The impact of wine yeast strains on the aromatic profiles of Sauvignon blanc wines derived from characterized viticultural treatments

by

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Declaration

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Summary

Grape must is a complex medium, and during wine production numerous biochemical pathways and metabolic reactions are taking place simultaneously to produce a specific taste and aroma. Microorganisms, specifically yeast, play a key role in the formation of metabolites formed during alcoholic fermentation. Sauvignon blanc, a well studied grape cultivar, is known to have a versatile range of aroma profiles ranging from “green” to “tropical”. It has been broadly stated that a “green” Sauvignon blanc can be created in the vineyard and a “tropical” Sauvignon blanc can be created by selecting a specific yeast strain, and that the balance between “green” and “tropical” characters is essential for the final aroma profile. Except for grape-derived varietal aromatic compounds such as methoxypyrazines (*green*), volatile thiols (*tropical*) and monoterpenes (floral), yeast derived volatile compounds including esters, higher alcohols, fatty acids and carbonyl compounds will also contribute to the final wine aroma.

The main aim of this study was to assess how viticultural treatment-derived differences in grape must, can impact on aroma production when this grape must is fermented with different commercial wine yeast strains. The viticulture treatment focused on light intensity modulated through canopy treatment. Volatile aroma differences were compared for canopy and yeast treatments, specifically focusing on the fermentation derived bouquet (esters, higher alcohols, volatile fatty acids, carbonyl compounds and monoterpenes).

Results showed significant differences between initial must compositions, including titratable acidity, malic acid and yeast assimilable nitrogen. The volatile aroma compounds were also significantly impacted although no noticeable effect on the overall fermentation kinetics was observed.

Depending on the yeast strain differences in volatile compounds varied. A clear vintage effect is noticeable between volatile compounds affected by the treatments. Data generated in 2012 shows clear differences between ethyl- and acetate esters and could clearly be grouped according to yeast strain through multivariate analysis.

Sensory evaluation results could clearly be distinguished according to canopy treatment and to a lesser degree according to yeast strain used. This indicates that although yeast has a more prominent impact on the fermentative bouquet that develops during alcoholic fermentation the overriding aroma is primarily derived from grape-derived metabolites which can be manipulated by canopy treatments. None the less the difference in fermentation bouquet does add to the complexity of the wine especially in the case of fermentation derived “tropical” aromas including guava and passion fruit. In some cases where shaded grapes had higher ester concentrations, the resultant wine also had higher aroma quality.

This study has contributed to a better understanding of the complex relationships between canopy manipulation and yeast selection on aroma formation. The analysis of volatile aroma

alone however is not enough to understand the final perception of wine taste and further in-depth studies of the viticultural and oenological factors is needed.

In particular, this project has focused on a single vineyard over only two vintages. The general validity of the conclusions derived from this study therefore will require additional data sets.

Opsomming

Druiwemos is ‘n komplekse medium en tydens wynbereiding is daar verskeie biochemiese weë en metaboliese reaksies wat gelyktydig plaasvind om ‘n spesifieke smaak en aroma te produseer. Mikro-organismes, veral gis, speel ‘n sleutelrol in die vorming van metaboliete tydens alkoholiese gisting. Sauvignon blanc, ‘n goed bestudeerde druifkultivar, besit ‘n veelsydige reeks aromaprofiele wat wissel van “groen” tot “tropies”. Oor die algemeen word dit voorgehou dat ‘n “groen” Sauvignon blanc in die wingerd geskep word, terwyl ‘n “tropiese” Sauvignon blanc geskep kan word deur ‘n spesifieke gisras te selekteer, en die balans tussen “groen” en “tropiese” karakters is noodsaaklik vir die finale aromaprofiel. Behalwe vir druifafgeleide kultivarafhanklike aromatiese verbindings soos metoksipirasiene (*groen*), vlugtige tiole (*tropies*) en monoterpene (*blomagtig*), sal gisafgeleide vlugtige komponente, waaronder esters, hoër alkohole, vetsure en karbonielverbindings, ook tot die finale wynaroma bydra.

Die hoofdoelwit van hierdie studie was om te bepaal hoe verskille in druiwemos wat afkomstig is van wynkundige behandeling ‘n impak op aromaproduksie kan hê wanneer hierdie druiwemos met verskillende kommersiële wyngisrasse gegis word. Die wynkundige behandeling het gefokus op ligintensiteit wat deur lowerbehandeling gereguleer is. Vlugtige aromaverskille is op grond van lower- en gisbehandelings vergelyk, met ‘n spesifieke fokus op die gistingsafgeleide boeket (esters, hoër alkohole, vlugtige vetsure, karbonielverbindings en monoterpene).

Die resultate het beduidende verskille getoon tussen aanvanklike mossamestellings, waaronder titreerbare suurheid, appelsuur en gis-assimileerbare stikstof. Daar was ook ‘n noemenswaardige impak op die vlugtige aromaverbindings, hoewel geen merkbare effek op die algehele gistingskinetika waargeneem kon word nie.

Die verskille in vlugtige verbindings het gewissel op grond van die gisras. ‘n Duidelike oesjaareffek was merkbaar tussen vlugtige verbindings wat deur die behandelings geaffekteer is. Data wat in 2012 gegenereer is, toon duidelike verskille tussen etiel- en asetaatesters en kon duidelik m.b.v. meervariantanalise volgens gisras gegroepeer word.

Die resultate van die sensoriese evaluering kon duidelik volgens lowerbehandeling onderskei word, en tot ‘n mindere mate volgens die gisras wat gebruik is. Dít dui daarop dat hoewel gis ‘n meer prominente impak het op die gistingsboeket wat tydens alkoholiese gisting ontwikkel, is die oorheersende aroma hoofsaaklik afgelei van druifafgeleide metaboliete wat deur lowerbehandelings gemanipuleer kan word. Nietemin dra die verskil in gistingsboeket by tot die kompleksiteit van die wyn, veral in die geval van gistingsafgeleide “tropiese” aromas, insluitend koejawel en grenadella. In sommige gevalle waar beskadude druiwe hoër esterkonsentrasies gehad het, het die gevolglike wyn ook ‘n hoër aromakwaliteit gehad.

Hierdie studie dra by tot ‘n beter begrip van die effek van die komplekse verhoudings tussen lowermanipulasie en gisseleksie op aromavorming. ‘n Analise van vlugtige aroma alleen

is egter nie voldoende om die finale persepsie van wynsmaak te begryp nie en bykomende diepgaande studies van die wingerdkundige en wynkundige faktore word benodig.

Hierdie projek het in die besonder gefokus op ‘n enkele wingerd oor slegs twee oesjare. Die algemene geldigheid van die afleidings wat van hierdie studie gemaak word, sal dus bykomende datastelle vereis.

This thesis is dedicated to my family

Hierdie tesis is opgedra aan my familie

~

Biographical sketch

Anke von Mollendorff was born on 21 September 1988 in Standerton, Mpumalanga, South Africa. She matriculated at Point High School, Mossel Bay in 2006. In 2007 Anke enrolled for a BscAgric-degree majoring in Viticulture and Oenology at Stellenbosch University. She obtained her degree in 2010 and in 2011 enrolled for the MscAgric-degree in Oenology at the same University.

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Preface

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

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Chapter 1

General introduction and project aims

1. General introduction and project aims

1.1INTRODUCTION

Sauvignon blanc is well-known for its versatile aroma profiles, ranging from “green” to “tropical”. Green character is usually associated with grass and green pepper notes whereas the tropical character is associated with passion fruit, grapefruit and even citrus aromas. However, it remains difficult to control and predict the final character of a wine from the quality of grapes and grape musts. This is mostly due to the complex wine matrix that undergoes numerous metabolic transformations that researchers are still far from fully understanding.

Grape-derived impact compounds such as terpenes, pyrazines, thiols are known to play a key role in Sauvignon blanc’s varietal aroma, while alcoholic fermentation conducted by yeast leads to the formation of aroma active secondary metabolites such as esters, higher alcohols and fatty acids. The level of aromatic metabolites produced during alcoholic fermentation depends on the availability of precursors. The must composition (*precursor availability*) depends on viticultural management practices as well as climatic conditions and the harvesting date (*ripening stage*). Ripeness as well as fruit exposure can also affect the aromatic profile of Sauvignon blanc wines (Marias *et al*., 2001). A study done in 2011 by Deloire, presented a berry aromatic model where berry hue was used as an indicator for white wine aroma style. A model specifically for Sauvignon blanc presented seven different berry hue stages during ripening, each correlating with an expected wine style **Table 1.1**. This Sauvignon blanc model can as a result be used as a guideline to determine the harvest date for a certain wine style.

Table 1.1 Seven berry hue stages each with a expected wine style for Sauvignon blanc (Deloire 2011)

Berry hue thresholds (in degrees)	Expected wine aromatic profiles
>90	Green/unripe
90 - 85	Green/asparagus
85 - 80	Asparagus/citrus
80 - 75	Asparagus/Tropical fruit/grapefruit/citrus
75 - 70	Tropical fruit
70 - 65	Fermentative/terpene
65 - 60	Phenolic/neutral/terpene

Yeast contributes greatly to wine aroma. The choice of yeast strain becomes an important factor for modulating wine during the primary fermentation stage. For this purpose *Saccharomyces cerevisiae* yeast strains are generally used. Through the years several researchers have investigated how different *Saccharomyces* yeast strains can be used to modify wine styles (Rapp 1998; Antonelli *et al*., 1999; Mateo *et al*., 2001; Dubourdieu *et al*., 2006; King *et al*., 2008;

Swiegers *et al*., 2009; Sumby *et al*., 2009 and Barrajón *et al*., 2011). These studies suggest that it is possible for winemakers to select yeast strains that lead to Sauvignon blanc wines being more “tropical” or “green”.

Aroma is a main consumer driver in the wine industry and it is essential that a more holistic approach is implemented to further broaden our knowledge on wine aroma. This study is part of an integrated project focusing on a viticulture light intensity treatment and its effect on the grape physiology (evaluated through transcriptomic, metabolic and chemical composition analysis), must composition, and microbial flora, as well as the finished wine aroma character and ageing potential. The viticultural treatment is carried out on a well characterized Sauvignon blanc block and includes a leaf removal treatment resulting in grapes having more light exposure (exposed treatment) as well as a no leaf removal treatment resulting in the grapes having less light exposure (shaded treatment). Both these treatments are applied more extreme than would normally be done during viticultural practises to ensure a broad range of effects. The focus of the research will be to provide a fully integrated and controlled research chain starting with characterised model vineyards and ending with a comprehensive chemical, sensory and quality assessment of the final product. This will improve our knowledge on two extreme treatment applications to create a range of trends which could become indicators for aroma modulation.

There are currently very few projects involving such an in-depth integrated approach. The aim for this specific study is to assess the impact that these two viticultural treatments will have on the grape must, fermentation kinetics and aroma compound production of two different commercial wine yeast strains, particularly in terms of esters, higher alcohols, volatile fatty acids, carbonyl compounds and monoterpenes.

1.2 PROJECT AIMS

This study forms part of an integrated project in the Institute of Wine Biotechnology at the University of Stellenbosch with the theme of *Metabolomics* and *Metrics* of vine, wine and wine organisms which aims to improve our knowledge through a holistic approach.

The main aims for this study were as follow:

(i) To assess the fermentation kinetics of different commercial wine yeast strains between viticultural shaded and exposed canopy treatment wines;

(ii) To evaluate the impact of different commercial wine yeast strains on the volatile aroma of viticultural shaded and exposed treatment wines focusing on fermentation metabolites including esters, higher alcohols, volatile fatty acids, monoterpenes, carbonyl compounds and;

(iii) Using multivariate data analysis to assess the broad range of impacts of yeast fermentation on differences in grape must related to viticultural treatments. This information will be used to create guidelines for canopy management in terms of aroma development for Sauvignon blanc wine styles.

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Chapter 2

Literature review

The impact of yeast on the varietal aroma composition of Sauvignon blanc: A Mini Review

2. LITERATURE REVIEW

The impact of yeast on the varietal aroma composition of Sauvignon blanc: A Mini Review

2.1 INTRODUCTION

Aroma is a major driver of consumer perception and liking, and a large number of studies have focused on understanding the multifaceted process of aroma development during grape ripening and winemaking. However, we are far from fully understanding these complex processes and our ability to control aroma production remains limited.

Microorganisms, specifically yeasts, play a vital role in the winemaking process. Although yeast strains are recognized for their main function of converting sugar into ethanol and carbon dioxide, the process includes a great number of other biochemical pathways resulting in hundreds of secondary metabolites which convert the aromatically dull must into an aromatic wine (Pretorius, 2000; Swiegers and Pretorius, 2005; Ciani *et al*., 2010). It is known that yeast metabolites formed during alcoholic fermentation could either enhance varietal aroma (*synergistic interaction*) or mask (*antagonistic interaction*) favourable aromas (Styger *et al*., 2011).

Generally for a wine fermentation, the origins of aroma compounds contributing to overall end aroma can be divided into four categories; (*1*) primary or grape aroma, (*2*) secondary grape aroma, (*3*) fermentation bouquet and lastly (*4*) maturation bouquet. These aroma compounds are further described in **Table 2.1** (Rapp, 1998).

This review will focus on the varietal aroma of Sauvignon blanc, revealing the impact compounds associated with grape-derived compounds as well as fermentative metabolites contributing to final aroma.

Table 2.1 Origin of aroma compounds present in wine (Adapted from Rapp, 1998)

Category	Definition	Sauvignon blanc aroma compounds
1. Primary or grape aroma	Aroma compounds as they are to be found in the undamaged plant cells of the grape	Methoxypyrazines
2. Secondary grape aroma	Compounds formed during the processing of the grapes; and by enzymatic, chemical and thermal reactions in grape must	Monoterpenes
3. Fermentation bouquet	Aroma compounds formed during alcoholic fermentation and malolactic fermentation	Esters, higher alcohols, volatile fatty acids, carbonyl compounds, volatile thiols
4. Maturation bouquet	Caused by chemical reactions during maturation of the wine in the bottle	-

In the past, fermentations occurred spontaneously. Today, yeast starter cultures are a cost-effective choice reducing the chances of the development of spoilage microorganisms and of unwanted aromas, and there are approximately 200 yeast starter cultures commercially available. These strains differ regarding fermentation kinetics as well as in their ability to produce aroma profiles (Sablayrolles *et al*., 2009). There has been a renewed interest on how these wine strains can be used to modulate wine aroma (Pretorius, 2000; Styger *et al*., 2011).

Besides the variability in the aroma profiles produced by different *S. cerevisiae* strains, many non-*Saccharomyces* species are currently being investigated for their possible contribution to winemaking as well as aroma, although many of these strains result in high concentration of acetic acid, acetaldehyde, acetoin, ethyl acetate as well as potential off-flavours linked to the presence of vinyl and ethyl phenols (Chatonnet *et al*., 1995; Ciani *et al*., 2010). Except for off-flavour profile the chance of stuck fermentation with non-*Saccharomyces* yeasts are high due to their reduced survival at high ethanol concentrations (Romano *et al*., 2003; Jolly *et al*., 2006). However, more studies are appearing with possible strategies to use these different yeast strains to improve aroma complexity.

Other *Saccharomyces* species such as *Saccharomyces bayanus*, *Saccharomyces paradoxus, Saccharomyces uvarum, Saccharomyces pastorianus* and *Saccharomyces kudriavzevii* are of interest, in particular for co-inoculation with different *S. cerevisiae* strains (Heard, 1999; Romano *et al*., 2003; Ciani *et al*., 2006; King *et al*., 2008; Ciani *et al.,* 2010).

The mechanisms by which yeasts can contribute to the outcome of alcoholic fermentation were summarised by Fleet (2003) and are listed in **Table 2.2**. Yeasts are present throughout the grape growing and winemaking process, initially in the vineyard (majority non-*Saccharomyces* yeasts), throughout alcoholic (mostly *Saccharomyces cerevisiae*) and malolactic fermentation and in certain cases even present in wine after packaging (spoilage yeasts).

Table 2.2 Seven mechanisms are listed by which yeast impact wine flavour (Fleet, 2003)

Mechanisms by which yeast impact wine flavour
1. Affect grape quality before harvest; biocontrol of moulds
2. Conduct alcoholic fermentation of grape juice into wine
3. Biocatalyse transformation of flavour neutral, grape components into flavour active components
4. Impact on wine flavour and other properties through autolysis
5. Bioadsorb components of grape juice
6. Cause spoilage during bulk storage of wine in the cellar and after packaging
7. Influence growth of malolactic and spoilage bacteria

The grape cultivar plays an essential role when it comes to final wine aroma. White wine cultivars generally emit more fresh and fruity aroma whereas red cultivars normally present nuances of berries, plums, and pepper. As shown in the **Table 2.2**, yeasts however play a role in the transformation of flavour neutral grape components into flavour active components (Fleet, 2003; Hernández-orte *et al*., 2008; Styger *et al*., 2011). This mechanism has been the interest of many researchers over the past few years revealing the origin of many unexplained aroma compounds contributing to certain varietal aromas. It is therefore important to further study the impact of yeast strains on the aroma profiles of specific cultivars.

Sauvignon blanc, a well studied cultivar, is known to have a versatile range of aroma profiles ranging from a “green” aroma including flavours of grass, capsicum, tomato leaf to a more “tropical” aroma including aroma flavours such as passion fruit, pineapple, gooseberry and citrus zest (Lacey *et al*., 1991; Marias, 1994).

Grape-derived compound groups relevant for Sauvignon blanc wines include methoxypyrazines, volatile thiols and terpenes. Methoxypyrazines are formed as secondary products from amino acids by plants and are associated with aromas such as bell pepper, grassy, vegetative, leading to “greener” wine styles (Swiegers *et al*., 2006). The most prominent methoxypyrazines present in wine is 2-isobutyl-3-methoxypyrazine (IBMP), 2-*sec*-butyl-3-methoxypyrazine (SBMP) and lastly 2-isopropyl-3-methoxypyrazine (IPMP) (King, 2010).

Volatile thiols present in Sauvignon blanc include 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and lastly 3-mercaptohexyl acetate (3MHA). The first two compounds are grape derived and are present as cystein and glutathione bound conjugate precursors in a non-volatile form in the grape must. This form can only be transformed to the aroma active compounds in the presence of yeast during alcoholic fermentation.

Terpene compounds are normally present in grape must in one of two forms, a free volatile form or a non-volatile sugar conjugated form (Gunata *et al*., 1985). The latter compound needs to be released from the bound form either enzymatically or through chemical hydrolysis. The bound forms are known as glycoconjugates and are hydrolyzed by an enzyme known as *β*-glucosidase releasing the volatile terpene (Hernández *et al*., 2003). Of these terpenes, monoterpenes namely linalool, citronellol and nerol usually contribute to the floral aroma mostly in Muscat cultivars but are also present at lower concentration in Sauvignon blanc wines (Ebeler, 2001).

Fermentation derived compounds refer to esters, higher alcohols, volatile fatty acids, carbonyls and sulphur containing compounds (including thiols). These compounds are formed by the fermenting yeast strains, and can be altered by parameters such as fermentation temperature, oxygen exposure as well as nutrient addition (Torija *et al*., 2003; Garde-Cerdán *et al*., 2008; Coetzee, 2011).

All these aroma compounds together only represent a small percentage of the remaining fraction (~ 1 %) of the total composition of the wine as 85 – 90 % of wine consists of water, 10 – 15 % of alcohol, and 0.4 -1 % of acids.

An aromatic model for white wine cultivars, derived from berry colour (hue) development during berry ripening in correlation with aroma profile, was reported in 2011 by Deloire. This model (**Figure 2.1**) predicts five different aroma classes during the ripening stage which could have an effect on the wine style of the end-product. These classes are dominated by specific impact compounds, (*1*) Methoxypyrazines (*vegetal*), (*2*) Thiols (*Tropical*), (*3*) Fermentative bouquet, (*4*) Terpene and lastly (*5*) neutral/phenolic aromas.

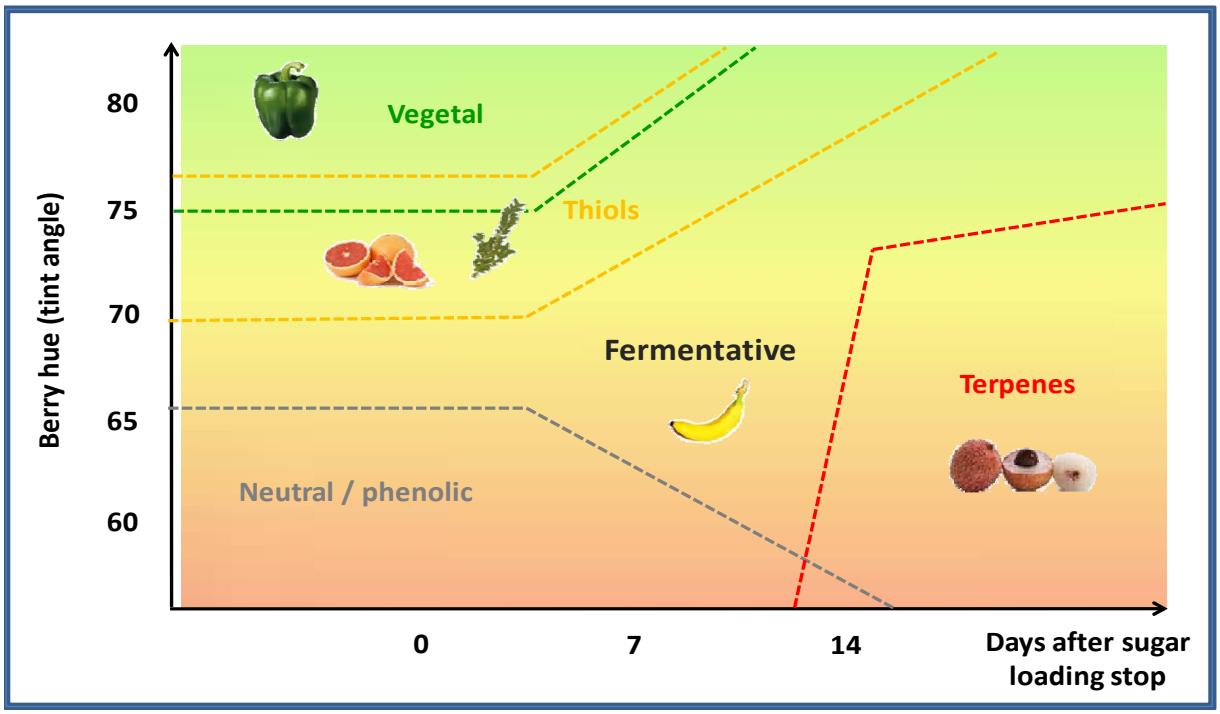

Figure 2.1 White cultivar berry aromatic model. Evolution of berry colour (hue) is used as a tool to predict the style of wine form vérasion to harvest (Deloire, 2011).

According to the aromatic model, Sauvignon blanc can be further divided into seven aromatic profiles according to the berry hue (**Table 2.3**) when harvested at different stages during the ripening period.

Table 2.3 The colour representation (hue) and expected style of wine for Sauvignon blanc aroma (Not specific for South African conditions) (Deloire, 2011).

Berry hue threshold (in degrees)	Expected wine aromatic profiles
>90	Green/unripe
90-85	Green/asparagus
85-80	Asparagus/citrus
80-75	Asparagus/Tropical fruit/grapefruit/citrus
75-70	Tropical fruit
70-65	Fermentative/terpene
65-60	Phenolic/neutral/terpene

As already mentioned yeast plays a pivotal role in releasing these varietal and fermentative aroma compounds. This review will include the origin of the five aroma classes formed during grape ripening as well as how they are affected and influenced by the presence of yeast to produce aromas demanded by the current consumer market. **Table 2.4** compiled from Moreno-Arribas and Polo (2009) gives an outline of the fermentative compounds as well as grape derived compounds (volatile and non-volatile forms) and how these varietal active-aromas are formed by the interaction with yeast.

Table 2.4 Interaction between yeast and grape compounds (Compiled from Moreno-Arribas and Polo, 2009).

Grape compound	Metabolism	Metabolite/product
Nutrients	***Catabolic/anabolic pathways*** →	***Fermentation bouquet***
Hexoses	Sugar metabolism (glycolysis / TCA pathway) & lipid metabolism	Esters, higher alcohols, acids, carbonyls
	Sugar metabolism	Polysaccharides
Amino acid, ammonium, peptides	Nitrogen metabolism	Higher alcohols, acids, carbonyls
Sulphate (Sulphite)	Sulphur metabolism	Volatile sulphur compounds
Flavour precursors	***Biotransformations*** →	***Varietal aroma***
Glycosides	Hydrolysis	Monoterpene, norisopreniods Aliphatics, benzene derivatives
Cysteinylated conjugates	Non-hydrolytic cleavage	Long-chain polyfunctional thiols
Non-conjugated secondary metabolites	Reduction, esterification, decarboxylation	Transformation products
Non-precursor flavour-active compounds	***Metabolism/Biotransformation*** →	***Flavour-active compounds***
Carboxylic acids	TCA-pathway	Carboxylic acids, transformation products
Phenolic compounds	(yeast metabolites)	Phenolic adducts and polymers

2.2 THE IMPACT OF YEAST ON SAUVIGNON BLANC'S VARIETAL AROMA

2.2.1 METHOXYPYRAZINES

Methoxypyrazines are known for their vegetative/herbaceous aroma contributing to Sauvignon blanc's "green" aroma profile. They are grape-derived, nitrogen-containing ring compounds situated in the skin and exocarp of the grape berry that form due to secondary amino acid catabolism (Marais, 1994; King, 2010). Three methoxypyrazines are detected in Sauvignon blanc, 2-isobutyl-3-methoxypyrazine (IBMP), 2-*sec*-butyl-3-methoxypyrazine (SBMP) and 2-isopropyl-3-methoxypyrazine (IPMP). IBMP is found in Sauvignon blanc at concentrations ranging between 0.4 – 44 ng/L (**Table 2.5**), the concentrations being mostly above its sensory detection threshold and contributing to the bell pepper aroma (Alberts *et al*., 2009).

A recent study by Pickering *et al*. (2008) suggested that IPMP has a lower sensory threshold value than IBMP and might play a more important role than previously thought. At low concentrations ranging between 8 - 15 ng/L methoxypyrazines contribute to pleasant aromas whereas concentration as high as 30 ng/L contribute to unripe character (Alberts *et al*., 2009; Pickering *et al*., 2010). Methoxypyrazines have very low sensory detection thresholds (**Table 2.5)**.

The accummulation of these compounds in grapes is generally susceptible to environmental parameters including micro climate, canopy management, soil, water content as well as terroir (Sala *et al*., 2004; Swiegers *et al*., 2006; Styger *et al*., 2011). Methoxypyrazines increase during berry ripening until véraison after which they start degrading especially in the presence of sunlight (Lacey *et al*., 1991). Cooler climates usually present a more “greener” style Sauvignon blanc due to the presence of higher methoxypyrazine concentrations (Lacey *et al*., 1991; Marias, 1994). The “green” character in the wine aroma can therefore be manipulated through viticulture practices including canopy management (*dense canopy*), training systems and pruning strategies (Marias, 1994; Sala *et al*., 2004; Swiegers *et al*., 2006). In the cellar methoxypyrazine concentrations can be enhanced by longer skin contact conditions releasing more methoxypyrazines situated in the skin cells (Marias, 1998).

Table 1.5 Methoxypyrazine compounds with their aroma profiles, sensory detection threshold as well as concentration ranges of South African (SA) Sauvignon blanc wines (Compiled from Lacey *et al*., 1991, Marais, 1994; Sala *et al*., 2004; Alberts *et al*., 2009; King *et al*., 2010)

Compound	Aroma	Aroma detection threshold[a]	Concentrations in SA* Sauvignon blanc (ng/L)
2-isobutyl-3-methoxypyrazine (IBMP)	*Bell pepper, green bean, herbaceous*	2 ng/L	0.4 - 44
2-*sec*-butyl-3-methoxypyrazine (SBMP)	*Earthy, asparagus, vegetal*	1 ng/L	0.03 - 3.2
2-isopropyl-3-methoxypyrazine (IPMP)	*pea, asparagus, vegetal*	2 ng/L	0.03 - 3.9

[a] Water

2.2.1.1 Impact of yeast on methoxypyrazines

While it has been shown that the concentration of methoxypyrazines can be manipulated before fermentation in the vineyard (Swiegers *et al*., 2006) the question whether the yeast strain has an impact on the final methoxypyrazine levels in wine remains to be clearly answered. Sala *et al*. (2004) reported that methoxypyrazine levels change during the fermentation process which was contradicted by Lund *et al*. (2009) who suggested that the concentration remains constant during alcoholic fermentation. Marais *et al*. (2001) reported that yeast strain did not play a large role in ibMP concentrations in wine. A study done by Pickering *et al*. (2008) investigated the

effect of commercial yeast strains on the concentration and sensory impact of ipMP in Cabernet Sauvignon and found that yeast strains vary in their ability to mask green aromas in wine.

Although a mechanism for the biosynthesis of methoxypyrazines by *Saccharomyces* yeast was hypothesised by Cheng *et al*. (1991) many years ago, no other studies have elucidated any mechanism that would suggest the biosynthesis of this compound by yeasts. The impact of yeast on methoxypyrazines remains unclear, and additional studies are needed to determine if wine yeast strains do have an influence on the concentrations during alcoholic fermentation and in the final wine.

2.2.2 VOLATILE THIOLS

Volatile thiols contribute to Sauvignon blanc's tropical aroma with flavour profiles of pineapple, gooseberry, citrus, passion fruit and grapefruit (Tominaga *et al*., 1998b, 2000).

Thiol precursors are situated in the skin and exocarp of the grape berry and form part of sulphur compounds that are mostly released during alcoholic fermentation (Swiegers *et al*., 2007; King, 2010). They have also been referred to as polyfunctional mercaptans (Swiegers *et al*., 2007; Benkwitz *et al*., 2012).

The three most prominent thiols present in Sauvignon blanc wines include 4-mercapto-4methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA). Concentration ranges in wine are listed in **Table 2.6**. 4MMP is the most prominent of the three and is usually detected above its sensory threshold (0.8 ng/L), contributing to an aroma of "box tree" and "blackcurrant". It has been established that 4MMP and 3MH are synthesised in the grape berry and their non-odorous precursors are present in the grape must. Precursors are in a cysteine-bound conjugate form and need to be cleaved in the presence of yeast for the non-aromatic thiol to become an active impact compound. These precursors include S-4-(4-methylpentan-2-one)-L-cysteine (Cys-4MMP) resulting in 4MMP and S-3-(hexan-1-ol)-L-cysteine (Cys-3MH) resulting in 3MH and were first describe in 1998 (Tominaga *et al*., 1998b; Swiegers *et al*., 2007).

3MHA is not present as a precursor in the grape must but is formed by the esterification of 3MH by the action of yeast ester-forming alcohol acetyltransferases encoded by the *ATF1* gene (Howell *et al*, 2005; Roland *et al*., 2010). These compounds are present in trace amount, but with very low sensory threshold values result in very high odour activity values (Tominaga *et al*., 1998a; Benkwitz *et al*., 2012). At low concentration these three compounds contribute to aromas of box tree, blackcurrant, passion fruit and grapefruit, but when present in high concentration may be responsible for undesirable flavours such as cat urine (Howell *et al*, 2005). Threshold values and aroma descriptors are listed in **Table 2.6**.

Table 2.6 Volatile thiols present in wine aroma and their perception threshold levels. Adapted from Tominaga *et al*., 1998b; Swiegers and Pretorius, 2005; Dubourdieu *et al*., 2006.

Compound	Aroma	Aroma detection threshold (ng/L)	Concentration in wine (ng/L)
4-mercapto-4-methylpentan-2-one (4MMP)	*Box tree, broom*	0.8 (12% w/w)*	0 – 30
3-mercaptahexylacetate (3MHA)	*Box tree, passion fruit*	4.0 (12% w/w)	1 – 100
3-mercaptohexan-1-ol (3MH)	*Grape fruit, passion fruit*	60 (12% w/w)	50 - 5000
4-mercapto-4-methylpentan-2-ol (4MMPOH)	*Citrus zest*	55 (12% w/w)	0 – 86

* Hydroalcoholic model solution (% w/w ethanol)

2.2.2.1 Impact of yeast on volatile thiols

The absence of tropical aromas in Sauvignon blanc grape must before fermentation is an indicator that the process is essential to amplify these varietal aromas in the wine (Swiegers *et al*., 2007). Indeed, yeast is responsible for degrading the S-cysteine conjugate bond to release the volatile aroma (Tominaga *et al*., 1998b).

Murat *et al*. (2001) determined the ability of four *S. cerevisiae* yeast strains to liberate volatile thiols from their cysteinlated precursors. Results showed significant differences in the production of 4MMP clearly indicating that yeast strain play a vital role in the volatile thiol production as yeast strains differ in their ability to release 4MMP.

A mechanism for the release of 4MMP was first suggested to be due to the action of yeast sulphur carbon *β*-lyases which involves a *β*-elimination reaction to release 4MMP from Cys-4MMP (Tominaga *et al*., 1998a). Howell *et al*. (2005) further investigated the mechanism of thiol transformation through a genetic strategy, which anticipated that carbon-sulphur lysases with *β*-elimination activity is involved in the transformation reaction. Four genes (B*NA3*, *CYS3*, *GLO1* and *IRC7*) influence the release of 4MMP concluding that the mechanism involves multiple genes. The mechanism for the release of varietal thiols during alcoholic fermentation is therefore due to the activity of *β*-lyase released by *S. cerevisiae* yeast (Roland *et al*., 2010).

Although yeasts are the key element of releasing these non-volatile aromas, only as little as 5 % of the pool of non-aromatic precursors are transformed (Swiegers *et al*., 2007). Enhanced conversion of 3MHA was demonstrated in 2006 by Swiegers *et al*., through co-inoculation of two yeast strains with complementary activities, one capable of releasing high levels of 4MMP and 3MH from the pool of precursors, and the second strain with a significant ability to transform 3MH to 3MHA. In a more recent study a wine yeast strain was engineered to enhance the conversion of cystein conjugate precursors into volatile thiols (Swiegers *et al*., 2007). The

modified strain enhanced the release of 4MMP and 3MH up to 25 times. Unfortunately as this yeast is genetically modified (GM) it remains commercially unavailable, nonetheless contributing to the knowledge of yeast strain importance.

King *et al*. (2008) demonstrated that the co-inoculation of two *Saccharomyces* yeast strains could modify the chemical and sensory profile of Sauvignon blanc, and that the choice of yeast strain might have a large impact on consumer acceptance of the aroma (King *et al*., 2010).

Zott *et al*. (2011) investigated fermentations with non-*Saccharomyces* yeast strains to evaluate their impact on the release of volatile thiols. The results showed that non-*Saccharomyces* yeast strains (*Metschnikowia pulcherrima, Torulaspora delbrueckii, Kluyveromyces thermotolerans*) in a controlled environment could improve thiol release in wines made from varieties containing S-cysteine conjugate precursors. Masneuf-Pomarède *et al*. (2006) showed there is a significant interaction between yeast and fermentation temperature which can influence the concentration of thiols in wine.

The pathways accepted for the release of 4MMP, 3MH and 3MHA during alcoholic fermentation is illustrated in **Figure 2.2** (Roland *et al*., 2010).

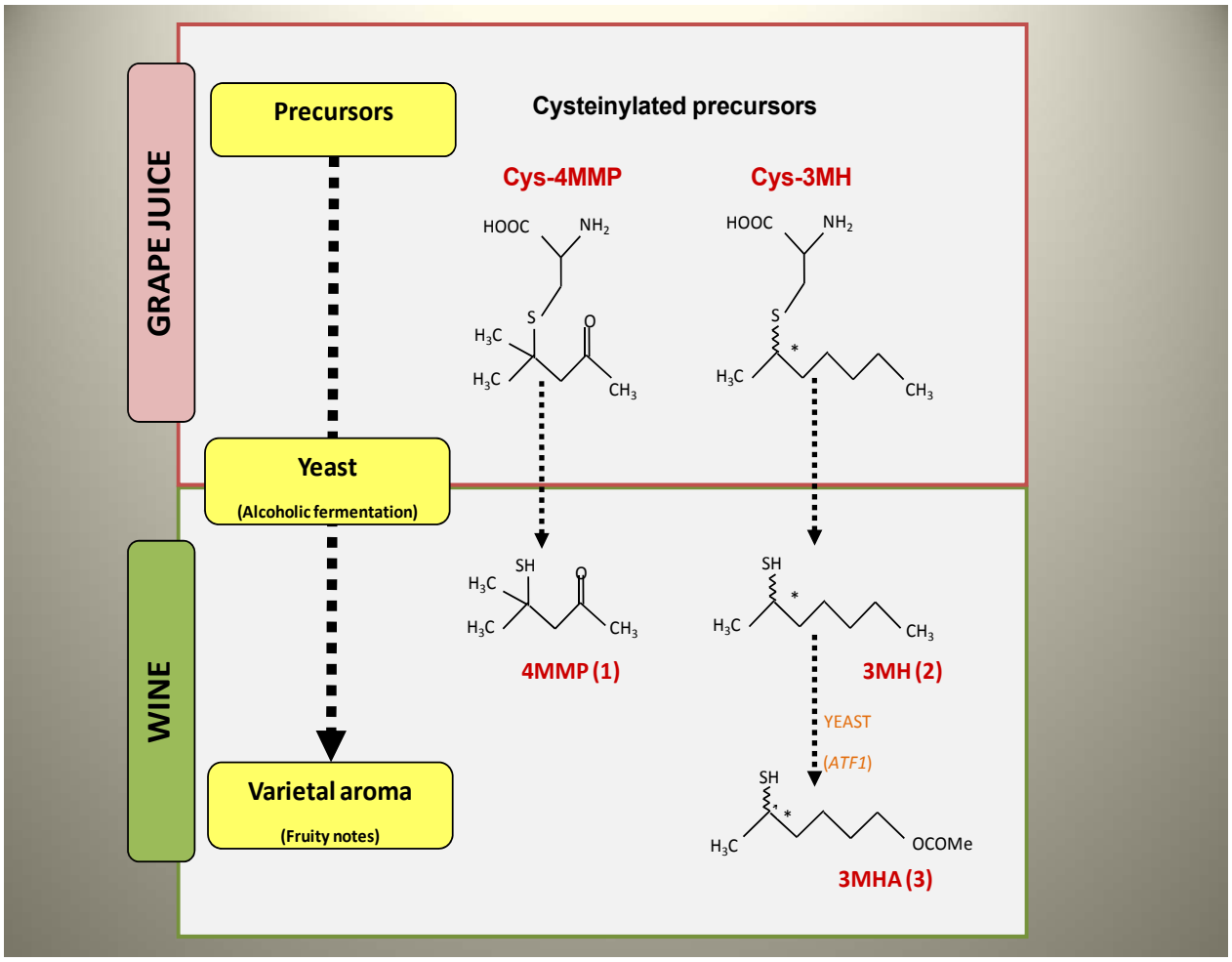

Figure 2.2 Different biogenesis pathways for 4MMP (1), 3MH (2) and 3MHA (3) during alcoholic fermentation (Addapted from Roland *et al*., 2010).

2.2.3 TERPENES

Terpenes are compounds belonging to secondary plant constituents (Mateo and Jiménez, 2000). They are 10-carbon compounds mainly situated in the skin and exocarp of the grape berry (King and Dickinson, 2000; King, 2010). These compounds are present in the grape must either in a free or bound form (Gunata *et al*., 1985). The bound form is generally most prominent as the ratio from free to bounds shifts to bound closer to the ripening stage with mature berries having higher concentrations (Mateo and Jiménez, 2000; Styger *et al*., 2011). The bound precursor is non-volatile and conjugated to a sugar moiety. This conjugated form referred to as a glycoconjugate needs to be cleaved in order for the volatile terpene to be released. The volatile aroma can be released chemically, through hydrolysis involving an acid-catalyzed reaction or via enzymatic release through glycosidase enzymes. Both these reaction occur during alcoholic fermentation although they are not specifically changed by yeast metabolism (Mateo and Jiménez, 2000). Due to acid hydrolysis being very slow (*being pH-dependent*) most bound forms are released by the action of glycosidase if the enzyme is present. If no enzyme is present the reaction will be slower if at all possible at wine pH (Sefton *et al*., 1998). Monoterpene alcohols mainly contribute to the varietal aroma of Sauvignon blanc wines, contributing to floral aromas (rose, overall floral). The concentration levels found in Sauvignon blanc wines are however much lower than in the floral Muscat cultivars (**Table 2.7**) were concentration levels are normally above detection threshold values (Sefton *et al*., 1994; Ebeler, 2001).

The most important monoterpenes include α-terpineol, linalool, geraniol, nerol, citronellol (**Table 2.7**) (Sefton *et al*., 1994; Styger *et al*., 2011). The grape must does contain *β*-glucosidase enzymes but this enzyme usually has low activity due to low concentration and the low pH levels. To enhance monoterpene release, additional commercial enzymes usually originating from fungi *(Aspergillus niger*) can be added to increase liberation activity.

Table 2.7 Aroma profiles of important monoterpenes and their corresponding detection thresholds measured in water (Adapted from King and Dickinson, 2000).

Compound	Aroma	Sensory threshold* (µg/L)	Concentrations in Sauvignon blanc (µg/L)	Concentrations in muscat cultivars (µg/L)
Geraniol	*Floral, rose like, citrus*	132	5	506
Citronellol	*Sweet, rose like, citrus*	100	2	nd
Linalool	*Floral, fresh, coriander*	100	17	455
Nerol	*Floral, fresh, green*	400	5	94
α-terpineol	*Lilac*	460	9	78

* water
nd = not detected

2.2.3.1 Impact of yeast on terpenes

During alcoholic fermentation some yeast can secrete glucosidases (Fleet, 2008; Styger *et al*., 2011). This leads to enzymatic hydrolysis involving two stages. The first stage includes the cleavage of the terminal sugar from *α*-L-rhamnosidase, *α*-L-arabinosidase or *β*-D-apiosidase to release rhamnose, arabinose or apiose and the corresponding glycoside. The second stage includes the liberation of the monoterpene through the action of a *β*-glucosidase releasing the volatile terpene (Mateo and Jimènez, 2000). The production of glucosidases by yeasts varies with species and strain, but data suggests that non-*Saccharomyces* yeasts are stronger producers of such enzymes than *S. cerevisiae* (Fleet, 2008). These non-*Saccharomyces* yeast strains include species of *Hanseniaspora*, *Debaryomyces* and *Dekkera*.

In 2000 King and Dickinson proved that monoterpene alcohols could be transformed by *S. cerevisiae*, *Torulaspora delbrueckii* and *Kluyveromyces lactis*. This study concluded that monoterpenoids present in wine should not be assumed to directly originate from the corresponding terpene due to the chances of biotransformation reactions between geraniol, citronellol, nerol, linalool and α-terpineol by yeast. Depending on the yeast strain, *S. cerevisiae* has been shown to modify the terpenic aroma through the production of citronellol from geraniol and nerol (Mateo and Jiménez, 2000). Furthermore, data by Carrau *et al*. (2005) suggest that *S. cerevisiae* can synthesis monoterpenes de novo in the absence of grape-derived precursors. Ugliana *et al*. (2006) and Hernández-Orte *et al*. (2008) confirmed that different yeast genera have different abilities to release aromatic aroma compounds from bound odourless precursors.

2.2.4 NEUTRAL/PHENOLIC AROMA COMPOUNDS

Phenolic compounds can generally be divided into non-flavonoids (*hydroxybenzoic & hyroxycinnamic acids*), flavonoids (*flavonols & flavanols*) and phenolic-protein-polysaccharide complexes (Basha *et al*., 2004). These compounds can have an effect on wine contributing to astringency, bitterness as well as being important to quality of the final wine including wine colour (Singleton, *et al*., 1975; Basha *et al*., 2004; Komes *et al*., 2007). Phenolic compounds also play a role in browning reactions. The total phenolic compounds present in white wines are generally lower than in red wines. Most phenolic compounds are situated in the skins and seeds of the grape berry. The general concentration of phenols present in wine with minimal amount of skin contact will range between 100 – 250 mg/L (Komes *et al*., 2007). It was reported by Smith and Waters (2012) that a difference in phenolic composition can have a textural influence on the final wine.

2.2.4.1 Impact of yeast on neutral/phenolic aroma

Phenolic “taste” or aroma is still not well defined. When harvesting in the phenolic/neutral stage during grape ripening it could cause the wine style to be less varietal and have more phenolic attributes including bitterness, astringency and in some cases depending on the pH also a “hot’’

attribute (Smith and Waters, 2012). This phase can be referred to as the absence of aroma. Phenols seem to have an influence on the perception of certain key volatiles in Sauvignon blanc (Lund *et al*., 2009).

Phenolic compounds can be transformed into off-flavour phenolic compounds that form due to the presence of spoilage yeasts such as *Brettanomyces*.

2.2.5 FERMENTATIVE AROMA COMPOUNDS PRODUCED BY YEAST

Fermentation derived compounds include esters, higher alcohols, volatile fatty acids, carbonyl compounds and sulphur-containing compounds. These compounds are not directly related to the central carbon metabolism but are produced as secondary metabolites and mostly derived from the metabolism of amino and fatty acids (reviewed in Styger *et al*., 2011). The different pathways are shown in **Figure 2.3**. Many data sets show that the concentrations of individual compounds is strongly dependent on the yeast strain that is conducting alcoholic fermentation, but other yeast and bacterial species that are naturally present in must may also contribute to the final aroma profile (Rossouw *et al*., 2008; Styger *et al*., 2011).

The formation and concentration levels of these secondary compounds is also dependent on must composition including amino acid differences, fermentation temperature, oxygen exposure and the list continues. This review will only focus on the impact of yeast.

The secondary metabolites produced during alcoholic fermentation are not considered to directly contribute to the varietal character of Sauvignon blanc, except possibly for some of the esters that may contribute to the specific fruity and tropical characters. These esters are strongly impacted by the yeast strain. The other compounds play a part in creating the vinous aroma present in wine and due to their synergistic interactions with other aromatic compounds are relevant for this discussion.

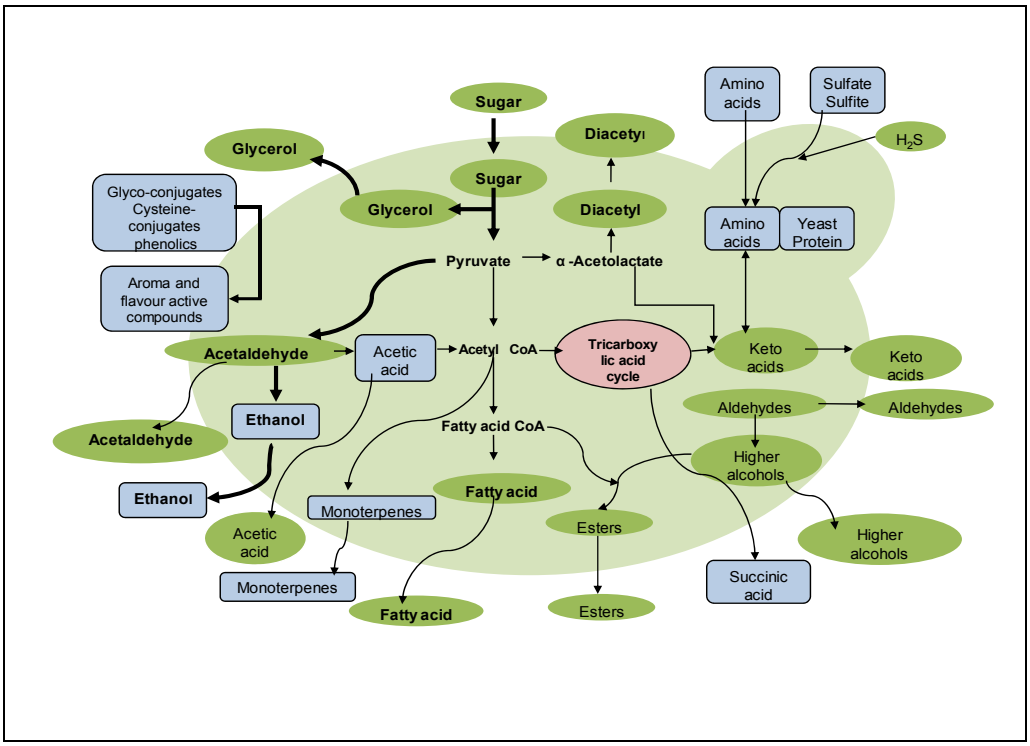

Figure 2.3 A schematic representation of derivation and synthesis of flavour-active compounds from sugar, amino acids and sulphur metabolism by wine yeast (adapted from Swiegers *et al*., 2005).

2.2.5.1. Esters

Esters are a group of compounds that are important especially for white wines including Sauvignon blanc as they can contribute to fresh and fruity flavours. Esters can be divided into two groups; acetate esters and ethyl esters. Ethyl esters consist of an alcohol group (ethanol) and an acid group which is a medium-chain-fatty-acid (MCFA), whereas acetate esters consist of an acid group (acetate) and an alcohol group either ethanol or a higher alcohol derived from amino acid metabolism (Saerens *et al*. 2008). Acetate esters are usually present at higher concentrations than ethyl esters and are associated with fruity aromas. Ethyl esters tend to contribute more to apple aromas (Saerens *et al*. 2008). The most abundant ester known is ethyl acetate with concentrations ranging to 85 mg/L in wine (**Table 2.8**) (Longo *et al*., 1992). In 1973 it was reported by Daudt and Ough that yeast strains have an impact on the formation of esters. 14 pure yeast strains were studied by Soles *et al*. (1982) they proved that if all other factors (pH, nitrogen, temperature) were kept constant the yeast strain became important in terms of the esters produced as significant differences between strains was obtained. In 2003 Plata *et al*. tested various wine yeast species for their ability to produce ethyl acetate and isoamyl acetate which are two important aromatic esters. They found that both compounds were dependent on the yeast strain used. **Table 2.8** lists a few esters their concentration ranges in wine as well as sensory detection thresholds.

Table 2.8 Some esters and their concentrations present in wine, odour thresholds (Lambrechts and Pretorius, 2000; Swiegers and Pretorius, 2005; Rossouw *et al*., 2008).

Compounds	Aroma	Concentration dry wine (mg/L)	Threshold (mg/L)
Ethyl acetate	Varnish, nail polish, fruity	85	12
Isoamyl acetate	Banana, pear	2.37	60 0.26*
2-Phenylethyl acetate	Rose, honey, fruity, flowery	0.21	1.8
Ethyl isovalerate	Apple, fruity Banana	nd – 0.7	
Isobutyl acetate	Banana	0.07	
Ethyl butanoate	Floral, fruity	0.01 – 1.8	0.4 (Beer)
Ethyl 2-methyl-butanoate	Strawberry, pineapple	nd - 0.9	
Hexyl acetate		0.14	0.67
Ethyl hexanoate	Apple, banana, violets	1.06	0.08
Ethyl octanoate	Pineapple, pear	2.11	0.58
Ethyl decanoate	Floral	0.56	0.5

*Percentage-above-chance-scores of 50% in grain spirit solution of 9.4 % (w/w)
nd= not detected

2.2.5.2 Higher alcohols

Higher alcohols are also known as fusel alcohols. Of the total content of aroma compounds in wine ranging from 0.8 -1.2 g/L, higher alcohols contribute to almost 50% of this range making it quantitavely the largest group of compounds (Longo *et al*., 1992; Rapp, 1998; Vilanova *et al*., 2010). Higher alcohols are produced from glucose anabolically and catabolically and from their corresponding amino acids including threonine (*1-propanol*), valine (*isobutanol*), isoleucine (*2-methyl-1-butanol*) and leucine (*3-methyl-1-butanol*) (Giudici *et al*., 1993; Herraiz and Ough, 1993). They are formed by the Ehrlich pathway, where amino acids are deaminated, α-keto-acids are decarboxylated and reduced to the correlating alcohol (Bell *et al*., 1979; Herraiz and Ough, 1993). The higher alcohol production will increase as the amino acids concentration in the grape must increases (Swiegers and Pretorius, 2005). In 1967 Rankine showed that wine yeast strains can differ in their higher alcohol production when he confirmed that *n*-propanol, isobutanol and isoamyl alcohol showed variation between the yeast strains studied. Isoamyl alcohol is usually the higher alcohol with the highest concentration present in wine at the end of alcoholic fermentation with concentrations ranging from 45 – 490 mg/L. Other well-known alcohols are listed in **Table 2.9**.

Some higher alcohols (hexanol and hexenol) have been known to contribute to grassy and herbaceous notes in wine (Marias, 1994; Vilanova *et al*., 2010). It is known that yeast strain differ in their production of higher alcohols and that too high production (> *400 mg/L*) can have a negative effect on the wine aroma whereas lower production (< *300 mg/L*) contributes to the complexity of the wine (Lambrechts and Pretorius, 2000).

Table 2.9 Some higher alcohols and their corresponding amino acids as well as concentration ranges in wine. Aroma and sensory threshold is also listed (Lambrechts and Pretorius 2000; Rossouw *et al*., 2008).

Compound	Corresponding amino acid	Aroma	Concentration in wine (mg/L)	Threshold (mg/L)
Propanol	Threonine/ 2-Amino-butyric acid	Stupefying	9 – 68 125	306 500ζ 800¤
Butanol	-	Fusel odour	0.5 – 8.5	50
Isobuyl alcohol	Valine	Alcoholic	9 -28 (100) 140	74 500 ζ 75.0* 200¤
Active amyl alcohol	Isoleucine	Marzipan	15 - 150	65¤
Isoamyl alcohol	Leucine	Marzipan	45 - 490	300 ζ 7.0* 70¤
Hexanol	-	-	0.3 -12	5.2* 4¤
Tyrosol	Tyrosine	Bees wax, honey like	-	-
Tryptophol	Tryptophan	-	-	-
Phenylethyl alchohol	Phenylalanine	Floral, rose	10 - 180	7.5* 125¤

*Percentage-above-chance-scores of 50 % in grain spirit solution of 9.4 % (w/w)
ζ In wine solution
¤ In beer

2.2.5.3 Volatile fatty acids

Studies done on the production of volatile fatty acids include the theory of medium-chain-fatty acids (MCFA) contribution to stuck or sluggish fermentations. Fatty acids associated with sluggish fermentations include decanoic acid and octanoic acid (Lafon-Lafourcade *et al*., 1984). These MCFA are produced as intermediates from the biosynthesis of long-chain fatty acids during alcoholic fermentation by the yeasts present (Lambrechts and Pretorius, 2000). Viegas *et al*. (1989) demonstrated that some fatty acids produced during fermentation are toxic and that the effect is amplified with the decrease in pH levels. The same study proved that the amount of fatty acids produced as well as released into the fermentation is yeast strain dependent. MCFA are produced during fatty acid biosynthesis from acetyl co-enzyme A (Herraiz and Ough, 1993). The volatile acid composition of wine is generally between 500 - 1000 mg/L and is dominated by acetic acid almost contributing to 90 % of the total volatile acids (Lambrechts and Pretorius, 2000). Other well-known fatty acids are listed in **Table 2.10** with the concentration ranges in wine as well as sensory threshold values. The yeast strain used can have an impact on the amount of acetic acid produced which can have an impact on the varietal aroma (Lambrechts and Pretorius, 2000).

Table 2.10 Some volatile fatty acids, their aroma description, concentration ranges in wine as well as thresholds (Lambrechts and Pretorius 2000; Rossouw *et al*., 2008).

Compound	Aroma	Concentration in wine (mg/L)	Threshold (mg/L)
Acetic acid	Vinegar, pungent	150 - 900	700 – 1000 100 – 125 400
Propionic acid	Rancid,slightly pungent	Traces	20.0*
Butyric acid	Pungent	Traces	2.2/4.0*
Isobutyric acid	Pungent,less than butyric acid	Traces	8.1*
Valeric acid	Unpleasant	Traces	
Isovaleric acid	Rancid, cheese, sweaty, at times putrid, stinky	< 3	0.7*
2-Methylbutyric acid	Sour, vinegar, cheese, sweaty	?	
Hexanoic acid	Rancid, fatty, pungent	Traces -37	8 8.8*
Octanoic acid	Oily, fatty, rancid, soapy, sweet, faint fruity, butter	Traces - 41	10 15*
Nonanoic acid		?	
Decanoic acid	Fatty, unpleasant, rancid, citrus, phenolic	Traces - 54	6

*Percentage-above-chance-scores of 50 % in grain spirit solutions of 9.4 % (w/w)

2.2.5.4 Carbonyl compounds

The most prominent carbonyl compound detected in wine is acetaldehyde as it contributes to maintain the redox balance during glycolysis. This compound can be present at levels ranging between 10 - 300 mg/L and has a sensory threshold value of 100 mg/L in wine. Carbonyl compounds are generally associated with aromas of apple, nutty and even citrus (Swiegers and Pretorius, 2005). Although acetaldehyde is generally associated with oxygen exposure and therefore due to oxidation specifically in white wines, it is known that some yeast strains that are sulphite-resistant produce higher levels of acetaldehyde (Casalone *et al*., 1992). Other carbonyl compounds (**Table 2.11**) that can contribute include diacetyl, acetoin and 2,3-pentadione. Acetoin can be produced in low concentrations by yeast while diacetyl and 2,3-pentadione are usually associated with wines that went through malolactic fermentation. Carbonyl compounds are usually of interest due to their low threshold values (**Table 2.11**) (Longo *et al*., 1992).

Table 2.11 Some carbonyl compounds their aroma descriptions, ranges in wine as well as sensory thresholds (Lambrechts and Pretorius 2000)

Compound	Aroma	Concentration in wine (mg/L)	Threshold (mg/L)
Acetaldehyde	Sour, green apple	10 – 300	100
Benzaldehyde	Bitter almond	0.3×10^2 – 4.1	
Butanal	Pungent	Traces	
Diacetyl	Buttery	0.05 – 5	0.15ζ 2 – 5*
Propanal	Similar to acetaldehyde	Traces	
Isobutanal	Slightly apple like	Traces	
Pentanal	Cocoa, coffee-like, slightly fruity, choking at high levels	Traces	
Isovaleraldehyde	Warm, herbaceous, slightly fruity, nut-like, acrid at high levels	Traces	
2-acetyltetrahydropyridine	Mousy taint	Traces	1.6×10^{-3}

*Values above which an off-flavour will result
ζBeer

2.2.5.5 Sulphur-containing compounds

Sulphur compounds can be divided into sulphides, polysulphides, thioesters, heterocyclic compounds and thiols (Swiegers and Pretorius, 2005). Except thiols that were already discussed in 2.2.2 the other compounds are generally associated with having a negative impact on wine releasing aromas like rotten egg, garlic and cabbage. In **Table 2.12** the main sulphur compound groups are listed with their aroma descriptors. *S. cerevisiae* plays a vital role in the production of volatile sulphur compounds and can produce these off-flavour volatiles from sulphur sources or precursors derived from grapes (Swiegers *et al*., 2007). Most of the sulphur compounds formed during alcoholic fermentation, especially H_2S, is associated with off-flavours and usually goes hand-in-hand with yeast nutrition. This shows that although yeast strains do play an important role in production of sulphur compounds. The winemaker can limit most of the production these sulphur odours by making sure the grape must is not nitrogen-deficient therefore minimizing the impact on the varietal aroma. Most yeast strains available for commercial use have been developed to minimize H_2S and off-flavour sulphur production and enhancing the favourable volatile thiol release.

Table 2.12 Sulphur compounds and their correlating volatile aroma produced during alcoholic fermentation by yeast strains (Compiled from Swiegers and Pretorius 2005; Swiegers *et al.,* 2007).

	Sulphur compounds	Volatile aroma	Aroma threshold (µg/L)
1.	H_2S	*Rotten egg aroma*	30 – 80
2.	Methanethiol	*Cooked cabbage*	0.3
3.	Dimethylsulfide and Dimethyltrisulfide	*Cabbage, cauliflower, garlic aromas*	25
4.	Methylthioesters	*Cooked cauliflower, cheesy, chives*	-
5.	Fruity volatile thiols	*Passionfruit, grapefruit, gooseberry, guava and box hedge*	(refer to **Table 2.6**)

2.3 CONCLUSION

Generally it is stated that a “green” Sauvignon blanc can be created in the vineyard and a “tropical” Sauvignon blanc can be created by selecting a specific yeast strain. The balance between “green” and “tropical” remains essential for the final aroma profile.

All data show that the choice of yeast strain or strains used during alcoholic fermentation does have a significant impact on the final aroma profile of Sauvignon blanc. It is also clear that yeast plays a role in the modulation of the varietal aroma compounds, specifically monoterpenes, volatile thiols and the overall fermentation bouquet. The effect of yeast on methoxypyrazines has not yet been conclusively elucidated. In **Figure 2.4** a schematic representation is made of the five aroma classes during ripening stage (previously described in **Figure 2.1**) and how yeast can impact on the character of such wines. The data indeed show that it is possible to shift from one aroma class to another through mechanism described in this review.

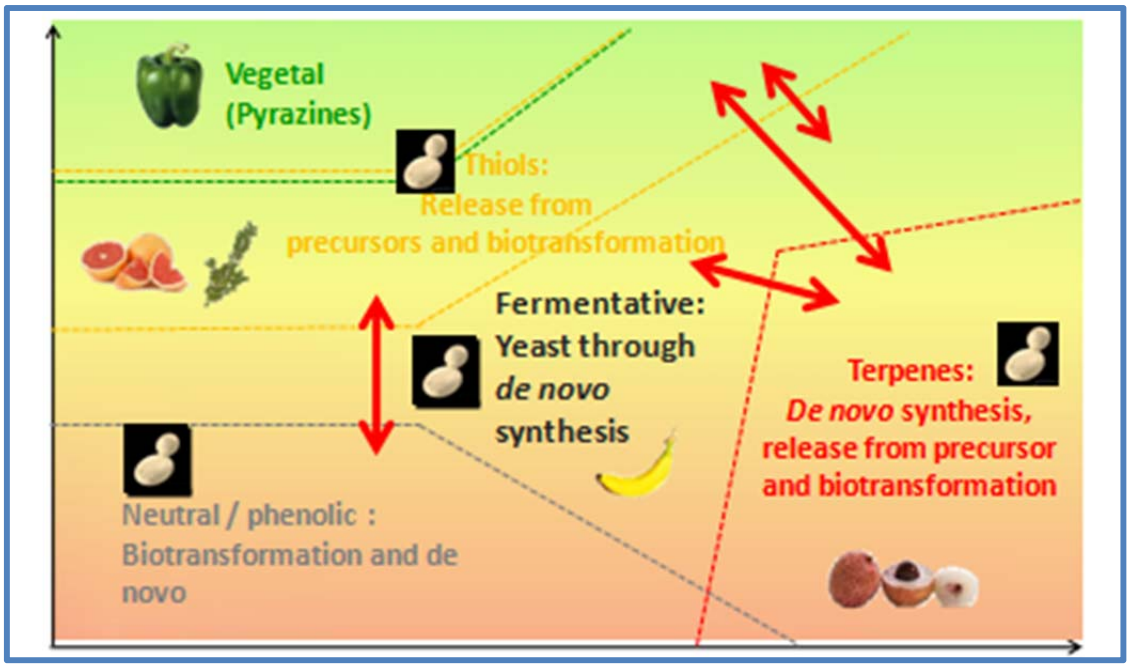

Figure 2.4 A Schematic representation showing how yeast can be used to shift between varietal aroma profiles.

Winemakers have access to information allowing an informed decision when choosing a yeast strain to create a certain wine style.

It is evident that yeast strains developed for commercial use have to comply with certain characteristical aspects listed in **Table 2.13**. Several companies supply dried preparations of highly specialised yeast strains that have specifically been selected to act as the basis of a desirable fermentation. Furthermore, and although Genetically Modified Organisms are not accepted in the global wine industry, tailoring yeasts to prevent off-flavours and promote favourable wine styles are certainly an option for the future.

Table 2.13 The specification that commercial yeast used for inoculation should comply with (Compiled from Regodon *et al*., 1997).

Selected yeasts should comply with the following:
1. ↓ Production of VA
2. ↑ Tolerance to alcohol
3. Ethanol production to quantity of sugar in must
4. Total fermentation of sugars
5. Good fermentation speed
6. Growth at high temperature
7. Resistance to SO_2
8. ↓SO_2 production
9. ↓ H_2O production
10. Facilitate settling after fermentation
11. ↓ Foaming
12. Killer phenotype
13. Good glycerol production
14. Limited production of higher alcohols

Taken together, the data clearly suggest that the choice of a specific yeast strains can be of help to achieve a specific wine style, winemakers have to take into account a multitude of other parameters such as vineyard management and fermentation management practices (temperature, oxygenation, nutrient additions) to ensure a favourable outcome.

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Chapter 3

Research results

The impact of wine yeast strains on the aromatic profiles of Sauvignon blanc wines derived from characterized viticultural treatments

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3. Research results

The impact of wine yeast strains on the aromatic profiles of Sauvignon blanc wines derived from characterized viticultural treatments

3.1 INTRODUCTION

Aroma is a major driver of consumer perception and liking, and a large number of studies have focused on understanding the multifaceted process of aroma development during grape ripening and winemaking. However, we are far from fully understanding these complex processes and our ability to control aroma production remains limited.

The importance of yeast has been summarised in a review by Fleet (2003). In this review, seven mechanisms are mentioned on how yeast can have an impact on wine flavour. Two of these mechanisms are of particular importance for this study, (i) the conversion of grape juice into wine, which, besides the conversion of sugars into alcohol by alcoholic fermentation, includes the production of a large number of secondary, aroma active metabolites, and (ii) the transformation of the non-volatile grape components into flavour active aroma compounds, namely thiols and terpenes.

Sauvignon blanc is known to be one of the cultivars which can produce a variety of wine styles ranging from fresh and crispy “green” to more rich and fruity “tropical” styles. The origin of aroma compounds leading to these aroma profiles can be divided into three main groups; grape-derived compounds, fermentation derived compounds as well as aroma compounds that form during bottle ageing. Sauvignon blanc is a wine not usually bought to be matured and for this reason the focus will be on the first two groups.

Grape-derived compounds can be divided into several groups, of which three are of particular importance for this varietal, methoxypyrazines, volatile thiols as well as terpenes. Methoxypyrazines are formed as secondary products from amino acids by plants and are associated with aromas such as bell pepper, grassy, vegetative, leading to “greener” wine styles (Swiegers *et al*., 2006). The most prominent methoxypyrazine is 2-isobutyl-3-methoxypyrazine (IBMP), which has a very low perception threshold and contributes to aromas of bell pepper. Two other compounds present at smaller concentrations include 2-isopropyl-3-methoxypyrazine (IPMP) and 2-*sec*-butyl-3-methoxypyrazine (SBMP) (King, 2010). Importantly, these compounds cannot be significantly modified by yeast and their final presence in wine is entirely dependent on viticultural practices and the processing of the grapes in the cellar.

Another family of grape derived compounds that adds to the varietal character of Sauvignon blanc is volatile thiols. Thiols enhance Sauvignon blanc’s “tropical” aromas such as passion fruit, grapefruit, gooseberry and box tree and when present in very high concentration, cat urine. The most prominent thiols present in Sauvignon blanc are 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and lastly 3-mercaptohexyl acetate (3MHA). The first two

compounds are grape derived and present in a non-volatile cystein bound and glutathione conjugates in grape must. This precursor can be transformed into an aroma active compound during alcoholic fermentation due to *β*-lyase activity in the presence of *S. cerevisiae* (Roland *et al*., 2010). Swiegers *et al.* (2006) showed that 3MHA is formed by the esterification of 3MH by wine yeast and that no cystein conjugate precursor for 3MHA can be found in the grape must.

Finally, terpenes are present in grape must in a free volatile or a sugar conjugated non-volatile form (Gunata *et al*., 1985). As with thiols, the aroma active free form needs to be released from the bound form either enzymatically or through chemical hydrolysis. The bound forms are known as glycoconjugates and are hydrolyzed by an enzyme known as *β*-glycosidase releasing the volatile terpene (Hernández *et al*., 2003). During alcoholic fermentation yeast secretes glucosidases (Fleet, 2008; Styger *et al*., 2011). Of these terpenes, monoterpenes, namely linalool, citronellol and nerol usually contribute to the floral aroma mostly in Muscat cultivars, but are also present at lower concentration is Sauvignon blanc wines (Ebeler, 2001).

The fermentation derived compounds that are de novo synthesised by yeast include esters, higher alcohols, volatile fatty acids, carbonyls and sulphur containing compounds. Their production is dependent on parameters such as the genetic background of the dominant yeast strain, the availability of precursors (including added yeast nutrients), fermentation temperature, oxygen exposure as well as fermentation stresses. Garde-Cerdán *et al*. (2008) investigated the effect of amino acid addition to nitrogen deficient must. The data showed that the formation of total esters could be enhanced by higher amino acid content. Two important esters, isoamyl acetate and 2-phenylethyl acetate, which contribute to a fruity aroma, were proven to be directly proportional to the increase of amino acid concentration. Higher alcohols did not seem to be much affected except for 2-phenylethanol and amino acid addition favoured total fatty acid production. Fermentation temperature plays a crucial role in volatile aroma fatty acid production. If fermentation temperatures are too low, sluggish or stuck fermentations can occur due to the presence of medium chain fatty acids (MCFA) especially octanoic and decanoic acids (Torija *et al*., 2003). As the productions of MCFA are depended on yeast strains, the yeast choice once again becomes relevant. From this study it is clear that must composition, especially the amino acid content can favour volatile aroma production.

Of the previously listed fermentation compounds, esters are by far the most important as they contribute to the fruity aroma of Sauvignon blanc (Verstrepen *et al*., 2003). These include esters of acetate as well as fatty acid ethyl esters, whose production is catalysed by alcohol acetyltransferases (AATases) (Verstrepen *et al*., 2003).

Higher alcohol production contributes to the background aroma and can have a positive or negative contribution depending on the concentration levels present. At optimum levels these alcohols can contribute to the complexity of the final wine.

Volatile fatty acids would have a negative effect portraying pungent aromas if it was not for the fact that they are almost always below their threshold level making the presence of fatty

acids a positive contribution (King, 2010). However, the ethyl esters of fatty acids offer pleasant, fruity aromatic compounds.

This data clearly demonstrate the importance of both viticultural and oenological practices, including the choice of yeast strain, when aiming for a specific, “green” or “tropical”, Sauvignon blanc wine style. Producing such wine styles does clearly not only depend on focusing on one aspect of the winemaking process but rather on complex interactions between the terroir, climate, viticulture treatments and cellar practises such as fermentation temperature control, yeast selection and sulphur additions (Dubourdieu *et al*., 2006; Swiegers *et al*., 2006, 2009; Roncoroni, *et al*., 2011).

For a winemaker, the fundamental question is how to achieve a desired style. In recent years many studies have aimed at determining the impact flavour/aroma compounds and investigating ways of enhancing their presence in wine (Murat *et al*., 2001; King *et al*, 2008; Barrajón *et al*., 2011; Bellon *et al*., 2011).

However, most studies focus only on individual aspects to improve wine styles and quality. The integrated study was therefore undertaken to address both the viticultural and oenological aspects by focusing the research on a controlled research chain starting with a characterized model vineyard and ending with a chemical, sensory and quality assessment of the final product. The viticultural treatment is carried out on a well characterized Sauvignon blanc block and includes a leaf removal treatment resulting in grapes having more light exposure (exposed treatment) as well as a no leaf removal treatment resulting in the grapes having less light exposure (shaded treatment). The treatment has an effect on the precursors formed in the grape resulting in must differences. During alcoholic fermentation yeast plays an important role in transforming the non-aromatic grape must into its aromatic form.

The aim for this specific study is to assess the impact of two different yeast strains on the aroma composition of wines fermented with grapes obtained from the two viticultural treatments in a characterized Sauvignon blanc vineyard as mentioned above. Particularly on fermentation kinetics and aroma compound production of esters, higher alcohols, volatile fatty acids, carbonyl compounds and monoterpenes as one part of the integrated research chain.

3.2 MATERIALS AND METHODS

3.2.1 WINEMAKING PROCESS

3.2.1.1 Experimental viticulture treatments 2011 and 2012

Sauvignon blanc (*Vitis vinifera* L.) grapes were obtained from Morningstar, a farm situated in Elgin, for both 2011 and 2012 vintages. The vines (clone 316 grafted on rootstock 101.14 Ruggeri) were planted in 2004 with row direction NW-SE (2.8m x 1.5m). A vertical shoot positioned (VSP) trellis system with two removable wires was used. The specific block is the subject of a viticultural research project focusing on light intensity and the effect on the grape

composition and final wine style. For both seasons grapes where harvested from the same rows to investigate the treatment effect over a period of two years. The viticulture treatment includes two canopy treatments, 100% exposed bunches morning side and 100% shaded bunches, as described in Kritzinger (2011) section 4.2.5.1. A total leaf and lateral removal in the bunch zone (40cm height from the cordon) was applied. Shaded treatment was a permanent thick canopy at the bunch zone during berry growth and ripening. The resultant light effect is described in **Table 3.1**.

Figure 3.1 illustrates the vineyard block layout. A checkers board layout was chosen to exclude any heterogeneity present in the block. The block consisted of 17 rows of which row 4 – 11 was harvested in 2011. The same rows were harvested in 2012 except for row 5 that was harvested separately for an additional experiment (*Panel resolution*) assessing the variation between the individual panels.

Table 3.1 Two viticulture treatments for Sauvignon blanc grapes obtained for winemaking

Viticulture treatment	Code	Light treatment
Leaf removal	LR	Exposed Canopy
No Leaf removal	NLR	Shaded Canopy

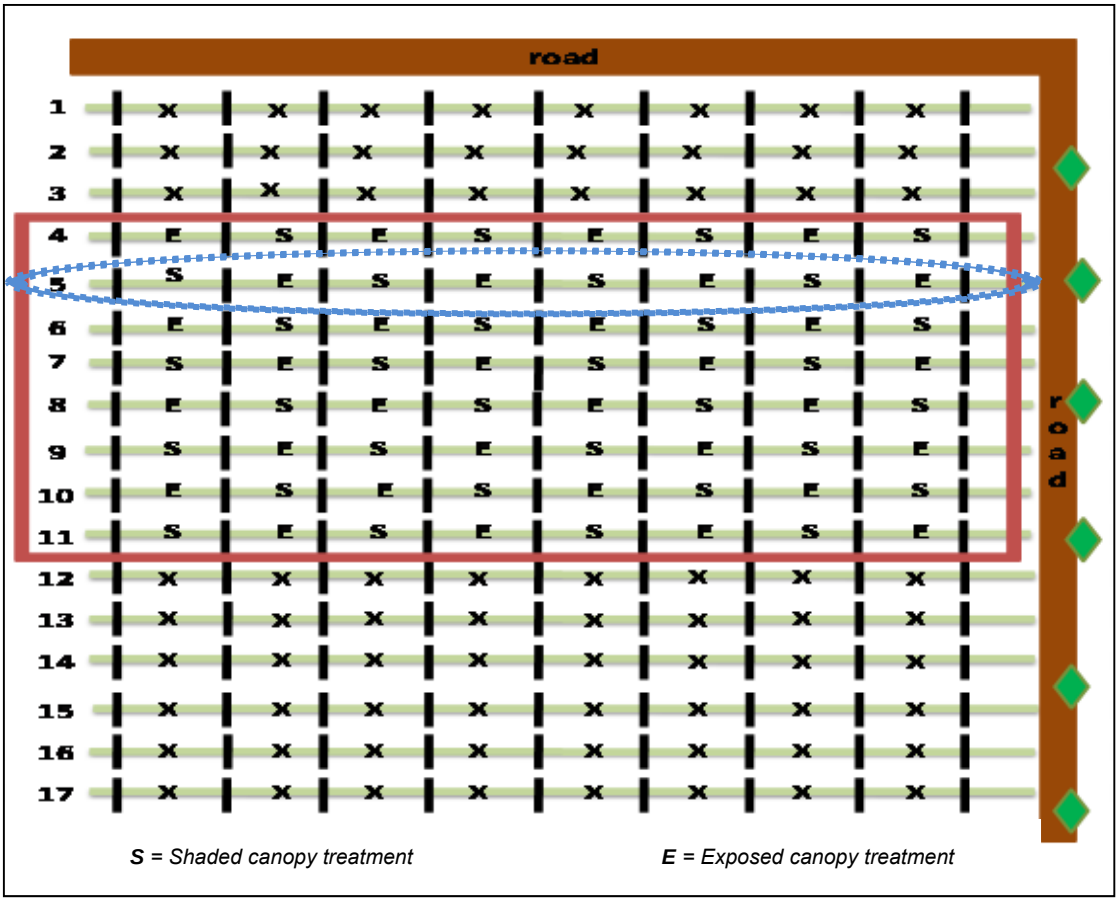

Figure 3.1 Sauvignon blanc vineyard block layout for 2011 and 2012 vintages consisting of shaded (S) and exposed (E) viticultural treatments. The red box indicates the rows harvested. Row 5 was harvested separately in 2012 for an additional experiment.

3.2.1.2 Harvest method for 2011 and 2012

The grapes were handpicked from both treatments and were kept separate for experimental purposes. For shaded bunches, the whole canopy was harvested whereas for the exposed canopy only bunches that were exposed were harvested, reducing the total amount of grapes harvested. Both treatments were harvested on the same date. Shaded grapes harvested in 2011 were 350.9 kg and in 2012 were 493.5 kg. Exposed grapes harvested in 2011 were 203.6 kg and in 2012 were 216.50 kg.

3.2.1.3 Experimental winemaking for 2011 and 2012

Throughout the winemaking process the shaded and exposed grapes were treated identically. Grapes were crushed and sulphur dioxide was added at 30 ppm. Juice was homogenized while using dried ice to keep oxygen exposure to a minimum. The grapes were pressed after which the juice from the shaded grapes was transferred to 300 L tank and that from the exposed grapes to a 90 L tank. A pectolytic enzyme (Rapidase® Vino super, DSM Oenology, The Netherlands) was added at 0.3 ml/L and the juice was left to settle overnight at 4 °C.

The juice from each treatment was divided and racked into six 20 L steel canisters which were prefilled with CO_2 gas to limit oxygen contact.

Experimental winemaking layouts for the two treatments were the same and consisted of inoculating with two different *Saccharomyces cerevisiae* wine yeast strains. For the 2011 vintage three canisters from each canopy treatment were inoculated with Cross Evolution® (Lallemand) and with VIN7 (Anchor Yeast Biotechnologies) respectively. For the 2012 vintage VIN7 was replaced by Lalvin DV10 which is classified as *Saccharomyces cerevisiae* var. *bayanus.* VIN7 was replaced due to poor transcriptomic results in 2011. The experimental codes are described in **Table 3.2** for the 2011 and 2012 vintage.

The yeast was rehydrated and inoculation was done at 0.3 g/L as the suppliers recommend. For both yeasts the experiment was done in triplicate. The canisters were placed at 15 °C where the fermentation took place.

After sugar concentration had decreased by 5°B, diammonium phosphate was added at 0.3 g/L.

Table 3.2 Combination between viticultural canopy treatments and yeast strains used in 2011 and 2012

Code 2011	***VIN7***	***Cross Evolution***
Shaded Canopy (NLR*)	SHVIN1	SHCE1
	SHVIN2	SHCE2
	SHVIN3	SHCE3
Exposed Canopy (LR*)	EXPVIN1	EXPCE1
	EXPVIN2	EXPCE2
	EXPVIN3	EXPCE3
Code 2012	***DV10***	***Cross Evolution***
Shaded Canopy (NLR*)	SHDV1	SHCE1
	SHDV2	SHCE2
	SHDV3	SHCE3
Exposed Canopy (LR*)	EXPDV1	EXPCE1
	EXPDV2	EXPCE2
	EXPDV3	EXPCE3

*NLR =no leaf removal
*LR = leaf removal

CO_2 gas was used to avoid oxygen contact during sampling. When the fermentation was dry (sugar concentration being < 5 g/L) the wine was racked into 4.5 L glass bottles and SO_2 was added to 70 ppm. Sugar was determined by using Fourier transform mid-infrared spectroscopy (FT-MIR) (WineScan FT 120, Foss Analytical, Denmark). After cold stabilization for one week at 4 °C the free sulphur was adjusted to 30 ppm. 4.5 L glass bottles of each replicate were first racked into a canister, homogenised then filtered and bottled into green 750 ml sterilized bottles. Bottles were sealed with screw cap closures. After bottling the wines were stored at 15 °C.

3.2.1.4 Small-scale winemaking for panel resolution 2012

The panel resolution experimental wines in 2012 were made to determine the range of internal variation within each vineyard canopy treatment. Instead of making wine as a batch from shaded and exposed grapes, one row (refer to **Figure 3.1** row 5) from the experimental layout was chosen and the panels were handpicked separately and small-scale wines were made. The row consisted of six panels being harvested, three shaded and three exposed panels in random order as set out in the vineyard layout (checkers board layout). The total kilograms harvested for each panel is shown in **Table 3.3**. Grapes from each panel were kept separately.

Crushing was by hand and dry ice was used to keep oxygen exposure to a minimum. A sulphur addition of 30 ppm, as well as an addition of pectolytic enzyme (Rapidase® Vino super, DSM Oenology, The Netherlands) at 0.3 ml/L was made and juice was left to settle overnight at 4 °C. The settled juice of each panel was divided and racked into three 500 ml Erlenmeyer flasks resulting in eighteen flasks, as each panel was done in triplicate. *Saccharomyces cerevisiae* yeast strain Cross Evolution® (Lallemand) was used to inoculate the eighteen flasks. Fermentation caps were used to prevent oxidation and the wines were left to ferment at 15 °C. Diammonium phosphate was added at 0.3 g/L on day three of alcoholic fermentation. Alcoholic

fermentation was monitored by percentage in CO_2 weight loss because of small volumes. When weight loss stabilised the fermentation was considered completed.

Table 3.3 Grapes harvested per panel.

Shaded panels	*Kilograms*	*Exposed panels*	*Kilograms*
SH1 (R*5-P*2)	8.0kg	**EXP1 (R5-P1)**	7.04kg
SH2 (R5-P4)	11.22kg	**EXP2 (R5-P3)**	7.24kg
SH3 (R5-P6)	10.20kg	**EXP3 (R5-P5)**	7.20kg

*R = Row
*P= Panel

3.2.1.5 Sampling procedure during alcoholic fermentation for batch fermentations

Sampling was done on the juice before sulphur addition and the juice was analyzed for free SO_2, pH and sugar. CO_2 gas was used to prevent oxidation during sampling. Samples were taken at specific stages of fermentation progress in small sampling bottles which were prefilled with CO_2 gas. After sampling the headspace were filled with CO_2 gas. All sampling points are shown in **Table 3.4**.

Table 3.4 Samples taken in the 2011 and 2012 vintages for analyses

	2011 Vintage				2012 Vintage			
Timepoint	***1/4***	***1/2***	***3/4***	***4/4***	***1/4***	***1/2***	***3/4***	***4/4***
Major volatiles	√	√	√	√	√	√	√	√
Carbonyl compounds				√				√
Monoterpenes								√
Malic acid				√	√	√	√	√
Citric acid								√

In addition to **Table 3.4**, sampling for monitoring the fermentations and samples for yeast cell counts were taken every day for the duration of the fermentation. Sugar and microbial analyses as well as major volatile analysis were done immediately and the rest of the samples were frozen at -20 °C until further analysis could be done.

3.2.1.6 Sampling procedure during alcoholic fermentation for panel resolution

The sampling procedure was done in the same manner as discussed in section 3.2.1.5. Samples were taken for the analysis of major volatiles and of malic acid at around halfway and at the end of alcoholic fermentation. For carbonyl compounds and monoterpenes only end of alcoholic fermentation samples were analysed.

3.2.2 Microbiological analysis

Microbiological analysis was only undertaken on the batch fermentations for 2011 and 2012 and not for the panel resolution experimental wines. Samples were taken daily during alcoholic fermentation as described in section 3.2.1.5. Samples were homogenized and a ten-fold serial dilution series was prepared by using sterile de-ionized water.

For microbiological purposes plating was done on Yeast Peptone Dextrose agar (Biolab, Merck, South Africa) containing 12 % ethanol and 0.015 % sodium metabisulphite. The addition of ethanol and sodium metabilsulphite suppresses most non-*Saccharomyces* yeast from growing. Plates also contained 50 mg/L chloramphenicol (Roche diagnostics GmbH, Mannheim, Germany) diluted in 1 ml 96 % ethanol which suppresses the growth of lactic acid bacteria, and 25 mg/L Kanamycin sulphate (Roche diagnostics GmbH, Mannheim, Germany) diluted in 1 ml de-ionized water which suppresses the growth of acetic acid bacteria.

Plating out was done daily for the length of the alcoholic fermentation to monitor yeast growth. The plates were incubated in aerobic conditions at 30 °C for 7 – 10 days. Plates were then counted to determine colony forming unites per millilitre (cfu's/mL).

3.2.3 Determining standard fermentation kinetics

Throughout the fermentation samples were taken every day and analysed by Fourier transform mid-infrared spectroscopy (FT-MIR) (WineScan F120, FOSS Analytical, Denmark). The instrument allows the quantification of ethanol, volatile acidity, total acidity, pH, malic acid, lactic acid, glucose, fructose, residual sugar and glycerol. Sample preparation involves 50 ml of wine samples being filtered through round filter paper with a diameter of 185 mm, graded at 20-25μm (Schleicher & Schuell, reference no. 10312714). The method used is described in Treurnicht 2011.

Although the whole spectral range is stored for each sample, only wavelengths 964 – 1532 cm^{-1}, 1716 – 2731 cm^{-1} and 3300 – 3500 cm^{-1} were selected for multivariate data analysis. The excluding of certain wavelengths is necessary to avoid noise caused by water absorption (Nieuwoudt *et al.* 2004).

3.2.4 Volatile aroma compounds

3.2.4.1 Major volatiles measured by GC-FID

Analysis was done on frozen samples for 2011 and on fresh samples in 2012. Analysing for major volatile aroma compounds (higher alcohols, esters and volatile fatty acids) in the wine was done by using gas chromatography with flame ionisation detection (GC-FID).

Five ml of wine sample was placed in Pyrex glass tubes. The internal standard, 4-methyl-2-pentanol (100 μl of 0.5 mg/l solution in 12 % v/v ethanol, 2.5 g/L tartaric acid, de-ionised water from a Mili-Q system, pH which is adjusted to 3.5 using 0.1 M NaOH) was added and the tube was closed to prevent evaporation of the internal standard. One ml of diethyl ether (Merck) is

added in a fume hood. The mixture is sonicated in an ultrasonic bath for five minutes. The wine/ether mixture is then centrifuged for three minutes at 4000 rpm. To prevent water from interfering with the extraction method a small spoon of dried $NaSO_4$ crystals are added to absorb water. The tube is vortexed and again centrifuged for three minutes at 4000 rpm. The ether layer is removed carefully with a micropipette and placed in a glass GC-vial with $NaSO_4$ to absorb any unwanted water. The extract is then taken off the salt using a micro pipette and placed in a glass insert in the vial and capped. The extracted sample is injected into the GC-FID. Each sample is injected in duplicate. The 39 compounds measured by this fast GC-FID method, validated by Malherbe *et al*. (2011) is listed in **Table 3.5.** The instrumental parameters for this method include initial temperature at 33 °C held for 8 minutes followed by a ramp of 21 °C min^{-1} up to 130 °C and held for 1.3 minutes. Another two ramps follow at the same rate, the first until 170 °C held for 1 minute and the second up to 240 °C held for 2.5 minutes. Post run occurs at 240 °C for 5 minutes. The front inlet has an injection volume of 1 µl using split mode. Split mode is used with a split ratio of 15:1 and split flow rate of 49.5 ml min^{-1}. Injector temperature is at 200 °C with initial pressure at 84.5 kPa with flow mode being constant. Column flow rate of 3.3 ml min^{-1} is maintained using hydrogen as carrier gas making runtime per sample 50 minutes. A J&W DB-FFAP column with dimension 60 m length x 0.32 mm i.d. x 0.5 µm film thickness (Agilent, Little Falls, Wilmington USA) is used. The detector used is a flame ionisation detector (Agilent, Little Falls, Wilmington USA) with temperature 250 °C.

Volatile compound peaks were integrated and quantified using the ratio of the peak area and internal standard peak area. This is made possible by calibration curves constructed for each compound with pure standards. The software used for integration was HP Chemstation software (Rev.B01.03 [204]).

Table 3.5 Major volatile compounds measured by GC-FID method

ESTERS	HIGHER ALCOHOLS	VOLATILE FATTY ACIDS
Ethyl Butyrate	Methanol	Acetic acid
Ethyl Hexanoate	Propanol	Propionic acid
Ethyl Lactate	Isobutanol	Isobutyric acid
Ethyl Caprylate	Butanol	Butyric acid
Ethyl Caprate	Isoamyl alcohol	Isovaleric acid
Diethyl Succinate	Hexanol	Valeric acid
Ethyl Acetate	2-Phenylethanol	Hexanoic acid
Isoamyl Acetate	4-Methyl-1-pentanol	Octanoic acid
Hexyl Acetate	4-Methyl-1pentanol	Decanoic acid
2-Phenylethyl Acetate	3-Ethoxy-1-propanol	
Ethyl Propionate	Pentanol	
Ethyl-2-methylpropanoate	1-Octen-3-ol	
2-Methyl-propyl acetate		
Ethyl-2-methylbutyrate		
Ethyl Isovalerate		
Ethyl-3-hydroxybutanoate		
Ethyl Phenylacetate		

3.2.4.2 Monoterpenes extraction by GC-FID

Monoterpene extraction was done by making use of solid phase extraction (SPE). The method used is described in Zietsman *et al*. (2011). **Table 3.6** lists the compounds quantifiable by this method.

Table 3.6 Monoterpenes quantifiably by GC-FID method

Limonene	**Linalyl acetate**	**Geraniol**
Fenchone	α-Terpeneol	α-Ionone
Linalooloxide	Citronellol	β-Farnesol
Linalool	Nerol	

3.2.4.3. Major Carbonyl compounds measured by GC-MS

Sampling for carbonyl compounds was done at end of alcoholic fermentation. Carbonyl compounds were determined by making use of gas chromatography mass spectrometry (GC-MS). The method used is described in Malherbe *et al.* (2012). In **Table 3.7** the compounds quantifiable by the method is listed.

Table 3.7 Compounds quantifiable by GC-MS

Diacetyl	Acetoin	Nonenal
2,3-Pentadione	Heptenal	Nonadienal
Hexenal	Octenal	
Octanal	Decanal	

3.2.5 Malic acid and citric acid analysis

The Arena XT 20 enzyme robot (Thermo Electron Oy, Finland) was used with enzyme kits for malic acid (Enzytec™ *Fluid* L-Malic acid Id-No: 5280. Thermo Fisher Scientific Oy, Finland distributed by R-Biopharm AG, Germany) and citric acid determination (Thermo Scientific Citric Acid Reference number: 984327, Thermo Fisher Scientific Oy, Finland) according to manufacturer's instructions.

3.2.6. Data analysis

Univariate analyses used Statistica V. 10 software (Statsoft Inc., Tulsa, OK). Factorial analysis of variance was conducted on the volatile aroma data, combining the two viticultural canopy treatments, yeast strains used and time point of sampling. For post-hoc analyses Fisher least significant difference (LSD) were used. All significant differences were interpreted on a 5 % significance level ($p < 0.05$). Multivariate data analyses were done on GC-FID data as well as spectra generated from the FT-MIR WineScan (FT120, FOSS Analytical, Denmark). Sensory data was combined with chemical data by using Principal Component Analyses (PCA) performed in The *Unscrambler* 9.2 Software (CAMO Process AS, Oslo, Norway).

3.2.7. Sensory evaluation of final wines

A panel of 10 judges was subjected to eight hours of highly intensive training on Sauvignon blanc aroma and taste characteristics using reference standards (**Table 3.8**). Panellists were asked to individually generate descriptors for the wines. Consensus was reached on the final wine descriptors as well as intensity levels. The panellist rated the wines intensity levels on a 100 mm line scale.

After the training sessions two formal testing sessions were held in a well-ventilated and temperature (20-22 °C) controlled sensory laboratory with isolated sensory booths. Each panellist was presented with six wines at a time. All wines were tested in triplicate. All three repeats were tested on the same day. Wines (25 ml) were presented in a randomized order using three digest codes in standard ISO tasting glasses covered with Petri-dishes. Water and crackers were available for palate cleansing in between replicate sessions. No reference standards were present during the formal tasting sessions.

Table 3.8 List of reference standards used during panel training

Attribute	Fresh Standards (served in petri dishes)	Standards in wine (Dosage per 50mL)	Supplier
Pineapple	One small wedge (4cm^2) of freshly cut pineapple	NA	Purchased fresh for Spar
Citrus	1/8 of a slice (9cm^2) of freshly cut grapefruit	6g	Purchased fresh for Spar
Green Pepper	One small piece (0.5 x 2cm)	1.5g	Purchased fresh for Spar
Passion Fruit	4 pips and a 1 cm^2 piece of skin	8 pips and 2 cm^2 piece of skin	Purchased fresh for Spar
Canned Beans/peas	2 beans and 5 peas	10mL of canned bean brine and 10mL of canned pea brine	Koo, Tiger Brands Ltd., Bryanston, South Africa
Guava	One slice with peel 5cm^2	NA	Purchased fresh for Spar
Dried Fruit	¼ dried apple ¼ dried pear ¼ dried apricot ¼ dried peach ¼ prune	NA	Safari
Apple Cider	25mL	NA	Huntersdry

*NA= not applicable

3.3 RESULTS

3.3.1 Winemaking

3.3.1.1 2011 and 2012 batch-fermentation

Analysis of the juices obtained from shaded and exposed canopy treatments showed that the sugar content at harvesting date was lower for the juice obtained from the shaded canopy vines, suggesting that sugar accumulation had been slower in these conditions (**Table 3.9**).

The pH levels did not show significant differences. Titratable acidity (TA) showed a significant difference between treatments. In 2011 the TA was 1.02 g/L lower in the exposed canopy vines juice, while for 2012 it was 1.61 g/L lower. Malic acid was 1.01 g/L lower in the exposed canopy vines juice in 2011 and 1.16 g/L lower in 2012. The yeast assimilable nitrogen (YAN) was slightly higher in the juice obtained from the shaded canopy vines.

Wines made from the 2011 and 2012 harvest were monitored daily throughout alcoholic fermentation by means of FT-MIR (WineScan FT 120, Foss Analytical, Denmark).

All four treatments of the 2011 harvest completed alcoholic fermentation (**Figure 3.9**). Shaded canopy wines fermented faster than the exposed canopy wines. SHCE finished in 13 days and SHVIN finished in 14 days, while the exposed canopy wines took 20 days for EXPCE and 21 days for EXPVIN to finish alcoholic fermentation. In both treatments, Cross Evolution fermented faster than VIN7.

Table 3.9 Juice analyses for Sauvignon blanc grapes after settling for 2011 and 2012 vintage

	Balling		*pH*		*TA (g/L)*		*Malic acid (g/L)*		*YAN (mg/L)*	
	2011	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Exposed	23.03	23.45	3.18	3.16	7.42	7.64	2.54	1.97	370	260
Shaded	22.03	22.54	3.16	3.11	8.44	9.25	3.55	3.13	380	300

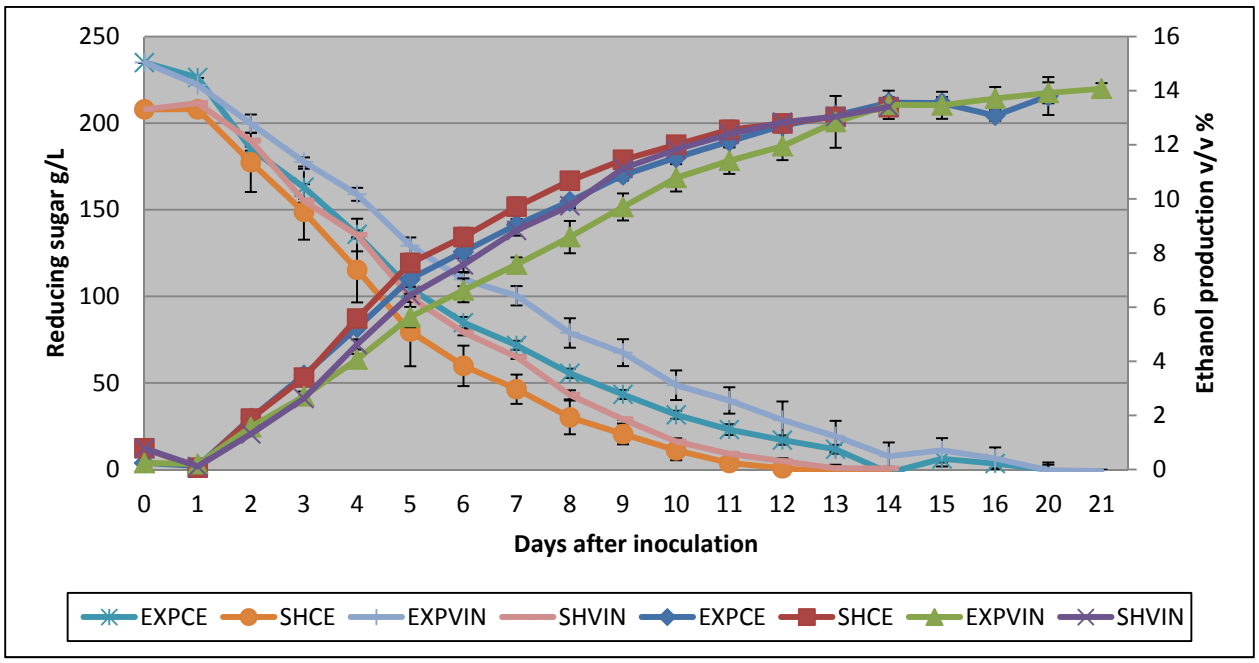

Figure 3.2 A fermentation graph showing sugar degradation and ethanol production during the 2011 vintage. Fermentation was monitored throughout alcoholic fermentation for 21 days. Data shown indicate the average changes in sugar (g/L) and ethanol (v/v %) of each treatment that was performed in triplicate. The relative standard deviation (RSD) is show in the graph.

For the 2012 harvest (**Figure 3.3**) all four treatments finished alcoholic fermentation in 25 days. There were no significant differences between the treatments except for a slight trend that the DV10 fermented faster and SHCE showed a slower start in fermentation rate.

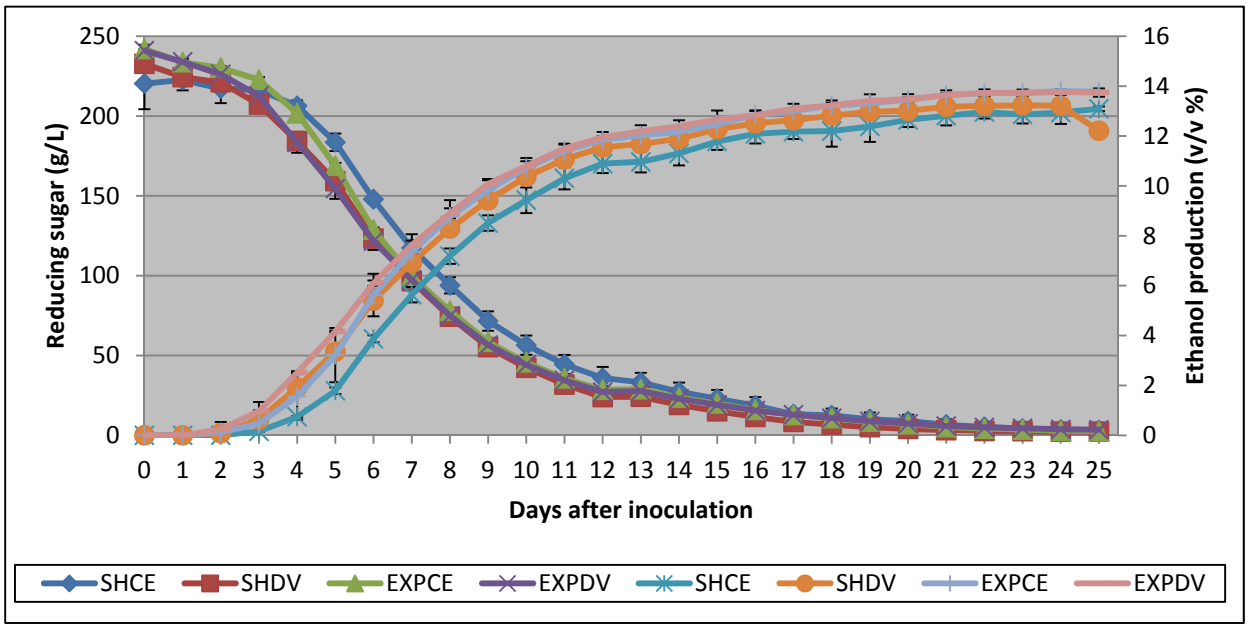

Figure 3.3 A fermentation graph showing sugar degradation and ethanol production during the 2012 vintage. Fermentation was monitored for 25 days until the end of alcoholic fermentation. Data shown indicates the average changes in sugar (g/L) and ethanol (v/v %) of each treatment that was performed in triplicate. The relative standard deviation (RSD) is shown in the graph.

3.3.1.2 2012 Panel resolution

The small scale panel resolution wines were weighed every day to monitor the weight loss. All the fermentations were dry after 25 days (**Figure 3.4**), and there were no differences in fermentation performance between the treatments in terms of the canopy treatments. When panel resolution fermentations are compared to the batch fermentation of 2012 it can be seen that fermentation performance was similar.

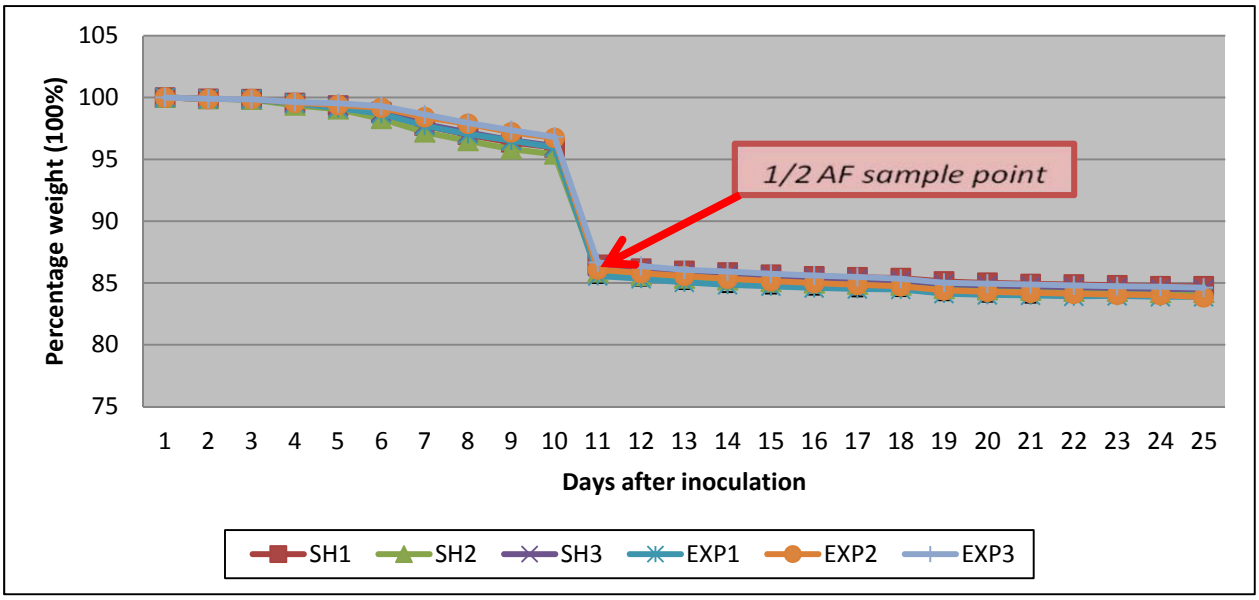

Figure 3.4 A fermentation curve of panel resolution wines monitored by measuring weight loss. The fermentations were monitored for 24 days until alcoholic fermentation was dry and the weight was stable for three days. On day nine, half alcoholic fermentation samples were taken which lead to a drop in overall weight as indicated. Data shown indicates the changes of each panel that was performed in triplicate.

3.3.2 Microbiological analysis

The fermenting musts were plated out daily during alcoholic fermentation to observe the yeast cell growth measured as colony forming units (cfu's/ml) (**Figures 3.5 and 3.6**). Both 2011 and 2012 was inoculated at 10^6 cells and increased to 10^8 after which the cell counts stayed more or less constant. In 2011, VIN7 had lower initial inoculation numbers than Cross Evolution and although SHVIN finished alcoholic fermentation first it could be the reason why EXPVIN took seven days longer.

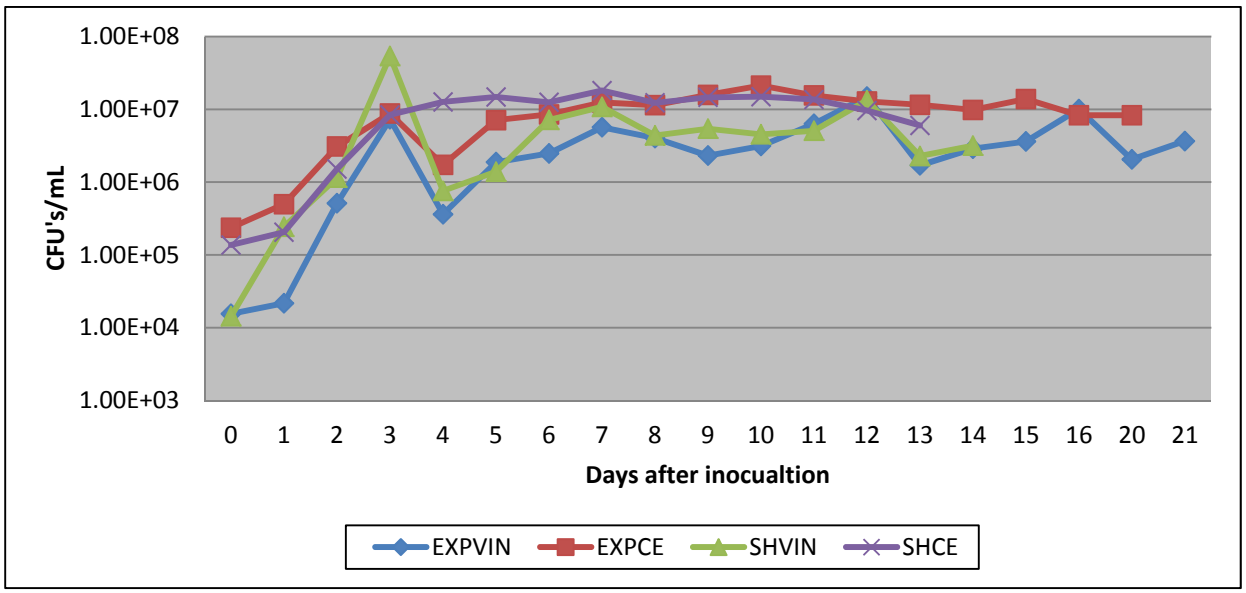

Figure 3.5 Yeast cell counts (cfu's/mL) of the four treatments in 2011. Each line represents the average of triplicate measures. Yeast growth was monitored throughout fermentation until fermentation was dry (sugar < 5 g/L).

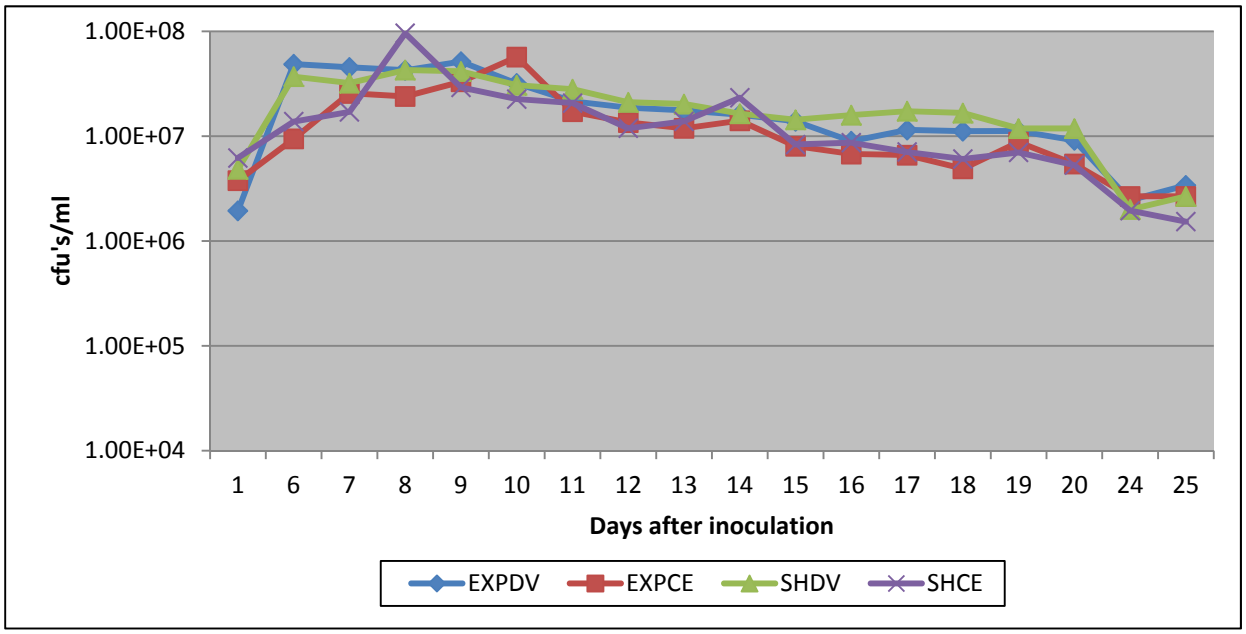

Figure 3.6 Yeast cell counts (cfu's/mL) of the four treatments in 2012. Each line represents the average of each treatment done in triplicate. Yeast growth was monitored throughout fermentation until fermentation was dry (sugar < 5 g/L). Days 2 – 5 is not included in the graph due to dilution errors which caused inconsistent cell counts.

3.3.3. Secondary metabolites

3.3.3.1 Volatile acidity

Volatile acidity showed similar trends in both vintages and for both yeast strains. Accumulation started on day 2 of fermentation, and acetic acid stabilised after day 6 for 2011 and day 13 for 2012. A vintage effect was obvious in terms of total VA produced; with 2011 leading to a 2 fold higher VA in all the VIN 7 treatments. Differences between yeast strains were also obvious, and were clearly the dominant factor explaining the differences observed in 2011. Vintage

differences are clear between 2011 and 2012 (**Figures 3.7 and 3.8**). The 2011 data showed variation between treatments with the highest concentration reaching up to 1 g/l whereas 2012 data the treatments were similar with the highest concentration level at 0.45 g/l. In 2011 the exposed viticultural treatment wines had higher volatile acidity (VA) concentrations whereas this trend was only seen in the EXPCE treatment in 2012. In 2011 VIN7 produced much higher VA levels than Cross Evolution whereas Cross Evolution produced higher concentrations than DV10 in 2012.

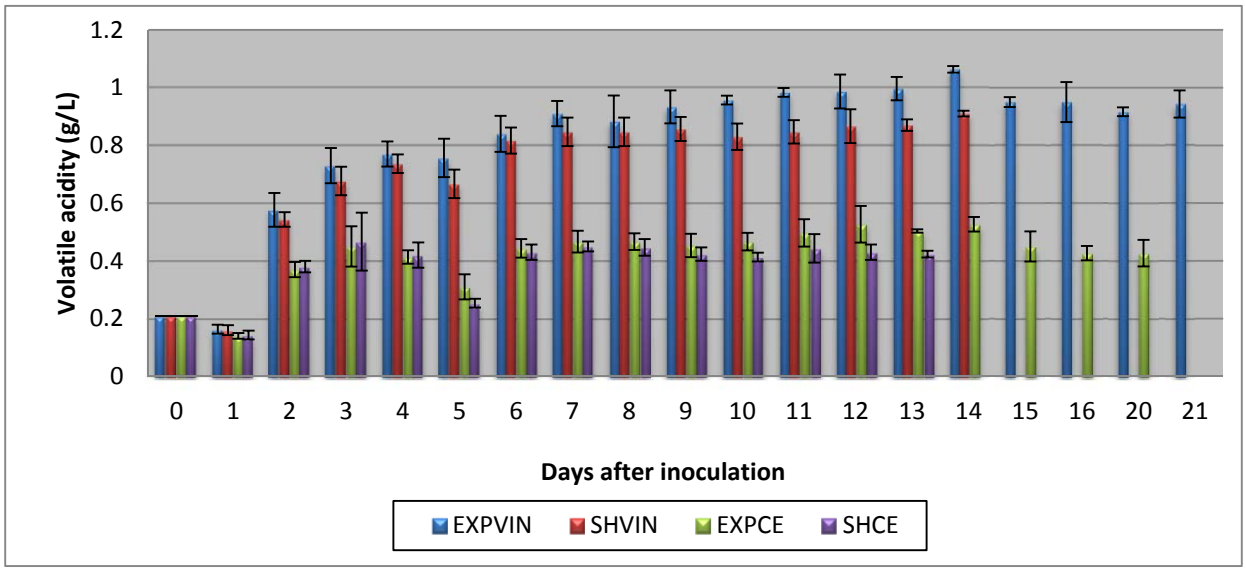

Figure 3.7 Volatile acidity for 2011 was measured by FT-MIR analysis. Data shown indicates the average changes observed for each treatment performed in triplicate. The relative standard deviation (RSD) is shown in the graph. (Samples were taken daily until day 16 and then only again on day 20 and 21).

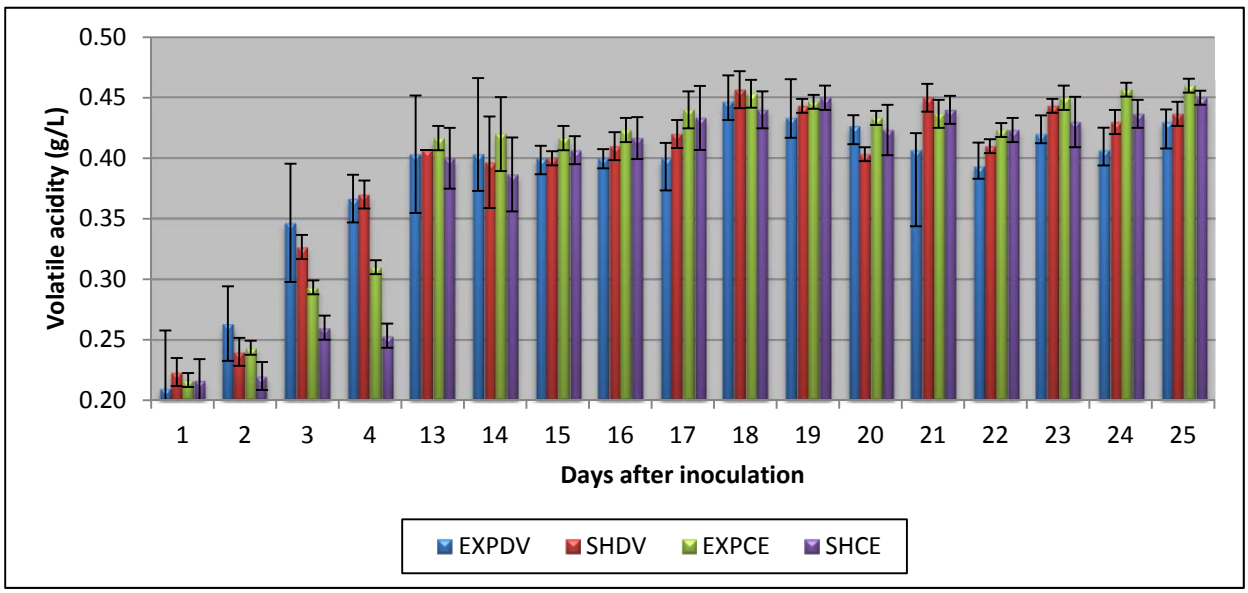

Figure 3.8 Volatile acidity for 2012 was measured until day 25 of alcoholic fermentation by FT-MIR analysis. Data shown indicates the average changes of each treatment that was performed in triplicate. The relative standard deviation (RSD) is shown in the graph. On day 12 the scanning program on FTMIR was changed form Must under fermentation to Dry white wine as sugar was < 30g/L. This led to a drop in VA measured and for this reason day 5 – 12 is excluded in the graph.

3.3.3.2 pH

In **Table 3.10** pH levels for the initial juice as well as end of alcoholic fermentation is listed. For 2011 and 2012 the pH of the juices ranged between 3.1 - 3.18 having no significant difference between treatments. During alcoholic fermentation pH of all the treatments dropped between 0.02 – 0.26 depending on the yeast strain used.

Table 3.10 The pH levels measured for 2011 and 2012 vintages for both the juice and end of alcoholic fermentation.

	2011				2012			
	SHVIN	**EXPVIN**	**SHCE**	**EXPCE**	**SHDV**	**EXPDV**	**SHCE**	**EXPCE**
JUICE	3.16	3.18	3.16	3.18	3.1	3.16	3.1	3.16
END AF*	3.01	3.16	3.03	3.12	2.99	2.92	2.98	2.90

*AF= alcoholic fermentation

3.3.4 Volatile aroma compounds

3.3.4.1 Major volatile compounds

Factorial ANOVAs were performed to determine the influence of canopy treatment, yeast and time point measured on the concentrations of the volatile compounds as well as the interaction between canopy*yeast, canopy*timepoint, yeast*timepoint and canopy*yeast*timepoint were also determined.

Comparing volatile aroma data of the two vintages (**Table 1A - 6A** in addendum A), specifically comparing fermentations done with Cross Evolution, it is clear that 2011 vintage was characterized by overall higher total ester and higher alcohol concentrations. In turn 2012 produced higher total volatile fatty acids which were mainly due to higher acetic acid concentrations. These differences can be due to a vintage effect.

Compounds that showed noticeable difference between vintages included ethyl lactate, ethyl caprate, ethyl acetate, ethyl-3-hydroxybutanoate, propanol, isobutanol, 3-ethoxy-1-propanol, acetic acid, hexanoic acid, octanoic acid and decanoic acid. Compounds that responded primarily to a single factor (*yeast or canopy*) were clearly dominated by yeast strain rather than canopy treatment for both vintages.

In 2011, 23 (**Table 3.11**) of the 39 compounds measured were influenced by the yeast treatment whereas only six compounds were influenced by the viticultural canopy treatment.

Table 3.11 Compounds influenced by the yeast and viticultural canopy treatment respectively for the 2011 vintage.

ESTERS		HIGHER ALCOHOLS		FATTY ACIDS	
Yeast	*Canopy*	*Yeast*	*Canopy*	*Yeast*	*Canopy*
Ethyl butyrate	Ethyl lactate	Propanol	Pentanol	Acetic acid	Acetic acid
Ethyl hexanoate	Ethyl caprate	Isobutanol		Butyric acid	Isobutyric acid
Ethyl lactate	Isoamyl acetate	Butanol		Isovaleric acid	
Ethyl caprylate		2-Phenylethanol		Hexanoic acid	
Diethyl succinate		3-ethoxy-1-propanol		Octanoic acid	
Isoamyl acetate		Pentanol		Decanoic acid	
Hexyl acetate					
2-Phenylethyl acetate					
2-Methyl-propyl acetate					
Ethyl-3-hydroxybutanoate					
Ethyl phenylacetate					

In 2012, 22 (**Table 3.12**) of the 39 compounds measured were influenced by the yeast treatments whereas 10 compounds were influenced by the viticultural canopy treatment.

Table 3.12 Compounds primarily influenced by the yeast and viticultural canopy treatment respectively for the 2012 vintage.

ESTERS		HIGHER ALCOHOLS		FATTY ACIDS	
Yeast	*Canopy*	*Yeast*	*Canopy*	*Yeast*	*Canopy*
Ethyl butyrate	Diethyl succinate	Methanol	Methanol	Isobutyric acid	Propionic acid
Ethyl hexanoate	Isoamyl acetate	Propanol	Propanol	Valeric acid	Isobutyric acid
Ethyl lactate	2-Methyl-propyl-acetate	Isobutanol	Isobutanol	Hexanoic acid	
Ethyl caprate		Butanol	2-Phenylethanol	Octanoic acid	
Diethyl succinate		Isoamyl alcohol	4-Methyl-1-pentanol	Decanoic acid	
Ethyl acetate		4-Methyl-1-pentanol			
Isoamyl acetate		3-ethoxy-1-propanol			
2-Methyl-propyl-acetate		Pentanol			
Ethyl phenylacetate					

For the 2012 panel resolution 17 compounds (**Table 3.13**) were influenced by the viticultural canopy treatment with Cross Evolution used as yeast strain.

Table 3.13 Compounds influenced by the viticultural canopy treatment for the 2012 panel resolution wines

CANOPY		
Esters	***Higher alcohols***	***Volatile fatty acids***
Diethyl succinate	Methanol	Propionic acid
Ethyl acetate	Propanol	Isobutyric acid
2-Phenylethyl acetate	Isobutanol	Isovaleric acid
2-Methyl-propyl acetate	Isoamyl alcohol	Decanoic acid
Ethyl-3-hydroxybutanoate	2-Phenylethanol	
Ethyl phenylacetate	Pentanol	
Ethyl caprate		

Results obtained for panel resolution wines measured over two time points are shown in **Table 7A** in addendum A.

3.3.4.1.1 2011

Compounds influenced significantly in the factorial ANOVAs are shown in **Table 9A - 10A** in addendum A with significant *p*-values indicated in red. In **Table 11A** and **12A** the compounds significant on a third and second order of interaction is shown.

Esters were the compound group mostly influenced by both treatments although yeast strain showed a greater impact.

The time points measured during alcoholic fermentation showed to be significant for all the compounds except for hexanol and 4-methyl-1-pentanol which did not show a significant change during the four time points.

Only one ester, diethyl succinate, (**Figure 3.9**) was significant for the interaction between canopy*yeast*timepoint and two esters (*ethyl lactate, 2-phenylethyl acetate*) were significant for the interaction between canopy*yeast.

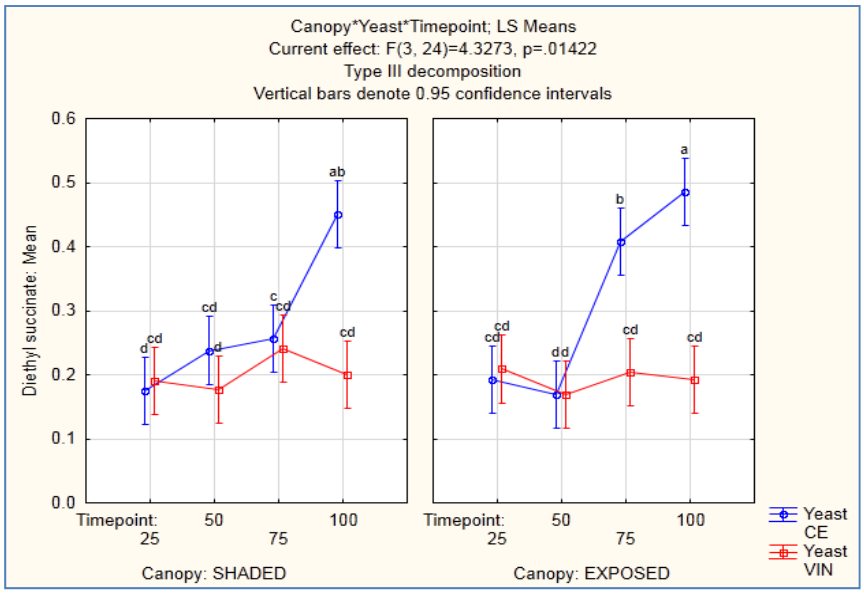

Figure 3.9 The figure shows the third order of interaction Canopy*yeast*timepoint for diethyl succinate the only volatile compound showing significant differences due to all three factors. Different letters within a figure denote significant differences at $p < 0.05$.

Yeast had the most influence (**Figure 3.9**), with Cross Evolution producing significantly higher concentrations for diethyl succinate than VIN7. Both viticultural treatments showed the same trend with VIN7 staying constant over the time points whereas Cross Evolution had an increase to the end of alcoholic fermentation.

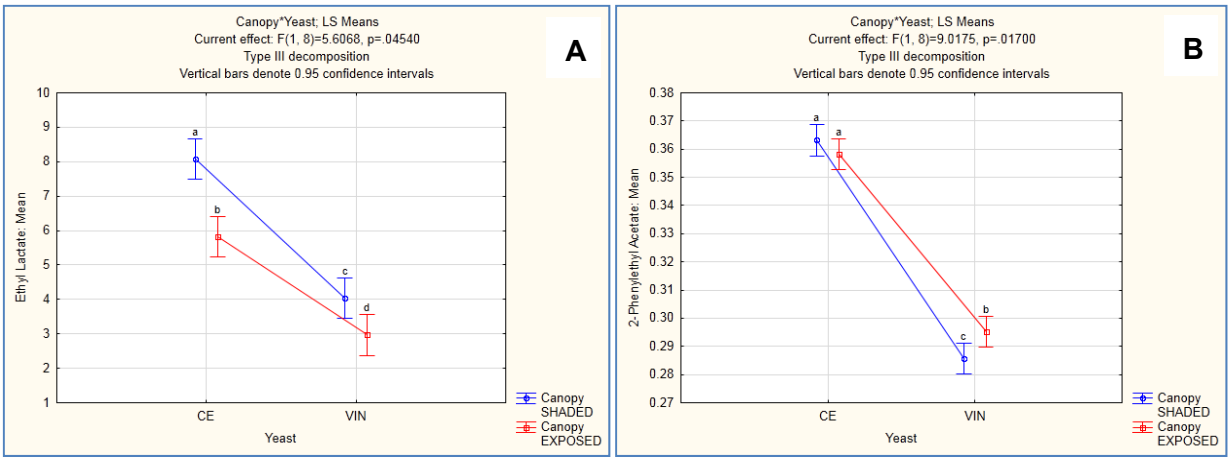

Figure 3.10 Secondary interaction between Canopy*yeast for the two esters (**A**) ethyl lactate and (**B**) 2-phenylethyl acetate the only compounds significantly affected by the two factors. Different letters within a figure denote significant differences at $p < 0.05$.

Ethyl lactate and 2-phenylethyl acetate (**Figure 3.10**) were influenced by the yeast strain with Cross evolution producing significantly higher concentrations than VIN7. The shaded treatment was significantly higher in ethyl lactate for both yeast treatments. For VIN 7, 2-phenylethyl acetate showed higher concentrations for the exposed treatment.

No significant interaction between canopy*yeast*timepoint was found for higher alcohols or volatile fatty acids although one fatty acid, acetic acid (**Figure 3.11**), was influenced by the canopy*yeast interaction.

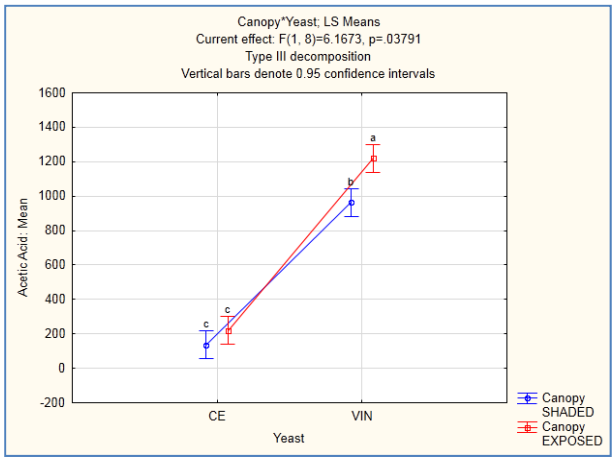

Figure 3.11 Shows the secondary interaction between canopy*yeast for the volatile fatty acid, acetic acid the only compounds significantly affected by the two factors. Different letters within a figure denote significant differences at $p < 0.05$.

Acetic acid (**Figure 3.11**) had significantly higher concentrations for VIN7. The exposed treatment had higher concentrations than the shaded treatment.

Total production of esters (**Figure 3.12**) throughout alcoholic fermentation for 2011 showed no noticeable differences. Both treatments did not affect the total ester production but did have an effect on certain individual esters as seen in the ANOVA analysis.

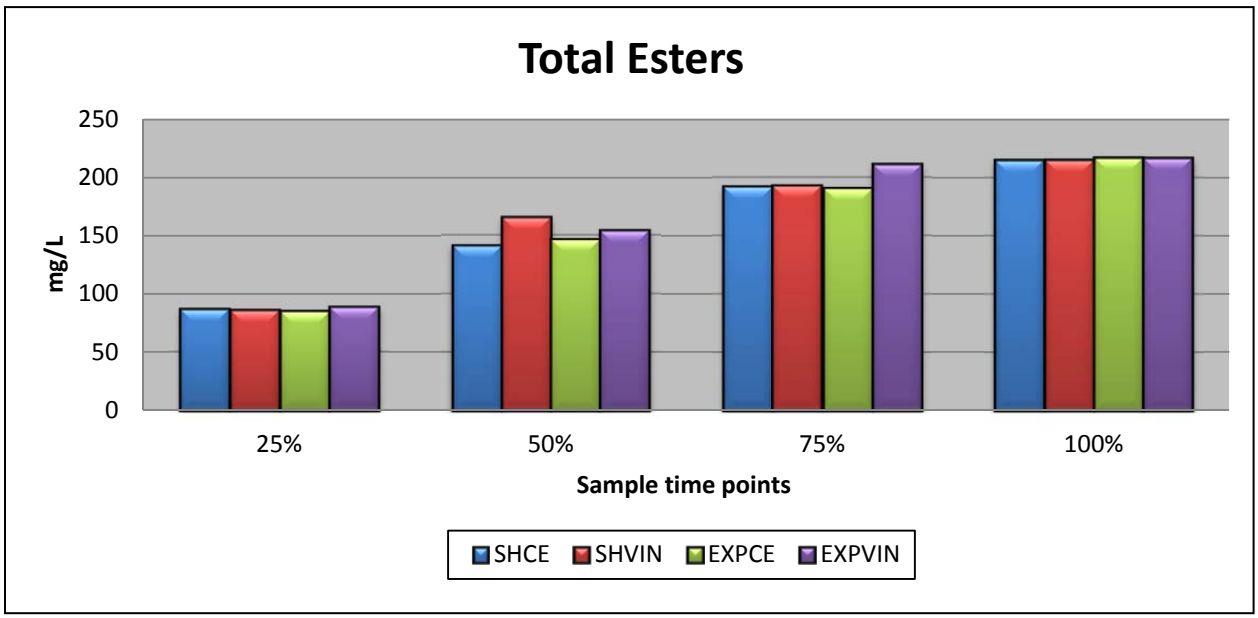

Figure 3.12 Total ester formation throughout alcoholic fermentation for the four time point sampled for each treatment. The data shown is the average of triplicate fermentations each analysed in duplicate through GC-FID.

Total higher alcohols (**Figure 3.13**) were influenced by the treatments, with Cross Evolution producing much higher concentrations than VIN7. The canopy treatment however did not affect the total higher alcohol production.

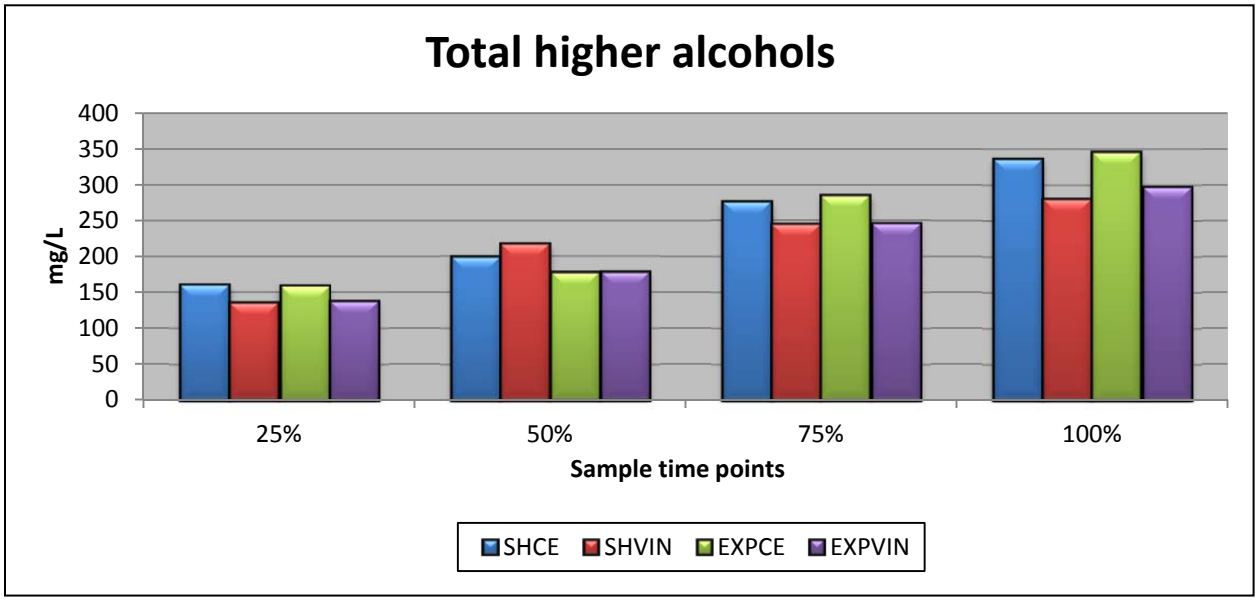

Figure 3.13 Total higher alcohol formation alcoholic fermentation for the four time point sampled for each treatment. The data shown is the average of triplicate fermentations each analysed in duplicate through GC-FID.

The total volatile fatty acids (**Figure 3.14**) showed the same trend as the total higher alcohols with Cross Evolution producing higher concentrations than VIN7.

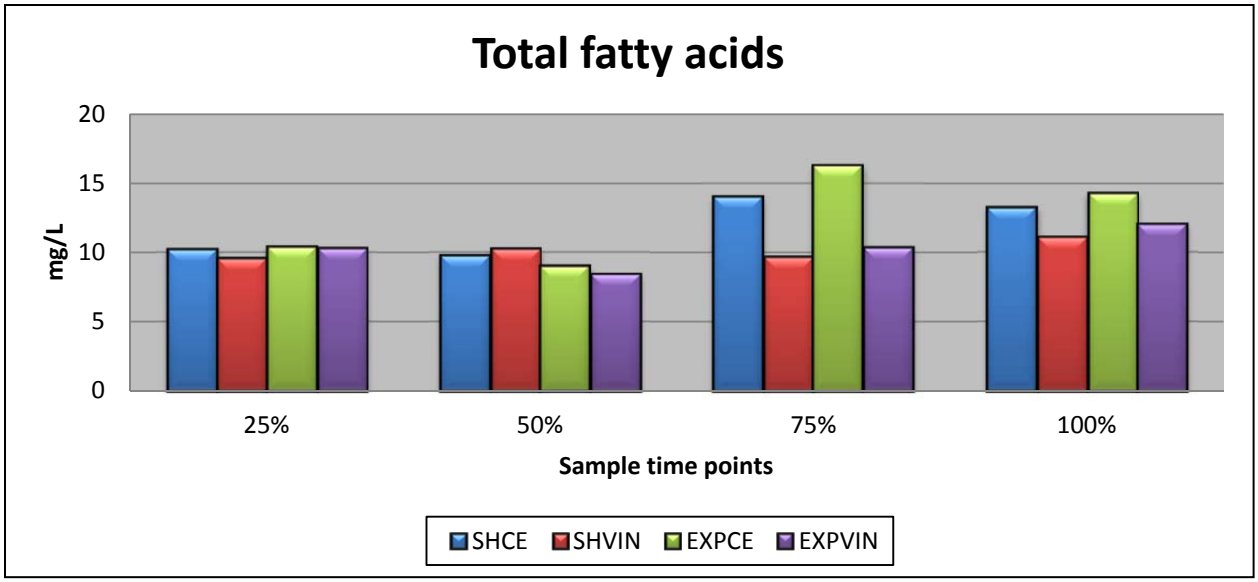

Figure 3.14 Total volatile fatty acid formation throughout alcoholic fermentation for the four time point sampled for each treatment (excluding acetic acid). The data shown is the average of triplicate fermentations each analysed in duplicate through GC-FID

3.3.4.1.2 2012

The compounds influenced significantly in the factorial ANOVAs are shown in **Table 13A-14A** in addendum A with significant *p*-values indicated in red.

Esters and higher alcohols were the compound groups mostly influenced in 2012 for both treatments although the yeast strain, as in 2011, had a greater influence. The time points measured during alcoholic fermentation showed to be significant for all the compounds except for hexanol, propionic acid and valeric acid which did not show a significant change during the four time points.

Nine esters showed significant interaction between canopy*yeast*timepoint whereas only one higher alcohol (*2-phenylethanol*) and one volatile fatty acid (*octanoic acid*) showed this interaction (refer to **Table 13A, 14A**).

The nine esters showing third order interaction can be categorized into two groups; ethyl esters (*ethyl butyrate, ethyl hexanoate, ethyl lactate, ethyl caprylate, ethyl caprate*) and acetate esters (*ethyl acetate, isoamyl acetate, hexyl acetate, ethyl phenylacetate*).

Three of the ethyl esters (*ethyl butyrate, ethyl hexanoate, ethyl caprate*) showed similar trends where DV10 produced significantly higher concentrations (**Figures 3.15**).

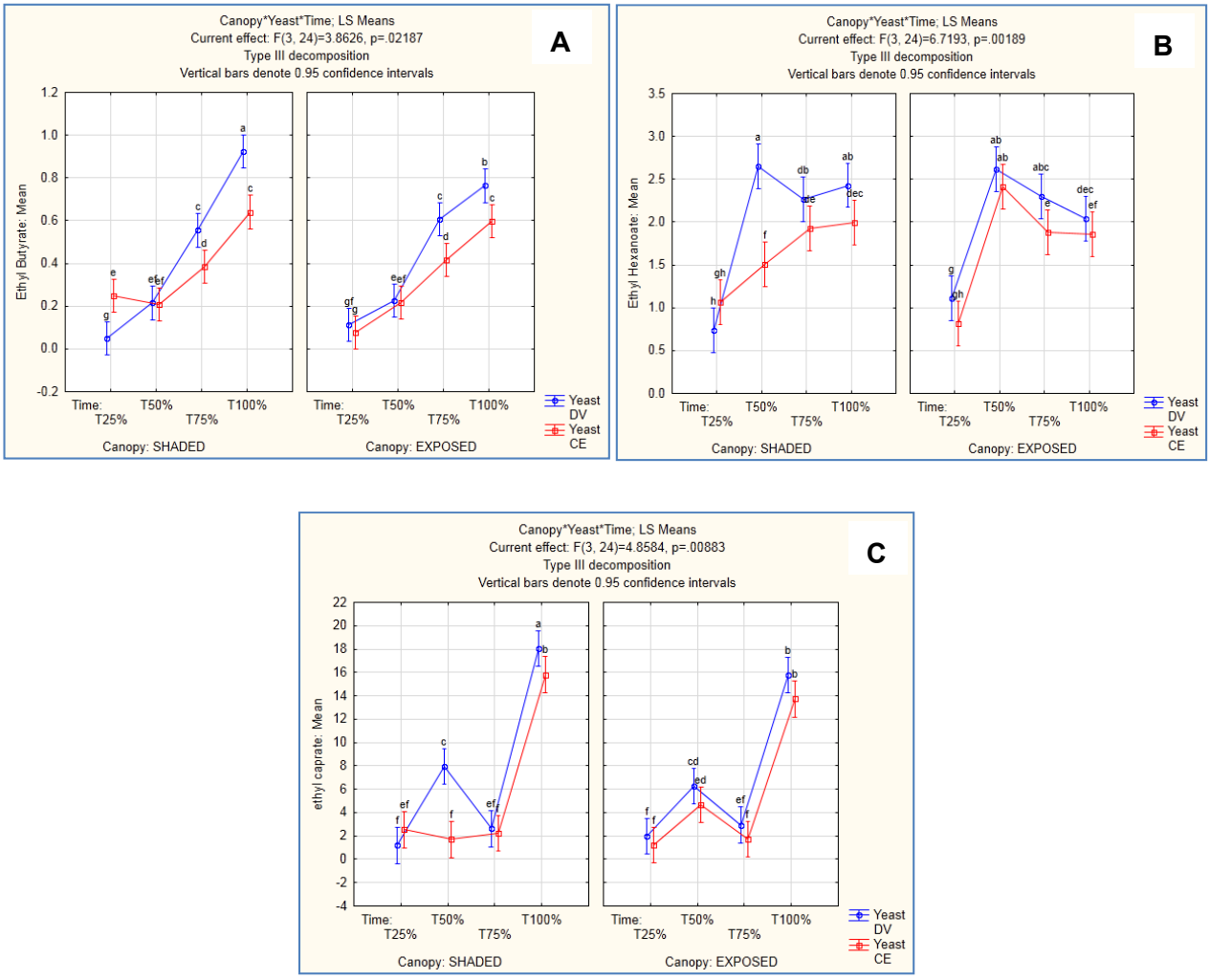

Figure 3.15 shows the third order of interaction between canopy*yeast*timepoint for the three ethyl esters, ethyl butyrate (**A**), ethyl hexanoate (**B**) and ethyl caprate (**C**).

Ethyl lactate and ethyl caprylate (**Figure 3.16**) were influenced by the canopy treatment with ethyl lactate having higher concentration in the exposed treatment whereas ethyl caprylate had higher concentrations for the exposed treatment.

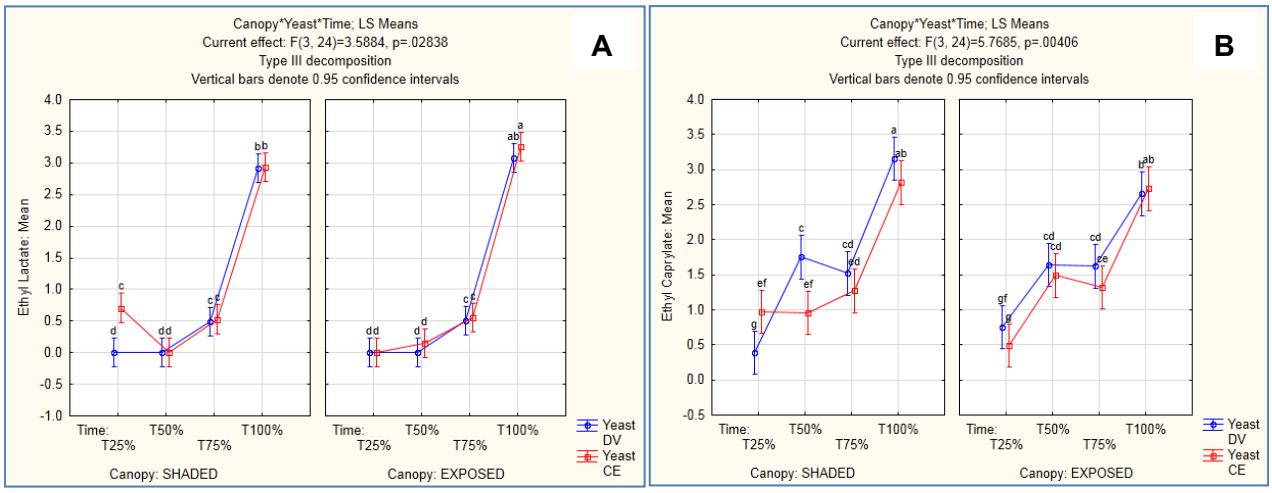

Figure 3.16 The two ethyl ester, ethyl lactate (**A**) and ethyl caprate (**B**) showing third order interaction between canopy*yeast*timepoint

Four acetate esters (*ethyl acetate, isoamyl acetate, hexyl acetate, ethyl phenylacetate)* showed similar trends with Cross Evolution producing significantly higher concentrations (**Figure 3.17**).

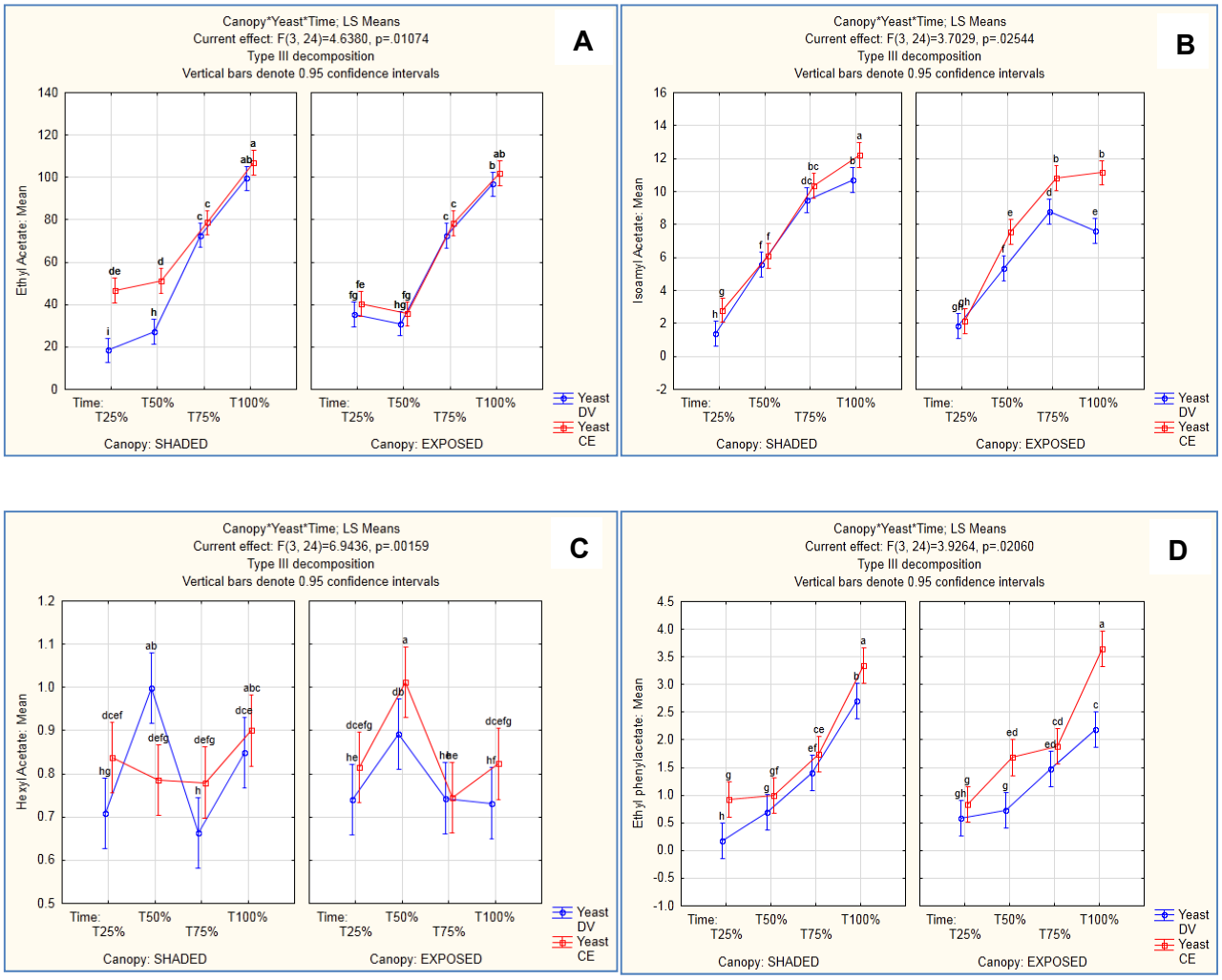

Figure 3.17 Shows the third order of interaction for the four acetate esters, ethyl acetate (**A**), isoamyl acetate (**B**), hexyl acetate (**C**) and ethyl phenylacetate (**D**).

The compounds influenced by the third order interaction in most cases had higher concentrations for the shaded treatments.

Compounds influenced by the second order interaction (canopy*yeast) included three esters (*diethyl succinate, ethyl acetate, isoamyl acetate*), three higher alcohols (*propanol, isoamyl alcohol, 2-phenylethanol*) and four volatile fatty acids (*acetic acid, propionic acid, isobutyric acid, isovaleric acid*) (**Table 16A** addendum A).

For all three esters Cross Evolution produced higher concentrations than DV10, with the shaded treatments having the highest concentration. For the three higher alcohols DV10 produced higher concentrations with the exposed treatment producing higher concentrations. The four volatile fatty acids showed variation without a definite trend.

Total production of esters (**Figure 3.18**) at the end of alcoholic fermentation for 2012 showed no noticeable differences although Cross Evolution had an influence during fermentation. Both treatments showed no effect on the total ester production but did have an effect on certain individual esters as shown in the ANOVA analyses.

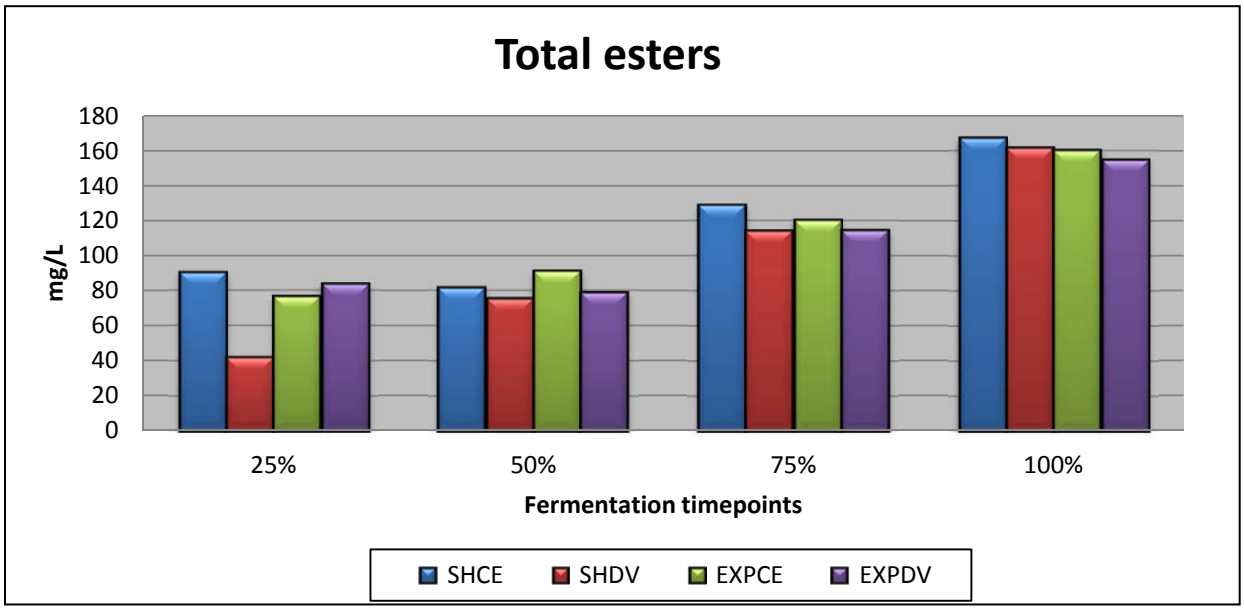

Figure 3.18 Total ester formation throughout the four time points of sampling for each treatment. The data shown is the average of triplicate fermentations each analysed in duplicate through GC-FID.

Total higher alcohols (**Figure 3.19**) were influenced by the yeast treatments with DV10 producing higher concentrations than Cross Evolution. The canopy treatment did not affect have an effect.

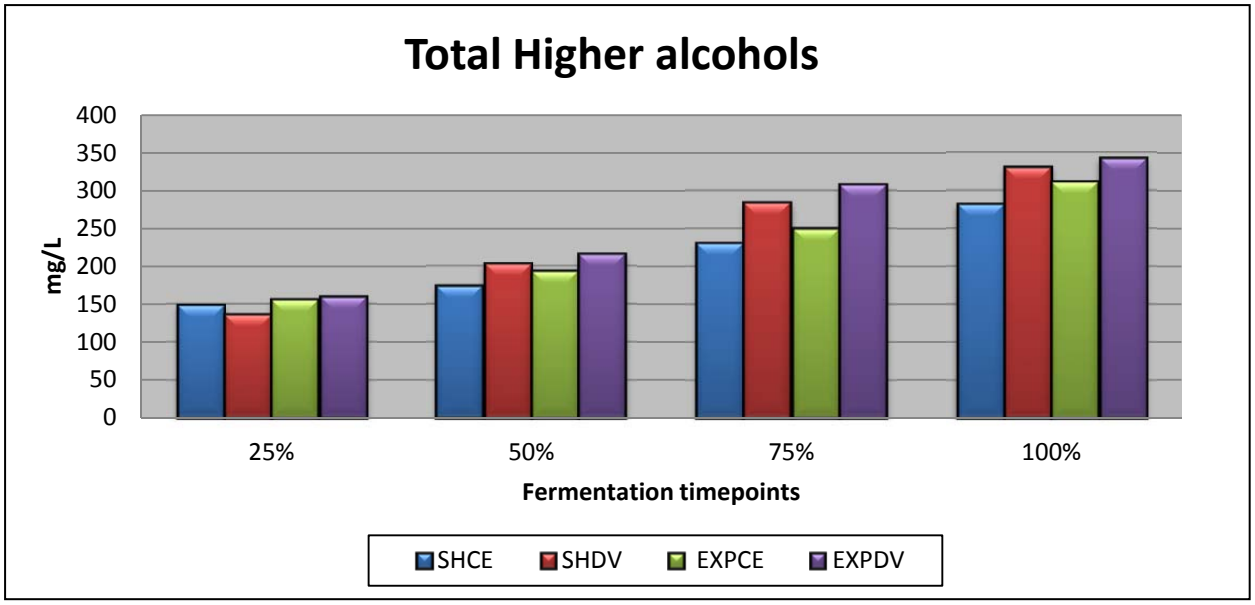

Figure 3.19 Total higher alcohol formation throughout the four time points of sampling for each treatment. The data shown is the average of triplicate fermentations each analysed in duplicate through GC-FID.

The total volatile fatty acids (**Figure 3.20**) showed more variation than the 2011 data with trends suggesting that canopy treatments had an influence.

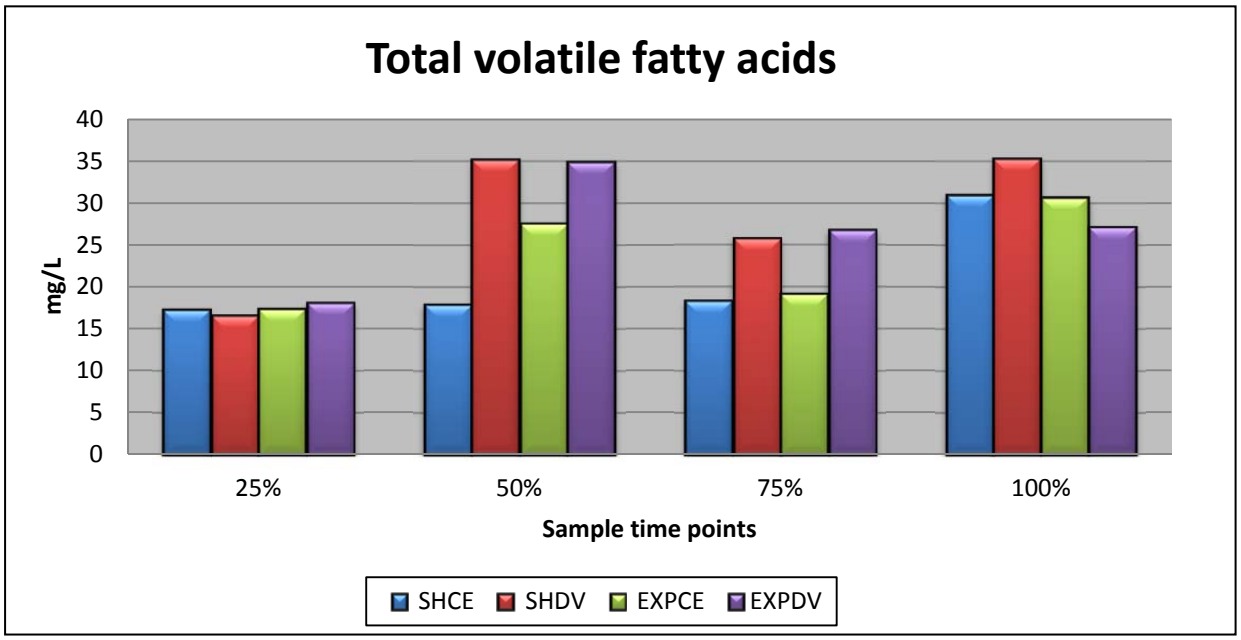

Figure 3.20 Total volatile fatty acid formation throughout the four time points of sampling for each treatment (excluding acetic acid). The data shown is the average of triplicate fermentations each analysed in duplicate through GC-FID.

3.3.4.1.3 2012 Panel resolution

A factorial ANOVA was performed to determine the influence of the viticultural canopy treatment, the time point analysed during alcoholic fermentation as well as the interaction between the canopy*timepoint. The compounds influenced significantly by these factors are shown in **Table 17 A – 18 A** in addendum A with the significant *p*-values indicated in red.

Overall the panels did show some variation between canopy treatments especially for ethyl hexanoate, ethyl lactate, ethyl acetate and ethyl caprate (**Table 7A addendum A**). Compared to the 2012 batch fermentation all the same compounds were significantly influenced for the treatments except for ethyl-3-hydroxybutanoate, 2-phenylethanol, isovaleric acid and decanoic acid that were significant in the panel wines, but not in the batch fermentation.

Esters and higher alcohols were the compound groups that were most influenced in 2012 by the canopy treatment.

The time points measured during alcoholic fermentation showed to be significant for all the compounds except for ethyl hexanoate and isobutyric acid which did not show a significant change in concentration during the two time points.

Six esters, six higher alcohols and four volatile fatty acids were influenced by the canopy treatment all, except for diethyl succinate, having the same trend were the shaded treatment produced significantly higher concentrations (**Table 20A** addendum A).

From the compounds mentioned four esters (*ethyl caprate, diethyl succinate, ethyl acetate, ethyl-3-hydroxybutanoate*) showed significant interaction between canopy*timepoint whereas only one higher alcohol (*pentanol*) and one volatile fatty acid *(decanoic acid*) showed interaction (**Table 19A** addendum A).

The total ester production (**Figure 3.21**) for the panel wines showed variance between panels although it was still clear that the shaded treatment produced higher concentrations than the exposed treatment.

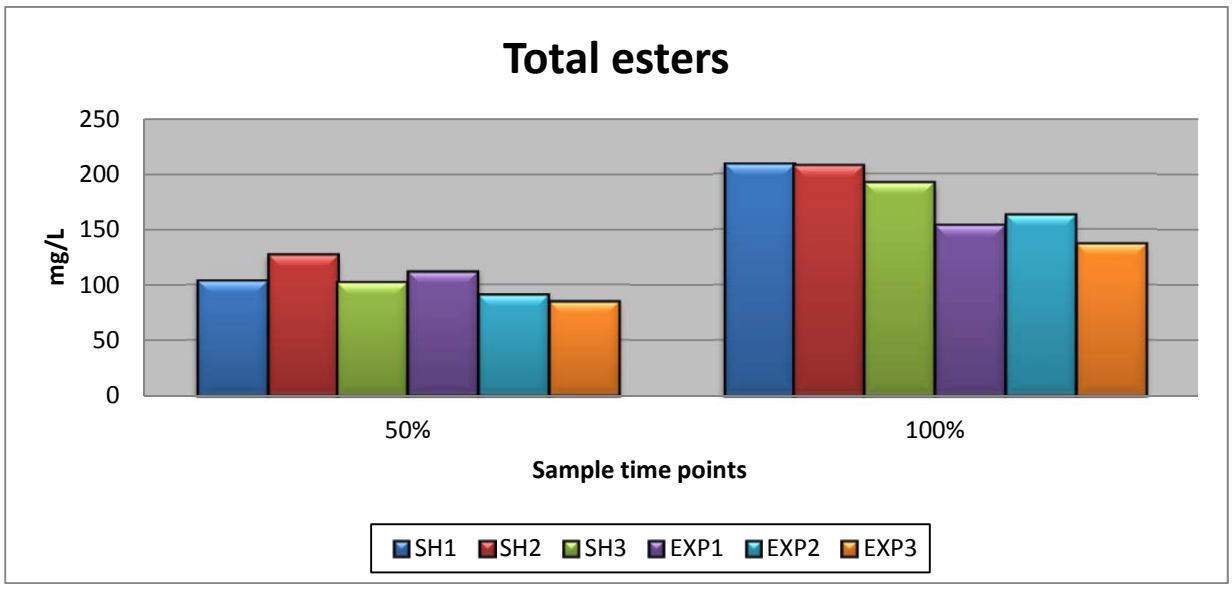

Figure 3.21 Total ester formation throughout the four time points of sampling for each treatment. The data shown is the average of triplicate fermentations each analysed in duplicate through GC-FID.

The total higher alcohols (**Figure 3.22**) showed less variation although shaded panels had slightly higher concentrations at the end of alcoholic fermentation.

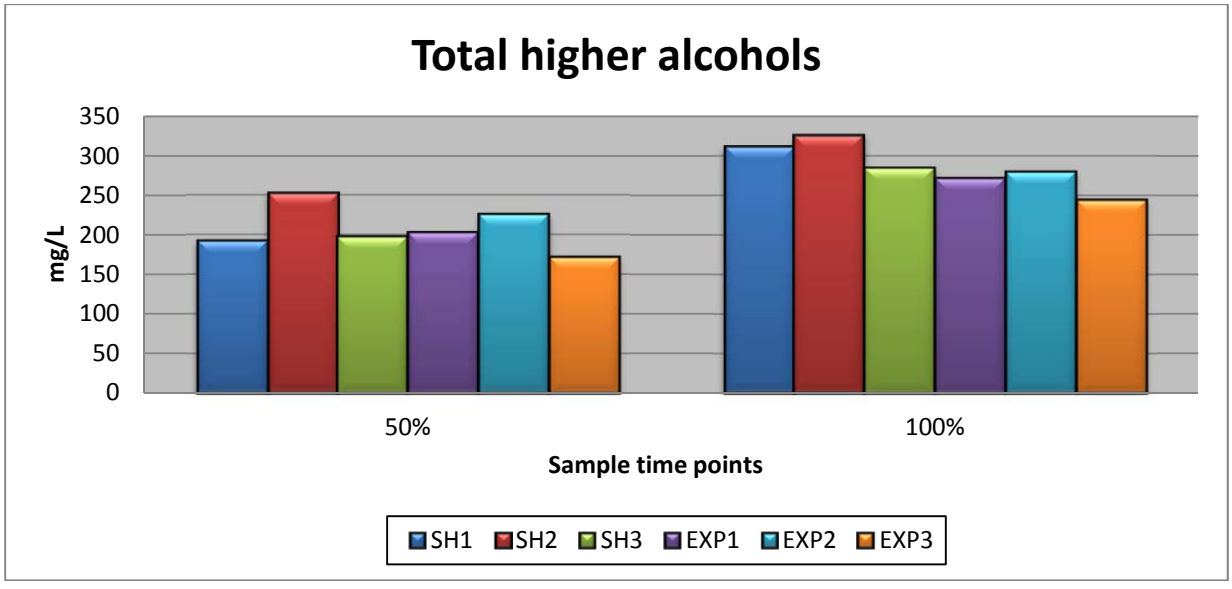

Figure 3.22 Total higher alcohol formation throughout the four time points of sampling for each treatment. The data shown is the average of triplicate fermentations each analysed in duplicate through GC-FID.

Total volatile fatty acids showed variation between panels without having a definite trend (**Figure 3.23**).

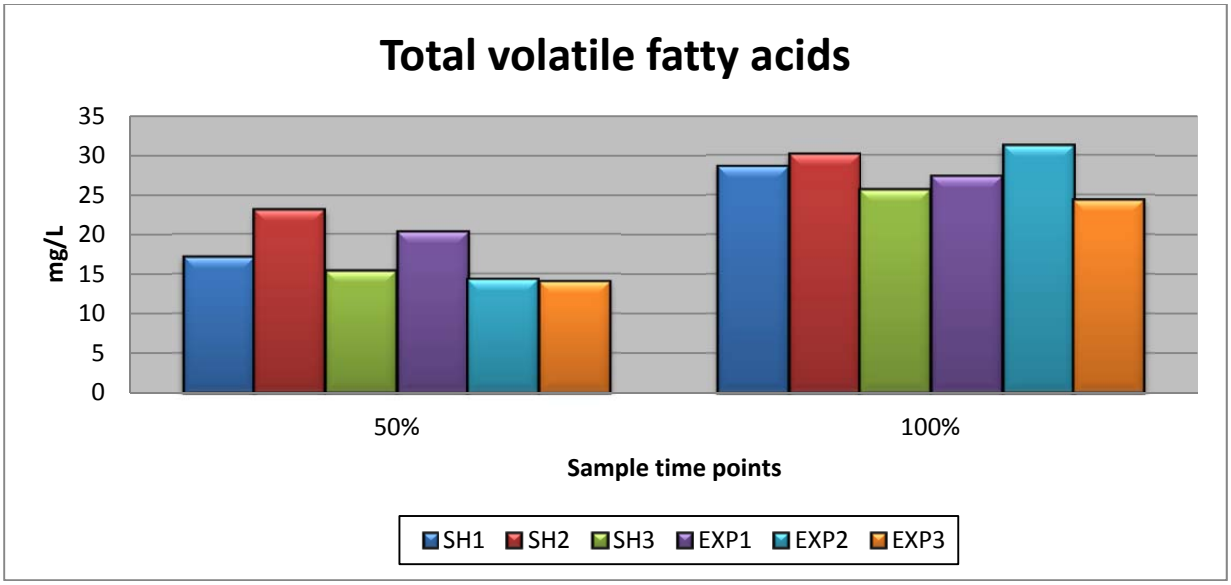

Figure 3.23 Total volatile fatty acid formation throughout the four time points of sampling for each treatment (excluding acetic acid). The data shown is the average of triplicate fermentations each analysed in duplicate through GC-FID.

3.3.4.2. Monoterpenes

3.3.4.2.1 2012

Table 3.14 lists the monoterpenes analysed. The compound with the highest concentration found in all four treatments was geraniol (*rose*). The three compounds, limonene, fenchone and β-ionone were not detected in any of the four treatments. α-terpineol (*lily of the valley*) was only detected in the exposed treatment and not in the shaded treatment. No trends were seen between canopy treatments but yeast had an influence on the concentrations produced within each canopy treatment. For the shaded treatment wines, DV10 in general produced higher concentrations for individual compounds whereas in the exposed treatment wines Cross Evolution produced higher concentrations. EXPDV had highest concentrations of linalyl acetate and α-terpineol. EXPCE had the highest concentrations of linalool (*rose*), nerol (*rose*) and β-farnesol. SHDV had the highest concentration of geraniol (*rose*) and α-ionone, whereas SHCE had the highest concentrations of linalooloxide and citronellol (*citronella*).

Total monoterpene (**Figure 3.24**) production for 2012 showed variation between treatment combinations. The two treatments that had the highest total monoterpene production were SHDV and EXPCE producing similar concentrations.

Table 3.14 Monoterpenes concentrations (μg/L) measured in 2012 Sauvignon blanc batch fermentations. Concentrations represent the average value of triplicate fermentation treatments analysed in duplicate by GC-FID. (standard deviations not shown) (nd: not detected).

	SHADED		EXPOSED	
Treatments	***Cross-evolution***	***DV10***	***Cross-evolution***	***DV10***
MONOTERPENES				
Limonene	nd	nd	nd	nd
Fenchone	nd	nd	nd	nd
Linalooloxide	13.941	8.610	8.122	7.811
Linalool	19.355	27.214	32.760	28.822
Linalyl acetate	3.206	3.742	3.133	3.905
α-terpineol	nd	nd	0.666	0.795
Citronellol	2.224	2.180	2.028	2.164
Nerol	85.961	188.987	190.713	90.535
Geraniol	1919.065	2526.09	2450.554	2012.263
α-ionone	24.183	24.300	15.585	17.320
β-ionone	nd	nd	nd	nd
β-farnesol	73.818	87.948	207.425	130.450
TOTAL	**2141.753**	**2869.071**	**2910.986**	**2294.065**

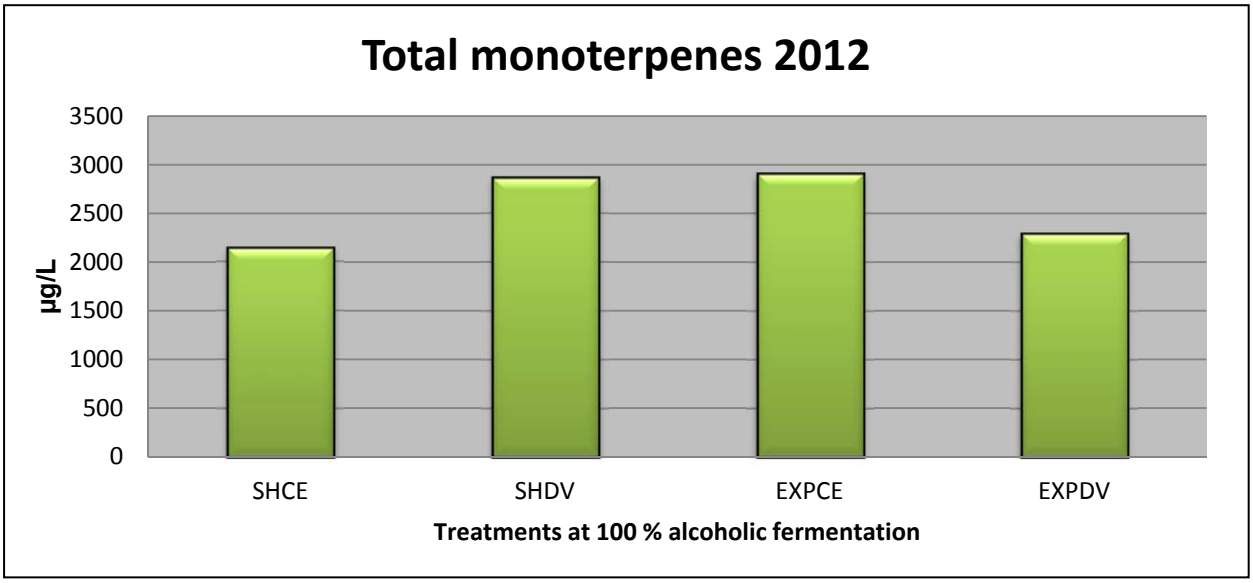

Figure 3.24 Total monoterpene concentrations produced by batch fermentation during 2012. Total concentrations represent averages of triplicate treatment fermentations each analysed in duplicate by GC-FID.

3.3.4.2.2 2012 Panel resolution

Single panel fermentations showed overall similar trends to the batch fermentation (**Table 3.15**). Geraniol was the compound with the highest concentration as in the batch fermentation. The same three compounds (*limonene, fenchone and β-ionone*) were not detected in the panel wines. Interestingly *α*-terpineol was not detected in any of the panel wines. Shaded treatments had similar concentrations with citronellol, geraniol and α-ionone being the highest in the shaded panels. Linalooloxide was only detected in the exposed treatments of the panel wines being different from the batch wines. SHCE had the highest total monoterpene production

correlating with the batch fermentation. Even though variation is visible between panels within a canopy treatment overall trends projected the same as the batch fermentation wines (**Table 3.15**).

Total monoterpene (**Figure 3.25**) production for panels produced during 2012 were similar except for shaded panel 1 and exposed panel 1 that had slightly higher concentrations. Although the above mentioned panels showed different concentration it is possible that variation between panels will be lost when panels are combined to make batch wine.

Table 3.15 Monoterpene concentrations (μg/L) measured in 2012 Sauvignon blanc panel resolution. Concentrations represent the average value of triplicate fermentation treatments analysed in duplicate by GC-FID. (standard deviations not shown) (nd: not detected).

	SHADED			EXPOSED		
Treatments	***PANEL 1***	***PANEL 2***	***PANEL 3***	***PANEL 1***	***PANEL 2***	***PANEL 3***
MONOTERPENES						
Limonene	nd	nd	nd	nd	nd	nd
Fenchone	nd	nd	nd	nd	nd	nd
Linalooloxide	nd	nd	nd	8.082	7.311	7.638
Linalool	28.794	23.906	25.763	40.793	24.608	28.064
Linalyl acetate	3.017	3.672	2.845	3.848	3.738	3.670
α-terpineol	nd	nd	nd	nd	nd	nd
Citronellol	2.268	2.344	2.222	3.183	1.631	1.755
Nerol	61.336	83.063	83.862	59.638	53.366	61.856
Geraniol	2696.227	2345.908	2236.664	2811.477	2273.248	2198.026
α-ionone	20.290	26.154	26.425	15.908	16.487	24.660
β-ionone	nd	nd	nd	nd	nd	nd
β-farnesol	62.295	81.186	78.470	71.345	64.770	66.186
TOTAL	**2874.227**	**2566.233**	**2456.251**	**3014.274**	**2445.159**	**2391.855**

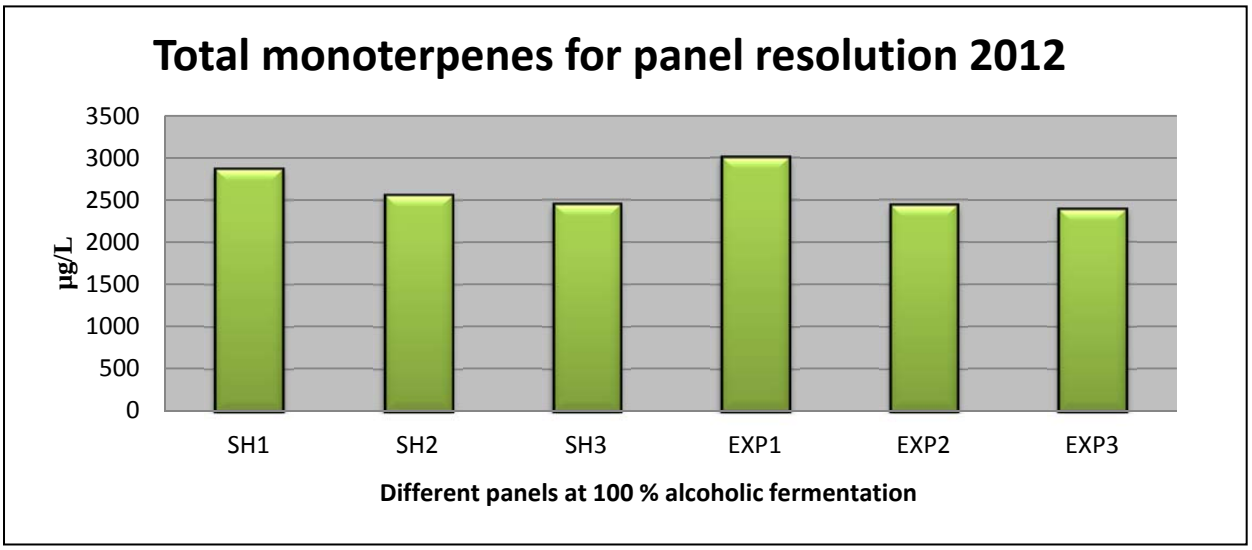

Figure 3.25 Total monoterpene concentrations produced by panel resolution fermentations during 2012. Total concentrations represent averages of triplicate treatment fermentations each analysed in duplicate by GC-FID.

3.3.4.3 Carbonyl compounds

3.3.4.3.1 2011

2011 analysis for carbonyl compounds included analysis for diacetyl, 2,3-pentadione and acetoin to investigate trends between treatments. From the above mentioned compounds only acetoin was detected. **Table 3.16** shows the highest concentration detected were 0.137 mg/L.

Total carbonyl production (**Table 3.16**) for 2011 showed the combination SHCE produced the highest concentration with Cross Evolution producing overall higher levels than VIN7. The canopy treatment did not affect the carbonyl production for VIN7 as similar concentrations were obtained. Cross Evolution did show differences between canopy treatments with the shaded treatment producing higher concentrations.

Table 3.16 Carbonyl compounds (mg/L) measured for Sauvignon blanc 2011 at end of alcoholic fermentation. Concentrations represent the average value of triplicate fermentation treatments analysed by GC-MS (standard deviations not shown) (nd: not detected).

	SHADED		EXPOSED	
	VIN7	***Cross evolution***	***VIN7***	***Cross evolution***
Carbonyl Compounds				
Diacetyl	nd	nd	nd	nd
2,3-pentadion	nd	nd	nd	nd
Acetoin	0.037	0.137	0.046	0.074
Total	**0.037**	**0.137**	**0.046**	**0.074**

3.3.4.3.2 2012

2012 analysis was done at two time points, half fermentation (50 %) as well as end of alcoholic fermentation (100%). For both these time points the analysis was not only done for the three compounds analysed in 2011 (*diacetyl, acetoin, 2,3-pentadione*) but an additional seven compounds were analysed (*hexenal, octanal, heptenal, octenal, decanal, nonenal, nonedienal*).

Acetoin, the only compound that was detected (**Table 3.17**), was only detected at the end of the fermentation and not on the 50 % fermentation sample. Higher levels of acetoin were detected in the shaded treatments. SHCE had the highest concentration. The rest of the carbonyl compounds were detected below their limit of quantification (LOQ).

Table 3.17 Carbonyl compounds (mg/L) measured for Sauvignon blanc 2012 at two time points during alcoholic fermentation. Concentrations represent the average value of triplicate fermentation treatments analysed by GC-MS (standard deviations not shown) (nd: not detected).

	SHADED				EXPOSED			
	Cross evolution		*DV10*		*Cross evolution*		*DV10*	
	50%	100%	50%	100%	50%	100%	50%	100%
Carbonyl Compounds								
Diacetyl	nd	nd	nd	nd	nd	nd	nd	Nd
2,3-pentadione	nd	nd	nd	nd	nd	nd	nd	Nd
Acetoin	nd	4.7601	nd	3.5401	nd	3.2801	nd	2.9340
Total	**nd**	**4.7601**	**nd**	**3.5401**	**nd**	**3.2801**	**nd**	**2.9340**

3.3.4.3.3 2012 Panel resolution

The 2012 panel resolution wines were analysed at end of alcoholic fermentation for the same compounds mentioned in 3.3.4.3.2.

The only compound detected was acetoin (**Table 3.18**). The panels showed variation within canopy treatments without any clear trends. The rest of the carbonyl compounds were detected below their limit of quantification (LOQ).

Table 3.18 Carbonyl compounds (mg/L) measured for Sauvignon blanc 2012 panel resolution at end of alcoholic fermentation. Concentrations represent the average value of triplicate fermentation treatments analysed by GC-MS (standard deviations not shown) (nd: not detected).

	SHADED			EXPOSED		
	SH1	*SH2*	*SH3*	*EXP1*	*EXP2*	*EXP3*
Carbonyl Compounds						
Diacetyl	nd	nd	nd	nd	nd	Nd
2,3-pentadione	nd	nd	nd	nd	nd	Nd
Acetoin	5.0448	3.5775	6.5996	3.2422	4.6655	6.2101
Total	**5.0448**	**3.5775**	**6.5996**	**3.2422**	**4.6655**	**6.2101**

3.3.5 Malic and citric acid

3.3.5.1 2011

Figure 3.26 shows malic acid decreased during alcoholic fermentation. The juice analysis (refer to **Table 3.2**) showed a difference in initial malic acid concentration between canopy treatment juices which is also visible in this figure. The yeast strains did not affect the amount of malic acid degraded during alcoholic fermentation as both yeast treatments within a canopy treatment had similar final concentrations.

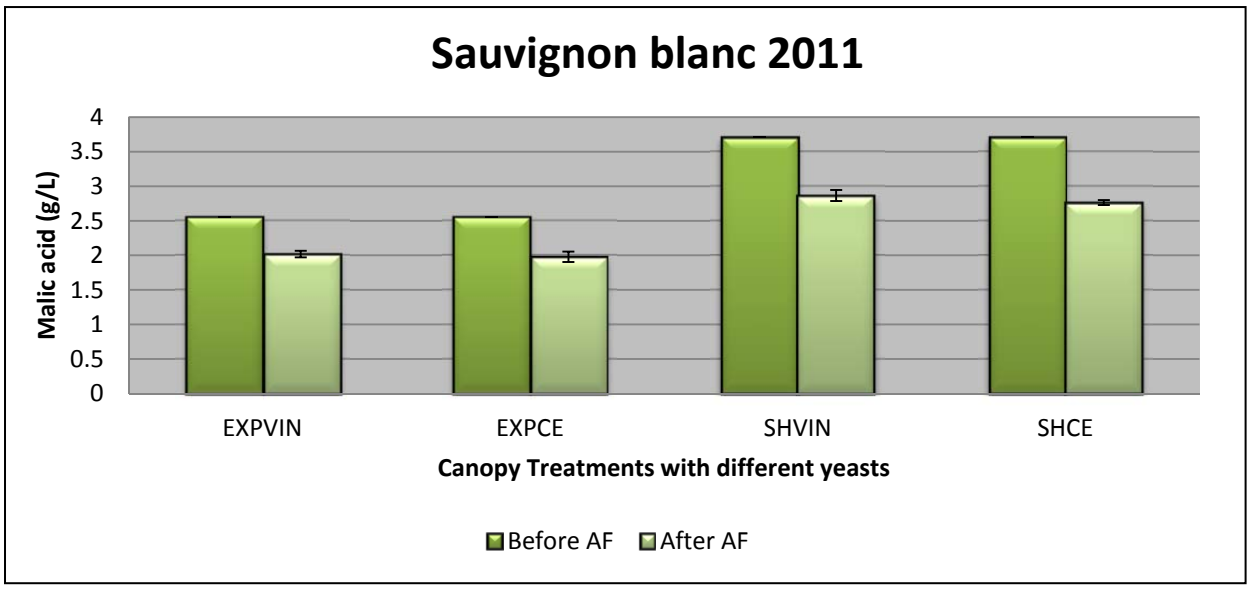

Figure 3.26 Malic acid measured in 2011 before and after alcoholic fermentation. Concentrations represent the average of triplicate treatments. Standard error bars are shown.

3.3.5.2 2012

In 2012 the fermentations were analysed for malic acid on four time points throughout alcoholic fermentation. Malic acid did slightly decrease during alcoholic fermentation. The initial malic acid concentration differed as seen in the juice analysis (refer to **Table 3.2**). There was a slight trend at the end of alcoholic fermentation that DV10 had higher malic acid concentrations than Cross Evolution (**Figure 3.27**). Although the initial malic acid is affected by the canopy treatment it is clear that the choice of yeast could play a potential role in the final concentrations of malic acid.

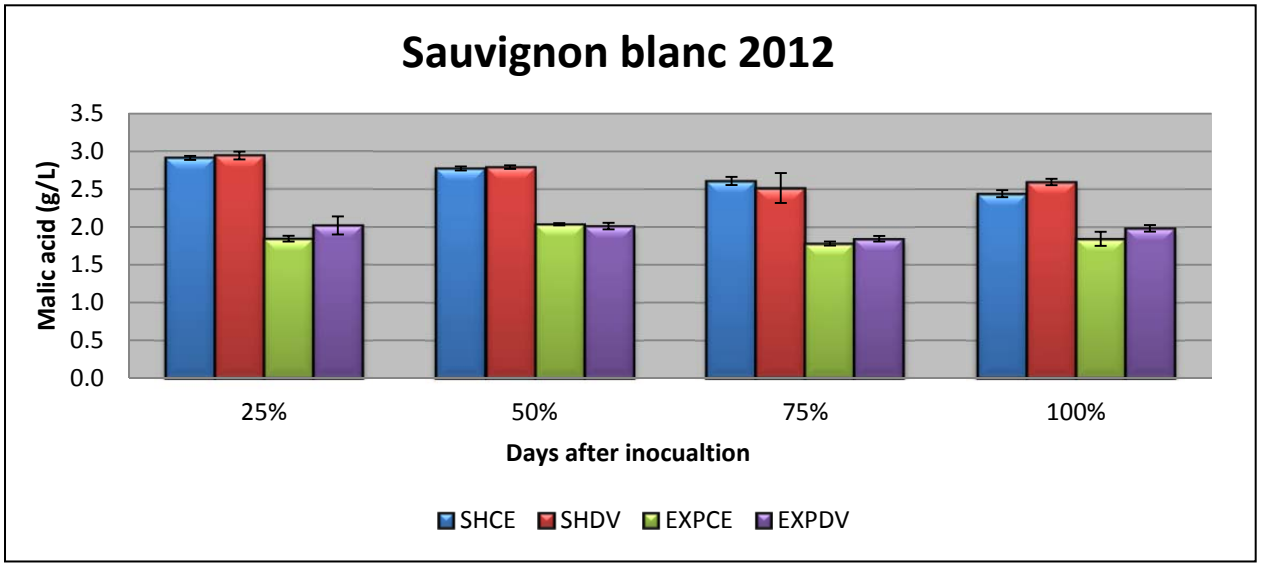

Figure 3.27 Malic acid measured in 2012 at four time point during alcoholic fermentation. Concentrations represent the average of triplicate treatments. Standard error bars are shown.

Citric acid analysis was done on the 2012 batch fermentation at end of alcoholic fermentation to investigate concentration differences between treatments. A difference in citric acid between the

canopy treatments was observed (**Figure 3.28**). The shaded canopy had almost 0.05 g/L more citric acid. No clear differences are visible between yeast combinations within treatments.

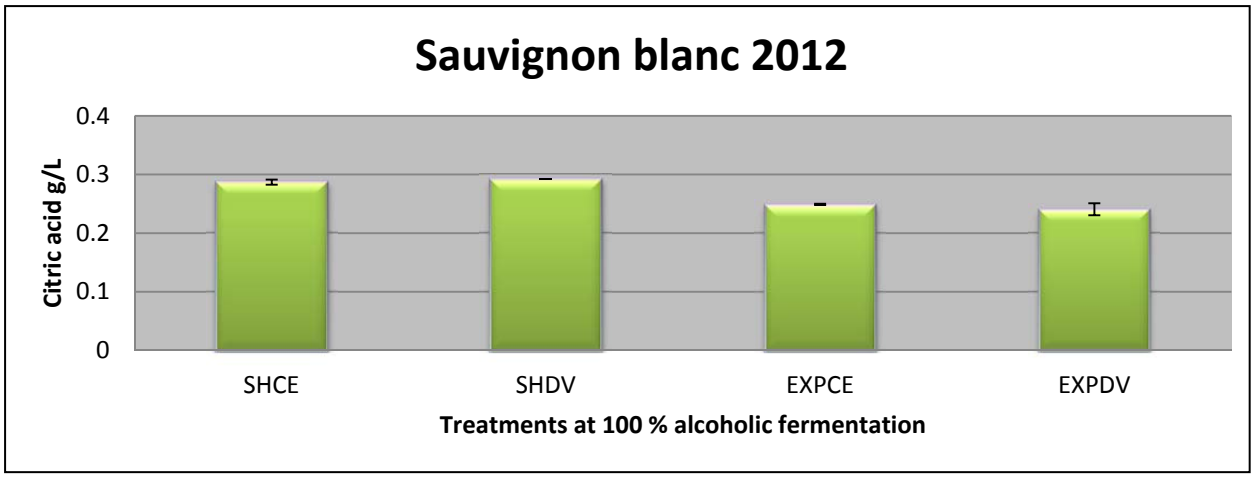

Figure 3.28 Citric acid measured in 2012 at end of alcoholic fermentation. Concentrations represent the average of triplicate treatments. Standard error bars are shown.

3.3.5.3 2012 Panel resolution

Analysis for malic acid done at two time points during alcoholic fermentation showed malic acid stayed constant. Variation between panels of the same canopy treatment could be observed (**Figure 3.29**). There is a trend that as the row precedes the malic acid decreased no matter what the panel treatment was (Row: SH1, EXP1, SH2, EXP2, SH3, EXP3). Overall the shaded treatment did have higher malic acid values.

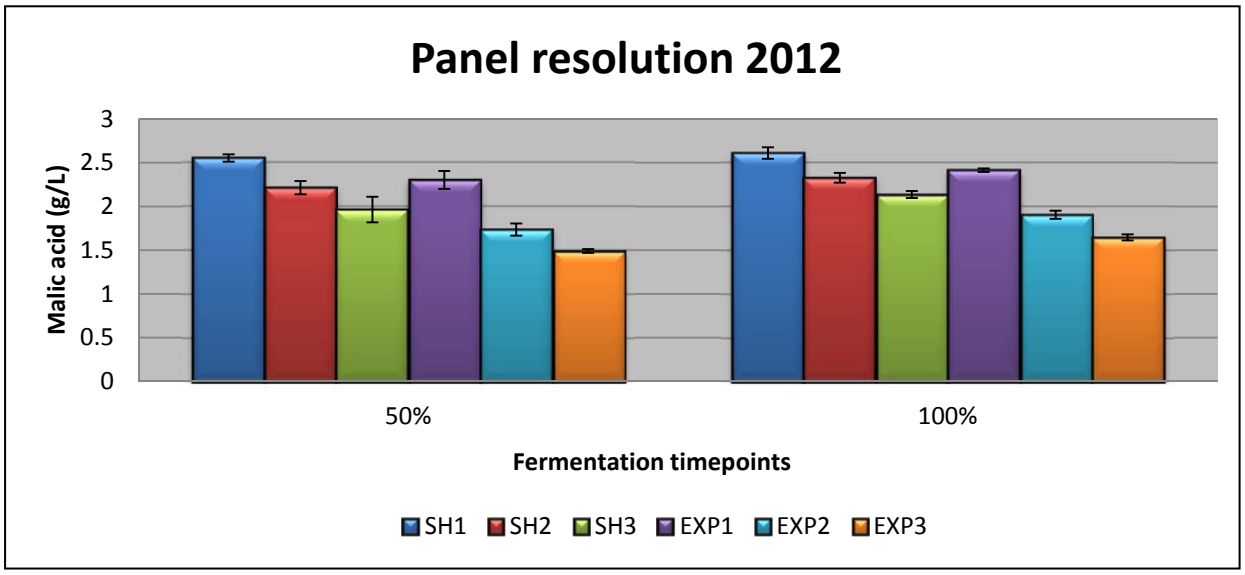

Figure 3.29 Malic acid measured in 2012 panel resolution wines at two time points during alcoholic fermentation. Concentrations represent the average of triplicate treatments. Standard error bars are shown.

3.3.6 Multivariate data analysis

3.3.6.1 GC-FID chemical data

3.3.6.1.1 2011 and 2012 Vintage

A PCA was constructed using chemical data obtained from GC-FID analysis for the 2011 and 2012 vintage. With this multi-dimensional profile of the chemical data it is possible to see any hidden trends between treatment combinations not visible by univariate data analysis.

A score plot of the different time points analysed show clear groupings between 25 % (blue), 50 % (red), 75 % (green) and 100 % (black) for both vintages (**Figure 3.30 A and Figure 3.31 A**). PC 1 for 2011 describes 48 % of the variance between the samples. There were no groupings for the different canopy treatments (**Figure 3.30 B**) although groupings between yeast strains to end of alcoholic fermentation are seen (**Figure 3.30 C**). PC1 for 2012 describes 39 % of the variance between samples. There were no groupings for canopy treatments, the same trend as 2011 (**Figure 3.31 B**). Although PC2 only describes 13 % of the variance, a separation between the two yeasts, DV10 (blue) and Cross Evolution (red) is clear (**Figure 3.31 C**). **Figure 3.30 D and Figure 3.31 D** shows the loading plot of compounds affecting the separation.

In both vintages the fermentation derived volatiles were mostly affected by yeast strain as groupings in 2011 formed between VIN7 and Cross Evolution and 2012 between DV10 and Cross Evolution.

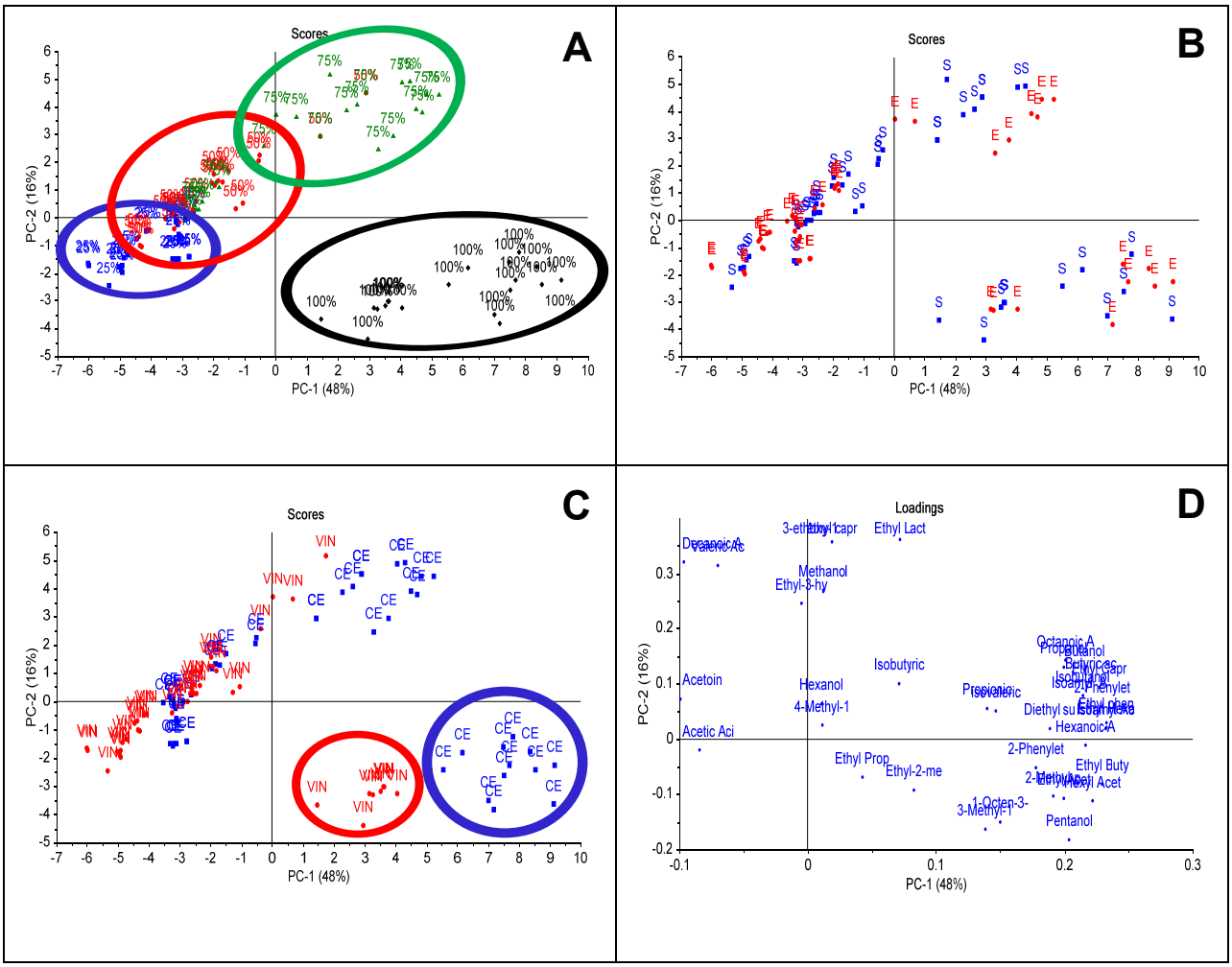

Figure 3.30 PCA Score plot (PC1 vs. PC2) of the batch fermentation treatments in 2011. PC1 explains 48 % of the variance between samples. The treatments separated along PC1 and discrimination is based on the GC-FID data. **A** show the separation by time points 25 %, 50 %, 75 % and 100 %. **B** shows the separation by canopy treatment. **C** shows the separation by yeasts and **D** shows the loadings plot.

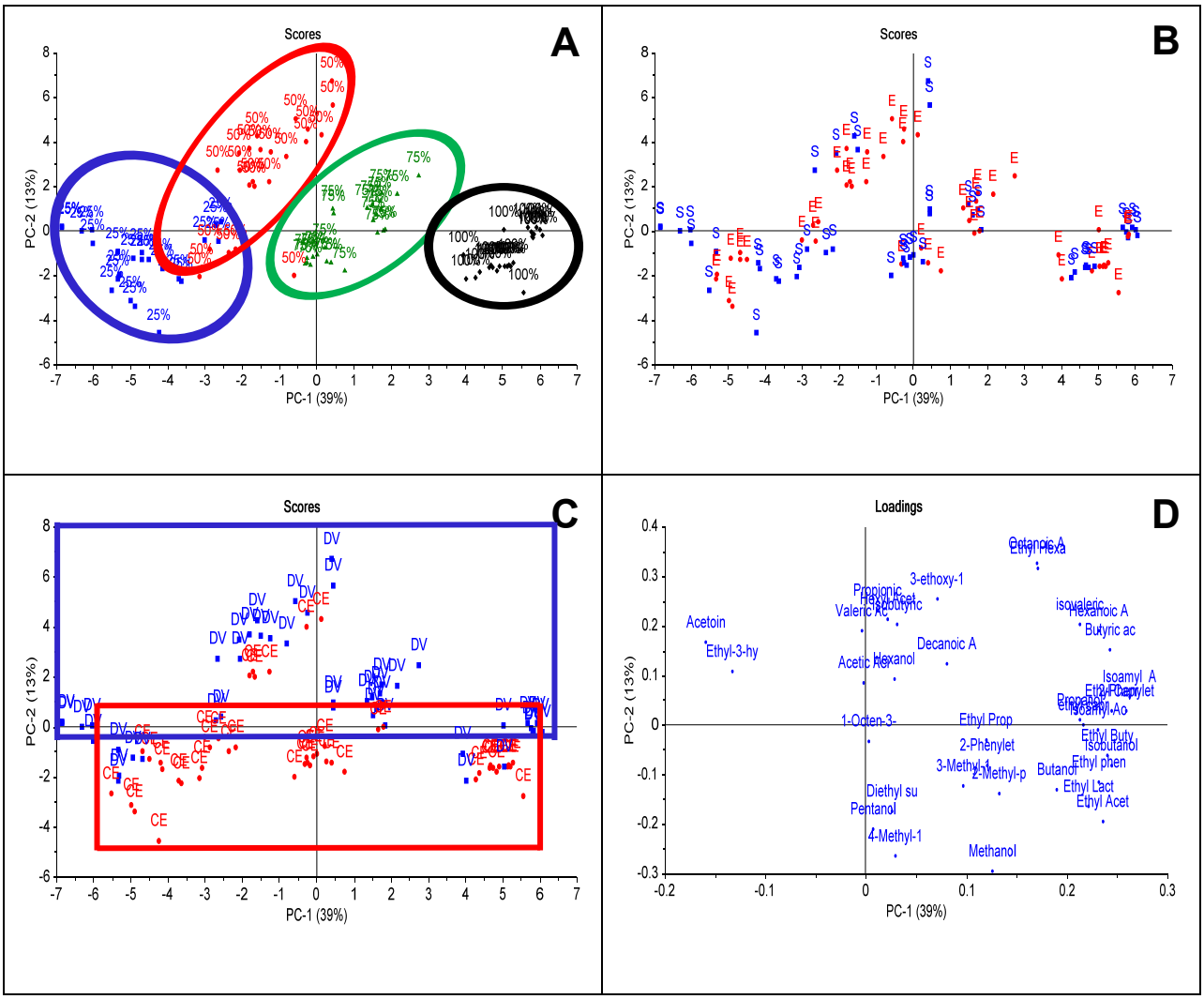

Figure 3.31 PCA Score plot (PC1 vs. PC2) of the batch fermentation treatments in 2012. PC1 explains 39 % of the variance between samples. The treatments separated along PC1 and discrimination is based on the GC-FID data. **A** show the separation by time points 25 %, 50 %, 75 % and 100 %. **B** shows the separation by canopy treatment. **C** shows the separation by yeasts and **D** shows the loadings plot.

3.3.6.1.2 2012 Panel resolution

PCA constructed for the two time points when chemical analysis was done. A score plot for the time points 50% (blue) and 100 % (red) had clear groupings (**Figure 3.32 A**). PC1 explains 59 % or the variance between samples. No clear groupings between the exposed and shaded panels were observed (**Figure 3.32 B**). Only one yeast strain was used for the panel resolution wines as a result no yeast score plot is shown. **Figure 3.32 C** shows the loading plot for the compounds analyses influencing the separations of the samples.

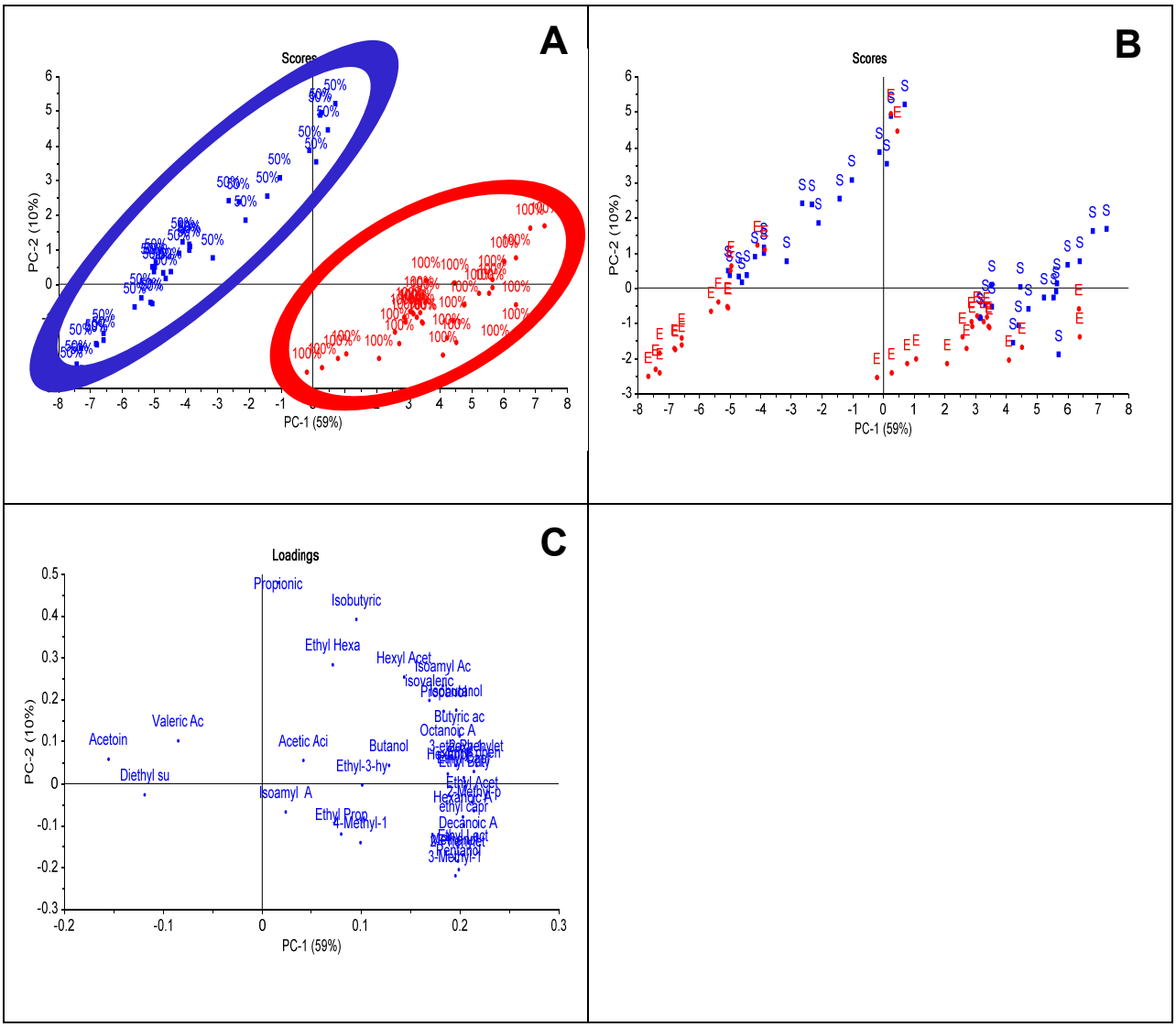

Figure 3.32 PCA Score plot (PC1 vs. PC2) of the panel resolution treatments in 2012. PC1 explains 59 % of the variance between samples. The treatments separated along PC1 and discrimination is based on the GC-FID data. **A** show the separation by time points 50 % and 100 %. **B** shows the separation by canopy treatment and **C** shows the loadings plot.

3.3.6.2 Fermentation spectral data

Spectral data was obtained by FT-MIR analysis. By importing the spectra data into *The Unscrambler* software, principal component analysis (PCA) was done. The PCA allows the investigation of differences on a day to day basis during alcoholic fermentation and possible differences between canopy and yeast combination treatments could be seen.

3.3.6.2.1 2011

A score plot presenting the days during alcoholic fermentation, ranging from day 1 to day 21, clearly shows how the fermentation proceeds over time (**Figure 3.33 A**). Groupings between samples are further apart at the beginning of fermentation, while towards the end of the fermentation the samples start to overlay, but still showing clear groupings of the two canopy treatments. On day 14 the fermentation samples for both the EXPDV and EXPCE samples

portrayed a different pattern to the shaded treatment samples. PC1 explains 66 % of the variance between samples.

The score plot for the canopy treatments clearly groups according to shaded (red) and exposed (blue) (**Figure 3.33 B**). Although no differences could be seen in the predicted values from the FT-MIR used for fermentation graphs (refer to **Figure 3.2**) it is clear in **Figure 3.33 B** that there are differences. **Figure 3.33 C** gives the loadings plot with all the wavelengths used for the FT-MIR analysis (excluding the wavelength were water absorbs). A score plot constructed for yeast strains did not show any groupings for the different treatments (data not shown).

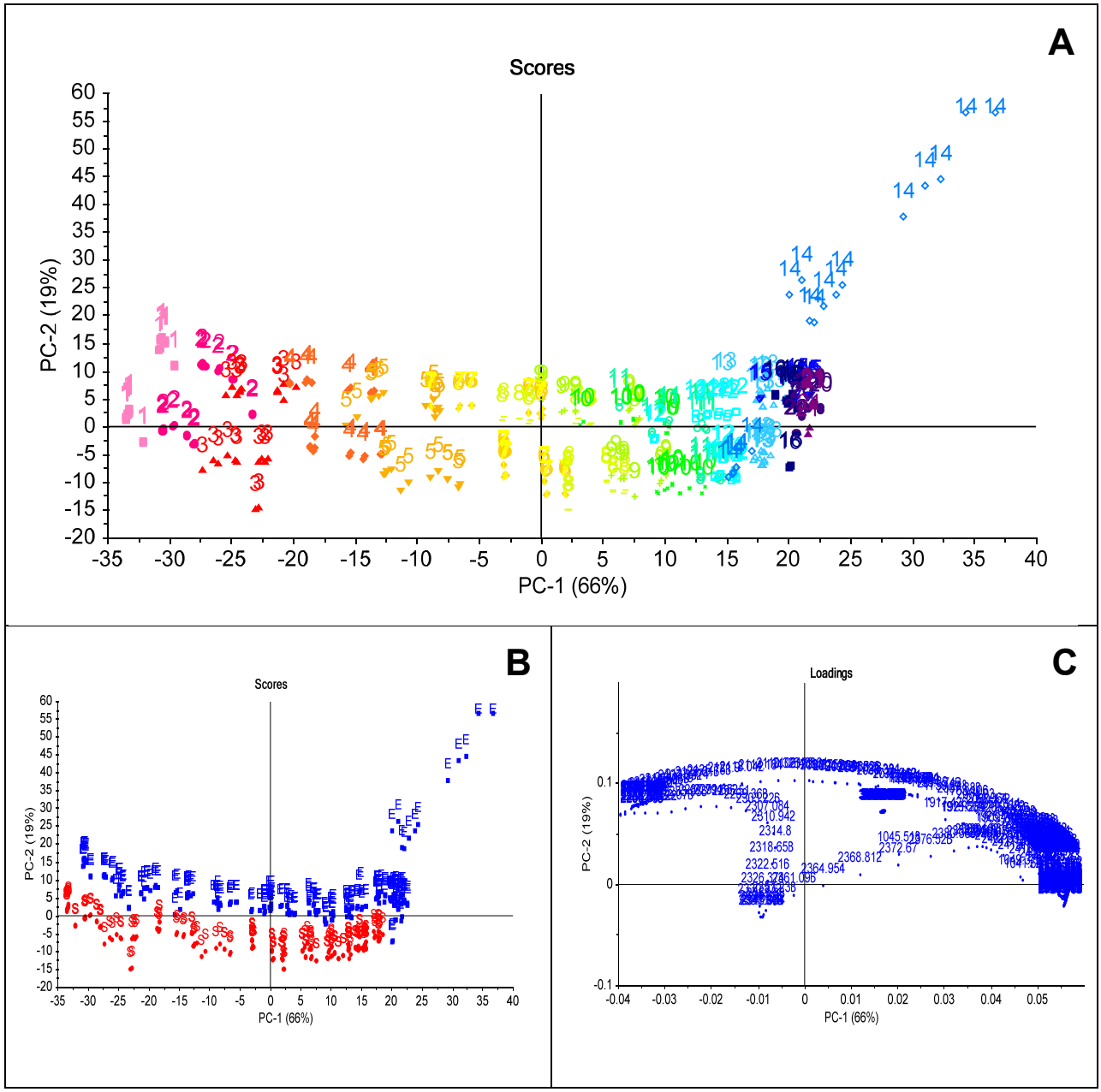

Figure 3.33 PCA Score plot (PC1 vs. PC2) of the 2011 spectral data. PC1 explains 66% of the variance between samples. The treatments separated along PC1 and discrimination is based on the FT-IR data. **A** shows the separation by days from 1 to 21. **B** shows the separation by canopy treatment and **C** shows the loadings plot.

3.3.6.2.2 2012

PCA constructed with the 2012 spectral data represents day 1 to day 26 of alcoholic fermentation (**Figure 3.34 A**). PC1 describes 69 % of the variance between samples. As in the 2011 data a clear graph (upside down fermentation graph) is seen with samples being further apart in the beginning of the fermentation overlaying more to the end. The score plot for the different canopy treatments clearly show groupings of the shaded (blue) and exposed (red) samples (**Figure 3.34 B**). **Figure 3.34 C** shows the loadings plot of all the wavelengths used for the FT-MIR analysis. A score plot for the yeast strains as mentioned in the 2011 data was also constructed but did not show any significant groupings (data not shown).

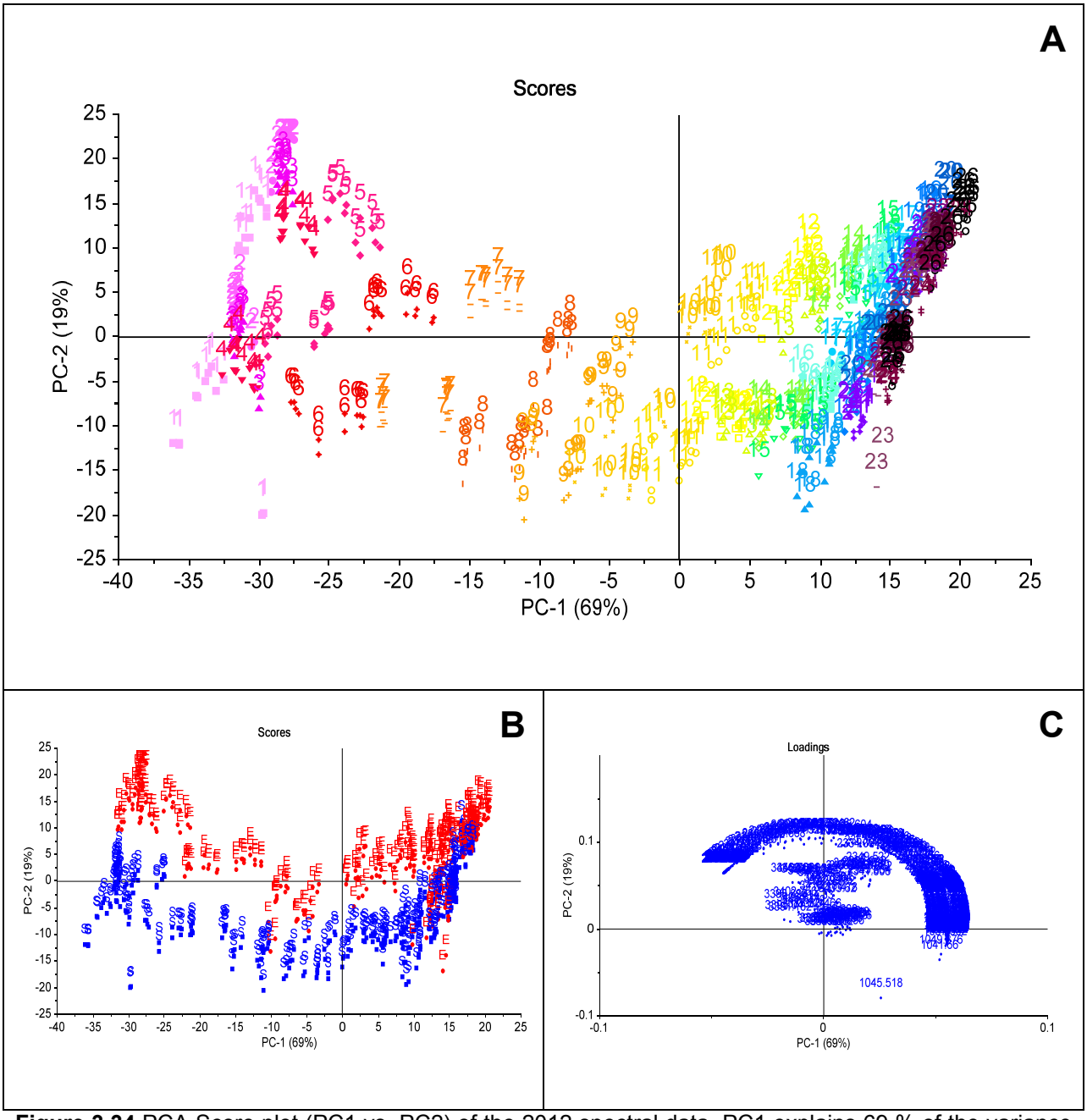

Figure 3.34 PCA Score plot (PC1 vs. PC2) of the 2012 spectral data. PC1 explains 69 % of the variance between samples. The treatments separated along PC1 and discrimination is based on the FT-IR data. **A** shows the separation by days from 1 to 26. **B** shows the separation by canopy treatment and **C** shows the loadings plot.

3.3.7 Sensory data

The sensory data was used in combination with chemical data obtained by GC-FID analysis. The data was imported into *The Unscrambler* software to further investigate any trends for grouping chemical compounds to sensory attributes observed during sensory evaluation of the treatments.

A bi-plot (**Figure 3.35**) was constructed including the wine samples analysed during the sensory evaluation, the attributes used to score the wines as well as the chemical analysis for the wine measured by GC-FID in 2012. PC1 describes 38 % of the variance between samples and gives clear groupings of the different canopy treatments, shaded (SH) treatments on the right and exposed (EXP) treatments on the left. PC 2 describes 31 % of the variance between samples and gives a clear grouping between the different yeast combinations, Cross Evolution and DV10.

The bi-plot shows that the combination SHDV correlated with attributes including, green pepper, pineapple, and citrus as well as scoring the highest in overall green and overall tropical. SHDV also had the highest astringency as well as sour attributes. From a chemical point of view the SHDV wines were divided by five ethyl esters including diethyl succinate, ethyl caprate, ethyl caprylate, ethyl hexanoate and two fatty acids, hexanoic acid and octanoic acid.

The combination SHCE could be described as more tropical, having attributes such as passion fruit, guava as well as canned beans. Chemical compounds associated with these wines include five acetate esters, isoamyl acetate, hexyl acetate, ethyl acetate, 2-methyl-propyl acetate, ethylphenyl acetate, two higher alcohols, pentanol, butanol and one fatty acid, acetic acid.

The combination EXPDV was not correlated with being high in any specific attribute. Chemical compounds associated with this treatment were mostly higher alcohols including 2-phenyethanol, 3-ethoxy-1-propanol, ethyl butyrate, hexanol, isoamyl alcohol, propanol, ethyl-3-hydroxybutanoate, ethyl propionate as well as fatty acids butyric acid, valeric acid and decanoic acid.

The combination with EXPCE was associated with attribute including apple cider, dried fruit as well as a sweet taste. Chemical compounds included esters, 2-phenylethyl acetate, 4-methyl-1-pentanol, 3-methyl-1-pentanol, ethyl lactate, higher alcohols included methanol, isobutanol, 1-octen-3-ol and fatty acids, isobutyric acid and propionic acid.

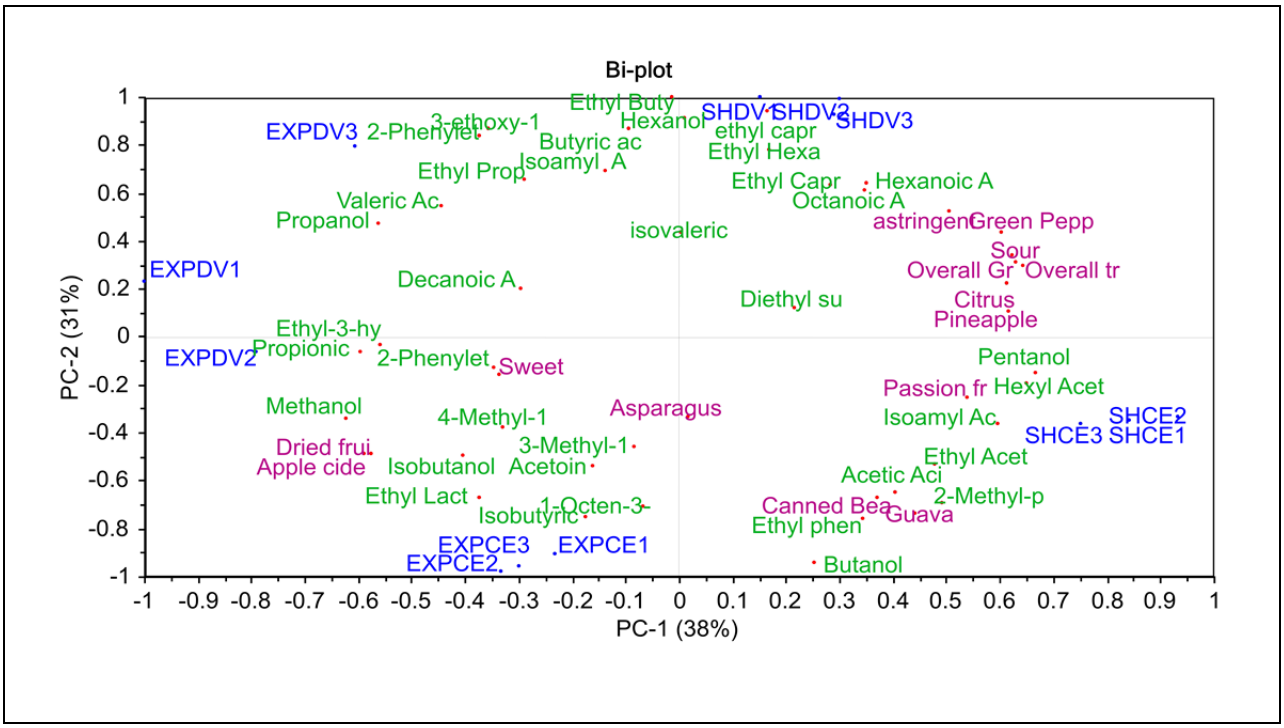

Figure 3.35 PCA bi-plot constructed from sensory and chemical data obtained in 2012. Loadings for chemical compounds indicated in green, and loadings for attributes indicated in purple. Scores for different wine treatments are indicated in blue.

Overall the two shaded viticultural treatment wines scored higher in the green attributes than the two exposed treatment wines. Interestingly the viticultural canopy treatment did have an effect on the sensory profile although not specifically on the fermentation derived volatiles. The shaded viticultural treatments scored high for overall green as well as tropical aromas whereas the exposed viticultural treatments were more associated with dried fruit or apple cider (**Figure 3.36**). Aroma could be grouped by canopy treatment rather than yeast strain. Yeast strains chosen did contribute to the differences in fermentation bouquet especially in contributing to aromas of guava and passion fruit. The main differences in “green” and “tropical” aroma could be the presence of methoxypyrazines and thiols.

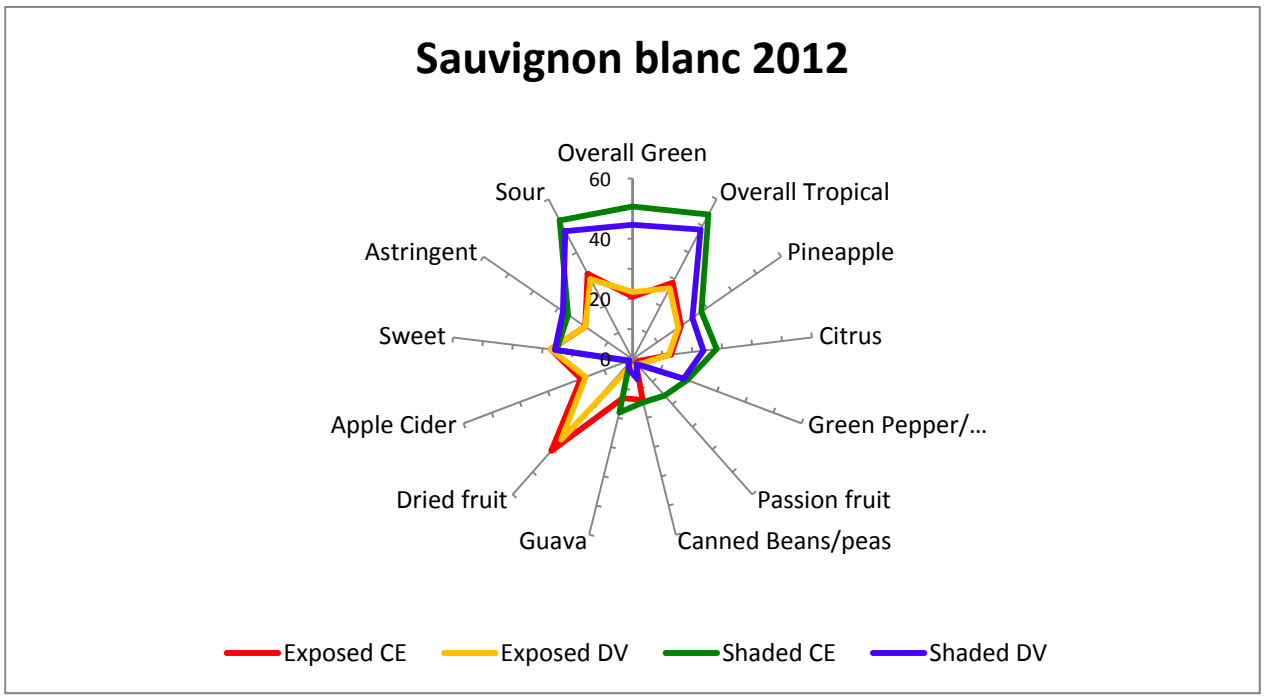

Figure 3.36 Spider plot indicating the effect canopy and yeast treatments on the different flavourings on the aroma profile. Shaded DV10 (Blue), Shaded Cross Evolution (Green), Exposed Cross Evolution (Red) and Exposed DV10 (Yellow).

3.4 DISCUSSION

As intended in the application of two different canopy treatments, initial juice compositions showed clear differences in terms of sugar content, titratable acid, and malic acid concentration. The shaded viticultural treatments juice also had a slightly higher YAN content which could be the reasons for the faster alcoholic fermentation rate in 2011. The difference in YAN can play a major role on the performance of yeast and also aroma due to much needed nitrogen source as well as amino acids acting as precursors for ester and higher alcohol formation. Ugliano *et al.* (2007) stated that only when YAN levels are lower than 150 mg/L will there be a chance of slow or stuck fermentation suggesting that both 2011 and 2012 had acceptable YAN levels.

The difference in juice composition confirms previous studies that viticultural canopy management does indeed have an effect on malic acid as well as a decrease in grassy character with leaf removal (Kozina *et al*., 2008; Lohitnavey *et al*., 2010).

All the treatments finished alcoholic fermentation indicating that initial must composition did not have a negative effect on the overall fermentation performance. Yeast strains however did differ in fermentation performance in 2011 where Cross Evolution fermented faster.

In both batch fermentations yeast had no cell growth problems. Volatile acid (VA) differences were seen over the two vintages with 2011 having higher concentrations than 2012. The exposed viticultural treatment wines had slightly higher VA with concentrations depending on the yeast strain used. It is known that VIN 7 produces more VA than other yeast strains; this

is confirmed in this study. The legal limit for volatile acidity is 1.2 g/L although it can be detected from 0.7 g/L. In both vintages the VA levels were below the threshold but in 2011, VIN7 could have had an influence on aroma properties of the wine. No significant differences in the pH of the wines were observed.

The fermentation derived volatile aroma concentrations showed differences between the two vintages. For both vintages more than half of the 39 volatile compounds measured were influenced by either the viticultural canopy or yeast treatment. From all the compounds influenced esters were the compound group that was mostly influenced specifically by the yeast treatment. Ester compounds influenced in both vintages by yeast treatment included ethyl butyrate, ethyl hexanoate, ethyl lactate, diethyl succinate, isoamyl acetate, 2-methyl-propyl-acetate and ethyl phenylacetate. A few ester compounds differed between the two vintages with ethyl caprylate, hexyl acetate, 2-phenyl ethylacetate and ethyl-3-hydroxybutanoate being significantly influenced in 2011 and ethyl caprate and ethyl acetate influenced in 2012. Ester production during alcoholic fermentation can therefore be highly strain dependent. Although individual esters were influenced, little impact was detected on the total ester production. Total higher alcohol and total volatile fatty acid production was primarily influenced by the yeast strains, correlating with previous study by Swiegers *et al*. (2009) showing variation between fermentation product profiles due to different yeast strains.

Monoterpene analysis showed no specific trends although interestingly α-terpinieol was only detected in the exposed viticultural treatment.

Carbonyl compounds were mostly under the limit of detection except for acetoin that was detected in the 2011 and 2012 vintage. Acetoin is a by-product of MLF but is also produced by yeast at low concentrations. As the odour threshold value for acetoin is 150 mg/L the amount detected would not have a significant effect on the aroma on its own but could contribute to the level of complexity (Romano *et al*., 1996).

Malic acid concentrations showed slight decrease throughout alcoholic fermentation also indicating differences between yeast strains. *Saccharomyces cerevisiae* species does not generally degrade malic acid during fermentation although some strains can consume a small amount (Coloretti *et al*., 2002; Torija *et al*., 2003). Acid plays an essential role in the final wine quality and therefore canopy management as well as yeast choice is important if it affects the malic acid outcome.

Citric acid concentrations were higher in the shaded than exposed treatments at end of alcoholic fermentation. Citric acid, a tri-acid, is present in low molar concentrations and only has a minimal affect on the titratable acidity and pH of the wine (Torija *et al*., 2003).

Panel resolution wines did show variation between treatments but importantly the overall trends were the same as the 2012 batch fermentation. This suggests that intra-vineyard heterogeneity has a reduced impact when compared to overall canopy treatments, at least when applied in such relatively extreme ways as described here.

As would be expected, multivariate data analysis showed clear groupings between different time points of analysis. Spectral analysis of the 2011 and 2012 vintages showed clear groupings of different days throughout alcoholic fermentation indicating compositional change over time. The spectra also showed clear groupings for the two viticultural canopy treatments. This could be due to the difference between initial must composition, especially the initial sugar. No groupings could be seen for the yeast strains used with the spectral data.

The bi-plot compiled by sensory descriptive analysis combined with chemical data both obtained from the 2012 batch fermentation, divided the four treatments each into a quadrant. Although chemical data did not show clear groupings for the different treatments there are clear associations between treatments and chemical compounds being significantly different. EXPDV is associated with 2-phenylethanol, propanol as well as isoamyl alcohol that showed significant higher levels in this case. SHDV is associated with ethyl caprate, ethyl hexanoate, diethyl succinate, ethyl butyrate that were all significantly higher in shaded DV10. EXPCE is associated with propionic acid as well as isobutyric acid which were significantly higher in this combination and lastly SCHE was associated with isoamyl acetate, ethyl acetate and ethyl phenylacetate that were all significantly higher in this combination.

The spider plot clearly shows the treatments resulting in four different wine aroma profiles indicating that viticultural canopy treatment combined with a certain yeast strain does affect the final wine profile. Although the yeast played a more significant role in the fermentation derived aroma profile for esters, higher alcohols and fatty acids it is clear that the end profile was more prominently impacted by canopy treatment than yeast strain. This could mostly be due to the other varietal aromas, thiols and methoxypyrazines that commonly dominate the aroma profile by contributing to “tropical” (thiols) and “green” (methoxypyrazines) flavours.

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Chapter 4

General discussion and conclusions

4. General discussion and conclusions

4.1 CONCLUDING REMARKS AND FUTURE WORK

Sauvignon blanc a cultivar originating from the Loire valley in France, is planted worldwide with increasing popularity. Depending on the consumer preference, many winemakers adapt winemaking techniques to produce wines of specific wine styles. Several reports have been made on “green” flavours being enhanced by viticulture treatments, and “tropical” aromas being improved by cellar practises, including specifically focusing on the choice of yeast strain (Swiegers *et al*., 2006; Swiegers *et al*., 2007). It is evident that aroma drives consumer liking and therefore studies increasing our knowledge on aroma development during grape ripening and winemaking are of great importance.

The overall aim of this integrated study was to assess the effect of two extreme viticultural treatments on the volatile aroma profile of Sauvignon blanc. This in-depth study assessed the treatment affects on grape must composition, microbial flora, transcriptomics, metabolic and chemical composition to create guidelines for wine styles from an integrated approach.

As expected the two canopy treatments resulted in different must compositions which is consistent with other reports of canopy management on grape must composition (Kozina *et al*., 2008; Lohitnavey *et al*., 2010).

It has been shown that some impact compounds are responsible for the sensorial properties found in Sauvignon blanc. These impact compounds include isobutyl-2-methoxypyrazine (IBMP) that induce characters of bell pepper and contribute to the green aspects as well as 4MMP (black currant), 3MH (passion fruit) and 3MHA (passion fruit, guava) that contribute to the tropical profile. These compounds were measured during the study to assess the viticultural and yeast impact as part of the integrated approach. Apart from these impact compounds low impact compounds such as esters, higher alcohols and fatty acids were also measured as they were also hypothesised to contribute to fruity character and enhance complexity.

Canopy treatments did show differences in methoxypyrazine content with the shaded treatment having a two-fold higher concentration than the exposed. These results are also consistent with findings in literature that environmental parameters including shaded canopies tend to increase methoxypyrazine concentrations specifically contributing to higher concentrations in warmer climate conditions (Lacey *et al*., 1991; Sala *et al*., 2004; Swiegers *et al*., 2006; Styger *et al*., 2011). Although literature on methoxypyrazine concentrations at the end of alcoholic fermentations are contradictory (Sala *et al*., 2004; Lund *et al*., 2009), in this study it was found that yeast strain did have an effect on the concentration in the final wine. Regardless to differences between canopy treatments Cross Evolution had slightly higher concentrations at end of alcoholic fermentation than VIN7 for 2011 vintage (Data not discussed in this thesis).

Interestingly the Cross Evolution treatments scored higher for canned beans/peas which could be consistent with Cross Evolution having higher IBMP concentrations.

Canopy treatments might have influenced the thiol precursors available in the grape must composition but the sensory data for this study shows a clear indication that no matter what the initial precursor availability of the must was it was dependent on the yeast strain to release aromas of guava. For this attribute the Cross Evolution treatments regardless of the canopy treatment scored much higher whereas in the DV10 treatments it was nonexistent. This can be ascribed to Cross Evolution being a yeast strain that has the ability to release greater amounts of 3MH as well as the conversion of 3MH to 3MHA (Swiegers *et al.*, 2009). For passion fruit, a thiol related aroma, the shaded Cross Evolution treatment scored the highest with all the other treatments scoring very low. This could be that the shaded canopy treatment potentially had higher precursor levels and that Cross Evolution had the ability to express greater release of 4MMP than DV10. This proves to be consistent with literature that yeasts strains are the key element for thiol release during alcoholic fermentation, but that canopy treatment does also have an effect on the thiols released during fermentation which could be correlated to the differences in initial must composition and precursor availability (Swiegers *et al*., 2007).

Although fruity aromas in wine can derive to a great extent from the presence of the mixture and different concentrations of esters produced during alcoholic fermentation, these volatile compounds have been thought to have little or no impact on the sensory properties in wine due to a masking effect. The masking effect in most cases, as hypothesised, is due to the principal contributors of Sauvignon blanc aroma (*methoxypyrazines and thiols*) and their low perception threshold resulting in high odour activity values (OAV). Benkwitz *et al*. (2012) took a holistic approach in measuring impact compounds that could contribute to Sauvignon blanc aroma. Except for the confirmation that volatile thiols and methoxypyrazines contribute greatly to the aroma and do project a masking effect, several fermentation esters where proven to be above their perception threshold. These esters, isoamyl acetate, ethyl butanoate, ethyl hexanoate, ethyl isovalerate and ethyl isobutyrate (perception thresholds **Table 2** in Benkwitz *et al*., 2012) except for the last two not being detected, were found to be above their perception thresholds in this study. These compounds are known to contribute to the fruity flowery and sweet characters of the wine. Fermentations done with DV10 showed to mainly be dominated by ethyl esters with the exposed canopy correlating with more sweet and apple cider aroma profiles confirming these statements. Isoamyl acetate was the one ester that was impacted by the canopy and yeast treatment for both vintages. Hexyl acetate, ethyl octanoate (ethyl caprylate) and ethyl decanoate (ethyl caprate) were also above their perception threshold values contributing to green grassy attributes.

Table 4.1 summarizes the factors that have been found in literature, as well as in the present study, to have a great effect on Sauvignon blanc wine aroma contributing to either “green” or “tropical” wine styles.

Table 4.1 Factors influencing aroma profiles of Sauvignon blanc resulting in "green" or "tropical" aromas being enhanced.

"Green" aroma profile		**"Tropical" aroma profile**	
Manipulating factors	***Reference***	***Manipulating factors***	***Reference***
Shaded canopy = less sunlight exposure	Marias *et al*., 2001 Sala *et al*., 2004 Swiegers *et al*., 2006	Exposed canopy = more sunlight exposure	Lacey *et al*., 1991 Marias *et al*., 2001
Harvesting date (*ripeness*) = earlier harvesting	Marias *et al*., 2001	Harvesting date (*ripeness*) = later harvesting	Marias *et al*., 2001
Berry hue colour (*in degrees*): > 90 – 85	Deloire, 2011	Berry hue colour (*in degrees*): 80 -70	Deloire, 2011
Longer skin contact = ↑ release of methoxypyrazines	Marias, 1998		
Choosing a yeast with less release of 4MMP and 3MH and less conversion to 3MHA (alkyl-methoxypyrazines suppress the impact of 3MHA Campo *et al* ., 2005)	Rapp, 1998 Antonelli *et al*., 1999 Mateo *et al*., 2001 Campo *et al*., 2005 Dubourdieu *et al*., 2006 King *et al*., 2008 Swiegers *et al* ., 2009 Sumby *et al*., 2009 Barrajón *et al*., 2011	Choosing a yeast with greater release of 4MMP and 3MH and greater conversion of 3MHA Or co-inoculation strategies with one yeast releasing 4MMP and one converting 3MH to 3MHA	Rapp, 1998 Antonelli *et al*., 1999 Mateo *et al*., 2001 Swiegers *et al*., 2006 Swiegers *et al*., 2007 Dubourdieu *et al*., 2006 King *et al*., 2008 Swiegers *et al*., 2009 Sumby *et al*., 2009 King *et al*., 2010 Barrajón *et al*., 2011
Yeast that increases methoxypyrazine concentration during AF*	Contradictory: Sala *et al*., 2004 Lund *et al*., 2009	The use of non-*Saccharomyces* yeast strains	Zott *et al*., 2011
		Increased fermentation temperature (23°C at the beginning of AF*	Swiegers *et al*., 2006
		Yeast strain enhancing fermentation derived esters especially acetate esters (*fruity*)	Saerens *et al*. 2008

*AF = Alcoholic fermentation

Except for the factors that can be manipulated by viticultural and oenological treatments a few environmental factors including climate, soil as well as terroir cannot be manipulated although they could have a profound effect on the aroma (Lacy *et al*., 1991; Marais *et al*., 1994, Sala *et al*., 2004; Swiegers *et al*., 2006; Styger *et al*., 2011).

This study has contributed to a better understanding of the complex relationships between canopy manipulation and yeast selection on aroma formation. The analysis of volatile aroma

alone however is not enough to understand the final perception of wine taste and further in depth studies of the viticultural and oenological factors is needed.

In particular, this project has focused on a single vineyard over only two vintages. The general validity of the conclusions derived from this study therefore will require additional data sets.

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ADDENDUM A

Factorial ANOVA data analysis

1. Major volatile aroma analysis for 2011 and 2012

A factorial ANOVA was performed to determine the influence of the viticultural canopy treatment, yeast strain used as well as the time point analysed during alcoholic fermentation on the volatile composition of the different treatments for 2011 and 2012 batch fermentations.

For 2011 and 2012 the interaction between canopy*yeast, canopy*timepoint, yeast*timepoint as well as canopy*yeast*timepoint was determined.

For the 2012 panel resolution wines the interaction between canopy*timepoint was determined.

Tables 1A – 3A lists the concentrations for esters, higher alcohols and volatile fatty acids measured over the four time points during alcoholic fermentation for 2011. **Table 4A – 6A** lists the concentrations for esters, higher alcohols and volatile fatty acids measured over the four time points during alcoholic fermentation for 2012. **Table 7A – 8A** lists the concentrations for esters, higher alcohols and volatile fatty acids measured over the two time points during alcoholic fermentation for the 2012 panel resolution wines.

Table 9A – 10A lists the *p*-values for factorial ANOVA done on the 2011 vintage. The red values indicate the single factors as well as the interactions that were significant on 5 % significance level ($p < 0.05$). **Table 11A** lists the compounds that had a significant third order interaction between canopy*yeast*timepoint for 2011.

Table 12A lists the compound that had a significant second order interaction between canopy*yeast for 2011.

Table 13A -14A lists the *p*-value for the factorial ANOVA done on the 2012 vintage. The red values indicate the single factors as well as the interactions that were significant on the 5 % significance level ($p < 0.05$). **Table 15A** lists the compounds that had a significant third order interaction between canopy*yeast*timepoint for 2012.

Table 16A lists the compounds that had a significant second order interaction between canopy*yeast for 2012.

Table 17A - 18A lists the *p*-values for the factorial ANOVA done on the 2012 panel resolution wines. The red values indicate the single factors as well as the interactions that were significant on the 5 % significance level ($p < 0.05$).

Table 19A lists the compounds that had a significant second order interaction between canopy*timepoint for panel resolution wines 2012 with **Figure 1A** showing the graphs.

Table 20A lists the compounds that were significant for the viticultural canopy treatment for 2012 panel resolution wines with the graphs shown in **Figure 2A - 4A**.

Table 1A Ester concentration (mg/L) measured at four time points during alcoholic fermentation in Sauvignon blanc during **2011** vintage. Concentrations represent the average of triplicate treatments each analyzed in duplicate by GC-FID (Standard deviations not shown) (nd = not detected).

	SHADED								**EXPOSED**							
	Cross Evolution				*VIN 7*				*Cross Evolution*				*VIN 7*			
	1/4	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**
ESTERS																
Ethyl Butyrate	0.049	0.180	0.290	0.654	nd	0.112	0.112	0.405	0.039	0.096	0.343	0.658	nd	0.050	0.125	0.424
Ethyl Hexanoate	0.657	1.054	1.706	2.337	0.402	1.018	0.869	1.521	0.586	0.80	1.856	2.405	0.351	0.516	0.776	1.514
Ethyl Lactate	4.144	10.238	12.495	5.464	2.225	5.363	6.764	1.759	3.394	5.926	9.818	4.123	1.978	3.508	5.631	0.747
Ethyl Caprylate	0.788	1.232	1.837	2.152	0.446	1.055	0.975	1.342	0.608	0.941	2.002	2.132	0.443	0.648	1.047	1.464
Ethyl caprate	2.978	4.583	5.512	1.457	1.905	3.558	3.139	1.400	1.457	2.223	4.424	1.510	2.191	1.436	3.902	1.541
Diethyl succinate	0.174	0.238	0.257	0.450	0.190	0.176	0.241	0.200	0.192	0.169	0.408	0.486	0.209	0.169	0.204	0.193
Ethyl Acetate	36.856	53.418	70.330	117.024	30.490	62.555	69.152	130.311	35.034	45.631	83.823	122.311	31.826	50.217	73.420	137.441
Isoamyl Acetate	1.906	4.047	8.825	13.041	0.862	4.320	4.769	8.225	1.690	2.890	9.237	12.508	0.698	1.950	4.324	7.407
Hexyl Acetate	0.646	0.654	0.743	0.930	0.613	0.692	0.636	0.881	0.681	0.639	0.785	0.951	0.619	0.622	0.668	0.884
2-Phenylethyl Acetate	0.304	0.319	0.340	0.490	nd	0.309	0.325	0.509	0.303	0.306	0.334	0.490	nd	0.305	0.332	0.543
Ethyl Propionate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd
Ethyl-2-methylpropanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd
2-Methyl-propyl acetate	0.210	0.214	0.234	nd	0.196	0.210	0.207	nd	0.208	0.208	0.231	0.310	nd	0.199	0.205	0.285
Ethyl-2-methylbutyrate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd
Ethyl isovalerate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd
Ethyl-3-hydroxybutanoate	37.135	65.719	86.587	54.657	48.331	85.839	109.979	58.283	40.096	85.625	73.387	53.685	50.071	94.082	120.460	55.164
Ethyl phenylacetate	1.128	1.893	3.418	4.574	0.370	1.348	1.576	2.875	0.951	1.252	3.443	4.354	0.325	0.780	1.568	2.933
TOTAL	**86.974**	**143.734**	**192.574**	**215.617**	**86.030**	**166.556**	**198.749**	**215.531**	**85.239**	**146.706**	**190.091**	**217.773**	**88.711**	**154.482**	**212.662**	**217.523**

Table 2A Higher alcohols (mg/L) measured at four time points during alcoholic fermentation in Sauvignon blanc during **2011** vintage. Concentrations represent the average of triplicate treatments each analyzed in duplicate by GC-FID (Standard deviations not shown) (nd = not detected).

	SHADED								**EXPOSED**							
	Cross Evolution				*VIN 7*				*Cross Evolution*				*VIN 7*			
	1/4	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**
HIGHER ALCOHOLS																
Methanol	43.265	49.832	49.777	40.731	39.058	50.302	50.221	39.376	41.927	48.973	53.536	46.907	41.976	48.641	51.807	41.383
Propanol	39.749	39.749	39.749	94.536	39.749	39.749	39.749	40.912	39.749	39.749	39.749	98.648	39.749	39.749	39.749	43.609
Isobutanol	13.333	16.433	23.061	24.429	8.693	16.935	19.810	24.354	13.748	14.766	24.129	25.352	8.910	13.171	20.300	26.219
Butanol	0.769	1.264	2.103	2.244	0.479	0.965	1.080	1.167	0.683	1.042	2.208	2.358	0.475	0.800	1.160	1.262
Isoamyl Alcohol	55.725	84.294	146.235	157.788	43.383	100.223	122.429	162.417	55.187	67.030	149.253	156.570	42.643	68.311	123.789	170.982
Hexanol	0.719	0.493	0.566	0.662	0.763	0.553	1.552	0.650	0.755	0.488	0.632	0.688	0.870	0.549	0.593	0.779
2-Phenylethanol	5.833	6.614	11.548	13.438	3.088	6.996	8.416	11.094	5.861	5.512	12.629	14.105	3.263	5.138	8.832	12.075
4-Methyl-1-pentanol	0.090	0.082	0.082	0.174	0.064	0.076	0.070	nd	0.097	0.092	0.093	nd	0.066	0.069	0.073	Nd
3-Methyl-1-pentanol	nd	0.096	0.123	2.026	nd	nd	0.093	1.007	nd	nd	0.133	1.047	nd	nd	nd	1.008
3-ethoxy-1-propanol	1.256	1.985	3.126	0.777	0.722	1.768	1.919	0.033	1.301	2.016	2.836	0.851	0.789	1.604	2.074	0.190
Pentanol	0.115	0.104	0.136	0.272	0.089	0.104	0.100	0.256	0.111	0.088	0.128	0.265	0.086	0.079	0.100	0.248
1-Octen-3-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd
TOTAL	**160.854**	**200.946**	**276.507**	**337.077**	**136.088**	**217.671**	**245.439**	**281.266**	**159.419**	**179.755**	**285.326**	**346.790**	**138.828**	**178.111**	**248.477**	**297.755**

Table 3A Volatile fatty acids (mg/L) measured at four time points during alcoholic fermentation in Sauvignon blanc during **2011** vintage. Concentrations represent the average of triplicate treatments each analyzed in duplicate by GC-FID (Standard deviations not shown) (nd = not detected).

	SHADED								**EXPOSED**							
	Cross Evolution				*VIN 7*				*Cross Evolution*				*VIN 7*			
	1/4	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**
FATTY ACIDS																
Acetic Acid	132.215	153.086	147.615	110.200	755.607	1091.114	1114.650	889.651	204.955	230.693	234.134	207.435	941.827	1263.337	1515.763	1156.238
Propionic Acid	1.057	0.817	1.601	1.441	1.271	0.936	0.929	1.261	1.295	0.804	1.748	1.645	1.476	0.817	1.305	1.462
Isobutyric Acid	1.001	0.909	1.226	1.020	1.161	1.054	1.002	1.065	1.239	0.959	1.513	1.344	1.406	1.056	1.308	1.406
Butyric acid	0.191	0.218	0.300	0.308	0.178	0.202	0.206	0.233	0.180	0.202	0.330	0.345	0.185	0.182	0.223	0.257
isovaleric acid	0.425	0.469	0.617	0.599	0.384	0.454	0.462	0.689	0.467	0.430	0.755	0.682	0.417	0.398	0.527	0.567
Valeric Acid	0.342	0.352	0.409	0.203	0.277	0.388	0.407	0.233	0.336	0.343	0.309	0.229	0.268	0.324	0.393	0.225
Hexanoic Acid	2.481	2.629	3.825	4.888	2.449	2.736	2.489	3.669	2.608	2.401	4.731	5.195	2.601	2.280	2.335	4.094
Octanoic Acid	2.786	2.790	4.074	4.042	2.297	2.869	2.476	3.204	2.655	2.465	4.884	3.992	2.387	1.993	2.614	3.269
Decanoic Acid	1.987	1.644	2.048	0.780	1.617	1.664	1.555	0.776	1.679	1.476	2.072	0.865	1.619	1.422	1.719	0.786
TOTAL	**142.485**	**162.914**	**161.715**	**123.481**	**765.241**	**1101.417**	**1124.176**	**900.781**	**215.414**	**239.773**	**250.476**	**221.732**	**952.186**	**1271.809**	**1526.187**	**1168.304**

Table 4A Esters (mg/L) measured at four time points during alcoholic fermentation in Sauvignon blanc during **2012** vintage. Concentrations represent the average of triplicate treatments each analyzed in duplicate by GC-FID (Standard deviations not shown) (nd = not detected).

	SHADED								**EXPOSED**							
	Cross Evolution				*DV10*				*Cross Evolution*				*DV10*			
	1/4	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**
ESTERS																
Ethyl Butyrate	0.249	0.206	0.383	0.640	0.049	0.215	0.555	0.925	0.078	0.216	0.416	0.597	0.113	0.226	0.605	0.763
Ethyl Hexanoate	1.070	1.508	1.924	1.996	0.739	2.651	2.262	2.430	0.812	2.414	1.879	1.853	1.109	2.622	2.295	2.039
Ethyl Lactate	0.707	nd	0.529	2.926	nd	nd	0.486	2.922	nd	nd	0.506	3.071	nd	0.150	0.556	3.262
Ethyl Caprylate	0.972	0.955	1.273	2.814	0.389	1.753	1.521	3.153	0.491	1.490	1.320	2.729	0.754	1.643	1.623	2.656
ethyl caprate	2.524	1.687	2.219	15.817	1.178	7.928	2.621	18.046	1.215	4.664	1.749	13.717	2.002	6.261	2.936	15.763
Diethyl succinate	0.186	0.676	0.576	0.321	0.078	nd	0.238	0.251	0.167	0.172	0.332	0.176	0.178	nd	0.180	0.261
Ethyl Acetate	46.764	51.120	78.682	107.01	18.426	27.093	72.654	99.547	40.243	35.654	78.351	101.975	39.105	31.050	72.452	96.762
Isoamyl Acetate	2.807	6.098	10.352	12.231	1.340	5.555	9.471	10.710	2.139	7.573	10.808	11.138	1.845	5.332	8.777	7.627
Hexyl Acetate	0.838	0.786	0.780	0.900	0.708	0.998	0.664	0.849	0.816	1.013	0.745	0.823	0.740	0.892	0.743	0.732
2-Phenylethyl Acetate	nd	0.170	0.196	0.390	nd	0.162	0.240	0.490	nd	0.164	0.196	0.397	nd	0.165	0.255	0.505
Ethyl Propionate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl-2-methylpropanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-Methyl-propyl acetate	0.208	0.177	0.182	0.225	nd	0.162	0.168	0.211	0.207	0.184	0.182	0.221	0.197	0.162	0.169	0.204
Ethyl-2-methylbutyrate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl isovalerate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl-3-hydroxybutanoate	34.413	22.134	30.212	18.802	26.057	29.892	21.417	19.922	33.653	36.392	22.817	20.943	37.706	31.713	22.470	20.779
Ethyl phenylacetate	0.929	0.990	1.737	3.351	0.169	0.688	1.410	2.701	0.840	1.680	1.883	3.654	0.578	0.732	1.476	2.189
TOTAL	**91.667**	**86.507**	**129.045**	**167.423**	**49.133**	**77.097**	**113.707**	**162.157**	**80.661**	**91.616**	**121.184**	**161.294**	**84.327**	**80.948**	**114.54**	**153.54**

Table 5A Higher alcohols (mg/L) measured at four time points during alcoholic fermentation in Sauvignon blanc during **2012** vintage. Concentrations represent the average of triplicate treatments each analyzed in duplicate by GC-FID (Standard deviations not shown) (nd = not detected).

	SHADED								**EXPOSED**							
	Cross Evolution				*DV10*				*Cross Evolution*				*DV10*			
	1/4	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**
HIGHER ALCOHOLS																
Methanol	43.950	38.215	44.623	49.719	39.631	30.211	41.507	51.084	46.566	31.736	47.077	55.883	48.202	33.260	47.325	58.016
Propanol	32.168	40.790	54.129	55.703	33.551	43.261	68.481	68.538	38.153	35.933	57.057	62.315	45.917	50.849	80.163	84.920
Isobutanol	9.255	11.400	14.427	16.494	6.339	9.568	14.694	16.691	10.396	11.951	15.438	17.658	7.155	10.908	15.279	17.122
Butanol	0.925	1.358	2.125	2.273	0.559	0.937	1.380	1.343	0.709	1.227	1.980	2.436	0.659	0.900	1.322	1.274
Isoamyl Alcohol	56.559	75.709	105.980	145.197	46.503	100.537	134.282	167.420	57.611	102.511	117.462	154.832	56.027	98.150	135.777	154.644
Hexanol	0.560	0.419	0.322	0.582	0.499	0.764	0.499	0.694	0.569	0.524	0.338	0.587	0.576	0.598	0.483	0.615
2-Phenylethanol	4.312	5.565	8.631	12.241	3.431	7.300	10.904	13.796	4.933	9.233	11.057	14.892	4.352	7.859	11.963	13.437
4-Methyl-1-pentanol	0.086	0.014	nd	0.080	0.025	nd	nd	0.070	0.093	nd	nd	0.087	0.085	nd	nd	0.075
3-Methyl-1-pentanol	nd	0.018	nd	0.160	nd	nd	nd	0.138	0.092	nd	nd	0.173	nd	nd	nd	0.138
3-ethoxy-1-propanol	1.045	0.704	1.046	1.280	6.164	10.394	13.037	11.761	1.118	1.116	1.169	1.386	6.437	13.894	15.919	14.493
Pentanol	0.126	nd	nd	0.122	0.032	nd	nd	0.113	0.089	nd	nd	0.110	0.084	nd	nd	0.095
1-Octen-3-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
TOTAL	**148.986**	**174.192**	**231.283**	**283.851**	**136.734**	**202.972**	**284.784**	**331.648**	**160.329**	**194.231**	**251.578**	**310.359**	**169.494**	**216.418**	**308.231**	**344.829**

Table 6A Volatile fatty acids (mg/L) measured at four time points during alcoholic fermentation in Sauvignon blanc during **2012** vintage. Concentrations represent the average of triplicate treatments each analyzed in duplicate by GC-FID (Standard deviations not shown) (nd = not detected).

	SHADED								**EXPOSED**							
	Cross Evolution				*VIN 7*				*Cross Evolution*				*VIN 7*			
	1/4	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**
FATTY ACIDS																
Acetic Acid	328.301	285.625	292.472	397.797	390.162	438.729	329.102	366.413	493.606	305.438	293.762	409.493	399.900	354.838	300.187	315.715
Propionic Acid	1.288	1.440	1.835	1.567	1.843	2.423	2.169	1.882	2.977	2.257	2.226	2.097	2.057	2.409	2.447	2.050
Isobutyric Acid	0.875	0.904	0.962	0.948	0.820	1.109	0.959	0.916	1.309	1.466	1.165	1.217	0.874	1.145	1.033	0.940
Butyric acid	0.268	0.290	0.335	0.364	0.240	0.319	0.356	0.407	0.243	0.332	0.335	0.369	0.251	0.333	0.365	0.386
isovaleric acid	0.467	0.608	0.591	0.736	0.460	0.762	0.733	0.846	0.538	0.759	0.656	0.800	0.493	0.719	0.731	0.750
Valeric Acid	0.260	0.295	0.283	0.235	0.446	0.400	0.462	0.279	0.232	0.386	0.320	0.273	0.515	0.391	0.183	0.452
Hexanoic Acid	3.786	4.690	4.741	7.597	3.298	6.458	6.967	8.379	2.801	5.483	5.122	7.336	3.410	7.016	7.214	7.129
Octanoic Acid	6.755	8.404	8.418	12.767	7.027	14.885	12.200	14.642	6.051	11.627	8.142	12.251	6.308	15.504	12.305	10.446
Decanoic Acid	3.732	1.418	1.197	6.869	2.326	8.986	1.931	8.012	2.791	5.663	1.245	6.397	3.859	7.454	2.484	5.020
TOTAL	**345.732**	**303.674**	**310.834**	**428.880**	**406.622**	**474.071**	**354.879**	**401.776**	**510.548**	**333.411**	**312.973**	**440.233**	**417.667**	**389.809**	**326.949**	**342.888**

Table 7A Esters (mgL) measured at two time points for each panel during alcoholic fermentation in Sauvignon blanc during **2012** vintage for the **panel resolution** wines. Concentrations represent the average of triplicate treatments each analyzed in duplicate by GC-FID (Standard deviations not shown) (nd = not detected).

	SHADED						**EXPOSED**					
	Panel 1		*Panel 2*		*Panel 3*		*Panel 1*		*Panel 2*		*Panel 3*	
	1/2	**4/4**	**1/2**	**4/4**	**1/2**	**4/4**	**1/2**	**4/4**	**1/2**	**4/4**	**1/2**	**4/4**
ESTERS												
Ethyl Butyrate	0.341	0.663	0.487	0.585	0.210	0.485	0.290	0.671	0.188	0.711	0.178	0.424
Ethyl Hexanoate	2.214	2.045	2.558	1.759	1.360	1.547	2.209	2.140	1.622	2.440	1.299	1.474
Ethyl Lactate	nd	2.823	1.442	2.343	0.883	1.799	1.472	1.694	0.907	2.114	0.804	1.568
Ethyl Caprylate	0.983	2.026	1.442	2.343	0.883	1.799	1.472	1.694	0.907	2.114	0.804	1.568
Ethyl caprate	2.424	12.572	3.592	15.047	2.172	11.393	5.303	11.246	2.634	12.198	1.700	9.147
Diethyl succinate	0.329	0.165	0.306	0.162	0.176	0.154	0.382	0.166	0.568	0.189	0.414	0.170
Ethyl Acetate	62.564	110.141	77.374	102.467	56.871	95.795	55.551	101.359	46.277	102.467	38.895	84.786
Isoamyl Acetate	7.859	10.888	12.103	10.531	6.538	8.293	8.116	9.792	4.880	10.668	6.538	7.383
Hexyl Acetate	0.769	0.815	0.868	0.815	0.627	0.733	0.777	0.765	0.634	0.747	0.627	0.705
2-Phenylethyl Acetate	0.171	0.376	0.195	0.405	0.180	0.376	0.174	0.351	0.165	0.364	0.166	0.373
Ethyl Propionate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl-2-methylpropanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-Methyl-propyl acetate	0.174	0.221	0.193	0.223	0.171	0.215	0.172	0.216	0.160	0.211	0.160	0.208
Ethyl-2-methylbutyrate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl isovalerate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl-3-hydroxybutanoate	25.079	63.337	25.513	67.289	32.351	66.447	36.872	20.720	33.182	25.913	34.381	26.675
Ethyl phenylacetate	1.160	3.450	2.494	3.479	1.123	2.795	0.969	2.481	0.336	2.980	0.355	2.020
TOTAL	**104.067**	**209.522**	**128.567**	**207.448**	**103.545**	**191.831**	**113.759**	**153.295**	**92.460**	**163.116**	**86.321**	**136.501**

Table 8A Higher alcohols and volatile fatty acids (mg/L) measured at two time points for each panel during alcoholic fermentation in Sauvignon blanc during 2012 vintage for the panel resolution wines. Concentrations represent the average of triplicate treatments each analyzed in duplicate by GC-FID (Standard deviations not shown) (nd = not detected).

	SHADED						**EXPOSED**					
	Panel 1		*Panel 2*		*Panel 3*		*Panel 1*		*Panel 2*		*Panel 3*	
	1/2	**4/4**	**1/2**	**4/4**	**1/2**	**4/4**	**1/2**	**4/4**	**1/2**	**4/4**	**1/2**	**4/4**
HIGHER ALCOHOLS												
Methanol	43.367	58.952	42.497	59.549	45.536	58.595	42.937	55.041	43.811	56.697	41.445	53.336
Propanol	44.712	68.581	57.803	70.728	50.358	59.600	47.000	57.241	44.636	54.052	35.821	47.583
Isobutanol	12.544	18.618	16.135	19.514	12.379	17.071	12.584	15.499	8.916	14.242	8.506	12.816
Butanol	1.005	1.382	1.961	2.368	1.605	2.225	0.878	1.142	0.863	1.620	1.366	2.279
Isoamyl Alcohol	83.056	148.969	122.019	157.746	79.775	133.417	91.402	130.028	122.019	138.382	79.775	116.652
Hexanol	0.441	0.703	0.439	0.638	0.261	0.497	0.521	0.660	0.335	0.602	0.234	0.464
2-Phenylethanol	6.713	13.356	10.760	14.124	7.009	12.083	6.933	11.137	4.602	12.861	4.417	10.076
4-Methyl-1-pentanol	nd	0.081	nd	0.074	nd	0.068	nd	0.087	nd	0.086	nd	0.082
3-Methyl-1-pentanol	nd	0.159	nd	0.169	nd	0.151	nd	0.154	nd	0.188	nd	0.153
3-ethoxy-1-propanol	0.866	1.503	1.282	1.683	1.087	1.610	1.064	1.338	0.855	1.679	0.649	1.416
Pentanol	nd	0.1268	nd	0.1276	nd	0.1080	nd	0.0982	nd	0.0972	nd	0.0965
1-Octen-3-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
TOTAL	**192.704**	**312.431**	**252.896**	**326.720**	**198.010**	**285.425**	**203.319**	**272.424**	**226.037**	**280.507**	**172.213**	**244.953**
VOLATILE FATTY ACIDS												
Acetic Acid	77.203	216.362	272.630	393.324	343.527	505.530	74.842	103.160	100.777	181.038	350.976	541.799
Propionic Acid	1.673	1.570	2.184	1.711	1.791	1.653	1.877	1.455	1.303	1.545	1.512	1.506
Isobutyric Acid	0.790	0.764	1.148	1.098	0.905	0.969	0.828	0.764	0.532	0.633	0.564	0.618
Butyric acid	0.304	0.381	0.353	0.374	0.289	0.339	0.333	0.367	0.260	0.405	0.252	0.326
isovaleric acid	0.436	0.663	0.682	0.755	0.508	0.683	0.454	0.530	0.302	0.467	0.357	0.546
Valeric Acid	0.250	0.196	0.267	0.235	0.291	0.251	0.279	0.214	0.257	0.235	0.250	0.230
Hexanoic Acid	4.567	8.043	5.832	7.637	3.972	6.648	5.359	8.299	3.953	9.395	3.706	6.831
Octanoic Acid	7.731	10.518	10.483	11.391	6.408	8.868	8.977	10.164	6.514	12.236	6.298	9.165
Decanoic Acid	1.456	6.529	2.290	7.021	1.303	6.323	2.250	5.629	1.256	6.408	1.165	5.221
TOTAL	**94.409**	**245.025**	**295.786**	**423.547**	**358.993**	**531.265**	**95.198**	**130.582**	**115.154**	**212.362**	**365.080**	**566.241**

Table 9A Probability values (*p*-value) for esters are given for **2011**. The effect of canopy treatment, yeast used as well as the time point sampled given separately. The interaction between the factors canopy*yeast, canopy*timepoint, yeast*timepoint as a combined effect on the volatile aroma compounds are shown. The third level of interaction between canopy*yeast*timepoint is also shown. Values in red show factors significantly affecting the wine volatiles at a 5% significance level.

	Canopy	*Yeast*	*Timepoint*	*Canopy*Yeast*	*Canopy*Timepoint*	*Yeast*Timepoint*	*Canopy*Yeast*Timepoint*
ESTERS							
Ethyl Butyrate	0.422663	0.000002	0.000000	0.986349	0.016346	0.000001	0.670838
Ethyl Hexanoate	0.050520	0.000004	0.000000	0.495668	0.005296	0.000050	0.835026
Ethyl Lactate	0.000182	0.000001	0.000000	0.045398	0.010177	0.001472	0.435379
Ethyl Caprylate	0.176808	0.000019	0.000000	0.612448	0.003431	0.000183	0.741824
Ethyl caprate	0.028027	0.062376	0.000000	0.136979	0.018545	0.201131	0.361412
Diethyl succinate	0.345956	0.000056	0.000000	0.137468	0.099423	0.000000	0.014216
Ethyl Acetate	0.415370	0.103366	0.000000	0.651922	0.000729	0.009322	0.700907
Isoamyl Acetate	0.015373	0.000021	0.000000	0.872900	0.031790	0.000203	0.931257
Hexyl Acetate	0.793961	0.000676	0.000000	0.320593	0.018258	0.011842	0.992370
2-Phenylethyl Acetate	0.371513	0.000000	0.000000	0.016996	0.003202	0.000000	0.059982
2-Methyl-propyl acetate	0.173633	0.016287	0.000000	0.350248	0.512505	0.470143	0.443678
Ethyl-3-hydroxybutanoate	0.735772	0.045178	0.000001	0.499506	0.965649	0.234745	0.713188
Ethyl phenylacetate	0.106083	0.000028	0.000000	0.460017	0.006170	0.000057	0.650140

Table 10A Probability values (*p*-value) for higher alcohols and volatile fatty acids are given for **2011**. The effect of canopy treatment, yeast used as well as the time point sampled given separately. The interaction between the factors canopy*yeast, canopy*timepoint, yeast*timepoint as a combined effect on the volatile aroma compounds are shown. The third level of interaction between canopy*yeast*timepoint is also shown. Values in red show factors significantly affecting the wine volatiles at a 5% significance level.

	Canopy	*Yeast*	*Timepoint*	*Canopy*Yeast*	*Canopy*Timepoint*	*Yeast*Timepoint*	*Canopy*Yeast*Timepoint*
HIGHER ALCOHOLS							
Methanol	0.426188	0.353266	0.000005	0.730088	0.465972	0.721450	0.603456
Propanol	0.271550	0.000000	0.000000	0.478128	0.000762	0.000000	0.140958
Isobutanol	0.494769	0.003840	0.000000	0.915588	0.003526	0.010064	0.923366
Butanol	0.377422	0.000002	0.000000	0.387795	0.018841	0.000011	0.468090
Isoamyl Alcohol	0.173510	0.185260	0.000000	0.724997	0.011187	0.067236	0.953159
Hexanol	0.585716	0.288759	0.453933	0.487940	0.512724	0.645194	0.430521
2-Phenylethanol	0.705468	0.000761	0.000000	0.850179	0.004624	0.046666	0.823740
4-Methyl-1-pentanol	0.417061	0.110009	0.601825	0.431151	0.373313	0.624552	0.366264
3-Methyl-1-pentanol	0.298905	0.227788	0.000000	0.364095	0.442709	0.403311	0.400948
3-ethoxy-1-propanol	0.517813	0.000010	0.000000	0.154299	0.173201	0.099549	0.649859
Pentanol	0.017266	0.000385	0.000000	0.734481	0.082522	0.141944	0.986850
FATTY ACIDS							
Acetic Acid	0.001222	0.000000	0.000269	0.037910	0.598917	0.001196	0.678654
Propionic Acid	0.157060	0.108700	0.000002	0.655068	0.043153	0.001065	0.859614
Isobutyric Acid	0.005824	0.744024	0.000667	0.844595	0.008117	0.012807	0.983028
Butyric acid	0.538579	0.000041	0.000000	0.765677	0.001080	0.000003	0.432064
isovaleric acid	0.639324	0.040847	0.000021	0.248224	0.250233	0.148151	0.548150
Valeric Acid	0.109176	0.717711	0.000000	0.819487	0.331983	0.099445	0.454588
Hexanoic Acid	0.596463	0.000331	0.000000	0.497772	0.024335	0.000015	0.155321
Octanoic Acid	0.517707	0.000357	0.000000	0.640282	0.007301	0.000246	0.438966
Decanoic Acid	0.295605	0.017743	0.000000	0.485062	0.064692	0.038437	0.522919

Table 11A Shows the values for esters that are significant on a third order interaction (Canopy*yeast*timepoint) level for aroma compounds analysed for **2011** harvest. The concentrations represent the average of triplicate treatments, done by GC-FID. Standard deviations are not shown. Different letters within a row denote significant differences at $p < 0.05$. No higher alcohols or fatty acids were significant

	SHADED								**EXPOSED**							
	Cross-Evolution				*VIN7*				*Cross-Evolution*				*VIN7*			
	1/4	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**
ESTERS																
Diethyl succinate	0.174^{d}	0.238^{cd}	0.257^{c}	0.450^{ab}	0.190^{cd}	0.176^{d}	0.241^{cd}	0.200^{cd}	0.192^{cd}	0.169^{d}	0.408^{b}	0.486^{a}	0.209^{cd}	0.169^{d}	0.204^{cd}	0.193^{cd}

Table 12A Shows the values of esters and fatty acids that are significant on a second order interaction (Canopy*Yeast) level for aroma compounds analysed for **2011** harvest. The concentrations represent the average of triplicate treatments, each done in duplicate by GC-FID. Standard errors are not shown. Different letters within a row denote significant differences at $p < 0.05$. No higher alcohols were affected by this order of interaction

	SHADED		**EXPOSED**	
YEAST STRAIN	***Cross Evolution***	***VIN7***	***Cross Evolution***	***VIN7***
ESTERS				
Ethyl lactate	8.0852^{a}	4.0277^{c}	5.8151^{b}	2.9657^{d}
2-Phenylethyl acetate	0.3631^{a}	0.2856^{c}	0.3581^{a}	0.2952^{b}
FATTY ACIDS				
Acetic acid	135.779^{c}	962.755^{b}	219.304^{c}	1219.291^{a}

Table 13A Probability values (p-value) for the effect of canopy treatment, yeast used as well as the timepoint sampled given separately for **2012** ester production. The interaction between the factors canopy*yeast, canopy*timepoint, yeast*timepoint as a combined effect on the volatile aroma compounds are shown. The third level of interaction between canopy*yeast*timepoint is also shown. Values in red show factors significantly affecting the wine volatiles at a 5% significance level.

	Canopy	*Yeast*	*Timepoint*	*Canopy*Yeast*	*Canopy*Timepoint*	*Yeast*Timepoint*	*Canopy*Yeast*Timepoint*
ESTERS							
Ethyl Butyrate	0.207128	0.002252	0.000000	0.397502	0.050991	0.000011	0.021868
Ethyl Hexanoate	0.405991	0.000697	0.000000	0.370160	0.005889	0.007258	0.001889
Ethyl Lactate	0.957593	0.031943	0.000000	0.434944	0.006565	0.204300	0.028376
Ethyl caprylate	0.871356	0.084930	0.000000	0.834856	0.088741	0.023134	0.004059
Ethyl caprate	0.347626	0.007727	0.000000	0.622177	0.036250	0.001123	0.008832
Diethyl succinate	0.027279	0.005641	0.026376	0.033909	0.112022	0.009418	0.355538
Ethyl Acetate	0.451319	0.000083	0.000000	0.004922	0.045697	0.021204	0.010740
Isoamyl Acetate	0.049401	0.000028	0.000000	0.034255	0.000169	0.027245	0.025437
Hexyl Acetate	0.923889	0.072480	0.000002	0.292809	0.042056	0.055006	0.001587
2-Phenylethyl Acetate	0.631339	0.638061	0.001107	0.641016	0.231161	0.432505	0.244608
2-Methyl-propyl acetate	0.038119	0.018086	0.000000	0.216446	0.009321	0.008124	0.112764
Ethyl-3-hydroxybutanoate	0.063814	0.249090	0.000023	0.351093	0.059286	0.046375	0.086934
Ethyl phenylacetate	0.160027	0.000068	0.000000	0.164732	0.201665	0.020799	0.020597

Table 14A Probability values (p-value) for the effect of canopy treatment, yeast used as well as the timepoint sampled given separately for **2012** higher alcohol and fatty acid production. The interaction between the factors canopy*yeast, canopy*timepoint, yeast*timepoint as a combined effect on the volatile aroma compounds are shown. The third level of interaction between canopy*yeast*timepoint is also shown. Values in red show factors significantly affecting the wine volatiles at a 5% significance level.

	Canopy	*Yeast*	*Timepoint*	*Canopy*Yeast*	*Canopy*Timepoint*	*Yeast*Timepoint*	*Canopy*Yeast*Timepoint*
HIGHER ALCOHOLS							
Methanol	0.026470	0.020689	0.000000	0.296877	0.043510	0.069341	0.075808
Propanol	0.000423	0.000010	0.000000	0.006334	0.006474	0.000026	0.727840
Isobutanol	0.008726	0.003354	0.000000	0.484877	0.120197	0.000314	0.938097
Butanol	0.088516	0.000000	0.000000	0.350200	0.027493	0.000000	0.055723
Isoamyl alcohol	0.078020	0.008174	0.000000	0.043590	0.345862	0.006729	0.085653
Hexanol	0.257524	0.101148	0.988945	0.550252	0.418998	0.264508	0.348301
2-Phenylethanol	0.000585	0.333225	0.000000	0.009073	0.151622	0.007825	0.031391
4-Methyl-1-pentanol	0.038119	0.018086	0.000000	0.216446	0.009321	0.008124	0.112764
3-Methyl-1-pentanol	0.320537	0.193429	0.000155	0.503695	0.388814	0.300570	0.286682
3-ethoxy-1-propanol	0.141413	0.000000	0.000000	0.228907	0.979107	0.000000	0.859498
Pentanol	0.949863	0.041375	0.000072	0.673237	0.949962	0.013566	0.947413
FATTY ACIDS							
Acetic Acid	0.931138	0.224867	0.000992	0.025123	0.094145	0.003037	0.740777
Propionic Acid	0.005808	0.183393	0.518630	0.034411	0.339745	0.296373	0.330376
Isobutyric Acid	0.000490	0.007217	0.008674	0.002445	0.359531	0.262786	0.280156
Butyric acid	0.254532	0.067249	0.000000	0.154807	0.430679	0.091233	0.326047
isovaleric acid	0.147060	0.055602	0.000000	0.016564	0.544812	0.115797	0.431147
Valeric Acid	0.667643	0.006507	0.368898	0.674790	0.711291	0.573792	0.723329
Hexanoic Acid	0.960645	0.000406	0.000000	0.897334	0.041966	0.000376	0.050661
Octanoic Acid	0.826684	0.000094	0.000000	0.109222	0.026937	0.000912	0.047724
Decanoic Acid	0.297628	0.045503	0.000067	0.723646	0.735927	0.169264	0.191761

Table 15A Shows the concentrations (mg/L) for esters, higher alcohol and fatty acids that are significant on a third order interaction (Canopy*yeast*timepoint) level for aroma compounds analysed for **2012** harvest. The concentrations represent the means of triplicate treatments, each done in duplicate by GC-FID. Standard deviations are not shown. Different letters within a row denote significant differences at $p < 0.05$.

	SHADED								**EXPOSED**							
	Cross Evolution				*DV10*				*Cross Evolution*				*DV10*			
	1/4	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**	**1/4**	**1/2**	**3/4**	**4/4**
ESTERS																
Ethyl Butyrate	0.2489^{e}	0.2061^{ef}	0.3827^{d}	0.6403^{c}	0.0493^{g}	0.2149^{ef}	0.5547^{c}	0.9247^{a}	0.0779^{g}	0.2163^{ef}	0.4157^{d}	0.5967^{c}	0.1132^{gf}	0.2259^{e}	0.6052^{c}	0.7631^{b}
Ethyl Hexanoate	1.0701^{gh}	1.5075^{f}	1.9239^{de}	1.9955^{dec}	0.7388^{h}	2.6514^{a}	2.2624^{db}	2.4297^{ab}	0.8116^{gh}	2.4141^{ab}	1.8789^{e}	1.8532^{ef}	1.1086^{g}	2.6216^{ab}	2.2947^{abc}	2.0392^{dec}
Ethyl Lactate	0.7066^{c}	0.000^{d}	0.5294^{c}	2.9258^{b}	0.000^{d}	0.000^{d}	0.4863^{c}	2.9221^{b}	0.000^{d}	0.000^{d}	0.5064^{c}	3.0712^{a}	0.000^{d}	0.1504^{d}	0.5563^{c}	3.2617^{ab}
Ethyl Caprylate	0.9715^{ef}	0.9552^{ef}	1.2725^{ed}	2.8137^{ab}	0.3887^{g}	1.7529^{c}	1.5208^{cd}	3.1531^{a}	0.4907^{g}	1.4899^{cd}	1.3203^{ce}	2.729^{ab}	0.7536^{gf}	1.6426^{cd}	1.6227^{cd}	2.6559^{b}
Ethyl caprate	2.5244^{ef}	1.6874^{f}	2.2187^{f}	15.8173^{b}	1.1777^{f}	7.9277^{c}	2.6215^{ef}	18.0460^{a}	1.2147^{f}	4.6636^{ed}	1.7490^{f}	13.7172^{b}	2.0021^{f}	6.2613^{cd}	2.9359^{ef}	15.7625^{b}
Ethyl Acetate	46.7638^{de}	51.1198^{d}	78.6816^{c}	107.0100^{a}	18.4260^{i}	27.0927^{h}	72.6538^{c}	99.5468^{ab}	40.2425^{fe}	35.6543^{fg}	78.351^{c}	101.9753^{ab}	39.105^{fg}	31.0495^{hg}	72.4517^{c}	96.7617^{b}
Isoamyl Acetate	2.8067^{g}	6.0977^{f}	10.3521^{bc}	12.2305^{a}	1.3998^{h}	5.5554^{f}	9.4707^{dc}	10.7104^{b}	2.1394^{gh}	7.5727^{e}	10.8084^{b}	11.1382^{b}	1.8453^{gh}	5.3322^{f}	8.7770^{d}	7.6271^{e}
Hexyl Acetate	0.8378^{dcef}	0.7857^{defg}	0.7796^{defg}	0.900^{abc}	0.7078^{hg}	0.9979^{ab}	0.6635^{h}	0.8490^{dce}	0.8156^{dcefg}	1.0126^{db}	0.7449^{be}	0.8232^{dcefg}	0.7403^{be}	0.8917^{db}	0.7434^{he}	0.7321^{hf}
Ethyl phenylacetate	0.9294^{g}	0.9902^{gf}	1.7370^{ce}	3.3513^{a}	0.1692^{h}	0.6879^{g}	1.4096^{ef}	2.7014^{b}	0.8396^{g}	1.6802^{ed}	1.8827^{cd}	3.6540^{a}	0.5784^{gh}	0.7319^{g}	1.4760^{ed}	2.1885^{c}
HIGHER ALCOHOLS																
2-Phenylethanol	4.3124^{hi}	5.5649^{h}	8.6307^{fg}	12.2405^{cd}	3.4306^{i}	7.3000^{g}	10.9041^{e}	13.7960^{ab}	4.9327^{h}	9.2334^{f}	11.0574^{ed}	14.8922^{a}	4.3515^{hi}	7.8589^{g}	11.9527^{ed}	13.4370^{cb}
FATTY ACIDS																
Octanoic acid	5.9623^{e}	8.4040^{d}	8.4183^{d}	12.7671^{cb}	7.0268^{de}	16.1100^{a}	11.4757^{c}	14.6423^{ab}	5.3002^{e}	5.3002^{c}	8.7212^{d}	12.2506^{c}	7.4378^{de}	15.5044^{a}	12.3312^{c}	11.5780^{c}

Table 16A Shows the concentrations (mg/L) of esters, higher alcohol and fatty acids that are significant on a second order interaction (Canopy*Yeast) level for aroma compounds analysed for **2012** harvest. The concentrations represent the means of triplicate treatments, each done in duplicate by GC-FID. Standard errors are not shown. Different letters within a row denote significant differences at $p < 0.05$.

	SHADED		**EXPOSED**	
YEAST STRAIN	***Cross Evolution***	***DV10***	***Cross Evolution***	***DV10***
ESTERS				
Diethyl succinate	0.4399^{a}	0.1629^{b}	0.2091^{b}	0.1567^{b}
Ethyl acetate	70.894^{a}	54.430^{b}	64.056^{b}	58.932^{c}
Isoamyl acetate	7.872^{a}	6.7874^{b}	7.915^{b}	5.900^{c}
HIGHER ALCOHOLS				
Propanol	45.6973^{c}	53.4573^{b}	48.36470^{c}	65.4625^{a}
Isoamyl alcohol	95.8618^{b}	112.1854^{a}	108.1039^{a}	111.1493^{a}
2-phenylethanol	7.687^{c}	8.858^{b}	10.029^{a}	9.400^{ab}
FATTY ACIDS				
Acetic acid	326.0488^{b}	381.1015^{a}	362.0746^{ab}	342.6595^{ab}
Propionic acid	1.5324^{b}	2.0791^{a}	2.3894^{a}	2.2406^{a}
Isobutyric acid	0.9223^{b}	0.9506^{b}	1.2891^{a}	0.9979^{b}
Isovaleric acid	0.6006^{b}	0.7001^{a}	0.6881^{a}	0.6733^{a}

Table 17A Probability values (p-value) for the effect of canopy treatment and time point sampled given for esters for the **2012 panel resolution** wines. The interaction between the factors canopy and timepoint separately on the volatile aroma compounds are shown. The second level of interaction between canopy* timepoint is also shown. Values in red show factors significantly affecting the wine volatiles at a 5% significance level

	Canopy	*Timepoint*	*Canopy*Timepoint*
ESTERS			
Ethyl Butyrate	0.515103	0.000000	0.076570
Ethyl Hexanoate	0.840882	0.992365	0.459157
Ethyl Lactate	0.100429	0.000000	0.806252
Ethyl Caprylate	0.327233	0.000000	0.169078
Ethyl caprate	0.392277	0.000000	0.021681
Diethyl succinate	0.031333	0.000758	0.043572
Ethyl Acetate	0.002230	0.000000	0.014825
Isoamyl Acetate	0.051896	0.000139	0.061627
Hexyl Acetate	0.080059	0.003395	0.884826
2-Phenylethyl Acetate	0.028430	0.000000	0.733462
2-Methyl-propyl acetate	0.002248	0.000000	0.069956
Ethyl-3-hydroxybutanoate	0.000000	0.000000	0.000000
Ethyl phenylacetate	0.000312	0.000000	0.377545

Table 18A Probability values (p-value) for the effect of canopy treatment and time point sampled given for higher alcohols and fatty acids for the **2012 panel resolution** wines. The interaction between the factors canopy and timepoint separately on the volatile aroma compounds are shown. The second level of interaction between canopy* timepoint is also shown. Values in red show factors significantly affecting the wine volatiles at a 5% significance level

	Canopy	*Timepoint*	*Canopy*Timepoint*
HIGHER ALCOHOLS			
Methanol	0.005305	0.000000	0.052321
Propanol	0.003727	0.000000	0.139270
Isobutanol	0.000438	0.000000	0.812861
Butanol	0.066201	0.000000	0.148273
Isoamyl Alcohol	0.018337	0.000000	0.259235
Hexanol	0.638876	0.000000	0.275090
2-Phenylethanol	0.006079	0.000000	0.101549
4-Methyl-1-pentanol	0.443740	0.000616	0.443740
3-Methyl-1-pentanol	0.442350	0.000000	0.442350
3-ethoxy-1-propanol	0.147949	0.000000	0.139652
Pentanol	0.000012	0.000000	0.000012
FATTY ACIDS			
Acetic Acid	0.435282	0.000004	0.749181
Propionic Acid	0.029464	0.018022	0.122034
Isobutyric Acid	0.000347	0.164509	0.874302
Butyric acid	0.344545	0.000002	0.085183
isovaleric acid	0.000555	0.000000	0.961745
Valeric Acid	0.868107	0.007354	0.678715
Hexanoic Acid	0.615868	0.000000	0.070834
Octanoic Acid	0.819654	0.000008	0.293205
Decanoic Acid	0.035198	0.000000	0.022315

Table 19A Shows the concentrations (mg/L) of esters, higher alcohols and fatty acids that are significant on a second order interaction (Canopy*timepoint) level for aroma compounds analysed for **2012** panel resolution. The concentrations represent the means of triplicate treatments, each done in duplicate by GC-FID. Standard errors are not shown. Different letters within a row denote significant differences at $p < 0.05$.

Canopy	***SHADED***		***EXPOSED***	
Timepoint	**1/2**	**4/4**	**1/2**	**4/4**
ESTERS				
Ethyl caprate	2.6244^{b}	13.2725^{a}	3.2123^{b}	10.8636^{a}
Diethyl succinate	0.2467^{b}	0.1604^{b}	0.4546^{a}	0.1749^{b}
Ethyl acetate	65.1492^{b}	103.2345^{a}	45.5865^{c}	96.2041^{a}
Ethyl-3-hydroxybutanoate	27.8126^{c}	64.7745^{a}	34.8114^{b}	24.4358^{c}
HIGHER ALCOHOLS				
Pentanol	0.0000^{c}	0.1193^{a}	0.0000^{c}	0.0973^{b}
FATTY ACIDS				
Decanoic acid	1.5910^{c}	6.7082^{a}	1.5570^{c}	5.7528^{b}

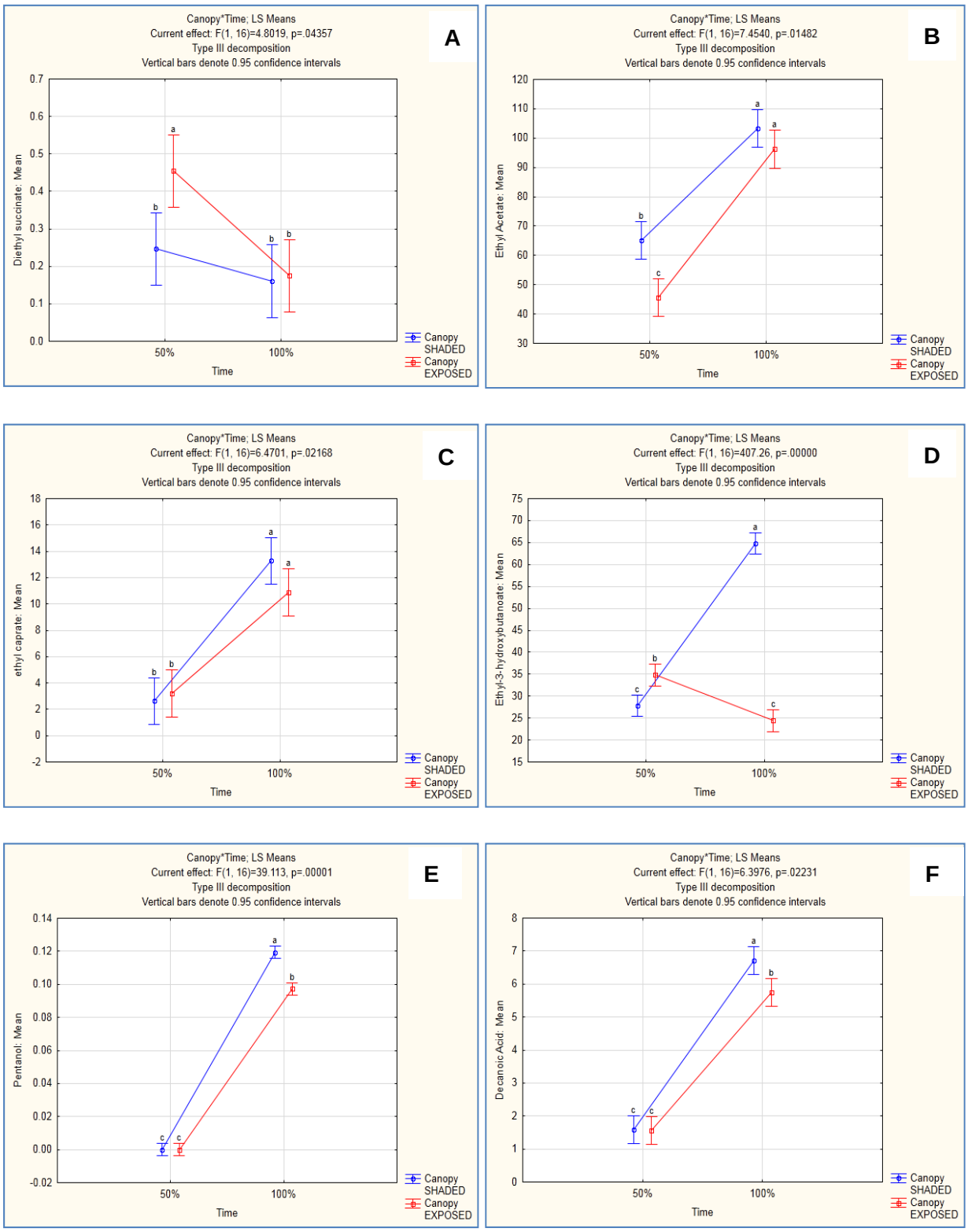

Figure 1A shows the second order of interaction for canopy*timepoint for the compounds diethyl succinate (A), ethyl acetate (B), ethyl caprate (C), ethyl-3-hydroxybutanoate (D), pentanol (E) and decanoic acid (F).

Table 20A Shows the values of esters, higher alcohols and fatty acids that are significantly influenced by the canopy treatment for compounds analysed for 2012 panel resolution. The concentrations represent the means of triplicate treatments, each done in duplicate by GC-FID. Standard errors are not shown. Different letters within a row denote significant differences at $p < 0.05$.

Canopy	*SHADED*	*EXPOSED*
ESTERS		
Diethyl succinate	0.204[b]	0.315[a]
Ethyl acetate	84.192[a]	70.900[b]
2-Phenylethyl acetate	0.2806[a]	0.2654[b]
2-Methyl-propyl acetate	0.1983[a]	0.1879[b]
Ethyl-3-hydroxybutanoate	46.294[a]	29.624[b]
Ethyl phenylacetate	2.3403[a]	1.5234[b]
HIGHER ALCOHOLS		
Methanol	51.2615[a]	48.8777[b]
Propanol	57.1256[a]	47.0067[b]
Isobutanol	15.6922[a]	12.0937[b]
Isoamyl alcohol	117.7851[a]	99.3191[b]
2-Phenylethanol	10.3758[a]	8.3375[b]
Pentanol	0.060[a]	0.050[b]
FATTY ACIDS		
Propionic acid	1.7262[a]	1.5328[b]
Isobutyric acid	0.9464[a]	0.6565[b]
Isovaleric acid	0.5974[a]	0.4428[b]
Decanoic acid	4.150[a]	3.655[b]

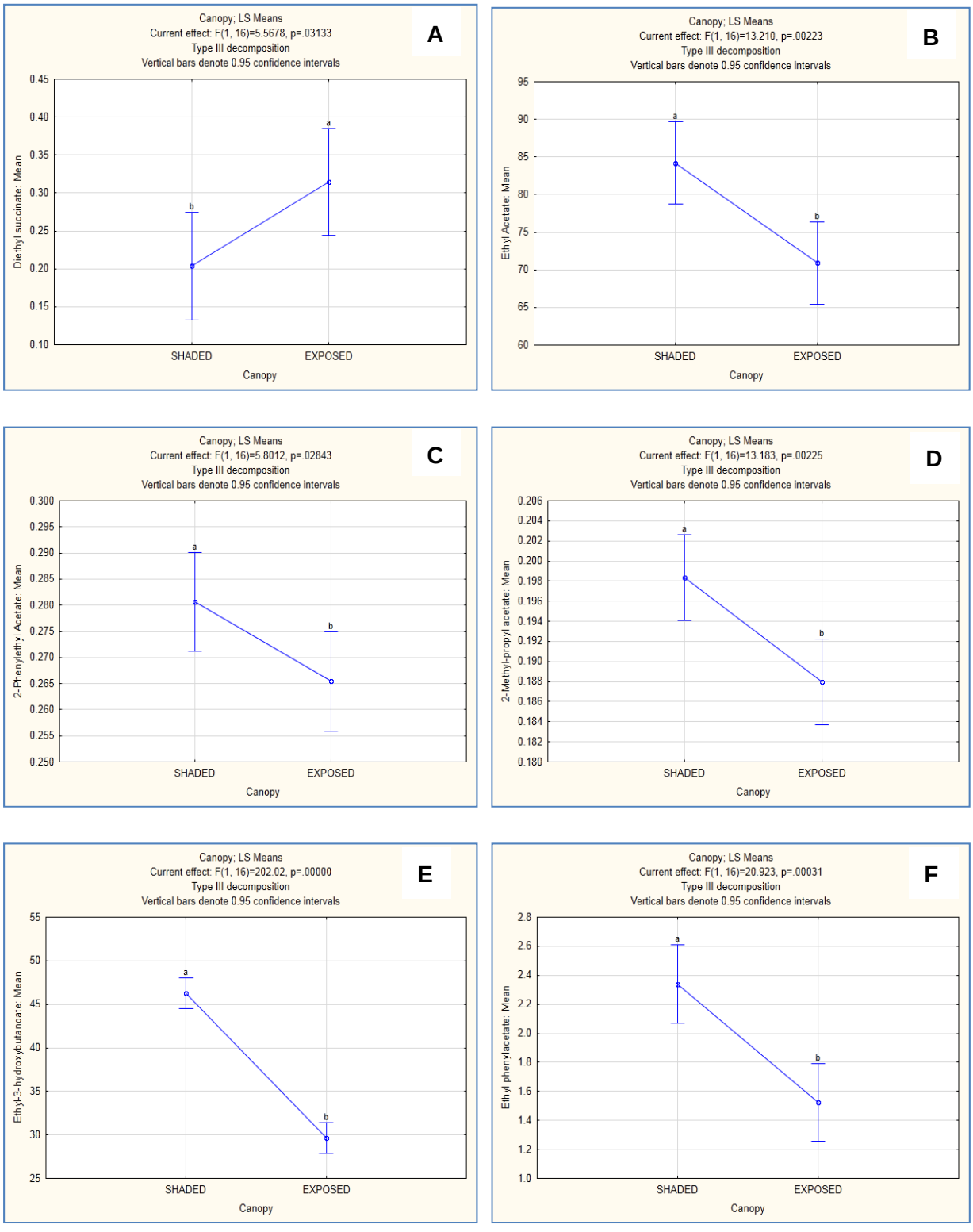

Figure 2A shows the influence of the viticulural canopy treatment for the compounds diethyl succinate (A), ethyl acetate (B), 2-phenylethyl acetate (C), 2-methyl-propyl acetate (D), ethyl-3-hydroxybutanoate (E) and ethyl phenylacetate (F).

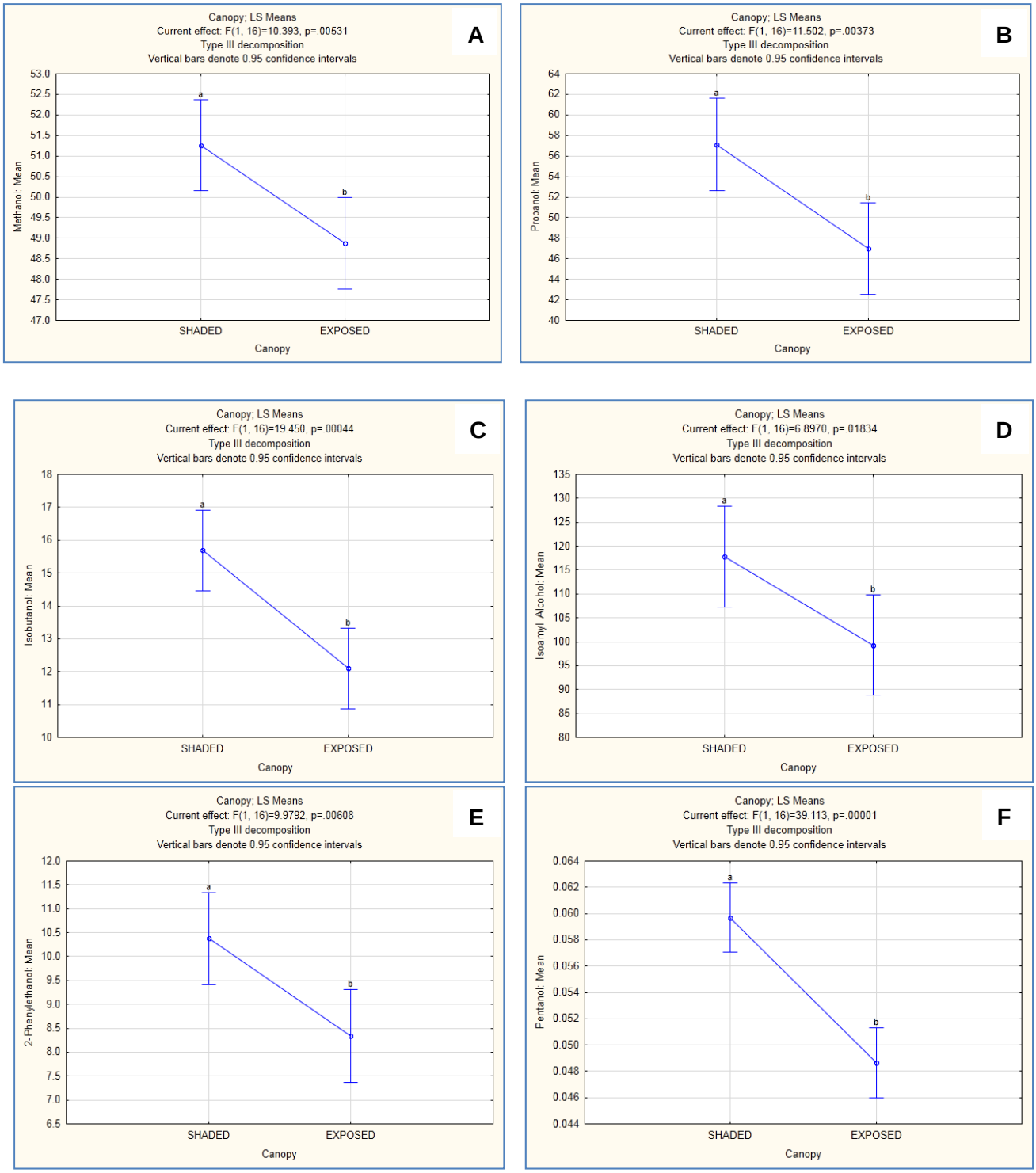

Figure 3A shows the influence of the viticulural canopy treatment for the compounds methanol (A), propanol (B), isobutanol (C), isoamyl alcohol (D), 2-phenylethanol (E) and pentanol (F).

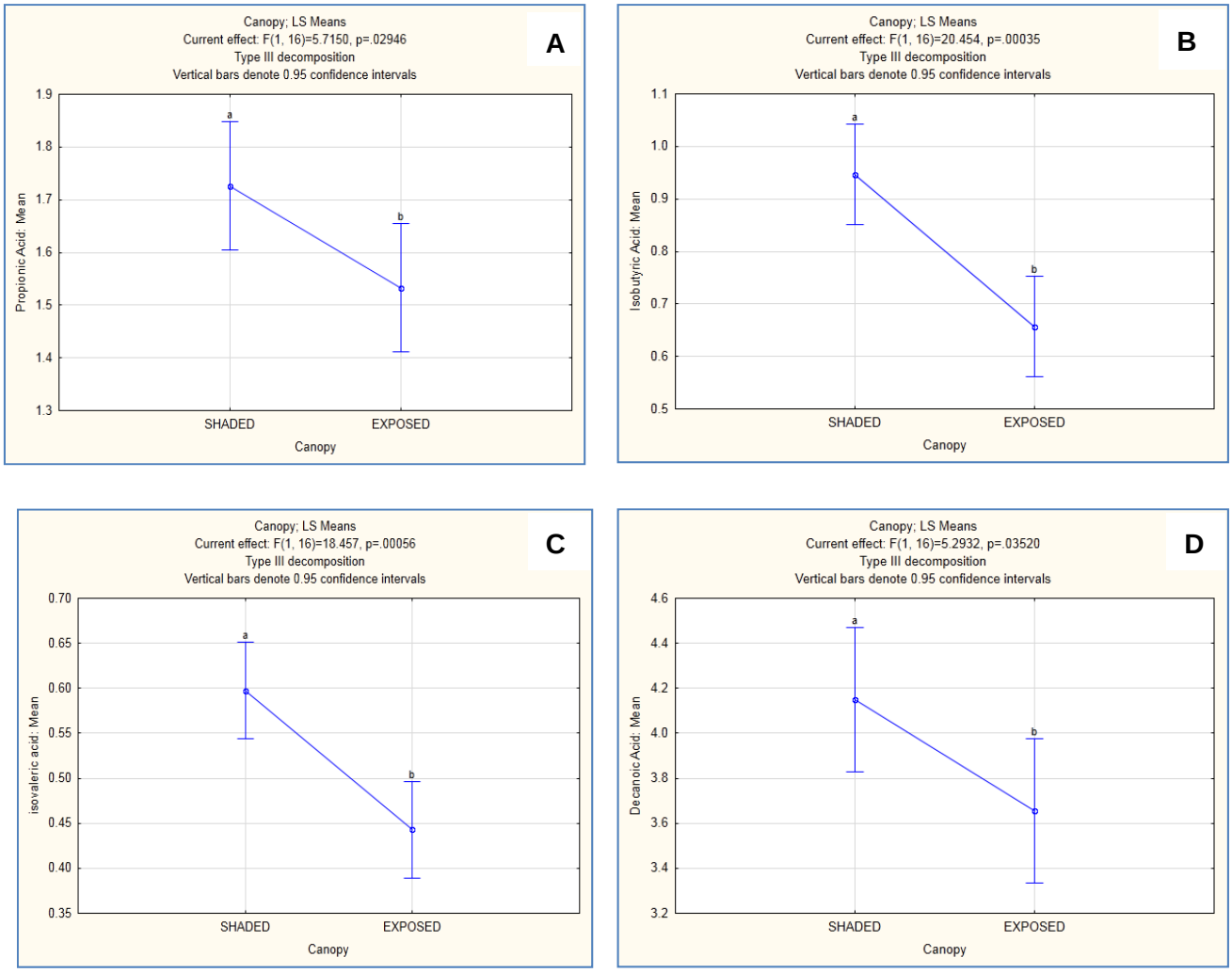

Figure 4A shows the influence of the viticulural canopy treatment for the compounds propionic acid (A), isobutyric acid (B), isovaleric acid (C) and decanoic acid (D).