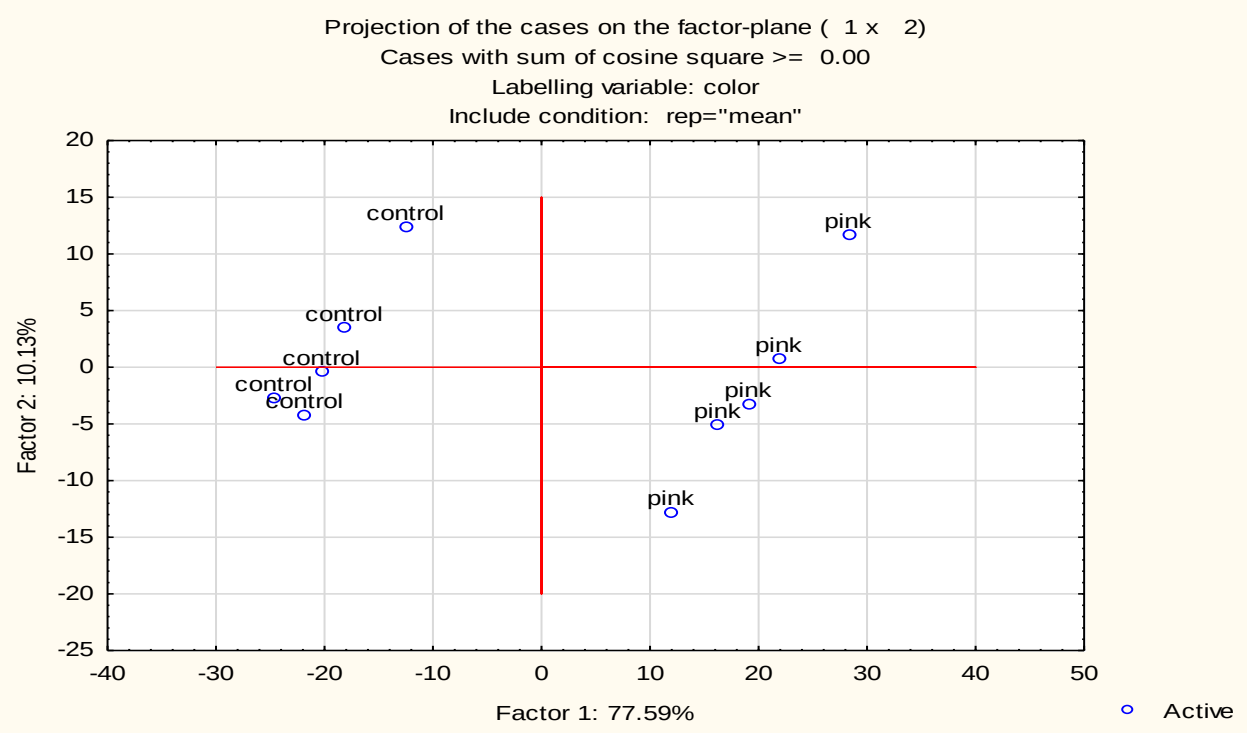

Fig. 4.3: PCA scoring to discriminate between wine types, based on the FTIR fingerprint region (929 - 3927 cm^{-1}).

Figure 4.4 depicts the raw FT-MIR spectra of control and pink wine samples in the region of the infrared medium spectrum (929–3900 cm^{-1}). The water absorption points, 1582–1698 and 2971–3627 cm^{-1} were removed. The area under the red oval circle depicts the spectra points 2299 to 2391 cm^{-1}. While there were significant differences from other spectra (as will be

depicted later), this area (2299 to 2391 cm^{-1}) showed a definitive difference between the pink and control wine samples, favouring the pink samples. Figure 4.5 depicts the spectra between the control and pink wine samples of wavenumbers 2299–2391 cm^{-1}. False-positive bands could be identified in this region due to atmospheric CO_2 in poorly adjusted and old equipment (2350 cm^{-1}). Samples stored at low temperatures with dissolved CO_2 can show a false positive band (2325 cm^{-1}), as well as absorption of gaseous N_2 (2330 cm^{-1}) (Socrates, 2001). Compounds fitting the absorption bands of 2299 to 2391 cm^{-1}, could be amines with $–NH_2^+$, $-NH^+$ and $C=NH^+$ (2250 – 2700 cm^{-1}) as well as mercaptans (2280 – 2330 cm^{-1}) (Socrates, 2001). This possible absorption of amines could indicate to compounds found in pinking of onions where amino acids bind with thiosulfates (Shannon *et al.*, 1967; Kubec *et al.*, 2004a, b; Dong *et al.*, 2010; Kubec & Velíšek, 2007; Kučerová *et al.*, 2011; Kubec *et al.*, 2015). In an extensive study done by Fragoso *et al.* (2011a, b), the possibility to use FT-MIR in the identification of phenolic compounds was investigated. Grape juice of different phenolic ripeness was analysed, and after the addition of catechin, gallic acid, tannic acid and malvidin-3-glucoside, and absorption between 1168–1457 cm^{-1} (gallic acid), 1133–1160 cm^{-1} (tannic acid) and 1238–1322 cm^{-1} (catechin) and 1373–1457 cm^{-1} (catechin), were examined. The team of Fragoso (2011a, b) also noticed that with an increase in malvidin-3-O-glucoside concentrations the spectra ranges could not be identified, and therefore suggested that the range for anthocyanins would probably fall between 1108–1457 cm^{-1}. This was confirmed by Culbert *et al.* (2015) after studying total phenolic compounds which absorbed between 900–1500 cm^{-1}. Statistical analysis showed a spectral difference between wavenumbers 1096–1373 cm^{-1}, which favours the control samples. The total phenolic concentration analysed by LC-MS (data not shown) showed that the control samples had a higher concentration of phenols than the pink samples. Therefore, a logical deduction could be made that total phenolic compounds do not influence the pinking of the wine samples. Janik *et al.* (2007) showed that pigmented compounds like anthocyanins are in the visible spectrum range between 400–700 nm (25000–14285 cm^{-1}). This could explain why no indication of anthocyanins was found as it falls outside of the spectra range used for this study.

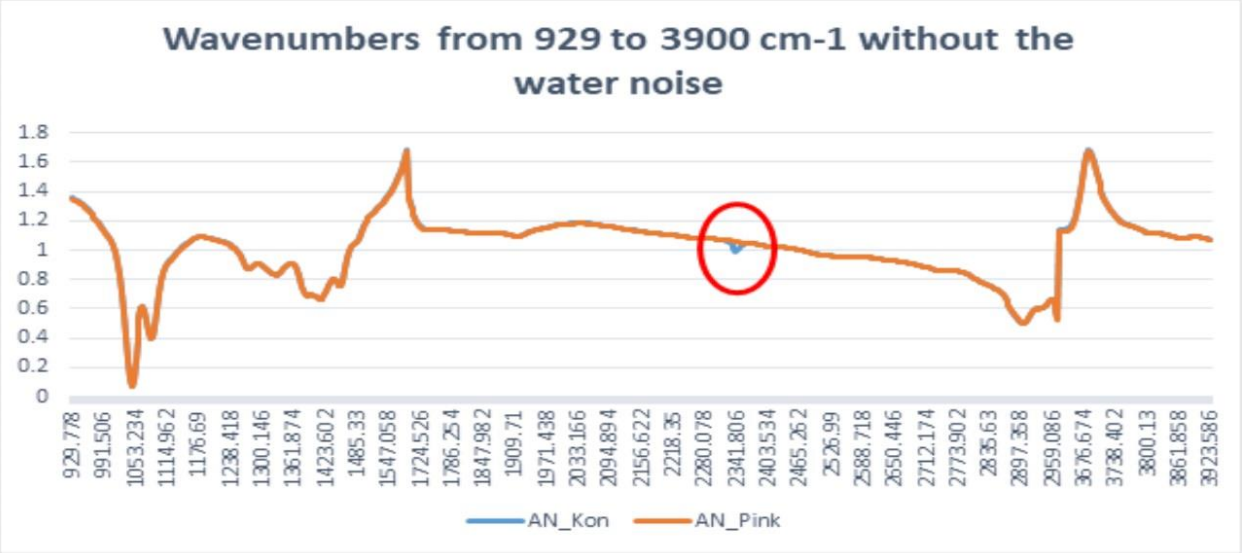

Fig. 4.4: The raw FT-MIR spectra of control and pink wine samples in the region of the infrared medium spectrum (929 – 3900 cm^{-1}). The water absorption points, 1582 – 1698 and 2971 – 3627 cm^{-1} were removed. The area under the red oval circle depicts the spectra points 2299 to 2391 cm^{-1}.

The AUC analysis indicated that 486 wavenumbers showed significant differences between the control and pinked samples. This could be grouped into nine groups of wavenumbers (Table 4.1) which differ in the number of spectral points in each group. Each of these groups also differs in their sensitivity to reference groups (either control or pink). By using the AUC's optimal cut-off point and testing the sensitivity and specificity of each wavenumber, these spectral groups can be identified as favouring the control samples or the pinked samples (Table 4.2).

Table 4.2: Spectral groups with their reference categories and the corresponding compound groups

Spectral groups (cm^{-1})	**Reference category**	**Assignments**
929–1026	K	C-O stretching & O-H out-of-plane tension of carboxylic acid, alcohols and organic acids
1053–1057	P	C-N stretching of amines
1064–1072	K	C-O stretching of primary alcohols
1095–1373	K	C-O stretching of secondary, tertiary alcohols, esters and aromatic esters, S=O stretching of sulphuric compounds
1466–2272	K	Symmetric & asymmetric in-plane bending of CH3
2303–2391	P	Amines and mercaptan
2411–2700	K	Not identified
2777–2789	K	Weak and broad O-H stretching of alcohols, strong O-H stretching of carboxylic acids
2874–2916	P	N-H stretching of amine salts

The spectral groups are depicted as wavenumbers (cm^{-1}); reference categories are K (control) and P (pinked) wine samples.

Identification of specific compounds using the frequencies is nearly impossible (Liu, 2006), although certain stretching and vibrating overtones of groups and functional groups are known. The frequency bands of 929–1026 cm^{-1} shown in Table 4.2, are associated with C=C bending of alkenes (Sigma-Aldrich), C-O stretching and O-H out-of-plane tension of carboxylic acid, alcohols and organic acids (Cocciardi *et al.*, 2005). These bands favour the control sample and the difference could be a slight difference between alcohol and organic acids of the control and pinked samples. Frequency bands 1053–1057 cm^{-1} are associated with C-H stretching of amines (Sigma-Aldrich). These bands favour the pinked samples and could show that amino acids may play a role in the pinking of white wines. The frequency bands of 1064–1072 cm^{-1} are more associated with C-O stretching of primary alcohols (Sigma-Aldrich). These bands favour

the control sample and the difference could be explained because of slight alcohol differences between the samples. Frequency bands 1095–1373 cm^{-1} encompass a broad range and it can be associated with a whole range of stretching, like C-O stretching of secondary and tertiary alcohols, esters and aromatic esters or S=O stretching of sulphuric compounds (Sigma-Aldrich). The frequency bands of 1466–2272 cm^{-1} are also a broad range of bands that can associate with a whole range of compounds, like the C=O stretching of acid halides, acetaldehyde and aliphatic ketones, as well as medium C-H bending of alkenes (Sigma-Aldrich) and symmetric and asymmetric in-plane bending of CH_3 (Silva *et al.*, 2014). The spectral group corresponding to 2303–2391 cm^{-1} is the only spectral group that showed a distinct differentiation between the control and pinked samples (Figure 4.4). The C-N triple bond of nitriles can, in most cases, peak (with varying intensity) around 2349 cm^{-1}. These C-N triple bonds can be associated with amines, amides, pyridines, nitriles and pyrroles, which is a constituent of 3-3'-dithio-2,2-dipyrrole which led to pinking in *Allium* species of garlic and onions (Hu *et al.*, 2010). The frequency bands of 2777–2789 cm^{-1} are more associated with strong O-H stretching of carboxylic acids or a weaker O-H stretching of alcohols (Sigma-Aldrich) which favours the control sample. The last frequency ranges of 2874–2916 cm^{-1} favour the pinking samples and are associated with C-H stretching of alkenes and strong N-H stretching of amine salts. This range, in conjunction with range 1053–1057 cm^{-1}, could also indicate an association with amino acids that may influence the pinking of white wines.

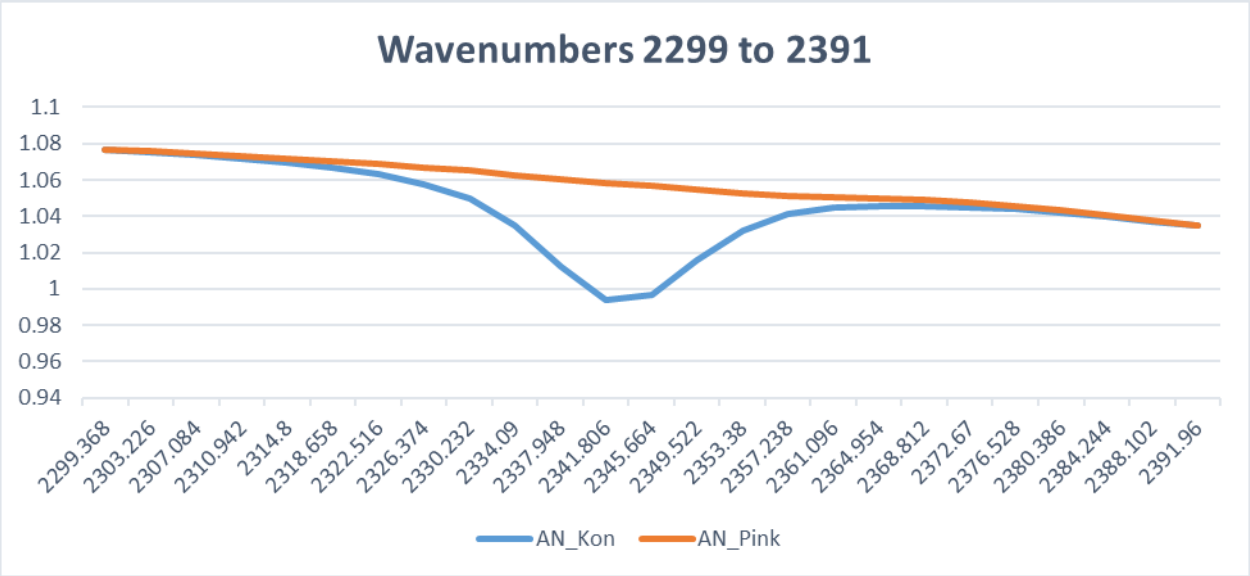

Fig. 4.5: A spectra plot graph depicting the control wines versus pink wine from wavenumbers 2299 to 2391 cm^{-1}.

4.3.6 Grape reaction product (GRP)

In Figure 4.6, the GRP concentration of the control sample is much higher than the pink sample. This can be ascribed to the oxidation of caftaric acid and the reduction by glutathione to form GRP. With the formation of H_2O_2 in the wine, the H_2O_2 oxidised to hydroxyl radical with the help of Fe^{2+}. This hydroxyl radical has then the potential to further oxidise the GRP to decrease its concentration (Cheynier *et al.*, 1989; Li *et al.*, 2008; Oliveira *et al.*, 2011; Danilewicz & Standing, 2018). During must oxidation, glutathione concentration decreases while the GRP concentration increases (Vallverdú-Queralt *et al.*, 2015). Under oxidation conditions, quinones can oxidise other substrates, like GRP or other phenolic compounds, which can lead to a decrease in GRP concentrations (Fracassetti & Tirelli, 2015).

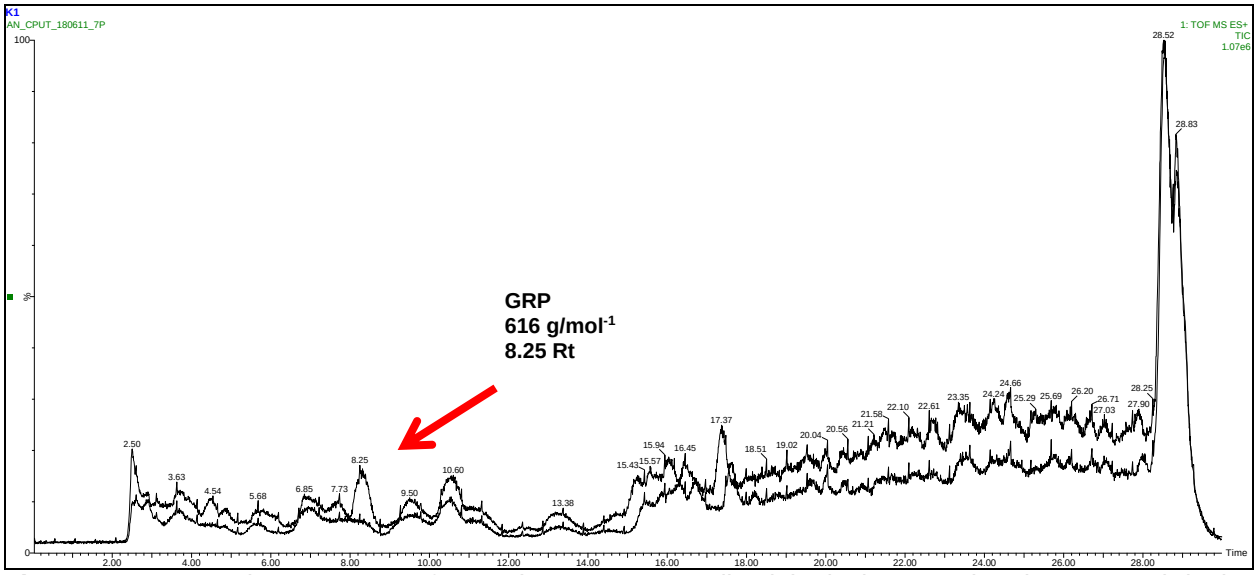

Fig. 4.6: A LC-MS chromatogram of control versus a naturally pinked wine sample. The top graph is the control and the bottom graph is the pink samples.

Glutathione (GSH) is a natural tripeptide present in grapes and wines. Its antioxidant properties are particularly useful during winemaking with white varieties like Sauvignon blanc (Kritzinger *et al.*, 2013; Pons *et al.*, 2015). Glutathione is normally found in the pulp and skins of grape cultivars, especially Sauvignon blanc. An increase in skin contact time for this cultivar could increase the glutathione concentration by as much as 55% (Pons *et al.*, 2015). Polyphenol oxidase (PPO) has the ability to oxidise caftaric acid and/or coutaric acid to form o-diphenols (Kritzinger *et al.*, 2013). Glutathione reacts with these o-diphenols to form 2-S-glutathionylcaftaric acid (GRP) which cannot be oxidised by PPO. Laccase, from *Botrytis cinerea*, can oxidise GRP back to o-diphenols, which in turn can lead to browning of white wines (du Toit *et al.*, 2007; Cejudo-Bastante *et al.*, 2010; Roland *et al.*, 2010; Kritzinger *et al.*, 2013; Gambuti *et al.*, 2016).

4.4 CONCLUSION

UV/Vis, FTIR and LC-MS analysis, and CIELab colour data of unpinked (control), naturally pinked and pink-induced wines showed spectral/colour differences in unpinked (control) and pinked samples. Compounds identified that could possibly be related to pinking in Sauvignon blanc include the monomeric anthocyanin petunidin-3-O-glucoside. Andrea-Silva et al. (2014) reported malvidin in Siria grapes (a native Portuguese cultivar) as the compound responsible for pinking. No other phenolic compounds associated with pinking in this study. The biggest absorption difference, however, correlated with amines. Amines could indicate the amino acids. In onions, certain amino acids (i.e. L-cysteine) (containing an amine group) can bind to thiosulfonates to form S-allylcysteine sulfoxides compounds that induce pinking in onions and garlic. Although amino acids have been extensively researched in wine, thiosulfonates and their forming compounds are unknown in wine and need further research. This would be a new research direction that is worthwhile investigating in the future. Further investigations and follow-up studies are needed to evaluate Sauvignon blanc from more South African regions and vintages to confirm the results of this study. The compounds responsible for pinking is, therefore, still at large.

4.5 LITERATURE CITED

Andrea-Silva, J., Cosme, F., Ribeiro, L.F., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Domingues, M.R.M. & Nunes, F.M., 2014. Origin of the pinking phenomenon of white wines. J. Agric. Food Chem. 62, 5651-5659.

Arapitsas, P., Oliveira, J. & Mattivi, F., 2015. Do white grapes really exist? Food Res. Inter. 69, 21-25.

Azuma, A., Kobayashi, S., Yakushiji, H., Yamada, M., Mitani, N. & Sato, A., 2007. VvmybA1 genotype determines grape skin color. Vitis. 46 (3), 154–155.

Baumgarten, G.F., 1987. The determination of alcohol in wines by means of near-infrared technology. S. Afr. J. Enol. Vitic. 8 (2), 75–77.

Bevin, C.J., Fergusson, A.J., Perry, W.B., Janik, L.J. & Cozzolino, D., 2006 Development of a rapid “fingerprinting” system for wine authenticity by mid-infrared spectroscopy. J. Agric. Food Chem. 54 (26), 9713-9718.

Boss, P.K., Davies, C. & Robinson, S.P., 1996. Anthocyanin composition and anthocyanin pathway gene expression in grapevine sports differing in berry skin colour. Aust. J. Grape Wine Res. 2, 163–170.

Cao, F., Wu, D. & He, Y., 2010. Soluble solids content and pH prediction and varieties discrimination of grapes based on visible–near infrared spectroscopy. Comp. Electr. Agric. 71S: S15–S18.

Cejudo-Bastante, M.J., Pérez-Coello, M.S. & Hermosín-Cutiérrez, I., 2010. Identification of new derivatives of 2-S-glutathionylcaftaric acid in aged white wines by HPLC-DAD-ESI-MS. J. Agric. Food Chem. 58 (21), 11483-11492.

Cheynier, V., Souquet, J.M. & Moutounet, M., 1989. Glutathione content and glutathione to hydroxycinnamic acid ratio in *Vitis vinifera* grapes and musts. Am. J. Enol. Vitic. 40 (4), 320–324.

Cocciardi, R.A., Ismail, A.A. & Sedman, J., 2005. Investigation of the potential utility of single-bounce attenuated total reflectance Fourier Transform Infrared spectroscopy in the analysis of distilled liquors and wines. J. Agric. Food Chem. 53 (8), 2803–2809.

Cosme, F. andrea-Silva, J., Filipe-Ribeiro, L., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Domingues, M.R.M. & Nunes, F.M., 2019. The origin of pinking phenomena in white wines: An update. 41st World Congress of Vine and Wine. BIO Web of Conferences 12, 1–5.

Cozzolino, D., Cynkar, W.U., Dambergs, R.G., Mercurio, M.D. & Smith, P.A., 2008. Measurement of condensed tannins and dry matter in red grape homogenates using near infrared spectroscopy and partial least squares. J. Agric. Food Chem. 56, 7336–7631.

Cozzolino, D., Kwiatkowski, M.J., Parker, M., Cynkar, W.U., Dambergs, R.G. & Gishen, M., 2004. Prediction of phenolic compounds in red wine fermentations by visible and near infrared spectroscopy. Anal. Chim. Acta. 513, 73–80.

Culbert, J., Cozzolino, D., Ristic, R. & Wilkinson, K., 2015. Classification of sparkling wine style and quality by MIR spectroscopy. Mol. 20, 8341–8356.

Danilewicz, J.C. & Standing, M.J., 2018. Reaction mechanisms of oxygen and sulfite in red wine. Am. J. Enol. Vitic. 69 (3), 189–195.

De Sanctis, F., Silvestrini, M.G., Luneia, R., Botondi, R., Bellincontro, A. and Mencarelli, F., 2012. Postharvest dehydration of wine white grapes to increase genistein, daidzein and the main carotenoids. Food Chem. 135, 1619–1625.

Díaz-Maroto, M.C., Schneider, R., & Baumes, R., 2005. Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. J. Agric. Food Chem. 53, 3503–3509.

Dong, Y., Wang, D., Li, M., Hu, X. & Zhao, G., 2010. One new pathway for Allium discoloration. Food Chem. 119, 548–553.

Du Toit, W.J., Lisjak, K., Stander, M. & Prevoo, D., 2007. Using LC-MSMS to assess glutathione levels in South African white grape juices and wines made with different levels of oxygen. J. Agric. Food Chem. 55 (8), 2765-2769.

Fawcett, T. 2006. An introduction to ROC analysis. Pattern Recogn. Lett. 27, 861–874.

Fournier-Level, A., Lacombe, T., Le Cunff, L., Boursiquot, J-M. & This, P., 2010. Evolution of the VvMybA gene family, the major determinant of berry colour in cultivated grapevine (Vitis vinifera L.). Heredity. 104, 351–362.

Fracassetti, D. & Tirelli, A., 2015. Monitoring of glutathione concentration during winemaking by a reliable high-performance liquid chromatography analytical method. Austr. J. Grape Wine Res. 21, 389–395.

Fragoso, S., Aceña, L., Guasch, J., Busto, O. & Mestres, M., 2011a. Application of FT-MIR spectroscopy for fast control of red grape phenolic ripening. J. Agric. Food Chem. 59, 2175–2183.

Fragoso, S., Guasch, J., Aceña, L., Mestres, M. & Busto, O., 2011b. Prediction of red wine colour and phenolic parameters from the analysis of its grape extract. Int. J. Food Sci. Technol. 46, 2569–2575.

Gambuti, A., Rinaldi, A., Romano, R., Manzo, N. & Moio, L., 2016. Performance of a protein extracted from potatoes for fining of white musts. Food Chem. 190, 237–243.

García-Beneytez, E., Cabello, F. & Revilla, E., 2003. Analysis of grape and wine anthocyanins by HPLC-MS. J. Agric. Food Chem. 51 (19), 5622-5629.

Giguere, P.A., 1983. Molecular association and structure of hydrogen peroxide. Textbook Forum. 60 (5), 399–401.

Hernández-Hierro, J.M., Nogales-Bueno, J., Rodríguez-Pulido, F.J. & Heredia, F.J., 2013. Feasibility study on the use of Near-Infrared hyperspectral imaging for the screening of anthocyanins in intact grapes during ripening. J. Agric. Food Chem. 61, 9804 − 9809.

Hu, D., Zhang, J., Zhang, T., Dong, J., Leng, X. & Zhao, G., 2020. Characterization of yellow pigments formed on reaction of 2-(1H-pyrrolyl) carboxylic acids with pyruvic acid in garlic greening model systems. Food Res. Int. 43, 915–921.

Imai, S., Akita, K., Tomotake, M. & Sawada, H., 2006. Identification of Two Novel Pigment Precursors and a Reddish-Purple Pigment Involved in the Blue-Green Discoloration of Onion and Garlic. J. Agric. Food Chem. 54 (3), 843–847.

International Organisation of Vine and Wine (OIV), 2012. Compendium of international methods of wine and must analysis. Vol. 1. Paris. 1–488.

Janik, L.J., Cozzolino, D., Dambergs, R., Cynkar, W. & Gishen, M., 2007. The prediction of total anthocyanin concentration in red-grape homogenates using visible-near-infrared spectroscopy and artificial neural networks. Analytica Chimica Acta. 594, 107–118.

Jeong, S-T., Goto-Yamamoto, N., Hashizume, K., Kobayashi, S. & Esaka, M., 2006. Expression of VvmybA1 gene and anthocyanin accumulation in various grape organs. Am. J. Enol. Vitic. 57 (4), 507–510.

Jones, T., 1989. Pinking of white table wines - further studies. Unpublished Master's thesis. University of California, Davis, CA, USA.

Kaffka, K.J. & Norris, K.H., 1976. Rapid instrumental analysis of composition of wine. Acta aliment. 5 (3), 267–279.

Kritzinger, E.C., Bauer, F.F. & du Toit, W.J., 2013. Role of glutathione in winemaking: A review. J. Agric. Food Chem. 61, 269–277.

Kubec, R., Hrbácočá, M., Musah, R.A. & Velíšek, J., 2004a. Allium discoloration: Precursors involved in onion pinking and garlic greening. J. Agric. Food Chem. 52 (16), 5089–5094.

Kubec, R., Hrbácočá, M., Musah, R.A. & Velíšek, J., 2004b. Allium discoloration: The nature of onion pinking and garlic greening. Czech J. Food Sci. 22 (Special Issue), 109–112.

Kubec, R. & Velíšek, J., 2007. Allium discoloration: The color-forming potential of individual thiosulfinates and amino acids: Structural requirements for the color-developing precursors. J. Agric. Food Chem. 55 (9), 3491–3497.

Kubec, R., Urajová, P., Lacina, O., Hajšlová, J., Kuzma, M. & Zápal, J., 2015. Allium discoloration: Color compounds formed during pinking of onion and leek. J. Agric. Food Chem. 63, 10192–10199.

Kučerová, P., Kubec, R., Šimek, P., Václavík, L. & Schraml, J., 2011. Allium discoloration: The precursor and formation of the red pigment in giant onion (Allium giganteum Regel) and some other subgenus Melanocrommyum species. J. Agric. Food Chem. 59, 1821–1828

Lee, E.J., Yoo, K.S. & Patil, B.S., 2010. Identification of candidate amino acids involved in the formation of pink-red pigments in onion (*Allium cepa* L.) juice and separation by HPLC. J. Food Sci. 75 (8), C684–C689.

Lee, J., 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants and wines by the pH differential method: collaborative study. J. AOAC Int. 88 (5), 1269–1278.

Li, Y., Brown, C.W. & Lo, S.-C., 1999. Near infrared spectroscopic determination of alcohols—solving non-linearity with linear and non-linear methods J. Near Infrared Spectrosc. 7, 55–62.

Li, H., Guo, A., & Wang, H., 2008. Mechanisms of oxidative browning of wine. Food Chem. 108, 1–13.

Liu, L., 2006. Geographical classification of wines using vis-NIR spectroscopy. Unpublished Master's thesis. School of Chemical Engineering. Shenyang Pharmaceutical University, China.

Lorenzo, C., Garde-Cerdán, T., Pedroza, M.A., Alonso, G.L. & Salinas, M.R., 2009. Determination of fermentative volatile compounds in aged red wines by near infrared spectroscopy. Food Res. Intern. 42, 1281–1286.

Mitani, N., Azuma, A., Fukai, E., Hirochika, H. & Kobayashi, S., 2009. A retrotransposon-inserted VvmybA1a allele has been spread among cultivars of Vitis vinifera but not North American or East Asian Vitis species. Vitis. 48 (1), 55–56.

Monagas, M., Bartolomé, B. & Gómez-Cordovés, C., 2005. Updated knowledge about the presence of phenolic compounds in wine. Critical Reviews in Food Science and Nutrition, 45, 85–118.

Nieuwoudt, H.H., Prior, B.A., Pretorius, I.S., Manley, M. & Bauer, F.F., 2004. Principal component analysis applied to Fourier Transform Infrared Spectroscopy for the design of calibration sets for glycerol prediction models in wine and for the detection and classification of outlier samples. J. Agric. Food Chem. 52 (12), 3726–3735.

Oliveira, C.M., Ferreira, A.C.S., De Freitas, V. & Silva, A.M.S., 2011. Oxidation mechanisms occurring in wines. Food Res. Int. 44 (5), 1115–1126.

Palma, M. & Barroso, C.G., 2002. Application of FT-IR spectroscopy to the characterisation and classification of wines, brandies and other distilled drinks. Talanta 58, 265–271.

Patz, C.-D., Blieke, A., Ristow, R. & Dietrich, H., 2004. Application of FT-MIR spectrometry in wine analysis. Anal. Chim. Acta 513, 81–89.

Pfanzagl, J. & Sheynin, O., 1996. Studies in the history of probability and statistics XLIV A forerunner of the t-distribution. Biometrika. 83 (4), 891-898.

Pons, A., Lavigne, V., Darriet, P. & Dubourdieu, D., 2015. Glutathione preservation during winemaking with *Vitis Vinifera* white varieties: Example of Sauvignon blanc grapes. Am. J. Enol. Vitic. 66 (2), 187–194.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2001). In Handbook of Enology. The chemistry of wine stabilization and treatments (Vol. 2). Chichester: John Wiley & Sons Ltd.

Roland, A., Vialaret, J., Razungles, A., Rigou, P. & Schneider, R., 2010. Evolution of S-cysteinylated and S-glutathionylated thiol precursors during oxidation of Melon B. and Sauvignon blanc musts. J. Agric. Food Chem. 58 (7), 4406–4413.

Shannon, S., Yamaguchi, M. & Howard, F.D., 1967. Precursors involved in the formation of pink pigments in onion purees. J. Agri. Food Chem. 15 (3), 423–426.

Shapiro, S.S. & Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika. 52 (3/4), 591-611.

Silva, S.D., Feliciano, R.P., Boas, L.V. & Bronze, M.R., 2014. Application of FTIR-ATR to Moscatel dessert wines for prediction of total phenolic and flavonoid contents and antioxidant capacity. Food Chem. 150, 489–493.

Simpson, R.F., 1977a. Oxidative pinking in white wines. Vitis. 16, 286–294.

Simpson, R.F., 1977b. Pinking in Australian white table wines. Austr. Wine Brew Spirit Rev. 56–58.

Simpson, R.F., Miller, G.C. & Orr, G.L., 1982. Oxidative pinking of white wines: recent observations. Food Tech. Austr. 34 (1), 44–47.

Singleton, V.L. & Esau, P., 1969. Phenolic substances in grapes and wine and their significance. Adv. Food Res. Suppl. 1, 1-282.

Singleton, V.L., Trousdale, E. & Zaya, J., 1979. Oxidation of wines I. Young white wines periodically exposed to air. Am. J. Enol. Vitic. 30 (1), 49–54.

Socrates, G., 2001. Infrared and Raman characteristic group frequencies: Tables and charts (3rd Ed.). John Wiley & Sons, Ltd. Chichester, New York, Weinheim, Toronto, Brisbane, Singapore.

South African Wine Laboratory Association (SAWLA). 2002. Methods of analysis for wine laboratories. Stellenbosch.

Terrier, N., Torregrosa, L., Ageorges, A., Vialet, S., Verriés, C., Cheynier, V. & Romieu, C., 2009. Ectopic expression of VvMybPA2 promotes proanthocyanidin biosynthesis in grapevine and suggests additional targets in the pathway. Plant Physiology. 149, 1028–1041.

This, P., Lacombe, T., Cadle-Davidson, M. & Owens, C.L., 2007. Wine grape (Vitis vinifera L.) color associates with allelic variation in the domestication gene VvmybA1. Theor. Appl. Genet. 114, 723–730.

Tobe, S.J., 1983. Pinking in table wines from white grapes. Unpublished thesis. University of California Davis

Vaimakis, V. & Roussis, L., 1993. Must oxigination and polyphenoloxidase inhibition and the oxidation of white wine. Lebensm. Wiss. Technol. 26(2), 133–137.

Vallverdú-Queralt, A., Verbaere, A., Meudec, E., Cheynier, V. & Sommerer, N., 2015. Straightforward method to quantify GSH, GSSG, GRP and hydroxycinnamic acids in wines by UPLC-MRM-MS. J. Agric. Food Chem. 63, 142–149.

Van Wyk, C., Louw, A. & Rabie, M., 1976. The effect of reductive wine making conditions on wine quality and composition. In Proceedings of the 11th International Oenological symposium, June 3-5, Sopron, Budapest, Hungary. 180–200.

Vianna, E. & Ebeler, S.E., 2001. Monitoring ester formation in grape juice fermentations using solid phase micro-extraction coupled with gas chromatography-mass spectrometry. J. Agric. Food Chem. 49, 589–595.

Yano, T., Aimi, T., Nakano, Y. & Tamai, M., 1997. Prediction of the concentrations of ethanol and acetic acid in the culture broth of a rice vinegar fermentation using near-infrared Spectroscopy. J. Ferm. Bioeng. 84 (5), 461-465.

Zoecklein, B., Fugelsang, K., Gump, B. & Nury, F., 1995. Phenolic compounds and wine color. In Wine analysis and production. New York: Chapman and Hall: 115–151.

Chapter 5

Sensory evaluation of pinked Sauvignon blanc wines

5. Sensory evaluation of pinked Sauvignon blanc wines

5.1 INTRODUCTION

Pinked wines are defined as white wines that show a pink colouration under certain winemaking conditions and were first described by Singleton and Esau in 1969. Many white cultivars are affected by the pinking phenomena, including Albariño, Chardonnay, Chenin blanc, Crouchen, Doradillo, Garnatxa blanca (Grenache), Grüner Veltliner, Macabeo, Muscat Gordo Blanco, Pálava, Palomino, Parellada, Pinot Gris, Riesling, Sauvignon blanc, Semillon, Síria, Sultana, Thompson Seedless, White Riesling and Xarel·lo (Simpson, 1977a; Tobe, 1983; Jones, 1989; Lamuela-Raventós *et al.*, 2001; Andrea-Silva *et al.*, 2014; Nel *et al.*, 2020). Simpson (1977a) described pinking as a troublesome discolouration that develops after normal winemaking production. Sometimes the discolouration only develops after bottling and storage, when certain winemaking processes lead to an influx of oxygen (Simpson, 1977a; Simpson *et al.*, 1982). The pink discolouration that takes place could be so slight that it cannot be noticed, or it could be confused with browning (Simpson, 1977b).

Anecdotal evidence suggests that the pinking of white wine does not influence the taste or the aroma of the wine (Simpson, 1980). Although extensive sensory research has been done on Rosé and Blanc de noir styled wines in terms of flavour, aroma, sweetness and colour (Amerine & Ough, 1967; Ellis & Kok, 1987; Huertas *et al.*, 2003; Gamasa *et al.*, 2009; Masson & Schneider, 2009 & Darici *et al.*, 2014), little work has been conducted on pinking in white wines.

A wine taster uses his/her senses (sight, smell and taste) to assess a wine. The wine taster extracts a lot of information from the colour of a wine, including the age, maturity and possible defects of the wine (Wang & Spence, 2019). It is for this reason that the colour of a wine is so important to a winemaker in judging the quality of a wine. Several aroma characteristics of the wine are assessed, like quality, the intensity of aroma and temporal attributes (i.e. the change of aroma over time, which influences both quality and intensity) (Jackson, 2009). These aroma attributes give clues to the wine taster of the finer nuances of the wine and what to expect when the wine is tasted. Two types of oral chemoreceptors are used when a wine is sensorial evaluated. The specialised receptors, that are situated in cavities of the taste buds, register the gustatory (taste) perception of sweet, sour, bitter, salt and umami (savoury). The free nerve endings generate the perception of astringency, dryness, burning, puckering and pain. The combination of the chemoreceptors in the mouth with the receptors in the nose produces the perception of flavour (Jackson, 2009). Pinking in white wines is related to oxidation (Singleton *et al.*, 1979; Simpson, 1980; du Toit *et al.*, 2006). Some cultivars, such as

Sauvignon blanc, are oxygen-sensitive (Day *et al.*, 2015) and are more prone to pinking (Simpson, 1977a, 1977b; Andrea-Silva *et al.*, 2014). Some of the chemical components key to oxygen-sensitive cultivars are strongly modulated by oxygen exposure, triggering a chain of chemical reactions and consequently altering the wine composition. For this reason, it would be expected that a trained wine taster could pick up notes of oxidation on pinked wines. Oxidation could also be a two-part process, where the wines of certain cultivars turn pink before turning brown (Simpson, 1977a; Lamuela-Raventós *et al.*, 2001). Lastly, anthocyanins could be synthesised during berry maturation, which could contribute to slight pink discolouration in the wine (Andrea-Silva *et al.*, 2014; Arapitsas *et al.*, 2015).

In studies done by Simpson (1977a, b), the minimum absorbency for a wine to be deemed as pink was established as 0.05 AU. In his pioneering study, Simpson found that by adding hydrogen peroxide (H_2O_2) and waiting for 24 hours, the wines with a high tendency to pink will have an absorbency of 0.05 units, and higher after the treated samples were subtracted from the absorbency of the control wine (Simpson, 1977a). Simpson's method was adopted by the wine industry worldwide and are still used today in all wine laboratories to test for pinking susceptibility. The norm is that when white wine is tested and the absorbency, after the addition of H_2O_2, is more than 0.05 AU measured at 500 nm, the wine is seen as susceptible to pinking.

This study is aimed to ascertain if a pinked white wine influences the sensory attributes of the wine. One experiment was designed to test the hypothesis that a wine taster cannot differentiate between the aroma of a pinked wine versus a control wine. A second experiment was designed to test the hypothesis that a wine taster cannot differentiate between the taste of a pinked wine versus a control wine. Experiments one and two were based on triangular tests. A third experiment tested the taster's ability to rank various pinked wines from the control sample to a heavily pinked wine, to establish the absorbency unit (AU), where wine is visually perceived as pink. A fourth experiment aimed to establish the wine taster's ability to identify an oxidised wine and a pinked wine. This study represents novel work as it is the first known reported investigation on the influence of pinking on the taste and aroma of a wine.

5.2 MATERIALS AND METHODS

5.2.1 Wines

The Sauvignon blanc wines for the sensorial analysis came from the vintage of 2018. Wine A was obtained from the ward of Constantia, Coastal region, Western Cape, South Africa. The

wines B & C were obtained from a cellar in the Robertson Valley district, Breede River Valley region, Western Cape, South Africa. The wines were untreated (no PVPP added) and the wine was filtered. The wines were sampled early in the morning directly from the tank. Cleaned green wine bottles (750 mL) were filled with CO_2 gas to prevent oxidation in the bottle before filling. The wines were immediately sealed with a cork after it was filled. The ambient temperature was about 20°C and the wine was transported early in the morning to keep it at the ambient temperature. The wine was refrigerated at a constant of 10°C in the lab. CO_2 gas was blown in the bottles before any filling and before the closing of bottles to prevent oxidation.

5.2.2 Testing for pinking susceptibility

According to the SASEV Methods of Analysis for Wine Laboratories (2002), a 0.072% (w/v) H_2O_2 solution (1.2 mL of 30% (w/v) H_2O_2 in 500 mL volumetric flask with distilled water) was used. For each wine sample, 6 x 25 mL sample bottles were filled with wine, of which three were kept as the control samples, and an addition of 1.00 mL of the 0.072% (w/v) H_2O_2 solution was added to the other three treatment bottles. The sample bottles were mixed gently and left for at least eight hours at 20°C in a dark cabinet. After eight hours, the samples were measured on a spectrophotometer at 500 nm, zeroed with the control sample (0 mL of H_2O_2 added) and, if the AU is above 0.05, the wine was seen as susceptible to pinking. Every sample was done in triplicate.

5.2.3 Sensorial evaluations

A series of experiments were designed to established a) if there are taste and aroma differences in pinked wines, b) at which point a pink discolouration can be visually perceived, and c) if a tasting panel can differentiate between an oxidised and pink wine.

5.2.3.1 Evaluating sensory differences between control and pink wines

Sauvignon blanc wine (wines A), with a high pinking susceptibility (AU_{500}), was used for the triangular tests. The triangular tests formed part of the experiment designed to test the hypothesis that a wine taster cannot differentiate between the aroma and taste of a pinked wine versus a control wine. CO_2 gas was blown on the control wine before sealing the bottles to prevent oxidation, while the other half was naturally pinked without the addition of hydrogen peroxide (H_2O_2). Natural pinking was induced by pouring the wine over in empty bottles to add oxygen to the wine to help with the natural pinking process. After the initial airing, CO_2 gas was

blown on top of the wine to minimize further oxygen contact until the wine pinked naturally after about three days. Control and test (pinked) wines were then presented to a trained panel.

A triangular test was conducted in black ISO/INAO (International Organization for Standardization, 1977) wine-tasting glasses. Fifty millilitres wine samples (wines A) were served at an ambient temperature of 20°C in the tasting glasses and covered with a petri dish for 1 h before tasting. Fourteen panelists had two control wines and one pink wine, while 14 panelists had one control and two pink wines. After taking a break the panels changed around and tasted the opposite wine combination. This process was done twice as the panelist had to firstly point out the odd sample on aroma (experiment 1) and after taking a break of one hour had to come back and identify the odd sample on tasting (experiment 2). The replicates were done in triplicate.

5.2.3.2 Rank the point of pinking perception

Sauvignon blanc wine (wines B), was used for the ranking experiment, which tested the taster's ability to rank various pinked wines from the control sample to a heavily pinked wine, to establish the point where a wine is perceived as pink. The wine (AU_{500} 0.034 nm) was then poured, under a blanket of CO_2, in 6 x 750 mL green glass bottles. Each bottle of wine was artificially coloured, with a Rosé wine (AU_{500} 0.210 nm) obtained from the same cellar in the Robertson valley, to obtain 0.01, 0.02, 0.03, 0.04 and 0.05 absorbency units (AU) at 500 nm using a VWR UV-1600 PC spectrophotometer (VWR UV-1600PC Spectrophotometer, Monitoring and Control Laboratories, South Africa). One bottle of the Sauvignon blanc wine, with no Rosé addition, was used as the control and one Rosé wine was used as the extremity for colour representation.

Clear ISO/INAO tasting glasses were used. Each sample set was randomised so that no panelists had the same sequence, and panelists had to rank the wine from the lowest to the most intense pink wine, also indicating at which minimum point they perceived the wine as pink (Figure 5.1). The samples were evaluated in individual tasting booths. Data collected at Stellenbosch University Sensory Laboratory were collected using paper ballots specifically designed by the sensory laboratory of Stellenbosch University (Addendum A).

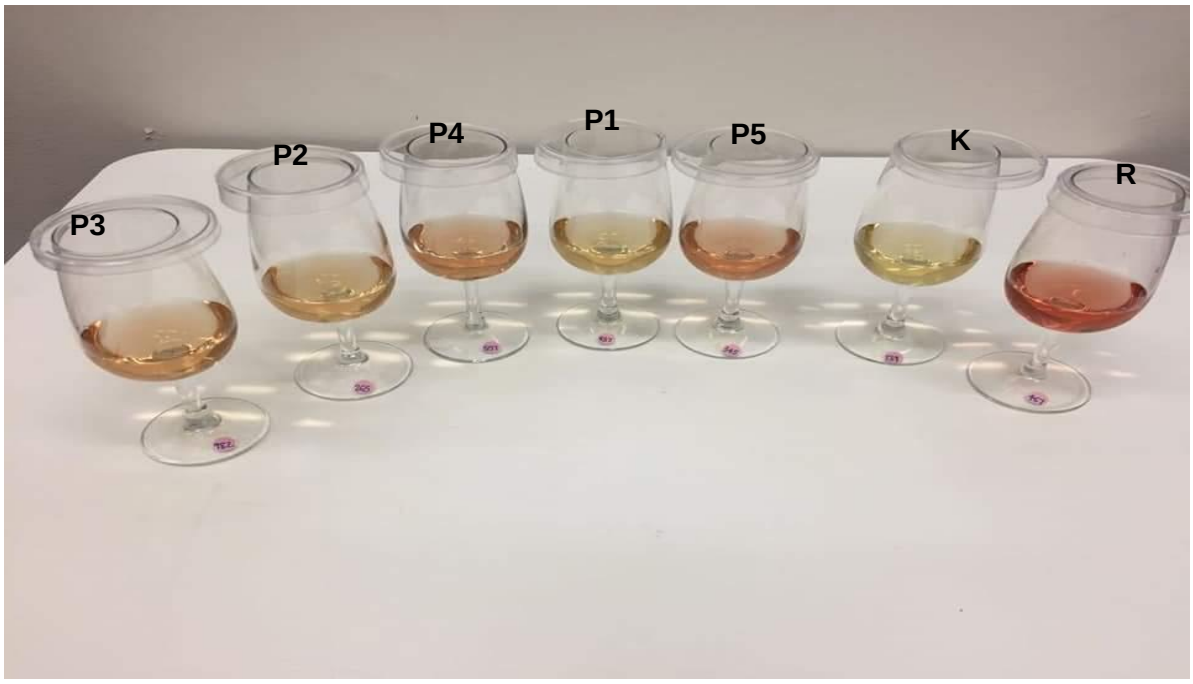

Fig. 5.1: Wines corresponding to 0.01(P1), 0.02 (P2), 0.03 (P3), 0.04 (P4) & 0.05 (P5) AU with a control (K) wine and a Rosé (R) wine.

5.2.3.3 Distinguishing between oxidised and pinked wines as wine faults

Sauvignon blanc wine (wines C) was used for the identification of pinking and oxidation as wine faults, forming part of the fourth experiment. Two bottles of the wine were artificially pinked with a Rosé wine (see *Rank the point of pinking perception*) to correlate with 0.02 AU and 0.03 AU. Two bottles of the wine were hyper-oxidized by blowing O_2 through the wine until a brown colour was obtained (AU_{420} – 0.171).

Clear ISO/INAO tasting glasses were used. Each sample set was randomised so that no panelist had the same sequence and every glass was covered with white paper so that panelist could not see the colour of the wine (Figure 5.2) before the evaluation started. The samples were evaluated in individual tasting booths and only one sample could be uncovered at a time. Data were collected using paper ballots specifically designed by the Sensory Laboratory of Stellenbosch University (Addendum B). There were two samples of control wines (K1 & K2), two samples of oxidised wine (Ox1 & Ox2) and two pink wine samples (P0.02 & P0.03). The panelists were asked if they could detect a colour difference in the wine and if so, what do they think was the reason for the colour difference. When a panelist could not distinguish between an oxidised and pink wine, they indicated it as *oxid/pink*.

Fig. 5.2: Example of sample preparation for distinguishing between oxidised and pinked wines as wine faults. The control, oxidised and pink wines were covered with white paper so that the panelist could not see the colour of the wine. The samples were evaluated in individual tasting booths and only one sample could be uncovered at a time.

5.2.4 Sensory evaluation panel

Sensorial evaluations were conducted in the Department of Viticulture & Oenology at Stellenbosch University (SU), Stellenbosch, Western Cape, South Africa. Sensory panel members were given non-monetary incentives for their participation. The panel consisted of 14 tasters (2 males and 12 females of which the youngest was 22 years and the oldest 62 years, the mean age is 43 years) that were trained by the University's sensory laboratory and were used for all the sensorial experiments. Panels were made up of trained members and consisted of industry professionals and industry experts (Brand, 2019). Every tasting was done in duplicate. The tasting took place in a white partitioned booth so that the panelists could not influence each other. The wine was given through a sliding door in front of the panelist or was covered with white paper.

5.2.5 Statistical analyses

Roessler tables for the minimum number of correct judgements to establish significance at various probability levels for a triangle test (one-tailed, p = 1/3) were used (Roessler *et al.*, 1978; Stone & Sidel, 1993). Statistical evaluation for the triangular test dictates that 15 of the 28 respondents had to correctly identify the sample for p = 0.05 to be of significant difference (Roessler *et al.*, 1978). For the other two sensorial evaluations, a cross-tabulation was used to compare judged classifications with the true classifications of the wines. Tests for an association between judged and actual classification were done using Chi-square and generalized Fisher exact tests by using Statistika 13.0 (TIBCO Software Inc., USA). Adjustments for repeated responses from the same judge were done using Rao and Scott (1981; 1987) adjustment.

5.3 RESULT & DISCUSSIONS

5.3.1 Evaluating sensory differences between control and pink wines

Anecdotal evidence (Simpson, 1980), claimed that when white wines turn pink, it does not influence the taste and aroma of the wine. As this was never tested before, a sensory evaluation was performed to challenge the anecdotal evidence as mentioned by Simpson (1980). As specified by the Roessler tables, 12 of the 14 panelists is the minimum number to agree on a triangular tasting, to be of a significant difference of p = 0.05 (Roessler *et al.*, 1978). Three Sauvignon blanc samples (two control and one pink, and one control and two pink) were presented to two groups of 14 professional panelists in triplicate in dark glasses. The panelists were asked to identify the anomalous sample from the three samples on aroma alone. In Table 5.1, only 4, 6 and 8 panelists respectively, could correctly identify the pink sample from the two control samples in the three tasting repeats. In the second line-up, 8, 9 and 8 panelists could correctly identify the control sample from the two pink samples. Cumulatively, 12, 15 and 16 panelists respectively could correctly identify the anomalous sample. Therefore, panelists could significantly correctly identify the anomalous sample from repeats 2 and 3, but not for repeat 1. Two conclusions can be made i.e. that it is possible for panelists to correctly identify this pinked wine from a normal wine based on aroma or that the panelists learned by experience. Some panelists, who correctly identified the anomalous sample, commented on their ballot papers that there was a slight indication of oxidation on the nose which lead to the conclusion that the panelists could identify pink wine on the aroma. This could be explained by the ingress of oxygen during the natural pinking process. This oxidation process can result in a change in aroma (Singleton *et al.*, 1980; Simpson *et al.*, 1982, 1983; Razmkhab *et al.*, 2002; du Toit *et al.*, 2006, Coetzee *et al.*, 2016b).

Table 5.1: Results from the triangular test depicting the number of assessors who, on aroma, identified the different sample correctly

Aroma						
	SB 1, n=14		**SB 2, n=14**		**SB 3, n=14**	
	Decisive	***Non-decisive***	***Decisive***	***Non-decisive***	***Decisive***	***Non-decisive***
2K1P	4	9	6	7	8	5
1K2P	8	7	9	6	8	7
Average	**6**	**8**	**7.5**	**6.5**	**8**	**6**

K = Control wine, P = pinked wine; Decisive = panel members correctly identifying the right sample; non-decisive = panel member that could not correctly identify the correct sample; n = the total amount of samples; Repeat 1 – 3 = Sauvignon blanc repeats.

The panelists were then asked to identify the anomalous sample on taste alone (experiment 2). In Table 5.2 only 6, 5 and 5 panelists respectively, could correctly identify the pink sample between the two control samples. Only 10, 8 and 7 respectively could correctly identify the control sample between two pink samples. Therefore, only for repeat 1 could the anomalous sample correctly be identified, while in repeats 2 and 3 the panelists were unsure (Roessler *et al.*, 1978). This means that the panelists could not correctly identify the pinked wine on taste alone.

Table 5.2: Results from the triangular test depicting the number of assessors who correctly identified, on taste, the anomalous sample

Taste						
	SB 1, n=14		**SB 2, n=14**		**SB 3, n=14**	
	Decisive	***Non-decisive***	***Decisive***	***Non-decisive***	***Decisive***	***Non-decisive***
2K1P	6	7	5	8	5	8
1K2P	10	5	8	7	7	8
Average	**8**	**6**	**6.5**	**7.5**	**6**	**8**

K = Control wine; P = pinked wine; Decisive = panel members correctly identifying the right sample; non-decisive = panel member that could not correctly identify the correct sample; n = the total amount of samples; Repeat 1 – 3 = Sauvignon blanc repeats.

According to Franco-Luesma *et al.* (2019), wine tasters can hesitate when tasting a wine. This hesitation can suggest that the taster does not pick up the oxidation notes and perceived the wine just as it is, but the panelists could pick up the oxidation notes if he/she is familiar with the oxidation notes. In this study, it became clear that the panelists hesitated in favour of the non-oxidised notes. The Pinking of white wine is preceded by the ingress of oxygen during the winemaking process or at bottling (Singleton *et al.*, 1979). The normal aroma of oxidation is associated with aldehydes and ketones (Waterhouse & Laurie, 2006; Coetzee *et al.*, 2016a). Therefore, two possible explanation is possible. First, the perception of an oxidation aroma could explain the correct identification of the panelist on aroma alone and secondly, that some familiarity could help the panelist to better identify a wine..

In conclusion, even though the pink wine, in these experiments, were briefly exposed to air to initiate pinking, as opposed to a control wine, which was kept under a CO_2 blanket, no significant differences could be picked up by the panelists in the taste of the wine, but in the aroma, there was a probability for a taster to identify a difference. This may prove the anecdotal evidence that there is no negative effect on the taste of pinked wines, but that some panelists may pick up aroma differences as stated by Simpson (1977a).

5.3.2 Rank the point of pinking perception

The pinking potential of white wines is measured at 500 nm on a spectrophotometer after the addition of H_2O_2 and compared against a control sample. When the difference in AU is 0.05 and above, the wine is considered as having the potential to pink (SASEV, 2002). Australia, America and various laboratories in Europe use the same specifications (personal communication with various laboratories). The AU for pinking susceptibility was established as 0.05 AU. The question is, at what AU the pink discolouration can visually be observed.

During the ranking exercise, the panelists could all arrange the samples in order from the lightest wine to the darkest wine. The panelists had to record the sample where they observed a definite colour change. At an AU of 0.01, the wine had a slightly darker hue but the panelists indicated that it was still within an acceptable range. It was only from 0.02 AU and higher than the panelist could start to distinguish between a pinkish wine and a control wine. At 0.03 AU, 53% of the panelists reported the colour change as pink. The value separating the indication point at which the panelists noticing the pink colour change at 0.03 AU (Figure 5.3). Using this data, it would be possible to fine-tune the pinking assay to include 0.03 AU as the cut-off absorbency for pinking susceptibility.

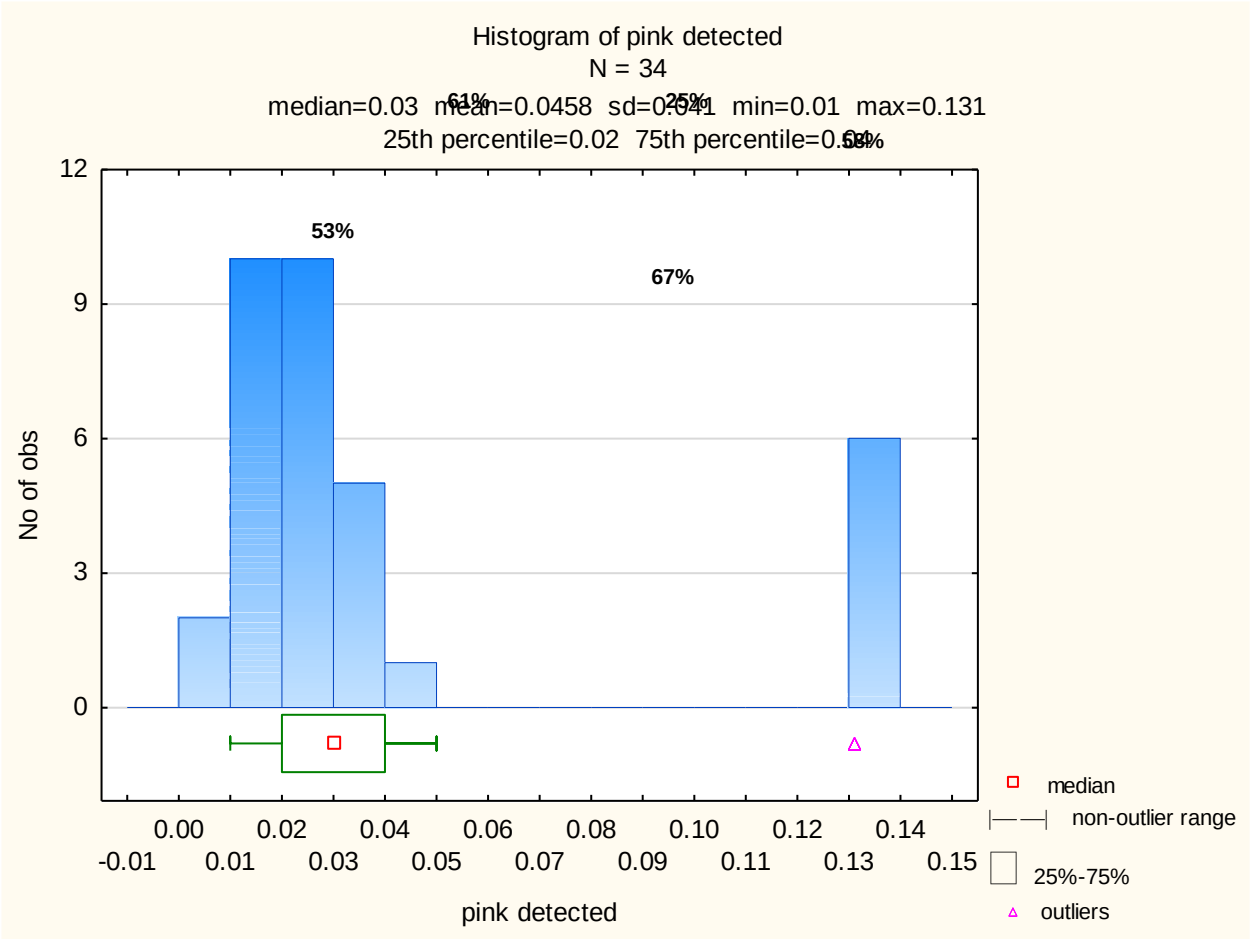

Fig. 5.3: A histogram depicting the median point at which the panelists noticed a differentiation in the colour of the wine samples. The bar on the far right indicates the Rosé wine.

Versari *et al.* (2008) stated that it is difficult to correlate the relationship between wine colour and its chemical composition. Hernández *et al.* (2011) also found that there is a general lack in agreement between absorbance measurements and visual colour assessment. This explains the reason why the panelists only start noticing the pink colour change at 0.03 AU.

5.3.3 Distinguishing between oxidised and pinked wines as wine faults

The comments made by some of the panelists in section *Evaluating sensory differences between control and pink wines*, made it clear that the aromas on the pink wine resembled oxidation. The hypothesis that a sensory panel can distinguish pinking from oxidised wines was tested in this experiment. A range of wines, which included control, oxidised and pinked wines were given a completely randomised order to a panel of trained tasters. They were asked to visually identify a wine fault, if any, in the wines given to them.

In Figure 5.4A the panelists' ability to correctly identify the control (untreated but filtered 2018 Sauvignon blanc) wine was a significant 52%, while 15% and 22% respectively incorrectly classified the oxidised and pinked wines as the control samples. Eleven percent of the panelists indicated a colour defect but were uncertain if the sample was pink or oxidised and rated the colour defect as oxidised or pink, i.e. category oxid/pink in Figure 5.4A. The ability of the panelists' classification of pink samples is indicated in Figure 5.4B. It became evident that a significant 67% of the panelists could correctly identify a pinked wine by default, while 8% thought that the control and oxidised samples were pink as well. Seventeen percent of panelists indicated a colour defect but was uncertain if the sample was pink or oxidised and rated the colour defect as oxidised or pink, i.e. category oxid/pink in Figure 5.4B. Panelists had difficulty in identifying oxidised samples. Fifty-three percent correctly identified the oxidised samples, but 29% thought that the control wine was oxidised as well, while only 7% thought that the pinked wine was oxidised (Figure 5.4C). Eleven percent of panelists indicated a colour defect but were uncertain if the sample was pink or oxidised and rated the colour defect as oxidised or pink, i.e. category oxid/pink in Figure 5.4C.

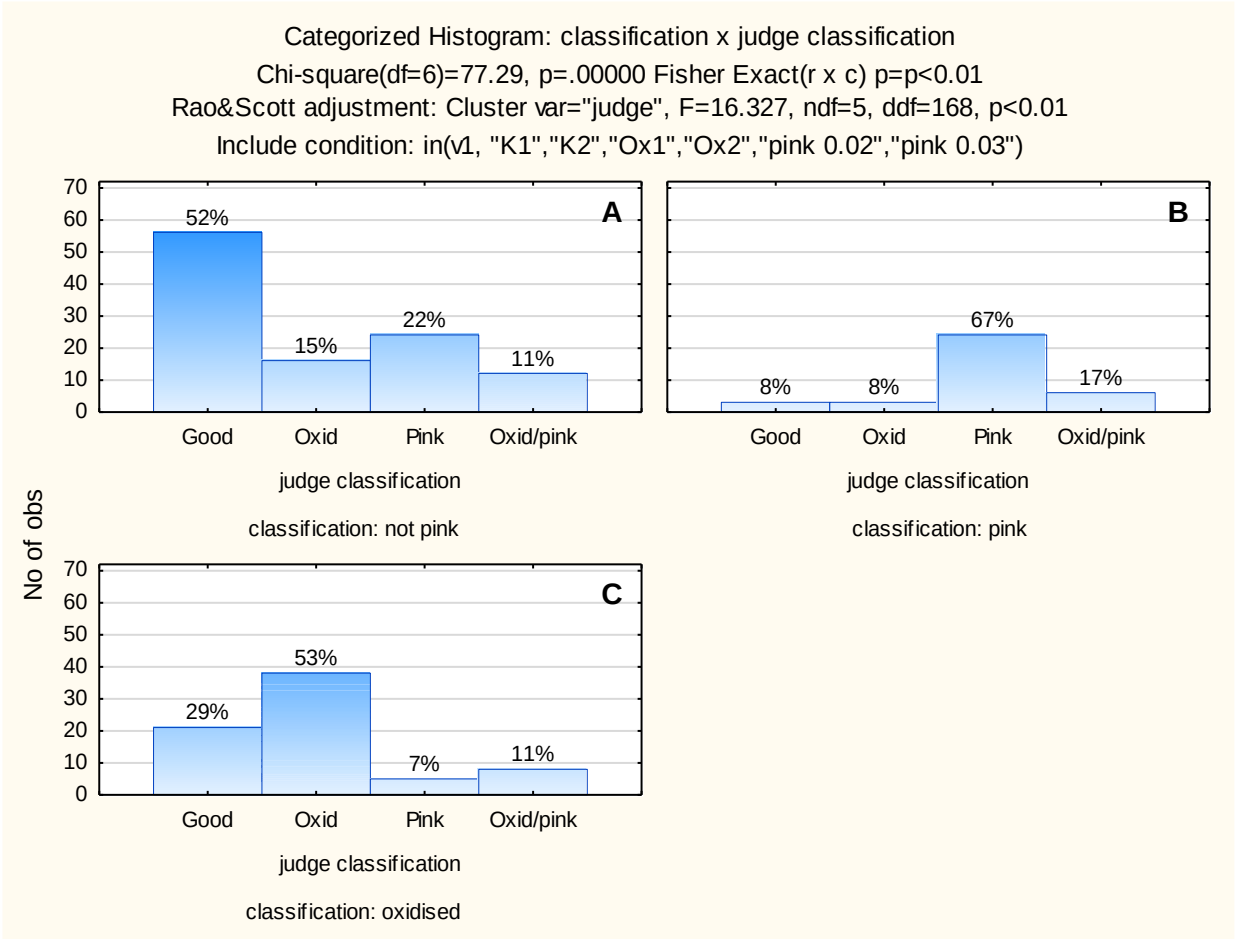

Fig. 5.4: Categorized histogram depicting the classification of the wine against the panelist classification. K1 – control wine (Good) 1; K2 – control wine 2; Ox1 – oxidised wine 1; Ox2 – oxidised wine 2; pink0.02 – pink wine 1 & pink0.03 – pink wine 2; Oxid/pink – the uncertainty of panelists whether a wine was oxidised or pink. A – The judges' classification against the control samples (Good); B – the judges' classification against pink samples; C – judges' classification against oxidised samples.

Even though the majority of panelists could correctly identify between the wine samples, there was still a high percentage of panelists that could not do this successfully. There could be various reasons for this, for instance, the background of the panelists (Grohmann *et al.*, 2018), how they are trained by the sensory lab personnel, whether a white or red wine is being tasted (Gawel & Godden, 2008), their understanding of wine and winemaking, and their experience in identifying wine faults. Wang & Prešern (2019) suggested that blind tasting training can improve the tasters' accuracy. Franco-Luesma *et al.* (2016) also suggest that the perception threshold differs between tasters and that some tasters are more sensitive to oxidation aromas than others.

5.4 CONCLUSION

There is no literature reported scientific evidence that pinking affects the flavour of wine, making this study the first scientific approach into the sensory impact of pinking. Anecdotal evidence suggests that pinking does not impact the flavour of a wine, however a trained panel in this study could differentiate differences in aroma but not taste. The number of panel members that could identify pinked wines based on aroma was, however, only marginally (based on the Roessler scale) more than the non-decisive tasters, indicating that the wine would pass the consumers lips without a second thought on the sensory and colour abnormality. A slight pink tint is not considered a fault among many international wine evaluators, however, the South African Wine and Spirit Board consider pinking in white wines as a wine fault.

The point at which the pink discolouration or hue could visually be observed by the trained panel is 0.03 AU, lower than the 0.05 AU specified by the assay for pinking potential. Therefore, instead of using 0.05 AU in the assay as prescribed by SAWLA , 0.03 can be used to detect earlier pinking susceptibility in wine.

The study showed that panellists had the ability to distinguish between control, pinked and oxidised wines, results useful in future for researchers, sensory panels, and judges of wine competitions to be trained in identifying oxidised from pinked wines as wine faults.

5.5 LITERATURE CITED

Amerine, M.A and Ough, C.C., 1967. Sweetness preference in Rosé wines. Am. J. Enol. Vitic. 18 (3), 121–125.

Andrea-Silva, J., Cosme, F., Ribeiro, L.F., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Domingues, M.R.M. and Nunes, F.M., 2014. Origin of the pinking phenomenon of white wines. J. Agric. Food Chem. 62, 5651−5659.

Arapitsas, P., Oliveira, J. and Mattivi, F., 2015. Do white grapes really exist? Food Res. Int. 69, 21–25.

Brand, J., 2019. Rapid sensory profiling methods for wine-workflow optimisation for research and industry applications. Doctor of Philosophy (Wine Biotechnology) thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Coetzee, C., Van Wyngaard, E., Šuklje, K., Ferreira, Antonio C. Silva., & Du Toit, W.J. 2016. Chemical and sensory study on the evolution of aromatic and nonaromatic compounds during the progressive oxidative storage of a Sauvignon Blanc wine. J. Agric. Food Chem. 64 (42), 7979−7993.

Darici, M., Cabaroglu, T, Ferreira, V. and Lopez, R., 2014. Chemical and sensory characterisation of the aroma of Çalkarası rosé wine. Austr. J. Grape Wine Res. 20, 340–346.

Day, M.P., Schmidt, S.A., Smith, P.A. and Wilkes, E.N., 2015. Use and impact of oxygen during winemaking. Austr. J. Grape Wine Res. 21, 693–704.

du Toit, W.J., Marais, J., Pretorius, I.S. and du Toit, M., 2006. Oxygen in must and wine: A review. S. Afr. J. Enol. Vitic. 27 (1), 76–94.

Ellis, L.P. and Kok, C., 1987. Colour changes in blanc de noir wines during ageing at different temperatures and its colour preference limits. S. Afr. J. Enol. Vitic. 18 (1), 16–22.

Gamasa, C.S., Hernández, B., de Santiago, J.V., Alberdi, C., Alfonso, S. and Diñeiro, J.M., 2009. Measurement of the colour of white and rosé wines in visual tasting conditions. Eur. Food Res. Technol. 229, 263–276.

Gawel, R. and Godden, P.W., 2008. Evaluation of the consistency of wine quality assessments from expert wine tasters. Austr. J. Grape Wine Res. 14, 1–8.

Grohmann, B., Peña, C. and Joy, A., 2018. Wine quality and sensory assessments: do distinct local groups of wine experts differ? J. Wine Res. 29 (4), 278–289.

Hernández, B., Sáenz, C., Alberdi, C., Alfonso, S. and Diñeiro, J.M., 2011. Colour Evolution of Rosé Wines after Bottling. S. Afr. J. Enol. Vitic. 32 (1), 42–50.

Huertas, R., Yebra, A., Pérez, M.M., Melgosa, M. and Negueruela, A.I., 2003. Color variability for a wine sample poured into a standard glass wine sampler. Color Res. Appl.28 (6), 473-479.

International Standard Organization 3591, 1977. Sensory analysis - Apparatus - Wine-tasting glass

Jackson, R.S., 2009. Wine Tasting: A Professional Handbook (2nd edition). Academic Press, Elsevier, California.

Jones, T., 1989. Pinking of white table wines: Further studies. Unpublished Master of Science thesis, University of California, Davis, CA, USA.

Lamuela-Raventós, R.M., Huix-Blanquera, M. and Waterhouse, A.L., 2001. Treatments for pinking alteration in white wines. Am. J. Enol. Vitic. 52 (2), 156–158.

Masson, G. and Schneider, R., 2009. Key compounds of Provence Rosé wine flavor. Am. J. Enol. Vitic. 60 (1), 116–122.

Mora, M., Urdaneta, E. and Chaya, C., 2018. Emotional response to wine: Sensory properties, age and gender as drivers of consumers' preferences. Food Qual. Prefer. 66, 19–28.

Nel, A.P., du Toit, W.J. and van Jaarsveld, F.P., 2020. Pinking of white wines - a Review. S. Afr. J. Enol. Vitic. 41 (2), 151-157.

Rao, J.N.K., and Scott, A., 1981. "The analysis of categorical data from complex sample surveys: Chi-squared tests for goodness of fit and independence in Two-Way tables," J. Am. Stat. Assoc. 76, 221–230.

Rao, J.N.K. and Scott, A.J., 1987. On Simple Adjustments to Chi-Square Tests with Sample Survey Data. Ann Stat. 15(1), 385–397.

Razmkhab, S., Lopez-Toledano, A., Ortega, J.M., Mayen, M., Merida, J. and Medina, M., 2002. Adsorption of phenolic compounds and browning products in white wines by yeasts and their cell walls. J. Agric. Food Chem. 50 (25), 7432–7437.

Roessler, E.B., Pangborn, R.M., Sidel, J.L. and Stone, H., 1978. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. J. Food Sci. 43 (3), 940–947.

Simpson, R.F., 1977a. Oxidative pinking in white wines. Vitis. 16, 286–294.

Simpson, R.F., 1977b. Pinking in Australian white table wines. Austr. Wine, Brew. Spirit Rev. November 28, 56–58.

Simpson, R.F., 1980. Some aspects of oxidative pinking of white wines. Austr. Grapegrower Winemaker 204 (12), 12–14.

Simpson, R.F., Miller, G.C. and Orr, G.L., 1982. Oxidative pinking of white wines: Recent observations. Food Tech. Austr. 34 (1), 44–47.

Simpson, R.F., Bennett, S.B. and Miller, G.C., 1983. Oxidative pinking of white wines: a note on the influence of sulphur dioxide and ascorbic acid. Food Tech. Austr. 35 (1), 34–36.

Singleton, V.L. and Esau, P., 1969. Phenolic substances in grapes and wine and their significance. Adv. Food Res. Suppl. 1, 1-282.

Singleton, V.L., Trousdale, E. and Zaya, J., 1979. Oxidation of wines I. Young white wines periodically exposed to air. Am. J. Enol. Vitic. 30 (1), 49–54.

Singleton, V.L., Zaya, J. and Trousdale, E., 1980. White table wine quality and polyphenol composition as affected by must SO_2 content and pomace contact time. Am. J. Enol. Vitic. 31 (1), 14–20.

Stone, H. and Sidel, J., 1993. Sensory evaluations practices. Academic Press, Orlando, FL.

SASEV. 2002. Methods of analysis for wine laboratories. Compiled by the South African Wine Laboratory Association. Stellenbosch. South Africa

Tobe, S.J., 1983. Pinking in table wines from white grapes. Unpublished MSc thesis, University of California, Davis, USA.

Versari, A., Boulton, R.B. and Parpinello, G.P., 2008. A comparison of analytical methods for measuring the color components of red wines. Food Chem. 106, 397–402.

Wang, Q.J. and Prešern, D., 2019. Does blind tasting work? Investigating the impact of training on blind tasting accuracy and wine preference. J. Wine Econ. 13 (4), 384–393.

Wang, Q.J. and Spence, C., 2019. Drinking through rosé-coloured glasses: Influence of wine colour on the perception of aroma and flavour in wine experts and novices. Food Res. Int. 126, 1–11.

ADDENDUM A

Flight

Judge name: ..... Date

Please rank the samples from the lightest colour to the darkest colour

139	*437*	*265*	*982*	*557*	*345*	*467*

Which sample is the first sample in the series that looks pink?

Comments:
The numbers in the first set of blocks are just an example of the randomized numbers that were allocated to the wine samples. Each panelist had his/her own set of randomized numbers. All the sample sets were also randomised so that no one panelist got the same number and sequence.

ADDENDUM B

Flight

Judge name..... Date

Please evaluate the samples one at a time. Take away the white carton of one sample at a time, immediately cover the sample again before assessing the next sample. Only one sample must be visible at a time.

Answer the following questions for all the samples.

Sample code	**Does the sample have a colour defect?**	**Yes**	**No**	**If yes, what was the colour defect?**
508				
600				
754				
645				
671				
594				

The numbers in the first column (Sample code) is just an example of the randomized numbers that were allocated to the wine samples. Each panelist had his/her own set of randomized numbers. All the sample sets were also randomised so that no one panelist got the same number and sequence.

Chapter 6

General discussions and conclusions

6. General discussions and conclusions

6.1 GENERAL DISCUSSION AND CONCLUSIONS

Literature on the influence of viniviticultural factors on pinking potential is not available. This study showed that certain viticultural and winemaking practices do influence the pinking potential of Sauvignon blanc wines. Sauvignon blanc was the cultivar of choice in this study, as it is the 3rd most planted white variety in South Africa, forms a large percentage of the white wine cultivars sold locally, dominates packaged exports, and since it is one of the oxygen- and pinking prone white wine cultivars.

In the determination of the effectiveness of antioxidants such as ascorbic acid in conjunction with SO_2 as curative agents for the prevention of pinking, ascorbic acid proved to be very effective in reducing the potential of a wine to pink. Ascorbic acid, however, needs to be used in conjunction with SO_2 to reduce H_2O_2 (a strong oxidising agent) formed from the dissociation of ascorbic acid. The H_2O_2 formed with ascorbic acid addition could lead to an induced pinking formation as well as browning and shorter shelf life (Bradshaw *et al.*, 2011; Barril *et al.*, 2016).

The number of fining agents that are available to the winemaker is large and this leaves the winemaker with a wide choice of fining agents to be used. Twenty-nine different fining agents were tested in this study at three different addition stages (settling, fermentation and fining) to see which ones were most effective in preventing pinking. The most effective fining agents at all winemaking stages for the prevention of pinking were ascorbic acid, and patatin, a potato-based protein. The other fining agents that can be recommended for use at certain winemaking stages for the prevention of pinking are combinations of PVPP, gelatine, and of potassium caseinate and silica. This study shows that other agents are equally if not more effective than PVPP, the agent mostly used in the wine industry, depending on the winemaking stage where it is used. PVPP works best in combination with gelatine, potassium caseinate and bentonite. Winemakers must make sure what the active ingredients of the product is and at what stage will it work the best.

Throughout this study for the different experiments that investigated winemaking processes that affect the pinking susceptibility of Sauvignon blanc, the influence of skin contact time on pinking susceptibility was evident. To prevent the potential for wines to pink, skin contact should be minimised, particularly under reductive winemaking conditions and higher grape ripeness levels.

Grape temperature at processing does play a role in pinking. Grapes at 4°C and 20°C were processed and the resulting wines evaluated for pinking potential. Wines from grapes processed at the lower grape temperature, showed pinking potential, whereas grapes

processed at ambient (20°C) temperature did not. In a separate, yet unpublished study, grapes from cooler areas showed pinking susceptibility whereas grapes from warmer areas did not. Wine cellars, particularly in cooler areas, need to monitor grape temperatures at harvest and at processing, to ensure processing of the grapes take place at ambient/cellar temperature to avoid pinking, particularly for pinking-susceptible cultivars.

The method of pressing also impacted on the potential of Sauvignon blanc to pink. To prevent the potential for wines to pink, grapes should be pressed after removal of the stems (destemming).

Pinking potential of the final wines increased significantly as the maturity of the Sauvignon blanc grapes increased, and grapes should not be harvested above 22°B if pinking is to be avoided.

UV/Vis, FTIR and LC-MS analysis, and CIELab colour data of unpinked (control), naturally pinked and pink-induced wines showed spectral/colour differences in unpinked (control) and pinked samples. Compounds identified that could possibly be related to pinking in Sauvignon blanc include the monomeric anthocyanin petunidin-3-O-glucoside. Andrea-Silva *et al.* (2014) reported malvidin in Siria grapes (a native Portuguese cultivar) as the compound responsible for pinking. Differences in concentrations of grape reaction product (GRP) between pinked wines and their non-pink counterparts were also detected. No other phenolic compounds associated with pinking in this study. The biggest absorption difference, however, correlated with amines. Amines could indicate amino acids. In onions, certain amino acids (i.e. L-cysteine) (containing an amine group) can bind to thiosulfonates to form S-allylcysteine sulfoxides compounds that induce pinking in onions and garlic (Kubec *et al.*, 2004a, b; 2011, 2015; Kubec, & Velíšek, 2007). Although amino acids have been extensively researched in wine, thiosulfonates and their forming compounds are unknown in wine and need further research. This would be a new research direction that is worthwhile investigating in the future. Further investigations and follow-up studies are needed to evaluate Sauvignon blanc from more South African regions and vintages to confirm the results of this study. The compounds responsible for pinking is, therefore, still at large.

There is no literature reported scientific evidence that pinking affects the flavour of wine, making this study the first scientific approach into the sensory impact of pinking. Anecdotal evidence suggests that pinking does not impact the flavour of a wine, however a trained panel in this study could differentiate differences in aroma but not taste. The number of panel members that could identify pinked wines based on aroma was, however, only marginally (based on the Roessler scale) more than the non-decisive tasters, indicating that the wine would pass the consumers lips without a second thought on the sensory and colour abnormality. A slight pink tint is not considered a fault among many international wine

evaluators, however, the South African Wine and Spirit Board consider pinking in white wines as a wine fault.

The point at which the pink discolouration or hue could visually be observed by the trained panel is 0.03 AU, lower than the 0.05 AU specified by the assay for pinking potential. Therefore, instead of using 0.05 AU in the assay as prescribed by SAWLA , 0.03 can be used to detect earlier pinking susceptibility in wine.

The study showed that panellists had the ability to distinguish between control, pinked and oxidised wines, results useful in future for researchers, sensory panels, and judges of wine competitions to be trained in identifying oxidised from pinked wines as wine faults.

6.2 CONCLUDING REMARKS AND FUTURE RECOMMENDATIONS

Practical guidelines and recommendations from this study for the prevention of pinking to enable winemakers to establish pinking risk practices, are:

- Harvest Sauvignon blanc grapes at lower ripeness levels (not above 22°B);
- Process grapes at ambient/cellar temperature; avoid very low grape temperatures at processing;
- Minimise skin contact time;
- Press grapes after removal of the stems (destemming) (no whole-bunch pressing);
- Although PVPP is generally used, the antioxidant agent ascorbic acid or patatin (a potato-based protein) are very effective at any of the winemaking stages to reduce or eliminate the risk of pinking. Other fining agents recommended for use at certain winemaking stages for the prevention of pinking are combinations of PVPP, gelatine, and of potassium caseinate and silica. Other agents can be equally if not more effective than PVPP, the agent mostly used in the wine industry, depending on the winemaking stage where used. PVPP works best in combination with gelatine, potassium caseinate and bentonite. Make sure what the active ingredients of the product is and at what stage will it work the best.
- Pinking is an oxidative event, and factors associated with oxidation such as metals (Fe and Cu) and oxygen associate with higher pinking potential.

- Practices such as exposing pinked wines to sunlight, has limited success, since measured colour reductions not always corresponded to the visual disappearance of pinking, questioning the effectiveness of sunlight (UV) as a post-pinking treatment.

- Use 0.03 AU when assaying for pinking potential, instead of 0.05 AU as prescribed by SAWLA, for the earlier detection of pinking susceptibility in wine.

Although differences in concentrations of petunidin-3-O-glucoside, grape reaction product (GRP), and amines between pinked wines and their non-pink counterparts were detected, the compounds responsible for pinking are still at large. Future investigations should focus on these compounds and thiosulfonates in wines. In onions, amino acids binding to thiosulfonates to form S-allylcysteine sulfoxide compounds has been reported to induce pinking in onions and garlic. Although amino acids have been extensively researched in wine, thiosulfonates and their forming compounds are unknown in wine and need further research. Further investigations and follow-up studies are needed to evaluate Sauvignon blanc from more South African regions and vintages to confirm the results of this study. Once compounds responsible for pinking in Sauvignon blanc has been identified, routine methods can be developed for the early identification of pinking compounds in must samples. Information can then be added to the practical guidelines and recommendations for the prevention of pinking, to enable winemakers to establish pinking risk practices.

6.4 LITERATURE CITED

Andrea-Silva, J., Cosme, F., Ribeiro, L.F., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Domingues, M.R.M. and Nunes, F.M., 2014. Origin of the pinking phenomenon of white wines. J. Agric. Food Chem. 62, 5651–5659.

Barril, C., Rutledge, D.N., Scollary, G.R. & Clark, A.C., 2016. Ascorbic acid and white wine production: A review of beneficial versus detrimental impacts. Aust. J. Grape Wine Res. 22, 169-181.

Bradshaw, M.P., Barril, C., Clark, A.C., Prenzler, P.D. & Scollary, G.R., 2011. Ascorbic acid: A review of its chemistry and reactivity in relation to a wine environment. Crit. Rev. Food Sci. 51, 479-498.

Kubec, R., Hrbácočá, M., Musah, R.A. and Velíšek, J., 2004a. Allium discoloration: Precursors involved in onion pinking and garlic greening. J. Agric. Food Chem. 52 (16), 5089–5094.

Kubec, R., Hrbácočá, M., Musah, R.A. and Velíšek, J., 2004b. Allium discoloration: The nature of onion pinking and garlic greening. Czech J. Food Sci. 22 (Special Issue), 109–112.

Kubec, R and Velíšek, J., 2007. Allium discoloration: The color-forming potential of individual thiosulfinates and amino acids: Structural requirements for the color-developing precursors. J. Agric. Food Chem. 55 (9), 3491–3497.

Kubec, R., Urajová, P., Lacina, O., Hajšlová, J., Kuzma, M. and Zápal, J., 2015. Allium discoloration: Color compounds formed during pinking of onion and leek. J. Agric. Food Chem. 63, 10192–10199.

Kučerová, P., Kubec, R., Šimek, P., Václavík, L. and Schraml, J., 2011. Allium discoloration: The precursor and formation of the red pigment in giant onion (Allium giganteum Regel) and some other subgenus Melanocrommyum species. J. Agric. Food Chem. 59, 1821–1828.