Effect of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* yeasts on the phenolic content and sensory attributes of Chenin blanc wines

by
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Declaration

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

Signed **Date**

Abstract

Wines contain a number of phenolic compounds, belonging to non-flavonoid and flavonoid complexes. Phenolic compounds in wine are responsible for wine colour, astringency, and bitterness. *Saccharomyces cerevisiae* yeast is normally used in winemaking but it has been proved to decrease the phenolic content in wines. Current research on the use of non-*Saccharomyces* yeast in winemaking has produced better quality wines than *S. cerevisiae* yeast therefore improving the sensory profile of wine. This study evaluated effect of *Torulaspora delbrueckii* yeast on the phenolic content of experimental wines derived from Chenin blanc grapes.

A reversed phase high-performance liquid chromatographic (RP-HPLC) method was used for the identification and quantitation of the phenolic compounds. The difference test method was used to determine the sensory attributes of wines. The data was subjected to analysis of variance to compare treatment differences between the wines and principal component analysis to establish possible correlation between the data sets. Furthermore, a gas chromatographic-flame ionization detection method (GC-FID) was used for the quantification of volatile compounds in the wines.

In this work, wines made with *T. delbrueckii* strain M2/1 had high concentration of (+)-catechin, caffeic acid, ferulic acid and *p*-coumaric acid in all studied vintages. Wines made with VIN13 had higher concentrations of flavan-3-ols, compared to wines made with M2/1 and 654. In sensory evaluation, M2/1 wines were prominent in astringency and complexity. Yeast strain M2/1, also attributed to body and complexity of the wine. However, in this study no correlations were observed between the phenolic content and sensory attributes and *vice versa*. The quality of wine cannot be concluded by chemical or sensory analysis alone, but the data sets are complementary.

Although the phenolic concentration of wines made with *S. cerevisiae* strain (VIN 13) and *T. delbrueckii* (M2/1) were similar in measured phenolic concentrations, they had different sensory attributes. Wines made during the 2013 vintage indicated the importance of the use of a strain with higher enzyme activity and high fermentation rate. There is minimal to no skin contact in white winemaking. Therefore, the use of a yeast strain with an increased enzyme activity can facilitate the extraction of phenolics from grape, resulting in wine with improved quality.

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To God be the glory, great things he has done

Dedication

I would like to dedicate this thesis to my parents who encouraged and supported me every step of the way, Ndithi maz' anethole.

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Glossary of terms

ssp.:	species
ca:	approximately
°B:	degree in Brix
°C:	degree Celsius
"*terroir*":	French term which refers to climate, soil and location
véraison:	The period when the grape berry skin colour changes from green to black. In this period the grapes become soft and take on the colours characteristic of the specific grape cultivar
Vitis vinifera:	Botanical/scientific name for grapes which wine is made from
Grape must:	Freshly pressed grape juice
Vinification:	The process of turning grape juice into win

Chapter I

Introduction to the study

1. Introduction

This study forms part of an existing project (Agricultural Research Council, chemical profiling of non-*Saccharomyces* wines) where wines were produced with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* yeasts (as the reference yeast). In the production process of wine, the alcohol fermentation process is important to the final resulting wine, as it produces secondary metabolites that enhance the sensory profile of wine (Swiegers *et al*., 2005). Therefore, the choice of a yeast strain that converts grape sugars to alcohol is important because it has an effect on the chemical composition, sensory properties and the quality of the wine (Swiegers *et al*., 2005; Ciani & Maccarelli, 1998).

Saccharomyces cerevisiae yeast is normally used for the production of commercial wine. However, it is known that this yeast decreases the concentration of phenolic in wine (Caridi *et al*., 2004), via the adsorption on the yeast cell wall. Therefore, the phenolic content of wine will decrease during the racking process when yeast is removed from resultant wine. The latest technology of using non-*Saccharomyces* yeasts for wine production (Jolly *et al*., 2014; Ciani *et al*., 2009; Renault *et al*., 2009) adds another variable to the investigation of non–*Saccharomyces* yeast in winemaking.

Phenolic compounds which include anthocyanins, flavonols, flavan-3-ols and phenolic acids are a part of the genetic makeup of *Vitis vinifera* spp. among other plants and are extracted from the grape must to the wine through the vinification process (Braidot *et al*., 2008). The presence phenolic compounds vary from cultivar to cultivar, e.g. anthocyanins are not present in white grapes. Phenolic concentration evolves as the grape berry ripens and matures (Alonso-Borbalán *et al*., 2003; Kennedy *et al*., 2000). The low content of phenolic compounds in white wine is a result of the white wine vinification process. Therefore, the phenolic content of wine correlates to the overall quality of wine because phenolics contribute to the astringency, bitterness, colour and mouth-feel of the wine (Kennedy, 2008; Lesschaeve & Noble, 2005).

1.1. Research statement

The purpose of this study is to identify and quantify the phenolic compounds affected by *T. delbrueckii* yeast during production and to study their sensory attributes in Chenin blanc wines.

1.2. Background to the research study

The wine industry in South Africa is an important agricultural entity as it contributes to the economy of the country through the buying and selling of products, offering employment opportunities and contributing to the tourism industry. Ongoing improvement of wine quality is imperative to the industry, as the success of the wine industry relies on the quality of wine. This includes microbiological aspect (yeast choice and performance), and chemical and sensory profiling. The yeast used to inoculate grape must can have an effect on the phenolic (flavan-3-ols, flavonols and phenolic acids) concentrations of the final wine (Tataridis *et al*., 2013). Grape must is normally inoculated with *S. cerevisiae* yeast in commercial winemaking. However, results from a reviewed article has shown that *S. cerevisiae* wine yeasts can cause a decrease on the flavonol and flavan-3-ol concentration of wine (Caridi *et al*., 2004). These compounds contribute to the sensory characteristics of wine particularly colour and astringency.

There has been an increasing research on the use of non-*Saccharomyces* yeasts in winemaking (Ciani *et al*., 2010; Jolly *et al*., 2006). This yeast species is dominant in the grape skin but decrease during spontaneous fermentation (Jolly *et al*., 2003b). It has been shown that the use of natural yeast in winemaking can produce wine with improved sensory properties (Ciani *et al*., 2010) but they are often responsible for stuck fermentation. The ARC Infruitec-Nietvoorbij, generates a large number of experimental wines, especially those derived from *T. delbrueckii* yeasts. These wines are currently subjected to superficial chemical analyses only, i.e. standard chemical analysis and volatile aroma compound analysis. The results arising from these analyses limit the conclusions that can be made regarding the overall quality of the wine. Sensory evaluation has shown that there are differences in aroma and mouth feel attributes among the wines. However, results from sensory evaluation alone cannot explain the differences observed. Therefore, the quantification of phenolic compounds could explain the differences observed between wines.

1.3. Aim and objectives

It has been reported that *T. delbrueckii* yeast produces improved quality wines compared to wines produced with *S. cerevisiae* (Jolly *et al*., 2014; Van Breda *et al*., 2012). The contribution of *T. delbrueckii* yeast to the increased wine quality is still undetermined. This study aimed to use a liquid chromatographic technique (HPLC) to evaluate the phenolic compound concentration differences in Chenin blanc wines produced by *T. delbrueckii* 654 and M2/1 strains and *S. cerevisiae* yeast strain VIN 13 (as the reference yeast). The following objectives are set to accomplish the aims of this study:

- To identify and quantify the phenolic compounds i.e. flavonols and flavan-3-ols affected by *T. delbrueckii* strains in Chenin blanc wines.
- To compare the sensory attributes and phenolic compound concentration of wines made with *T. delbrueckii* yeast strains and *S. cerevisiae* yeast strain using multivariate statistical analysis.
- To correlate the chemical and sensory data.

1.4. Hypothesis and assumptions

The proposed project hypothesis is that the use of non-*Saccharomyces* yeast improves the sensory profile of wine. The cell wall of the selected yeast can decrease the phenolic content in the wine by adsorbing the phenolic compounds.

1.5. Delimitations

To set the research borders the delimitation are as follows:

- Determination of the phenolic content and sensory evaluation will be done on wines that were produced during 2011, 2012 and 2013 vintages. The project does not include the winemaking process.
- Analysis will only be done on Chenin blanc wines.
- The study will only be focusing on flavan-3-ols, flavonols and phenolic acids in Chenin blanc wine.

Chapter II

Reviewed Literature

2. Literature review

2.1. Winemaking process

The winemaking process for red and white wine is different. Once the grapes for white wine production pressed, the juice is allowed to settle without skin contact. During this stage, any solids that are in the juice after pressing will gradually settle at the bottom of the tank resulting in a clarified grape juice. The clarified juice (supernatant) is transferred to another tank for fermentation. This process, in which the clarified juice is removed from the solids is known as racking. The clarified juice is then inoculated with yeast. During the process of fermentation, the yeast converts the grape sugars into carbon dioxide, heat and alcohol. Yeast by-products are also formed during fermentation. The fermentation process for white wine is done over a longer period of time than that of red wine production. The longer fermentation time is required to keep the fermenting juice at a low temperature (10-15°C) in order to prevent the juice from spoiling. White grape juice lacks the natural preservatives which is found in the grape skin and therefore the juice is more prone to oxidation and browning. Keeping the juice at a low temperature, prevents spoilage, but keeps the activity of the yeasts low. The fermentation process will therefore take longer (Ribéreau-Gayon *et al*., 2006). When fermentation is complete, the wine is racked off the yeast lees and then cold stabilised at 0°C for at least two weeks. Figure 2.1 illustrates the winemaking process of red and white wine.

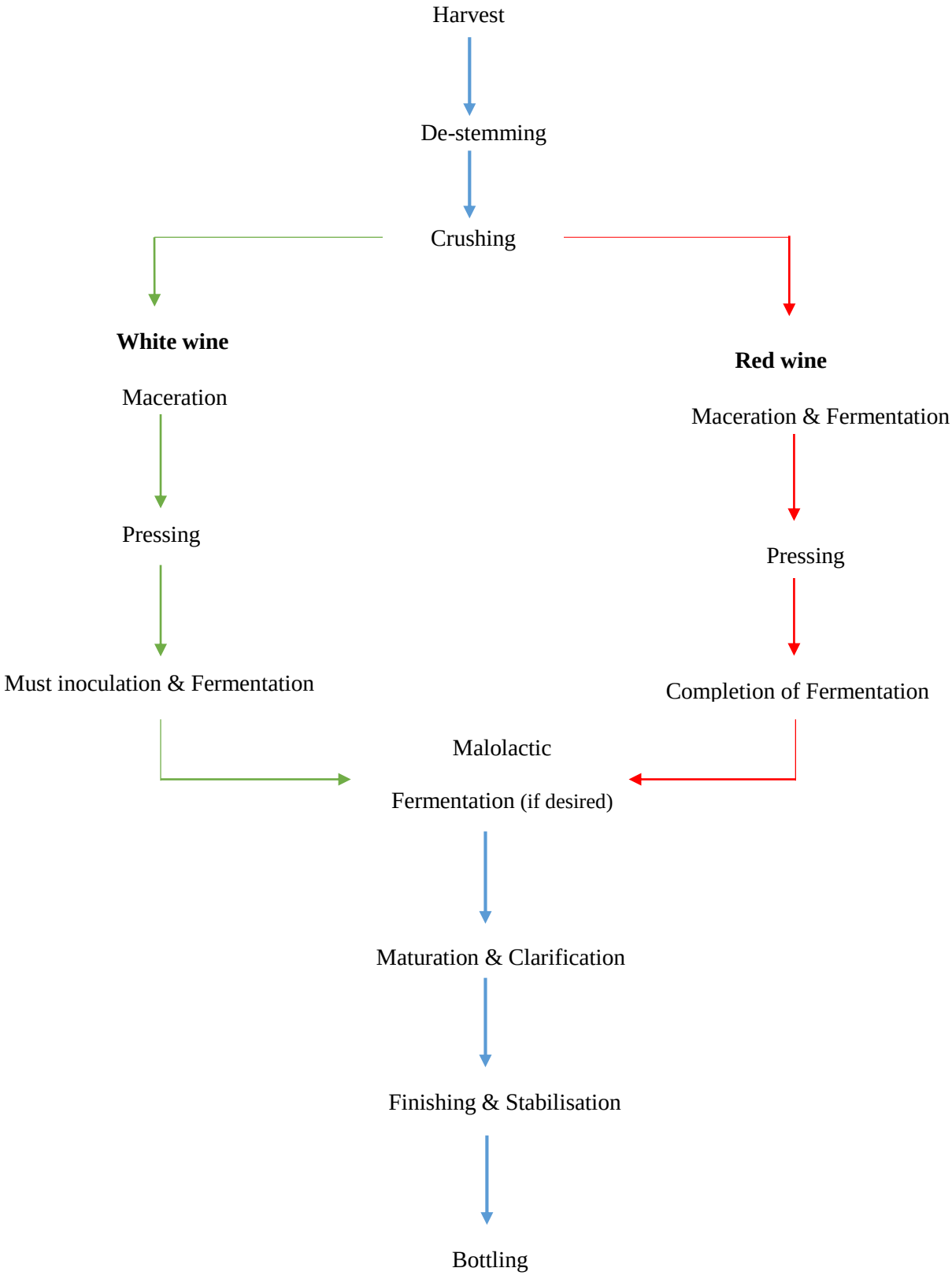


Figure 2.1. Flow diagram for red and white winemaking (ARC standard protocol for winemaking)

2.2. South Africa Chenin blanc wine

Chenin blanc grapes are economically the most important wine grape cultivar grown in South Africa and comprise approximately 26% of all planted grape vines (Floris, 2011). Chenin blanc was thought of as a "workhorse cultivar" in South Africa and was mainly used for the production of brandy and bulk wine blends (Chenin blanc Association, 2010). It was subsequently discovered to be a versatile grape cultivar, capable of being used in the production of high quality wines of many different styles, including noble late harvest, sparkling wines, dry white wines, sherries and brandies (Potashinik & Winkler, 2013; Marais, 2003).

The "Chenin blanc association" has conducted research on Chenin blanc in South Africa and has made an effort to improve the quality of Chenin blanc wines. South Africa is internationally recognised as an emerging producer of world class Chenin blanc wines (Fridjhon, 2006). A review of the published scientific research on Chenin blanc wines, showed that the chemical profiling of Chenin blanc wine has focused mostly on the volatile composition (De Kock, 2015; Lawrence, 2012). However, limited information is available on the composition of phenolic compounds found in Chenin blanc wines.

It is important for a wine producing country to produce wines with improved quality, in order to continue increasing its market share abroad. It has therefore become a matter of importance to chemical profile Chenin blanc wines. Most chemical profiling studies on Chenin blanc wines focused on chemical compounds present in wines made with the *S. cerevisiae* yeast. De Beer *et al*. (2005), compared the changes of phenolic compositions in experimental wines made from Chenin blanc and Chardonnay during bottle ageing. The study showed that there were no significant differences in the flavan-3-ol and flavonol content of Chenin blanc wines between 0 and 12 months of bottle ageing. This observation however, was different for Chardonnay, e.g. Chardonnay, due to cultivar related differences. A simultaneous inoculation of Chenin blanc must with *Candida pulcherrima* and *S. cerevisiae* were investigated by Jolly *et al*. 2003b. Jolly and co-workers showed that the simultaneous inoculation of Chenin blanc must produce wines with improved overall quality, compared to wines made with *S. cerevisiae* only. This means that the selection of a yeast strain able to complete fermentation, can improve the wine quality. A subsequent study was conducted where forty-four *T. delbrueckii* yeast

strains were used in the production of Chenin blanc wines (van Breda *et al*., 2011). The purpose of the investigation was to identify a strain(s) with the potential to produce wines with improved quality. Seven out of the forty-four yeast strains investigated, (206, 301, 654, 704, M2/1, M2/27 and M2/15) had the potential to complete fermentation on their own and/or in a combination of inoculation. However, strain 654 and M2/1 showed greater potential with regards to fermentation rate and metabolites produced, compared to other strains.

2.3. Wine microbiology

The winemaking process starts at the vineyard, continues throughout fermentation and maturation. The final product is therefore affected by various aspects such as the viticultural and oenological practices (Garrido & Borges, 2013). The choice of yeast strain for grape must inoculation plays an important role in the formation of the yeast derived compounds by producing and excreting metabolites during its growth and yeast cell autolysis (Kennedy, 2008).

The role of the yeast in winemaking has been studied since 1866, when Louis Pasteur first explained the bio-conversion of grape to wine. However, up to date there are still many areas, e.g. production of extracellular metabolites, that are not fully understood (Pretorius, 2000), especially those of non-*Saccharomyces* yeast strains. The Old World countries e.g. European countries have observed that non-*Saccharomyces* yeasts give the wine the authentic character because non-*Saccharomyces* yeasts are naturally present on the grape skin surfaces (Renault *et al*., 2009). It is with this reason, that non-*Saccharomyces* yeast is receiving a lot of attention in both the Old and New world winemaking countries.

2.4. Yeast classification

The classification of yeast is based on the sexuality of the yeasts. The teleomorphic is the sexual state that produces ascospores and anamorphic is the asexual type that does not produce ascospores (Jolly *et al*., 2014). *Saccharomyces* and non-*Saccharomyces* yeasts are habitual terms used by wine microbiologist when referring to the genus with different species.

In the past decades it was generally accepted that all non-*Saccharomyces* yeasts became inactive and died after the start of alcoholic fermentation due to the increasing ethanol concentration and added SO_2, for which non-*Saccharomyces* yeast are sensitive to. Non-*Saccharomyces* yeasts were also considered as spoilage yeast since they were thought to produce negative traits (Tataridis *et al*., 2013; Jolly *et al*., 2006).

2.4.1. *Torulaspora delbrueckii*

Torulaspora delbrueckii yeasts are classified among Crab-tree negative and/or Crab-tree positive organisms (Rodicio *et al*., 2009) but with lesser sensitivity to ethanol, compared to *S. cerevisiae.* The biomass production of *T. delbrueckii* yeast remains higher under limited oxygen conditions, compared to *S. cerevisiae*. This is due to their ability to withstand high concentrations of solutes (Alves-Araujo *et al*., 2007). Fermentation trials have proven that the effect of selected *T. delbrueckii* yeast strains on aroma compounds is positive, producing wines with pronounced sensory complexity and floral or fruity aroma (Tataridis *et al*., 2013; Ciani *et al*., 2009).

This yeast has been reported to have a positive effect on the taste and aroma of wine (Domizio *et al*., 2011; Ciani *et al*., 2005), and in exhibiting low production of acetaldehyde and acetone ((Pacheco *et al*., 2012) even in high-sugar must (Bely *et al.*, 2008), due to its high fermentation purity. The usage of *T. delbrueckii* under standard conditions in combination or sequential culture with *S. cerevisiae*, has been suggested as a strategy to reduce the acetic acid content of wine (Bely *et al*., 2008; Ciani *et al*., 2005).

2.5. Effect of yeasts on the phenolic content of wine

Phenolic compounds in the grape must interact with the by-product of yeast known as mannoprotein (Caridi, 2007). These mannoproteins can decrease the phenolic content by adsorbing them on the yeast cell wall. The concentration of phenolic compounds in the wine can be affected by the yeast adsorption activity (Domizio *et al*., 2014). Therefore, it can be expected that the fermentation derived compounds may have an effect on the mouth-feel character of wine.

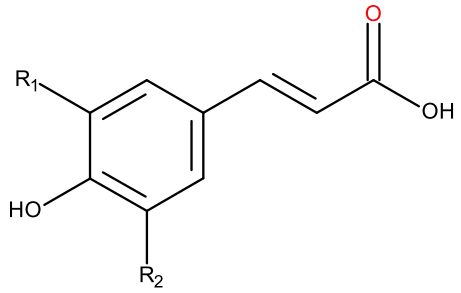
The above mentioned observations were derived from investigations carried out using the conventional *S. cerevisiae* yeast for winemaking. Limited information is available on the effect of *T. delbrueckii* yeast on the phenolic compound concentrations in Chenin blanc wines.

2.6. Phenolic compounds in white wine

The phenolic composition of wine depends on the grape cultivar. The winemaking processes determine the degree of extraction of phenolics from the grape must and subsequent reaction evolutions of phenolics and reactions with other compounds. Flavonoid compounds are responsible for wine quality such as wine colour (Harbertson, 2006), astringency, mouth-feel, bitterness and wine stability (Kennedy, 2008; Minussi *et al*., 2003). The phenolic content of white grape cultivars differs from red grape cultivars (Ribéreau-Gayon *et al*., 2006). White wine is composed of phenolic compounds found in the flesh of the grape such as hydroxycinnamic acids, hydroxybenzoic acids, flavan-3-ols, and flavonols. Red wine is mostly composed of anthocyanins, flavan-3-ols and tannins, which are present in grape skin and grape seed in addition to the phenolics found in white wine (Mattivi *et al*., 2006). This research will only focus on the phenolics in white wine.

2.6.1. Hydroxycinnamic acids

Hydroxycinnamates are the representative class of phenolic acids found in grapes and wine. The most referenced compounds are caffeic-, *p*-coumaric-, ferulic-, and sinapic acids as shown in figure 2.2 (Garrido & Borges, 2013). These acids are present in wine in both the *cis*- and *trans*- forms, but the *trans*- form is more stable, and therefore more dominant ((Pozo-Bayón *et al*., 2003). Hydroxycinnamic and their tartaric esters are the main class of phenolics acids in white wines and the main class of non-flavonoid in red wines (Vanzo *et al*., 2007). These compounds are known to be partially responsible for the astringency in both grapes and wine (Kallithraka *et al*., 2009).

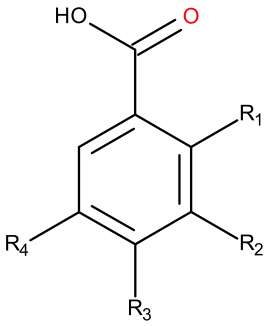


Compound name	**R_1**	**R_2**
p-Coumaric acid	H	H
Caffeic acid	OH	H
Ferulic acid	OCH_3	H

Figure 2.2. Hydroxycinnamic acids present in *Vitis vinifera* spp. (Kennedy, 2008)

2.6.2. Hydroxybenzoic acids

The most dominant hydroxybenzoic acids in white wine are gallic-, gentisic- and *p*-hydroxybenzoic acids and are mainly found as conjugated esters and glycosides in grapes (Fig. 2.3). Unlike the hydroxycinnnamic acids, the hydroxybenzoic acids are found in their free form and at low concentrations (Baderschneider & Winterhalter, 2001). Gallic acid of all the above mentioned is the most important acid in wine, because it is the precursor of all hydrolysable tannins and is present in condensed tannins.

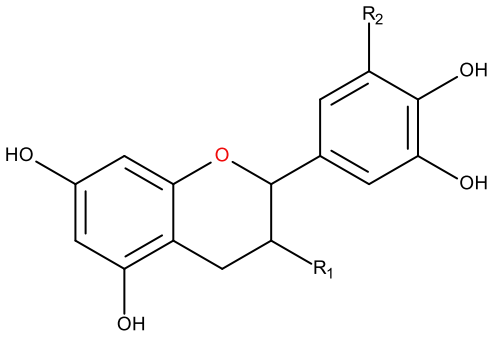


Compound name	**R_1**	**R_2**	**R_3**	**R_4**
Gallic acid	H	OH	OH	OH
p-Hydroxybenzoic acid	H	H	OH	H
Protocatechuic acid	H	OH	OH	H

Figure 2.3. Hydroxybenzoic acids present in *Vitis vinifera* spp. (Castillo-Muñoz *et al*., 2007)

2.6.3. Flavan-3-ols

Flavan-3-ol compounds were initially characterized in the 1920s in plants (Freudenberg, 1924) and later quantified in grapes and wine (Garrido & Borges, 2013; Betés-Saura *et al*., 1995; Singleton & Esau 1983). The major flavan-3-ol monomers found in grapes are formed from four sub-units, i.e. (+)-catechin, (-)-epicatechin, (-)-epigallocatechin-3-gallate and (-)-epicatechin-3-gallate as shown in figure 2.4 (Garrido & Borges, 2013; Kennedy, 2008). High concentrations of these compounds are found in the flesh of grapes. This work has also shown that flavan-3-ol monomers are produced before *véraison* as well as during fruit ripening (Ribéreau-Gayon *et al*., 2006; Rodriguez *et al*., 2006; Romeyer *et al*. 1986). Most flavan-3-ol compounds are present in the grape seed, longer extraction times are required at higher temperature. Higher alcohol concentrations lead to greater extraction of flavan-3-ols (Kennedy *et al*., 2005). These compounds are responsible for bitterness in wine and may also have some association with astringency, particularly (+)-catechin. Flavan-3-ols also have antioxidant properties and stabilizing abilities depending on the molecular structure.



Flavan-3-ol monomers	**R_1**	**R_2**
(+)-Catechin	OH	H
(-)-Epicatechin	OH	H
(-)-Epigallocatechin-3-gallate	OH	OH
(-)-Epicatechin-3-gallate	R_3	H

R_3- substituent

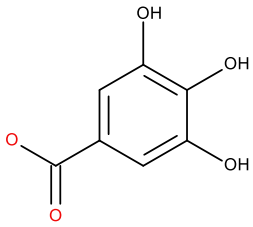
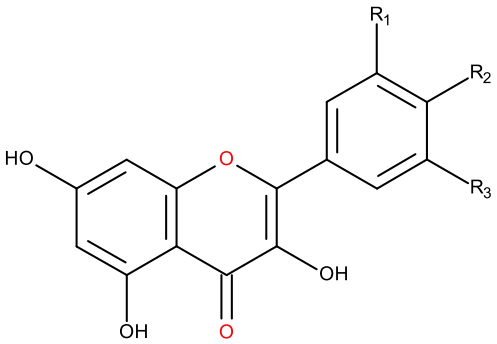


Figure 2.4. Flavan-3-ols present in *Vitis vinifera* spp. (Castillo-Muňoz *et al*., 2007)

2.6.4. Flavonols

Flavonols are present in the grape skin as flavonol glycosides and they are extracted during the pressing process of white grapes (Jeffery *et al*., 2008). Their free aglycones are produced from the hydrolysis of the glycosidic bond by enzymes or the acidic conditions of the wine. Quercetin represents the majority of the flavonol content of wine, followed by kaempferol and isorhamnetin. The flavonols myricetin, laricitrin, and syringetin are not present in all white grape cultivars because of the absence of the enzyme 3, 5-hydroxylase in certain white grape cultivars (Jeffery *et al*., 2008; Mattivi *et al*., 2006). The presence of flavonols in wine is important because of their colour and health properties, especially in white wine (Williamson & Manach, 2005).



Flavonol monomer	**R_1**	**R_2**
Kaempferol	H	H
Quercetin	H	OH
Isorhamnetin	H	OCH_3

Figure 2.5. Flavonols present in *Vitis vinifera* spp. (Castillo-Muňoz *et al*., 2007)

2.7. Sensorial contribution of flavan-3-ol and flavonol to wine quality

Flavan-3-ols and flavonols contribute to organoleptic characteristics of wines such as colour, astringency, and bitterness. There is a proportional relationship between high quality wines and the flavonoid composition of wines (Fanzone *et al*., 2012; Fernandez *et al*., 2011). During winemaking and wine aging, the flavonoid compounds interact with other wine constituents, such as mannoproteins and polysaccharides, contributing to the wine stability and improving the sensory perception of wines (Lorrain *et al*., 2013; Fanzone *et al*., 2012).

2.7.1. Effect of phenolic compounds on astringency and bitterness perception of wine

Bitterness and astringency are two important attributes of wine mouth-feel which is indicative of the wine quality (McRae & Kennedy, 2011). Bitterness is defined as a taste sensation, mostly elicited at the back of the tongue, whereas astringency, is a tactile sensation in which a drying, puckering feeling is perceived throughout the oral cavity. In wine, astringency and bitterness are produced primarily by flavonoid compounds most of which are extracted from the grape skin and seeds during fermentation (McRae & Kennedy, 2011; Kennedy *et al*., 2005).

Monomeric flavan-3-ols are bitter, but after the polymerisation of flavonoid compounds, the astringency increases more than the bitterness (McRae & Kennedy, 2011; Oberholster, 2008). The molecular conformation of flavonoids affects the sensory properties, e.g. (-)-epicatechin is more astringent and bitter than its chiral isomer (+)-catechin (Oberholster, 2008). The perception of both bitterness and astringency are also affected by wine components such as ethanol, pH, polysaccharides and acidity (Mc Rae & Kennedy, 2011; Demiglio & Pickering, 2008; Fontoin *et al*., 2008). Increasing the wines' viscosity and/or pH, decreases the intensity of astringency, whereas little or no effect on bitter taste is experienced. Increased concentrations of ethanol enhance the intensity of bitterness in wines, but has no effect on the perception of astringency (Oberholster, 2008).

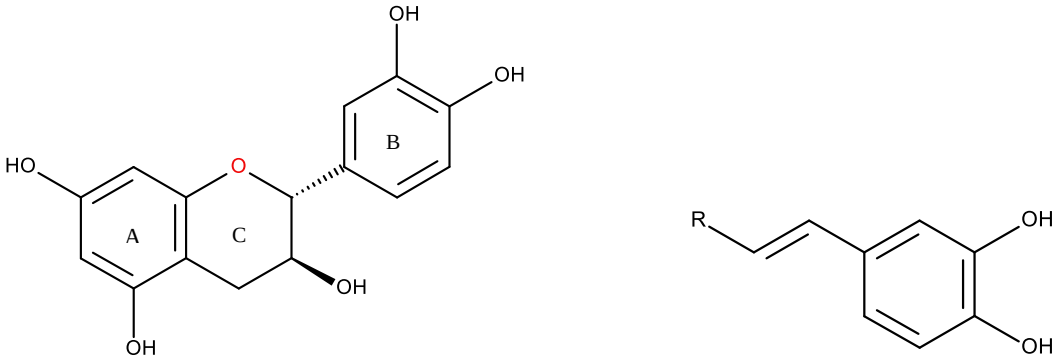
Increased astringency and/or bitterness is not always a desirable attribute in wine. However, astringency in the presence of other compounds giving rise to wine quality is desirable and the reactions leading to this improved wine are important for the wine industry.

2.7.2. Effect of phenolic compounds on the colour of white wine

The browning of white wine after bottling is a results of the oxidation of phenolics to quinones, which in turn is polymerised to form macromolecules with a typical yellow-brown hue (Scollary, 2004). The reaction starts during early stages of winemaking until the end of the ageing stages (Li *et al*., 2008). It was thought that this reaction converted hydroxycinnamic acid esters (caffeic acid) present in the wine, to the corresponding quinones (Scollary, 2004; Singleton, 1987). However, it was subsequently confirmed that there was no significant correlation between caffeic acid concentration and the potential of wine to undergo oxidative coloration. This related to flavan-3-ol content in the wine (Li *et al*., 2008; Sioumis *et al*., 2006).

Catechin is characterised by having one aromatic ring (Fig. 2.6) with a phloroglucinol functionality ring A and a second aromatic ring B with a catechol functionality (Scollary, 2004). Caffeic acid possesses only one aromatic ring, similar to the B ring of (+)-catechin, showing catechol functionality. The *ortho*-hydroxy group (Figure 2.7), in the catechin structure with catechol-type functionality, undergoes an

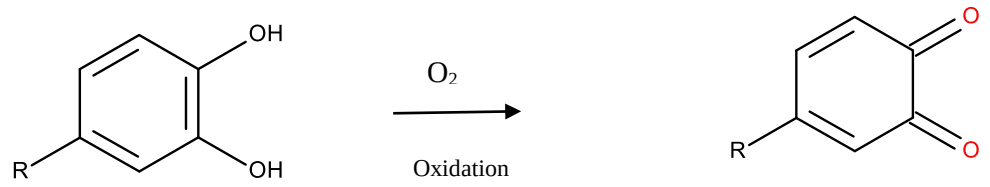
enzymatic reaction known as polyphenol oxidase producing the *ortho*-quinone compound. The *ortho*-quinone compound then reacts with other wine components to form coloured polymers.



(+)-Catechin

Caffeic acid

Figure 2.6. (+)-Catechin and caffeic acid molecular structures (Scollary, 2004)



Ortho-hydroxy phenolic

Ortho-quinone

Figure 2.7. Conversion of an *ortho*-hydroxyphenolic compound to the corresponding *ortho*-quinone (Scollary, 2004)

Polyphenol oxidase activity decreases after fermentation and oxidative browning is correlated to polyphenol chemical oxidation (Scollary, 2004) even though catechin autoxidation was shown to produce the same products as enzymatic oxidation (Li *et al*., 2008). Flavan-3-ols play an important role in the latter process and a study has shown that the process of converting flavan-3-ols into yellow xanthylium pigments produces compounds that contribute to white wine colour (Es-Safi *et al*., 2000). Therefore, the browning of white wine is a result of polyphenol oxidase and chemical oxidation of flavan-3-ols.

2.8. Flavonoid degradation

Flavan-3-ols are considered as the most unstable phenolics, regarding the non-enzymatic degradation processes (Li *et al*., 2008; Fernández-Zurbano *et al*., 1998). During fermentation flavan-3-ol compounds, i.e. catechins can undergo partial cleavage into lower molecular weight phenolic units. The process is mainly due to the increase of temperature under storage conditions (Fig. 2.8). These compounds can react with aldehydes present in the wine, which are produced by the yeast as a by-product (Lopez-Toledano *et al*., 2004) yielding coloured compounds (Fig. 2.9). In evaluating the contribution of yeast to colour change in fermented beverages, the condensation reaction between catechin and acetaldehyde was studied (Lopez-Toledano *et al*., 2004). Previous investigations showed that the yeasts retain the oligomers produced in the reaction, although they have no inhibitory effect on the condensation reaction (Garrido & Borges, 2013; Lopez-Toledano *et al*., 2004). Therefore, this type of reaction can contribute to the colour change observed in white wine after bottling.

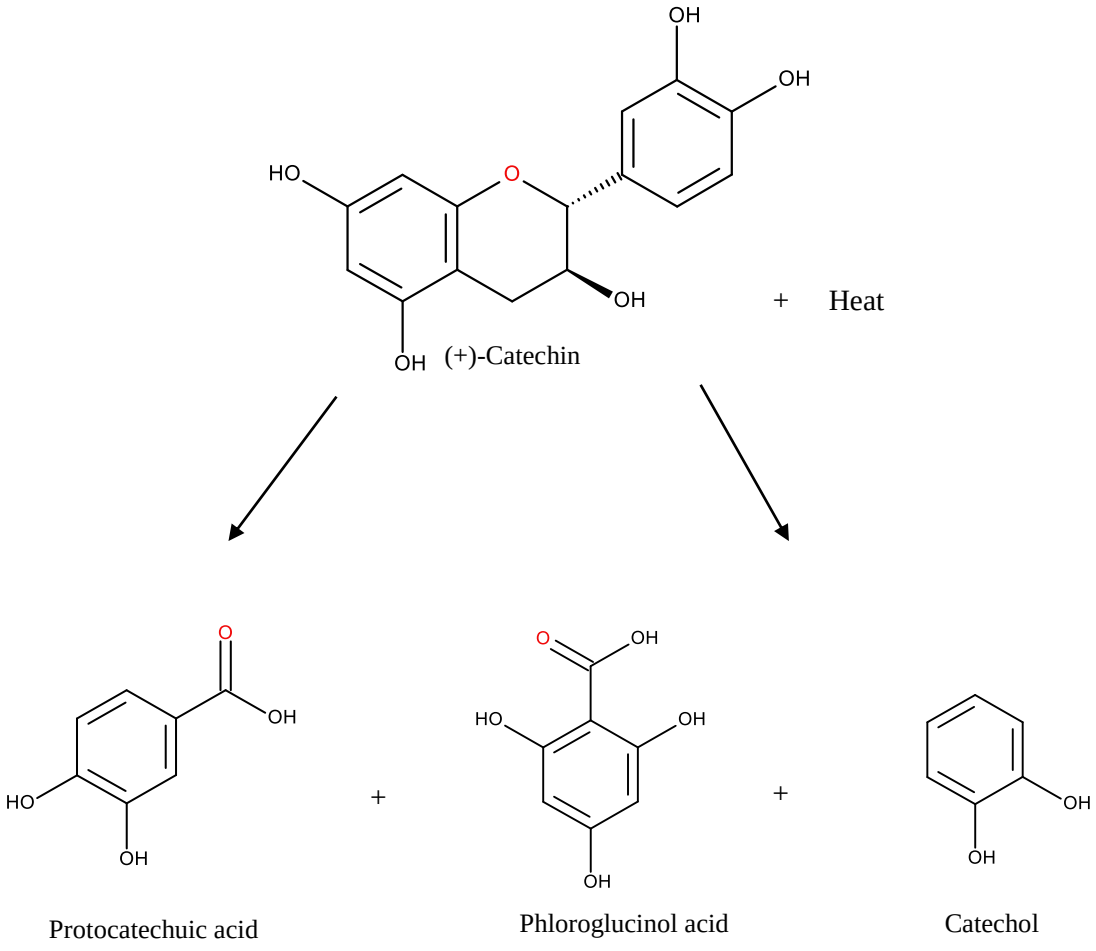


Figure 2.8. Degradation reaction of (+)-catechin in wine (Garrido & Borges, 2013)

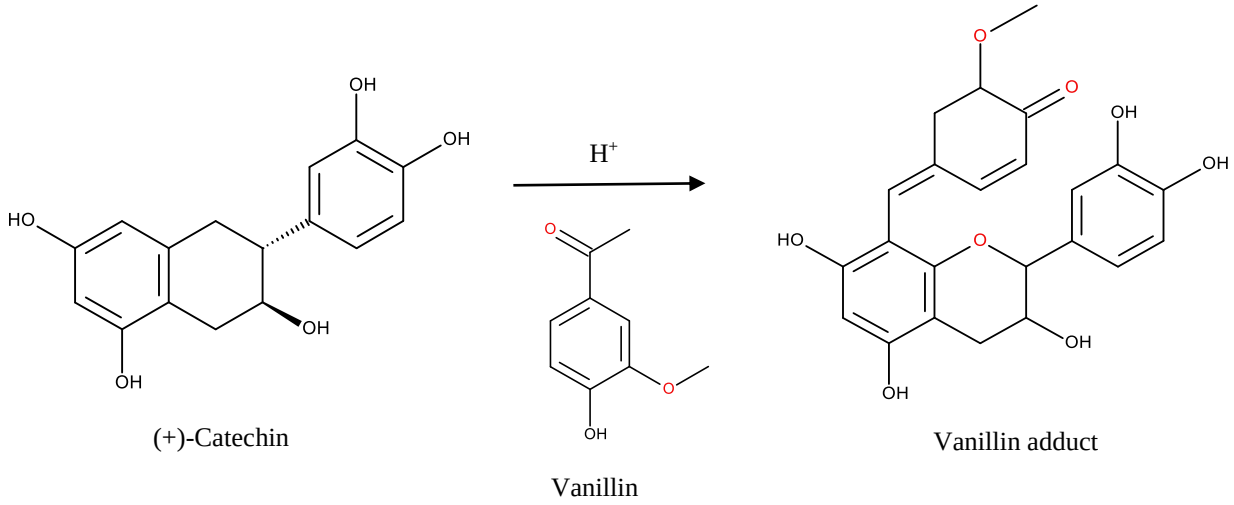


Figure 2.9. Vanillin and (+)-catechin reaction with the corresponding product (4-[[2-(3,4-dimethylphenyl)-3,5-dimethyl-chroman-8-yl] methylene]-6-methoxy-cyclohex-2-en-1-one), responsible for yellow colour in white wine (Garrido & Borges, 2013)

2.9. Analysis of phenolics in white wines

The concentration of phenolic compounds in white wine is low due to the winemaking process of white wine and as such are often not studied (Jeffery *et al*., 2008), even though they play an important role in the quality of wine. There is little detailed information about concentrations of phenolics in South African Chenin blanc wines. However, there are reports in the literature about the antioxidant activity of flavan-3-ols and flavonols compounds in Spanish and Californian white wines (Makris *et al*., 2006; Frankel *et al*., 1995).

2.9.1. Spectrophotometric methods used for analysis of phenolics

A number of spectrophotometric methods for the quantification of phenolic compounds in grapes and wine have been developed (Herderich & Smith, 2005; Schofield *et al*., 2001). However, these assays are based on different principles and are used to determine different structural groups present in phenolic compounds. The most suitable method for the determination of the total phenolic compound concentration in wine or wine extract is the measurement of absorption at *ca* 280 nm for flavan-3-ols and *ca* 360 nm for flavonols, with an appropriate sample dilution. The absorbance value is based on the characteristic absorption of the benzene cycles that absorb at 280 nm or 360 nm wavelength(s) values. This method presents a number of advantages, including short analysis time and reproducibility. However, certain molecules, such as cinnamic acids, have no maximum absorption at these wavelengths. Other non-flavonoid compounds such as amino acids, which also have benzene ring absorb at 280 nm causing an interference in the absorption (Lorrain *et al*., 2013).

A second method for minimising the interference was developed to determine the phenolic content. Folin-Ciocalteu method, which is based on reductive properties of phenols (Lorrain *et al*., 2013; Schofield *et al*., 2001). This method uses the reduction of phosphomolybdic acid to a blue coloured complex by phenolic compounds in alkaline conditions. This method remains non-specific for phenolics since some phenolic groups present in extractable proteins or reducing substances such as ascorbic acid can also participate in the reduction reaction (Lorrain *et al*., 2013). Furthermore, possible interference from other readily oxidised substances such as sulphites and sulphur dioxide that is present in wine as a result of fermentation processes may lead to overestimation of the phenolic content (Agatonovic-Kustrin *et al*., 2015; Lopez-Velez *et al*., 2003). Šeruga *et al*. (2011) applied the Differential Pulse Voltammetry (DPV) method in an attempt to determine the phenolic content in wine. This analytical method is routinely used in the determination of phenolics in food samples. The study showed that the DPV technique was more sensitive and selective in determining the total phenol content, in comparison to the other spectrophotometric methods.

2.9.2. Chromatographic methods used for analysis of phenolics

Liquid chromatographic techniques are widely used for both separation and quantification of phenolic compounds in wine. The aspects of compound separation in wines have already been extensively reviewed (Agatonovic-Krustin *et al*., 2015; Herderich & Smith, 2005; Flammini, 2003; Merken *et al*., 2000) and the chromatographic separation for grape and wine phenolics is continuously improving. Various detection methods have been applied in combination with HPLC for phenolic compound determination using UV-Vis (ultra violet visible), photodiode array (DAD), fluorescence and mass spectrometry. However, UV detection remains the most popular and commonly used quantitation method (De Villiers *et al*., 2012; Robbins & Scott, 2004; Stevofa *et al*., 2003), because of the natural absorbance of phenolic compounds in the UV region. Flavonols show two absorption bands maxima in the 350 nm–370 nm regions, while flavan-3-ols show two bands at 210 nm and 278 nm (Merken *et al*., 2000).

The detection and separation of phenolics nowadays is based on the use of HPLC coupled to photo diode array detection (DAD) at different wavelengths (De Villiers *et al*., 2012; Mozetič *et al*., 2006). This type of detection allows maximum absorbance of phenolic compounds and controls peak purity and identification of compounds by means of visible spectra and retention times but mass spectroscopy detection is often applied. This mode of detection (mass spectroscopy) is gaining popularity as it provides specific identification of the eluted compound with molecular weight and fragments.

2.10. Fermentative aromas produced by the yeast

The yeast can affect the aromatic flavour of the wine through the anabolic and/or catabolic pathways (Lambrechts & Pretorius, 2000). The biosynthesis of aroma compounds in wine is important, because a large percentage of the total aroma compounds are derived from the fermentation process. There are two groups of aroma compound namely thiols and terpenes. Volatile thiols are mostly affected by '*terroir*'. Thiols are normally found in the skin of the grapes as non-aromatic monoterpenes (Swiegers *et al.*, 2005). An enzyme is sometimes added during fermentation to facilitate the breakdown of the glycosidic bond of monoterpenes thereby releasing terpenes as aromatic compounds (Ferreira *et al*., 2001).

However, studies have shown that certain non-*Saccharomyces* yeasts have the ability to produce *β*-glycosidase enzyme (Giovani & Rosi, 2005; Ferreira *et al*., 2001) that can catalyse the hydrolysis process. Therefore, the excretion of this enzyme by certain non-*Saccharomyces* yeast can be a useful tool in enhancing the aroma compounds of wine without an addition of exogenous enzymes.

Fermentation aroma compounds includes esters and higher alcohols which are produced as secondary metabolites from the metabolism of amino acids and fatty acids. Higher alcohols are known to impart aromatic complexity and fruity notes when their concentrations are less than 300 mg/L. They can be perceived as pungent odours at concentrations above 300 mg/L (Swiegers & Pretorius, 2005; Lambrechts & Pretorius, 2000). The presence of higher alcohols in wine has been investigated (Moreira *et al.*, 2008; Rojas *et al.*, 2003). The investigation showed that wines made with non-*Saccharomyces* yeasts were higher in production of higher alcohols, compared to wines made with *S. cerevisiae* yeast. However, wines made with the combination of both non-*Saccharomyces* and *S. cerevisiae* yeast strains had lowest concentrations of higher alcohols. Some non-*Saccharomyces* yeast strains have been described as being proficient in the production of esters with *T. delbrueckii* producing high concentrations of ethyl caporate (Manzanares *et al.*, 2011). It is important to note that there is no reference for ester production by yeast, as with all other metabolites. However, the production of esters during fermentation is species and strain dependent, among other contributing factors (Lambrechts & Pretorius, 2000).

Table 2.1 lists some of the sensorial-active compounds present in wine, including their associated sensory aroma attributes and their origin (Ugliano & Henschke, 2009). A large number of the aroma compounds are produced by yeast through metabolism of the sugars in the grapes. Research has shown that some of the most important compounds for Chenin blanc wine are 3-mercaptohexan-1-ol and 3-mercaptohexylacetate (thiols), acetate esters, monoterpenes, higher alcohols and volatile fatty acids. These compounds make a positive contribution to Chenin blanc wine aroma profile (Lawrence, 2012; Van Antwerpen, 2012).

Table 2.1. Sensorial compounds in wine, produced as results of yeast metabolism (Ugliano & Henschke 2009)

Volatile compounds	Origin	Wine sensory attribute
Acetate esters	**A**	Flowery, fruity
Fatty acids	**A**	Flowery, fruity
Higher alcohols	**A, B**	Alcohol, herbaceous
Volatile acids	**A**	Sour, sweat, cheese
4-mercapto- methylpentanone	**B**	Box tree
3-mercaptohexan-1-ol	**B**	Green mango, box tree
3-mercaptohexyl acetate	**B**	Tropical fruit

A: compounds produced through yeast metabolism; **B**: compounds present in grapes as non-volatile precursors

2.11. Sensory analysis of wine

Sensory evaluation has been defined as a scientific method used to evoke, measure, analyse and interpret the response of humans to products as perceived through the senses of sight, smell and taste (Dzung & Dzua, 2003). Sensory evaluation functions as risk reduction mechanism for researchers and marketing managers. Wine sensory evaluation is usually done by a panel of trained judges. The training is acquired through experience (most judges are winemakers) and attending wine tasting courses.

There are different tests used for sensory analysis, but the "Difference" test is the most used test (van Breda *et al*., 2012; Boulton *et al*., 1995). It allows the judges to detect the differences between the wine samples based on their characteristics. This test is the simplest tests where judges are given wine samples and asked to identify dominant characteristics. These characteristic are identified and a percentage score is given on a line scale.

Literature studies show the importance of the determination of the phenolic content in wine because these compounds contribute to overall quality of wine. It is important for the wine industry to understand the different processes of winemaking. Literature has little information on the content of phenolic compounds in Chenin blanc wine but it is the most planted cultivar in South Africa. Most of the research in white grape cultivars and consequently wines, has been conducted on Sauvignon blanc, Zinfandel and Spanish cultivars (Rodriguez *et al*., 2006; Frankel *et al*., 1995). De Beer *et al*. (2005) studied the changes

in the phenolic composition and antioxidant activity in Chenin blanc wines where they focused on the total phenolic composition and the antioxidant activities but not on the contribution each phenolic compound has with regards to the mouth-feel and colour of the wine. Additionally, elucidation of phenolic compounds was conducted in wines produced with *S. cerevisiae* yeasts. It was noticed that the yeast used to produce wines has an effect on the phenolic concentration in the final wine. Caridi *et al*. (2004) shows that *S. cerevisiae* yeast lowers the phenolic concentration in the final wine.

There is no standard measurement for the concentration of phenolics in wine because each grape cultivar has a different phenolic compound profile. However, the content of phenolics in wine has effect on the overall quality of wine. Wines with low phenolic content have less intensity on mouth-feel whereas wines with high phenolic content are bitter and astringent.

The organoleptic characteristics of wine are affected by a number of chemical compounds that are grape derived, fermentation derived or formed during ageing and storage (Fanzone *et al*., 2012). The current world-wide interest in the positive role of non-*Saccharomyces* yeasts in wine production, shows a need for a comprehensive study of the chemical profiling of Chenin blanc wines produced by *T. delbrueckii* yeast, as its use has proven to make improved quality wines (van Breda, 2011). This study sought to identify and quantify the phenolic compounds in wine produced by *S. cerevisiae* and *T. delbrueckii* strain(s) which consequently effect of the sensory character of wine.

Grape and wine phenolic research is a recent occurrence, and through it wine production has realised the complexity of colour, flavour, and astringency in wine. Phenolic compounds in wine depend on many physical and chemical factors. This therefore drives studies on how the aforementioned factors directly and indirectly affect phenolic content in wine, consequently affecting the sensory profile of the wine. Phenolic compounds in grapes and wines are affected are known to be affected by "*terroir"*. The effect *"terroir"* has on the phenolic content in wines has been investigated by Lampíř & Paulošek (2013). The investigation showed no significant differences in the phenolic content between wines of the same region, in two years. However, Ali and co-authors studied the metabolic constituents of grapevine and grape-derived products. Results from the study confirmed that phenolic compounds were a genetic part of the grapes, but the phenolic content in the final wines was affected by chemical and

biochemical reductions, during winemaking (Ali *et al*., 2010). The yeast chosen for inoculation of grape must is responsible for the chemical reactions that can affect the total phenolic content in wine (Domizio *et al*., 2014; Kennedy, 2008). *Saccharomyces cerevisiae* yeast is yeast used for commercial winemaking. This yeast was proven to decrease the phenolic content of wine by adsorbing of the phenolic compounds on the yeast cell wall (Caridi *et al*., 2004). However, these studies were conducted in red wine and are therefore bias for phenolic content in white wine, due to the different phenolic composition between white and red grapes and winemaking processes.

White wine is mostly made with no skin or minimal skin contact (as discussed in section 2.1). Most phenolic compounds found in white wine are found on the skin of the grapes. A recent study conducted on different winemaking techniques for Chenin blanc showed no significant differences between phenolic concentration in wines made with no skin contact and wines made with skin contact before fermentation (Aleixandre-Tudo *et al*., 2015). However, the total phenolic content in wines fermented with grape skins were higher, compared to phenolic content in wine made with no skin contact. This observation was made in Chenin blanc wines made with commercial yeast strains (*S. cerevisiae*), therefore the chemical and biochemical of non-*Saccharomyces* yeast is still undetermined. This then adds another variable on the effect of *T. delbrueckii* yeast strains and its effect on the secondary metabolites produced by the yeast during winemaking.

Oenological practices can change the chemical composition of wine and its sensory properties, such as flavour, astringency and colour. In recent years, research on the production of South African Chenin blanc using the non-conventional yeast strains in winemaking has produced wines with improved overall quality and complexity (Van Breda *et al*., 2012; Jolly *et al*., 2006; Jolly *et al*., 2003). Several studies have been published in literature about the effect of phenolic compounds on the sensorial properties of wine (Kennedy, 2008; Lesschaeve & Noble, 2005) but there is limited information on the effect of *S. cerevisiae* and *T. delbrueckii* strains on the phenolic compounds. Understanding the chemical effect of yeast strain during winemaking will enable effective use of the non-*Saccharomyces* yeast which will produced wines with improved flavour and overall quality.

Chapter III

Identification and quantitation of phenolic and volatile compounds

3. Methodology

3.1. Introduction

The overall quality of wine is a result of interactions between various chemical compounds (Wansbrough *et al*., 1998). In excess of more than 200 chemical compounds (by products) have been identified in wine. These compounds are derived from grapes and are extracted during fermentation, maturation and storage of wine (Kennedy, 2008). The viticulture and winemaking processes can affect the concentration of these compounds in the final product. These compounds include the non-volatiles and volatile compounds in wine. The improvement of wine quality includes many aspects such as viticultural practises, yeast choice, yeast performance, winemaking processes and the release of secondary metabolites (during fermentation) that can contribute towards the wines flavour and aroma.

The recent interest in using non-*Saccharomyces* yeast in wine production was shown by van Breda *et al*. (2012). Wines were produced by non-*Saccharomyces* yeasts. These results showed that non-*Saccharomyces* yeast, i.e. *T. delbrueckii* yeast strains (654 and M2/1), produced wines with improved quality when compared to the "normal yeast" *S. cerevisiae* (van Breda *et al*., 2012). The wines were subjected to standard oenological chemical analysis, i.e. pH, volatile acidity, glycerol and percentage ethanol. The wines were also analysed for their volatile aroma profiles. However, the results emanated from these analyses limit the conclusions drawn regarding the differences in the wine quality. Sensory analysis results obtained showed differences in the aroma and mouth-feel attributes of the wines, but the sensory data alone could not explain the sensory differences observed between the wines. A previous investigation has shown that the yeast used to inoculate grape must has an effect on the final phenolic concentrations in wine (Ivanova *et al*., 2011; Mangani *et al*., 2011). Results from literature study have also shown that *S. cerevisiae* yeasts have negative effect, i.e. decreases the phenolic content of wine (Caridi *et al*., 2004). Phenolic compounds are secondary metabolites present in wines that contribute to the sensory profiles of wine particularly colour and mouth-feel attributes (Garrido & Borges 2013; McRae & Kennedy, 2011; Lesschaeve & Noble 2005).

There are many publications on the identification and quantification of phenolic compounds in wine (Castillo-Muňoz *et al*., 2010; Borbalán *et al.*, 2003; Stefova *et al*., 2003). However, high performance liquid chromatographic techniques (HPLC) are recognised as the most efficient and convenient method to quantify phenolic compounds in a sample matrix such as grapes extract and wine (Lorrain *et al*., 2013; Burin *et al*., 2011; Mozetič *et al*., 2006, Betés-Saura *et al*., 1996). Liquid chromatographic application allows separation and identification of phenolic compounds in wines using a different detection method from which UV Photo Diode Array Detector (DAD) is the most suitable and popular. This detection allows maximum absorbance of each group of phenolics, control of peak purity and identification of peaks by means of visible spectra and retention times.

Most studies on South African Chenin blanc wines focused on the quantification of volatile aroma compounds and not the non-volatile compounds, which is phenolics that could affect the mouth-feel properties of wine. This study firstly focuses on identifying and quantifying the phenolic compounds which are possibly affected by *S. cerevisiae* and *T. delbrueckii* strains and also compares the chemical data and sensory data using multivariate statistical analysis. In addition, this study also, investigates a possible correlation between the two sets of data to better understand how sensory attributes are affected by phenolic compounds.

3.2. Wine microbiology

3.2.1. Yeasts cultures

The two strains of *T. delbrueckii* yeasts and one of *S. cerevisiae* were used in this study are listed in Table 3.1. The two *T. delbrueckii* natural isolates were obtained from the Agricultural Research Council Infruitec-Nietvoorbij (ARC Infruitec-Nietvoorbij) microbiological culture collection. The natural yeast isolates were previously collected over three years from various regions in the Western Cape, South Africa. These isolates were identified (Jolly *et al*., 2003a) and stored under cryo-preservation at -80°C. A commercial *S. cerevisiae* wine yeast (strain VIN 13, AnchorBio-Technologies, Cape Town, South Africa) was also used.

Table 3.1. Yeast strains used in this study

Strain	Identification	Isolation material	Region	Year isolated	Concentration [1]cfu/mL
VIN13	*S. cerevisiae*	NK[2]	Anchor biotechnologies	NA[3]	$1X10^6$
654	*T. delbrueckii*	Must	Robertson	1995	$2x10^6$
M2/1	*T. delbrueckii*	Must	Robertson	1998	$2x10^6$
VIN13+654	co-inoculation	NA	NA	NA	
VIN13+M2/1	co-inoculation	NA	NA	NA	

[1]cfu= colony forming unit; [2]NK= not known; [3]NA= not applicable

3.2.2. Small scale winemaking

Chenin blanc wines were made according to the ARC Nietvoorbij approved winemaking protocol, at the Infruitec-Nietvoorbij experimental wine cellar. This project does not report on the winemaking process. Wine samples emanated from a previous project (van Breda *et al*., 2012). Initially, Chenin blanc wines were made with a number of *T. delbrueckii* yeast strains. However, van Breda *et al*. (2012) subsequently discovered that only two yeast strains (M2/1 and 654) showed potential for the use as single inoculant yeasts in experimental making of Chenin blanc wine compared to Chenin blanc wine produced by *S. cerevisiae* (reference yeast VIN13). The must aliquots were inoculated with yeast culture (Table 3.1). The inoculum concentration for wines made with combination of yeast strains was $1x10^6$ cfu/mL for *S. cerevisiae* strain and $2x10^6$ cfu/mL for *T. delbrueckii* strains The inoculation of *T. delbrueckii* strains in combination treatments was performed 24 hrs, after inoculation of *S. cerevisiae*. Yeast treatments used for the production of Chenin blanc wines were VIN13, 654, M2/1, VIN13+654 and VIN13+M2/1. The wines were made according to the following procedure:

The small-scale wine fermentations were performed in duplicate for 2011 and in triplicate for 2012 and 2013 vintages. Di-ammonium hydrogen phosphate (0.50 g/L) and SO_2 (50 mg/L) were added to the inoculated Chenin blanc must. The clarified must aliquots (18 L) were placed in 20 L stainless steel canisters fitted with fermentation caps. Fermentations were conducted at 15°C and monitored by CO_2 weight loss. The fermentations were allowed to continue until there was no reduction of residual sugars observed, i.e. < 2 g/L. Where fermentation was not completed within 32 days, it was stopped. Residual sugar analyses were done on all wines to confirm the end of the fermentation. Wines were racked off the yeast lees after fermentation and the free SO_2 adjusted to 35 mg/L. Bentonite (0.75 g/L) was added, and the wines were cold-stabilised at 0°C for two weeks. The wines were filtered and transferred to 750 mL bottles according to standard practices for white wine production. The wines were stored at 14°C after bottling, and they were tasted on the year of analysis.

Table 3.2. Yeast strain used for the production of Chenin blanc wines

Vintage	Yeast treatments					Number of samples
	VIN13	654	M2/1	VIN13+654	VIN13+M2/1	
2011	2	2	2	2	2	10
2012	3	3	3	3	3	15
2013	3	3	3	3	3	15
Total	8	8	8	8	8	40

3.2.3. Oenological parameters

The wines were analysed for percentage alcohol, volatile acidity (VA) and glycerol using a Winescan at Institute for Wine Biotechnology, Stellenbosch University. The Rebelein method for residual sugar analysis (RS) and the Ripper method for sulphur dioxide (SO_2) was used as the prescribed methods of the South African Wine Laboratories Association (Anon., 2002).

3.3. Chromatography

3.3.1. Standards and reagents

Phenolic standards which include gallic acid, (+)-catechin, (-)-epicatechin, epigallocatechin-3-gallate, caffeic acid, ferulic acid, *p*-coumaric acid, rutin, quercitrin and quercetin were purchased from Sigma-Aldrich, South Africa. Isoquercitrin standard was purchased from Extraynthese in France (Table 3.3). Acetonitrile, *Ortho*-phosphoric acid and methanol (HPLC grade) were purchased from Merck® South Africa. De-ionised water used was supplied through a Modulab® water purification system, supplied by Separations®.

Table 3.3. Phenolic standards used for RP-HPLC DAD analysis

Compound	Catalogue no.	Purity	Supplier
Flavan-3-ols			
(+)-Catechin	43412	≥ 99%	Sigma Fluka, South Africa
(-)-Epicatechin	E1753	HPLC grade	Sigma Chemical Co., South Africa
Epigallocatechin-3-gallate	E4143	≥95%	Sigma Aldrich, South Africa
Phenolic acids			
Gallic acid	14291-5	97%	Sigma Aldrich, South Africa
Caffeic acid	60018	99%	Sigma Fluka, South Africa
p-Coumaric acid	C9008	HPLC grade	Sigma Chemical Co., South Africa
Ferulic acid	46278	99%	Sigma Fluka, South Africa
Flavonols			
Rutin[1]	R5143	95%	Sigma Aldrich, South Africa
Isoquercitrin	9006	HPLC grade	Extrasynthese, France
Quercitrin	Q3001	85%	Sigma Aldrich, South Africa
Quercetin	Q4951	≥95%	Sigma Aldrich, South Africa

[1]Rutin= Glycosidic derivative of Quercetin

3.3.2. Preparation of mobile phases for HPLC analysis

Eluent A

An aliquot of 15 mL *Ortho*-phosphoric acid (85%) was measured using a graduated pipette (10 mL). The measured acid was added to approximately 500 mL of de-ionised water using a volumetric flask (1000 mL). An aliquot of 485 ml of de-ionised water was added to bring to a volume of 1000 mL. The solution was stirred for approximately 10 minutes using a magnetic stirrer plate. The suspension was filtered through a 0.22µm nylon membrane filter using a Millipore Vacuum Flask System. The filtrate was placed in an ultrasonic bath for 10 minutes. The pH of the eluent was verified as *ca* 1.35.

Eluent B

An aliquot of 15 mL *Ortho*-phosphoric acid (85%) was measured using a graduated pipette (10 mL). The measured acid was added to 185 mL of de ionised water. The water-acid mixture was added to 800 mL of acetonitrile in a Schott bottle (1000 mL). The solution was stirred for approximately 10 minutes using a magnetic stirrer plate. The suspension was filtered through a 0.22µm nylon membrane filter using a Millipore Vacuum Flask System. The filtrate was placed in an ultrasonic bath for 10 minutes. The pH of the eluent was verified as *ca* 1.25.

3.3.3. Reverse phase - high performance liquid chromatographic conditions

The RP-HPLC determination of phenolic compounds was performed by using Chemetrix Separation Product®. The system was equipped with an auto-sampler and a photodiode array detector. The polymer reverse phase analytical column (PLRP-S 100A, 5µM, 250 x 6.6 mm) with polystyrene divinylbenzene as a stationary phase was supplied by Polymer Laboratories®. Analysis was carried out at room temperature.

A gradient elution programme was used to elute the compounds of interest. Eluent A (mobile phase A) consisted of water/phosphoric acid (985:15 v/v) with a pH of *ca*. 1.35 and eluent B (mobile phase B) consisted of water/phosphoric acid/acetonitrile (185:15:800 v/v/v) with a pH of *ca.* 1.25. The gradient programme (Table 3.4) was used for separation and elution of the phenolic compounds. The column and the system were equilibrated for 20 minutes before and after each run to revert to the starting conditions. The flow rate was 1 mL/min.

Table 3.4. Gradient programme for HPLC-DAD phenolic compound separation

Times (min)	Eluent A (%) composition	Eluent B (%) composition	Flow Rate (min)
0	94	6	1
73	69	31	1
78	38	62	1
86	38	62	1
90	94	6	1

3.3.4. RP-HPLC analysis of Chenin blanc wine

The identification of the phenolic compounds was confirmed by their relative retention times and UV-visible absorption characteristics (Stefova *et al.*, 2003; De Villiers *et al.*, 2011). The method used for the analysis of phenolics was described by Waterhouse *et al.*, 1999. This method is endorsed by the *Office International de la Vigne et du Vin* (OIV, Resolution Oeno 22/2003). A wine sample aliquot of 2 ml was filtered through a 0.45 µm pore size nylon membrane syringe filter. A 50 µl aliquot of the filtrate was injected onto the analytical column via the HPLC auto injector. Results were extrapolated from calibration curves based on their spectral data and retention times, and expressed in milligrams per litre. Replicates from the same year of production were analysed on the same day.

3.4. Method validation

3.4.1. Calibration curves

Stock solutions of standards were prepared by dissolving a standard in eluent A and methanol (MEOH concentration < 5%). The working standard solutions were prepared by sequential dilutions of the stock solutions with eluent A. Each working solution was placed in an ultrasonic bath for approximately 1 minute before further dilutions to obtain homogeneity. Concentrations of the analytes were calculated from chromatogram peak areas on the basis of calibration curves. The method linearity was assessed by means of linear regression of the concentration of analyte injected versus peak areas. Injection of the standards was done in triplicate (see appendices A-K).

The method was validated in terms of linearity, precision, sensitivity, detection and quantification limits. Calibration curves for each compound with the respective correlation coefficient were calculated by least-squares linear regression analysis of the peak area of each analyte (R^2). Precision of the method was evaluated based on intraday and interday repeatability and was assessed by replicates (n = 6) measurements from each sample. Variance between repetitions was expressed as a percentage relative standard deviation (%RSD). The limits of detection and quantification were calculated by using the residual standard deviation of a regression line for each compound (equation 3.1 to 3.6) (Singh, 2013). Flavan-3-ols, flavonols and phenolic acids concentrations in the wines were determined by using the

area response of each individual wine compound by extrapolation in the corresponding calibration curves.

$$Sa = \frac{Serror}{intercept}\ X\ 100 \qquad 3.1.$$

$$YLOD = a + 3Sa \qquad 3.2.$$

$$YLOQ = a + 10Sa \qquad 3.3.$$

$$YLOD = b\ XLOD + a \qquad 3.4.$$

therefore

$$XLOD = \frac{YLOD - a}{b} \qquad 3.5.$$

$$XLOQ = \frac{YLOQ - a}{b} \qquad 3.6.$$

Where:
Sa: standard deviation of the regression
$S_{error:}$ Standard error of the intercept
LOD: Limit of detection
LOQ: limit of quantitation
Y: denotes absorbance (mAu)
X: denotes concentration (mg/L)
a: is the intercept of the calibration curve
b: the slope of the calibration curve

3.5. Sensory evaluation

A panel of 12 trained wine judges was used to evaluate the wines' sensorial attributes. The tasting of the wines was carried out over four consecutive days to eliminate tasting of replicates in one batch. The wines were presented to judges in a randomised order. Sensory analysis involved the evaluation of flavour, body/viscosity, acidity, astringency and complexity. The panel was supplied with a wine sample(s) and a tasting sheet that allowed tasters to evaluate the wine on a 10 cm unstructured line scale (Annexure A). All panel members were required to sign a consent form as part of the Cape Peninsula University of Technology's (CPUT) ethical standards policy.

3.6. Quantitation of volatile compounds

The quantitation of volatile compounds in the wine samples were conducted using a gas chromatograph coupled with flame ionisation detector (GC-FID). Sample preparation and GC-FID method were carried out according to Louw *et al*. (2009). The samples were injected in duplicate. The wine samples were submitted to the Central Analytical Facilities of the University of Stellenbosch for analysis.

3.7. Statistical analyses

Data from the analysis of Chenin blanc wines emanating from the application of a high-performance liquid chromatographic method (chemical) as well as the sensory data were subjected to analysis of variance (ANOVA) and principal component analysis (PCA).

3.7.1. Analysis of variance (ANOVA)

Analysis of variance is a statistical method to analyse measurements, which are subjected to different treatments (SAS, 2000). The purpose of analysis of variance is to establish significant differences between factors and treatments. ANOVA divides the variability among all the data into one component due to variability among group means (due to treatment) and another component that is due to variability within the groups (also called residual variation). Variability within groups is quantified as the sum of squares of the differences between each value and its group mean. This is the residual sum-of-squares. Variation among groups (due to treatment) is quantified as the sum of the squares of the differences

between the group means and a mean (the mean of all values in all groups). The data is then homogenised for the size of each group, this becomes the sum-of-squares.

Each sum-of-squares is associated with a certain number of degrees of freedom (df, calculated from number of samples and number of treatments), and the mean square (MS) is calculated by dividing the sum-of-squares by the appropriate number of degrees of freedom. The quotient gives the variance between data sets.

3.7.2. Principal component analysis (PCA)

Principal component analysis using (XLSTAT 2010 add-on statistical software on Excel 2010) was also conducted on the same data sets to establish clustering, correlation, association and "groupings" between treatments and variables of the wine samples, i.e. their relationships with the treatments (yeast/yeast combinations) and phenolic compound concentrations.

The purpose of the application of principal component analysis is to reduce the complexity of multivariate data into a principal component space. Principal component analysis is also a dimension reducing technique. The first q principal component ($q<p$), which explains the highest percentage of the variation in the variables, is always chosen. Eigenvalues, percentage variability and percentage cumulative variance for each principal component in the data are determined. The principal components are donated as C1 to C15 or PC1 to PC2, depending on the number of variables.

Variance in the data can be explained by more than two principal components, i.e. C1 to C15. However, the first two principal components, i.e. C1 and C2 are usually chosen, since it is easier to interpret a two-dimensional plot compared to other dimensional plots. The PCA results are reported on vector diagrams (bi-plots). The vector diagrams describe the relative positions and loadings of the variables in relation to treatment. The first two factor scores (C1 and C2) and two factor loadings are used to plot the vector diagrams. The axes (x and y or PC1 and PC2) represent the principal components and descri be the degree of variability in the data.

Chapter IV

Results and discussion

4. Results and discussion

4.1. Oenological parameters measured in Chenin blanc wines

The total soluble solids of grape must in (°B) for the three vintages prior to inoculation was slightly different (Table 4.1). Considering the effect of climate variation among vintages, the difference in total soluble solid concentrations in the musts was expected. Variation in climatic conditions, i.e. prevailing wind, diurnal temperature, precipitation and *"terroir"* did not affect the primary metabolites of yeasts. Non-*Saccharomyces* yeasts which are known for their low production of alcohol (Contreras *et al*., 2015) can divert the carbon (in glucose) from ethanol formation to other metabolites, e.g. glycerol. For all studied vintages (2011-2013) the concentration of ethanol in wines produced with *T. delbrueckii* yeast strains (654 and M2/1) was slightly lower than ethanol concentrations in wines produced with *S. cerevisiae* yeast strain, VIN 13. Consequently, the yield of glycerol in wines produced by low alcohol production yeast strains was higher, compared to wines made with *S. cerevisiae* (Table 4.2). Studies on the concentration of glycerol have proven that the presence of glycerol in wine contributes to the mouth-feel and complexity of wine (Ciani & Comitini, 2011). However high levels of glycerol (10 - 15 g/L) in wine have an effect on the production of acetic acid (Nieuwoudt *et al*., 2002). *Torulaspora delbrueckii* yeast strains 654 and M2/1, produced metabolites that are desired for improved wine quality and quality control, which are low production of alcohol and low to moderate production of volatile acids and glycerol concentrations, compared to *S. cerevisiae* yeast (Table 4.2). It was observed that the low production of ethanol by *T. delbrueckii* yeast strains was inversely proportional to glycerol production.

Table 4.1. Oenological parameters in Chenin blanc base must

Vintage	pH	[1]TA (g/L)	[2]TSS [3](°B)
2011	3.41	7.9	21.6
2012	3.49	7.5	20.4
2013	3.41	8.1	20.5

[1]TA= Titratable acidity; [2]TSS =Total soluble solids; [3]°B= Degree brix

Table 4.2. Oenological chemical parameters measured in Chenin blanc wines indicating vintages and treatment effect

Oenological parameters	2011					2012					2013				
	Yeast treatments					Yeast treatments					Yeast treatments				
	VIN13	654	M2/1	VIN13 + 654	VIN 13 + M2/1	VIN13	654	M2/1	VIN13 +654	VIN13 +M2/1	VIN13	654	M2/1	VIN13 + 654	VIN13 +M2/1
[1]VA (g/L)	0.245 ±0.02[5]	0.345 ±0.01	0.356 ±0.01	0.245 ±0.01	0.26 ±0.02	0.33 ±0.02	0.37 ±0.09	0.41 ±0.06	0.32 ±0.03	0.32 ±0.03	0.24 ±0.11	0.34 ±0.05	0.35 ±0.02	0.25 ±0.01	0.26 ±0.03
[2]TA (g/L)	5.79 ±0.05	5.68 ±0.02	5.66 ±0.01	5.81 ±0.01	5.71 ±0.02	5.41 ±0.08	5.41 ±0.10	5.39 ±0.13	5.37 ±0.10	5.39 ±0.15	6.09 ±0.01	5.96 ±0.01	5.97 ±0.02	6.03 ±0.04	6.09 ±0.07
[3]Alc (%)	13.0 ±0.04	11.395 ±0.38	11.43 ±0.41	12.93 ±0.42	12.98 ±0.03	12.46 ±0.06	12.06 ±0.61	11.95 ±0.45	12.36 ±0.09	12.31 ±0.02	12.06 ±0.05	12.03 ±0.04	11.94 ±0.04	12.0 ±0.04	11.94 ±0.08
[4]Gly (g/L)	6.85 ±0.01	7.025 ±0.28	6.69 ±0.25	7.46 ±0.54	6.85 ±0.29	5.80 ±0.22	6.30 ±0.70	6.93 ±0.95	5.84 ±0.32	5.73 ±0.11	7.02 ±0.09	7.33 ±0.22	7.19 ±0.07	6.90 ±0.11	7.01 ±0.15
Sugars (g/L)	1.71 ±0.21	26.98 ±5.73	26.9 ±0.01	2.73 ±0.01	1.93 ±0.23	1.82 ±0.10	8.23 ±11.2	10.57 ±8.65	2.07 ±0.44	1.91 ±0.19	1.68 ±0.03	2.35 ±0.48	2.49 ±0.51	1.89 ±0.39	1.59 ±0.33

[1]VA=Volatile acidity; [2]TA=Total acidity; [3]Alc= Ethanol; [4]Gly= Glycerol; [5](±Standard deviation of the replicate samples)

4.2. Method validation results

The HPLC method was validated by determining the linearity, peak purity, limits of detection and quantification and precision. Peak purity and precision of the elution times were determined for qualitative evaluation of the method. The method showed good repeatability. This was confirmed with the calculation of variance between six repetitions. Results are expressed as %RSD. Averaged coefficients of variations were 0.376, 3.89, 0.92, 2.89, 0.27 and 2.04% for gallic acid, (+)-catechin, (-)-epicatechin, epigallocatechin-3-gallate, caffeic acid and *p*-coumaric acid, respectively. The low %RSD values suggest the high precision of the method.

Linearity, limit of detection and limit of quantitation were determined for quantitative purposes (Table 3.4). The low LOD and LOQ values indicate the possible quantitation of each phenolic compound studied. The R^2 for all phenolic compounds studied was greater than 0.998, thus confirming the linearity of the method. Therefore, the HPLC method used in this study is effective for the identification and quantification of phenolic compounds in wine.

Table 4.3. Regression equation for calibration curves, regression coefficients, limits of detection and quantitation determination.

Phenolic compounds	Regression equation(s)	[1]R^2	[2]LOD (mg/L)	[3]LOQ (mg/L)
Flavan-3-ols				
Gallic acid	y= 39.44x - 22.798	0.9998	0.0613	0.2045
(+)-Catechin	y= 8.526x – 10.316	0.9997	0.1244	0.4147
(-)-Epicatechin	y= 13.308x – 16.758	0.9989	0.0130	0.0434
Epigallocatechin-3-gallate	y= 19.151x – 13.898	0.9998	0.0628	0.2096
Phenolic acid				
Caffeic acid	y= 70.046x – 56.086	0.9997	0.0375	0.1025
p-Coumaric acid	y= 115.7x – 249.82	0.9981	0.0168	0.0560
Ferulic acid	y= 75.85x – 103.54	0.9996	0.0155	0.0517
Flavonols				
Rutin[4]	y= 11.063x + 6.092	0.9994	0.1476	0.4922
Isoquercitrin	y= 12.359x +19.397	0.9986	0.2363	0.7877
Quercitrin	y=14.134x +18.331	0.9982	0.2699	0.8999
Quercetin	y=11.352x – 7.4785	0.9993	0.3574	0.7598

[1]R^2=coeffiecient; [2]LOD=Limit of detection; [3]LOQ=Limit of quantitation; [4]Rutin=Glyosidic derivative of Quercetin

Two flavan-3-ols and four phenolic acids were quantified in the wines (Fig. 4.1-4.6). Quercetin was the only flavonol identified in the studied wines but was below the limit of detection. Rutin and isoquercitrin were not detected in the wines. It is known that the flavonol concentration in wine decreases over time, due to the hydrolysis of their glycosides during storage, which results in the precipitation of their aglycone (De beer *et al*., 2005; Zafrilla *et al*., 2003). The levels of (+)-catechin, (-)-epicatechin, gallic acid, caffeic acid, *p*-coumaric acid and ferulic acid ranged from 5.90-16.11 mg/L, 5.65-15.99 mg/L, 10.64-14.74 mg/L, 2.51-13.89 mg/L, 3.95-7.078 mg/L and 2.51-3.89 mg/L, respectively. These differences in concentrations are due to vintage affects. These differences were expected, as there are many factors affecting the biosynthesis pathway of phenolics (Pérez-Magarino & González San-José, 2006). These factors include, diurnal temperatures, prevailing wind, precipitation, soil type and

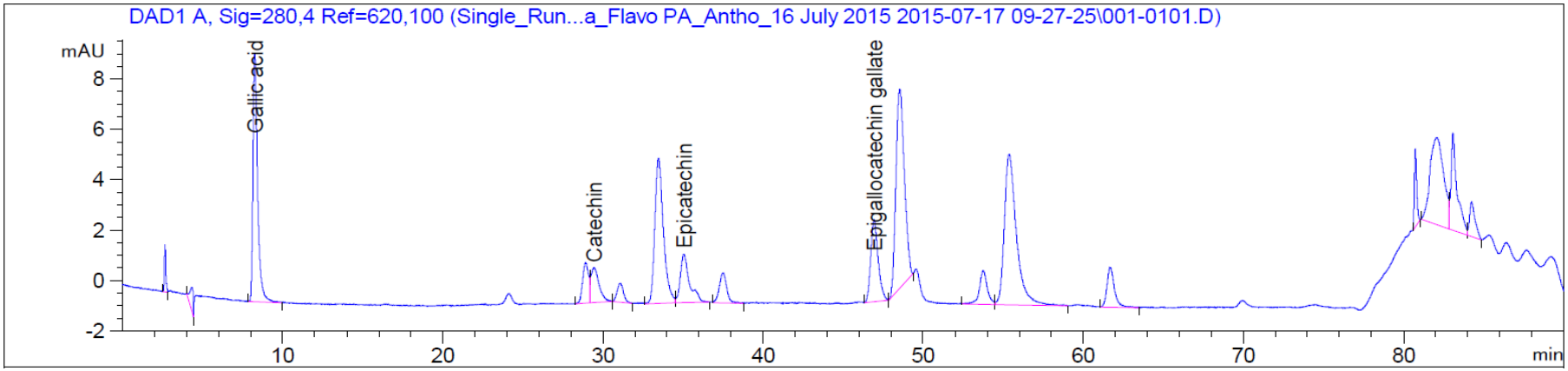


Figure 4.1. HPLC-DAD chromatogram of standards showing calibrated peaks at 280 nm

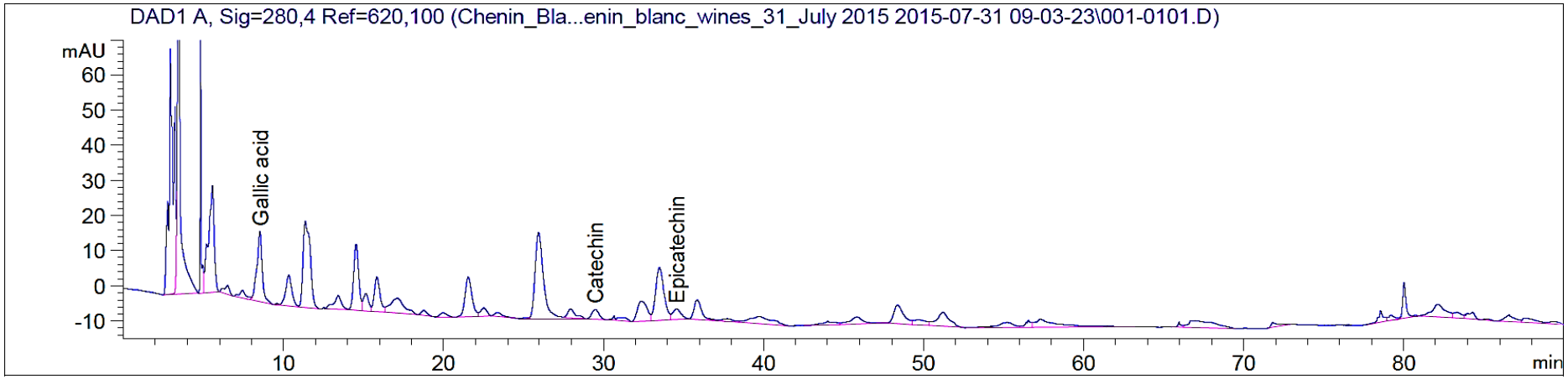


Figure 4.2. Chenin blanc wine HPLC-DAD chromatogram showing quantified flavan-3-ols at 280 nm

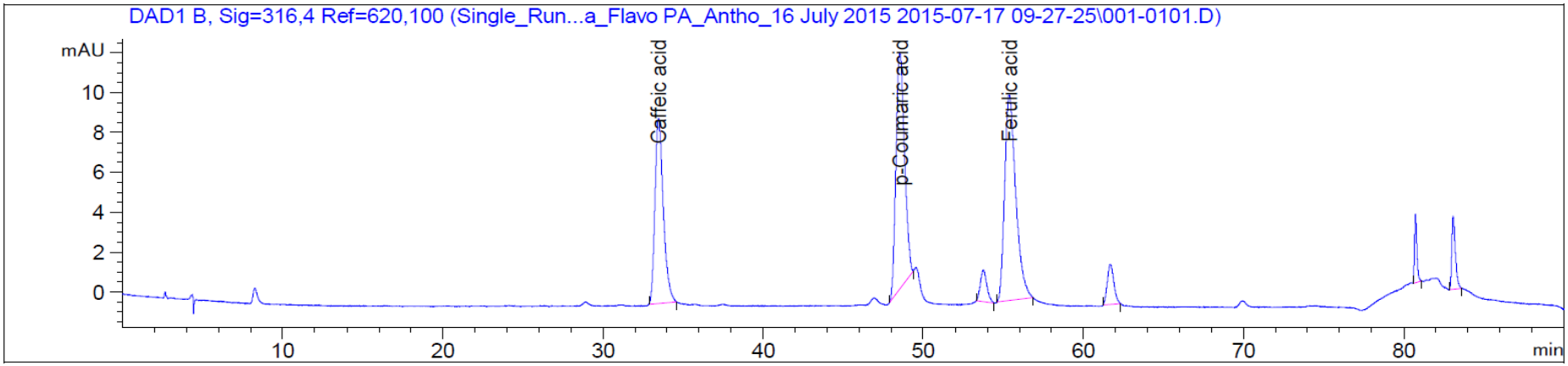


Figure 4.3. HPLC-DAD chromatogram of standards showing calibrated peaks at 316 nm

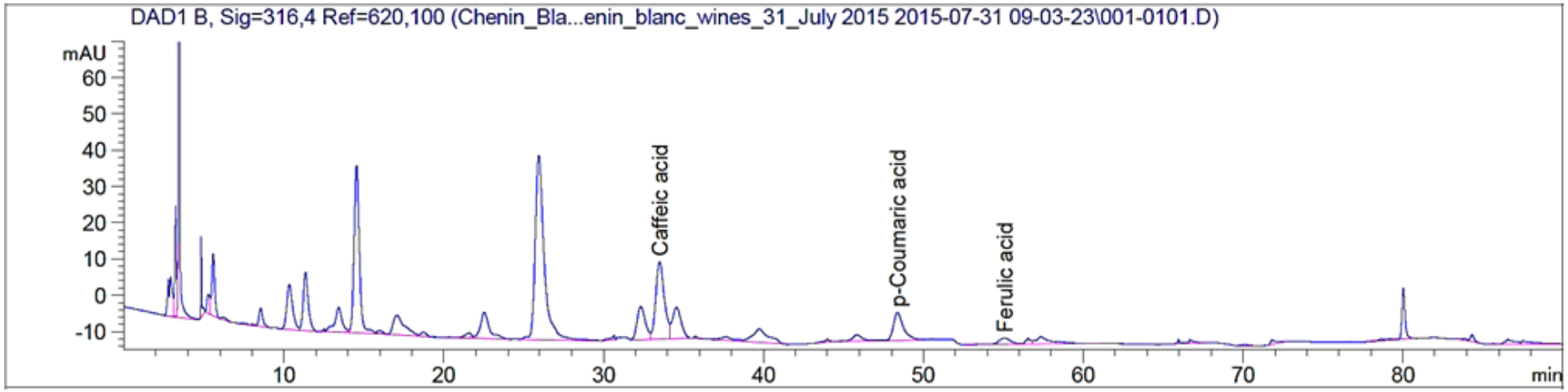


Figure 4.4. Chenin blanc wine HPLC-DAD chromatogram showing quantified phenolic acids at 316 nm

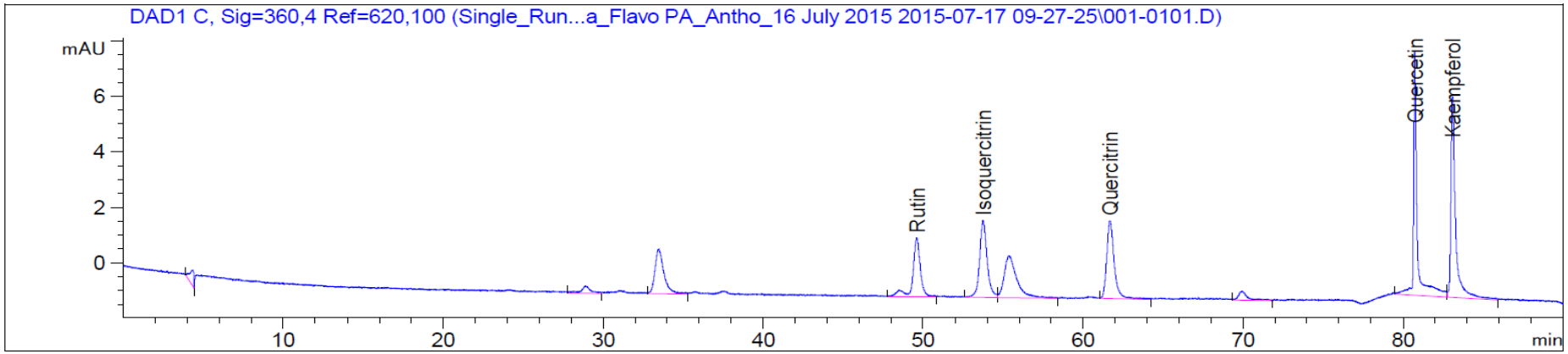


Figure 4.5. HPLC-DAD chromatogram of standards showing calibrated peaks at 360 nm

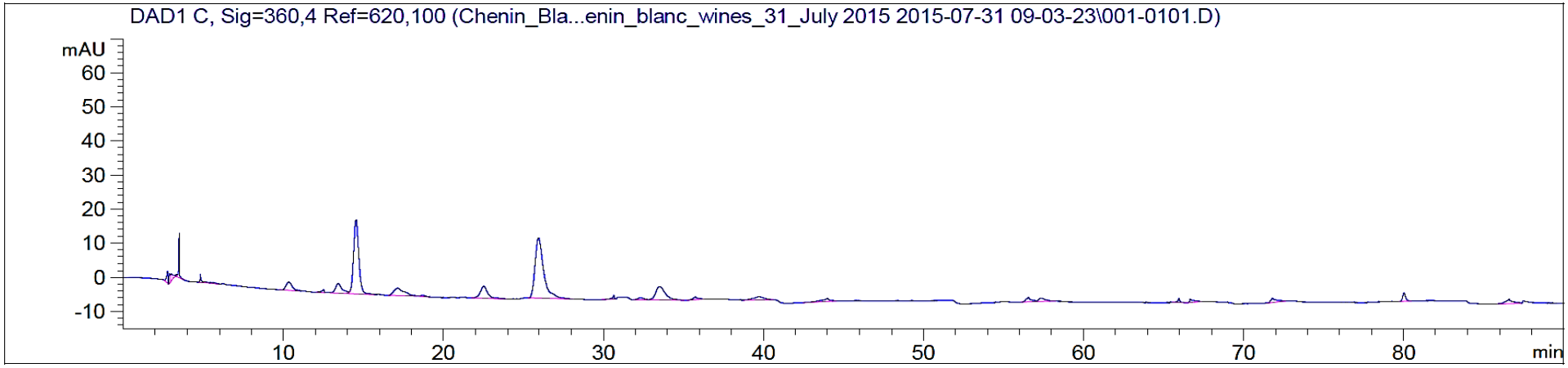


Figure 4.6. Chenin blanc wine HPLC-DAD chromatogram at 360 nm (no flavonol compounds detected)

4.3.1. Concentration of phenolic compounds in 2011 Chenin blanc wines

Results in Table 4.4 show significant differences between gallic acid and (+)-catechin concentrations in wines made with VIN13, 654 and M2/1 yeasts. Wines made with the combination of VIN 13 and 654 strains, were significantly different from wines made with VIN13 strain but showed correlation with wines made with 654 strain. This observation however was different for wines made with M2/1 strain. Wines made with M2/1 were significantly different from wines made with the combination of VIN13 and M2/1 and correlated with wines made with VIN13 strain. This could be due to the different fermentation rate between the *T. delbrueckii* strains and their compatibility with VIN13 strain during fermentation. No significant differences were observed in (-)-epicatechin and phenolic acid concentrations in this vintage.

Table 4.4. Yeast effect on the identified phenolic compounds in Chenin blanc wines made during 2011

Phenolic compounds	Yeasts treatments				
	VIN13	**654**	**M2/1**	**VIN13+654**	**VIN13+M2/1**
Flavan-3-ols					
Gallic acid	10.646^{b}	12.138^{a}	11.476^{ab}	10.741^{a}	10.712^{b}
(+)-Catechin	12.057^{a}	5.903^{c}	10.004^{ab}	6.915^{bc}	12.057^{a}
(-)-Epicatechin	5.645^{a}	6.922^{a}	5.706^{a}	6.288^{a}	6.110^{a}
EGCG[1]	ND[2]	ND	ND	ND	ND
Phenolic acids					
Caffeic acid	2.519^{a}	3.326^{a}	3.642^{a}	3.206^{a}	4.005^{a}
p-Coumaric acid	4.487^{a}	3.954^{a}	3.979^{a}	4.525^{a}	4.522^{a}
Ferulic acid	2.820^{a}	2.491^{a}	2.862^{a}	2.905^{a}	2.888^{a}
Flavonols					
Rutin	ND	ND	ND	ND	ND
Isoquercitrin	ND	ND	ND	ND	ND
Quercitrin	ND	ND	ND	ND	ND
Quercetin	BDL[3]	BDL	BDL	BDL	BDL

Superscript letters (a, b and c) next to the values indicate the significant differences between values.
[1]EGCG=Epigallo-3-catechin gallate; [2]ND=Not detected; [3]BDL=below detection limit

4.3.2. Concentration of phenolic compounds in 2012 Chenin blanc wines

There were no significant differences in wines made with VIN13 and M2/1 strains (Table 4.5) for gallic acid and (+)-catechin concentrations. The concentration of gallic acid and (+)-catechin was slightly lower in wines made with M2/1 strain compared to wines made with VIN13 strain. Significant differences were observed in wines made with *T. delbrueckii* strains. Wines made with 654 strain were lower in gallic acid and (+)-catechin concentration compared to wines made with M2/1 strain. Wines made with single strains, i.e. VIN13, 654 and M2/1 were not significant different in (-)-epicatechin concentration. The phenolic acid concentrations in the wines showed no significant difference between yeast strains. However, wines made with VIN13 had high caffeic and *p*-coumaric acid whereas wines made with M2/1 had high ferulic acid.

Table 4.5. Yeast effect on the identified phenolic compounds in Chenin blanc wines made during 2012

Phenolic compounds	Yeast treatments				
	VIN13	**654**	**M2/1**	**VIN13+654**	**VIN13 +M2/1**
Flavan-3-ols					
Gallic acid	12.279^{b}	15.519^{a}	13.574^{b}	13.458^{b}	12.760^{a}
(+)-Catechin	15.187^{a}	8.448^{b}	15.345^{a}	11.779^{b}	9.427^{b}
(-)-Epicatechin	9.304^{ab}	9.307^{ab}	8.900^{ab}	9.818^{b}	10.697^{a}
EGCG[1]	ND[2]	ND	ND	ND	ND
Phenolic acid					
Caffeic acid	11.829^{a}	11.155^{a}	12.982^{a}	12.700^{a}	13.891^{a}
p-Coumaric acid	7.078^{a}	5.707^{a}	6.098^{a}	6.994^{a}	6.907^{a}
Ferulic acid	2.929^{c}	2.659^{c}	3.805^{a}	3.297^{abc}	3.485^{ab}
Flavonols					
Rutin	ND	ND	ND	ND	ND
Isoquercitrin	ND	ND	ND	ND	ND
Quercitrin	ND	ND	ND	ND	ND
Quercetin	BDL[3]	BDL	BDL	BDL	BDL

Superscript letters (a, b and c) next to the values indicate the significant differences between values.
[1]EGCG=Epigallo-3-catechin gallate; [2]ND=Not detected; [3]BDL=below detection limit

4.3.3. Concentration of phenolic compounds in 2013 Chenin blanc wines

Significant differences were observed between the flavan-3-ol compounds quantified in wines made with pure culture yeast strains, i.e. VIN13, 654 and M2/1 strains (Table 4.6). Wines made with VIN 13 were lower in (+)-catechin but high in (-)-epicatechin and gallic acid. Wines made with 654 and VIN13+654 strains had higher concentration of gallic acid and (-)-epicatechin but low (+)-catechin. However, wines made with M2/1 and VIN13+M2/1 had high concentration of (+)-catechin, compared to wines made with 654 and VIN13+654 strains. Results show that VIN13 and 654 strains affected (-)-epicatechin and gallic acid whereas M2/1 strain affected (+)-catechin, in 2013. There were no significant differences observed in *p*-coumaric- and caffeic. Ferulic acid was high in wines made with VIN13.

Table 4.6. Yeast effect on the identified phenolic compounds in Chenin blanc wines made during 2013

Phenolic compounds	**Yeast treatments**				
	VIN13	**654**	**M2/1**	**VIN13+654**	**VIN13 +M2/1**
Flavan-3-ols					
Gallic acid	14.740^{a}	13.121^{ab}	13.116^{b}	14.706^{a}	13.647^{b}
(+)-Catechin	9.148^{c}	12.277^{ab}	15.884^{a}	10.055^{bc}	16.106^{a}
(-)-Epicatechin	15.996^{a}	12.875^{abc}	9.468^{c}	12.872^{ab}	10.910^{bc}
EGCG[1]	ND[2]	ND	ND	ND	ND
Phenolic acids					
Caffeic acid	8.628^{a}	10.373^{a}	11.881^{a}	9.226^{a}	10.406^{a}
p-Coumaric acid	6.781^{a}	6.506^{a}	6.556^{a}	6.546^{a}	6.592^{a}
Ferulic acid	5.139^{a}	3.139^{b}	3.646^{b}	3.581^{b}	3.408^{b}
Flavonols					
Rutin	ND	ND	ND	ND	ND
Isoquercitrin	ND	ND	ND	ND	ND
Quercitrin	ND	ND	ND	ND	ND
Quercetin	BDL[3]	BDL	BDL	BDL	BDL

Superscript letters (a, b and c) next to the values indicate the significant differences between values.
[1]EGCG=Epigallo-3-catechin gallate; [2]ND=Not detected; [3]BDL=below detection limit

Significant differences ($p < 0.05$) between the treatments were observed for all the identified compounds (Table 4.4-4.6) in wine made from grapes harvested from all three vintages except for hydroxycinnamic acids (HCA), *p*-coumaric acid and caffeic acid. These findings confirm the observation of Monagas *et al*. (2005) which showed that although HCA concentration is susceptible to change during winemaking, their concentration does not change significantly during storage. However, this observation is an exception to ferulic acid, as was found in this study. Wines made with M2/1 and wines made with VIN13+M2/1 proved higher in caffeic acid concentration, compared to other yeast treatments. It was also observed that wines made with VIN13 and a combination of *T. delbrueckii* strains *S. cerevisiae* were higher in *p*-coumaric acid concentration, compared to wines made with the *T. delbrueckii* only. Gallic acid was the predominant phenolic acid found in all wine samples and was present as an acyl of flavan-3-ols. High concentration of (+)-catechin and (-)-epicatechin were also noted. However, the gallate of catechin, i.e. epigallocatechin-3-gallate was not detected in any wines. The concentration of (+)-catechin in wines made with VIN13 and M2/1 strains was higher than in wines inoculated with strain 654. The difference in the concentrations of catechin indicates that there are strain differences (fermentation rate) within *T. delbrueckii* yeasts. Wines made from grapes harvested during 2013 showed differences in concentration from wines made from grapes harvested during 2011 and 2012 with regards to residual sugars. The concentration of (+)-catechin in wines made with 654 strain for 2013 vintage was higher than the wines made with the same yeast for vintage 2011 and 2012. This difference can be a result of the low residual sugar content measured in wines for this 2013 (section 4.1). The metabolic pathway of the yeast includes the excretion of a pectinase enzyme that breaks down the grape tissue for the extraction of phenolics (Manzanares, 2000). In 2013 most of the sugar was fermented by the yeasts, hence higher (+)-catechin concentrations in the wines.

4.4. Yeast effect on the phenolic compounds over three vintages

During wine ageing there are several chemical reactions which modify both the chemical and sensory characteristics of the wine (Balga *et al*., 2014). The effect of the yeast strains used during winemaking of Chenin blanc wines was the same for all vintages studied. Results show that wines made with VIN13 strain had higher total flavan-3-ol and phenolic acid content, compared to wines made with *T. delbrueckii* strains (654 & M2/1), see figures 4.7-4.8. The total phenolic content in wines made with VIN13 strain was comparable to the content of phenolics in wines made with M2/1 strain. However, wines made with 654 strain had the lowest phenolic content. Consequently, wines made with combination of VIN13 and M2/1 (VIN13+M2/1) had higher total phenolics compared to wines made with combination of VIN13 and 654 strains.

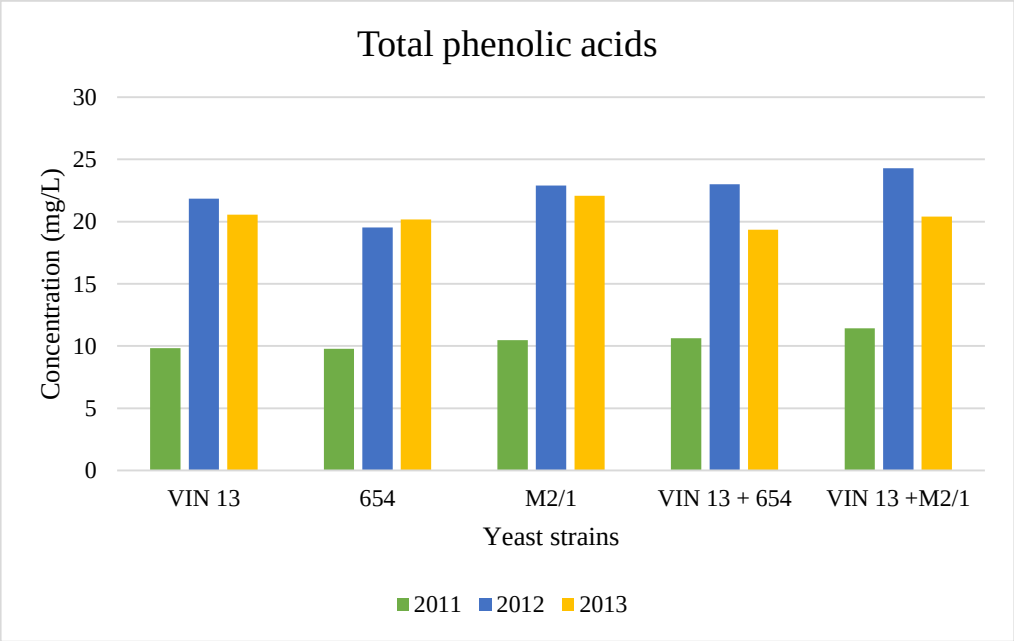


Figure 4.7. Vintage differences between total phenolic acid concentrations

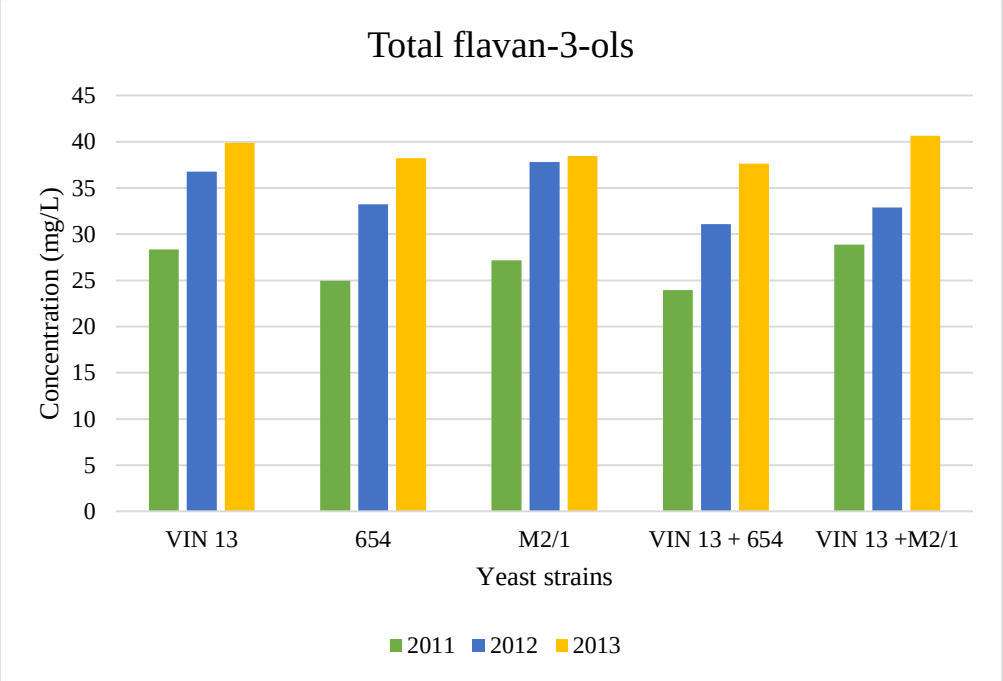


Figure 4.8. Vintage differences between total flavan-3-ol concentrations

4.5. Application of PCA on flavan-3-ols and phenolic acids data

4.5.1. Chenin blanc wines made during 2011

Principal component analysis (Fig. 4.9) for phenolic compound variables was applied to the percentage in weight of each compound in relation to the total content of the measured phenolics. Principal component yielded two principal components (PC1 and PC2) explaining 81.71% of the total variance in the two dimensions (F1 and F2) with 62.45% and 19.26% explained by PC1 and PC2, respectively. The bi-plots are an indication of a relationship between variables based on the angle between vectors (less than 90°). Figure 4.9 shows a high concentration of caffeic acid and gallic acid in wines made with M2/1 strain. Wines made with *T. delbrueckii* yeast are related to gallic acid, but 654 strain wines were higher in gallic acid concentration. Phenolic compounds (+)-catechin, *p*-coumaric acid and ferulic acid were highest in wines made with VIN13 but were low in all the compounds related to the *T. delbrueckii* strains. The clustering of wines made with VIN13 and wines made with combination yeast strains shows relationship between VIN13, VIN13+654 and VIN13+M2/1.

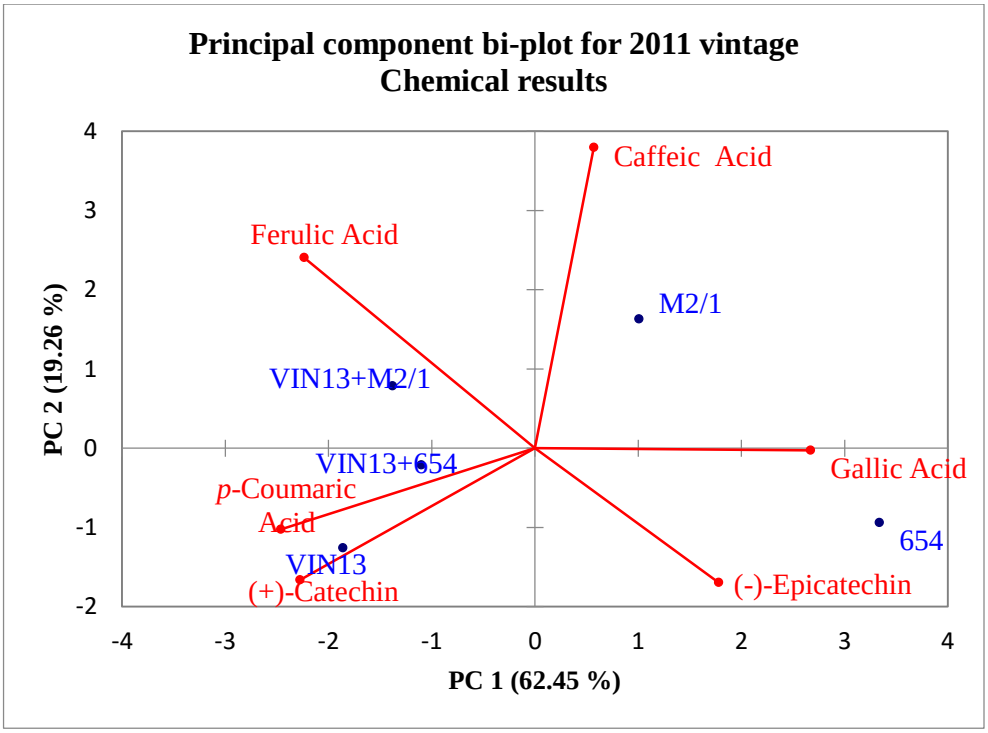


Figure 4.9. Vector diagram (bi-plot) of relative positions and loadings of six phenolic compound variables used in PCA for Chenin blanc wines made from grapes harvested at 21.6°B subjected to five different yeast treatments

4.5.2. Chenin blanc wines made during 2012

Principal component analysis (Fig. 4.10) for phenolic compound variables was applied to the percentage in weight of each compound in relation to the total content of the measured phenolics The total variance of the data explained for the vintage was 74.69% in the first two dimensions (F1 and F2) with 50.36% and 24.33% explaining PC1 and PC2, respectively. Wines made with VIN13 were highest in (+)-catechin and *p*-coumaric acid but lowest in gallic acid. However, wines made with 654 strain were lowest in concentration of (+)-catechin and *p*-coumaric acid. Wines made with combination of 654 and VIN13 strains correlated with wines made with VIN13. Ferulic acid, (-)-epicatechin and caffeic acid were high in wines made with VIN13+M2/1.

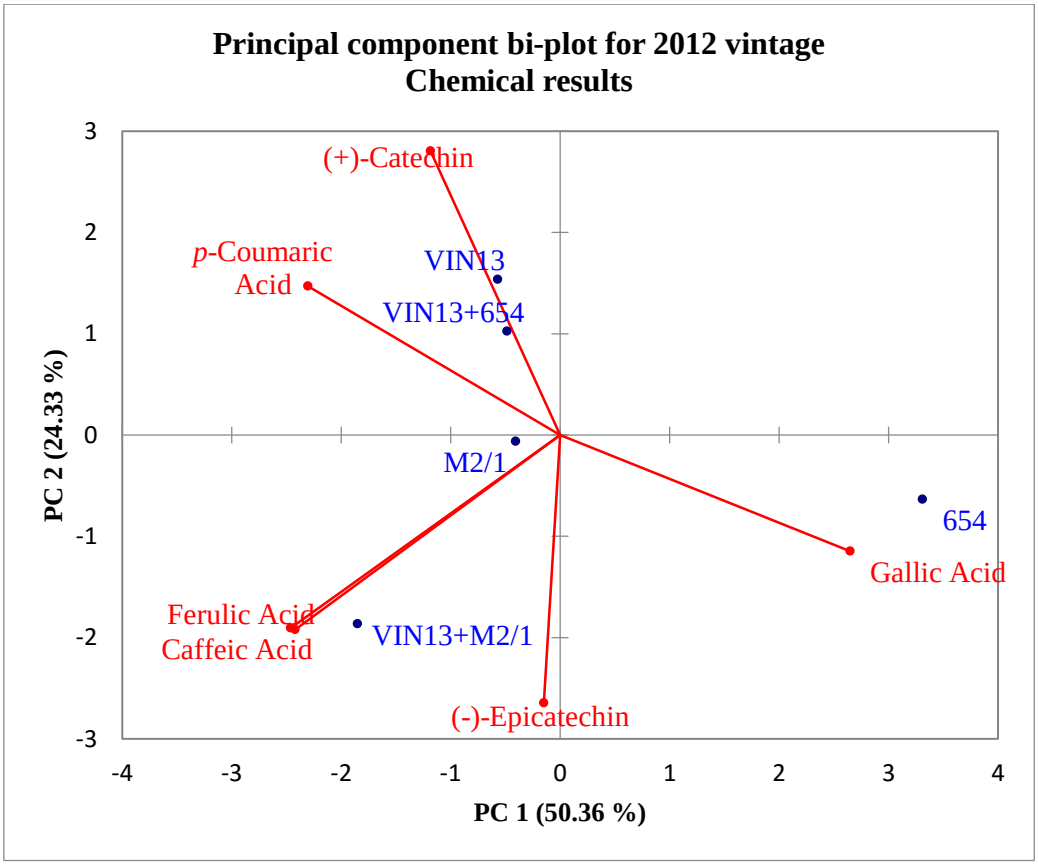


Figure 4.10. Vector diagram (bi-plot) of relative positions and loadings of six phenolic compound variables used in PCA for Chenin blanc wines made from grapes harvested at 20.4°B subjected to five different yeast treatments

4.5.3. Chenin blanc wines made during 2013

Principal component analysis (Fig. 4.11) for phenolic compound variables was applied to the percentage in weight of each compound in relation to the total content of the measured phenolics. Principal component analysis yielded two components in the first two dimensions (F1 and F2) with PC1 (81.18%) and PC2 (14.98%) explaining the total variance of 96.16%. The eigenvectors showed that VIN13+654 and VIN13+M2/1 had opposite correlations. Wines made with combination of VIN13 and 654 strains were high in (-)-epicatechin and gallic acid but lowest (+)-catechin and caffeic acid. However, wines made with VIN13+M2/1 strains were higher in caffeic acid and catechin but lower in (-)-epicatechin and gallic acid. Wines made with VIN13 were highest in ferulic and *p*-coumaric acid and were associated with high (-)-epicatechin and gallic acid concentrations.

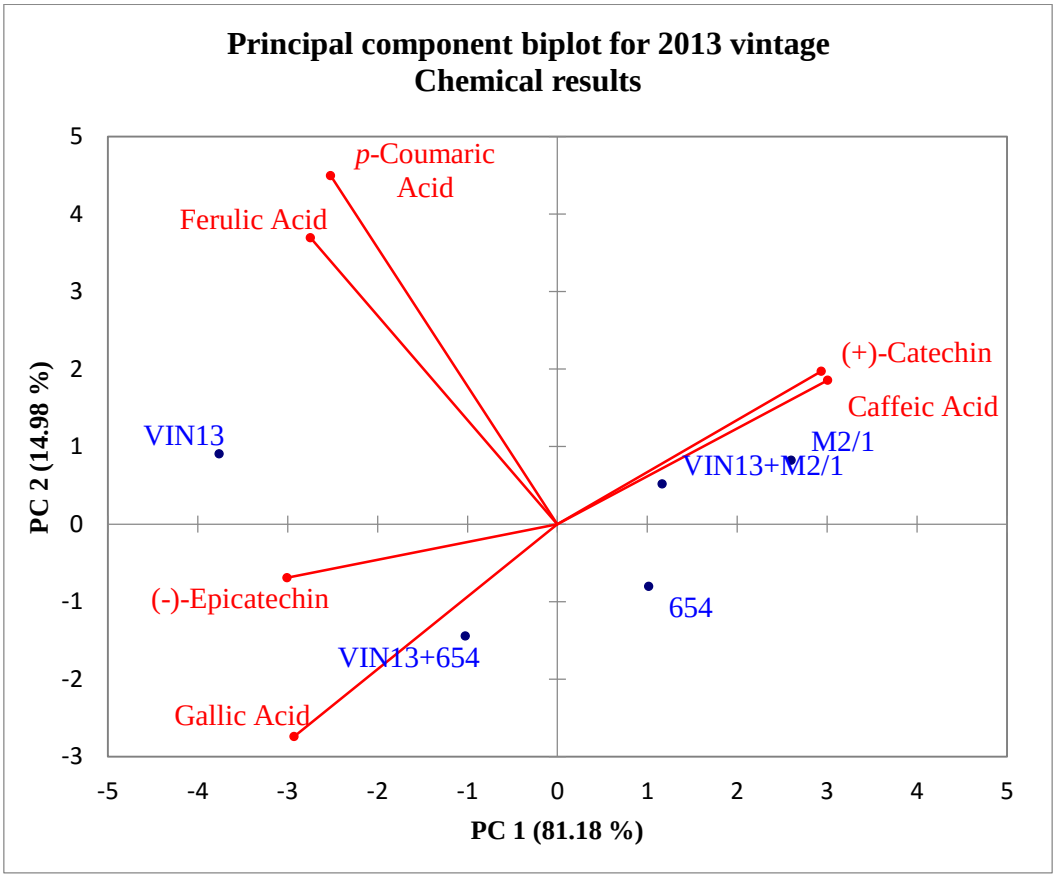


Figure 4.11. Vector diagram (bi-plot) of relative positions and loadings of six phenolic compound variables used in PCA for Chenin blanc wines made from grapes harvested at 20.5°B subjected to five different yeast treatments

Wines made with the VIN13 were high in *p*-coumaric acid, ferulic acid and (+) catechin for 2011 and 2012 vintages (Table 4.4-4.5). The high concentration of (+)-catechin in wines produced by *S. cerevisiae* strain compared to wines made with *T. delbrueckii* strain is due to the ability of *S. cerevisiae* yeast in excreting enzymes that can facilitate the extraction of flavan-3-ols from the grape must (Huynh *et al*., 2014; Arévalo-Villena, *et al*., 2011). The concentration of (+)-catechin in the wines vary with vintage, but it was observed that wines made with VIN13 were higher in (+)-catechin compared to wines made with 654, M2/1, VIN13+654 and VIN13+M2/1 yeast strains. Wines made with the combination of *S. cerevisiae* (VIN13) and *T. delbrueckii* (654 and/or M2/1) yeast strains had high concentrations of compounds associated with VIN 13, i.e. (+)-catechin, *p*-coumaric and ferulic acid, and were lower in compounds associated with the respective *T. delbrueckii* strains i.e. gallic acid and (-)-epicatechin. The aforementioned observation was made in wines made during 2011 and 2012. However, wines made with *T. delbrueckii* strains (654 and M2/1) were higher in (+)-catechin and caffeic acid compared to wines made with VIN13, VIN13+654 and VIN13+M2/1 in wines made during 2013. The measured residual sugar differences in wines made during 2013 and wines made during 2011 and 2012 could explain the different effect, the yeast have on the phenolic compounds.

4.6. Gas chromatography-flame ionisation detection (GC-FID)

Wine aroma consists of numerous volatile compounds; however, 17 major volatile compounds were quantitated in the studies wines. The quantified volatiles are affected by the yeast during winemaking (as discussed in literature) and simultaneously affect the sensory profile of wine. Analysis of variance was applied to the GC-FID data to establish significant differences between the treatments, i.e. different yeasts and yeast combinations used in Chenin blanc winemaking. Values ($p < 0.05$) at 95% confidence between the yeast treatments showed differences in volatile compounds. The concentrations of volatile compounds (Table 4.7), does not show notable differences between the vintages.

Esters cannot be interpreted as an isolated group of compounds because their formation and maturation is a dynamic process. Certain esters are known for their positive fruity aromas but they need to be in chemical equilibrium with their corresponding fatty acids and higher alcohols. An ethyl (acetate) ester was found as a main ester in Chenin blanc wines with levels less than 150 mg/L. Levels > 150 mg/L is indicative of spoilage character. Ethyl acetate ester is a product of acetic acid and ethanol interaction. A correlation between ethanol percentage and acetic acid was observed. There were minimal differences between ethanol percentages for all treatments and all three vintages as reported in Table 4.4. However, *T. delbrueckii* yeast strain M2/1 produced higher concentrations of acetic acid, compared to VIN13 yeast strain. This correlated with high concentration of acetyl acetate produced by M2/1 and 654 strains. Significant differences of acetic acid concentrations were observed between the *T. delbrueckii* yeast strains and the reference yeast (VIN13). Wines made from co-inoculated grape must had higher isoamyl and 2-phenyl acetate concentrations, compared to wines made from single inoculated grape must. Ciani & Comitini (2015), investigated the yeast to yeast interaction between *S. cerevisiae* and *T. delbrueckii*. They concluded that the interaction between the yeast species releases short chain fatty acids that react with ethanol which results in acetate esters production.

Ethyl esters are a product of long chain fatty acids and higher alcohols. There was no trend observed for ethyl esters. However, wines made during 2012 and 2013 showed significant differences between the treatments for most compounds. In contrast wines made during 2011 showed no significant differences between treatments in terms of volatile compounds. This could be due to the spontaneous hydrolysis

which leads to a decrease of ethyl esters overtime (Patrianakou & Roussis, 2013). Subsequently, the decrease of ethyl ester concentrations in wine during ageing and storage are related to different hydrolysis esterification equilibria, which could result in negligible differences between the effects of yeast strains. Higher alcohols showed increased concentrations in wines produced by *T. delbrueckii* yeast, with no significant difference between yeast strains. The volatile concentrations detected in the studied wines were similar to those reported by De Kock, (2015) except for methanol. However, the concentrations of methanol found were within the acceptable limit of 250 mg/L in white wine (OIV, 2011).

Table 4.7. Concentration of major volatile compounds quantifies in Chenin blanc wines (mg/L)

Volatile compounds	2011					2012					2013				
	Yeast treatments					Yeast treatments					Yeast treatments				
	VIN13	654	VIN13 +654	M2/1	VIN 13 +M2/1	VIN13	654	VIN 13 +654	M2/1	VIN 13 +M2/1	VIN13	654	VIN 13 +654	M2/1	VIN 13 +M2/1
Acetate esters															
Ethyl acetate	27.86^{a}	28.01^{a}	28.04^{a}	28.05^{a}	28.05^{a}	42.08^{a}	42.62^{a}	41.63^{a}	49.28^{a}	44.65^{a}	25.22^{b}	38.51^{a}	25.06^{b}	38.97^{a}	43.33^{a}
2-Phenyl acetate	2.762^{a}	2.743^{a}	2.739^{a}	2.737^{a}	2.736^{a}	2.971^{a}	2.630^{a}	2.933^{a}	2.898^{a}	3.265^{a}	2.139^{b}	2.129^{b}	2.245^{b}	1.846^{c}	3.616^{a}
Isoamyl acetate	0.638^{a}	0.644^{a}	0.646^{a}	0.646^{a}	0.646^{a}	1.387^{a}	0.961^{ab}	1.514^{a}	1.230^{a}	1.844^{a}	0.481^{ab}	0.419^{b}	0.543^{a}	0.375^{b}	0.495^{a}
Hexyl acetate	ND[1]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl esters															
Ethylphenyl acetate	1.149^{a}	1.149^{a}	1.149^{a}	1.149^{a}	1.149^{a}	1.161^{a}	1.162^{a}	1.156^{a}	1.162^{a}	1.158^{a}	2.139^{b}	2.129^{b}	2.245^{b}	1.845^{c}	3.617^{a}
Ethyl caprate	0.208^{a}	0.206^{a}	0.205^{a}	0.204^{a}	0.205^{a}	0.157^{a}	0.145^{a}	0.167^{a}	0.159^{a}	0.159^{a}	0.148^{ab}	0.136^{ab}	0.156^{a}	0.126^{b}	0.149^{a}
Ethyl caprylate	0.942^{a}	0.922^{a}	0.918^{a}	0.916^{a}	0.916^{a}	0.858^{ab}	0.706^{b}	0.891^{a}	0.707^{b}	0.906^{a}	0.561^{b}	0.594^{bc}	0.405^{bc}	0.405^{c}	0.674^{a}
Ethyl butyrate	0.503^{a}	0.502^{a}	0.502^{a}	0.502^{a}	0.502^{a}	0.493^{a}	0.493^{a}	0.496^{a}	0.499^{a}	0.544^{a}	0.410^{b}	0.597^{a}	0.432^{b}	0.588^{a}	0.612^{a}
Ethyl-3-hydroxybutanoate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.578^{a}	1.543^{a}	1.561^{a}	1.498^{b}	1.221^{c}
Diethyl succinate	6.896^{a}	6.756^{a}	6.721^{a}	6.710^{a}	6.710^{a}	3.079^{a}	2.857^{a}	2.932^{a}	2.505^{a}	2.828^{a}	2.523^{a}	2.115^{a}	2.511^{a}	2.088^{a}	3.357^{a}

Superscript letters (a, b and c) indicate the significant differences between treatments within season, according to least significant figures (p<0.05); [1]ND=Not detected

Table 4.7. Concentrations of major volatile compounds quantified in Chenin blanc wines (mg/L) (Continued)

Volatile compounds	2011					2012					2013				
	Yeast treatments					Yeast treatments					Yeast treatments				
	VIN13	654	VIN13 +654	M2/1	VIN13 +M2/1	VIN13	654	VIN13 +654	M2/1	VIN13 +M2/1	VIN13	654	VIN13+ 654	M2/1	VIN13 +M2/1
Higher alcohols															
Isobutanol	18.90^{a}	18.94^{a}	18.95^{a}	18.95^{a}	18.95^{a}	31.94^{a}	17.79^{b}	34.83^{a}	17.32^{b}	34.92^{a}	19.91^{a}	42.42^{a}	19.88^{a}	43.05^{a}	46.11^{a}
Propanol	39.55^{a}	39.84^{a}	39.92^{a}	39.94^{a}	39.94^{a}	14.62^{ab}	24.16^{a}	14.70^{ab}	19.74^{ab}	13.47^{b}	ND	ND	ND	ND	ND
Isoamyl alcohol	153.3^{a}	152.9^{a}	152.8^{a}	152.8^{a}	152.8^{a}	118.8^{a}	140.0^{a}	122.2^{a}	123.5^{a}	120.2^{a}	141.4^{b}	141.8^{b}	137.1^{b}	141.2^{b}	190.2^{a}
Butanol	1.118^{a}	1.123^{a}	1.124^{a}	1.124^{a}	1.124^{a}	1.440^{ab}	0.737^{b}	1.185^{ab}	0.866^{b}	1.260^{a}	0.774^{b}	0.595^{c}	0.783^{a}	0.562^{c}	0.933^{a}
Methanol	51.57^{a}	52.72^{a}	52.74^{a}	52.76^{a}	52.76^{a}	40.58^{a}	41.02^{a}	41.18^{a}	39.99^{a}	39.04^{a}	0.873^{a}	0.873^{a}	0.875^{a}	0.876^{a}	0.258^{b}
Pentanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acetic acid	141.2^{a}	141.6^{a}	141.8^{a}	141.8^{a}	141.8^{a}	133.6^{b}	211.7^{a}	132.4^{b}	230.9^{a}	129.4^{b}	126.3^{c}	217.0^{ab}	128.3^{c}	200.4^{b}	264.2^{a}

Superscript letters (a, b and c) indicate the significant differences between treatments within season, according to least significant figures (p<0.05); [1]ND=Not detected

4.7. Sensory evaluation of South African Chenin blanc wines

4.7.1. Treatment effect on sensory attributes of Chenin blanc wines made during 2011

Sensory data was analysed using ANOVA to establish significant differences between yeast strains (Table 4.8). Significant differences between treatments were observed in astringency, acidity and flavour but there were no significant differences in body and complexity attributes. Wines made with VIN13 scored high in astringency and were significantly different from wines made with 654 and M2/1 strains. Wines made with the combination of *S. cerevisiae* and *T. delbrueckii* strain(s) (VIN13+654 and VIN13+M2/1), scored high in acidity and were significantly different from wines made with single strains (VIN13, M2/1 and 654). However, there were no significant differences between wines made with *T. delbrueckii* yeast strains.

Table 4.8. Comparison of sensory attributes in Chenin blanc wines made during 2011

Sensory attributes	2011				
	Yeast treatments				
	VIN13	654	M2/1	VIN 13 + 654	VIN 13 + M2/1
Flavour	54.077^{c}	63.577^{a}	60.115^{b}	54.846^{c}	54.846^{a}
Body	54.385^{a}	56.231^{a}	55.345^{a}	55.038^{a}	53.577^{a}
Astringency	25.538^{a}	14.731^{b}	15.192^{b}	23.923^{a}	24.769^{a}
Acidity	45.385^{ab}	37.038^{b}	41.462^{b}	52.962^{a}	53.115^{a}
Complexity	45.154^{a}	53.077^{a}	53.038^{a}	49.122^{a}	48.769^{a}

Different superscript indexes a and b on the same line indicate significant difference between the different treatments according to least significant figures (p<0.05)

4.7.2. Treatment effect on sensory attributes of Chenin blanc wines made during 2012

There were no significant differences between the yeast strains for all measured sensory attributes in wine made during 2012 (Table 4.9). However, wines made with 654 strain scored higher in both body and complexity compared to wines made with M2/1, VIN13 and combination thereof. Astringency and body were lower in wines made with *T. delbrueckii* yeast strains (654 and M2/1) compared to wines made with *S. cerevisiae* strain (VIN13). The low stringency in wines made with *T. delbrueckii* yeast strains shows that astringency is associated with *S. cerevisiae* yeast strain because wines made with *S. cerevisiae* and wines made with the combination strains (VIN13+654 and VIN13+M2/1), scored higher than wines made with respective *T. delbrueckii* strains.

Table 4.9. Comparison of sensory attributes in Chenin blanc wines made during 2011

Sensory attributes	2012				
	Yeast treatments				
	VIN13	654	M2/1	VIN 13 + 654	VIN 13 + M2/1
Flavour	53.179[a]	56.974[a]	55.564[a]	54.590[a]	52.385[a]
Body	50.744[a]	52.000[a]	51.897[a]	53.564[a]	50.795[a]
Astringency	21.436[a]	18.769[a]	19.026[a]	21.333[a]	20.462[a]
Acidity	49.615[a]	47.667[a]	45.925[a]	45.564[a]	46.056[a]
Complexity	48.128[a]	50.103[a]	49.164[a]	47.239[a]	44.677[a]

Different superscript indexes a and b on the same line indicate significant difference between the different treatments according to least significant figures ($p<0.05$)

4.7.3. Treatment effect on sensory attributes of Chenin blanc wines made during 2013

Significant differences in astringency were observed between wines made with *S. cerevisiae* strain (VIN13) and wines made with *T. delbrueckii* strains (654 and M2/1). However, there was no significant difference in astringency scores between wines made with *T. delbrueckii* strains. Wines made with VIN13 scored higher in astringency compared to wine made with 654 and M2/1 strain(s). The lower scores of astringency in wine made with *T. delbrueckii*, suggests species variability between *S. cerevisiae* strain and *T. delbrueckii* strains in extracting phenolic compounds from grape must. Wines made with *S. cerevisiae* had higher scores of flavour, acidity and complexity. However, there were no significant differences between the yeast strains for flavour and complexity.

Table 4.10. Comparison of sensory attributes in Chenin blanc wines made during 2013

Sensory attributes	2013				
	Yeast treatments				
	VIN13	654	M2/1	VIN13+654	VIN13+M2/1
Flavour	55.265[a]	54.280[a]	54.947[a]	53.611[a]	52.551[a]
Body	56.573[a]	53.966[ab]	54.838[ab]	54.932[ab]	50.885[b]
Astringency	25.496[a]	22.641[ab]	23.863[ab]	20.921[b]	24.060[ab]
Acidity	53.893[a]	51.900[a]	52.543[a]	51.876[a]	51.543[a]
Complexity	48.420[a]	46.816[a]	48.120[a]	48.316[a]	47.274[a]

Different superscript indexes a and b on the same line indicate significant difference between the different treatments according to least significant figures ($p<0.05$)

4.8. Yeast effect on sensorial attributes over three vintages

Sensory evaluation can be subjective. However, most decisions with regards to wine quality rely on the sensory evaluation as it consequently affects the economic implication of wine. Sensory evaluation is also a final measurement used for judging the winemaking method by winemakers. There are a number of sensory characteristics in wine evaluation however, this study focused mainly on the attributes that are mostly affected by yeast. Most key phrases used to describe the flavour of wine are floral, fruity and nutty, etc. Wines made with *T. delbrueckii* strain had high scores in flavour for all studied vintages and were also high in acetate ester concentration (Table 4.7). the higher scores of flavour in wines made with *T. delbrueckii* (654 and M2/1) compared to *S. cerevisiae* yeast (VIN13) wines could be a result of species variations.

The body of wine which is often referred to as viscosity of wine in the mouth was also measured. The wines' body is mainly affected by the percentage alcohol, higher percentage alcohol results full bodied wines (Gawel *et al*., 2007). Wines under 12.5% alcohol content are known to be light to medium bodied. The measured alcohol content in all wines was less than 12.5%. The wines scored less than 60% in body. Literature states that wines with higher ethanol percentage are more bodied (Gawel *et al*., 2007) which was true for wines made in 2013, for all treatments. In contrast to literature, the study showed wines made with 654 and M2/1 high in body, in 2011 and 2012. This suggest that even though alcohol is primary responsible for the body of wine other metabolites such as glycerol can also affect the body of wine. Acidity scores in the wines was 45-50%. This shows that the studied wines were balanced. However, the different treatments used during winemaking affected the acidity scores. Wines made with *T. delbrueckii* yeast strain had lower acidity than *S. cerevisiae* and co-inoculated wines.

4.9. Analysis of sensorial attribute variables using principal component analysis

4.9.1. Chenin blanc wines made during 2011

Principal component analysis for sensory attribute variables (Fig. 4.12) was applied to the percentage in weight of each sensory attribute in relation to the total number of attributes measured. Principal component analysis explained 95.01% of the total variance in the data through the first two dimensions (F1 and F2), with 85.88% and 9.13% explained by PC1 and PC2 respectively. A correlation between wines made with *T. delbrueckii* strains was observed. The wines (654 and M2/1) scored high in flavour and body attributes. However, wines made with combination of *S. cerevisiae* and *T. delbrueckii* strains (VIN13+654 and VIN13+M2/1) scored high in astringency and acidity.

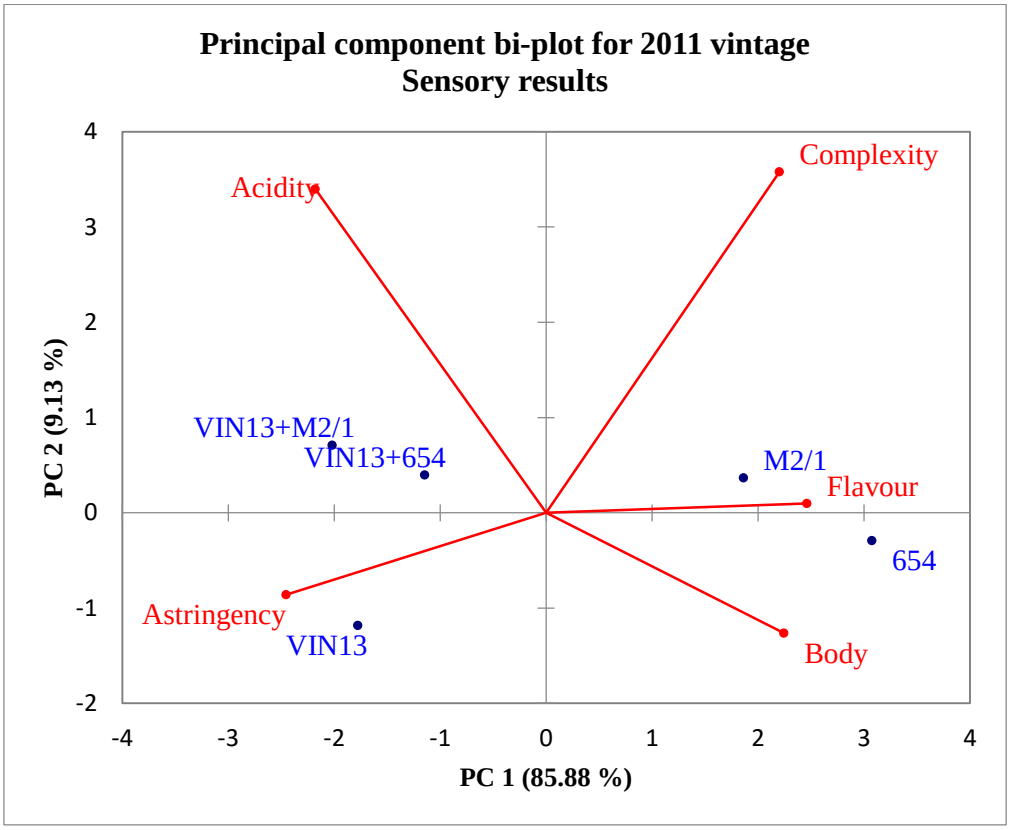


Figure 4.12. Vector diagram (bi-plot) of relative positions and loadings of five sensory attribute variables used in PCA for Chenin blanc wines subjected to five yeast treatments

4.9.2. Chenin blanc wines made during 2012

Principal component analysis for sensory attribute variables (Fig. 4.13) was applied to the percentage in weight of each sensory attribute in relation to the total number of attributes measured. Principal component analysis explained 80.55% of the total variance in the data through the first two dimensions (F1 and F2), with 49.60% and 30.95% explained by PC1 and PC2, respectively. Astringency and acidity were scored high in wines made with VIN 13 and wines made from combination of VIN13 and 654 strains (VIN13+654) whereas wines made with 654 yeast strain scored high in flavour and complexity attributes. Wines made with the combination of VIN13 and M2/1 strain (VIN13+M2/1) and wines made with M2/1 strain were low in all measured attributes.

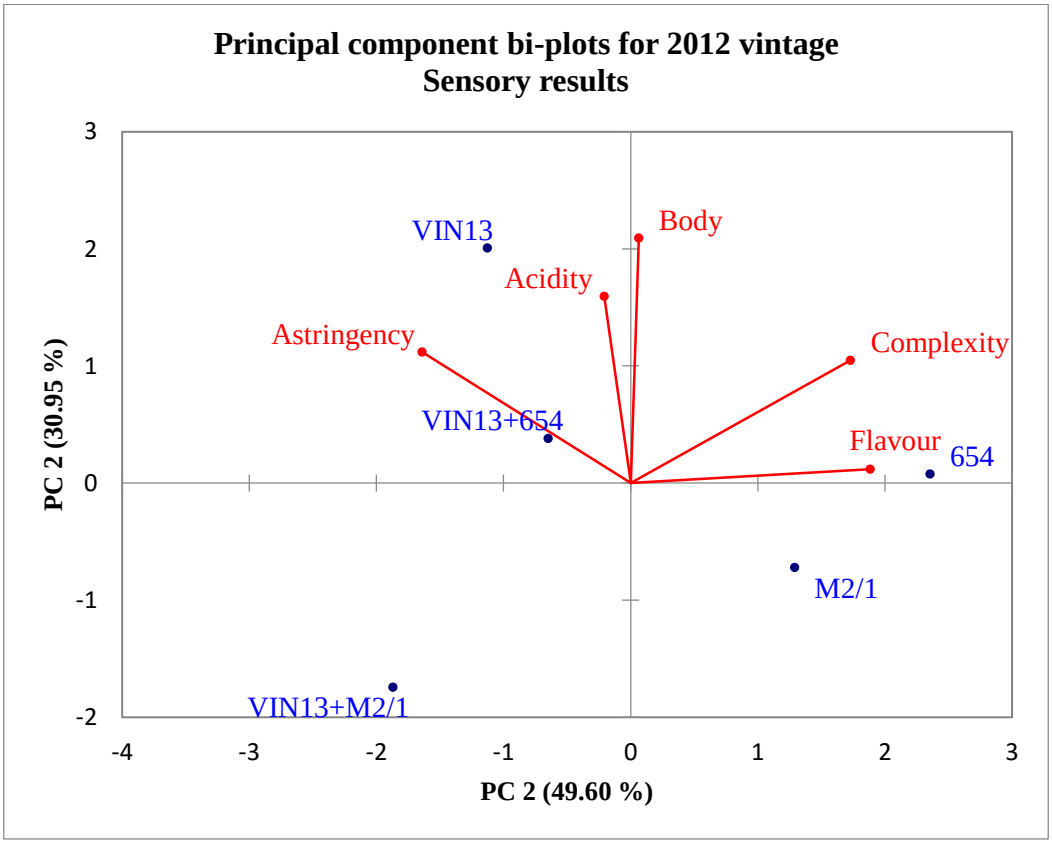


Figure 4.13. Vector diagram (bi-plot) of relative positions and loadings of five sensory attribute variables used in PCA for Chenin blanc wines subjected to five yeast treatments

4.9.3. Chenin blanc wines made during 2013

Principal component analysis for sensory attribute variables (Fig. 4.14) was applied to the percentage in weight of each sensory attribute in relation to the total number of attributes measured. Principal component analysis explained 78.03% of the total variance in the data through the first two dimensions (F1 and F2), with 56.59% and 21.44% explained by PC1 and PC2, respectively. Wines made with M2/1 and VIN13 strains scored high in acidity, body and astringency. However, wines made with combination of VIN13 and M2/1 strains (VIN13+M2/1) scored low in acidity and body. Wines made with 654 strain were low in all measured attributes.

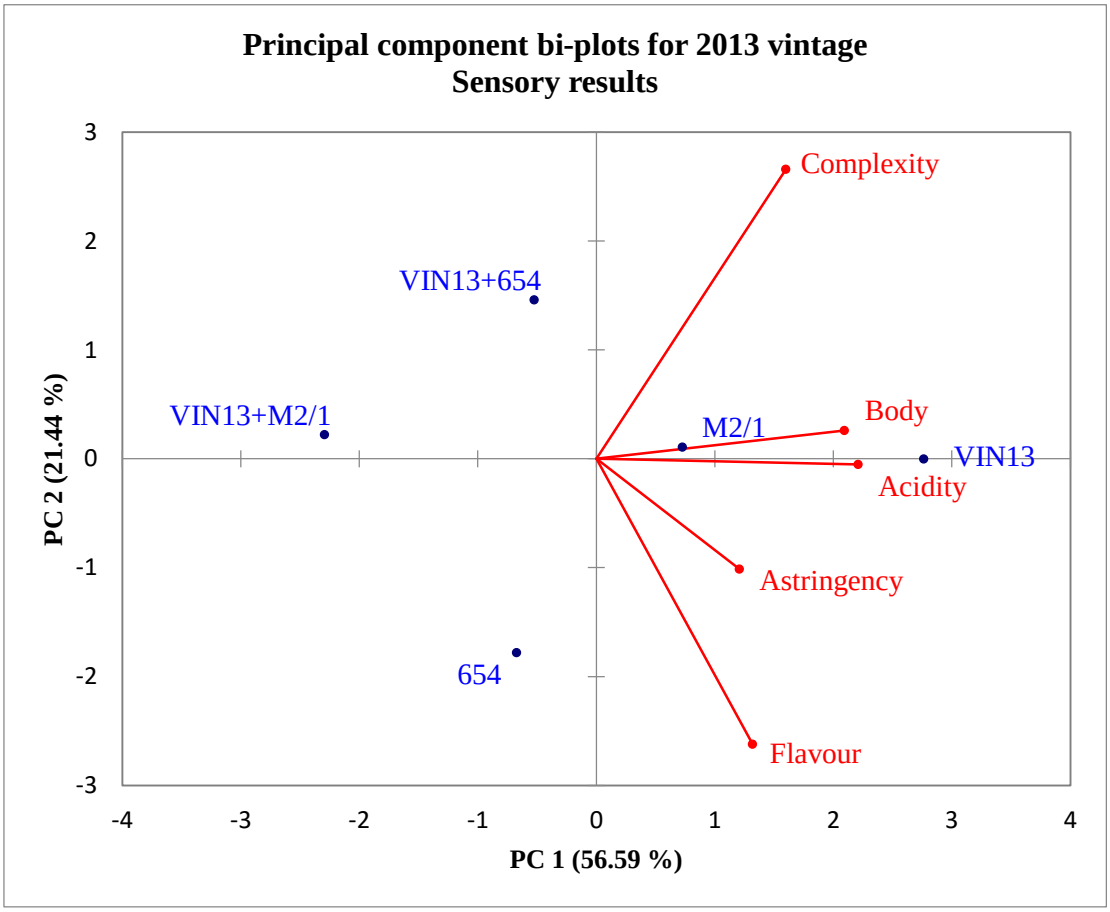
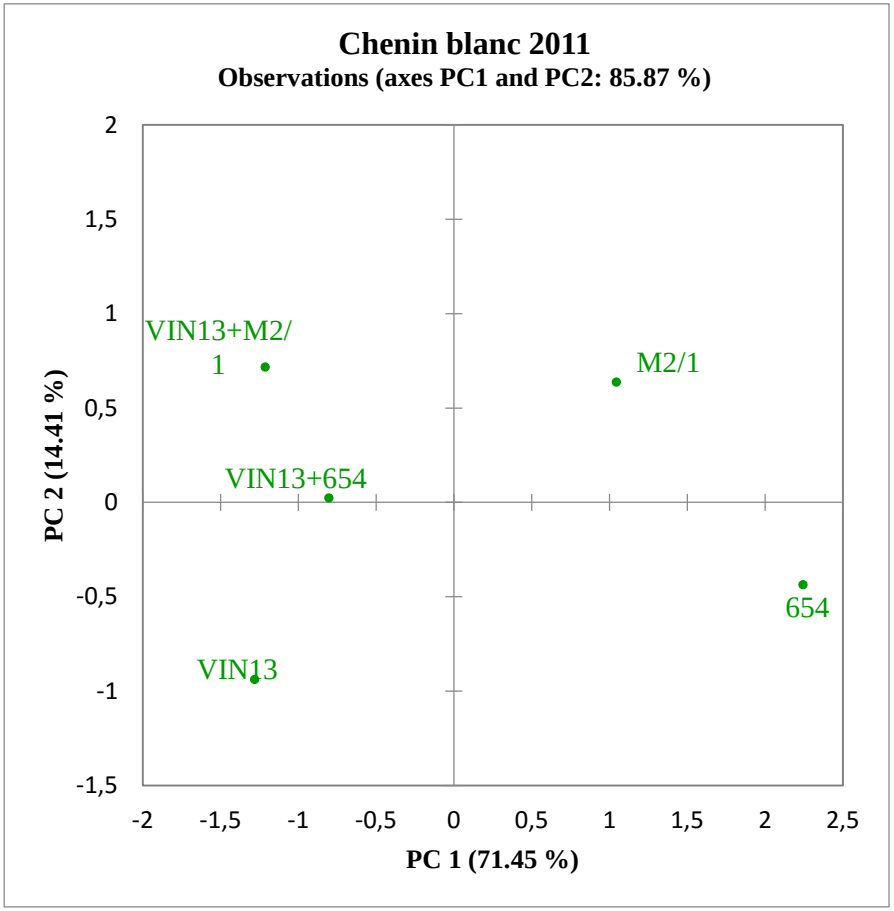


Figure 4.14. Vector diagram (bi-plot) of relative positions and loadings of five sensory attribute variables used in PCA for Chenin blanc wines subjected to five yeast treatments

A trend was observed in the PCA bi-plots of wines made during 2011 and 2012. Wines made with *T. delbrueckii* yeast strains (654 and M2/1) scored highest in body and flavour attributes but lowest in astringency. Conversely wines made with *S. cerevisiae* (VIN13) and wines made with combination of *S. cerevisiae* and *T. delbrueckii* strains (VIN13+654 and VIN13+M2/1) showed prominence in astringency. *Saccharomyces cerevisiae* (VIN13) yeast showed a contribution to the astringency attribute. This observation proves that differences between yeast species exist and similarities between yeast strain. It also shows the different contribution made by yeast species and/or yeast strains to the overall quality of wine. Wines made during the 2013 vintage, were different in this regard. There were correlations between wines made with VIN13 and M2/1 yeast strains. The wines showed prominence in acidity, body and astringency attributes. There are differences in the metabolites produced by *S. cerevisiae* yeast strain and *T. delbrueckii* yeast as their fermentation rates are different (Velázquez *et al*., 2015; Bely *et al*., 2008), however wines made with these yeast during 2013 scored similar sensory in the measured attributes.

4.10. Correlation between chemical and sensory data

Multi-factor analysis (MFA) applied on the chemical and sensory data showed no correlation between the sensory and chemical variables, and the effect of yeast on the quantified phenolic compounds. The MFA for phenolic compound variables and sensory attribute variables was applied to the percentage in weight of each compound in relation to the total content of the measured variables. The total variance explained for the studied vintages was more than 70%. Chemical and sensory results obtained have shown differences between wines made during 2011 and 2012 and wines made during 2013 vintage. The results obtained also showed similar observations on the MFA plots. The MFA plots for 2011 and 2012 (Fig. 4.15-4.16) showed clustering of wines made with *T. delbrueckii* (left A/C quadrant). The wines made with VIN13 yeast strain also clustered with wines made with a combination of *S. cerevisiae* and *T. delbrueckii* yeast strains (right B/D quadrants). Wines made with combination of yeast strain, i.e. VIN13+654 and VIN13+ M2/1 and wines made with VIN13 yeast strain only, were correlated with acidity and astringency whereas wines made with *T. delbrueckii* yeast strain (654 and M2/1) were correlated with body, complexity and flavour attributes. However, there were no correlations between the yeast treatments used, chemical data and sensory attributes except, for high (+)-catechin in wines made with VIN13 strain, which resulted to high astringency scores.



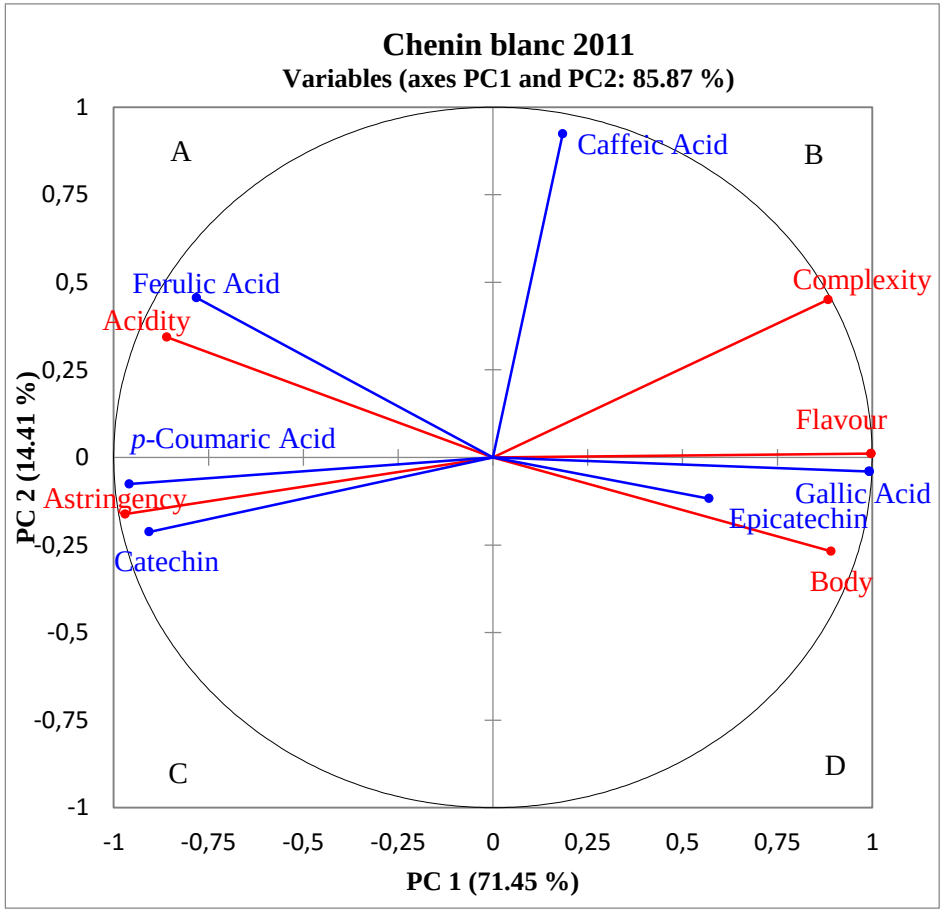


Figure 4.15 . Multi factor analysis bi-plots for Chenin blanc wines made during 2011, correlating the effect of the yeast(s) on the chemical (blue) and sensory (red) results obtained

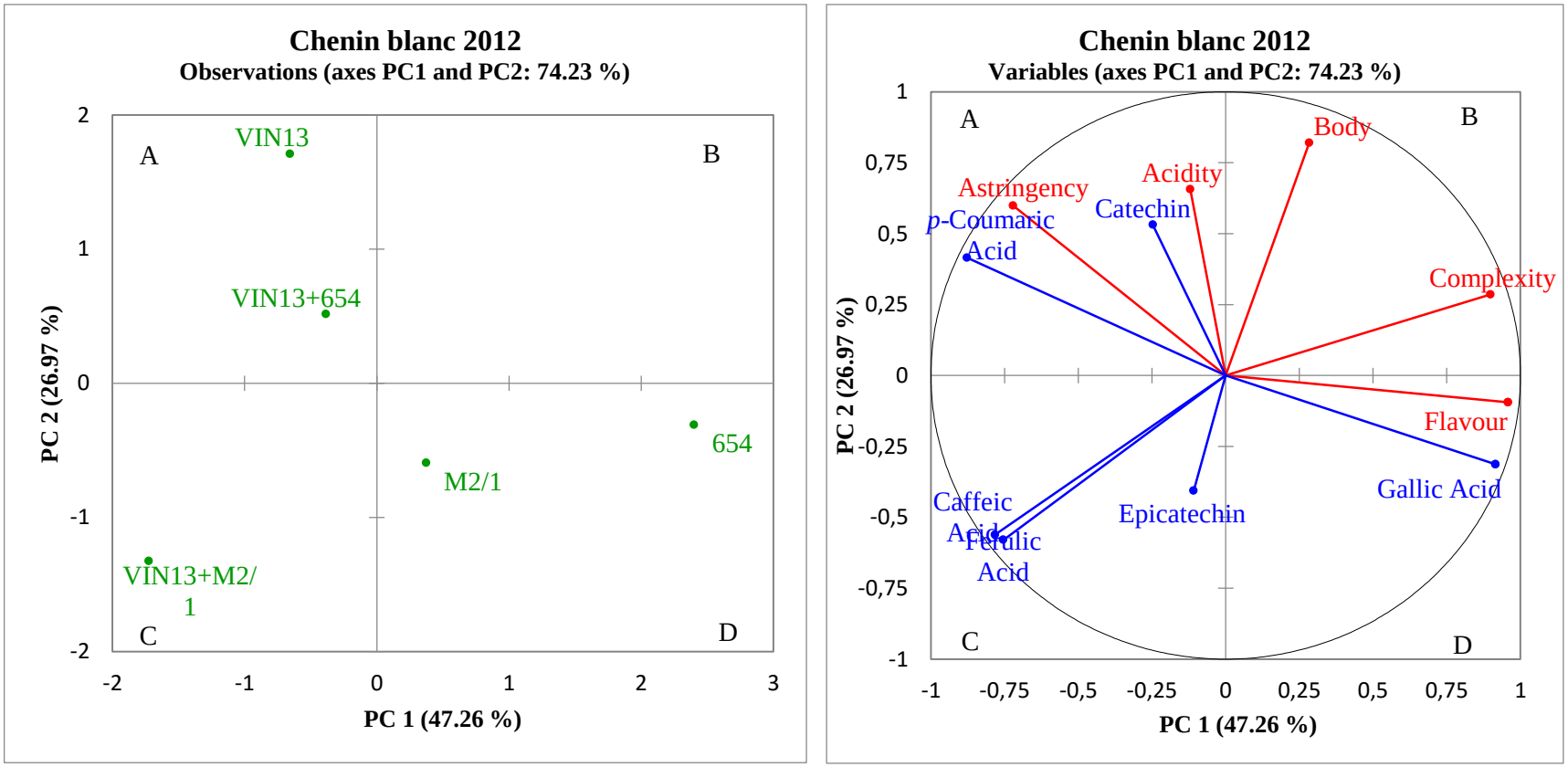


Figure 4.16. Multi factor analysis bi-plots for Chenin blanc wines made during 2012, correlating the effect of the yeast(s) on the chemical (blue) and sensory (red) results obtained

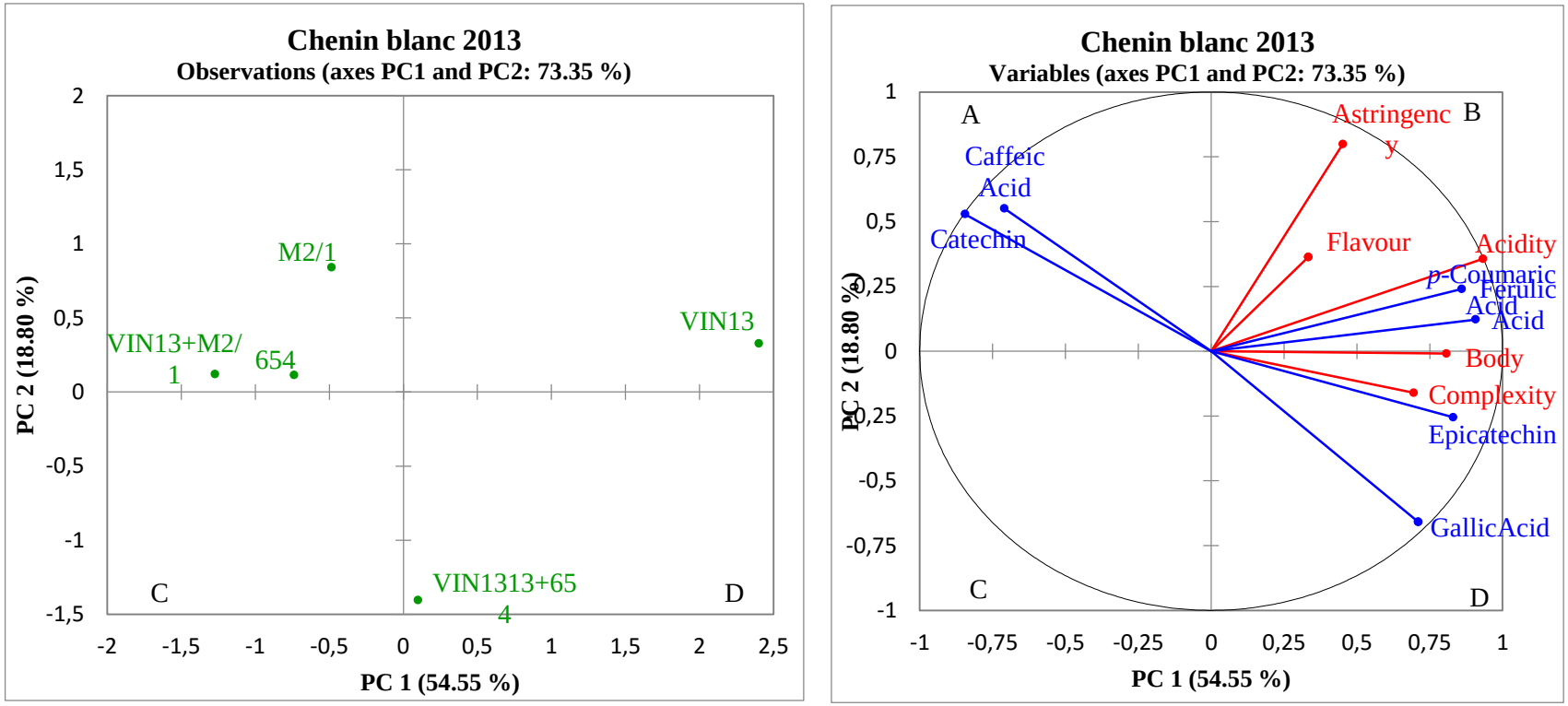


Figure 4.17. Multi factor analysis bi-plots for Chenin blanc wines made during 2013, correlating the effect of the yeast(s) on the chemical (blue) and sensory (red) results obtained

4.11. General Discussion

Caridi *et al*. (2004) showed that *S. cerevisiae* contributed to the decrease of phenolic compounds in wine, due to the modification of the cell wall. Mannoproteins found in the cell wall contain phosphate, pyruvate or glucuronic acid with a negative charges electrostatic. Therefore, positively charged phenolics, specifically anthocyanins will be attracted, and thus adsorbed by the yeast cell. This phenomenon was not observed in this study because phenolics found in white wines are neutral.

The results obtained in this study showed that the yeast strain does affected the final phenolic concentration in the finished wines. Phenolics are part of the genetics of the grape cultivar (Downey *et al*., 2004), the yeast used to inoculate grape must produces enzymes (during their multiplication) that breaks down the grape skin cell wall for extraction of the phenolics (Huynh *et al*., 2014; König *et al*., 2009). This is done through hydrolysis of the ester bond that link phenolics to the cell wall of the plant. The release of enzymes by the yeast also catalyses the breakage of the glycosidic bond that release phenolics in free form. *βeta*-glucosidase is the enzyme known for this reaction. Vernocchi *et al*. (2011) has shown that *β*-glucosidase activity is endowed in non-*Saccharomyces* yeast but not in *S. cerevisiae* yeast. This enzyme is also sensitive to the presence of high glucose concentration during fermentation. Wines made with *T. delbrueckii* yeast strains during 2011 and 2012 had low concentration of flavan-3-ols and high residual sugar concentration, i.e. greater than 2 g/L (see section 4.1). The low concentration of flavan-3-ols could be due to the low fermentation rate of the yeast which resulted in high residual sugar in the wines. The total soluble solids of the musts were similar for studied vintages (Fig 4.1), however the fermentation rate of *T. delbrueckii* strains was different for the three vintages. *Torulaspora delbrueckii* strains had a higher fermentation rate in 2013. This can be attributed to different environmental conditions during fermentation. The multiplication of a yeast strain during fermentation therefore results in improved enzyme activity, which results in better extraction of phenolics.

The phenolic profile of *Vitis vinifera* spp. is genetically establish (De Beers *et al*., 2005; Downey *et al*., 2004). However, it is well known that a yeast can affect the concentration of phenolics in wine, but limited information is available on the individual phenolics found in South African Chenin blanc wine. This study focused on the effect *T. delbrueckii* yeast strain has on the phenolics concentration in Chenin blanc wines. Phenolics that were not affected by the yeast include hydroxycinnamic acids caffeic acid and *p*-coumaric acid. These findings confirm the observation of Monagas *et al*. (2005), which showed that although HCA concentrations can change during winemaking, their concentration does not change significantly during storage.

It was noted that the fermentation rate of a yeast strain is directly proportional to the extraction of phenolic from the grape must. *Saccharomyces cerevisiae* yeast is known for a higher fermentation rate, compared to *T. delbrueckii* (Lea & Piggott, 1995). Therefore, wines made with *S. cerevisiae* had higher concentration of flavan-3-ol compounds, compared to wines made with *T. delbrueckii* yeast, in 2011 and 2012. However, the oenological parameters result in section 4.1 showed that *T. delbrueckii* strains M2/1 had higher fermentation rate in 2013, compared to 2011 and 2012. The fermentation rate of *T. delbrueckii* strain affected the phenolic concentration in wines made during 2013. Wines made with M2/1 strain had high flavan-3-ol concentration. Therefore, the high fermentation rate of *T. delbrueckii* yeasts strain (M2/1), resulted in increased concentration of (+)-catechin, caffeic acid, (-)-epicatechin and gallic acid in the wines.

The most common phenolic compounds found in white wine were also present in South African Chenin blanc wines produced by the different yeast but the concentrations varied. The concentrations of phenolics in the Chenin blanc wines in this study were higher than the concentration reported in the literature (Makris *et al*., 2006; Frankel *et al*., 1995). Viticultural practices, soil type, precipitation, prevailing wind, diurnal temperatures as well as winemaking processes have an effect on the phenolic concentrations in the grape (Hunter & Bonnard, 2011; Rodríguez-Montealegre *et al*., 2006). Phenolic compounds in wines are known to increase during fermentation but decrease during finning, maturation and aging because they tend to precipitate with proteins and yeast hulls (Ali *et al*., 2010). Concentrations of phenolic compounds quantified in this study did not differ among vintages. However, wines made

during 2013, vintage had highest concentrations of all identified compounds. Wines made during 2012 vintage had higher concentrations of phenolics compared to wines made during 2011 vintage. However, these concentrations are a representation of phenolics in the finished wine. This study did not include identification of phenolics during fermentation and finning processes.

The study of volatile compounds showed that the final aromatic composition of wine is dependent on the yeast strain. The formation of volatile compounds is linked to yeast metabolism, which is characterised by different enzyme activities. Literature search has shown that non-*Saccharomyces* yeasts have a higher hydrolytic activity, compared to most *Saccharomyces* yeast. This activity is responsible for releasing volatiles such as higher alcohols, esters, acids, and carbonyl compounds (Sadineni *et al*., 2012; Strauss *et al*., 2001). However, the concentration of volatile compounds produced by *S. cerevisiae* yeasts strains and *T. delbrueckii* yeasts strains used in this study did not prove to be significantly different in concentration for all wines studied over the three vintages.

Wine sensorial evaluation in the wine industry is important and is used to ensure that the wine reflect the typical characteristics of the grape cultivar it is made from. Judging the quality of wine is subjective while sensory evaluation is objective. Sensorial attributes in wine are a result of the chemical composition of the wine, e.g. phenolics, glycerol and acids. The same yeast strains (VIN13, 654, VIN13+654, M2/1 and VIN13+M2/1) were used for production of Chenin blanc wines for all the studied vintages. There was however difference in winemaking processes during 2013 vintage. Wines made with VIN13 yeast affected astringency more in comparison to other yeast strains. However, wines made with M2/1 were also associated with astringency. Astringency in wine is known to be affected by phenolic acids (McRae & Kennedy, 2011; Oberholster, 2008), i.e. significant concentration of flavan-3-ols. Significant differences between wines made with *S. cerevisiae* strain (VIN13) and wines made with *T. delbrueckii* strains (654 and M2/1) wines show the effect of yeast strains on the perception of astringency in wine.

The sensory evaluation of the studied wines showed that wines made with *T. delbrueckii* strains (654 and M2/1) were higher in body, compared to wines made with *S. cerevisiae* strain (VIN13). Ethanol is known as the contributor to the body of wine (Gawel *et al*., 2007). Contrary to literature, wines with

lower percentage ethanol had higher body, compared to wines with higher percentage ethanol. This suggests that metabolites produced by *T. delbrueckii* yeast (654 and M2/1) strains during fermentation affected the body of wine more than metabolites produced by *S. cerevisiae*. Therefore, the wines' body is determined by a number of factors such as percentage alcohol and extracts which includes non-volatile phenolics, glycerol, sugars and acids (Neto *et al*., 2014; Kennedy, 2008).

Wines made with *T. delbrueckii* had lower perceived acidity compared to wines made with the *S. cerevisiae*. Acidity in wine is affected by the presence of organic acids that precipitate protons on the tongue (De Klerk, 2010). The primary contributor of acidity in wine is tartaric acid. However, succinic acid, is a by-product of the metabolism of nitrogen by yeast cells during fermentation and can contribute to perceived acidity. A study conducted by Taillandier *et al*. (2014) showed that *T. delbrueckii* consumed less nitrogen than *S. cerevisiae*. However, this study showed no notable differences between the concentration of succinic acid in the wines (data not shown), but the low perceived acidity in wines made with *T. delbrueckii* can be correlated to low metabolism of nitrogen by *T. delbrueckii* strains.

Chapter V

Concluding remarks

5. Conclusion

The ability of a yeast strain to fermenting the sugar present in the grape must is related to the chemical compounds present in the final wine, i.e. phenolic compounds, glycerol, and alcohol content. The fermentation rate of yeast affects a number of metabolites produced during fermentation which subsequently affect the sensory properties of wine.

There were two flavan-3-ols and four phenolic acids quantified in South African Chenin blanc wines. Wines made with *S. cerevisiae* yeast strain had higher concentration of flavan-3-ols compounds, compared to wines made with *T. delbrueckii* yeast strains. However, wines made with M2/1 yeast strain had similar concentrations of (+)-catechin, caffeic acid, ferulic acid and *p*-coumaric acid in 2011 and 2012 but higher concentration in 2013, compared to wines made with VIN13 yeast strain. The high content of phenolic compounds in 2013 wines suggests that the ability of yeast strain in fermenting sugars is proportional to the total phenolic content in the wine.

Sensory evaluation showed that wines made with *T. delbrueckii* strains were higher in flavour, body and complexity and low in astringency and acidity, compared to wines made with *S. cerevisiae* strain. Phenolic compounds are known to affect the sensory attributes of wine. In this study only (+)-catechin correlated with astringency, other quantitated phenolics showed no correlation with sensory attributes. Phenolic compounds are known to affect the sensory attributes of wine. However, this study no correlations were observed between the phenolic content and sensory attributes and vice versa. The quality of wine cannot be concluded by chemical or sensory analysis alone, but the data sets are complementary.

5.1 Recommendations

The chemical compounds responsible for aroma and mouth-feel attribute in wine, i.e. flavan-3-ols and phenolic acid are affected by the presence of enzymes secreted by the yeast during fermentation known as pectinase and β-glucosidase. These enzymes extract and release of phenolic compounds in their free

form. They are also responsible for the hydrolytic activity which releases thiols in grapes as aromatic compounds. Therefore, the use of a yeast strain(s) with increased pectinase and β-glucosidase activity may produce wines with higher phenolic and aromatic compounds, compared to yeast strains used in the study. The extraction of phenolic compounds by microbial organism during fermentation includes several pathways, i.e. glycosylation, deglycosylation and methylation of the phenolic compounds However, there is a lack of knowledge on the extraction of phenolic compounds by the enzymes during grape must fermentation. Future research should focus on understanding the excretion of enzymes by the yeast used to inoculate grape must can be useful in the identification and quantitation of phenolics in Chenin blanc wine.

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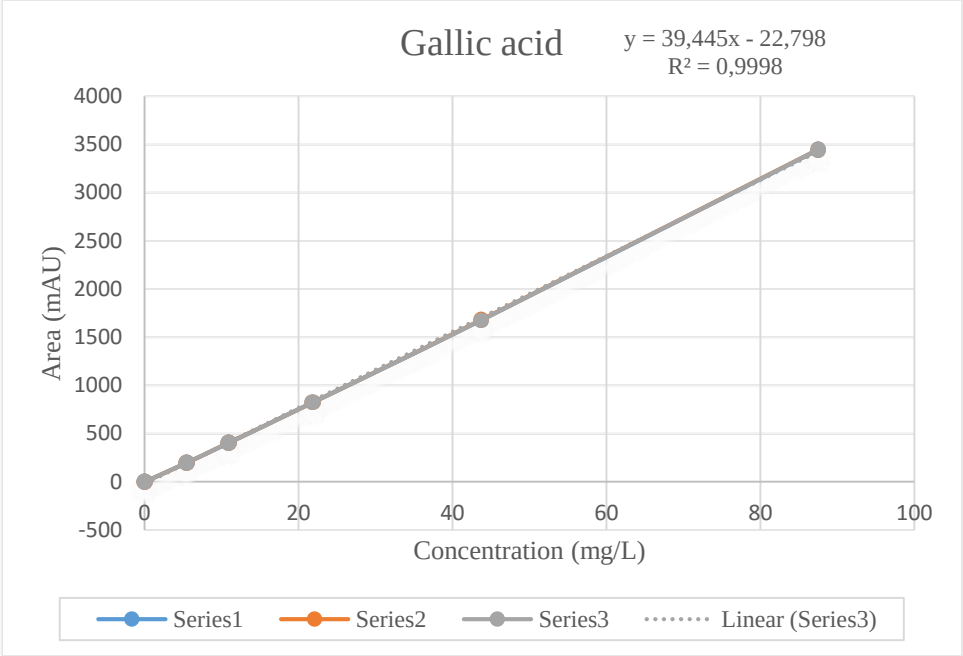
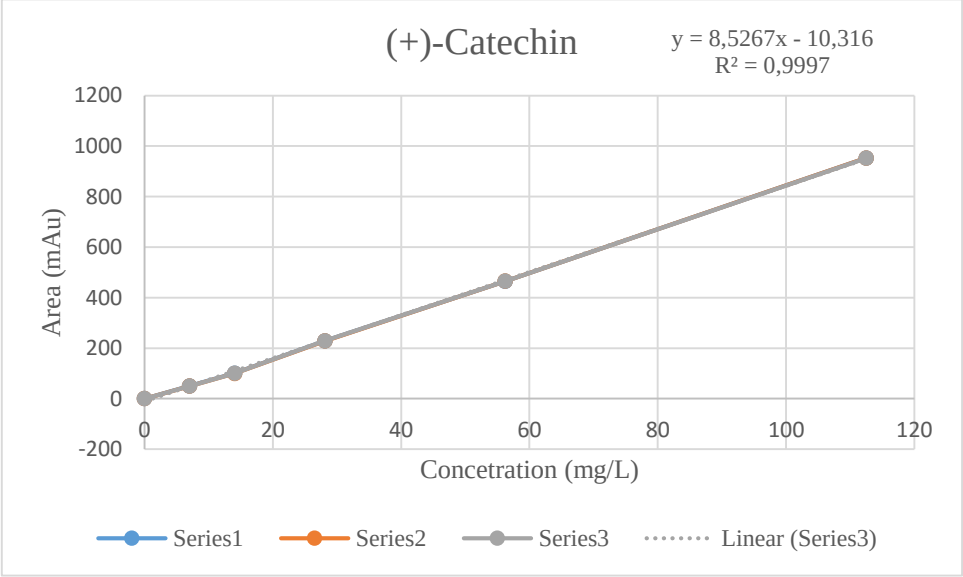
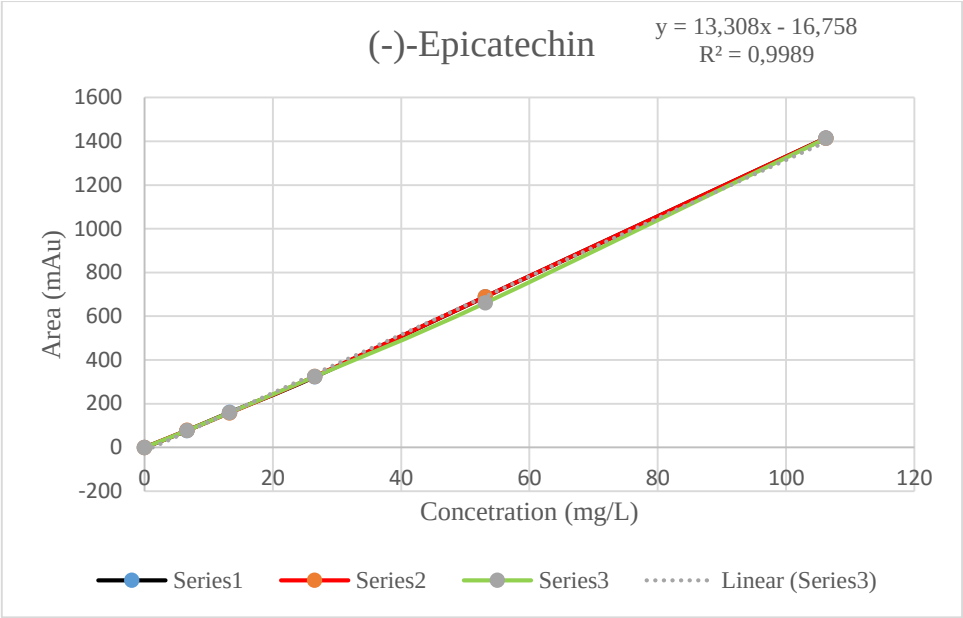
Appendices



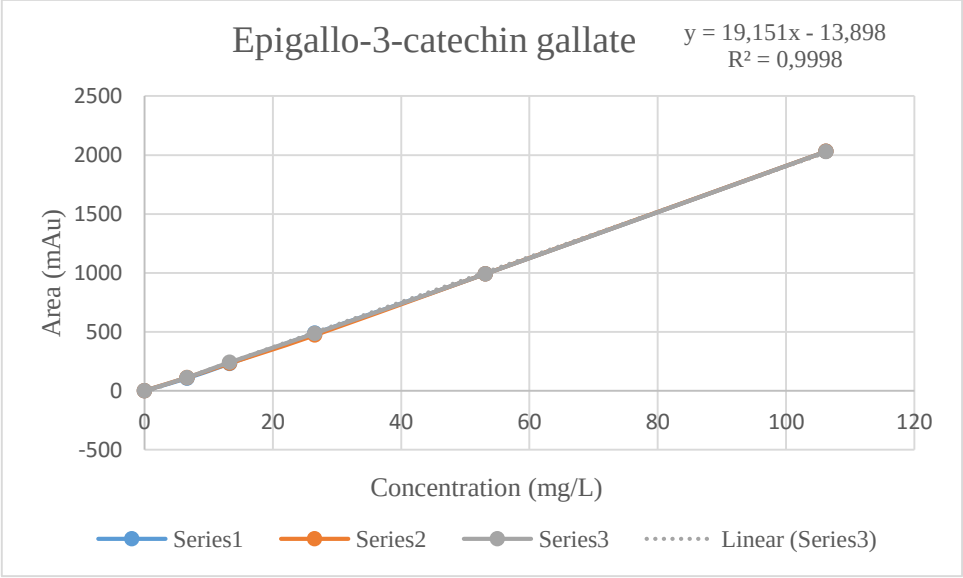
Appendix A: Linear standard curve for gallic acid



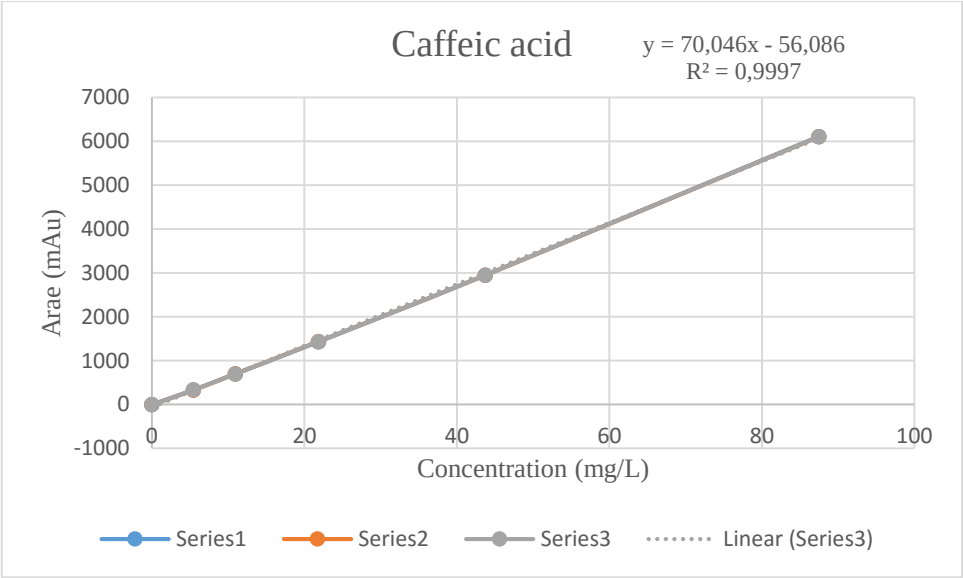
Appendix B: Linear standard curve for (+)-Catechin



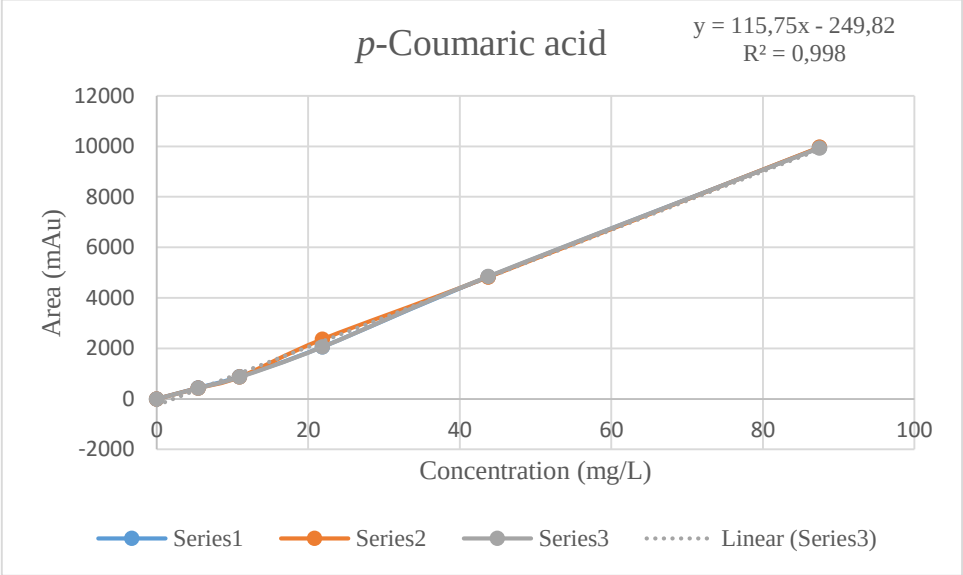
Appendix C: Linear standard curve for (-)-Epicatechin



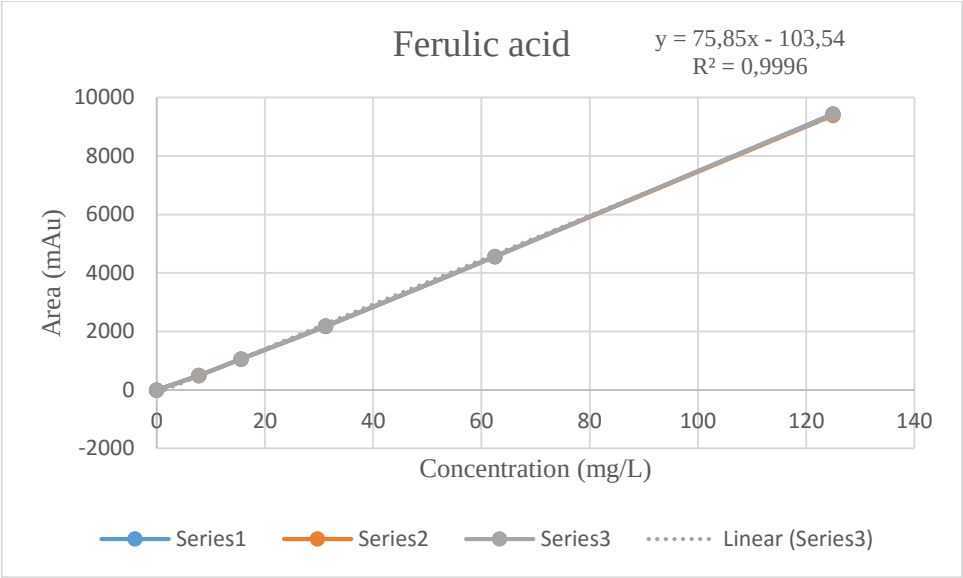
Appendix D: Linear standard curve for Epigallo-3-catechin gallate



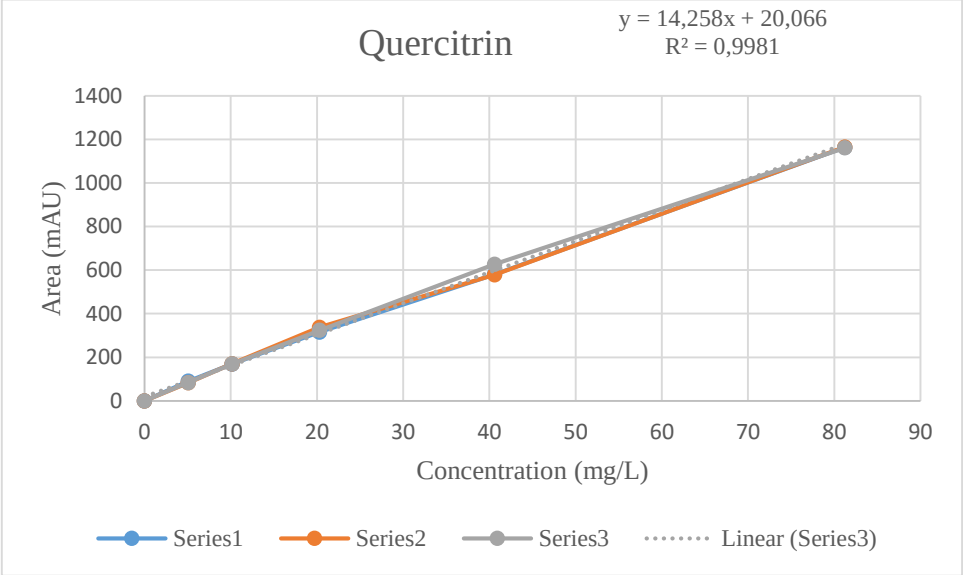
Appendix E: Linear standard curve for Caffeic acid



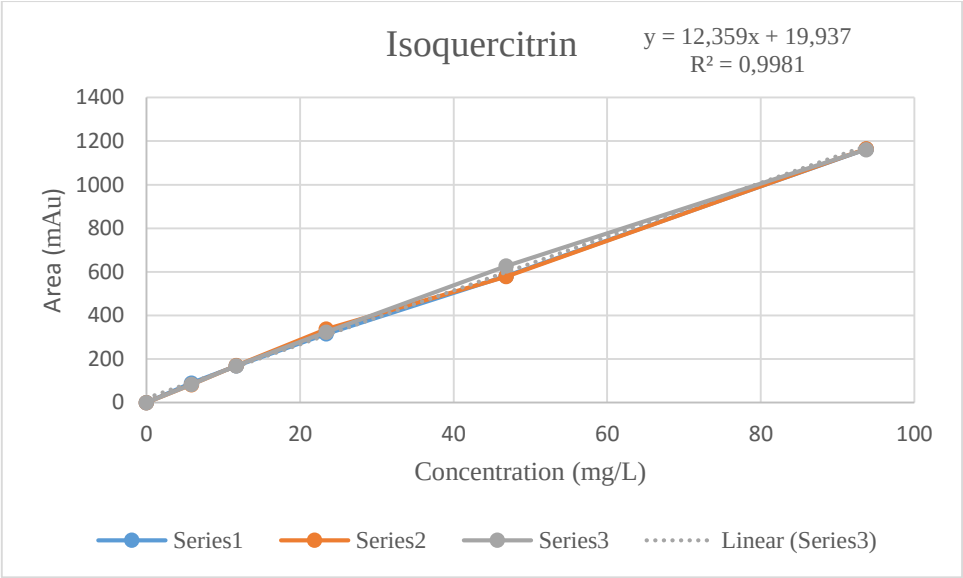
Appendix F: Linear standard curve for *p*-Coumaric acid



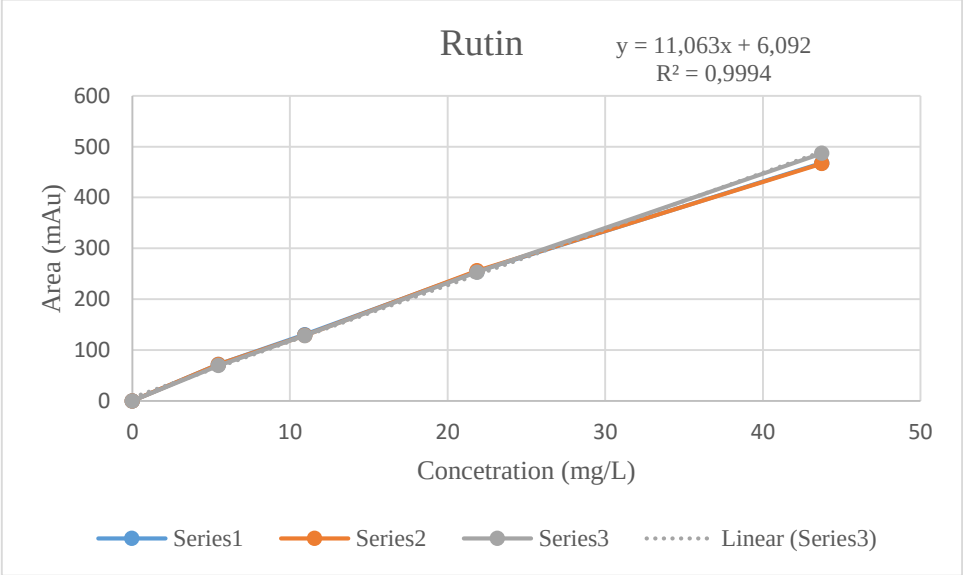
Appendix G: Linear standard curve for Ferulic acid



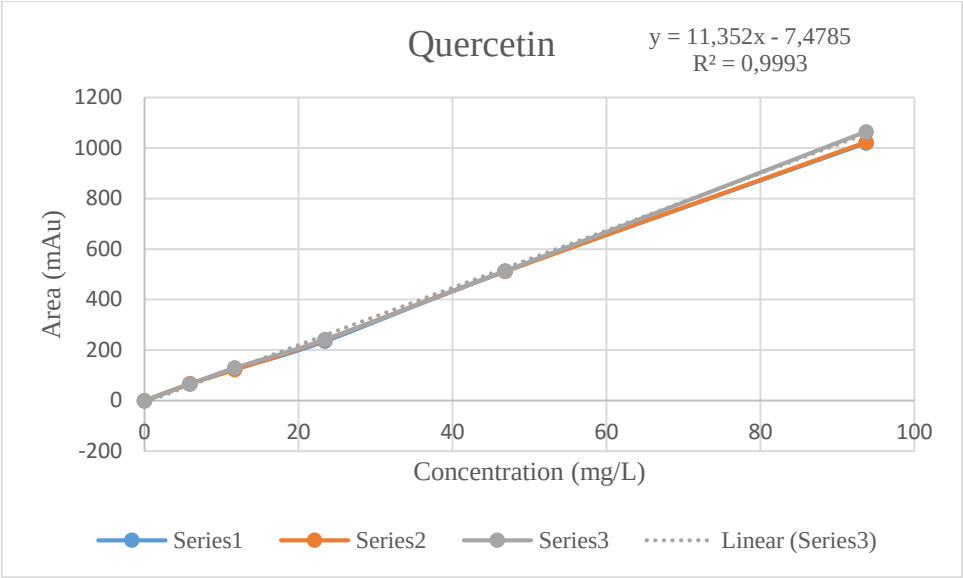
Appendix H: Linear standard curve for Quercitrin



Appendix I: Linear standard curve for Isoquercitrin



Appendix J: Linear standard curve for Rutin



Appendix K: Linear standard curve for Quercetin

Annexure A

***Cultivar*: Chenin blanc** ***Date*:** Cb16

Judge: _____

Judge the wine on the line-scale.

***Wine number*:** _____

Flavour	*Less*	*More*
Body	*Thin*	*Full*
Astringency	*Less*	*More*
Acidity	*Too little*	*Too much*
Complexity	*Less*	*More*

Comments: _____

Annexure A: Sensory tasting sheet