

# **Amelioration of smoke taint in wine using commercially available and legally permissible additives**

by

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## Declaration

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## Summary

The changes experienced in climate in many parts of the world have led to an increase in incidences of wildfire, and it has been predicted that these events will become more prevalent over time. All fires release volatile compounds into the atmosphere, and if they occur near vineyards where grapes are ripening, smoke taint may be detected in wines made from these grapes. Smoke taint is a critical issue for wine producing regions of the world as smoky and unpleasant flavours and aromas are perceived in affected wines, and this may have serious economic implications for producers. A number of researchers have tried to understand smoke taint, and their research has shown that volatile phenols (VP) are chemical compounds responsible. Additional research has revealed that although guaiacol, 4-ethylguaiacol, and 4-methylguaiacol were originally identified as chemical markers of smoke taint, other VPs such as cresols, eugenol, and phenol derivatives also play a role in causing smoky and ashy flavours. Strategies to eliminate the problem have ranged from washing the grapes and harvesting by hand, to minimising skin contact and choosing yeast and bacteria for minimal impact, and marketing wines for early release. These techniques work but do not eliminate an important underlying issue: glycoconjugates. Glycoconjugates or glycosides (VPs bound to sugars) are compounds that act as precursors of smoke taint produced as a detoxification by-product by vines. Glycosides can be hydrolysed by acid and enzymes, which means wines have the potential to increase available VPs in the wine, despite great care being taken to minimise VPs.

This study expands on previous strategies that have been used to ameliorate smoke taint by using commercially available and legally permissible products in South Africa and exploring their effectivity at different dosage levels. Grapes in this study were harvested and deliberately smoked in crates using a bee-smoker, which produced smoke generated from fynbos (indigenous vegetation) and pine needles. Activated charcoal, oak extract, polymer powder were used in the first part of this study to try and ameliorate the taint during winemaking. GC-MS analysis of treated wines and controls revealed that only activated charcoal at elevated levels decreased VPs chemically. Sensory analysis of treated wines and controls by a trained panel using Descriptive Analysis showed that oak extract did increase levels of eugenol and consequently increased the 'woody' attribute, thus somewhat masking the smoke aroma. None of the treatments were able to remove the smoke aroma and flavour satisfactorily, primarily because of ashy flavour on the palate, likely due to in-mouth enzymes hydrolysing VP-glycosides. Building on the data and knowledge accumulated during the first part of the study, the second part of the study attempted to reduce levels of volatile phenol glycosides by using  $\beta$ -glucosidases before treatment application for removing free volatiles ("release-and-remove"). The treatments used after the enzyme hydrolysis were activated charcoal, polymer powder, yeast hulls, and mannoproteins. Chemically, GC-MS showed that there were sharp

increases of VPs after the addition of enzymes, and some success in subsequent removal of the free volatiles. Further work is needed to determine the optimum levels of treatment. The data in this study showed potential for  $\beta$ -glucosidases to be used in the winemaking process, not only to release VPs (for later removal) but to increase the expression of fruity aromas in the wine. Enzymes may help to release other compounds that contribute to wine flavour, thus masking some of the smoke taint.

This study contributes to the improved understanding of methods that can be used for the removal or treatment of smoke taint, but the need for further work was highlighted. The use of  $\beta$ -glucosidases followed by multiple finings could be an option for producers after a fire incident has occurred near a vineyard during ripening of grapes.

## Opsomming

Die klimaatsveranderinge wat in baie dele van die wêreld ervaar word, het gelei tot 'n toename in die gevalle van veldbrande en daar is voorspel dat sulke gebeure met tyd sal toeneem. Alle brande stel vlugtige verbindings in die atmosfeer vry en as hulle naby wingerde plaasvind waar druiwe ryp word, kan 'n rooksmaak in die wyn geproe word wat met hierdie druiwe berei is. 'n Rooksmaak is 'n kritiese kwessie vir die wynproduserende dele van die wêreld omdat rokerige en onaangename geure en aromas in die geaffekteerde wyne bespeur kan word en dit kan ernstige ekonomiese implikasies vir die produsente inhou. 'n Aantal navorsers het probeer om die rooksmaak te begryp en hulle navorsing toon dat vlugtige fenole (VF) die chemiese verbindings is wat daarvoor verantwoordelik is. Bykomende navorsing het getoon dat alhoewel guajakol, 4-etielguajakol en 4-metielguajakol aanvanklik geïdentifiseer is as chemiese merkers van die rooksmaak, speel ander VF's, soos kresole, eugenol en fenol derivate ook 'n rol in die veroorsaking van rokerige en asgeure. Strategieë om die probleem uit te skakel, wissel van die was van die druiwe en oes met die hand tot die vermindering van dopkontak, om gis en bakterieë met 'n minimale impak te kies en bemarking van die wyn vir vroeë vrystelling. Hierdie tegnieke werk wel, maar skakel nie 'n belangrike onderliggende kwessie uit nie: glikokonjugate. Glikokonjugate of glikosiede (VF's verbind aan suikers) is verbindings wat optree as voorlopers van die rooksmaak wat geproduseer word as 'n detoksifikasie byproduk van die wingerdstokke. Glikosiede kan deur suur en ensieme gehidroliseer word, wat beteken dat wyn die potensiaal het om die beskikbare VF's in die wyn te verhoog, ten spyte van sorg wat geneem word om VF's te verminder.

Hierdie studie brei uit op vorige strategieë wat gebruik is om rooksmaak te verminder deur kommersieel beskikbare en wetlik toelaatbare produkte in Suid-Afrika te gebruik en hulle doeltreffendheid by verskillende dosisse te ondersoek. Die druiwe vir hierdie studie is geoes en opsetlik met behulp van 'n rookpomp in kratte gerook, met rook wat deur fynbos (inheemse plantegroei) en dennenaalde gegenerer is. Geaktiveerde steenkool, eik-ekstrak en polimeerpoeier is in die eerste deel van die studie gebruik om te probeer om die rooksmaak tydens wynbereiding te verminder. GC-MS analise van die behandelde wyne en kontroles het getoon dat slegs geaktiveerde steenkool teen verhoogde vlakke die VF's chemies kon verminder. Sensoriese analise van die behandelde wyne en kontroles deur 'n opgeleide paneel m.b.t. beskrywende analise het getoon dat die eike-ekstrak die vlakke van eugenol verhoog het en gevolglik die 'houtagtige' eienskap verhoog het, wat in 'n mate die rook-aroma verbloem het. Geen van die behandelings kon die rook-aroma en geur doeltreffend verwyder nie, hoofsaaklik as gevolg van die asgeure in die palet, moontlik as gevolg van binnensmondse ensieme wat VF-glikosiede hidroliseer. Op grond van die data en kennis wat tydens die eerste deel van die studie verkry is, het die tweede deel van die studie gepoog om die vlakke van vlugtige fenolglikosiede te verminder deur gebruik te maak van  $\beta$ -glukosidases

voor die toepassing van die behandeling om vry vlugtige verbindings te verwyder. Die behandelings wat ná ensiemhidrolise gebruik is, was geaktiveerde steenkool, polimeerpoeier, gisdoppe en mannoproteïene. Chemies het GC-MS getoon dat daar skerp toenames in VF's was ná die byvoeging van ensieme, en 'n mate van sukses in die gevolglike verwydering van vry vlugtige verbindings. Meer werk word benodig om die optimum vlakke van die behandeling te bepaal. Die data in hierdie studie het die potensiaal getoon vir  $\beta$ -glukosidases om in die wynbereidingsproses gebruik te word, nie net om VF's vry te stel nie (vir latere verwydering) maar ook om die uitdrukking van vrugtige aromas in die wyn te verhoog. Ensieme kan help om ander verbindings vry te laat wat 'n bydrae kan maak tot wyngneur en wat kan help om 'n mate van die rooksmaak te verbloem.

Hierdie studie dra by tot 'n verbeterde begrip van metodes wat gebruik kan word vir die verwydering of behandeling van rooksmaak, maar 'n behoefte aan verdere werk is uitgelig. Die gebruik van  $\beta$ -glukosidases gevolg deur veelvoudige brei is 'n moontlike opsie vir produsente nadat daar 'n brand naby 'n wingerd was terwyl die druiwe besig was om ryp te word.

## Isifinyezo

Kuleminyaka eyedlule, izindawo lapho kutshalwa khona izithelo zamagilebhisi zivelelwa izinhlekelele zemililo eduze nazo futhi kubikwa ukuthi zisazoqhubeka lezi zigameko. Lokhu kuchaphazela iwayini elivutshwe ngalamagilebhisi ngoba liba nephunga elingemnandi lentuthu (smoke-taint). Izindawo ezihaqwa ilezi zigameko, yilezo ezitholakakala ezindaweni ezinesimo sezulu esishisa kakhulu futhi okunganethi ehlobo okubalelwa kuzo i-Australia, i-Melika, i-Spain, i-Ningizimu Afrika, kanye namazwe asezwenikazi i-Ningizimu Amelika. Yonke imililo ikhiqhiza imvubela yamakhemikhali ahamba ngomoya. Yilapho ke, uma ukuthi lezi zigameko zenzeke eduze namasimu amagilebhisi iphunga lentuthu liye litholakale ewayinini. Leli phunga lentuthu lingudaba olubucayi, ngoba kukhahlamezeka nezomnotho kanye naso isiphuzo sewayini ngoba sigcina sesinuka kabi bese singaphuzeki. Ongoti nochwepheshe sebhola bathola ukuthi amakhemikhali abandakanyekayo i-guaiacol, ne-4-ethylguaiacol, Kanye ne-4-methylguaiacol, baphinde bathola ukuthi ama-cresols, ne-eugenol, kanye nemikhiqizo yama-phenol kuyimbangela ekunukeni kwentuthu.

Izingcubabuchopho kulomkhakha seziqhamuke nezindlela zokugwema leli phunga okubalwa kuzo uguhlamba amagilebhisi, ukuwavuna ngezandla, ukuwagcina isikhathi esifushane exubene namakhasi, ukukhetha imvubelo efanele, kanye nokukhangisa ukuze asheshe athengwe amawayini. Lezi zindlela ziyasebenza kepha ziphelela endleleni uma sekufikwa kwenye inkiyankiya lapho amakhemikhali entuthu ekwisibopho nezinhlobonhlobo zikashukela (glycoconjugates). Lesi sibopho singandisa amakhemikhali entuthu uma singahlukaniswa i-esidi kanye nama-enzyme. Izitshalo zikhiqhiza lezibopho ngoba zizivikela ekuhaqweni amakhemikhali entuthu yomlilo.

Ucwaningo lwethu lwandisa kulwazi oselukhona ngezindlela zokukhuculula amakhemikhali entuthu kwayini kusetshenziswa izinongo nemikhiqizo okusemthethweni nokutholakala simahla. Siye savuna amagilebhisi sase siwathuntelanisa ngentuthu yomlilo owakhiwe nge- *fynbos*, (okuyizitshalo semvelo eKapa) sase sakha iwayini ngawo. Imikhiqizo yokuhlaza iwayini esiyisebenzisile kwisigaba sokuqala ngamalahle ahluziwe, uketshezi lwesihlahla se-oak, kanye nemvuthu kapulasitiki. Sisebenzise i-GC-MS ukuhlola amakhemikhali entuthu atholakale emvubelweni, lapho sithole khona ukuthi amalahlale alehlisile izinga lamakhemikhali entuthu. Siphinde saba nethimba labaqeqeshelwe ukunambitha baphinde banuke ukudla. Bona bathole ukuthi uketshezi lwenyusa izinga lokunuka kwezinkuni ewayinini. Bathe bangalizwa emlonyeni leli wayini bathola ukuthi linambitheka okomlotha kanye nentuthu. Isigaba sesibili besibhekene ngqo neziphopho zamakhemikhali entuthu kushukela. Lapho sisebenzise amalahlale ahluziwe, impuphu kapilasitiki, izigujana zemvubelo kanye nama-mannoprotheni. Emuva kokuhlaza iwayini ngalemikhiqizo, kutholakale ukwehla kwamakhemikhali entuthu amazinga ehluke. Ngaphambi kokukhuculula kufakwe ama-enzyme, okunguwo abahlukanisi bezibopho. Imiphumela iveze ukuthi

ama-enzyme ayalenyusa izinga lamakhemikhali entuthu, okuyinto enhle. Kusho ukuthi iziphopho ziyencipha. Ama-enzyme abe nomthelela omuhle wokunyusa izinga lokunuka kamnandi kweyayini, lokho kuchaza ukuthi angahlukanisa izibopho ngaphambi kokuhlanzwa kweyayini aphinde anyuse izinga lokunambitheka kwalo.

Lolu cwaningo lwengeza kulwazi oselukhona ngezindlela zokuhlaza iwayini emuva kokungcoliseka ngamakhemikhali entuthu. Ukusetshenziswa kwama-enzyme kunganomthelela omuhle kwiwayini elikhahlamizekile ukuze lithengiseke lisesemnandi.



This thesis is dedicated to my family for their love, support, and encouragement to pursue my dreams and to Marianne McKay who became a source of strength to journey forward when hope was lost.

## **Biographical sketch**

Nongcebo Portia Langa was born on 09 November 1993, in Pietermaritzburg. She matriculated from Pietermaritzburg Girls' High School in 2011 and in 2012 she began studying at Stellenbosch University. Born and raised in KwaZulu Natal, which is sugarcane country, wine was a foreign concept to her until it became a passion. She completed her undergraduate degree in Viticulture and Oenology in 2014 and obtained a Wine Marketing certificate in 2017, Nongcebo commenced her MSc of Agriculture in Oenology at Stellenbosch University.

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## Preface

This thesis is presented as a compilation of five chapters. Each chapter is introduced separately and is written according to the style of the South African Journal of Oenology and Viticulture.

**Chapter 1** Introduction and project aims

**Chapter 2** Literature review

**Chapter 3** Research results

Amelioration of smoke taint in red wine using permissible fining treatments

**Chapter 4** Research results

The effect of post-fermentation enzyme treatments and fining on amelioration of smoke taint

**Chapter 5** Discussion and conclusions

**Appendices** **Appendix A:** Table of sensory training standards

**Appendix B:** 2017 DA tasting sheet

**Appendix C:** Tasting sheets for 2018 rapid method

**Appendix D:** Volatile phenols results in 2017 (Y1)

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# Chapter 1: General Introduction and Project aims

## 1.1 General Introduction

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In recent years, grape growing areas have seen an increase in veld fires which have resulted in smoke taint in wines produced from these regions. Areas affected by veldfires in the last decade have Mediterranean climates (Kelly *et al.* 2012) with hot, dry summers, and include Australia, the United States, Spain, South Africa, and South American countries. In 2003, the first serious economic impact due to smoke-taint was recorded in Australia (Høj *et al.* 2003). Australian researchers have since been the pioneers in this regard and have made noticeable strides in acquiring knowledge in this field.

The Western Cape is the main grape growing and wine making region in South Africa. The majority of devastating fires have been in 2015, 2014, and 2016 according to Global Fire Watch (<https://fires.globalforestwatch.org>) data of reported fire over the years. Strydom *et al.* (2016) found that for the period of 2003 to 2013, mountain fynbos was responsible for 9.26% of fires recorded in South Africa and the Western Cape experienced the highest frequencies of recorded fires from January to April. The losses due to veldfires in proximity to vineyards have badly affected wine producers, which is why methods of ameliorating the issue have been under investigation.

The exposure of grapes to veldfire smoke results in flavours and aromas that are unpleasant in wine, collectively called 'smoke taint' (Kennison *et al.* 2007). Smoke produces volatile phenols (VPs) which are associated with different aromas and tastes in wine (Høj *et al.* 2003). Different sources of fires will result in varied combinations of produced volatile phenols that are associated with smoke taint (Kelly *et al.* 2012). The volatile phenols enter the grapes through three pathways namely; the berries, leaves and roots (Ugrekheldze *et al.* 1997). The berries then metabolise these volatile phenols in order to reduce the toxicity of the volatiles by chemically bonding them to sugars and storing them in the berries, making them less soluble in water (Korte *et al.* 2000; Kennison *et al.* 2008). The compounds formed are glycoconjugates (Kennison *et al.* 2008) and will remain in the grapes and the grape juice until external influences such as acidity, enzymes, bacteria and yeasts start interacting with them.

Smoke taint is associated with certain flavours which are pungent and unpleasant. These include 'smoky', 'earthy', 'leathery', 'smoked meats', 'tarry', and 'rubbery' aromas which are accompanied by 'ashy', 'smoky', and 'green' flavours (Høj *et al.* 2003; Kennison *et al.* 2007; 2009; Whiting & Krstic 2007; Hayasaka *et al.* 2010; 2013; Parker *et al.* 2012). These flavours have been linked to volatile phenols (Kennison *et al.* 2007) and thresholds have been determined. The chemical compounds that have been used as markers for smoke taint are guaiacol 4-methylguaiacol and 4-ethylphenol (Kennison *et al.* 2007).

Little has been published on the use of a wide range of commercially available products on the removal of smoke taint on both aroma and taste of wine. The available research mainly focuses on

the removal of one to six compounds (Kennison *et al.* 2007; 2009; Parker *et al.* 2012; 2013). The issue of the release of VPs from glycoconjugates into the wine over time has not received a lot of attention, although some research has been carried out on bottle-aged wines (Singh *et al.* 2011, Hayasaka *et al.* 2013). A better understanding of the effects of amelioration methods, and management of VPs and glycosides could benefit the wine industry and help produce wines of better quality after fire and smoke incidents.

## 1.2 Project aims

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The aim of this study was mainly to investigate the use of legally permissible and commercially available products in South Africa on the removal of smoke taint in wines that were affected by smoke. A further aim was to attempt to reduce volatile phenols in finished wine by treating smoke-tainted wine with  $\beta$ -glucosidase enzymes to release VPs, followed by fining and bottling.

The specific aims and their objectives were as follows:

### **1.To investigate the use of permissible additives for reduction of smoke taint**

- (i) To deliberately smoke grapes after harvest with the aim of producing smoke tainted wine for treatment purposes,
- (ii) To investigate the use of activated charcoal, polymer powder, and an oak extract and to determine the effective dosage levels of each treatment on the removal of VPs,
- (iii) To analyse and quantify the selected aroma compounds in the different wines using gas-chromatography, and
- (iv) To test the effect of treatments on sensory attributes and selected chemical compounds.

### **2.To investigate the use of $\beta$ -glucosidase enzymes in reduction of smoke taint**

- (i) To produce wine made from smoke-affected grapes,
- (ii) To investigate the use of  $\beta$ -glucosidase enzymes to release VPs,
- (iii) To test the efficacy of activated charcoal, polymer powder, yeast hulls, and mannoproteins on the removal of these VPs,
- (iv) To analyse and quantify the selected VPs in the different wines using gas-chromatography, and
- (v) To investigate the effect of treatments on sensory attributes.



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## Chapter 2: Literature Review

### 2.1 Introduction

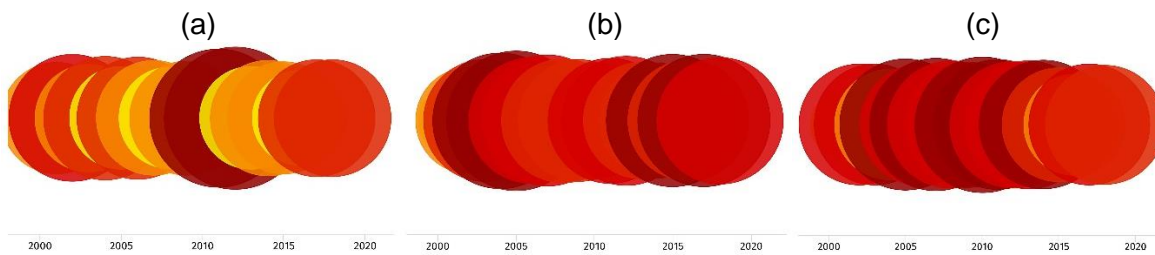
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Over the ages, fire has become an integral part of human existence, providing heat for warmth and cooking, and protection. Cooking food has led to an improvement in the safety of the human diet which in turn led to an increase in brain size of humans. Fires also provide light at night, and heat which allowed our ancestors to ward off predators, as well as means for earlier humans to be able to inhabit harsher environments. It has also been theorised that fire had a significant role to play in social and behavioural of humans by encouraging social circles and gatherings around the fires (Gowlett 2010). Fire is undoubtedly an irreplaceable resource for human survival (Gowlett 2016), but studies concerning its history and impact are surprisingly scarce.

Fire can also have devastating effects on society, environment, and economy when it spreads rapidly in uncontrolled manner (Strydom *et al.* 2016). Human and animal lives, and entire ecosystems can be lost. According SA fire loss statistics 2014 (<http://www.fpsa.co.za/journals/sa-national-fire-statistics>), over 800 human lives were lost due to fire events and the damage sustained amounted close to R2 billion in the year 2014. Strydom *et al.* (2016) suggested that there are two possible scenarios for fire formation due to climate change. The first scenario is that due to a warming climate, air temperatures will rise, heat waves and drought become more severe, plant material in the environment will dry at higher rates, leading to drier fuels for fires therefore an increase in fire occurrences (Strydom *et al.* 2016). Scenario two outlined by the authors is one in which the warming climate results in an increase in rainfall which will increase vegetation growth, leading to heavier fuel loads available which will increase fires and rates of speed from which they spread (Strydom *et al.* 2016). Both scenarios indicate that fires will become more devastating over time.

The Western Cape is the main grape growing and wine making region in South Africa. The majority of devastating fires have been in 2015, 2014, and 2016. Strydom *et al.* (2016) found that for the period of 2003 to 2013, mountain fynbos was responsible for 9.26% of fires recorded in South Africa and the Western Cape experienced the highest frequencies of recorded fires from January to April, which is the pre-harvest / harvest period for grapes in the Western Cape. In recent years, grape growing areas which are mostly found in Mediterranean climate (Kelly *at al.* 2012) have seen an increase in bushfires which have resulted in the taint in grapes and wine produced. The wine making regions that have been increasingly affected over the years are Australia (Figure 2.1a), America (Figure 2.1b), Spain, South Africa (Figure 2.1c), and South American countries (not shown). The year 2003 in Australia had the first noticeable loss of income as a result of smoke/fire impact on vineyards which was recorded, and thus spurred research into smoke taint. Australia have taken the lead in this regard and have made noticeable strides in acquiring knowledge in this

field. The losses recorded have negatively impacted producers (Høj *et al.* 2003), which is why methods of ameliorating the issue have been under investigation.



**Figure 2.1:** Illustration of recorded fires over the years from 2011 to 2018 in wine producing areas of (a) Australia, (b) USA, and (c) South Africa (Global Fire Watch 2018)

### 2.1.1 Chemical compounds associated with smoke taint

“Taints are unpleasant odours or tastes resulting from contamination of a food by some foreign chemical with which it accidentally comes into contact.” (Baigrie 2003). Smoke taint is then the amounting flavours that are unpleasant in wine due to the exposure of grapes to bushfire smoke. Smoke taint is a well-known issue that has been explored by numerous authors in reviews over the years (Krstic *et al.* 2015). Therefore, this review will not be comprehensive on smoke taint but will be limited to issues that pertaining to the removal of smoke taint in wine as well as the sensory effects of volatile compounds.

Smoke and ash result from the combustion of flammable material, and in the specific case of smoke taint, from the burning of vegetation near vineyards. Smoke - contains volatile phenols which are produced through the pyrolysis of lignin, and are associated with particular aromas and tastes in wine (smoke taint). Different sources of smoke (for example, different types of burning vegetation) will result in varied combinations of volatile phenols. Moreover, there are variables that have been identified as having an effect on the pyrolysis of lignin; these include the composition lignin, age of vegetation, state of decay, temperature, and oxygen availability (Kelly *et al.* 2012).

Smoke taint is associated with certain flavours which are pungent and unpleasant. These include wine descriptors such as ‘smoky’, ‘earthy’, ‘leathery’, ‘smoked meats’, ‘tarry’, and ‘rubbery’ aromas which are accompanied by ‘ashy’, ‘smoky’, and ‘green’ flavours on the palate (Høj *et al.* 2003; Kennison *et al.* 2007; 2009; Whiting & Krstic 2007; Hayasaka *et al.* 2010a; 2013; Parker *et al.* 2012). These flavours have been linked to their chemical counterparts (Kennison *et al.* 2007) and thresholds have been determined (Table 2.1) and their glycoconjugates. The chemical compounds that have been mainly associated with smoke taint are guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol and eugenol (Kennison *et al.* 2007). The volatiles are usually quantified through GC-MS analysis (Wilkinson *et al.* 2011; Singh *et al.* 2012), and their glycoconjugates by LC-MS (Hayasaka *et al.* 2010a). Sensory analysis usually uses descriptive analysis (DA) because of its repeatability (Martin *et al.* 2000; Lotong *et al.* 2002). Other methods of sensory evaluation such as sorting (Cartier *et al.* 2006) have been investigated against DA and were found to be effective at producing similar results even in untrained panellists.

**Table 2.1:** Volatile phenols and their aroma descriptors (source: De Vries *et al.* 2016)

Compound	Aroma descriptors	Odour Threshold ( $\mu\text{g/l}$ )	Reference
Guaiacol	Smoky, sweet, medicinal	7.5-23	Ferreira <i>et al.</i> 2000 Parker <i>et al.</i> 2012
2,6-Dimethylphenol	Medicinal, phenolic	570	Escudero <i>et al.</i> 2007
4-Methylguaiacol	Ashy, toasted, vanilla-like	65	Kennison <i>et al.</i> 2009
o-cresol	Band-aid, medicinal, smoky	62	Parker <i>et al.</i> 2012
Phenol	Sickeningly sweet, irritating	7100	Parker <i>et al.</i> 2012 Panzeri, 2013
4-Ethylguaiacol	Smoke spicy, toasted	110	Kennison <i>et al.</i> 2009
m-cresol	Dry, tar, medicinal-leathery	20	Parker <i>et al.</i> 2012
p-cresol	Band-aid, phenol-like	64	Parker <i>et al.</i> 2012
2,3-Dimethylphenol	Phenolic	500	Verschueren. 1983
Eugenol	Clove	6	Escudero <i>et al.</i> 2007
4-Ethylphenol	Barnyard, horsey, phenolic	605	Kennison <i>et al.</i> 2009
4-Vinylguaiacol	Clove, curry	40	Parker <i>et al.</i> 2012
3,4-Dimethylphenol	Sick sweet, medicinal	1200	Burdock 2010

The chemical thresholds have also been determined for the compounds associated with smoke taint (Table 2.1). Some of these compounds and aromas can also be linked to other taints like so-called 'brett' (off-odour associated with *Brettanomyces* contamination of wine) (Chatonnet *et al.* 1992; Lisanti *et al.* 2017) and 'greenness' (van Eeden 2009) because of the increase in alcohol (Kennison *et al.* 2007) which has an affect on the perceived 'green' character (Goldner *et al.* 2009). *Brettanomyces* and *Dekkera* yeast activities in wine result in the production of 4-ethylguaiacol and 4-ethylphenol (Chatonnet *et al.* 1990), compounds which are also linked to smoke taint. Guaiacol, 4-methylguaiacol, and eugenol are produced through the pyrolysis of oak lignin during the toasting process, so they are also associated with oak wood maturation (Kennison *et al.* 2008).

### 2.1.2 Transfer to the berries and wine

Volatile phenols enter grapes through three pathways. The first is via diffusion through the berry skin, second is by absorption through the leaves (Krstic *et al.* 2015) and the third route is uptake through the root system from affected groundwater (which is less likely in the Western Cape due to the dry climate in summer). A number of factors have been shown to play a role in uptake of VPs including the duration and intensity of smoke exposure (Kennison *et al.* 2008), thickness of berry skins and the grape varietal (Sheppard *et al.* 2009; Singh *et al.* 2011), although Kelly *et al.* (2014) indicated that cultivar differences did not play a significant role.

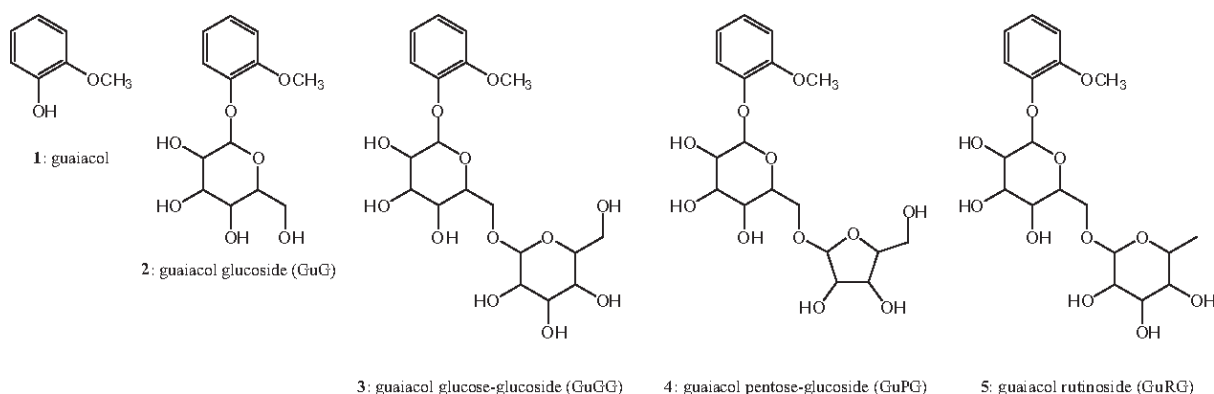
Sheppard *et al.* (2009) found that even short exposure of an hour pre- and post-harvest smoke on grapes could result in perceivable taint during sensory evaluation of wine. Grape berries have been found to be mostly susceptible to smoke uptake seven days post veraison (Kennison *et al.* 2009). This research was further expanded upon by Kennison *et al.* 2011, who investigated the effect of smoke exposure at key phenological stages. The study found that smoke exposure carry-over effects are only limited to physiological responses such as yield and bunch number and VPs are

not transferred to the next generation of grapes. Moreover, if the vines are exposed prior to flowering, then the resulting smoke taint will be low compared to the stages from fruit-set to harvest, as the berries are more likely to take up VPs than leaves or flowers. The reasons cited for this was that the source-sink relationship between leaves and berries, which plays a significant role. In earlier phenological stages there are no berries to store the products of smoke absorbed. After berry set, the increase in berry size causes an increase in the ability of the plant to store VPs absorbed by the leaves (Kennison *et al.* 2011). Also, the berries themselves are directly responsible for some absorption. The berries are able to reduce toxicity of the volatile phenols by making them soluble in water through the addition of a glucose group to the VP.

An earlier study by Kennison *et al.* (2007) concluded that even after harvest, berries were still susceptible to smoke taint as they continued metabolising VPs after harvest. The VPs are then bound to sugars in the berry in a process called glycosylation as it's a detoxification mechanism (Korte *et al.* 2000; Kennison *et al.* 2008). The compounds formed are called 'glycoconjugates' or 'glycosides' and will remain in the grapes and the grape juice until external influences such as acidity, enzymes, bacteria and yeasts start interacting with them (Sarry *et al.* 2004).

### 2.1.3 Glycosides

A number of studies of glycosylation of volatile phenols in grapes have been conducted over the years. Hayasaka *et al.* (2010) found the glycoconjugates formed after the application of liquid guaiacol to vines between guaiacol and glucose, and disaccharides identified as glucose-glucoside, pentose-glucoside and rutinose could be detected in leaves and/or fruits. In another study, the most abundant glycosides found in fruit were glucose-pentose disaccharides, followed by rutinoses (Pardo-Garcia *et al.* 2017) as shown in Figure 2.2.



**Figure 2.2:** Illustration of the most abundant glycoconjugates in wine. (Pardo-Garcia *et al.* 2017)

Glycosylation was shown to occur 10 to 14 days after smoke exposure and the glycosylated products were mostly formed in the skin and pulp (Dungey *et al.* 2011). This was further emphasised by a study done by Pardo-Garcia *et al.* 2017, where after foliar application of guaiacol, elevated levels of glycoconjugates were observed after 10 days of application. These compounds

can also be found in small amounts in wines not made from smoke-exposed grapes (Dungey *et al.* 2011; Ristic *et al.* 2011; Fudge *et al.* 2011).

Glycosides are hydrolysed through acid and enzyme catalysed hydrolysis during and after winemaking through various processes (Kennison *et al.* 2008). Ristic *et al.* (2011) evaluated the extraction of glycoconjugates into wine using different grape processing techniques. Fermenting red to dryness, crushing then destemming white wines, and whole bunch pressing of white wines resulted in 85%, 25%, and 18% extraction of glycoconjugates, respectively.

Yeast and bacterial contribution have been observed in the release of smoke VPs from their bound state (Kennison *et al.* 2008; Dungey *et al.* 2010; Ristic *et al.* 2011). The glycosidase activity has resulted in increases of VPs after fermentation compared to those observed before fermentation (Hayasaka *et al.* 2010). *Saccharomyces cerevisiae* yeast species have been found to exhibit  $\beta$ -glucosidase activity at low levels while non-*Saccharomyces* genera such as *Candida* and *Dekkera* (Sarry *et al.* 2004) have been found to express  $\beta$ -glucosidase activity when cultured on a suitable medium. *Botrytis cinerea* has been found to increase the presence of  $\beta$ -glucosidases in the wine but these are inhibited by a compound (glucono-d-lactone) that is found in mould contaminated juices (Gunata *et al.* 1989). The preparations which are used for pectic and hemicellulose enzymes in juice clarification also contain a high number of  $\beta$ -glucosidases which are isolated from *Aspergillus* spp. (Sarry *et al.* 2004). *Oenococcus oeni* has also been found to present  $\beta$ -glucosidase activity (Boido *et al.* 2002, Grimaldi *et al.* 2000). Some of the  $\beta$ -glucosidases can be inhibited by high levels of glucose while those that have been isolated from wine grapes have shown resistance (Sarry *et al.* 2004). *Lactobacillus plantarum* has been under investigation for its  $\beta$ -glucosidase activity (Sestelo *et al.* 2004), where abiotic stresses were investigated and it was found that pH at 5 and temperature of 45°C were ideal for enzyme activity.

$\beta$ -Glucosidase enzymes are involved in the breakdown of the glycosidic bonds between sugars and volatile phenols and, in winemaking, are mainly used for enhancing aroma (Baffi *et al.* 2013a). Glycosides have been shown to persist in wine during the winemaking process. A study by Kelly *et al.* 2012 found that 72-87% of smoke derived volatile phenols exist in glycoconjugated form at bottling and after 19 months of wine ageing it was found that 70% of VPs remain bound.

The long-term implication of glycoconjugates present in wine at bottling is the re-release of VPs from their bound glycoconjugate form during maturation and bottle-ageing. Acid hydrolysis occurs in the bottle at wine pH over time. In lab conditions, intentional acid hydrolysis was carried out, and it was found that a total of 92% of smoke glycosides had been eliminated with low levels of free VPs being observed as they were said to have decomposed (Hayasaka *et al.* 2010a). A contrasting study showed that low levels in the increase of VPs are observed over a period of 5-6 years and they concluded that the intensity of the perceived smoky aromas is cultivar dependent (Ristic *et al.* 2017).

It has also been found that in-mouth enzymes contribute to the release of the VPs. The sensory effect of glycoconjugates has been assessed and although each panellist's experience of intensity

was different, the in-mouth release occurred in all cases (Parker *et al.* 2012; Mayr *et al.* 2014). This was attributed to the presence of in-mouth bacterial microflora or epithelial cells for being sources of  $\beta$ -glucosidases. Moreover, high glucose levels have been found to hinder the activity of  $\beta$ -glucosidase enzymes in the mouth which is why the potential of smoke taint cannot be achieved by tasting of berries (Hemingway *et al.* 1999; Parker *et al.* 2012; Mayr *et al.* 2014).

## **2.2 Viticultural and oenological amelioration**

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### **2.2.1 Viticultural amelioration**

Studies over the years have determined that smoke is a complex mixture of gases that can have phytotoxic effects on plants (Ristic *et al.* 2016). These gases can cause leaf necrosis and inhibit photosynthetic abilities of the vines through the hindrance of stomatal conductance (Kennison *et al.* 2009; Ristic *et al.* 2016). The total soluble solids and yield were found to decrease in the fruit harvested from smoked exposed vines depending on the number of smoke applications (Kennison *et al.* 2008). However, the negative effects of smoke exposure on the grapevine is also influenced by the grape varietal, type of smoke and the duration of smoke (Ristic *et al.* 2016; Calder *et al.* 2010).

Several techniques of amelioration have been investigated in viticulture. Leaf removal is a practice performed during the growing season to control canopy density and regulate bunch exposure (Ristic *et al.* 2013). The effect of leaf removal pre- and post- smoke application was investigated (Kelly *et al.* 2012), and results showed that smoking without leaf removal yielded similar VPs in wines made from exposed grapes, as post-smoke leaf removal. However, leaf removal before the smoke event yielded the highest concentration of VP in the wine. The same study found that glycoconjugate levels in the wines were similar for all three treatments. Defoliation pre-smoking produced wines with intense smoky, ashy, burnt rubber and bitterness attributes, leaf removal post-smoking application reduced the intensity of cold ash and ashy aftertaste without affecting the expression of fruit aroma and flavour.

The influence of fruit maturity was also evaluated by Kennison *et al.* (2011) and Ristic *et al.* (2015). It was shown that harvesting between 16-20 and 22-25°Brix did yield differences, but these differences were between cultivars. Certain cultivars (Sauvignon Blanc and Chardonnay) may exhibit high levels of smoke associated characteristics after early harvest and another (Merlot and Shiraz) may not. This was observed in both red and white cultivars (Ristic *et al.* 2015). Harvesting later in the season was also shown to increase fruit expression in some cultivars such as Chardonnay, which may have had a masking effect on smoke aromas. In contrast, Shiraz was shown to exhibit smoke taint aromas irrespective of ripeness stage (Ristic *et al.* 2015).

### **2.2.2 Oenological/winemaking interventions**

Previous research has looked at different oenological and winemaking solutions to try and eliminate volatile phenols associated with smoke taint. The following techniques and practices

have been evaluated and some conclusions were made but these methods still fall short at solving the whole problem. Hand harvesting (Whiting & Krstic, 2007) has been recommended for its gentler approach in handling of bunches. By limiting skin breaks which allow for the release of juice, skin contact with grape juice is limited therefore extraction is slowed down. The exclusion of leaf material (Whiting & Krstic, 2007; Simos 2008) was shown to prevent the extraction of VPs from leaves into the wine/juice. Washing grapes (Høj *et al.* 2003) helps with removal of ash from the surface but VPs would have been absorbed at that stage. Keeping fruits cool after harvesting (Whiting & Krstic 2007; Simos 2008) and processing at  $\leq 10^{\circ}\text{C}$  provided less extraction of VPs from the skin. Whole bunch press (Simos 2008; Ulrich 2009) was more effective in reducing extraction of VPs in white wines as less skin contact is needed post-fermentation compared to red wines. Minimising skin contact (Kennison *et al.* 2008; Simos 2008; Ristic *et al.* 2011) at any point of the wine making process allowed for decreased extraction from the skin of a high number of VPs. Yeast selection (Ristic *et al.* 2011) was found to affect smoke related aromas, flavours and chemistry of wine. Masking of smoke aromas was investigated with addition of oak and tannins, this increased the complexity of the wine (Fudge *et al.* 2011). Reverse osmosis (Fudge *et al.* 2011) was found to remove VPs but other wine components were also removed. It was further found that smoke taint may return through hydrolysis if treated using reverse osmosis as glycoconjugates still remain. Because of the glycosylation of VPs, marketing for early release was suggested (Simos 2008; Ulrich 2009; Fudge *et al.* 2011; Singh *et al.* 2011) which makes sense for white wines and wines with minimal skin contact but would prove to be a less effective strategy on red wines which are fermented on skins.

### **2.3 New research products in experimental phase**

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Chemistry has been spear-heading research into developing products that may be suitable for the removal of VPs via adsorption. A filter membrane called “Molecular Imprinted Polymer” (MIPs) has been developed that be engineered to have sites that are molecular specific for binding thus the extraction of the targeted molecules from wine (Teixeira *et al.* 2015). A study sought to design such a membrane to extract VPs from wine, and it was very effective with 50-60% reduction rate of VPs. However, the study also showed that other non-volatile phenols were removed significantly by this treatment (Teixeira *et al.* 2015).

A cork extract suberin was researched by Gallardo-Chacon *et al.* (2015), for its ability to remove 4-ethylphenol and 4-ethylguaicol. The results showed a decrease of the compounds by 45- 71% when treated with suberin, the wide variation was attributed to different wine matrices. Suberin is a water insoluble biopolymer that serves as protection for plants against environmental damage and it represents approximately 37% w/w of 3g cork sample (Gallardo-Chacon *et al.* 2015).

Some work has shown that phenolic compounds, sulphur products and aroma compounds can be adsorbed by yeast lees (Chassagne *et al.* 2005; Mazauric *et al.* 2005) which suggest that this substance can be used to remove undesirable flavours. Other studies (Chassagne *et al.* 2005;



Pérez-Serradilla & Castro 2008; Pradelles *et al.* 2008) worked on the capability of *Saccharomyces cerevisiae* cells on the sorption of 4-ethylphenol and they showed that adsorption was greatly influenced by yeast strain, medium and mode of culture, and yeast cell wall nature and composition. So, with use of the lees drying process in three different ways, it was found that between 61.5% and 192% sorption was achieved in the adsorption of 4-EP (Pradelles *et al.* 2009). The potential of  $\beta$ -glucosidases (1,4- $\beta$ -D-glucoside glucohydrolases, EC 3.2.1.21) in wine has been explored as an enhancer of wine aroma through the hydrolysis of glucoside precursors, especially terpene release (Sarry *et al.* 2004 ; Baffi *et al.* 2012). In wines affected by smoke, this application means the release of bound VPs (Parker *et al.* 2012).

Mannoproteins have been studied and it was determined that they interact with volatile aromas (Lubbers *et al.* 1993). Mannoproteins are released during yeast autolysis or at fermentation and can interact with phenolic compounds, improving colour stability and decreasing astringency (Chatonnet *et al.* 1991; Pérez-Serradilla *et al.* 2008). Vidal *et al.* (2003) estimated that mannoproteins make up 35% of the total polysaccharides in red wines.

## 2.4 Conclusion

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A lot of research has gone into understanding smoke taint and its effects on wine. Through the investigation of volatile phenol compounds responsible, to identifying microorganisms that play a role and the understanding of the chemical interactions, the understanding of the issue is becoming ever so clearer, but more work is still needed. Studies still need to evaluate and/or develop potential products to eliminate volatile phenols completely, both in their bound and free forms as smoke taint can persist even after treatment. In the context of the South African wine industry, a study that focuses on the removal of smoke taint in wine using products that are available locally and are legal in the wine legislature has not been done. Studies on amelioration have only been done on Pinot noir, Merlot, and Cabernet Sauvignon (Fudge *et al.* 2011; 2012) cultivars, therefore more research is needed for other cultivars that are grown abundantly in South Africa like Chenin blanc and Pinotage. The effect of fining using the experimental products developed in new research have not yet been quantified in a wine matrix whether it be natural or synthetic. Linking sensory flavours to specific glycoconjugates is research that still needs to be done so that strategies specific to the removal of those glycosides can be devised.

## 2.5 References

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## Chapter 3: Amelioration of smoke taint in red wine using permissible fining treatments

### 3.1 Introduction

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Smoke taint leads to flavours of 'smoky, burnt', 'burnt rubber', 'ashtray', 'cold ash', 'smoked meats', 'smoked foods', 'leather', 'disinfectant/hospital', 'medicinal', 'earthy' aromas (Høj *et al.* 2003, Kennison *et al.* 2007; 2009; Whiting & Krstic 2007; Hayasaka *et al.* 2010; 2013; Parker *et al.* 2012) with "an excessively drying back-palate and retronasal ash character" (Hayasaka *et al.*, 2013) that are unpleasant in wine due to the exposure of grapes to bushfire smoke (Kennison *et al.* 2007). Smoke produces volatile phenols which are associated with different aromas and tastes in wine (Parker *et al.* 2012). The berries then metabolise these volatile phenols for reduction of the volatiles' toxicity by making them soluble in water (Korte *et al.* 2000).

Smoke taint is associated with certain flavours which are pungent and unpleasant. These flavours have been linked to chemical counterparts and odour detection thresholds have been determined in various matrices as listed in Chapter 2 Table 2.1. The chemical compounds that are associated with smoke taint are guaiacol, 4-methylguaiacol, and 4-ethylphenol (Kennison *et al.* 2007). These are usually quantified through the use of GC-MS methods of analyses (Wilkinson *et al.* 2011; Singh *et al.* 2012; De Vries *et al.* 2016). Over time, other volatile compounds have been identified as contributors to the smoke taint; guaiacol, 4-vinylguaiacol, phenol, o-cresol, m-cresol, p-cresol, and 4-methylsyringol, (Parker *et al.* 2012,2013; De Vries *et al.* 2016);

Studies into the effects of amelioration on smoke-taint during winemaking are very limited and seem to give some contradictory results. The earliest study to investigate amelioration of smoke taint evaluated techniques such cold maceration, fermentation on skins, fermentation with different yeast strains and the addition of oak chips and tannins (Ristic *et al.* 2011). The logic applied to using cold maceration for smoke-exposed grapes was that the typical process decreases the extraction of aromatic and phenolic compounds compared to normal on-skin fermentation, so the same should apply with smoke related VPs. The overall phenolic concentration was indeed reduced, but wines made from the smoked grapes displayed increased brown hue. This was enhanced by some yeast strains which, according to Ristic and co-workers (2011), were unable to produce secondary alcoholic fermentation metabolites which are present in the formation of anthocyanin pigments.

Yeast strain selection was found to have effects on VA production, titratable acidity, and extraction of wine phenolics such as anthocyanins (Ristic *et al.* 2011). Yeast strains were also found to affect the  $\beta$ -glucosidase activity by increasing guaiacol concentrations. The yeast strains that were selected in the study conducted by Ristic and co-workers (2011) showed little to no  $\beta$ -glucosidase activity. This study showed that yeast strain selection is important in the winemaking process if  $\beta$ -glucosidase activity is what is sought after.

Oak chips and tannin added during the winemaking process can significantly reduce the perception smoke-related sensory attributes (Ristic *et al.* 2011). This was found to be because of the masking effect that toasting of oak has on the wine by contributing flavours such as vanillin, acetovanillone, and syringaldehyde (Ristic *et al.* 2011; Kelly *et al.* 2015). It was also found that toasted oak chips increased the perceived fruit aroma compared to the control, which is unexpected. Oak aging and maturation are well-known to increase concentrations of guaiacol and 4-methylguaiacol (Ristic *et al.* 2011).

The use of reverse osmosis and solid phase adsorption by Fudge *et al.* (2011) was investigated as a potential solution to the removal of volatiles associated with smoke taint. Reverse osmosis is a filtration process across a semi-permeable membrane against a concentration membrane (Paulsen *et al.* 1985). In the wine industry, reverse osmosis is frequently used to change alcoholic content, VA, and acidity, although there is little formal research on these applications. Reverse osmosis has been shown to remove 4-EG and 4-EP associated with wines affected by *Brettanomyces* when used in conjunction with solid phase adsorption (Ugarte *et al.* 2005), reducing VPs by more than 67% after a three-hour treatment. This method also removed some desirable wine aroma as it did not discriminate between compounds selected for removal and those that contribute to the wine positively. Sensory studies have found significant differences in the removal of smoke taint related flavours, but also that smoke taint can gradually increase over time in the wines because of hydrolysis of glycoconjugates (Kennison *et al.* 2008).

Commercial fining agents have also been investigated to determine their efficacy in treating smoke taint in wine. Previous studies have used some of the agents in the removal of volatile phenols associated with 'brettiness' (Lisanti *et al.* 2008) and greenness (Pickering *et al.* 2006) successfully. A study by Fudge *et al.* (2012) showed that fining agents (Table 3.1, number 1-7) were least effective against smoke volatile phenols due to their affinity to other phenols in wine. It was observed that there were losses of colour and flavour. Activated carbon was found to be the most effective as it removed 58-71% of VPs and enhancing the expression of fruity characteristics after application, but this treatment is generally considered a 'last resort' as it is well-known to affect colour, aroma and enhance oxidation (Zoecklein 1990). Activated carbon has the ability to adsorb compounds of low polarity, so depending on the type of charcoal used and dosage, aroma and colour losses can be observed (Lopez *et al.* 2001).

**Table 2.1:** Fining agents used in previous studies percentage removal of volatile phenols (adapted from Fudge *et al.* 2012)

	<b>Fining agent</b>	<b>Removed VPs</b>	<b>Amount removed</b>
1	egg albumin		
2	potassium caseinate		
3	isinglass		
4	bentonite		
5	PVPP		
6	gelatine		
7	yeast cell walls		
8	Silica sol/activated carbon	VPs	3-14%
9	Synthetic mineral	Syringol 4-MG Guaiacol and cresols	58% 29% 13%
10	Activated carbon	VPs	58-71%

Yeast hulls have been investigated for the removal of 4-ethylphenol by Pradelles *et al.* (2009). These authors found that 61.5% to 192% removal was found, depending on the drying process of the yeast cells and yeast strains. The increase in surface area of the yeast cells from the damage sustained through the drying processes resulted in the greatest removal of 4-ethylphenol.

Polyethylene terephthalate (PET) is food grade plastic that is used mainly for packaging a variety of foods. Many studies have looked at the impact PET has on aroma profiles of wines and it has been found that the differences in manufacturing of PET like incorporating oxygen scavengers (Dombre *et al.* 2014) can have minimal effect on wine aromas. Moreover, in the process to reduce the environmental footprint of using plastic, recycled PET is preferred. However, it has been found that compounds trapped in the plastic matrix can desorb into wine therefore affecting the wine aroma packaged using the PET (Dombre *et al.* 2014).

The glycoconjugated forms were not shown to be affected by fining in any of the studies. However, in research conducted by Lisanti *et al.* (2017), PVPP and deodorant activated charcoal resulted in a significant decrease in 4-ethylphenol and 4-ethylguaiacol of 11 to 18% in naturally contaminated wines and the 'fruity' and 'berry' aromas were increased with the use of these treatments, probably as a result of the removal of the masking effect. Strategies to remove all free volatile phenols from the wine before the wine is released for sale will ignore the pool of potential smoke-taint precursors, and by the time the wine is opened and consumed, a significant level of free volatile phenols may have built up in the bottle (Singh *et al.* 2011).

In order to prevent this from happening, it is necessary to have wine-making strategies that can deal with both free volatiles and glycosides. The treatments used were activated charcoal, oak extract and polymer powder. In literature, they have been proven to remove VPs at varying degrees as well as preferred VPs for removal (see Chapter 2). In South African wine legislation, the use of tannin (if not 'foreign to wine') and charcoal is permitted while the use of polyethylene

terephthylene (PET) is only approved for use in bottling wine as containers (SAWIS, Liquor products act 60 of 1989).

### 3.2 Aims of the project:

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The aims of this aspect of the project were:

- A. To test the efficacy of three legal additives on deliberately smoke-tainted wines for removal of volatile phenols and smoke taint
  - i) To test efficacy of additives at two different levels (one level recommended by the manufacturer and the second level will double the initial dosage) on VP removal and/or reduction.
  - ii) To chemically and sensorially analyse treated and untreated wines for success in reduction of taint in comparison with controls.
- B. To investigate the potential for hydrolysis as a strategy for removing glycosides.
  - i) To establish the potential for smoke-affected wines to manifest a taint after slow acid hydrolysis of precursors during bottle-aging.
  - ii) To carry out a complete enzyme hydrolysis and monitor VPs before and afterwards in order to determine the concentration of glycolysated precursors, and the potential for smoke-taint development.
- C. To investigate the effects of amelioration treatments over time, the project wines made in Year 1 (Y1) will be retested in Year 2 (Y2) for volatile phenols and effect on attributes.

### 3.3 Materials and Methods

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The project was carried out during the 2017 (Y1) and 2018 (Y2) seasons. The grapes were harvested for both seasons from Welgevallen experimental farm 157 m above sea level (-33.939847, 18.865590). The block has a North-South direction on a horizontal surface. *Vitis vinifera* L. cv. Shiraz cultivar grapes were used, clone SH9C which was grafted onto 101-14 Mgt (*Vitis riparia* x *Vitis rupestris*). The vines were planted in the year 2000 with a 2.7 m by 1.5 m spacing. Trellising is a seven-wire vertical shoot positioning. The vines were irrigated with a pressure compensated drip system.

Sampling was done weekly and grape parameters were measured. These are pH, TA and balling to gauge the ripening process and help in determining the harvest date.

#### 3.3.1 Smoke treatment and winemaking

The grapes were hand-harvested when the sugar levels had reached 23-24 ° Balling. The grapes were separated into food grade plastic containers for smoking, and individual crates weighed. The containers (Addis, South Africa) were clear plastic and could be closed and sealed, with volumes ranging from 10 L to 40 L.





**Figure 3.1:** Smoking of grapes after harvest in plastic crates

In order to generate smoke, a commercial bee-keeping smoker was filled with a mixture of dried pine-needles and *fynbos* which was then set alight. The species used were chosen to mimic the bush fires in proximity to Western Cape (De Vries *et al.* 2016) vineyards, and included dried leaves, flowers, stems and twigs of pine trees (*Pinus radiata*), blombos (*Metasia muricata*), Pincushion (*Leucospermum codiofolium*), Erica (*Erica verticillata*), Restio (*Thamnochoryus insignis*), and Imphepho (*Helichrysum petiolare*). On the day of harvest the containers that had been selected for the smoke treatment had smoke applied for 30 seconds each until the air inside the containers were opaque (Figure 3.1). The smoke applications were done twice on the day of harvest within 2 hours after the completion of harvesting and twice 24 hours later. This was to ensure that the smoke taint was pronounced enough for detection by the trained panellists. Kennison *et al.*, (2009) found that multiple applications of smoke yielded cumulative effect that could be detected by panellists. In between smoke events, containers were stored in a laboratory at 18°C.

Grape processing was carried out at the Stellenbosch University experimental cellar, following standard winemaking protocols. Prior to processing, grapes were moved from the laboratory to the 4°C cold room, where they were stored for three days. In order to ensure homogeneity of smoke treatment, grapes bunches from all the containers that had been smoked were carefully divided between the containers before crushing and destemming. The crushing and destemming happened using CDS Vintec® (Paarl, South Africa) crusher.

SO<sub>2</sub> was added at crushing and destemming. Fermentation was carried out in 20L food-grade plastic (Polypropylene) buckets for the smoked treatments and 60L bucket for the unsmoked control. An extraction enzyme (Laffort® Lafase® HE Grand Cru, Bordeaux Cedex, France) was added to all buckets (smoked and unsmoked) at a dose rate of 4g/100kg in order to increase juice yield. The yeast used was Lallemand (Montreal, Canada) Lavlin QA23® 30 g/hL with the aid of Anchor® Fermaid K® at 40 g/hL yeast nutrient at inoculation. The buckets were stored at 25°C to ferment.

Three days after the initial yeast inoculation the wines were inoculated with Anchor® co-inoculant® at 1 g/hL to start malolactic fermentation (MLF). The MLF process was monitored once every two weeks by the Chemical Analysis Laboratory (Department of Viticulture & Oenology, Stellenbosch

University). Malic acid concentrations were measured by enzyme robot (Arena 20XT enzyme robot, Institute for Wine Biotechnology, Stellenbosch University). After the completion of MLF at < 0.1 g/L malic acid present, the wines had SO<sub>2</sub> added.

Pressing of the wines commenced six days after yeast inoculation using a pneumatic press (Speidel® hydropress, Ofterdingen, Germany) and up to 2 bars of pressure was applied. The wines had finished alcohol fermentation. The wine was then transferred into 4.5 L Distell® glass containers (Stellenbosch, South Africa) and kept in 20°C room to finish malolactic fermentation.

The wines were racked off the lees one month after yeast inoculation, and SO<sub>2</sub> was added giving the total of SO<sub>2</sub> added as 90 mg/L. Potassium metabisulphite (SO<sub>2</sub>) (Ever® srl, Pramaggiore, Italy) which was diluted to a concentration of 2.5% was used in the winemaking process.

The wines were filtered using Pall Corporation® Filtersystems GmbH Seitz K300® filter sheets, in a Wine Machinery® wall mounted filter. Bottling took place in parallel with each batch filtered. Bottles used were Consol® 750 ml green bottles. The bottles were screw-capped using Guala Closure Group® caps.

The wines were stored in crates in the 15°C room until sensory evaluation and chemical analysis. Samples of grapes, juice and wine were taken throughout the winemaking process to be used for chemical analysis and enzyme hydrolysis.

**Table 3.2:** Summary of treatments applied during winemaking

Sample code	Treatment	Trade name	Year of addition	Stage of application	Dosage
Control	None (unsmoked/clean grapes)	-	-	-	-
C-smoke	None: Smoked grapes	-	-	-	-
T1L1	Liquid tannin (oak extract)- level 1	WLT 150 Oakwood®	2017	Before alcoholic fermentation and before bottling	2 ml/L and 0.5 ml/L
T1L2	Liquid tannin (oak extract)- level 2				5 ml/L and 1 ml/L
T2L1	Activated Charcoal Level 1	Geosorb®	2017	During alcoholic fermentation	25 g/hL
T2L2	Activated Charcoal Level 2				45 g/hL
T3L1	Polymer powder: Level 1	Not registered	2017	Before bottling	3 g/L
T3L2	Polymer powder: Level 2				3 g/L on day 1 3 g/L on day 2

### 3.3.2 Treatment 1: Oak extract

Stoak® Technologies (Diep River, Cape Town, South Africa) “WLT 150 Oakwood” wood extract was added to the wine prior to alcoholic fermentation. The extract is described by the manufacturer as ‘a dark reddish brown with cocoa, vanilla, aged, cognac, woody, aged, spirits sensory aromas’ (Stoak® Technologies WLT 150 blend brochure), and was used for its potential masking effect on smoke taint. The liquefied extract was added directly to the juice and the fermentation occurred

with the tannin in the mixture. This additive is a concentrated hydroalcoholic extract of toasted American oak. The extract was added to wines at two different dosage levels: 2 ml/L ('Level 1') and 5 ml/L ('Level 2') before yeast inoculation. A second addition was made before filtration of 0.5 ml/L ('Level 1') and 1 ml/L ('Level 2'). The first application had three months contact time before filtration and the second application had one month contact time.

### **3.3.3 Treatment 2: Activated charcoal**

The charcoal product used for Treatment 2 was Laffort® Geosorb® (food grade granulated activated carbon). This product is recommended by the manufacturer for removal of smoke and other taints, and for moderation of colour (Geosorb® product data sheet). The treatment was applied, as per the recommended guidelines from Laffort®, one day after yeast inoculation. The dosage was 25 g/hL ('Level 1') and 45 g/hL ('Level 2'). The Geosorb® was rehydrated for 4 hours before being added directly into the fermenting juice.

### **3.3.4 Treatment 3: Polymer powder**

Treatment 3 used a finely ground powder of PET plastic that is recommended for the removal of taint compounds (specifically cork taint) via the mechanism of adsorbency of volatiles on contact. This treatment was applied post alcoholic fermentation, after racking but before bottling, as recommended by the manufacturer. 'Level 1' of the application was 3g/L on day 1. 'Level two' of the application was 3 g/L on day 1 and another dose of 3 g/L 24h later (day 2). The product was sieved off the wines after six hours of contact per application. However, it was seen that small particles remained behind, and the wines therefore had to be filtered.

### **3.3.5 Sensory Evaluation**

The sensory method chosen to be used for the first set of experimental wines was Descriptive Analysis (DA) (Lawless & Heymann 2010) and was conducted one month after bottling and one year after bottling.

The panel comprised of 8 females with ages ranging from 25 to 60 years, who had previous experience on evaluating wines made on smoke taint projects. The assessors were experienced evaluators of sensory products, were of legal drinking age, and were available for tastings at designated times. Panel training was conducted three weeks with three sessions of two hours each week. During each training session eight wines from the experiment were used in duplicate. The aroma standards that were used are listed in appendix A.

During the first year of the study, sensory training was conducted over three weeks with sessions on Monday, Wednesday and Friday. Eight wines were used in duplicates per session making a total of 16 wines. The wines were split into groups of four per two-hour session. This was the maximum value that could be used to limit panel fatigue and saturation (Solomon, 2006). The mouth cleansing regimen was rinsing with pectin solution (Earth products, apple pectin) then with sparkling water (Spar, sparkling spring water) then eating a cracker (Bakers, cream crackers) and

lastly rinsing with still water. The wines were at room temperature of 20°C when poured. 25 mL was poured and covered with petri dishes. A three-digit code was assigned to each glass. The panellists smelled and tasted the wines and came up with descriptors. The descriptors were narrowed down, and aroma standards were prepared. The panellist agreed on the final list of attributes to be used in the tasting's tests (Appendix B).

The chosen attributes to be used in the DA evaluation were narrowed down and grouped together during the training sessions. Appendix B shows the attributes used in the test sessions. The panellists had to rate each attribute on a line scale using Compusense® (Ontario, Canada) programme. Both the aroma and taste attributes were evaluated. The mouth cleansing regime was the same in each session.

The second year of the study, DA was carried out on the 2017 wines in a similar fashion as the previous year. The training of the panel took four days over two weeks for two hours, utilising the same panel members as the previous year as well as two new panel member additions, a female of fifty years of age and a male of approximately thirty years. The shorter period of training was because of the panellists had experience in smoke taint evaluation from the previous intensive sessions of training.

The actual sensory testing was carried over 3 days in a week (Monday, Wednesday, and Friday), with each day evaluating each biological replicate. Only two technical replicates were used on each day, as panel members became easily fatigued from the strong odours and flavours of the smoked wines. The mouth cleansing for the second year was conducted without the use of pectin because of shortages from the suppliers of the product. The cleansing process then included the use of sparkling water, crackers, and then still water. Other alternative mouth cleansers like carrots, whole milk, cucumbers, and mozzarella cheese (Vickers *et al.* 2007; Jaffe *et al.* 2017) would have imparted strong flavours to the mouth, and may have affected evaluation of the smoked wines.

The wines were poured (25 mL) into black International Standardisation Organisation (ISO) sensory evaluation glasses and covered with petri dishes while the content equilibrated to room temperature (20°C). The panellists during the evaluation agreed to the addition of an attribute, caramel, in the second year as it was more pronounced. They evaluated the attributes on a line scale for both aroma and taste as above in the previous year.

### **3.3.6 Chemical analyses**

Grapes samples were collected after smoking, and then frozen before being macerated and prepared for GC-MS analysis. Juice, must, and wine samples were collected throughout the winemaking process. Chemical analyses of volatile phenols were carried out by Central Analysis Facilities (CAF) using the Gas chromatography–mass spectrometry (GC-MS). The samples were taken before and after crushing and destemming, during alcoholic and malolactic fermentations, pressing, bottling and sensory evaluation in the first year of study. In the second year of the study,

the samples were taken before and after crushing and destemming, before enzyme application, and at bottling.

The compounds tested for were: guaiacol, 2,6-dimethylphenol (2.6DMP), 4-methylguaiacol (4MG), o-cresol, phenol, 4-ethylguaiacol (4EG), m-cresol, p-cresol, 2,3-dimethylphenol (2.3DMP), eugenol, 4-ethylphenol (4EP), 4-vinylguaiacol (4VG) and 3,4-dimethylphenol (3.4DMP).

Sample preparation had two methods for solid and liquid contents (grapes, juice and wine). The grapes were homogenised using a hand-held homogeniser. Fifty grapes were used, randomly selected from the crates. Five grams of the homogenate was measured for each analysis. The homogenated samples were transferred to 20 mL GC-MS headspace glass vials (Separations, Randburg, South Africa). A further 5 mL of MilliQ water (ultra-pure distilled water, Millipore, Bedford, MA, USA) was added to each vial and then vortexed (Vortex-Genie® 2; Scientific Industries Inc., NY, USA) for 30 seconds. Subsequently, 2.5 mL of 20% sodium chloride (NaCl) solution (Merck, Germany) was added as well as 100 µL of the phenol internal standard (anisole-d8: methoxybenzene-d8; Sigma, St. Luis, MO, USA) prepared in the CAF facility. The sample was then vortexed and loaded into the GC-MS machine. Stock solutions of pure compounds (all reference standards supplied by Sigma-Aldrich/Merck, KGaA, Darmstadt, Germany), were diluted for calibration purposes, creating an 8-point calibration series from 25 to 1000 µL/L.

Liquid sample preparation (juice or wine) required 10 mL of sample. After transferring the sample into the GC-MS vial, 2.5ml of 20% NaCl and 100 µL of the phenol internal standard of 100 µL/L concentration were added into the same vial and vortexed to be loaded into the GC-MS machine. Analysis of VPs was performed using a Thermo Scientific trace 1300 gas chromatograph (Anatech, coupled to a Thermo Scientific TSQ 8000 Triple Quadrupole Mass (Anatech Instruments (Pty) Ltd, RSA. The MS-detector was set for acquisition in single reaction monitoring (SRM) mode.

Vials were incubated in the auto-sampler for 5 minutes at 50°C, after which a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (Supelco, Bellafonte, PA, USA) was exposed to the headspace of the vial for a further 30 minutes at the same temperature. After exposure, the fibre was injected, and ten minutes were allowed for desorption of compounds of interest. The injector was operated in splitless mode. The total run time of the method was 30.54 minutes. Wines were analyzed by GC-MS according to a modified version of a previously described method (De Vries et al. 2016).

### 3.3.7 Enzymatic Hydrolysis

The method used was taken from a pilot study carried out during the previous year (2015) (unpublished) and adapted from a method developed by Kennison *et al.* (2008).

Model wine was made using the method by Wildenradt and Singleton (1974). A solution containing 12% ethanol was made up in Milli-Q water (ultra-pure distilled water, Millipore, Bedford, MA, USA). Tartaric acid (5 g/L) was added. The mixture was then adjusted to pH 3.5 using NaOH.

Two slightly differing methods were used to prepare berries and juice samples for analysis.

For berries, a sample of random 50 berries were chosen and homogenised, and 5 g of the homogenate was transferred into a test tube. For the purposes of reporting the method, this will be called 'test tube 1'. Five mL of model wine was added to another test tube ('test tube 2'). Both test tubes were put into a heating block at 30°C. Following this, 50 mg  $\beta$ -Glucosidase enzyme (Sigma-Aldrich®, South Africa) was measured into an Eppendorf tube (2 mL, Sigma-Aldrich®, South Africa) and set aside to be added to each test tube of berry sample to be analysed. Separating the grape and enzyme was done because of the consistency of the homogenised grape which would not have allowed for optimal mixing of the enzyme into the solution.

For wine, 5mL wine sample and 5 mL model wine were added into the same test tube and put into the 30°C heating block to heat up to 30°C. After this, 12.5 mg enzyme was measured out and set aside for each test tube of wine sample to be analysed.

The measured enzyme aliquots were stored in a 4°C fridge until use 24 hours before GC-MS analysis, the pre-weighed enzymes were added into the wine mixture in the heating block by quantitatively rinsing enzyme from the Eppendorf tubes with the wine solution into a separate clean, warm glass test tube. For berries, the enzymes were quantitatively transferred into 'test tube 1' using the model wine solution in 'test tube 2'. The wine and berry samples were then vortexed and kept in the heating block for 24 hours. After 24 hours, the solutions were transferred into 20mL head space vials. The test tubes were rinsed using 2.0 ml 20% NaCl solution and added to the corresponding vials. The internal standard (100  $\mu$ l) was added as previously, and the samples were vortexed and loaded for GC-MS analysis.

### **3.3.8 Data analysis**

Panel performance was monitored, and data was analysed using TIBCO Statistica™ (Statistica 10, Statsoft Inc., Tulsa, USA) with the help of the Stellenbosch University Statistical Analysis department. The aroma and flavour attribute correlations to treatments were achieved by using one-way Analysis of Variance (ANOVA) using Least Squares (LS) means tests for each attribute. Overviews over the two years of DA on the effects of treatments on the experimental wines are achieved by Principal Component analysis (PCA) biplots from Statistica™ where attributes are correlated to treatments from assessors' ratings. Chemical analysis of volatile phenols made use of Kruskal-Wallis Analysis of Variance (ANOVA) to test the effects of treatments on VPs in the wines. A confidence level of 5% was used to determine significant differences and those attributes and volatile phenols are reported on in the following work.

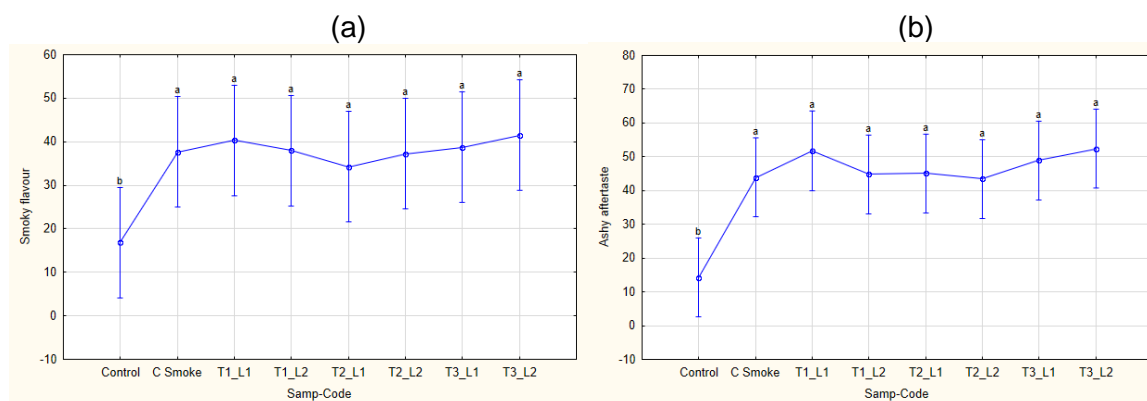
### 3.4 Results and Discussion

#### 3.4.1 PART A: Amelioration Treatments

##### 3.4.1.1 Sensory effects of Amelioration treatments:

###### a) General effects:

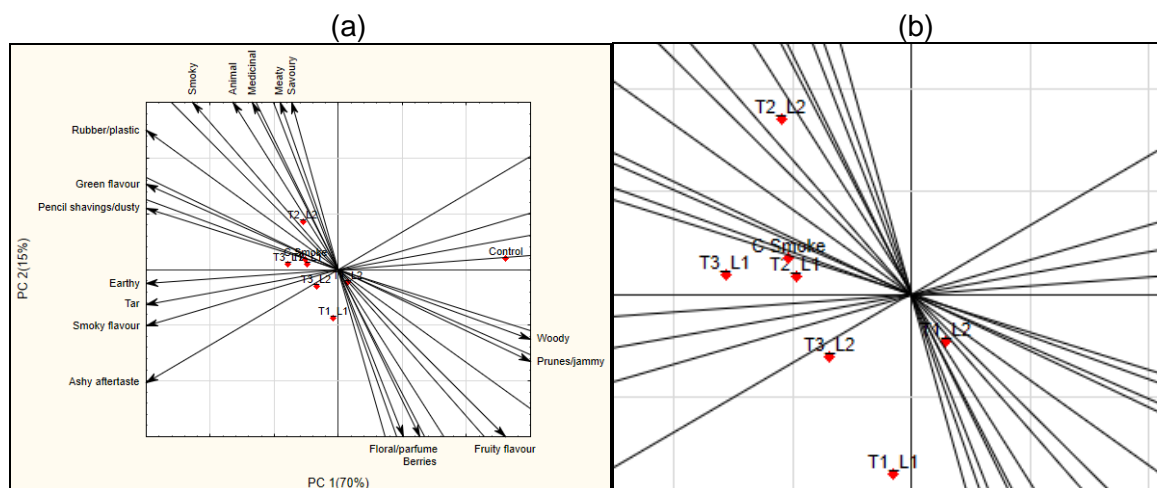
Results obtained from sensory and chemical analyses of the wines are discussed in this section in terms of general sensory effects, and then on a per treatment basis. Chemical results follow. Sample names used throughout the discussion are “c” = control (wine made from unsmoked grapes), “smoked control” (wine made from smoke grapes, untreated), T1 (oak extract at levels L1 and L2), T2 (activated charcoal at levels L1 and L2), and T3 (polymer powder at levels L1 and L2). Descriptive analysis was done for sensory results and GC-MS was used for chemical evaluation of the volatile phenols associated with smoke taint in wine.



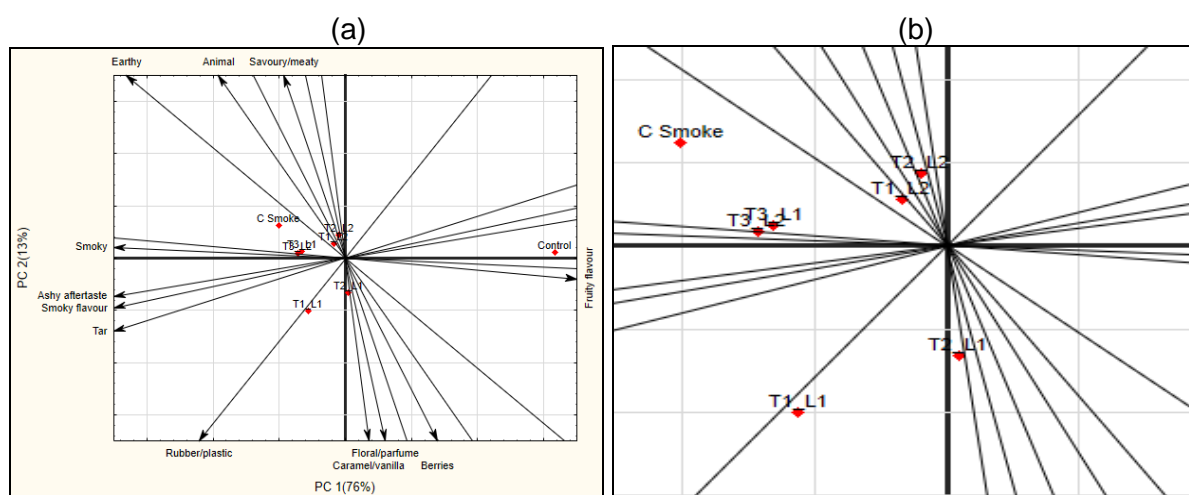
**Figure 3.2:** LS Means diagrams (Type II decomposition) showing panel scores of Y1 (2017) in intensity of the (a) ‘Smoky flavour’ and (b) ‘ashy aftertaste’ attributes in controls and treatments (T1- T3) at two different levels (L1 and L2);  $p < 0.001$ ; Vertical bars denote 95% confidence

Sensory analysis of aroma and taste attributes showed insignificant differences between treatments under each attribute. However, smoky flavour and ashy aftertaste (Figure 3.2) showed significances between the unsmoked controls and the rest of the treatments. This trend was also observed in the second year of sensory evaluation of the wines. The percentage observations remained within the same ranges as well. A trend that could also be observed in the second year, is the decrease in the detection of these attributes’ intensities when treated by activated charcoal compared to the intensities of the first year.

The flavour profile (Figure 3.2) shows what was expected, which was the unsmoked control wines being significantly less smoky and ashy compared to all the other wines. These results also allude to the underlying issue of glycoconjugates (Kennison *et al.* 2008), where chemically there were some decreases in VPs, and sensory analyses did show presence of ‘fruity’, ‘floral’, ‘woody’ etc. aromas but the wines that had grapes smoked were still perceived as smoky and ashy when tasted.



**Figure 3.3:** PCA of all sensory attributes in the first year (Y1) of the study (2017) a) showing attributes and b) detail showing separation of samples



**Figure 3.4:** PCA scores of all sensory attributes one year after bottling (Y2) for 2017 wines a) showing attributes and b) detail showing separation of samples

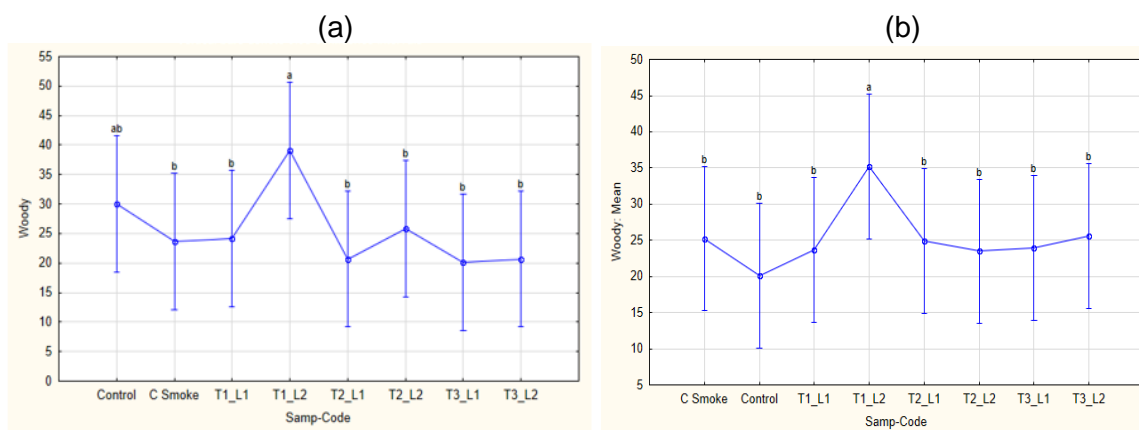
The PCA (Figure 3.3) from year one (Y1) of the study explains 85% of the variation in the dataset, along the two principal components (PC1 = 70%; PC2 = 15%). The unsmoked controls separate out along PC1 towards the positive attributes of 'berries', 'fruity flavour', 'woody', 'prunes/jammy' and 'floral/perfume' attributes from the other smoked treatments which are on the opposite side of the spectrum with the 'smoky' and unpleasant aromas and flavours.

The PCA (Figure 3.4) from year two (Y2) shows the unsmoked control related to fruity flavour while all the other treatments are clustered around the origin. The smoked control and the rest of the treatments are still clustered towards 'earthy', 'animal', 'smoky,' and savoury/meaty' attributes. The implication here may be that the wines are too young to get a clearer picture as a previous study only did a follow-up sensory analysis study after three years of wine being in the bottle (Singh *et al.* 2011) which showed a significant increase in guaiacol in 5 to 6 years (Ristic *et al.* 2017). The PCA of Y2 showed an improved separation of the data of 89% compared to the previous year's PCA of Y1 which had an 85% separation. This also shows complete consistency of the panel as the treatments separate along the same attributes in both years of tasting.

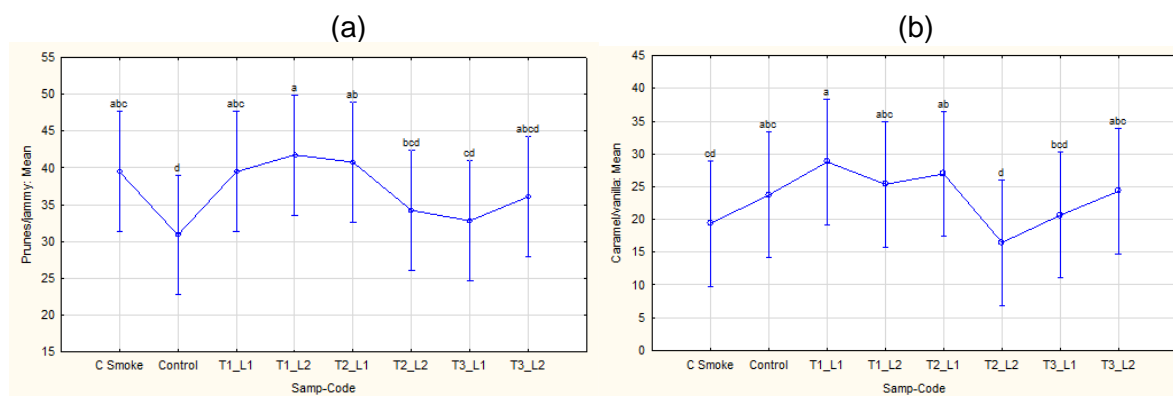


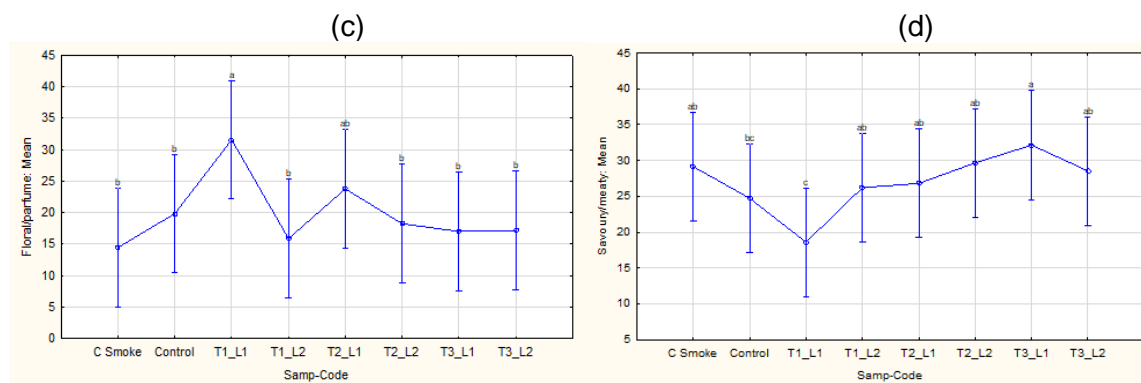
## b) Sensory effect of T1: Oak Extract

None of the wines had any form of oakwood treatment during the winemaking process, except the T1 wines where it was added to elucidate its effect on smoke taint. The oak extract used in the study was chosen for its masking abilities and the complexity it brings to the wine. Overall, in the study T1, contributed to an increase in VPs as well as an increase 'smokiness' and 'woodiness' of the wines. T1 at L2 showed the most significant differences with the 'woody' attribute which was consistently high (Figure 3.5(a) and Figure 3.5(b)) in both years of the study. This treatment (L2) had total oak extract added of 6 mL/L compared to L1 which had 2.5 mL/L total added. The 'woody' attribute was characterised as dry wood, oaky and toasted wood by the panel during training sessions. There were 38% in Y1 and 43% in Y2 perceived differences between the smoked control and oak extract at L2. This shows an improvement in distinction of the samples by the assessors and may also allude to the release of more VPs during the ageing process.



**Figure 3.5:** LS Means diagrams (Type II decomposition) showing panel scores of the 'woody' aroma attribute in controls and treatments (T1- T3) at two different levels (L1 and L2); Vertical bars denote 95% confidence intervals. a) Y1 ( $p=0.02$ ); b) Y2 ( $p=0.03$ )





**Figure 3.6:** LS Means diagrams (Type II decomposition) showing panel scores of (a) 'Prunes/jammy' ( $p=0.02$ ), (b) 'caramel/vanilla' ( $p=0.02$ ), (c) 'floral/perfume' ( $p=0.03$ ), and (d) 'savory/meaty' aroma ( $p=0.03$ ) attribute in Y2 in controls and treatments (T1- T3) at two different levels (L1 and L2);  $p= 0.02$ ; Vertical bars denote 95% confidence intervals.

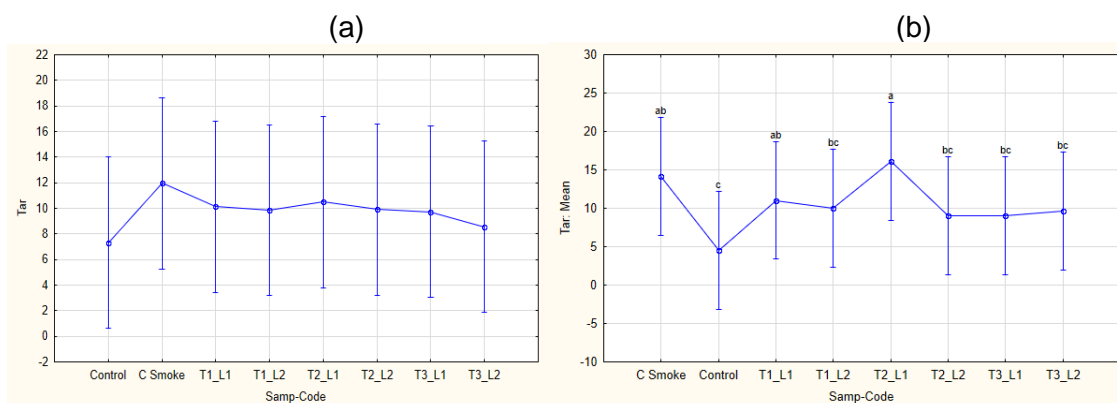
The wines were evaluated by the sensory panel twice, using exactly the same sensory methodology, one year apart (Y1 and Y2). The wines showed an increase in the number of attributes that were significantly different at  $p=0.05$  between the treatments after one year in bottle at Y2. In Figure 3.6 the (a) 'prunes/jammy' and (b) 'caramel/vanilla' remained consistently higher than the unsmoked and smoked controls. While (c) 'floral/perfume' attribute was significantly increased with the addition of the oak extract at L1 and (d) 'savory/meaty' was decreased while the olfactory interaction of compounds (Parker *et al.* 2012) may be related to the sensory results of 'prunes/jammy', 'caramel/vanilla', 'floral/perfume', and 'savory/meaty'. The high perceived 'floral/perfume' (Figure 3.6(c)) resulted in low perceived savory/meaty (Figure 3.6(d)) attribute for oak extract at L1. The 'floral/perfume' attribute has similar characteristics to the 'varnish' aroma described by Chatonnet *et al.* (1990) as being present in oak aged red wines with high levels of lactones. Oak extract at L1 had highest level of 'caramel' (Figure 3.6(b)), which is known to be associated with toasted oak (Cutzach *et al.* 1997) thus explaining the increase of 'caramel/vanilla' in this oak extract treatment compared to the smoked control. Oak extract at L1 also has some association with 'floral/perfume' and 'berry' attributes in the first year. And in the PCA (figure 3.4) of Y2, the oak extract L1 shows close relations to the 'rubber/plastic' attribute. This indicates that high levels ( $>$  odour detection threshold) of guaiacol, 4-ethylphenol, 4-methylguaiacol, and 4-ethylguaiacol can lead to an increase in unpleasant aromas (Kennison *et al.* 2009). Moreover, a significant decrease is witnessed at L2 for 'floral/perfume'. This means that the masking effect of the oak extract at higher levels then recommended may mask the expression of other aromas.

### c) Sensory effects of T2: Activated Charcoal

Activated charcoal is a fining agent used for its adsorption capabilities in wines and is versatile in its removal of unwanted compounds in wine. The results obtained in this study showed a general trend of activated charcoal at L2 resulting in significant decreases of aroma observations both positive and negative, this is because of the chemical properties of this fining agent which allows

for indifference in adsorption of volatile phenols (Jackson 2008). In figure 3.6, 'prunes/jammy', 'floral/perfume', and 'caramel/vanilla' all had decreases with the addition of 45 g/hL of activated charcoal.

The 'tar' (Figure 3.7) attribute showed significant differences of the unsmoked control from all the other treatment by being perceived less. Treatment 2 at L1 which is activated charcoal was only significantly different to five out of the eight treatments by being perceived the highest in tar aroma present. This however did not interfere with the 'floral/perfume' (Figure 3.6(c)) attribute which was increased with the addition of activated charcoal at L1.



**Figure 3.7:** LS Means diagrams (Type II decomposition) showing panel scores of intensity of the 'tar' attribute in controls and treatments (T1- T3) at two different levels (L1 and L2); Vertical bars denote 95% confidence; a) Y1 ( $p=0.888$ ) b) Y2 ( $p<0.001$ )

Comparison between the two years of sensory showed a decrease in maximum observed 'smoky' and 'woody' attributes by the panel when activated charcoal was added. For the 'woody' character, the introduction of the 'caramel/vanilla' attribute indicates an increase in distinction of aromas from toasted wood extract over time. The 'smoky' aroma may have been affected by the increase of positive aromas perceived by the panel which resulted in the decrease of 'smokiness' observed generally. The 'smoky flavour' had a slight decrease and 'ashy aftertaste' had a slight increase in the second year of the study compared to the first year. The decrease of the 'smoky flavour' may have resulted from the decrease of available glycoconjugates that can be released in the mouth (Parker *et al.* 2012; Mayr *et al.* 2014). The increase in ashy aftertaste may be attributed to the increase sensitivity of the panel to the compounds that relate to ashy aftertaste as they became more experienced. This effect was observed by Bende & Nordin (1997) where they found that a trained panel could distinguish between aromas and flavours, as well as be able to identify each attribute and name it compared to untrained individuals. Therefore, with increased experience in training, assessors become better at identifying attributes, and become more sensitised to it.

#### d) Sensory results of T3: Polymer powder

Treatment 3, polymer powder, had little to no effect on sensory results. The smoke-associated attributes still remained at high levels as can be seen with 'smoky flavour' and 'ashy aftertaste' (figure 3.2) where the perceived levels were even higher than the smoked control. The PCAs

(figure 3.3 & figure 3.4) both show T3 being more associated with 'smoky' and 'ashy aftertaste'. Figure 3.6 shows an increase in 'savory/meaty' as well as slight decrease in 'floral/perfume' and 'caramel/vanilla'. All of these results are not significant, and it is clear that this treatment had little effect in decreasing smoke taint or altering the aroma profile of the wines for the better.

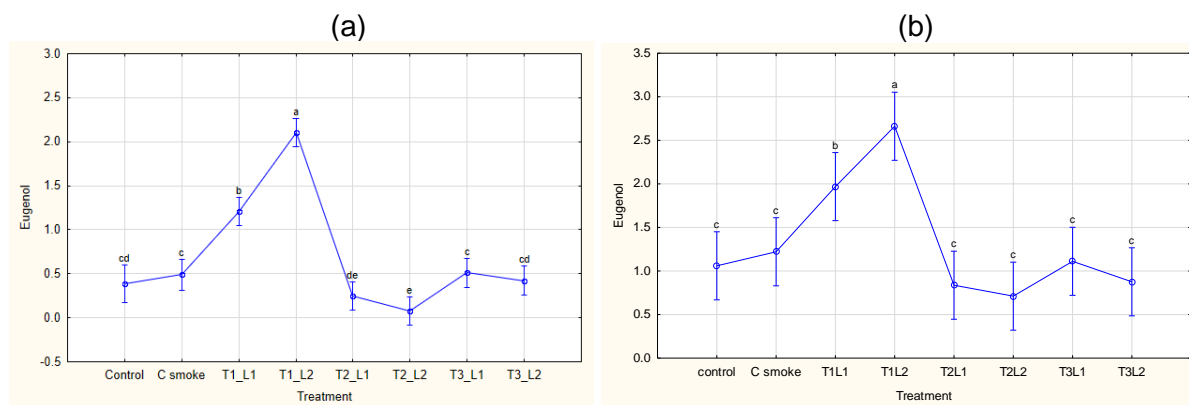
#### **3.4.1.2 Chemical results of amelioration treatments:**

Thirteen volatile phenol compounds were evaluated by GC-MS. Two of the compounds (4-vinylguaiacol and 3,4-dimethylphenol) proved very difficult to detect, possibly as they may have been unable to bind to the fibre used (Mokwena, 2018, personal communication). Ideally, these compounds could be reevaluated using HPLC-MS methods but because of budget and logistical constraints, this was not accomplished. Therefore, these two compounds were omitted from statistical evaluation and excluded from reporting.

Chemical analysis of the same samples of wine as was used in sensory analysis, showed significant differences in VP concentrations of the unsmoked controls compared to the smoked wines; both smoked controls and treated wines. This trend was seen in both years where the oak extract has the highest increase in VPs and activated charcoal had the highest decrease. Polymer powder showed negligible change in VPs, be it an increase or decrease compared to the smoked control wines.

##### **a) Chemical effects of T1: oak extract**

Eugenol (Figure 3.8) showed significant differences for T1 (oak extract) at L2 compared to the other treatments. Eugenol is twice as high at L2 compared to level one as a result of the high amount of oak extract added v/v. This result is very significant, with  $p < 0.001$ . Eugenol is a well known wood component that contributes to increased 'oakiness' in the wine (Singleton 1995). This can be correlated to the sensory results which indeed did show high levels of perceived 'woody' (Figure 3.5(a) and Figure 3.5(b)) attribute in the wines assessed. Measurements of eugenol (Figure 3.8) one year after bottling (Y2) showed a similar graph trend to the previous measurements one year prior. The noticeable difference is the increase in concentration per treatment which had a range of 22% to 69% of eugenol present in the wine. This probably resulted from the release of volatile phenols from their glycosides.

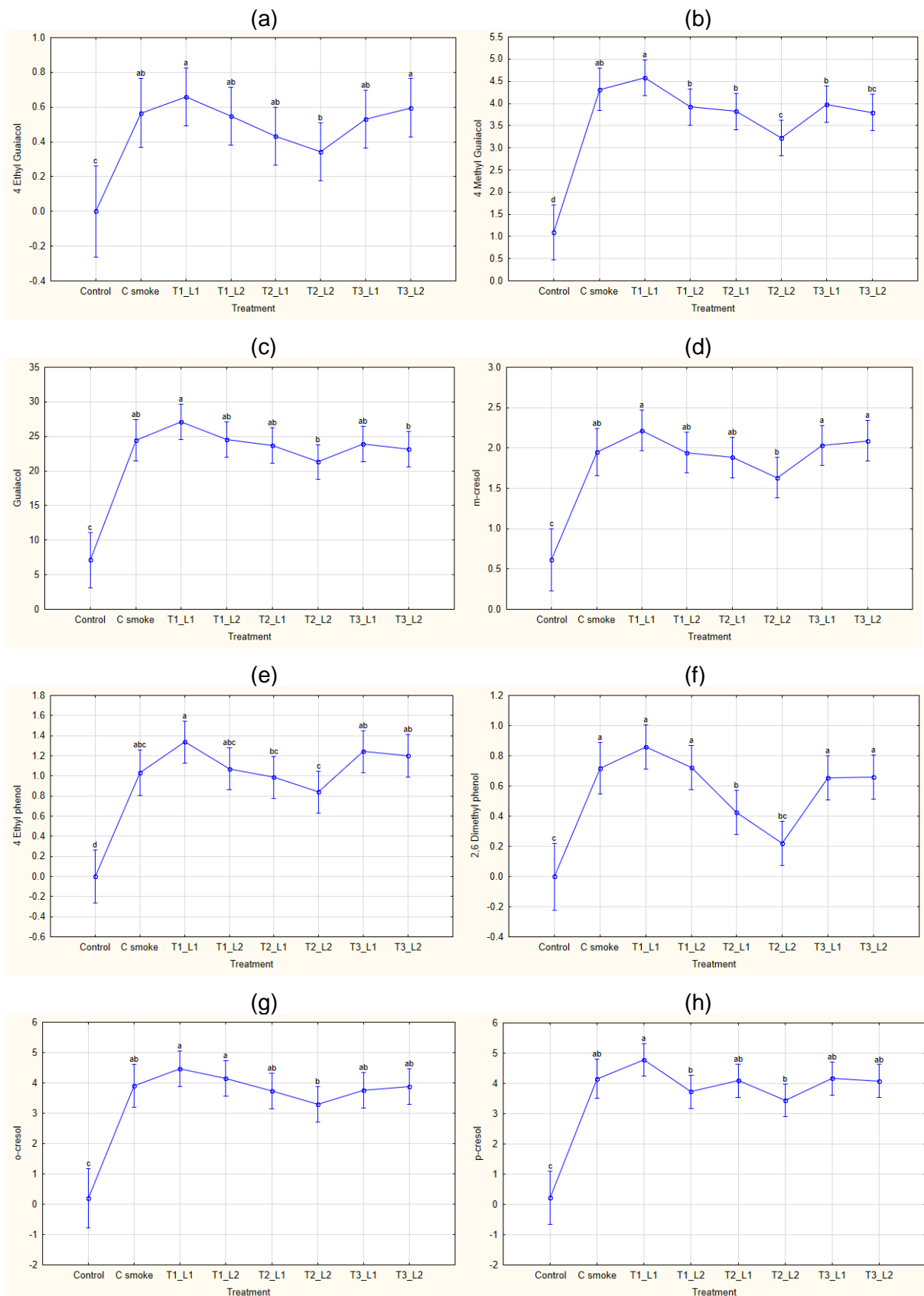


**Figure 3.8:** LS Means diagrams (Type III decomposition) concentration (µg/L) of eugenol in controls and treatments (T1- T3) at two different levels (L1 and L2); Vertical bars denote 95% confidence (a) Y1 (p<0.001) (b) Y2 (p<0.02)

The increase in eugenol levels may explain the improvement in performance of the panel in distinguishing between the smoked control and T1L2 when it came to the 'woody' attribute which saw a 5% increase in scores. This increase in 'woody' may have led to the decrease in 'floral/perfume' (Figure 3.6(c)) attribute.

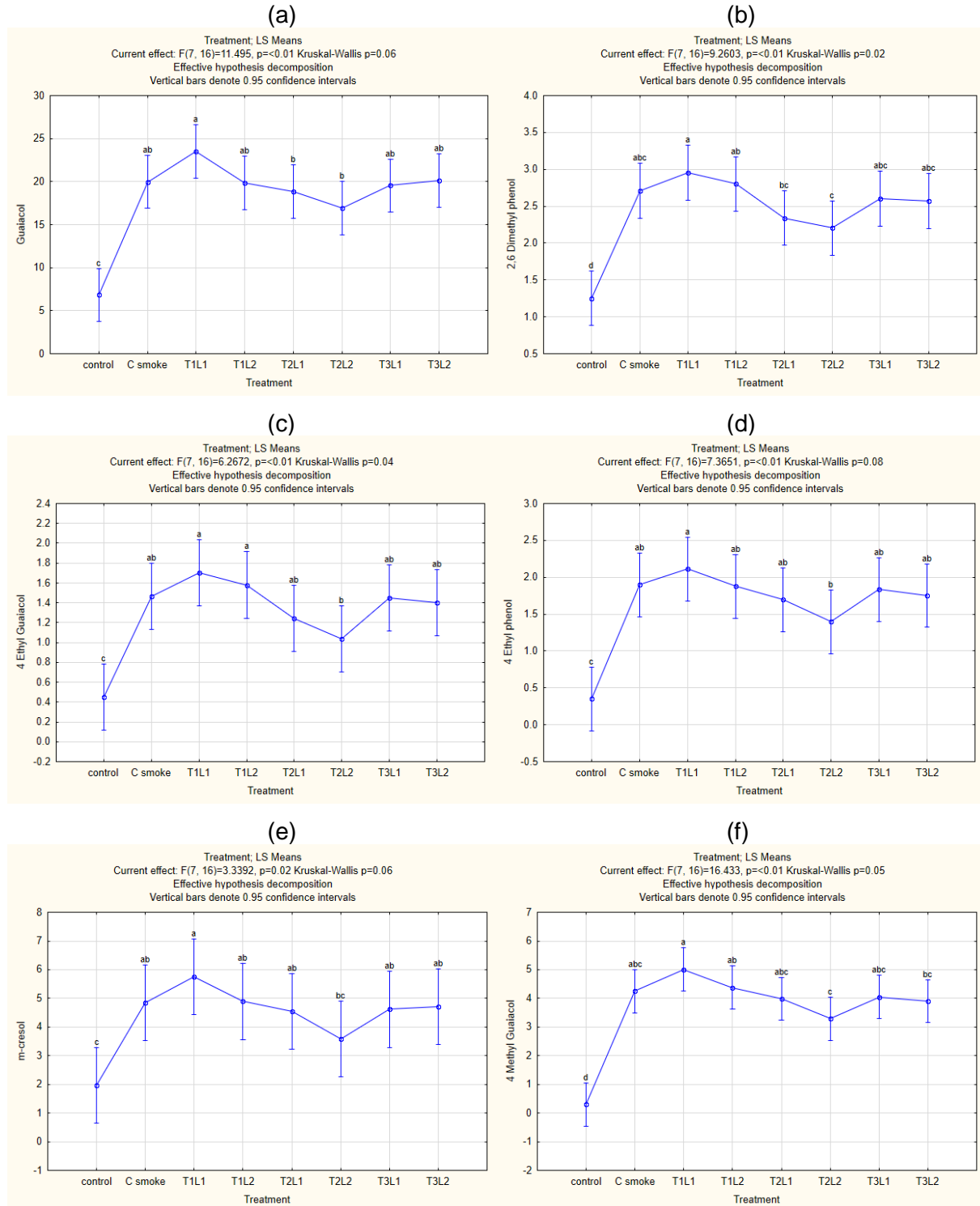
#### b) Chemical effects of T2: Charcoal treatment

As can be seen in figures (Figure 3.9a-h), the unsmoked control ("control") shows significantly less guaiacol, 4-methyl guaiacol, m-cresol, o-cresol, p-cresol, 4 ethyl phenol, and 2,6 dimethyl phenol at the p=0.01 level and 4-ethyl guaiacol at p=0.05. The odour detection threshold of 7.5-23 µg/L (Ferreira *et al.* 2000; Parker *et al.* 2012) for guaiacol is exceeded in the case of the smoked control and all the smoked treatments (Figure 3.9(c)). Likewise, the unsmoked control sample 4-methylguaiacol (Figure 3.9(b)) shows results of less than 0 which are below the detection threshold of 65 µg/L (Kennison *et al.* 2009). 4-ethylguaiacol (Figure 3.9(a)) also shows significantly low levels of compared to the detection threshold of 110 µg/L (Kennison *et al.* 2009). Parker *et al.* 2012 showed that 20 µg/L, 64 µg/L, and 62 µg/L were the detection thresholds for m-cresol (figure 3.9(d)), p-cresol (Figure 3.9(h)), and o-cresol (Figure 3.9(g)), respectively, and all the wines measured below those values. 4-ethylphenol has a detection threshold of 605 µL (Kennison *et al.*, 2009) and 2,3-dimethylphenol (Figure 3.9(f)) has 500 µg/L (Verschuere 1983) and all these compounds at different treatments measured (Appendix D) below those threshold values (Table 2.1). These wine matrices provide further evidence that the combination of volatile phenols and other wine compounds can result in smoke taint even if the odour thresholds are not reached as it was shown with the measurement of ethylguaiacol and ethylphenol which were below odour threshold but still presented the *Brettanomyces* characteristic in sensory (Romano *et al.* 2009)



**Figure 3.9:** LS Means diagrams (Type II decomposition) showing concentrations (µg/L) in Y1 of VPs measured per treatment using GC-MS (a) 4-ethyl guaiacol (p=0.01); (b) 4-methyl guaiacol (p<0.001); (c) guaiacol (p<0.001); (d) m-cresol (p<0.001); (e) 4-ethyl phenol (p<0.001); (f) 2,6 dimethyl phenol (p<0.001); (g) o-cresol (p<0.001); (h) p-cresol (p<0.001); in controls and treatments (T1- T3) at two different levels (L1 and L2); p= 0.00; Vertical bars denote 95% confidence

Moreover, Figure 3.9 (a-h) show that T2 at L2 has an effect of consistently decreasing the VPs compared to the other treatments. This was because 45 g/hL was used compared to 25 g/hL of level one. The recommended dosage by the manufacturer is between 10 g/hL to 45 g/hL. This is further illustrated by the sensory results where smoky flavour and ashy aftertaste had a slight decrease compared to other treatments. The different effects that activated charcoal has on sensory results versus chemical results can be attributed to the presence of glycoconjugates that increased the perceived smoke in the wine even if there was a decrease chemically of VPs.



**Figure 3.10:** LS Means diagrams (Type II decomposition) showing concentration ( $\mu\text{g/L}$ ) in Y2 of VPs measured per treatment using GC-MS one year after bottling (a) guaiacol (b) 2,6 dimethyl phenol (c) 4-ethyl guaiacol (d) 4-ethyl phenol (e) m-cresol (f) 4-methyl guaiacol in controls and treatments (T1- T3) at two different levels (L1 and L2);  $p < 0.001$ ; Vertical bars denote 95% confidence

The data (Figure 3.10 a-f) demonstrates similar graph trends of Y2 compared to Y1 after bottling the wines in terms of VPs available (Appendix E). There were slight increases in some of the VPs, but most remained at the same levels. This further illustrates that over time VPs can be released in the bottle at varying rates (Singh *et al.* 2011). The levels measured correspond to those found in literature for VPs.

Literature has found that fining with charcoal can have an effect on bound volatile compounds by decreasing them, the decrease is higher when compared to settling, using pectin enzymes, and using a mixture of bentonite, casein, silica gel during settling stage of the winemaking process, (Moio *et al.* 2004). This may explain why there is a low concentration of VPs for the wines treated with activated charcoal when compared to the other treatments.

### 3.4.2 PART B: Hydrolysis Experiment:

This additional experiment was carried out in order to elucidate whether enzyme hydrolysis would reveal the effect of 'hidden' VPs in the form of glycoconjugates. Results from before versus after treatment with the enzyme (Table 3.4) showed substantial increases in VPs after cleaving these compounds from their glycoconjugates precursors.

**Table 3.3:** Volatile phenol levels (average of machine duplicates) before and after enzyme hydrolysis of Y1 wine.

Sample Label	Guaiacol $\mu\text{g/L}$	2,6 Dimethyl phenol $\mu\text{g/L}$	4 Methyl Guaiacol $\mu\text{g/L}$	o-cresol $\mu\text{g/L}$	phenol $\mu\text{g/L}$	4 Ethyl Guaiacol $\mu\text{g/L}$	m-cresol $\mu\text{g/L}$	p-cresol $\mu\text{g/L}$	2,3 Dimethyl phenol $\mu\text{g/L}$	Eugenol $\mu\text{g/L}$	4 Ethyl phenol $\mu\text{g/L}$	
control grape	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	before
smoked grape	2.86	0.24	0.25	0.72	9.27	0.05	0.23	0.14	0.14	0.00	0.05	
control juice	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
smoked juice	0.36	0.00	0.00	0.04	0.00	0.02	0.00	0.00	0.00	0.00	0.00	
control	7.49	1.12	0.54	1.43	3.43	0.19	0.81	0.56	0.00	0.72	0.14	
C smoke	24.96	1.23	4.41	4.71	40.61	0.96	2.18	4.64	0.32	0.77	1.24	
T1L2	24.67 $\pm$ 1.54	1.24 $\pm$ 0.13	4.41 $\pm$ 0.36	4.23 $\pm$ 0.23	38.07 $\pm$ 2.7	0.94 $\pm$ 0.02	2.16 $\pm$ 0.14	4.02 $\pm$ 0.29	0.25 $\pm$ 0.01	2.37 $\pm$ 0.10	1.21 $\pm$ 0.02	
T2L2	20.98 $\pm$ 1.02	0.69 $\pm$ 0.05	3.29 $\pm$ 0.15	3.45 $\pm$ 0.18	4.60 $\pm$ 1.29	0.66 $\pm$ 0.04	1.82 $\pm$ 0.13	3.64 $\pm$ 0.18	0.16 $\pm$ 0.02	0.37 $\pm$ 0.03	1.02 $\pm$ 0.05	
T3L2	25.05 $\pm$ 1.58	1.26 $\pm$ 0.07	4.20 $\pm$ 0.52	4.47 $\pm$ 0.42	43.92 $\pm$ 4.9	0.96 $\pm$ 0.10	2.40 $\pm$ 0.28	4.73 $\pm$ 0.51	0.29 $\pm$ 0.05	0.73 $\pm$ 0.04	1.37 $\pm$ 0.16	
control grape	3.39	0.03	0.28	0.55	2.96	0.10	0.27	0.21	0.26	0.38	0.23	
smoked grape	44.38	0.04	12.86	10.32	39.75	1.56	8.78	11.83	0.61	0.52	0.64	
control juice	1.38	0.03	0.18	0.20	1.56	0.10	0.07	0.08	0.25	0.34	0.23	
smoked juice	17.28	0.03	5.31	5.37	21.78	0.74	2.57	4.26	0.50	0.40	0.43	
control	18.73	0.09	2.10	2.83	181.27	0.92	3.74	2.43	0.49	2.01	0.47	
C smoke	80.19	0.06	37.13	18.31	48.62	5.66	15.72	24.12	1.89	2.43	1.71	
T1L2	79.36 $\pm$ 12.22	0.07 $\pm$ 0.02	37.7 $\pm$ 7	18.63 $\pm$ 3.15	53.90 $\pm$ 12.3	5.45 $\pm$ 1.07	15.71 $\pm$ 2.70	23.37 $\pm$ 4.2	1.89 $\pm$ 0.34	3.51 $\pm$ 0.50	1.67 $\pm$ 0.26	
T2L2	77.04 $\pm$ 12.01	0.05 $\pm$ 0.01	36.60 $\pm$ 6.62	18.12 $\pm$ 2.95	54.07 $\pm$ 11.7	4.99 $\pm$ 0.97	15.76 $\pm$ 2.16	24.13 $\pm$ 3.9	1.90 $\pm$ 0.13	1.66 $\pm$ 0.17	1.60 $\pm$ 0.20	
T3L2	88.23 $\pm$ 13.10	0.06 $\pm$ 0.01	43.29 $\pm$ 7.35	20.73 $\pm$ 3.33	55.56 $\pm$ 6.54	6.35 $\pm$ 1.02	18.53 $\pm$ 2.99	27.01 $\pm$ 3.1	1.92 $\pm$ 0.25	2.11 $\pm$ 0.21	1.83 $\pm$ 0.17	

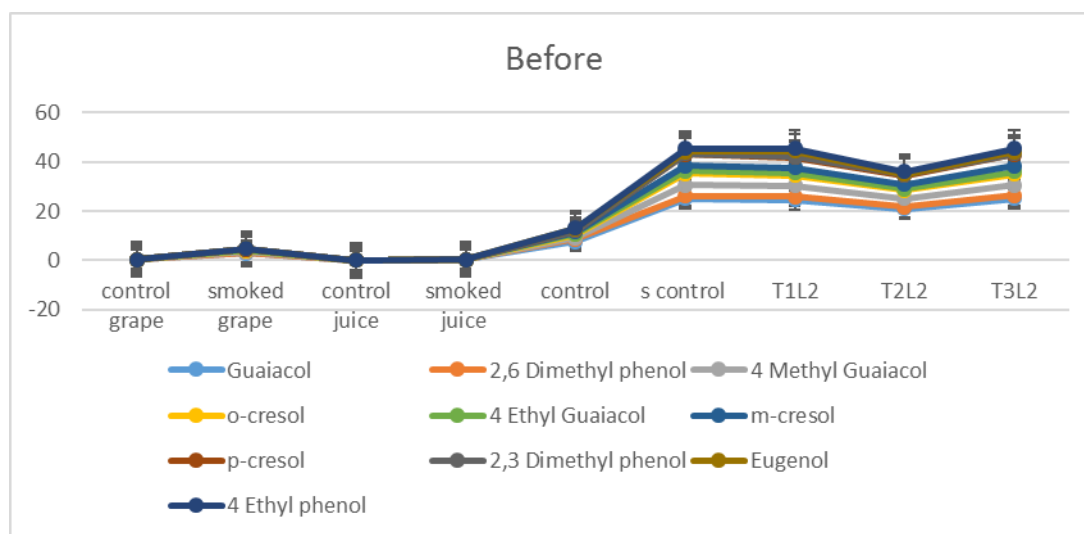


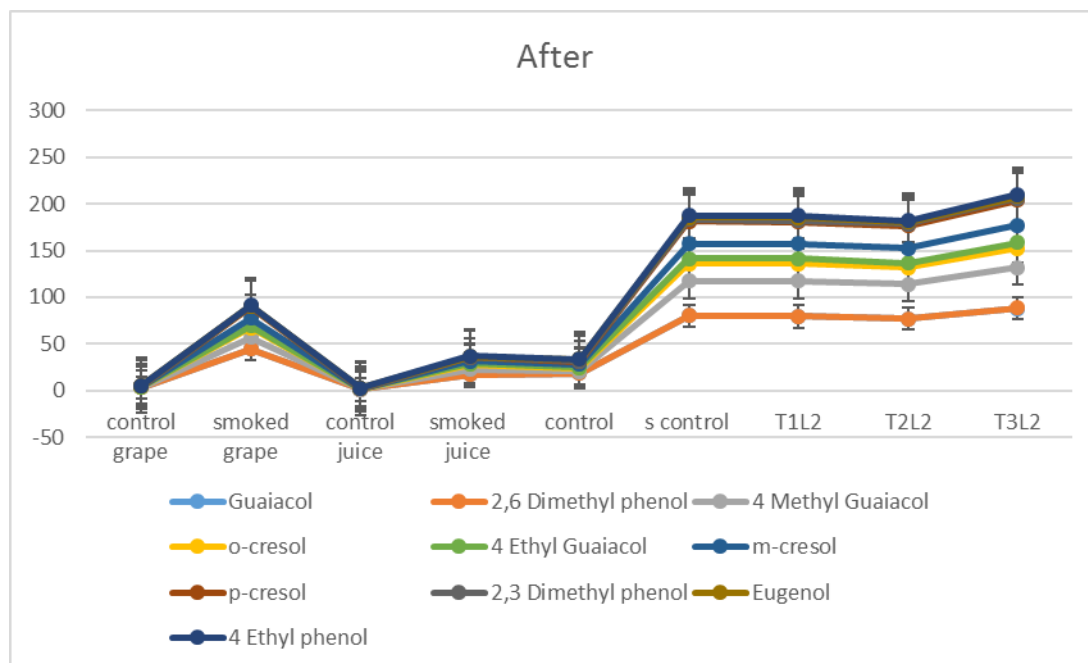
**Table 3.4:** Percentage changes of volatile phenol levels (average of machine duplicates) from before to after enzyme hydrolysis of Y1 wine.

Sample Label	Guaiacol	4 Methyl Guaiacol	o-cresol	phenol	4 Ethyl Guaiacol	m-cresol	p-cresol	2,3 Dimethyl phenol	Eugenol	4 Ethyl phenol
control grape	91	100	100	100	100	100	100	100	100	100
smoked grape	94	98	93	77	97	97	99	78	100	92
control juice	100	100	100	100	100	100	100	100	100	100
smoked juice	98	100	99	100	98	100	100	100	100	100
control	60	74	49	98	80	78	77	100	64	70
C smoke	69	88	74	16	83	86	81	83	68	28
T1L2	69	88	77	29	83	86	83	87	33	27
T2L2	73	91	81	36	87	88	85	91	78	36
T3L2	72	90	78	21	85	87	82	85	65	25

Significant increases of between 21 to 100% change of VPs after enzyme treatment (Table 3.4) are revealed, in the case of guaiacol causing increases far above the odour detection threshold of 23 µg/L to 88 µg/L (Table 3.3). Some of the VPs experienced 100% increase because of their non-existence before the treatment. This is further illustration of the presence of glycosides and their potential to increase VP levels in the wine.

Figure 3.11 shows VP concentrations across the wine production process, and also clearly demonstrates the much higher levels in post-enzyme treated wines.





**Figure 3.11:** (a) Before and (b) after enzyme application measurements of VPs

Thus, these results show the potential for smoke taint to develop intensely in the bottle as a result of VPs being released by acid hydrolysis over time, and measuring in excess of their detection thresholds when the bottle is opened for consumption. Smoke taint precursors/glycoconjugates remain in the wine until acid or enzyme hydrolysis occurs gradually over time (Fudge *et al.* 2011; Singh *et al.* 2011; Ristic *et al.* 2016; 2017). For producers, these results mean that wine will be contaminated by smoke taint over time even if it is treated before bottling. This has serious implications for winemaking with smoke-contaminated juice and grapes, and supports the requirement for additional investigations.

### 3.5 Conclusions

The projected increases in the number of wild fires has seen the need to come up with solutions to the issues that are associated with wildfires such as smoke taint in wine. Three different commercial treatments were selected on the basis of anecdotal or published smoke taint-reduction properties, and applied during the winemaking process, in the experimental cellar at the Department of Viticulture and Oenology at Stellenbosch University. For quantifying associated volatile phenols in treated and untreated samples, a GC-MS method was used, in conjunction with sensory evaluation by descriptive analysis at one month and again, one year after bottling.

This first part of this experiment aimed to ameliorate smoke taint in wines using commercially available products after the smoke incidence has occurred. This was applied in the South African wine industry context by using products that are locally available. The first aim of this part of the project was to test the efficacy of three legal additives on deliberately smoke-tainted wines for

removal of volatile phenols and smoke taint. The reduction aspect focussed on either removal or masking of smoke-related compounds in wines as well as testing two dosage levels of each treatment. The products used were an oak extract (for masking of smoke taint), activated charcoal and a polymer powder for the removal of smoke taint.

In this study, only guaiacol measured above odour threshold (Table 2.1, Chapter 2) and sufficient smoke taint was generated for easier detection by the panel. The levels of VPs generated are above those that can be found in the industry which means the study may need to be carried out on naturally tainted wines. There is thus a strong possibility that at those levels the treatments may have a significant decrease in VPs measured and smoke detected.

The data showed that activated charcoal was successful at removing fruity as well as undesirable attributes in the wine. This resulted in low perceived positive aromas such as 'floral/perfume' and an increase in aromas such as 'savory/meaty'. This treatment was marginally effective in decreasing smoke related aromas. The oak extract was successful in increasing 'woody' attribute and introducing the 'caramel/vanilla' attribute. However, this increase was overshadowed by the fact that the smoke aromas remained at high levels. Chemical analysis showed that activated charcoal at L2 had the highest decreases of VPs throughout compared to the smoked control.

The results showed that the oak extract at double the manufacturer's recommended level made the wine more 'woody', 'oaky' and 'caramel'. However, with the increase of these positive characteristics, the wine still remained smoky. In the first year, there was little distinction in sensory results between the smoked treatments except the 'woody' attributes. The second year of analysis yielded much more promising results with the PCA showing a stronger differentiation between treatments compared to the first year with activated charcoal a shift towards being associated with positive attributes of 'floral', 'caramel' and 'berries'. Eugenol measured the highest in the oak extract treatment and that can also be linked to the increased 'woody' attribute in sensory analysis. Chemical analysis showed similar trends in the first and second year of analysis. Activated charcoal at L2 had the biggest effect in the decrease of volatile phenols in the wine. This however stripped the wine of many aromas, as has been shown by other authors (López et al. 2001) and as revealed by the sensory results where low levels of any aroma attribute are detected.

Although there were some differences found between treatments regarding the aroma of the wine, none of the treatments had an effect on the flavour (palate) of the wine. This agrees with findings by Wilkinson *et al.* (2011) and Mayr *et al.* (2014), in which the majority of VPs were found to be stored in glycosylated forms in the wines and could be released by in-mouth enzymes.

The second aim of this study was thus to investigate the potential for hydrolysis followed by fining as a strategy for removing glycosides. This was done by establishing the potential for smoke-affected wines to manifest a taint after slow acid hydrolysis of precursors during bottle-aging and to carry out a complete enzyme hydrolysis, and monitor VPs before and afterwards in order to determine the concentration of glycosylated precursors, and the potential for smoke-taint development. The increase in significantly detectable attributes shows the potential for the wine to

reveal more aroma changes over time. The inclusion of hydrolysis in the study was to measure severity of glycoconjugates' effects on the wines during ageing. The increase in VPs which shows high risk potential. A possible recommendation from the results obtained would be to use activated charcoal at relatively high levels to remove smoke taint aroma after treatment with enzymes, and then add oak extract to increase positive aromas. The glycoconjugates still remain the main issue and further research still needs to be conducted in this area to decrease these compounds without compromising the quality of the wines. Chapter 4 of this study focuses on the use of enzymes during the winemaking process, with the aim of releasing volatile phenols and thus decreasing the quantity of glycoconjugates in the bottled wine.

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## Chapter 4: The effect of post-fermentation enzyme treatments and fining on amelioration of smoke taint

### 4.1 Introduction

Grape exposure to smoke from burning of wildfires ('veldfires') around vineyards results in wines that have 'smoky', 'barbeque', 'meaty', 'ashy' and 'burnt' characteristics that are collectively known as smoke taint (Høj *et al.* 2003). Table 4.1 outlines attributes and thresholds associated with some of the compounds that have been linked to smoke taint.

**Table 4.1:** Volatile phenol attributes and odour detection thresholds (ODTs) as found by previous workers

Compound	Aroma descriptors	ODT ( $\mu\text{g/l}$ )	Reference
Guaiacol	Smoky, sweet, medicinal	7.5-23	Ferreira <i>et al.</i> 2000 Parker <i>et al.</i> 2012
2,6 Dimethylphenol	Medicinal, phenolic	570	Escudero <i>et al.</i> 2007
4-Methylguaiacol	Ashy, toasted, vanilla-like	65	Kennison <i>et al.</i> 2009
o-cresol	Band-aid, medicinal, smoky	62	Parker <i>et al.</i> . 2012
phenol	Sickeningly sweet, irritating	7100	Parker <i>et al.</i> 2012 Panzeri 2013
4-Ethylguaiacol	Smoke, spicy, toasted	110	Kennison <i>et al.</i> 2009
m-cresol	Dry, tar, medicinal-leathery	20	Parker <i>et al.</i> 2012
p-cresol	Band-aid, phenol-like	64	Parker <i>et al.</i> 2012
2,3-Dimethylphenol	Phenolic	500	Verschueren 1983
Eugenol	Clove	6	Escudero <i>et al.</i> 2007
4-Ethylphenol	Barnyard, horsey, phenolic	605	Kennison <i>et al.</i> 2009
4-Vinylguaiacol	Clove, curry	40	Parker <i>et al.</i> . 2012
3,4-Dimethylphenol	Sick sweet, medicinal	1200	Burdock 2010

Although the olfactory contribution of smoke taint has been well documented, the issue associated with smoke exposure of grapes that has not received sufficient attention in the literature is the presence of glycoconjugated forms of volatile phenols (Kennison *et al.* 2008), as they cannot be detected in the aroma of the wine. Their hydrolysis can lead to in-mouth release of volatile phenols (VPs) and associated 'ashy'/'burnt' flavours on the palate.

Glycoconjugated forms arise as a result of VPs being taken up by the vines through the leaves and berries (Krstic *et al.* 2015), and then detoxified by being bound to sugars in a process called glycosylation (Korte *et al.* 2000; Kennison *et al.* 2008; Hartl *et al.* 2017). The glycoconjugates or glycosides will remain in the grapes and the grape juice until external influences such as acidity, enzymes, bacteria and yeasts start interacting with them (Sarry *et al.* 2004). These versions are water-soluble and cannot be fined or filtered (Harborne 1984; Korte *et al.* 2000; Kennison *et al.* 2008). They remain in the wine where they can be cleaved in the bottle by acids and enzymes thus increasing the level of volatile phenols in the wine during bottle maturation.

$\beta$ -Glucosidases are enzymes responsible for the release of VPs from sugars (Kennison *et al.* 2008). These enzymes are used by winemakers for aroma enhancement by breaking down of the glycosidic bonds between sugars and volatiles (mainly terpenes) in wine and ideally should be active under wine conditions: low temperatures, low pH, high glucose, and high ethanol (Baffi *et al.* 2013a). In wine the presence of  $\beta$ -glucosidases has been found to be influenced by yeast (Villena *et al.* 2007) and bacteria (Grimaldi *et al.* 2000) and commercial preparations of fungal origin have been mainly used in wine (Villera *et al.* 2007). The  $\beta$ -glucosidase activity of yeast and bacteria may thus result in wines high in VPs after fermentation (Kennison *et al.* 2008; Dungey *et al.* 2011; Ristic *et al.* 2011). As previously mentioned, the effects of glycoconjugates being present in wine are thus 'ashy' and 'smoky' flavours that can be detected on the palate as a result of in-mouth release that occurs due to the presence of these enzymes in the mouth (Parker *et al.* 2012; Mayr *et al.* 2014).

Strategies have been devised to try and limit VPs in wine and grapes, as well as limiting VPs released during the wine making process by different authors and have been summarised in the work done by Brodison *et al.* (2013). The use of activated charcoal is for fining purposes and for its abilities to adsorb compounds in the wine but it is impartial on what gets removed (Zoecklein 1990)- refer to Chapter 3 for activated charcoal and PET.

Yeast autolysis is an important oenological stage of winemaking. As the yeast cells lyse, they release cellular component into the wine which can contribute to flavours. Yeast cell walls/hulls and mannoproteins are some of the by-products of autolysis (Pérez-Serradilla *et al.* 2008). Mannoproteins are cell wall proteins which can enhance protein and colour stabilisation in wine. The capacity of mannoproteins for adsorption of aroma compounds has been attributed to the presence of high protein proportions. Yeast hulls have been characterised as fermentation activators because they fix toxic fatty acids and contribute sterols, and unsaturated long-chain fatty acids (Ribereau-Gayon *et al.* 2007). Yeast hulls have been investigated for the removal of 4-ethylphenol by Pradelles *et al.* (2009). These authors found that 61.5% to 192% removal could be measured depending on the drying process of the yeast cells and yeast strains. The increase in surface area of the yeast cell through damage from the drying processes resulted in significant removal of 4-ethylphenol.

These strategies mostly aim to remove free VPs, and keep VPs in their glycoconjugated form so that the wine can be marketed for early release. However, none of these treatments deal with the direct removal of glycoconjugates before bottling, and there is little research in this area.

The aim of this part of the project was thus to explore the success of strategies for releasing VPs from their glycoconjugates before wine was fined, bottled and sold, preventing unpleasant smoke-taint related occurrences for consumers at a later stage.



**Aims of the project:**

1. To hydrolyse volatile phenols (VPs) and their sugar moieties (glycoconjugated VPs) through the addition of commercial  $\beta$ -glucosidase enzymes post-fermentation.
2. To apply four fining treatments in order to remove liberated VPs after the  $\beta$ -glucosidase enzyme treatment. Treatments to be tested include activated charcoal, polymer powder, yeast hulls and mannoproteins.
3. To monitor results through chemical and sensory evaluation of the treated wines and unsmoked controls.
4. To make recommendations for winemaking and future studies based on the results of these trials

**4.2 Materials and Methods**

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The project on amelioration of smoke taint project was carried out during 2017 and 2018 seasons. The work discussed in this chapter of the study was carried out in 2018. The grapes were harvested from Welgevallen experimental farm 157m above sea level (-33.939847, 18.865590). Shiraz cultivar grapes were used, clone SH9C which was grafted on 101-14 Mgt (*Vitis riparia* x *Vitis rupestris*). The vines were planted in the year 2000 with a 2.7m by 1.5m spacing trellised on a seven-wire vertical shoot positioning. The vines were irrigated with a pressure compensated drip system. The block has a North-South direction on a horizontal surface.

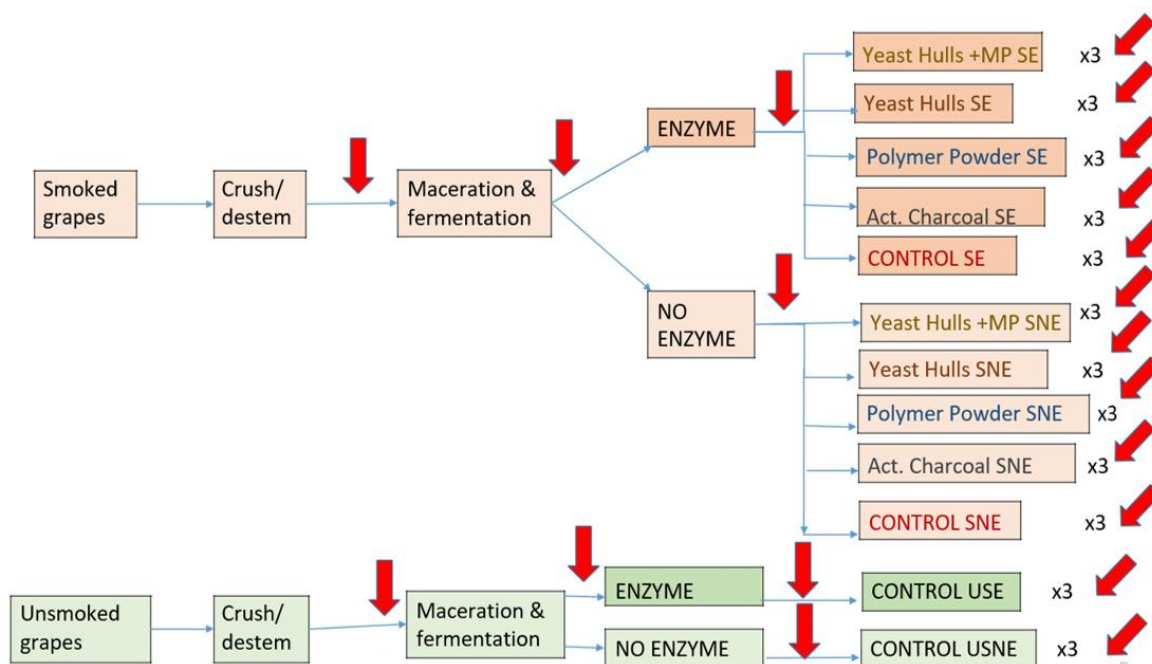
**4.2.1 Smoke treatments:**

The grapes were harvested on 08/03/2018 at 22-23 ° balling. Grapes (526 kg) were hand-harvested with each treatment replicate assigned 16 kg. As outlined in Chapter 3, Section 3.1.2, grapes were smoked in 40-60L clear plastic containers for 30 seconds using a beekeeping smoker (Agrimark, Stellenbosch). Grapes were smoked within two hours of harvesting, stored at 18 ° C in a laboratory, and smoked again 24 hours after harvest.

The grapes were stored in the Stellenbosch experimental cellar overnight at 20 ° C for logistical reasons and risk of contaminating other University experiments with smoke.

**4.2.2 Amelioration treatment experimental design:**

Experimental treatments followed the protocol shown in Figure 4.1. Smoked, and unsmoked grapes were subjected to exactly the same winemaking, enzyme and fining treatments. Smoked and unsmoked wines were separated into two batches post-fermentation, and one batch in each case was subject to enzyme treatments. The other batch in each case was simply fined, as outlined below. Samples were taken throughout the winemaking process for analysis of volatile phenol content by GC-MS.



**Figure 4.1:** Experimental protocols for enzyme-related treatments for smoked and unsmoked Shiraz grapes and wine (SE= smoked enzyme treatment; SNE = Smoked grapes, no enzymes; USE = Unsmoked grapes, enzyme treatments; USNE = Unsmoked grapes, no enzymes. Red arrows indicate samples taken at this point for GC-MS analysis.)

#### 4.2.3 Winemaking:

Grape processing commenced with dividing the smoke-treated grapes from each smoked crate evenly between fermentation buckets in order to minimise variability between them. The grapes were crushed and destemmed and SO<sub>2</sub> was added before fermentation. Winemaking followed standard experimental cellar protocols for the Department of Viticulture and Oenology. Inoculation with yeast *Saccharomyces cerevisiae* Lallemend (Montreal, Canada) Lavlin QA23<sup>®</sup> was carried out after this, which was within 48 hours of harvesting. This yeast was chosen in a pilot study for this project and it also has high β-glucosidase activity. Inoculation for malolactic fermentation (MLF) with Anchor Co-inoculant™ (Anchor, South Africa) was carried out three days after yeast inoculation.

Alcoholic fermentation took a total of five days in a temperature-controlled (25°C) environment. It was monitored twice daily by taking Balling measurements until less than -1 ° B. Punch downs were carried out three times a day to ensure maximum extraction of VPs from the skins. Pressing was carried out after the completion of alcoholic fermentation, and the wines were transferred to 4.5L glass containers to finish MLF. MLF was finished within a month of inoculation in a temperature-controlled room at 20°C. 3 g/L of enzyme Oenobrand (Montpellier, France) Rapidase™ Revelation Aroma were added to the wines after MLF had been measured below 0.5 g/L. After this, sulphur dioxide levels were checked, and SO<sub>2</sub> was added. Before the application of treatments, the individual wines were transferred to one container to homogenise them, and an

additional SO<sub>2</sub> was added. The final concentration of SO<sub>2</sub> that was added was 115 mg/L. The wines were allowed to stand for 3-5 days after treatment application.

#### 4.2.4 Enzyme treatments

The enzyme treatment (Oenobrand's Rapidase™ Revelation Aroma enzyme mixture) was applied as per the experimental protocol and allowed to stand for seven days before treatment application. This treatment is said to be “a microgranulated pectolytic enzyme preparation with the four essential α and β-glucosidase activities” on the Rapidase™ product sheet.

Bottling commenced 3-5 days after treatment application. The wines were racked off the lees and had 40mg/l of SO<sub>2</sub> added at bottling and were filtered through the Pall Corporation Filtersystems (GmbH) Seitz K300 filter sheets. The wines were then kept at 15 °C room until sensory evaluation.

#### 4.2.5 Fining treatments

The treatments applied were (activated charcoal or polymer extract or Extraferm® (Oenobrand's Montpellier, France) or Extraferm™+Mannoproteins) and the wines were kept at 25°C because of logistical reasons after treatment application for 3-5 days. Table 4.2 illustrates the treatments used, stage of application and dosage levels.

**Table 4.2:** Treatments applied to 2018 vintage wines post enzymatic hydrolysis

Sample code	Treatment	Sample labels	Trade name	Stage of application	Dosage
Control	None: unsmoked (clean) grapes/wine	Unsmoked control	-	-	-
C smoke	None: Smoked control	Smoked control	-	-	-
-	Enzyme	None; applied to all the wines	Rapidase® Revelation Aroma	After MLF	3g/L
Act. Char. NE Act. Char. ENZ	Activated Charcoal	Activated Charcoal	Charbon actif Plus GR®	Before bottling	50g/L
Powder NE Powder ENZ	Polymer powder	polymer powder	Not registered	Before bottling over 3 days	3g/L
Yeast hulls NE Yeast hulls ENZ	Yeast hulls	Extraferm	Extraferm®	Before bottling	40g/hL
Yeast hulls+ MP NE Yeast hulls+MP ENZ	Yeast hulls + mannoproteins	Extraferm + MP	Extraferm®	Before bottling	40g/hL 40mL/L

##### 4.2.5.1 Fining treatment 1: Activated charcoal (Act. Char. NE; Act. Char. ENZ)

The activated charcoal was applied to a wine that had finished both alcoholic and malolactic fermentations. The product used was Laffort© Charbon actif Plus GR® which was a different product than that which was used in the first year of the study. 50g/hL was measured out and

rehydrated for 2 hours before addition. The mixture was then added directly into the wine in 4.5L glass containers and was kept in the 25 °C for five days before filtration and bottling.

#### **4.2.5.2 Fining treatment 2: Polymer Powder (Powder NE; Powder ENZ)**

The treatment was applied to wine that had undergone alcoholic and malolactic fermentations. This application regime different to that of Y1 in chapter 3. 3 g/L of the powder was added at 0 hours, then sieving-off the powder at 24 hours and 3 g/L added again at 24 hours, and then sieving-off at 36 hours. The wine was mixed twice a day to ensure that enough contact between it and the PET was obtained. The wines were stored at 25 °C.

#### **4.2.5.3 Fining treatment 3: Yeast Hulls (Yeast hulls NE; Yeast hulls ENZ)**

Extraferm® is the powder of yeast cell hulls was used as the fourth treatment. 40g/hL was added to the wine and allowed to stand in the wine for five days. The product was from Anchor®.

#### **4.2.5.4 Fining treatment 4: Extraferm ® + Mannoproteins (Yeast hulls+ MP NE; Yeast hulls+MP ENZ)**

40g/hL of Extraferm™ were added, plus 40ml/L of mannoproteins were added after MLF had finished as recommended by the manufacturer. These products were provided by Oenobrand® (Montpellier, France).

### **4.2.6 Sensory training and testing**

Fifteen individuals were in the 2018 panel with ages ranging from 21 to 60. There were 14 females and 1 male.

Sensory training was carried out as outlined in Chapter 3 of this thesis. The panel was well-trained in a range of sensory methods and had previous experience with smoke taint. Aroma and taste attributes were selected by consensus by the panel during training sessions. They were the same as were used for the DA study in chapter 3: 'berries', 'prunes/jammy', 'floral/perfume', 'savory/meaty', 'woody', 'pencil shaving/dusty', 'smoky', 'earthy', 'tar', 'medicinal', 'animal', 'rubber/plastic', and 'caramel/vanilla'.

For sensory testing purposes, a combination of sensory methods was used for this section of the study, due to the increase in the number of wines to be evaluated. Although DA has been shown in to be reliable, detailed and reproducible (Lawless and Heymann 2010), panel fatigue influenced the results of the previous study (Chapter 3). Thus, rapid sensory methodology was chosen for this study in order to avoid panel fatigue. A combination of rapid sensory mapping (grouping samples according to the similarities and differences) (Cartier et al. 2006) and PSP (polarised sensory positioning) which gives the panellists a certain number of attributes to choose from when evaluating the dissimilarities of wines (Teillet et al. 2010) was chosen.

The tests were carried over 2 weeks (6 days) with each session taking 2 hours. Two sessions out of the six represented a biological replicate.

For sensory testing, 25 mL of the wines were poured into black ISO-standard tasting glasses at room temperature, 20°C, and covered with petri dishes. Each wine was given a three-digit code. Two flights were poured (one for smelling and the other for tasting) from the same bottle. The panellists were also given sparkling water, crackers and still water, in that order, for mouth rinsing between tasting samples. In Appendix B, designs for the aroma and taste tests are shown.

#### **4.2.7 Chemical analyses**

GC-MS was used to obtain chemical data of volatile phenols as outlined in Chapter 3 of this thesis.

#### **4.2.8 Data analysis**

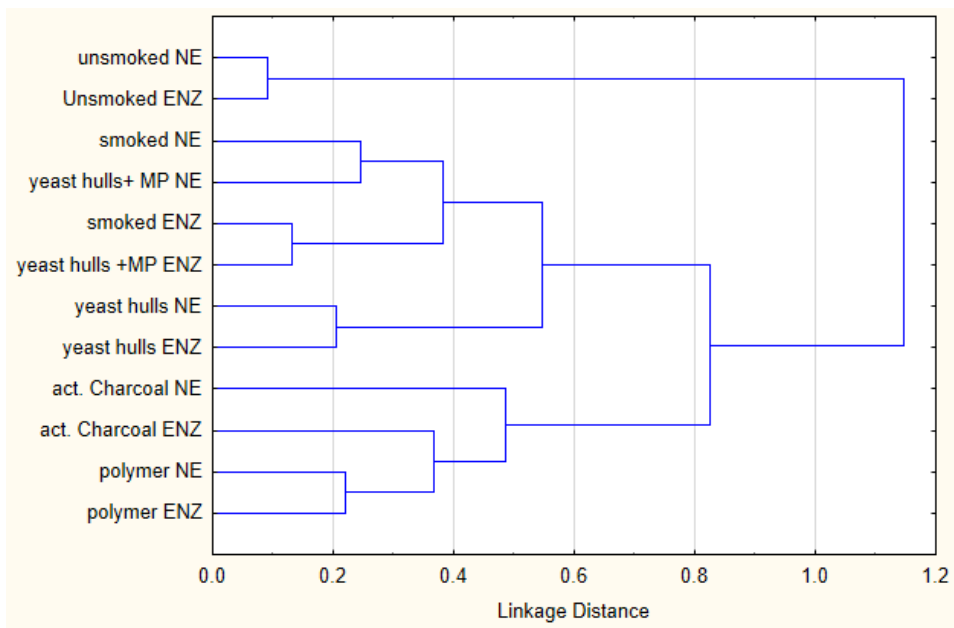
The sensory data was captured into Excel (Microsoft, Redmond, USA). All data analysis for chemistry and sensory was done using the Statistica (TIBCO Data Science, Palo Alto, USA) programme with the assistance from Stellenbosch University Statistical department. Chemical data analysis made use of the same methods as discussed in Chapter 3. For sensory analysis of the rapid method, cluster analysis using the Ward's method on STATISTICA was used to give dendrogram responses and correspondence analysis. Chi-square analysis using Rao & Scott adjustment yielded the histograms of responses per treatment against each attribute and that data set was condensed on Excel.

### **4.3 Results and Discussion**

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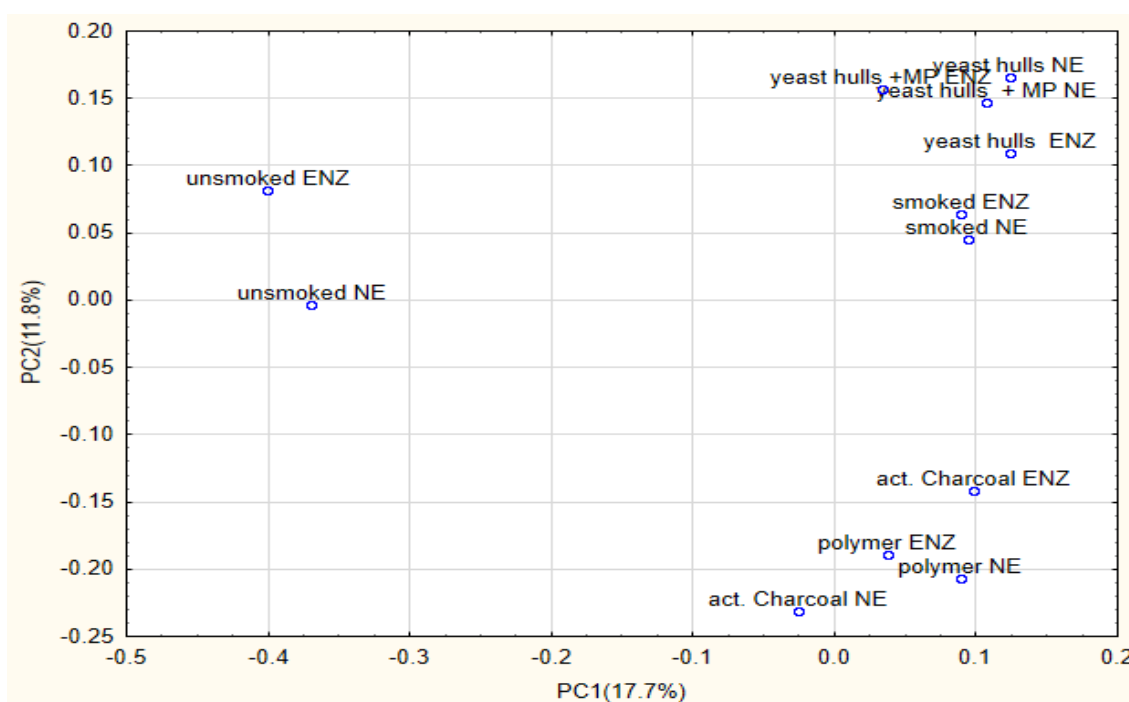
#### **4.3.1 Sensory Results:**

Cluster analysis for overall sensory evaluation data of the sorting exercise for sample aroma (Figure 4.2) indicated that three distinct groupings were formed. In this type of analysis, the further the linkage is from 0, the more differences there are between samples. Unsmoked wines formed a distinct cluster clearly separating from other treatments. The second cluster contains activated charcoal and the polymer powder. The third cluster grouped all the other treatments.



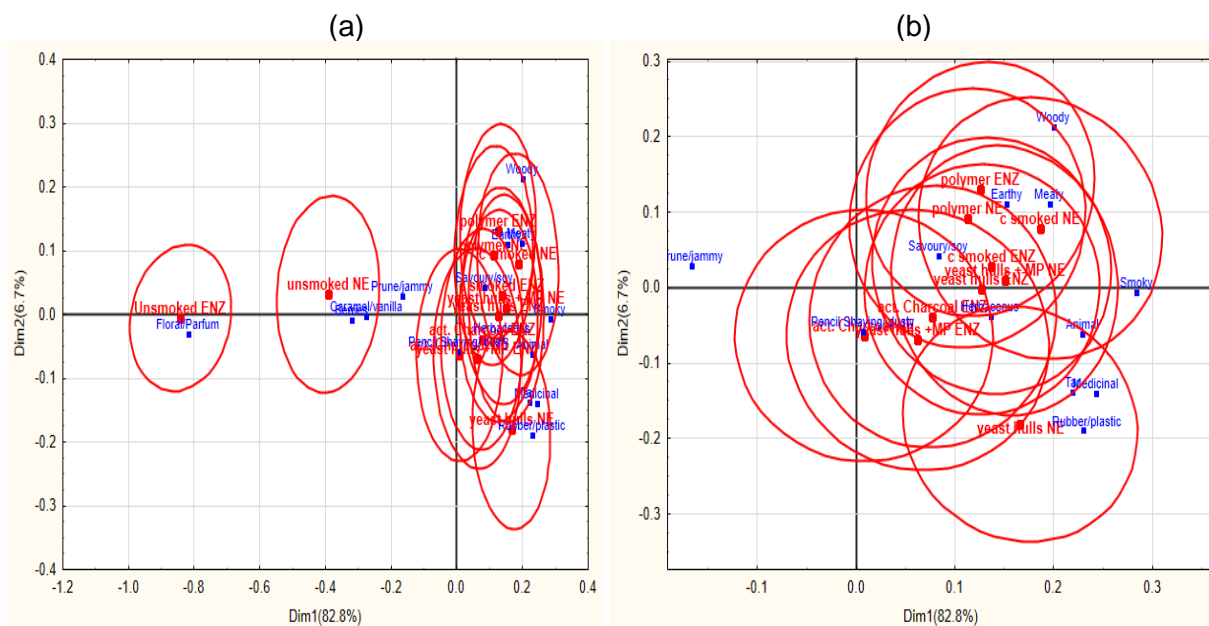
**Figure 4.2:** Dendrogram of sensory responses for the sorting exercise (aroma) generated by agglomerated hierarchical cluster (AHC) analysis. (NE = no enzyme, ENZ = enzyme treatment)

This result was supported by the Principle Component Analysis (Figure 4.3) which showed separation along both principle components for the three groups, although PC1 and PC2 only explain 30% of the variability within the dataset. This data indicates how closely related some of the treatments were to each other in terms of their lack of effect on the attributes as perceived by the panel during sorting. All the smoked treatments remained grouped on the positive side of PC1, whereas the controls were positioned together on the negative side of PC1. In terms of the second principal components, the yeast hulls treatments were grouped with the smoked controls, indicating that they did not cause a significant difference in terms of the perceived aroma of the samples. The charcoal and polymer powder treatments did separate out from the smoked controls (smoked ENZ and smoked NE) along PC2 indicating that these samples showed different attributes. These attributes are possibly 'woody', 'earthy', 'savory/soy', and 'pencil shaving/dusty' from Figure 4.4, although the groupings are not completely clear.



**Figure 4.3.** Principle Component Analysis biplot showing treatments and controls for the sensory sorting exercise (aroma) of samples. (NE = no enzyme, ENZ = enzyme treatment)

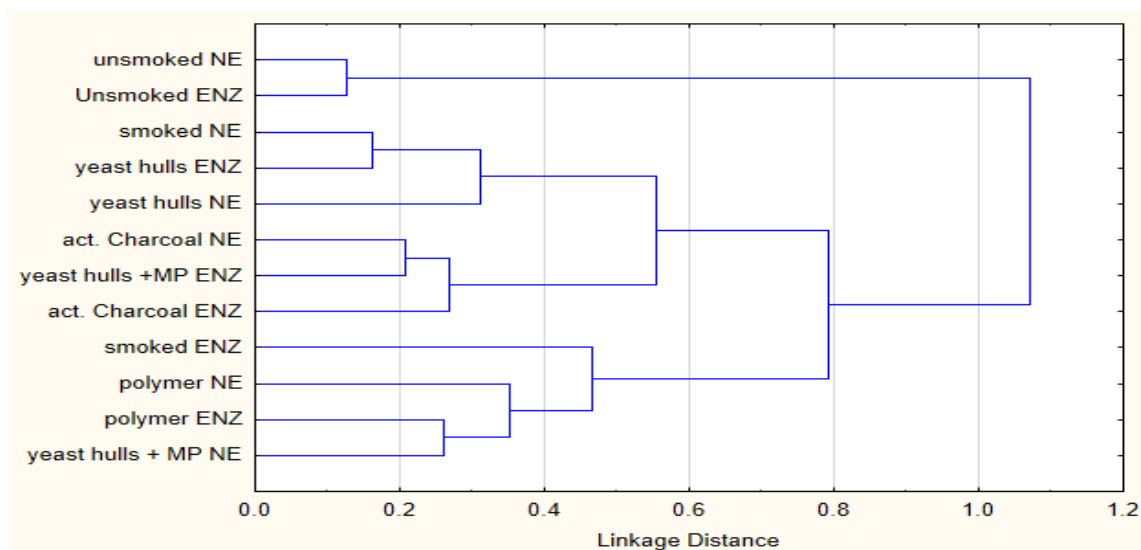
Figure 4.4 showed results of the correspondence analysis of the aroma sorting exercise. These results emphasized how closely associated the treatments were, with little separation between them. Although 83% of the variation in the dataset is explained by the separation along Dimension 1, this is between unsmoked controls with enzymes which is positioned on the negative side of Dimension 1, closely associated with 'floral/perfume'. The unsmoked control (without enzymes) is associated with 'berries', 'prunes/jammy' and 'caramel/vanilla', more to the centre of Dimension 1. These are all generally positive descriptors for red wine. The third group is a cluster of all the other treatments and attributes, including most of the negative aroma attributes like 'earthy', 'meaty', 'herbaceous', 'medicinal', 'animal' and 'pencil shavings', on the positive side of Dimension 1.



**Figure 4.4:** Correspondence Analysis of treatments a) showing all treatments and attributes b) detail showing separation of samples in the cluster. Red circles denote 95% confidence intervals (NE = no enzyme, ENZ = enzyme treatment)

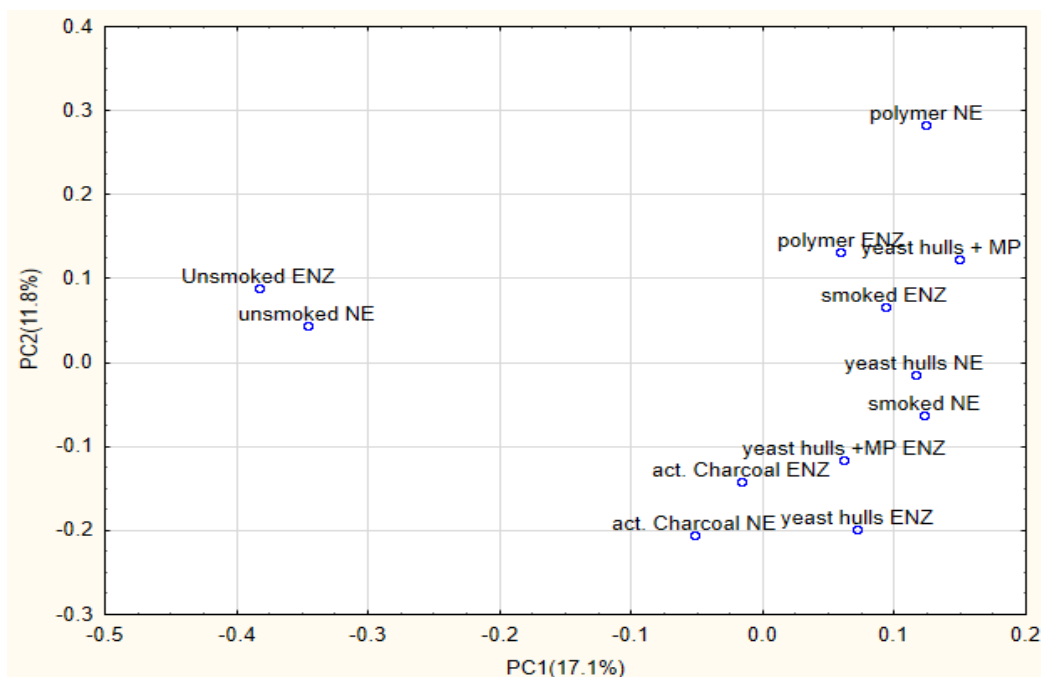
Although it is difficult to separate the attributes in this cluster, it can be seen that the smoky attribute is opposed along Dimension 1, and is furthest separated from the unsmoked control wines. Along Dimension 2, the 'woody', 'earthy', 'meaty', 'savory/soy' attributes are opposed to the 'rubber/plastic', 'herbaceous', 'smoky', 'pencil shaving/dusty', 'animal', 'tar', 'medicinal', and 'rubber/plastic' attributes in the negative quadrant of Dimension 2.

A cluster diagram of the taste/flavour (palate) sensory data (Figure 4.5) is presented. Figure 4.5 shows three groups at 0.6 linkage distance, indicating three major groupings in the samples regarding taste.



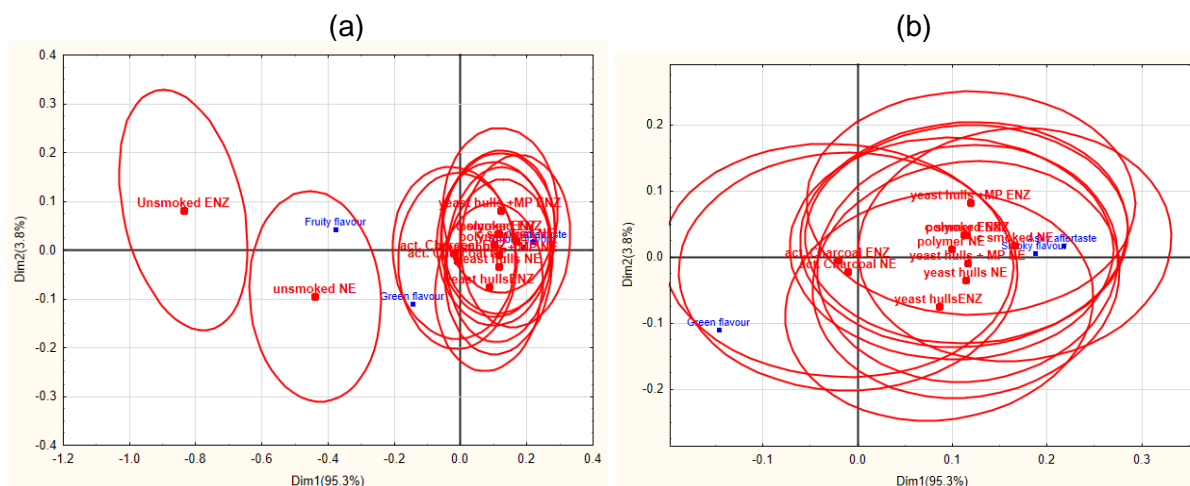
**Figure 4.5:** Dendrogram of responses generated by agglomerated hierarchical cluster (AHC) analysis of wine taste data. (NE = no enzyme, ENZ = enzyme treatment)





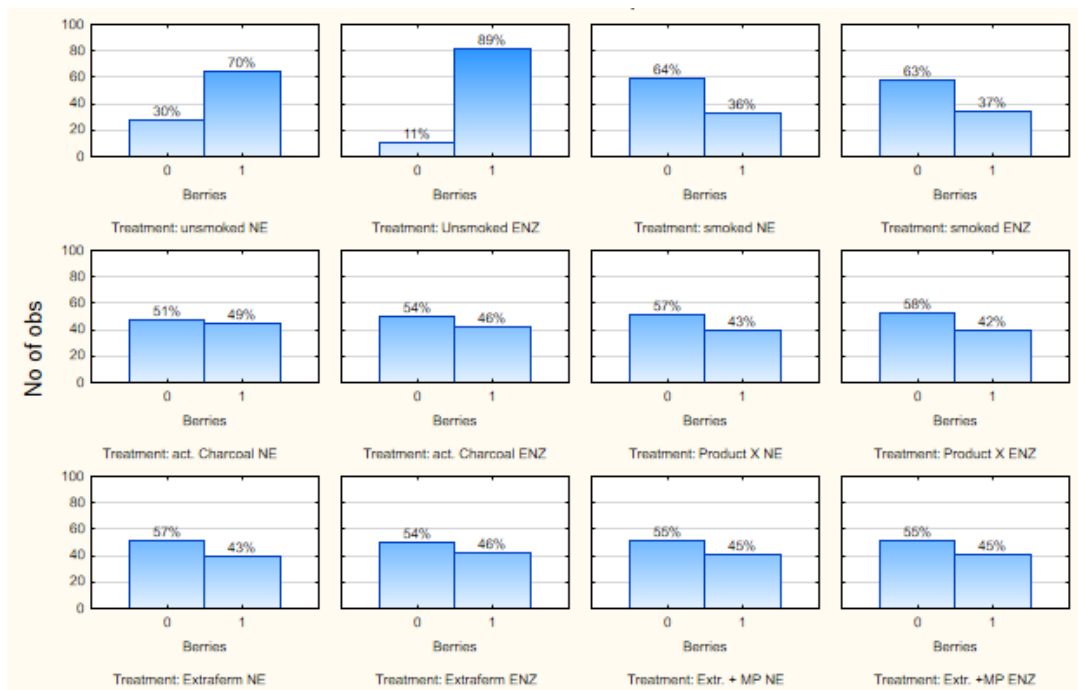
**Figure 4.6:** Principle Component Analysis Biplot for sensory data associated with taste/flavour of samples. (NE = no enzyme, ENZ = enzyme treatment)

This result was again emphasized by the Principle Component Analysis (Figure 4.6) which showed separation along both principle components for the two groups, although PC1 and PC2 only explain 29% of the variability within the dataset. This data indicates how closely related the treatments were to each other in terms of their lack of effect on the attributes as perceived by the panel during sorting. All the smoked treatments remained grouped on the positive side of PC1, whereas the controls are positioned together on the negative side of PC1. In terms of the second principal components, the yeast hulls, activated charcoal, polymer powder with enzymes and mannoproteins are grouped with the smoked controls, indicating that they did not cause a significant difference in terms of the perceived aroma of the samples. The polymer powder without enzyme does separate out slightly from the smoked controls (smoked ENZ and smoked NE) along PC2 indicating that these samples showed different attributes. These attributes are not clear in figure 4.7 as all the treatments are clustered between 'green flavour', and 'smoky flavour' with 'ashy aftertaste'



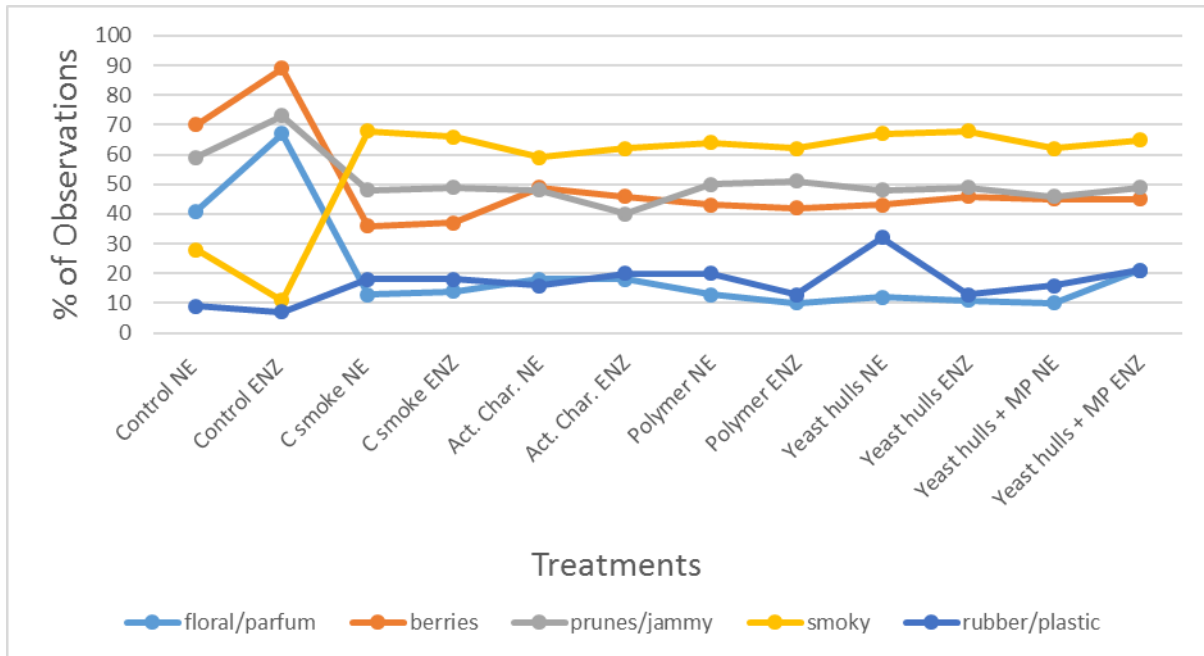
**Figure 4.7:** Correspondence Analysis of samples and taste attribute data a) showing all treatments and attributes b) detail showing separation of samples in the cluster. Red circles denote 95% confidence intervals. (NE = no enzyme, ENZ = enzyme treatment)

It is difficult to separate the samples according to the treatments in this cluster analysis (figure 4.7), however, it can be seen that the ‘green flavour’ is opposed along Dimension 2, and is furthest separated from the yeast hulls + mannoprotein wines. The unsmoked control that was not subjected to enzyme treatment (‘unsmoked NE’) is most associated with ‘fruitiness’ activated charcoal (NE and ENZ) was closest to ‘green flavour’ compared to the other smoked treatments. The ‘smoky flavour’, and ‘ashy aftertaste’ attributes were associated with the third cluster of treatments which had smoke applied.



**Figure 4.8:** Categorised histograms of data of observations of ‘aroma’ modality for ‘berries’ in rapid method sensory using chi-squares at significance  $p < 0.01$  using Rao & Scott adjustment. (NE = no enzyme, ENZ = enzyme treatment)

Figure 4.8 shows the percentage observations of 'berries' for each treatment. The unsmoked control with enzymes had the highest observations of 89% compared to the smoked control without enzymes which had 36% observations. This indicates that enzymes had an effect in releasing fruity/berry aroma from precursors during wine processing, which would be consistent with release of terpenoids from their glycosides. The data was significant at a  $p < 0.01$  level.

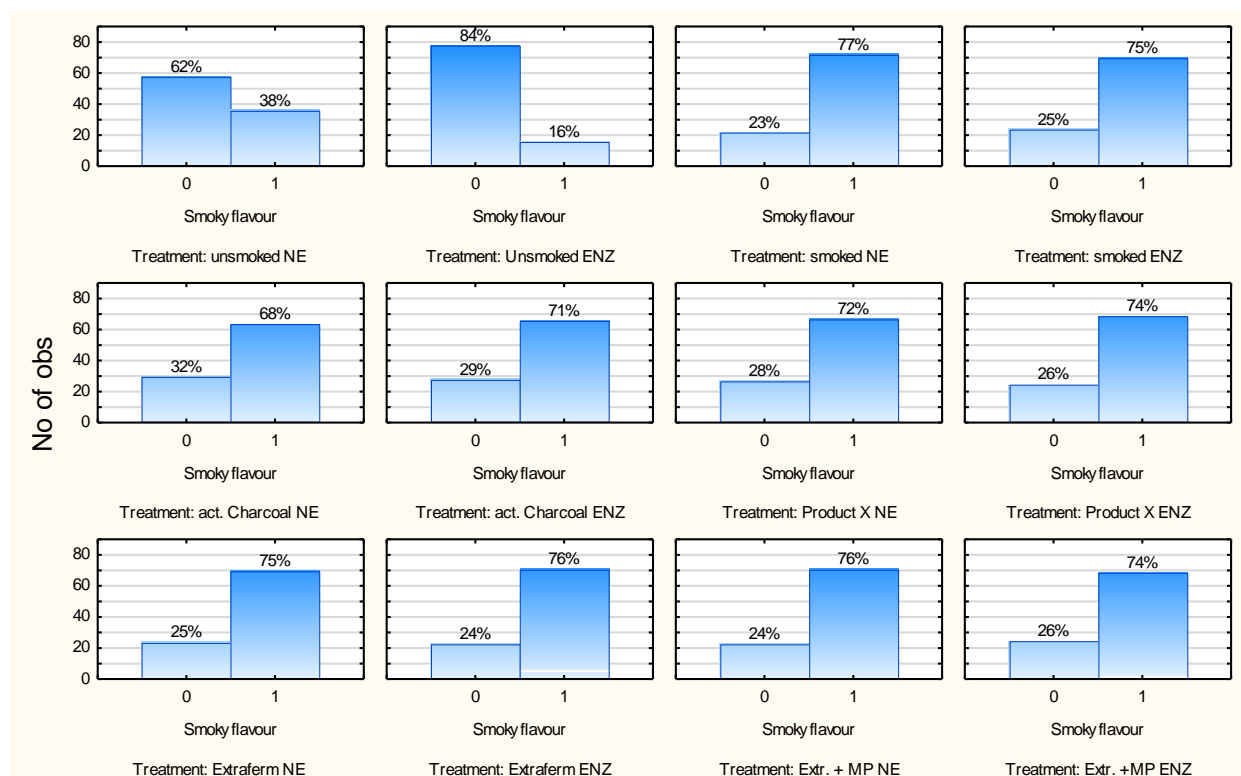


**Figure 4.9:** Condensed data from chi-squares comparing 'berries', 'floral/perfume', 'prunes/jammy' to 'smoky' and 'rubber/plastic' aromas.  $p < 0.01$  for the attributes used in this graph. (NE = no enzyme, ENZ = enzyme treatment)

Figure 4.9 represents condensed data previously shown in figure 4.8 for five aroma attributes in a group of 'fruity' ('fruity', 'floral/perfume', 'prunes/jammy') which are positive and 'smoky' ('smoky' and 'rubber/plastic') which are negative characteristics. The unsmoked control showed an increase in fruitiness and a decrease in smokiness after the addition of the enzyme. The application of smoke had the highest effect on 'floral/perfume' which was not perceived at high levels for any of the treatments. The smoked control has high levels of 'smoky' and a slight decrease after enzyme addition, this is followed by an increase of berries', 'floral/perfume', 'prunes/jammy'. Activated charcoal behaved the opposite to smoked control where with enzyme addition, decrease in positive aromas and an increase in negative aromas is represented in the data. The polymer powder treatment after enzyme addition, only increased 'prunes/jammy' and decreased the other attributes. Yeast hulls before enzyme treatment had the highest 'rubber/plastic' observations which decreased after enzyme treatment compared to smoked control (NE and ENZ). This may be because fining the wine plus an increase of 'smoky', 'berries', 'prunes/jammy' overshadowed the 'rubber/plastic' aroma. Yeast hulls + mannoproteins had all of the attributes increase after enzyme addition but the negative aromas were still lower than the smoked control and the positive aromas were higher.

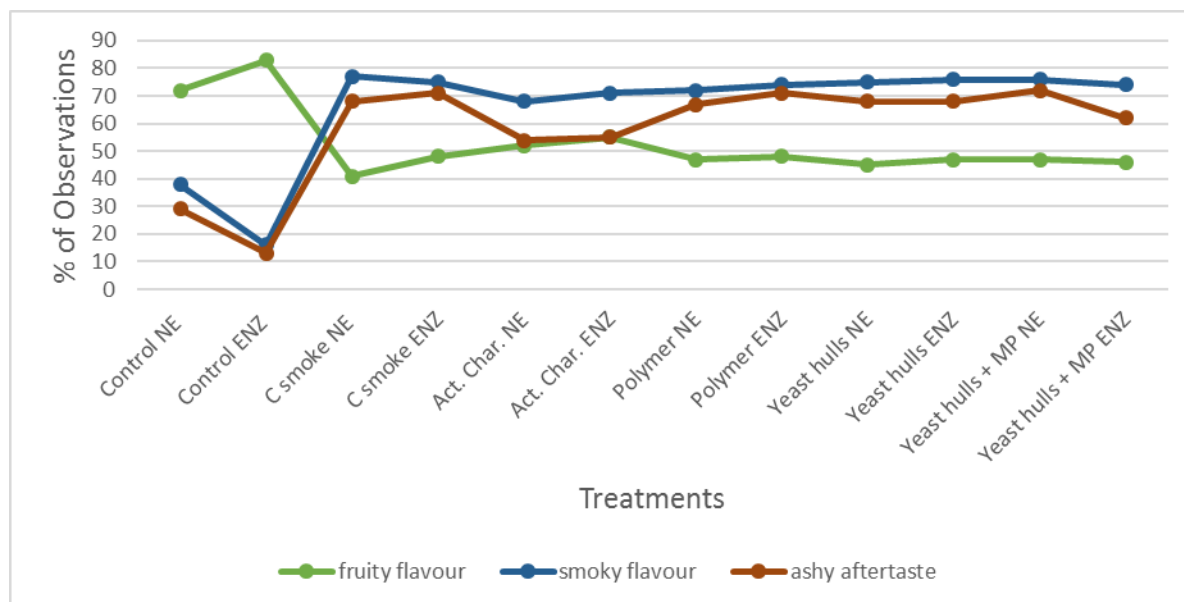
These results did confirm what has been said in literature on the potential of  $\beta$ -glucosidases as enhancers of aroma improvement in the wine (Mateo *et al.* 1997; Sarry *et al.* 2004; Villena *et al.* 2007; Reynolds *et al.* 2010; Parker *et al.* 2012; Baffi *et al.* 2013; Hjelmeland *et al.* 2015). There was an increase in perceived overall fruitiness with the addition of the enzyme.

Figure 4.10 shows the percentage observations of 'smoky flavour' for each treatment. The unsmoked control with enzymes had the lowest observations of 16% compared to the smoked control without enzymes which had 77% observations. This data set illustrates the effects of each treatment on the 'smoky flavour' attribute. The data was significant with  $p < 0.01$ .



**Figure 4.10:** Categorised histograms of data of observations of 'taste' modality for 'smoky flavour' in rapid method sensory using chi-squares at significance  $p < 0.01$  using Rao & Scott adjustment. (NE = no enzyme, ENZ = enzyme treatment)

Figure 4.11 shows condensed data of all histograms obtained from the panel using chi-squares with significant results of  $p < 0.01$ . The unsmoked control and the smoked control had an increase in 'fruity flavour' and a decrease in 'smoky flavour', and 'ashy aftertaste' after the enzymes were added. This shows that a decrease of in-mouth release of VPs from their glycoside is possible with the addition of enzymes even if no fining treatment was applied to smoked wines. This decrease in glycosides did not increase the 'smoky' aromas observed (figure 4.9) but this may be because the release of fruity aromas brought complexity to the wine which suppressed the 'smoky' aroma.



**Figure 4.11:** Condensed data from chi-squares comparing 'fruity flavour', 'smoky flavour', and 'ashy aftertaste' flavours.  $p < 0.01$  for the attributes used in this graph. (NE = no enzyme, ENZ = enzyme treatment)

Activated charcoal provided the highest decrease in 'ashy aftertaste' compared to all the treatments. 'Smoky flavour' was increased after enzymes, indicating that the panel perceived higher levels of VPs in the treated samples. Yeast hulls with enzymes also decreased 'ashy aftertaste' and 'smoky flavour' was also decreased by this treatment, which is promising. Although polymer powder provided a general decrease in negative flavours, an increase was still observed after enzyme addition and the 'fruity flavour' was also decreased.

#### 4.3.2 Chemical analyses

Chemical analysis was carried out using GC-MS at CAF at Stellenbosch University. The samples were analysed after filtration and bottling. Ten volatile phenols were quantified in triplicate using the method outlined in Chapter 3. The VPs analysed were guaiacol, 2,6-dimethylphenol, 4-methylguaiacol, o-cresol, phenol, 4-ethylguaiacol, m-cresol, p-cresol, eugenol, and 4-ethylphenol. Due to issues experienced during the later phases of the instrumental analysis, the treatment of yeast hulls + mannoproteins with enzymes had only one sample quantified and therefore this could not be included in statistical analysis.

Table 4.3 shows the results obtained from analysis of the wines after bottling.

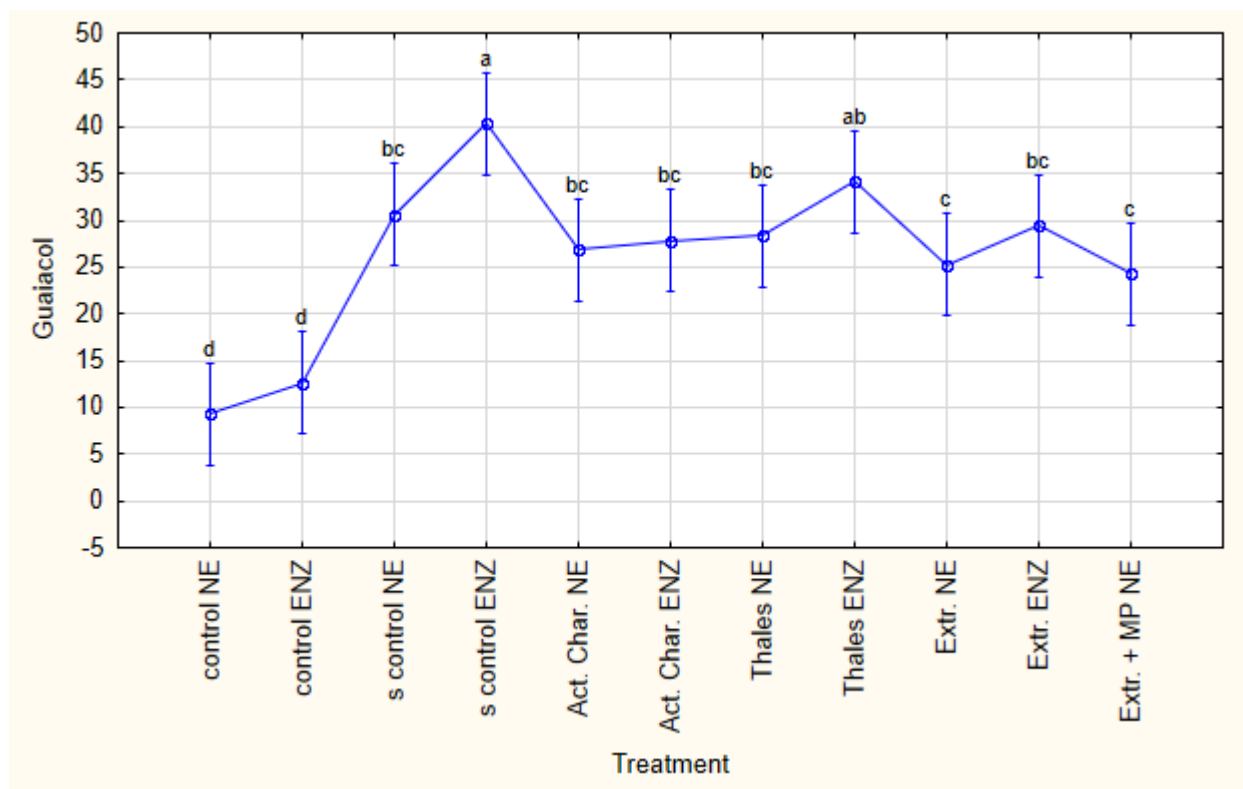
The use of enzymes in the study was to release the VPs from their glycosides before treating with fining products. The smoked control ENZ constantly showed the highest levels of VPs and unsmoked control NE presented the lowest levels of VPs. From these results it can be seen that enzymes were able to release 3.32  $\mu\text{g/L}$  more guaiacol in unsmoked controls than was present in the original samples, and also released 9.76  $\mu\text{g/L}$  more guaiacol from smoked controls.

**Table 4.3:** Volatile phenol results for analysis by GC-MS (averages of instrumental triplicates)

Treatment	Guaiacol µg/L	2,6- Dimethyl phenol µg/L	4 Methyl Guaiacol µg/L	o-cresol µg/L	phenol µg/L	4 Ethyl Guaiacol µg/L	m-cresol µg/L	p-cresol µg/L	Eugenol µg/L	4 Ethyl phenol µg/L
Control NE	9.29 ± 1.32	1.24 ± 0.16	0.36 ± 0.06	0.99 ± 0.28	0.92 ± 1.66	0.45 ± 0.08	1.52 ± 0.21	0.92 ± 0.18	1.08 ± 0.11	0.64 ± 0.09
Control ENZ	12.61 ± 0.56	1.11 ± 0.10	0.60 ± 0.05	1.95 ± 0.20	4.31 ± 0.52	0.41 ± 0.02	2.70 ± 0.27	1.43 ± 0.08	1.88 ± 0.30	0.85 ± 0.06
C smoke NE	30.58 ± 0.36	1.79 ± 0.03	7.09 ± 0.18	6.19 ± 0.25	42.64 ± 0.57	1.70 ± 0.03	7.69 ± 0.32	3.46 ± 0.06	1.12 ± 0.07	2.92 ± 0.06
C smoke ENZ	40.34 ± 11.24	3.35 ± 1.60	11.31 ± 4.98	9.33 ± 4.01	93.81 ± 12.45	2.87 ± 1.60	16.78 ± 7.82	12.35 ± 6.77	3.33 ± 2.60	6.58 ± 3.60
Act. Char. NE	26.87 ± 0.48	1.57 ± 0.02	5.71 ± 0.09	5.13 ± 0.03	40.69 ± 1.11	1.21 ± 0.02	6.07 ± 0.74	3.22 ± 0.10	0.75 ± 0.01	2.13 ± 0.06
Act. Char. ENZ	27.86 ± 1.01	1.63 ± 0.11	6.08 ± 0.50	5.81 ± 0.54	64.13 ± 2.93	1.21 ± 0.08	10.05 ± 0.62	5.16 ± 0.44	1.00 ± 0.08	3.00 ± 0.13
Polymer NE	28.34 ± 0.70	1.72 ± 0.08	6.43 ± 0.20	5.81 ± 0.14	39.56 ± 1.92	1.55 ± 0.08	7.76 ± 0.29	3.34 ± 0.20	1.05 ± 0.08	2.60 ± 0.15
Polymer ENZ	34.09 ± 5.57	2.95 ± 2.12	8.45 ± 2.28	7.06 ± 0.70	85.86 ± 7.91	1.90 ± 0.51	12.62 ± 1.66	8.80 ± 5.28	1.67 ± 0.58	4.35 ± 1.28
Yeast hulls NE	25.24 ± 0.66	1.52 ± 0.07	6.14 ± 0.26	5.45 ± 0.19	34.76 ± 0.77	1.43 ± 0.09	7.20 ± 0.45	2.95 ± 0.23	0.98 ± 0.07	2.40 ± 0.16
Yeast hulls ENZ	29.44 ± 8.19	2.85 ± 2.06	7.27 ± 2.80	5.98 ± 1.96	57.44 ± 1.78	1.68 ± 0.60	9.11 ± 4.97	6.50 ± 6.71	1.37 ± 0.83	3.39 ± 1.98
Yeast hulls + MP NE	24.26 ± 0.62	1.45 ± 0.09	5.64 ± 0.15	4.98 ± 0.18	42.01 ± 10.45	1.26 ± 0.10	6.62 ± 3.10	3.56 ± 0.78	1.03 ± 0.04	2.66 ± 0.33
Yeast hulls + MP ENZ	24.94	1.43	5.67	5.13	48.77	1.21	8.73	4.19	1.1	3.01

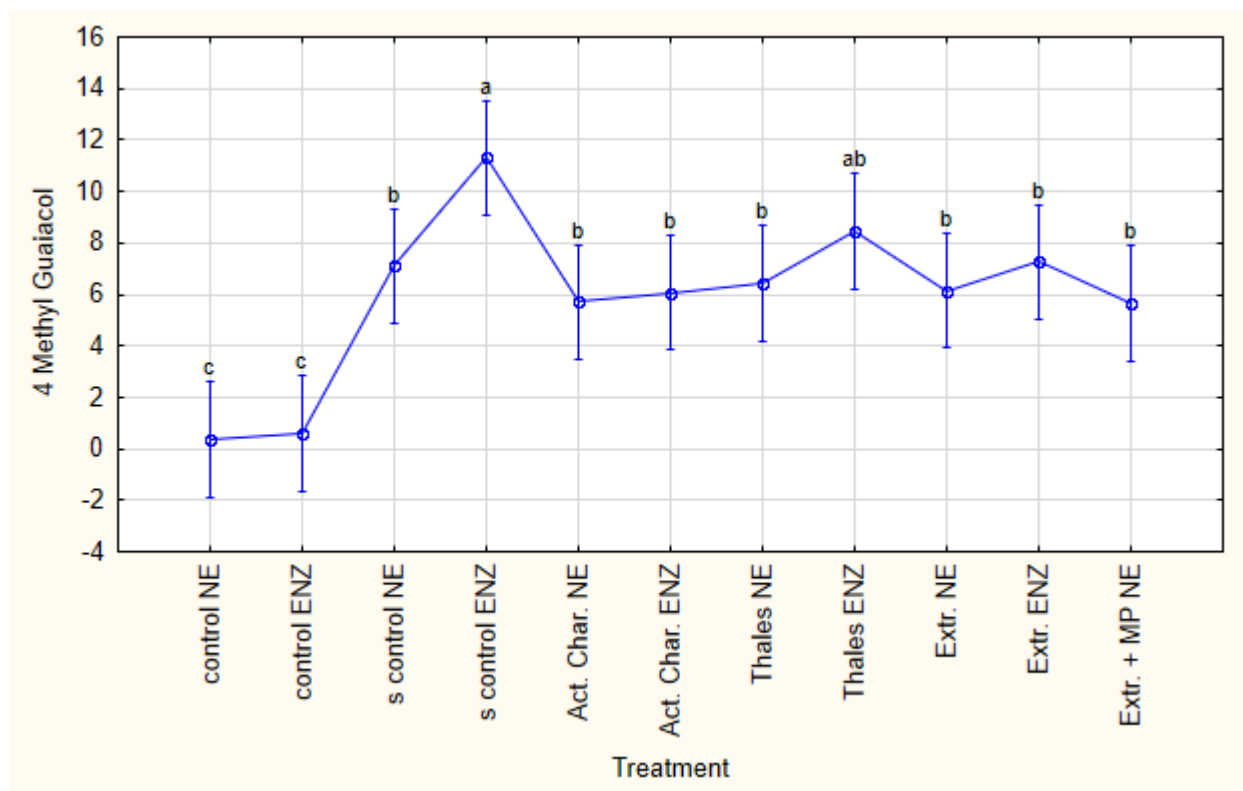
There was a clear general trend of treatments decreasing the number of VPs present both with and without enzyme addition when compared to smoked controls. Another overall trend observed was the increase in available VPs after the treatment with enzymes, indicating that the levels of fining agent might need adjusting for the higher levels of VPs released by the enzymes. The only exception was the yeast hull treatment which had a decrease in VPs after the addition of enzymes (figures 4.12-4.16).

The changes at different stages of winemaking of VPs were monitored and are presented in full in Appendix F. The levels remained low in grapes. Guaiacol was 0.87 µg/L for unsmoked control (grapes) and 2.64 µg/L for smoked control (grapes) because most of the VPs are in their bound form at this stage (Kennison *et al.* 2008; Ristic *et al.* 2011). The increase was then observed after the completion of both alcoholic and malolactic fermentations (guaiacol: 10.53 µg/L unsmoked control; 25.86 µg/L smoked control). Decreases were then observed after the application of treatments which were activated charcoal, polymer powder, yeast hulls, and yeast hull + mannoproteins.



**Figure 4.12:** LS Means diagrams (Type II decomposition) showing chemical results for guaiacol in controls and treatments; Vertical bars denote 95% confidence intervals. ( $p < 0.01$ ) (ENZ= treated with enzymes; NE = not treated)

Guaiacol (figure 4.12) demonstrated a trend observed for all treatments, where treatments with enzyme added had higher levels than those without. The smoked control with enzymes had the highest level of guaiacol above  $35 \mu\text{g/L}$ , which is above the ODT of  $23 \mu\text{g/L}$  (Ferreira *et al.* 2000 and Parker *et al.* 2012). The treatment that had the highest decrease post fining was the mix of yeast hulls and mannoproteins without enzymes, guaiacol was below  $25 \mu\text{g/L}$ . The polymer powder treatment showed elevated levels of guaiacol above  $30 \mu\text{g/L}$  in the wines that had enzymes- higher than the wines that did not. In literature the re-release (Dombre *et al.* 2014) of VPs into wine can be experienced with this product. In this case, addition was very carefully monitored, and time was kept constant for the polymer powder treatment (NE and ENZ). This may then indicate the decrease of the efficacy of the treatment with increased VP concentration, so a higher dosage may be effective in the removal of VPs.

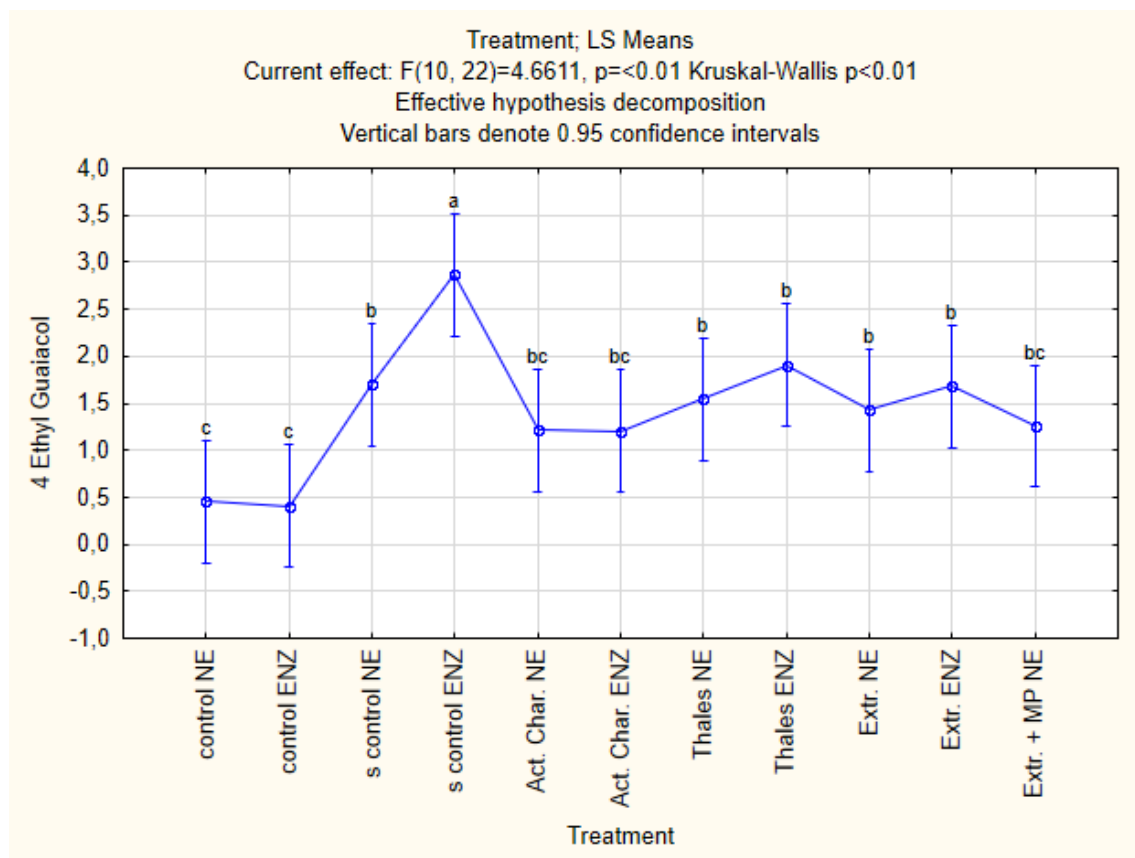


**Figure 4.13:** LS Means diagrams (Type II decomposition) showing chemical results for 4-methylguaiacol in controls and treatments; Vertical bars denote 95% confidence intervals. ( $p < 0.01$ ). (ENZ= treated with enzymes; NE = not treated)

The same trends were observed for 4-methylguaiacol (Figure 4.13) as were shown for guaiacol. Smoked controls had the highest measured 4-methylguaiacol concentrations while polymer powder ENZ had the third highest measured levels. There was a trend for increasing levels of 4-methylguaiacol after the addition of the enzyme in a treatment except for yeast hulls which decreased from above 7.3  $\mu\text{g/L}$  NE to below 5.6  $\mu\text{g/L}$ .

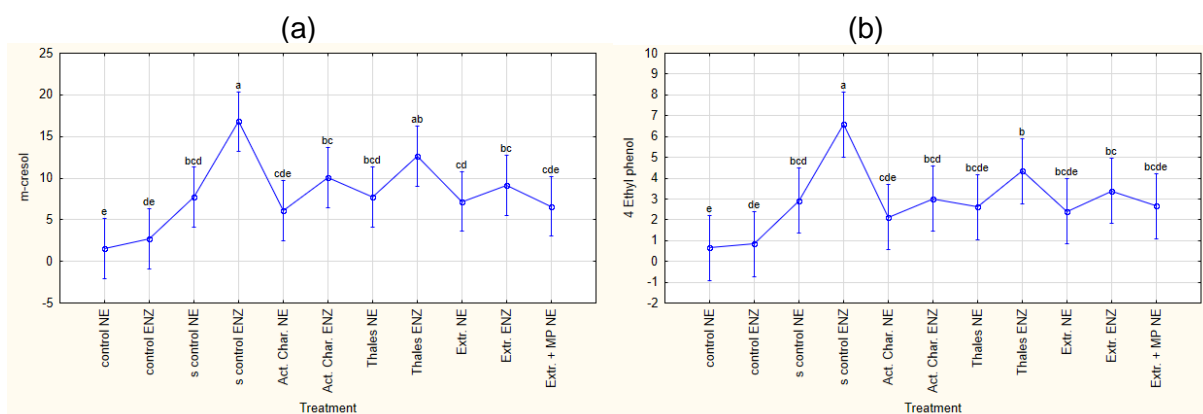
The data can be linked to sensory results (figure 4.11) where an increase in ashy aftertaste was experienced with the addition of the enzyme for all treatments except for yeast hulls and yeast hull + mannoproteins. 4-methylguaiacol has been correlated with the increase in ashy aftertaste alongside guaiacol, 4-methylsyringol, phenol, o-cresol, and m-cresol (Parker *et al.* 2012).

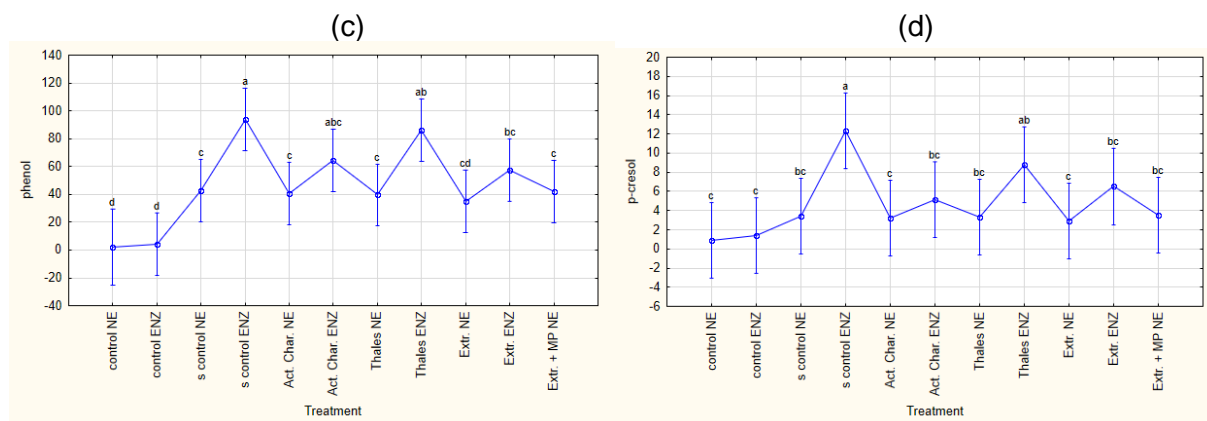




**Figure 4.14:** LS Means diagrams (Type II decomposition) showing chemical results for 4-ethylguaiacol in controls and treatments; Vertical bars denote 95% confidence intervals. ( $p<0.01$ ). (ENZ= treated with enzymes; NE = not treated)

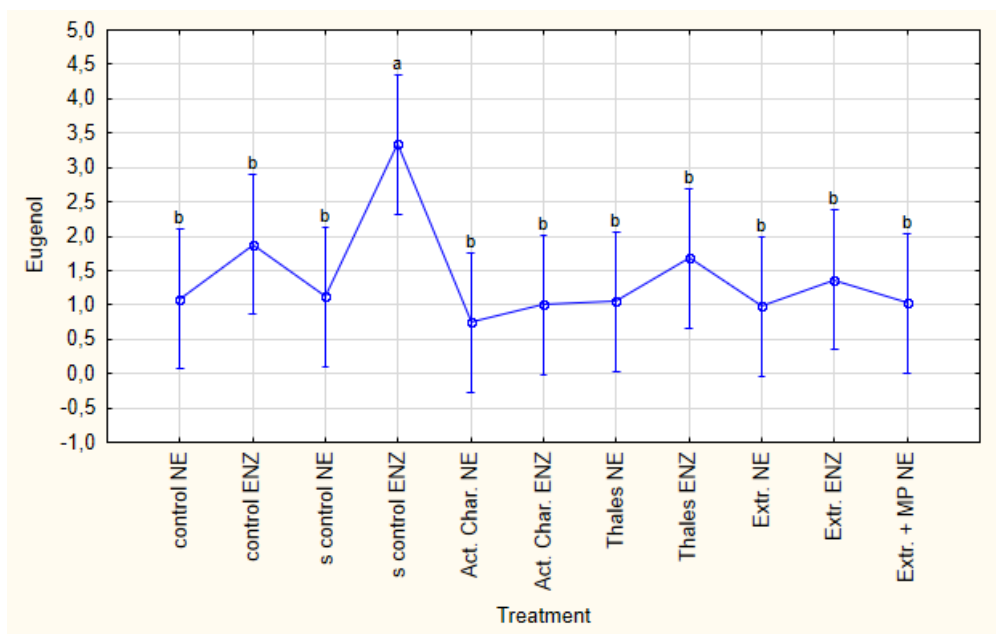
Activated charcoal showed the highest decrease in 4-ethylguaiacol (Figure 4.14). This can be linked to figure 4.11, where the 'smoky flavour' and 'ashy aftertaste' experienced a decrease. 4-ethylguaiacol provides smoke, spicy and toasted aromas to wine (Kennison *et al.* 2008) and a decrease in 'smoky flavour' was observed in activated charcoal while an increase in 'smoky flavour' was seen with the increase in this compound for the other treatments.





**Figure 4.15:** LS Means diagrams (Type II decomposition) showing chemical results for (a) m-cresol, (b) 4-ethylphenol, (c) phenol, and (d) p-cresol in controls and treatments; Vertical bars denote 95% confidence intervals. ( $p < 0.01$ ). (ENZ= treated with enzymes; NE = not treated)

Phenol, 4 ethyl phenol, m-, and p-cresol showed extremely high increases when enzymes were added (Figure 4.15). Concentrations of double and higher are shown in Table 4.3 between no enzyme additions to enzyme addition. m-cresol, 4-ethylphenol, phenol, and p-cresol had a change of 1.52 µg/L to 2.7 µg/L; 0.64 µg/L to .085 µg/L; 0.92 µg/L to 4.31 µg/L; 0.92 µg/L to 1.43 µg/L for unsmoked control and the changes for unsmoked control were 7.69 µg/L to 16.78 µg/L; 2.92 µg/L to 6.58; µg/L; 42.64 µg/L to 93.81 µg/L; 3.46 µg/L to 12.35 µg/L, respectively. Phenol has sickeningly sweet, irritating aromas (Parker *et al.* 2012; Panzeri 2013), m-cresol has dry, tar, and medicinal-leathery aromas (Parker *et al.* 2012), p-cresol has band-aid, phenol-like aromas (Parker *et al.* 2012) and 4-ethylphenol has barnyard, horsey, phenolic aromas (Kennison *et al.* 2009). Most of these were not observed significantly in sensory evaluation but the phenolic and sweet character might have contributed to the 'berries', prunes/jammy', and 'floral/perfume' (Figure 4.9). Yeast hulls had the highest decrease of all the treatments. By adding enzymes, the yeast hulls showed a slight decrease in the treatment that had enzymes added. Literature has explored the adsorptive abilities of yeast lees and in turn yeast hulls (Ribereau-Gayon *et al.* 2007; Pradelles *et al.* 2008; Reynolds 2010; Kheir *et al.* 2013) as detoxifying agents in fermentations. The result displayed here where a decrease in VPs is seen after the addition of yeast hulls further illustrates the efficacy of yeast hulls as VP fining agents.



**Figure 4.16:** LS Means diagrams (Type II decomposition) showing chemical results for eugenol in controls and treatments; Vertical bars denote 95% confidence intervals. ( $p < 0.01$ ). (ENZ= treated with enzymes; NE = not treated)

Eugenol shows an increase after enzyme addition for all treatments (figure 4.16). The controls both smoked and unsmoked showed the highest increases. While the treatments of activated charcoal, polymer powder, yeast hulls and mannoproteins had general decreases for both enzyme and no enzymes compared to the controls but slight increases per pair of treatments. The eugenol levels had little effect in the aroma results as 'woody' did not produce significant results when the chi-square test was done of the data. As eugenol is associated with cloves and spice (Escudero *et al.* 2007), this might have contributed to the fruity and sweet aromas observed by the panel (figure 4.9)

Overall it appeared that ENZ treatments increase the release of VPs which may be associated with the smoke taint attributes. The fining treatments did not however, seem to sufficiently decrease levels of guaiacol, 2,6-dimethylphenol, 4-methylguaiacol, o-cresol, phenol, 4-ethylguaiacol, m-cresol, p-cresol, eugenol, and 4-ethylphenol except for yeast hulls. It may also be the case that the enzymes continued hydrolysing glycolysates during the post-bottling period, once the fining agents had been removed. The dosage of fining agents may also not have been adequate to deal with the higher levels of VPs after release by enzymes. The dosage levels may either be increased per application or kept the same and the number of repeat applications could be increased. This however, has implications on costs. The producer would then have to weigh the cost of losing a product because of smoke taint against the cost of treating a tainted wine.

#### 4.4 Conclusions

Volatile phenols (VPs) are well known culprits when it comes to smoke taint in wine but their bound precursors (glycoconjugates) pose a greater threat to the quality of the wine as they are not

perceived during the winemaking process. Thus the removal of these glycoconjugated precursors may be the differentiating factor between wine that is palatable and wine that is not after a fire incident. It has been well documented in literature that slow acid hydrolysis and enzyme hydrolysis are the two main processes from which VP can be released from their glycoconjugated forms. Acid hydrolysis will act on glycolysates during bottle-aging, releasing VPs which can then be perceived by consumers. It has long been believed that marketing the wines for earlier release after a smoke incident may bring about better returns of investments and prevent in-bottle release of VPs.

In this study, the aims were to test commercial  $\beta$ -glucosidases enzymes as hydrolysis agents for the early release of VPs, and the efficacy of subsequent removal of the VPs by fining using legal winemaking additives.

To address the first aim of the study, viz. to hydrolyse volatile phenols (VPs) and their sugar moieties (glycoconjugated VPs) through the addition of commercial  $\beta$ -glucosidase enzymes, post-fermentation, smoked and unsmoked wines were treated with commercial enzymes after fermentation. Released VPs were monitored chemically and effects of treatments were assessed sensorially in all treated and untreated wines. From the data obtained the enzymes were able to release a significant number of VPs from their bound forms with a range that was up to 80% increase for smoked controls. Control wines were associated with positive fruity aroma attributes, and fruity flavour attributes, whereas there were significant increases in berries', 'floral/perfume', 'prunes/jammy' attributes after enzyme treatments.

The second aim was to apply four fining treatments to remove liberated VPs after the  $\beta$ -glucosidase enzyme was added into the wine. Treatments included activated charcoal, polymer powder, yeast hulls and mannoproteins. Results were monitored through chemical and sensory evaluation of the treated wines and unsmoked controls. The treatments were able to decrease the VPs to a certain extent but complete or significant removal was not achieved because smoke aroma and taste were still perceived even after the treatments were applied. Activated charcoal has the biggest effect in aroma, flavour, and VPs by having the highest decrease compared to the other treatments. The mixture of yeast hulls and mannoproteins show promising results for removal of VPs after enzyme treatment, and may provide another alternative to decreasing aroma and taste. However, the chemical results were inconclusive because of issues with instrumental analysis in the later stages of the project.

This study provides a practical and affordable way to speed the process of hydrolysis, but the efficacy of fining treatments needs to be tested further. The increase in fruitiness with addition of enzymes was observed, and this also brought a decrease in perceived smoke aroma. It is thus recommended for winemaking and future studies based on the results of these trials that the experiment be repeated over a longer period so all aspects can be explored. Keeping the wine longer before bottling may allow for  $\beta$ -glucosidase activity to complete, decreasing chances of further release in the bottle. Therefore, the determination of the time frame of which wines can be

kept is important, while also treating the wines. Aspects such as changing fining treatment dosages or increasing application frequencies need additional research.

## 4.5 References

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## Chapter 5: Discussion and conclusions

### 5.1 General discussion and conclusions

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Ever since the publication by Høj *et al.* in 2003, where the first recorded smoke taint in wine was reported, there have been strides to understand the phenomenon. Smoke taint is characterised by ‘smoky’, ‘burnt’, ‘burnt rubber’, ‘ashtray’, ‘cold ash’, ‘smoked meats’, ‘smoked foods’, ‘leather’, ‘disinfectant/hospital’, ‘medicinal’, ‘earthy’ aromas (Høj *et al.* 2003, Kennison *et al.* 2007; 2009; Whiting & Krstic 2007; Hayasaka *et al.* 2010; 2013; Parker *et al.* 2012) with “an excessively drying back-palate and retronasal ash character” (Hayasaka *et al.* 2013). A number of volatile phenols (VPs) are responsible for these flavours and aromas and strategies have been investigated to remove these compounds and related smoke taint flavours (Høj *et al.* 2003; Whiting & Krstic, 2007; Kennison *et al.* 2008; Simos 2008; Ulrich 2009; Fudge *et al.* 2011; Ristic *et al.* 2011; Singh *et al.* 2011). It has also been determined that most of the VPs are stored in wine as glycoconjugated moieties (Kennison *et al.* 2008; Hayasaka *et al.* 2010a; Dungey *et al.* 2011; Ristic *et al.* 2011).

In this study, a South African (SA) context for smoke taint was explored, using commercially available additives that are legally permissible according to current SA legislation for fining.

In the first part of the study, covered in Chapter 3 of this thesis, the efficacy of three legal additives on deliberately smoke-tainted wines for removal of VPs and smoke taint was tested. Commercial treatments were selected on the basis of anecdotal or published smoke taint-reduction properties. The products used were an oak extract (for masking of smoke taint), activated charcoal and a polymer powder for the removal of smoke taint. It was, of course, important to test efficacy of additives at different levels, so two levels were chosen: one level recommended by the manufacturer and the second level double the initial dosage. The reduction aspect focussed on either removal or masking of smoke-related compounds in wines.

In order to assess the efficacy of the treatments, wines were made from deliberately smoked grapes, and then controls and smoked wines were treated and analysed chemically and sensorially for success in reduction of taint in comparison with unsmoked controls. To investigate the effects of amelioration treatments over time, the project wines made were retested in a year later for VPs and effect on aroma and taste attributes. For quantifying VPs in treated and untreated samples, an existing GC-MS method was used, in conjunction with sensory evaluation by Descriptive Analysis (DA) at one month and again, one year after bottling.

In order to check the potential for smoke-affected wines to manifest a taint after slow acid hydrolysis of precursors during bottle-aging, an additional part to the first study was to investigate the potential for hydrolysis as a strategy for removing glycosides. This was achieved by carrying out a complete enzyme hydrolysis on all experimental wines, and monitoring VPs before and afterwards in order to determine the concentration of glycoconjugated precursors, and the potential for smoke-taint development.

Results (chemical as well as in sensory analysis) of the fining experiments showed that activated charcoal was most successful at removing undesirable and smoky attributes in the wine and also provided the greatest decrease in concentrations of VPs. Chemical analysis showed that activated charcoal at the higher level had the highest decreases of VPs throughout compared to the smoked control. This treatment, however, also removed fruitiness. This resulted in lower perceived levels of positive aromas such as 'floral/perfume' and an increase in aromas such as 'savory/meaty'. Thus, because of the stripping effect of the charcoal treatment (López *et al.* 2001), it appeared that a number of flavour compounds that contribute to better wine quality were removed.

The oak extract was successful in increasing 'woody' attribute and introducing the 'caramel/vanilla' attributes, especially at double the manufacture's recommended level. However, these positive aspects were obscured by the fact that the smoke aromas and available VPs remained at high levels. In fact, none of the treatments had an effect on the taste of the wine, which remained 'smoky' and 'ashy'. Although there were some differences found between smoked and unsmoked treatments regarding the aroma of the wine, none of the smoked treatments had an effect on the flavour (palate) of the wine. This agrees with findings by Wilkinson *et al.* (2011) and Mayr *et al.* (2014), in which the majority of VPs were found to be stored in glycosylated forms in the wines and could be released by in-mouth enzymes. An additional aim of the first study was thus to investigate the potential for release of VPs from glycosides, and for smoke-affected wines to manifest a taint after slow acid hydrolysis during bottle aging. Through carrying out a complete enzyme hydrolysis, and monitoring VPs before and afterwards in order to determine the concentration of glycosylated precursors, it was shown that VPs increased in the smoked wines. The increase in VPs showed extremely high risk potential for wines to develop smoke taint even after thorough fining.

The second year of analysis of wines made during the first part of the project yielded promising results with the PCA showing a stronger differentiation between treatments compared to the first year with activated charcoal showing a shift towards being associated with positive attributes of 'floral', 'caramel' and 'berries'. Eugenol measured the highest in the oak extract treatment and could be linked to the increased 'woody' attribute in sensory analysis. The VPs still remained at varying levels in comparison to their odour thresholds after one year in bottle.

Bound smoke taint precursors pose a greater threat to the quality of the wine and their removal can be the differentiating factor between wine that is palatable and wine that is not after a fire incident. The second major aim of the overall project was related to the need to completely hydrolyse glycosylated precursors (glycoconjugated VPs) in wine in order to ensure no 'late development' of smoke taint to be perceived by consumers. As shown in Chapter 3, VPs in smoked wine could be hydrolysed from sugar moieties through the addition of commercial  $\beta$ -glucosidase enzymes post-fermentation. Chapter 4 of the thesis further elucidated the 'release and remove' strategy that had been suggested by the results in Chapter 3.



To hydrolyse volatile phenols (VPs) and their sugar moieties (glycoconjugated VPs) through the addition of commercial  $\beta$ -glucosidase enzymes post-fermentation, smoked and unsmoked wines were treated with commercial enzymes after fermentation. In the second study, four fining treatments were applied to the enzyme treated wine in order to remove liberated VPs after the  $\beta$ -glucosidase enzyme treatment. Treatments tested included activated charcoal, polymer powder, yeast hulls and mannoproteins. Results were again monitored through chemical (GC-MS) and sensory evaluation of the treated wines and unsmoked controls. As the study in Chapter 3 had indicated, the panel experienced fatigue during the lengthy DA process, and thus the wines were assessed using a combination of rapid sensory mapping and polarised sensory positioning.

From the data obtained, it was shown that the enzymes were able to release a significant number of VPs from their bound forms with a range that was up to 80% increase for smoked controls. Control wines were again associated with positive fruity aroma attributes, and fruity flavour attributes, and there were significant increases in 'berries', 'floral/perfume', 'prunes/jammy' attributes after enzyme treatments, showing that these enzymes can have a positive effect on wine quality. In the smoked wines, the  $\beta$ -glucosidase enzymes were shown by GC-MS to have released VPs. The fining treatments were able to decrease these VPs to a limited degree, but significant removal was not achieved because smoke aroma and taste were still perceived by sensory evaluation even after the treatments were applied. Chemical analysis also showed that VPs were present after fining. In terms of efficacy, activated charcoal again had the biggest effect on aroma, flavour, and VPs by having the highest decrease compared to the other treatments. The mixture of yeast hulls and mannoproteins showed promise in removing smoky taste and flavours. Through personal communication from Du Plessis (2019), recommendations can be made on the choice of yeasts, bacteria and type of wine to be made as these play an important role in the release VPs. Rosé wines can be made from red grapes as lower VPs have been found and no MLF is needed for such wines as bacteria has an influence on VPs present in the wine. Rosé wines can be made for early release by using yeast that prevent or limit the release of VPs. If the aim is to release more VPs then the use of the yeast that was chosen for this study would be sufficient. In this study, the use of QA23 and co-inoculation yeast did have an influence and that is why these organisms were chosen in the pilot study.

With any study, experience and lessons allow for insights thus wisdom to know what can be done differently or improved upon. An additional aim of this work was to try and make recommendations for winemaking and future studies based on the results. The levels of VPs generated by the smoke treatments in these experiments were higher than those that likely to be found in the industry after natural fire events, which means the study could be repeated on naturally tainted wines. There is thus a strong possibility that at those 'normal' smoke taint levels the treatments may have a significant decrease in VPs measured and smoke taint detected by consumers. It is thus recommended for winemaking and future studies based on the results of these trials that the

experiment be carried out on a longer period so all aspects can be explored. Based on this study, keeping the wine in the cellar for a longer period before bottling will allow for changing treatment dosages whether it be to increase or decrease depending on the needs of the wines or blending or the use of reverse osmosis to eliminate VPs. As an alternative increasing application frequency of treatments may remove VPs gradually as they develop and get released into the wine. Therefore, a possible study would be the determination of the time frame for which wines can be kept in the cellar for treatments. Moreover, once enzyme treatments have been applied, the use of activated charcoal at relatively high levels to remove smoke taint aroma could be tested, and then adding oak extract to increase positive aromas of woodiness and caramel/vanilla to provide complexity may be recommended.

This study has the potential to expand to product development of products to specifically target volatile phenols and their glycoconjugates. For example, a product that may show some potential as a treatment to target VPs is suberin from cork. Also, filtration systems and materials with adsorptive capabilities could be investigated.

This current amelioration of smoke taint project has been carried out in the South African context as there have been a limited number of studies on this subject matter. This work expanded on the research mostly done in Australia by Anthea Fudge which looked at reverse osmosis, solid phase adsorption and commercial fining agents as means of smoke taint reduction and removal of VPs.

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## APPENDICES

### Appendix A

**Table of sensory training standards**

	<b>Aroma</b>	<b>Standard</b>
1	Caramel	Moir's, essence caramel flavour
2	Honey	Spar, honey choice grade
3	Vanilla	Woolworths, vanilla essence and vanilla paste mix
4	Polish	Kiwi, black quality shoe polish
5	Raw meat	Spar, steak fillet
6	Medicinal	Jean Lenoir : Le Nez du Vin faults
7	Banana	Jean Lenoir : Le Nez du Vin
8	Tobacco	Domingo, 100% whole leaf tobacco natural
9	Cinnamon	Robertsons, cinnamon
10	Rubber	Elastic bands
11	Earth	Jean Lenoir : Le Nez du Vin faults
12	Liquorice	Jean Lenoir : Le Nez du Vin
13	Nutty	Jean Lenoir : Le Nez du Vin
14	Jammy	Freshers, strawberry jam
15	Red berries	Jean Lenoir : Le Nez du Vin
16	Prunes	Jean Lenoir : Le Nez du Vin
17	Cooked veg	Cooked green beans
18	Balsamic vinegar	Olyvenbosch, balsamic vinegar
19	Strawberries	Jean Lenoir : Le Nez du Vin
20	Black pepper	Robertsons, black pepper
21	Soy	Vital, soy sauce
22	Leather	Jean Lenoir : Le Nez du Vin faults
23	Barnyard	Jean Lenoir : Le Nez du Vin faults
24	Floral/violet	Jean Lenoir : Le Nez du Vin
25	Mushrooms	Jean Lenoir : Le Nez du Vin
26	Muscat	Jean Lenoir : Le Nez du Vin
27	Musk	Jean Lenoir : Le Nez du Vin
28	Ashy	Ash from ashtray
29	Green olives	Tuna marine, green olives in traditional brine
30	Plums	Jean Lenoir : Le Nez du Vin
31	Smokey	Burnt cork
32	Roasted coffee	Coffee beans with hot water
33	Dark berries	Jean Lenoir : Le Nez du Vin
34	Tar	Creosote
35	Pencil shavings	Staedtler HB shavings
36	Woody	Oak chips

## Appendix B

### 2017 DA tasting sheet

Judge _____	Date _____
<b>Aroma</b>	
Berries	none----- -----intense
Prunes/Jammy	none----- -----intense
Caramel/Vanilla	none----- -----intense
Floral/Perfume	none----- -----intense
Savoury/Soy	none----- -----intense
Woody	none----- -----intense
Meaty	none----- -----intense
Pencil shavings/Dusty	none----- -----intense
Smoky	none----- -----intense
Earthy	none----- -----intense
Tar	none----- -----intense
Medicinal	none----- -----intense
Animal	none----- -----intense
Rubber/Plastic	none----- -----intense

**Berries:** red and dark berries

**Prunes:** Prunes, raisins, jammy, tobacco

**Savoury:** savoury, balsamic, soy sauce

**Woody:** toasted oak, vanilla pod

**Meaty:** raw meat, metallic

**Smoky** smoky, ashy

**Animal:** barnyard, leather, musk

**Earthy:** earthy, mushrooms, mouldy



# Appendix C

## Tasting sheets for 2018 rapid method

Name .....

Date .....

**Tasting Instructions:**

1. Please **TASTE** the 12 wine samples presented
2. Sort the samples according smoke flavour and aftertaste
3. Provide 3-5 descriptors to explain and characterise the groups
  - a. Please use the provided list for taste description

None	Low	Medium	High/Intense
Smoky flavour and aftertaste	Smoky flavour and aftertaste	Smoky flavour and aftertaste	Smoky flavour and aftertaste
Sample numbers	Sample numbers	Sample numbers	Sample numbers
Descriptors	Descriptors	Descriptors	Descriptors

Please check that you sorted all the wines by ticking off the codes: 631, 461, 638, 850, 210, 519, 129, 891, 785, 269, 625, 908

Name .....

Date .....

**Tasting Instructions:**

1. Please **SMELL** the 12 wine samples presented
2. Sort the samples according smoky aroma
3. Provide 3-5 descriptors to explain and characterise the groups
  - a. Please use the provided list for aroma description

None	Low	Medium	High/Intense
Smoky aroma	Smoky aroma	Smoky aroma	Smoky aroma
Sample numbers	Sample numbers	Sample numbers	Sample numbers
Descriptors	Descriptors	Descriptors	Descriptors

Please check that you sorted all the wines by ticking off the codes: 440, 953, 119, 957, 354, 926, 874, 876, 968, 654, 761, 398

## Appendix D

### Volatile phenols results in 2017 (Y1)

Treatment	Guaiacol	2,6-dimethyl phenol	4-ethylguaiacol	o-cresol	phenol	4-ethylguaiacol	m-cresol	p-cresol	eugenol	4-ethylphenol	4-vinylguaiacol
control	7,10±0,35	0	1,08±0,06	0,19±0,02	3,20±0,04	0	0,61±0,03	0,22±0,02	0	0,39±0,04	0
s control	23,91±2,14	0,66±0,17	4,25±0,32	3,81±0,31	40,20±4,65	0,53±0,14	1,92±0,16	4,11±0,24	0,13±0,05	0,49±0,09	1,03±0,15
T1L1	27,08±0,52	0,86±0,06	4,58±0,11	4,47±0,23	46,55±0,47	0,66±0,07	2,22±0,13	4,78±0,22	0,16±0,01	1,21±0,11	1,34±0,09
T1L2	24,56±2,80	0,72±0,07	3,92±0,44	4,14±0,61	38,51±5,46	0,55±0,10	1,94±0,25	3,72±0,71	0,06±0,03	2,11±0,24	1,07±0,18
T2L1	23,68±1,60	0,42±0,07	3,82±0,24	3,73±0,31	39,78±2,81	0,43±0,06	1,88±0,17	4,09±0,28	0,08±0,03	0,24±0,02	0,99±0,08
T2L2	21,31±0,92	0,22±0,08	3,22±0,28	3,30±0,23	35,4±2,92	0,34±0,09	1,63±0,13	3,44±0,29	0,01±0,01	0,08±0,03	0,84±0,17
T3L1	23,88±0,80	0,65±0,06	3,98±0,19	3,75±0,22	40,17±2,33	0,53±0,04	2,03±0,16	4,16±0,27	0,14±0,01	0,51±0,03	1,24±0,07
T3L2	23,12±1,72	0,66±0,11	3,77±0,17	3,89±0,52	40,98±0,33	0,60±0,20	2,09±0,12	4,08±0,15	0,08±0,05	0,42±0,06	1,20±0,20

## Appendix E

### Volatile phenols results in 2018 (Y2)

Treatment	Guaiacol	2,6-dimethyl phenol	4-ethylguaiacol	o-cresol	phenol	4-ethylguaiacol	m-cresol	p-cresol	eugenol	4-ethylphenol	4-vinylguaiacol
control	6,83±0,75	1,25±0,08	0,30±0,033	1,40±0,42	4,93±2,45	0,45±0,10	1,95±0,63	0,95±0,30	1,06±0,18	0,35±0,05	2,69±0,66
s control	19,98±1,17	2,71±0,14	4,25±0,53	3,44±0,75	47,08±8,91	1,46±0,13	4,85±0,83	4,01±1,41	1,22±0,26	1,90±0,35	4,19±2,25
T1L1	23,49±4,55	2,95±0,51	5,01±0,84	3,97±0,92	53,77±13,27	1,70±0,36	5,76±1,60	3,85±0,74	1,97±0,44	2,11±0,42	3,21±0,56
T1L2	19,87±1,46	2,80±0,16	4,38±0,54	3,32±0,76	34,39±4,61	1,58±0,15	4,89±0,76	3,64±1,62	2,66±0,37	1,88±0,40	4,70±2,89
T2L1	18,87±0,97	2,34±0,14	3,98±0,36	3,87±0,30	40,63±3,94	1,24±0,10	4,53±0,31	3,62±0,76	0,84±0,08	1,70±0,18	2,87±0,33
T2L2	16,90±1,39	2,20±0,20	3,29±0,32	2,77±0,73	35,20±3,30	1,04±0,18	3,59±0,28	2,97±0,70	0,71±0,24	1,40±0,60	2,41±0,35
T3L1	19,55±1,64	2,60±0,04	4,04±0,51	3,39±0,31	40,00±5,42	1,45±0,15	4,62±0,44	3,19±0,42	1,11±0,12	1,83±0,24	3,15±0,21
T3L2	20,10±1,91	2,57±0,35	3,90±0,48	2,73±1,18	41,06±7,00	1,40±0,40	4,70±1,26	3,57±0,63	0,88±0,19	1,75±0,28	3,12±0,43



## Appendix F

### Pre-treatment with fining products VP changes

Treatment	Guaiacol	2,6 Dimethyl phenol	4 Methyl Guaiacol	o-cresol	phenol	4 Ethyl Guaiacol	m-cresol	p-cresol	Eugenol	4 Ethyl phenol	4 Vinyl Guaiacol
control grapes	1,00 ± 0,10	0,97 ± 0,06	0,01 ± 0,01	0,06 ± 0,05	n/a	0,14 ± 0,01	n/a	n/a	0,17 ± 0,05	0,06 ± 0,01	0,01 ± 0,01
s control grapes	2,48 ± 0,13	1,25 ± 0,17	0,06 ± 0,01	0,67 ± 0,05	n/a	0,18 ± 0,01	n/a	n/a	0,14 ± 0,01	0,18 ± 0,01	0,05 ± 0,07
control juice	0,56 ± 0,03	0,43 ± 0,05	n/a	n/a	n/a	0,14 ± 0,00	n/a	n/a	0,05 ± 0,01	0,05 ± 0,00	2,78 ± 0,32
s control juice	2,57 ± 0,30	0,80 ± 0,12	0,56 ± 0,12	0,36 ± 0,13	1,34 ± 0,91	0,39 ± 0,02	0,01 ± 0,01	0,04 ± 0,04	0,16 ± 0,05	0,17 ± 0,02	5,02 ± 1,33
control before enzyme	11,7 ± 15,04	1,24 ± 0,56	0,51 ± 4,59	1,85 ± 2,28	6,37 ± 26,33	0,65 ± 1,18	2,19 ± 2,83	1,14 ± 3,11	1,50 ± 0,23	0,79 ± 1,39	7,66 ± 0,66
s control before enzyme	43,35 ± 1,92	2,45 ± 0,10	10,43 ± 0,56	7,13 ± 0,47	60,14 ± 4,32	3,05 ± 0,16	9,10 ± 1,14	6,71 ± 0,81	1,87 ± 0,08	3,78 ± 0,17	8,75 ± 0,43
control no enzyme	11,74 ± 0,20	1,23 ± 0,03	0,47 ± 0,02	1,53 ± 0,04	5,89 ± 0,36	0,60 ± 0,02	2,46 ± 0,21	1,12 ± 0,16	1,41 ± 0,04	0,84 ± 0,07	8,51 ± 0,37
control enzyme	15,28 ± 1,00	1,13 ± 0,04	0,73 ± 0,08	2,32 ± 0,28	10,30 ± 1,28	0,64 ± 0,01	4,29 ± 0,66	2,10 ± 0,34	2,44 ± 0,34	1,13 ± 0,14	18,51 ± 3,48
s control no enzyme	40,16 ± 1,10	2,09 ± 0,05	9,474 ± 0,22	7,63 ± 0,20	56,90 ± 2,61	2,32 ± 0,08	11,77 ± 0,21	4,72 ± 0,38	1,61 ± 0,04	3,67 ± 0,09	8,98 ± 0,35
s control enzyme	39,56 ± 1,09	2,08 ± 0,09	9,48 ± 0,26	7,36 ± 0,32	82,89 ± 2,94	2,09 ± 0,07	15,99 ± 1,55	6,63 ± 0,19	1,77 ± 0,06	4,20 ± 0,14	11,49 ± 0,26