# Factors influencing the style of brandy

by

**Nina Valleska Bougas**
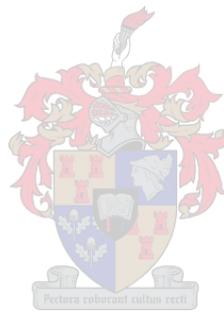
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# Declaration

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# Summary

Brandy producers in South Africa can produce three categories of brandy, and within those categories, varies styles in order to cater for diverse consumer needs. Thus they have divergent opinions as to what comprises a good base wine and distillate for their individual end-product style in mind. Due to the fact that brandy is the largest locally produced spirit in South Africa, it is crucial to investigate the factors that influence the production of brandy as better understanding and control of these processes leads to the production of a brandy that is more consistent in quality and in style.

The factors known to influence the quality of brandy are the grape cultivar, vinification techniques, distillation and the oak maturation process; however limited research has been done on the factors contributing to the style of brandies. Understanding and identifying the factors that contribute to the style of brandy, will allow for better control and manipulation of the process to ultimately produce a spirit product with a desired style classification. In order to do so, one must distinguish as to what differentiates (chemical and sensory analyses) the two most divergent styles of brandy (in this study referred to as class one and class five three-year barrel matured brandy).

Over 104 three-year old barrel matured brandy samples were collected and analysed using GC-FID and HPLC analysis over a three year period. Of these samples, only 7 were classified as a class one three-year barrel matured brandy and 23 were found to be classified as class five three-year barrel matured brandy. The results obtained showed that the class one samples were found to contain a higher concentration of total higher alcohols, higher ratio of higher alcohols vs. esters, higher alcohols vs. acids and aldehydes vs. esters, but a lower level of total esters and acids. The class five samples contained a higher concentration of total esters, furans, lactones and acids, but a lower level of total higher alcohols and ratio of higher alcohols vs. esters.

The factors investigated that possibly influence the style of brandy were: the base wine prior to distillation, yeast strain, fermentation temperature, condenser water temperature and barrel age. Results of the base wine analysed prior to distillation show that those samples that scored a sensory score of one could produce a class one three-year barrel matured brandy, and base wines that scored a sensory score of five would yield a class five three-year barrel matured brandy. Alchemy 1 yeast was found to produce elevated levels of total esters and could by default result in a class five three-year barrel matured brandy. Similarly if the wine was fermented at 24ºC it would result in the increased production of higher alcohols and total acids and in-turn could yield a class one three-year barrel matured brandy.

The results obtained show that the different condenser water temperatures do not necessarily influence the concentration of esters and higher alcohols. However, if a lower condenser water temperature (8ºC) was employed, it could yield a class one barrel matured brandy sample. These distillates are associated with a higher concentration of total higher alcohols and even though they may be positively associated with higher levels of esters, the esters could be removed during the head fraction in the distillation process.

The results also show that during the maturation process that the use of *18 year old barrels* for the maturation of three-year barrel matured brandy samples could possibly produce a class one three-year barrel matured brandy and the use of *new barrels* could yield a class five three-year barrel matured brandy sample.

It is recommended that analysing the base wine prior to distillation using GC-FID to identify the chemical composition is the most important step in determining the outcome of the style of brandy.

# Opsomming

Brandewyn produsente in Suid-Afrika kan drie kategorieë brandewyn produseer, en binne hierdie kategorieë sekere style om voorsiening te maak vir die diverse behoeftes van die mark, en dus het hulle uiteenlopende menings oor wat 'n goeie basis wyn en distillaat behels vir hul individuele eindproduk se spesifieke styl. As gevolg van die feit dat brandewyn die belangrikste spiritus produk is wat deur die Suid-Afrikaanse bevolking verbruik word, is dit noodsaaklik dat dié faktore wat die produksie van brandewyn beïnvloed ondersoek word om 'n beter begrip van, en beheer oor hierdie prosesse verkry kan. Dit sal lei tot die produksie van 'n brandewyn van konstante in qualitiet en in style.

Die faktore wat bekend is om die gehalte van brandewyn te beïnvloed, is die druifkultivar, wynbereidingstegnieke, distillasie en die hout verouderings proses, maar daar is beperkte navorsing oor die faktore wat bydra tot die styl van n brandewyn.  Om die faktore wat bydra tot die spesifieke styl van n brandewyn te identifiseer en te verstaan, kan dit lei tot 'n beter beheer en manipulasie van die prosess om uiteindelik n brandewyn met 'n gewenste styl te produseer. Ten einde dit te kan doen, moet 'n mens kan bepaal wat die twee uiterste style van brandewyn (wat in die studie sal bekend wees as klas een en klas vyf drie-jaar vat veroude brandewyn) van mekaar onderskei (chemiese en sensoriese analise).

Meer as 104 drie-jaar vat verouderde brandewyn monsters is ingesamel en ontleed met behulp van GC-FID en HPLC analyliese oor 'n tydperk van drie jaar. Van die 104  monsters, is slegs 7 as 'n klas een drie-jaar vat verourde brandewyn geklassifiseer en 23 as klas vyf drie-jaar vat verourde brandewyn geklassifiseer. Die resultate het getoon dat die klas een monsters 'n hoër konsentrasie van die totale hoër alkohole, 'n hoër verhouding van hoër alkohole teenoor esters het, hoër alkohole teenoor sure en aldehiede teenoor esters, maar 'n laer vlak van totale esters en sure. Die klas vyf monsters het 'n hoër konsentrasie van die totale esters, furanen, laktone en sure, maar 'n laer vlak van totale hoer alkohol en verhouding van hoër alkohole teenoor esters.

Die faktore wat ondersoek was wat moontlike 'n invloed op die styl van brandewyn kon hê, was die basis wyn voor distillasie, die gisras, die fermentasie temperatuur, die kondensor water temperatuur en die vat ouderdom.  Die ontledings van die basis wyn voor distillasie het gewys dat die monsters wat 'n sensorise telling van een behaal het, moontlik kan lei tot 'n klas een drie-jaar vat verouderde brandewyn, en basis wyne wat 'n sensorise telling van vyf gekry het weer 'n klas vyf drie-jaar vat verouderde brandewyn sou oplewer. Alchemie 1 gis ras het 'n verhoogde vlak van totale esters geproduseer wat tot 'n klas vyf drie-jaar vat verouder brandewyn gelei het  As die basis wyn gefermenteer is teen $24^0$C sal dit lei tot die verhoogde produksie van hoër alkohole en totale sure wat 'n klas een drie-jaar vat verouderde brandewyn kan oplewer.

Die resultate verkry, toon dat die verskillende kondensor water temperature nie noodwendig die konsentrasie van esters en hoër alkohole beïnvloed het nie, maar dit wil voorkom dat indien 'n laer kondensor water temperatuur ($8^0$C) gebruik is, kan dit 'n klas een vat verouderde brandewyn oplewer. Dit hou verband met 'n hoër konsentrasie van die totale

hoër alkohole en selfs al word die distillate positief geassosieer met hoër vlakke van esters, kan die esters verwyder word tydens die verwydering van die voorloop in die distillasie proses.

Die resultate toon ook dat die gebruik van 18 jaar oue vate vir die veroudering van brandewyn moontlik 'n klas een drie-jaar vat verouderde brandewyn kan oplewer en die gebruik van nuwe vate kan 'n klas vyf drie-jaar vat verouderde brandewyn lewer.

Dit word egter aanbeveel dat die basis wyn voor distillasie met GC-FID chemies ontleed word om die samestelling van die wyn te identifiseer  Die resultate bly die beste aanduiding van die styl van die brandewyn.

This dissertation is dedicated to my parents and fiancé for their love, support and continuous guidance.

# BIOGRAPHICAL SKETCH

Nina Bougas was born in Cape Town, South Africa on 7 March 1984. She matriculated at Parel Vallei High School, Somerset West in 2001 and enrolled at Stellenbosch University in 2002. She obtained a BScAgric-degree in Viticulture and Oenology in 2005 and an MScAgric (Oenology) degree in March 2009. She then enrolled for her PhD (Agric) in Oenology in April 2010.

# ACKNOWLEDGEMENTS

# PREFACE

This dissertation is presented as a compilation of eight chapters.  Each chapter is introduced separately and is written according to the style of the journal South African Journal of Enology and Viticulture to which Chapter 3, Chapter 4, Chapter 5, Chapter 6 and Chapter 7 is/was submitted for publication.

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# Chapter 1

# INTRODUCTION AND PROJECT AIMS

# 1. INTRODUCTION AND PROJECT AIMS

## 1.1 INTRODUCTION

Brandy is one of the most important spirits consumed by the South African population (Holtzkamp, 2012) and South Africa is one of the largest brandy producing countries in the world and falls 8th in the global market.

Brandy producers in South Africa produce three categories, and within those categories various styles in order to cater for diverse consumer needs, and thus have divergent opinions as to what comprises a good base wine and distillate for their individual end-product style in mind (Toerien, 2008).

The three different categories of brandy are known as blended, vintage or pot still brandy (Wine and Spirits Control Act No 47 of 1970). Each of these types of brandy has their own role within brandy. Depending on the category of brandy being made, the 3 year pot still brandy is either bottled as is (pure pot still brandy) or used as part of a blended or a vintage brandy.

By law, blended brandy must contain at least 30% of pot still brandy matured for a minimum of 3 years (South African Liquor Products Act No 60 of 1989). The remainder of the brandy is made up of neutral wine spirit, and this must not exceed 70% of the final blend. Blended brandy is diluted to 43% v/v as it is intended to be drunken with a mixer (le Roux, 1997).

Pot still brandy is by far the more complex brandy of the three styles and is considered the richest, fruitiest and most layered in style. By South African law, pot still brandy must contain at least 100% of pot still brandy matured for a minimum of 3 years. Vintage brandy must have at least 30% pot still brandy that is matured for at least 8 years and a maximum of 60% column still spirit aged for at least 8 years. South African brandy is said to hold its' own and rival some of the best Cognac's in the world (Toerien, 2008).

Brandy production is a multi-step process, with each step playing an important role in the overall profile and characteristics of the product. These steps include, the type of grapes used, the fermentation process, vinification techniques, distillation and the maturation period (Léaute, 1990).

In South Africa the main grape varieties used for the production of brandy are Chenin blanc and Colombard (Toerien, 2008), and the main yeast types used for vinification are 228 and VIN 13. It is important to select a yeast type that produces low levels of sulphur dioxide and higher alcohols as these can negatively affect the quality of the base wine. The alcohol concentration of the base wine is found to be between 8-11% v/v and levels of volatile acidity and total phenolic content must be below 0.7 g/L and 250 mg/L respectively (Steger and Lambrechts, 2000).

Distillation takes place in copper pot stills (Toerien, 2008). The type of pot stills that are used in South Africa include the well-known Savalle pot which has a capacity of 1000 L, the

Hermann pot which can hold 4000 L, together with some locally-designed stills that have a capacity of up to 22000 L (Toerien, 2008).

Distillation in copper pot stills is a slow, two phased process that creates a complex and multi-layered spirit (Ledauphin, 2006). In the first phase, the base wine is distilled into low wine. The alcohol concentration of the low wine is between 28-30% alcohol by volume (v/v) and this is essentially a concentration process resulting in the removal of a large proportion of water and soluble solids from wine (Léaute, 1990). The second stage of the distillation involves distilling the low wine into brandy. Three fractions of the liquid are then drawn in sequence. These fractions are known as the heads, hearts and the tails respectively (Carnacini, 1989). It is however the heart fraction that is of importance as it is rich in desirable aromas and flavour compounds and it is this fraction that is retained for maturation (Léaute 1990; Ledauphin, 2006).

After the distillation process the distillate is then placed in oak barrels to mature. These barrels vary with regards to their size and type of wood used, however by law in South Africa brandy must be matured in oak barrels (not exceeding 340 L) for a minimum of 3 years (South African Liquor Products Act No. 60 of 1989).

Previous research has been conducted to determine factors that influence the quality of brandy, but little has been investigated regarding the factors contributing to the style of brandies. To understand and identify factors that contribute to influencing the style of brandy, will allow for better control and manipulation to ultimately produce a spirit product with a desired style classification (Jack, 2003).

## 1.2 PROJECT AIMS

The factors that are known to influence brandy quality include the type of cultivar, yeast strain, vinification techniques, malolactic fermentation, the distillation process as well as the maturation period (barrel usage and degree of toasting) (Bougas, 2009; du Plessis *et al.,* 2002; Steger and Lambrechts, 2000). However, there is little information regarding the factors that influence the style of brandy, thus making it an important topic to research. This study forms an integral part of an extensive research program aimed at understanding the factors that influence the style of brandies to ensure a consistent product for the consumer and to be able to develop new brandy styles. The information obtained in this study could be used to design a prediction tool which can be used to deduce and ensure the outcome of a specific style of brandy. The specific aims of the project are:

1.  Analyzing three-year barrel matured brandy samples and determining their chemical composition using GC-FID (Gas chromatography flame ionization detector) and HPLC (High pressure liquid chromatography)

2.  Identifying those groups of compounds (namely esters, higher alcohols, acids and carbonyl and wood compounds) that are responsible for the separation of the brandy samples into different style classifications.

3.  Analyzing the chemical composition of commercial base wines and determining the correlation between the chemical profile and the known sensory score (as determined by Distell's internal brandy panel).

4.  Determining the effect of different vinification techniques such as fermentation temperature and yeast on the chemical composition of base wine prior to distillation.

5.  Determining the effect of different condenser water temperatures (8, 12 and 20ºC) on the volatile composition of the resultant distillate.

6.  Analyzing the volatile and non-volatile chemical composition of three-year brandy samples matured in barrels of different age and determining if there is a correlation between barrel age and the three-year brandy style classification.


## 1.3 LITERATURE CITED

du Plessis, H.W., C.L.C Steger., M. du Toit., M. Lambrechts. 2002. The occurrence of malolactic fermentation in brandy base wine and its influence on quality. J. Appl. Micro. 92: 1005-1013.

Holtzkamp, E. 2012. Liquor consumption patterns in South Africa. South African Wine Industry Information and Systems.

Jack, F. 2003. Development of guidelines for the preparation and handling of sensory samples in the Scotch Whisky industry. J. Inst. Brew. 119, 2: 114-119.

Léaute, R. 1990. Distillation in Alambic. Am. J. En. Vitic. 41, 1: 90-108.

Ledauphin, J. 2006. Gas chromatographic quantification of aliphatic aldehydes in freshly distilled Calvados and Cognac using 3-methylbenzothiazolin2-one. J. Chrom. A. 1115: 225-232.

Le Roux, J. 1997. Van Ryn's advanced brandy course. The Van Ryn Wine and Spirit Company.

Rose, A. 1977. Alcoholic beverages. London; New York: Academic Press.

Steger, C.L.C and M. Lambrechts. 2000. The selection of yeast strains for the production of premium quality South African brandy base products. J. Ind. Micro. Biotech. 24, 6: 431-440.

South African Wine Industry Information and Systems. 2008

South African Liquor Products Act No 60 of 1989.

Toerien, W. 2008. Firewater. Quivertree publications. Cape Town. South Africa.

# Chapter 2

# LITERATURE REVIEW

## Cognac, Armagnac and brandy

# 2. Cognac, Armagnac and brandy

## 2.1 INTRODUCTION

Brandy is a spirit made from fruit juice or fruit pulp and skin of grapes. There are many types of brandy on the market today, each with their individual style, varying with regards to the aroma and flavour and all round organoleptic perception (Gold, 1972). This chapter will focus on brandies that are made from grapes.

There are many words that are used to describe brandy. Most of them originate from Arabic, and it was probably the Arabs who introduced the technique of distilling to Europe. The first European documentation pertaining to distilling dates back to the 13th century and it was the Spanish that coined the term "*aqua vitae*" meaning the water of life (Bertrand, 2003). By the 16th century, both the Germans and Dutch had references to what was known as "*brantwein*" or "*brandewijn*" meaning burnt wine or wine that was like fire (Gold, 1972). Brandy was however used more for medicinal purposes at this time rather than for recreational purposes.

Distillation is said to have originated in China in 3000 BC. However, there are also indications that the Greeks were distilling by 1000 BC and the Romans by 200 BC as documented in the works of Hippocrates and Pliny. For centuries, the art of distilling remained firmly in the hands of the alchemist and they were responsible for many improvements in the art of distilling. It was only around the 15th century that the enjoyment of distilled spirits as beverages came to be widely appreciated. Today the spirit and alcoholic beverage industries operate in almost every country of the world and are one of the most diverse industries due to the tremendous variety of beverages and spirits that they produce (Rose, 1977).

## 2.2 BRANDY

### 2.2.1 Brandy in a global spirit context

According to Datamonitor (June 2010), the global spirits market is estimated to be around 19 billion litres. Brandy (including Cognac and Armagnac) is the 4th largest global spirits category at around 1 billion litres, with a cumulative average growth rate of 2.1% since 2004.

The top markets for brandy are: India, Philippines, Russia, Brazil, Germany, USA, Chile, South Africa and the Ukraine (Datamonitor 2010). France produces Cognac, Armagnac and French brandy. However, most French brandy is exported to Russia and Eastern Europe and is therefore reported under the sales of brandy in Russia. It should also be noted that most of the "brandy" produced in India, the Philippines and Brazil is so-called pseudo-brandy (cane spirit based with flavourants).

Grape-based brandy producing countries all have a significantly sized wine industry that provides the base wine for brandy production. Brandy production and consumption tends to

be highly regionalized (with the exception of Cognac which is known and consumed globally) and production methods thus differ significantly.

## 2.2.2 Production of brandy

A wide variety of grape cultivars are used to produce Cognac, Armagnac and brandy. Cognac is made mainly from the cultivars Ugni Blanc, Folle Blanche; Armagnac with Folle Blanche, Ugni Blanc and Baco, and brandies produced in other countries use Colombard, Chenin Blanc and the Airen grape variety, however there are many other cultivars used (le Roux, 1997). The grape cultivar used will yield a brandy with a distinct and unique character and taste profile that will contribute to the eventual taste of the brandy (Quady and Guymon, 1973).

The wine that is made for the production of brandy (known as base wine) differs significantly from table wine. The grapes are picked early to ensure high acid concentrations and lower sugar levels (18 to 20° Balling) (Toerien, 2008). The addition of sulphur dioxide is common practice with the production of table wine; however addition of sulphur during the production of base wine for brandy varies. In South Africa the level of total sulphur in the base wine must be lower than 20 mg/L as the reaction with sulphur and the copper in the pot stills used for the distillation results in the formation of copper sulphate (Steger and Lambrechts, 2000) which is considered undesirable. Sulphur can also cause erosion of the distilling equipment made from copper. In other countries such as Spain the base wine is transported long distances geographically from point of winemaking to point of distillation and therefore sulphur is used to protect the base wine from oxidation (Bertrand, 2003). The yeast sediment that forms during the fermentation of the base wine is either included or not in the distillation process depending on the style of brandy produced. It acts as natural preservative by preventing oxygen from reacting with the flavour compounds in the wine. Distillation in copper pot stills is a slow, two phased process that creates a complex and multi-layered spirit (Ledauphin *et al.,* 2006). In the first phase, the base wine is distilled into low wine. The alcohol concentration of the low wine is between 28-30% alcohol by volume (v/v) and this is essentially a concentration process resulting in the removal of a large proportion of water and soluble solids from wine (Léaute, 1990). The second stage of the distillation involves distilling the low wine into brandy. Three fractions of the liquid are then drawn in sequence. These fractions are known as the heads, hearts and the tails respectively (Carnacini, 1989). It is however the heart fraction that is of importance as it is rich in desirable aromas and flavour compounds and it is this fraction that is retained for maturation (Léaute 1990; Ledauphin *et al.,* 2006).

Although the main apparatus used for the production of brandy, Cognac and Armagnac is the pot still, distillation can also be carried out using a column still (continuous distillation). This apparatus is composed mainly of stainless steel and not copper, although some stills are modified to consist of both materials (Kister, 1992). Distillation takes place continuously and not in batches as with the case of pot still distillation and the distillate obtained is refined, has a higher alcohol concentration (approximately 90% abv) and is less aromatic than that obtained from pot still distillation (Kister, 1992; Hilmen, 2000). The decision made as to what

apparatus should be used for the production of brandy is dependent on the style of brandy desired.

After the distillation process the distillate is then placed in oak barrels to mature. These barrels vary with regards to their size, type of wood used and the length of the maturation period that is determined by the laws and regulations that govern each specific country. The brandy is then blended with distilled water, to decrease the alcohol concentration, and additives such as caramel, sugar and oak infusion may in certain instances be added. Again these additions are dependent on the legal classifications of each country (Toerien 2008; South African Brandy Foundation; Bureau National Interprofessionel du Cognac 2010).

**2.2.3 South African brandy**

South Africa is one of the largest brandy producing countries in the world and falls 8th in the global market with total sales of over forty eight million litres (Holtzkamp, 2012) . Considering the total South African population and the fact that only a small percentage is exported makes brandy one of the most important spirits consumed in the country (South African Wine Industry Information and Systems 2009). Local statistics indicate that brandy is one of the most purchased spirit beverage, second to whiskey and is therefore such an integral part of the South African landscape (Toerien, 2008). Its success is probably due to its versatility, as brandy can be enjoyed on its own, over ice, with mixers or in cocktails and is suited for all occasions and seasons (Toerien, 2008).

The South African brandy industry adopted the French distillation technique known as "*Methode Charentaise*", using mainly the grape varieties Chenin Blanc and Colombard. The alcohol concentration of the base wine is found to be between 8-11% v/v and levels of volatile acidity and total phenolic content must be below 0.7 g/L and 250 mg/L respectively. For the production of South African brandy, the main yeast types used are 228 and VIN 13 and it is important to select a yeast type that produces low levels of sulphur dioxide and higher alcohols as these can negatively affect the quality of the base wine. The base wine is then evaluated organoleptically against quality criteria as determined by each company. The selected base wines are then distilled to obtain the spirit needed for aging (Toerien 2008; Steger and Lambrechts, 2000).

Distillation takes place in copper pot stills (Toerien, 2008). The type of pot stills that are used in South Africa include the well-known Savalle pot which has a capacity of 1000 L, the Hermann pot which can hold 4000 L, together with some locally-designed stills that have a capacity of up to 22000 L (Toerien, 2008). South African brandy is made up of not only pot still brandy, but also neutral wine spirits. This neutral spirit also makes a contribution to overall end product. South Africa was the first country to use neutral spirits derived from grapes (Toerien, 2008). All of the brandies must be matured in oak barrels (not exceeding 340 L) and matured for a minimum of 3 years (South African Liquor Products Act No. 60 of 1989).

Brandy producers in South Africa produce many styles in order to cater for diverse market needs, and thus have divergent opinions as to what comprises a good base wine and distillate for their individual end-product style in mind (Toerien, 2008).

In South Africa there are three main types of brandy: blended, vintage and pot still. Distinctly different from each other in style, they nevertheless share a common South African identity (Snyman, 2010). Each of these styles of brandy varies greatly with regard to their organoleptic profile and is firstly dependent on legal classification (Toerien, 2008). Depending on the style of brandy being made, the 3 year pot still brandy is either bottled as is (pure pot still brandy) or used as part of a blended or a vintage brandy (Snyman, 2005).

Blended brandy must contain at least 30% of pot still brandy matured for a minimum of 3 years in barrels. The remainder of the brandy is made up of neutral wine spirit, and this must not exceed 70% of the final blend. Blended brandy is diluted to 43% abv as it is intended to be enjoyed in a long drink with a mixer and ice or in cocktails (Snyman 2005).

Pot still brandy is by far the most complex brandy of the 3 types and is generally the richer, fruitier and smoother (Toerien, 2008; Léaute, 1990). By South African law, pot still brandy must contain at least 100% of pot still brandy matured for a minimum of 3 years. Many pot still brandies are matured for much longer periods. Pot still brandies are bottled at a minimum concentration of 38% v/v and are ideally enjoyed neat or over ice. Vintage brandy must have at least a minimum of 30% potstill brandy matured for at least 8 years, a maximum of 60% column still spirit matured for at least 8 years and a maximum of 10% wine spirits (unmatured). Due to the blend composition of vintage brandies, these tend to typically have a more dominant wood maturation flavour profile and a drier finish on the palate. They are also bottled at a minimum alcohol concentration of 38% v/v and are also enjoyed neat or over ice.

South African brandies are distinguished by their often overt fragrances of stone fruit like apricots, peaches and pears, whereas Cognacs are elegant, restrained and have an underlying minerality of flavour that is the reflection of the chalky soils in which the grapes originate (Snyman, 2010).

Figure 2.1 illustrates the Brandy Aroma wheel that was developed by Jolly and Hattingh (2000) and is used to describe the sensory attributes found in South African brandies. This aroma wheel can be used as a tool when evaluating South African brandies (Jolly and Hattingh, 2000).
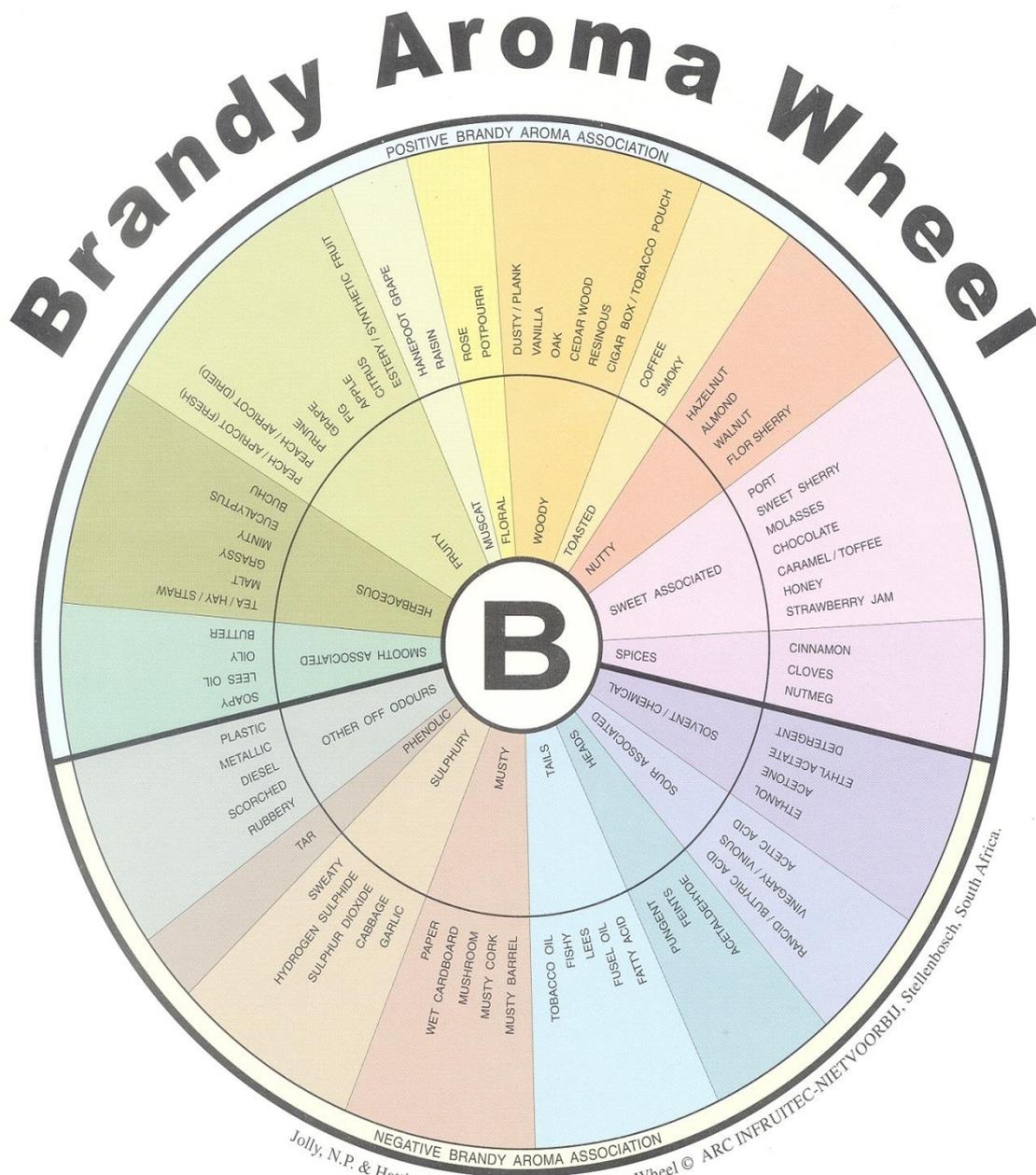
**Figure 2.1. Descriptors of a general profile that is used when evaluating South African brandies (Jolly and Hattingh, 2000).**

Distell is the single largest brandy producer in South Africa and produces a number of different products. Distell produces Klipdrift which is South Africa's most popular brandy and the only South African brandy to feature on the 2010 impact Global list of premium spirits as well as another well-known brandy Richelieu, which is also a blended brandy. Distell also

produces premium pot still and vintage brandies such as the Van Ryn's range. South African brandy is often referred to as "the world's finest" with an impressive and consistent track record of quality accolades generated from the most respected international sprits competitions. Over the past 12 years, South African brandies have won the coveted title of "Worldwide Best Brandy" at the prestigious International Wine and Spirit Competition 9 times (International Wine and Spirits Competition).

### 2.2.4 Cognac

The brandies produced in Cognac have a long-standing and universal reputation for their elegance and refinement and are often symbols of status and prestige (Faith, 1992). The Cognac sub regions (crus) are classified or defined by their terroir (predominantly aspect and very importantly soil structure and content). The two crus highest in chalk content are Grande and Petite champagne and these areas are known to produce Cognacs that require longer ageing and yield refined and elegant characters. Bon bois, Fin bois, Bois Ordinaires and Borderies have low chalk content and are generally used to produce younger, more upfront Cognacs (Decree 1938, Bureau National Interprofessionel du Cognac, 2010). The Borderies crus is the smallest of all six crus and these Cognacs reach optimum quality after a shorter ageing period than Cognac from the Grande and Petite champagne area. These areas of Cognac production are controlled by the AOC (Appellation d' origine controlee) and any brandies (produced by the "*Methode Charentaise"*) that are made outside these areas cannot legally be classified as Cognac. The main Cognac producers globally are Hennessy, Martell and Rémy Martin respectively (Bureau National Interprofessionel du Cognac, 2010).

Cognac generally does not refer to age claims on its labels and relies on a system of well known acronyms that refer to the age of the youngest component in the blend. VS (Very Special) is the youngest Cognac that has to be aged for at least 2 years; VSOP (Very Special Old Pale) at least 4 years and the oldest, usually called XO (Extra old), is at least 8 years old. Many Cognacs contain components much older than the minimum age and can originate from different "crus" thus creating a harmonious and complex blend where the sum is greater than its parts.

Cognac is distilled using the wine produced from the cultivars Ugni Blanc, Folle Blanche, St. Emillion and a small amount of Colombard that are grown in specific areas in Cognac (Bureau National Interprofessionel du Cognac, 2010). These cultivars produce a wine that is slightly acidic, harsh, with an alcohol percentage of 9-11% v/v but are however also fruity in nature (Bureau National Interprofessionel du Cognac, 2010).

Distillation starts in October, a mere month after the usual date of harvest and continues until the 31st of March (Spenser, 1983). For the production of Cognac all the distillations are carried out in copper pot stills and law stipulates that the distillation process must be conducted using a naked flame for the heat source (in the past the heat source was a wooden fire, these days natural gas is used) (Toerien, 2008; Léaute, 1990). Distillation is carried out using the "*Methode Charentaise*" technique whereby these copper pot stills are used using batch distillation to produce the distillate of Cognac (Faith, 1992).

Two successive distillations yield the Cognac, where the first produces the low wines (*brouillis*) containing 26-30% v/v. The second step is where the low wines are re-distilled and collected in three different fractions (Léaute, 1990).

The first fraction is known as the heads and constitutes about 1% of the original amount of low wine used. The second fraction is the brandy heart (*coeur*) where the alcohol concentration increases to produce a distillate with an average of 70% v/v. Thirdly, the tails (*seconde*) are recovered which contain the remaining alcohol in the charge (Faith 1992). The head and tail fractions are added back to the pot still to be redistilled to recover any remaining alcohol. The amount of these two fractions added is subject to the distillers' discretion and the final desired style of the Cognac (Léaute, 1990).

The special qualities that make up Cognac are not only due to the distillation process but are also a combination of the soil, climate and cultivars (Faith, 1992).

Once the heart has been distilled it is adjusted to 60% v/v with distilled water or matured without dilution and transferred to limousin oak barrels of volumes between 270 and 450 L, for not less than 2 years (Bureau National Interprofessionel du Cognac 2010). The barrels must be of very good quality as they must maintain their structure to last for 40 to 50 years (Grossman, 1964).

By law the minimum alcohol concentration of Cognac is governed by country specific regulations i.e. if you export Cognac you must comply with the law of the country you wish to sell in. Once they have undergone the minimum maturation time all additives are prohibited except distilled water, sugar, caramel and oak infusions for the final adjustment and blending (Decree 1986, Bureau National Interprofessionel du Cognac, 2010).

The Cognac gradually changes from a colour-less fluid to yield a liquid that takes on a beautiful amber tone, a change in the taste and perfume to ultimately produce a finished Cognac product (Grossman, 1964).

The quality of Cognac's aging is measured by its "*rancio*" taste (hydrolysis of fatty acid esters together with oxidation and transformation into ketones). The formation of the "*rancio*" taste during aging is accompanied by the continual extraction of tannins, slow oxidation, and various chemical reactions (Ferrari *et al.,* 2004).

Figure 2.2 is a diagram of the Cognac aroma wheel that was developed during the Second International Cognac Summit organized by the Bureau National Interprofessionel du Cognac (Lenoir, 2009).

Acacia          Almond

                                        Apricot
                        Wild carnation

Hawthorn        Grape

                        Orange              Banana

Butter    Iris    Blossom
                                            Lemon
Honeysuckle                        Lime tree

                    SPRING        SUMMER

Orange
blossom
                                                Hay

                        Cognac
Cedar                   Harmony              Passion
wood                                         fruit

Oak
wood                                         Mango

                    WINTER        AUTUMN
Glazed
fruits
                                        Dried apricot

Litchi
                                        Butterscotch

Coffee                      Cinnamon

Hazelnut            Cigar box

Leather                     Cloves

Walnut
                Humus/oak
Smoky           moss        Ginger

Toast           Wild        Coconut
                mushrooms

Black                       Nutmeg

**Figure 2.2. The cognac aroma wheel (Lenoir, 2009).**

What makes this aroma wheel unique when compared to other brandy aroma wheels such as the South African brandy wheel is that it is categorized by seasons (Lenoir, 2009). The reason for linking the terms with seasons is that any Cognac can be appreciated differently in different circumstances. By using this link, it makes it easier for the public to understand Cognac and its complex aromas and flavours. Everyone can relate to seasons and Cognac's palette of flavours fits the season cycle perfectly (Lenoir, 2009).

## 2.2.5 Armagnac

Armagnac is said to be oldest distilled spirit in France and also possibly the world. There is evidence that distillations took place in the 12th Century in the Gascony region of southwest France (Davies, 2003). Seven hundred years later, Armagnac is still drunk largely as a digestif. Although Armagnac has nearly always been in the shadow of its northern neighbour Cognac, it is now evolving into a contemporary and highly sophisticated brandy with Pernod Ricard being its multinational ambassador. Pernod Ricard owns Marquis de Montesquiou and Comte de Lauvia, the two main producers of Armagnac in France (Serjeantson, 2010; Davies, 2003).

This spirit is produced in the region known as Armagnac in France, which is south east of Bordeaux. Three demarcated regions exist, namely Haut Armagnac (chalky soils) in the east, Ténarèze (calcareous soils), in the centre and Bas Armagnac (sandy soils) in the west (Serjeantson, 2010).

The sandy soils of Bas Armagnac, also known as "black Armagnac" produce the best spirit reminiscent of plums and prunes and approximately 5127 hectares are planted under vine. The chalk/clay soils of Tenareze yield a fine floral product and the Haut Armagnac (white Armagnac) contributes only 7% of grapes for distillation and produces approximately fifteen million bottles of Armagnac per year (Serjeantson, 2010).

The four principal grapes used for the production of this brandy are namely Folle Blanche, Ugni Blanc, Baco and Colombard (Bertrand, 2003). Most Armagnacs are a blend of two or more of these grape types, although some producers do also make single varietal Armagnacs (Serjeantson, 2010).

Estate Armagnac is produced using a modified pot still which has an additional column still attached; however the vast amount of commercial Armagnac today is produced in column stills (known as *Verdier systeme*). These stills work continuously throughout the harvest, which officially begins in September and must be completed before the 30th of April (Venter 1996; Bertrand, 2003).

Most of these column stills have five plates and produce a spirit of approximately 60% v/v, rich in congeners and fruity flavours that need a long maturation period. The strength of the final spirit is directly related to the number of plates in the still, the maximum being fifteen. The head fraction is recovered at the beginning of the distillation process and the heart fraction is collected until the alcohol content ranges between 52-63% v/v. The remaining liquid is not used and is discarded (Serjeantson, 2010).

Unlike in the case of brandy, the wine used is distilled only with the fine yeast lees and not the total lees content and generally speaking there are four times fewer fatty acid ethyl esters in Armagnac than in cognac. Therefore it is likely that the quality of Armagnac comes from the specific nature of the soils and the aromas of the vines (Bertrand, 2003).

The ageing process is critical to the finished product, since the oak is one of the main factors responsible for the flavour of Armagnac (Serjeantson, 2010). Traditionally local Gasçon Oak was used for the maturation process; however Limousin and Troncais are becoming increasingly popular with barrel volumes varying between 420 and 500 L.

Commercially it is sold according to its age with *Trois etoiles* representing an Armagnac of more than two years old, *VSOP* after five years of maturation and *Hors d'age, Napoleon and XO* containing Armagnac that is older than six years (Venter, 1996). Before Armagnac is sold, it is reduced to a strength of 40 - 48% v/v and can then be used for the formation of liqueurs and the base of cocktails (Tritton, 1972).

Armagnac can be described as robust, earthy with complex floral aromas, in some instances being complemented with notes of wood and prunes. Armagnac's palette is ever changing and alternates between a light, lively fruitiness to a complex brandy with notes of roses, dried fruit, berries, marmalade, cedar wood and potpourri (Venter, 1996).

## 2.2.6 Spanish brandy

Today Spain is still a major brandy drinking and producing country. There are two main Catalonian producers, namely Torres and Mascaro and these are strongly influenced by the French methods (Heckle, 2003). Almost all the wine for the production of Brandy de Jerez is produced elsewhere in Spain from the Airen grape variety in La Mancha and Extremadura as the local Sherry grapes are too valuable to divert into Brandy production (Beverage Testing Institute 2010). Brandy de Jerez is made mainly by the Sherry houses that are situated around the city Jerez al de Frontera.

Distillation of Brandy de Jerez is conducted in column stills and, like the production of the wine, is carried out elsewhere and then transported to Jerez for aging. Brandy de Jerez is aged in old sherry casks by following the traditional dynamic system (*Soleras y Criaderas*) and sometimes additionally the static system (*Aňadas*). This solera system entails a series of old barrels each of them holding a slightly older spirit than the one above to it. Brandy is then racked off from the last barrel (no more than a third of the volume is removed) and then replenished with brandy drawn from the next barrel in line all the way down the Solera line to the first barrel which contains newly distilled spirit (Toerien, 2008). The result of such a system blends a variety of different vintages together and also speeds up the maturation system (Beverage Testing Institute, 2010).

Their best selling brand is called Torres, a solera matured pot still brandy. However there is a Penedés brandy that comes from the Penedés area in Catalonia which is modeled on the cognacs of France. This brandy is made from a mixture of regional grapes as well as locally grown Ugni Blanc from Cognac and it is distilled in copper pot stills. The resulting brandy is heartier than Cognac, but leaner and drier than Brandy de Jerez (Beverage Testing Institute,

2010). The only drawbacks are that the system is costly and that second hand sherry casks are becoming increasingly difficult to find (Heckle, 2003).

By law, basic Brandy de Jerez is matured in oak casks made from American oak (*Quercus alba*) of at least 500 L for a minimum of six months and these barrels must have previously contained Sherry as this is what gives Brandy de Jerez its unique characteristics (Snyman, 2010). Other classifications of Spanish brandy such as Reserva and Gran Reserva must be matured for one year and a minimum of three years respectively. The best Reserva and Gran Reserva are matured for a length of twelve to fifteen years (Beverage Testing Institute, 2010).

According to the Decree, No. 2484/1974 of 9 August 1974 as stipulated in the Special regulations for the production of Spanish brandy, brandy may be sold between 35 and 45% v/v – most are sold at 38 % v/v and the premium brands at 40% v/v.

Spanish brandy is sweeter and "earthier" when compared to other brandies such as Cognac and Armagnac with sensory descriptors such as ripe fruit, lots of sherry notes (acetaldehyde), dried apricots, raisin, spicy oak and some sweet floral notes are the main attributes that are used to best describe Spanish brandy (International Wine and Spirit Awards Competition). The acetaldehyde characters are a result of the addition of $SO_2$ to the wine prior to distillation (Heckle, 2003).

However the general trend has been moving away from the rich, dark, and sweet brandies of the past to less sweet brandies with lower alcohol levels (Heckle, 2003).

## 2.2.7 American brandy

Brandy that is produced in America is distilled using selected grapes such as Flame Tokay and Thompson Seedless mainly from the California region (Guymon, 1974). America produces many different types of brandies, but for the purpose of this chapter only the regulations pertaining to grape brandy will be mentioned. American law stipulates that the distillate obtained must not be greater than 80% v/v and the alcohol concentration of the final brandy must not be less that 40% v/v (Electronic code of Federal regulations, 2010). Korbel, Christian Brothers, P. Masson's Grande Amber, and E and J Gallo's E and J are market leaders. Recently small, quality-oriented batch distillers such as Germain Robin, R M S Distillery (both in California) and Dry Creek Distillery (in Oregon) have been gaining market share. All cater to small, sophisticated niche markets that value quality over price (Berberoglu, 2010).

The pot still is used for the production of brandy, however the continuous still is preferred in California as the final outcome of the distillate is cleaner but at the same time contains the highly sort after congeners. The brandy distillate that is produced is taken off as a side-stream from the horizontal plate in the middle or upper region of the concentrating section. This type of distillation is also more efficient, and produces a product that is more uniform (Guymon, 1974).

## 2.3 FACTORS INFLUENCING THE PRODUCTION OF BRANDY

### 2.3.1 Grape Cultivars

There is still a great deal of debate as to what the most desirable characteristics are in grapes specifically aimed for the production of brandy distillates (Nykänen, 1986). No particular variety has been universally shown to be the best. The specific terroirs in each brandy producing country influence the choice of cultivar as well as the style of brandy required. The main types of cultivars that are used in the production of brandy include Chenin Blanc, Colombard (mainly for South African brandy) Folle Blanche, Ugni Blanc, Baco and Palomino (in case of Armagnac and Cognac) and in America, Thompson seedless and Flame Tokay (le Roux, 1997).

A few studies have however focused on the requirements of grape cultivars for the production of base wine for brandy.

Studies conducted by Guymon (1969) concluded that the optimal grape variety for the production for brandy distillates is a white variety that displays a pleasing aroma, good tonnage and is also resistant to mould, rot and oxidation.

Lafin *et al.,* (1964) recommended the St. Emillion cultivar which is better resistant to *Botrytis cinerea* and has a higher acid content. Fruit containing a high phenolic content has also been shown to result in poor quality distillates (Toerien, 2008). Further studies conducted by Quady and Guymon (1973) indicate that there is a good correlation between quality of brandy and grapes that are fruity and aromatic versus grapes that are overripe and oxidized.

The effect of maturity level on the cultivar is also a factor to consider as the quality from Thompson Seedless was little affected by maturity while that from French Colombard decreased with increasing maturity. According to Lafon (1964) high quality distillates have never been made from wines of more than 10.5% v/v. The South African brandy industry also makes use of this quality criterion (Toerien, 2008).

### 2.3.2 Yeast strain

The majority flavour compounds are formed during fermentation by the yeast. These compounds include volatile organic acids, alcohols, aldehydes and esters. The production and the amount of these compounds found in the wine are yeast strain dependent (Nykänen, 1986).

Lurton *et al.,* (1995) extensively studied the influence of yeast from the cognac area on the composition of wine spirits. A highly significant effect of yeast strain was found for 43 compounds believed to be important for Cognac. The most interesting strains for the production of quality spirits had a high ester concentration along with low aldehyde and hexanol levels with a moderate to low levels of higher alcohols.

Riponi *et al.,* (1996) evaluated the behaviour of 15 *Saccharomyces* strains, which were well known for wine production but had not been evaluated for brandy production. They showed that the yeast produced wines that were significantly different and that the yeast could be classified on a high level into three groups, also based on work done by previous authors. The choice of the yeast strain used for the production of brandy base wine was however company dependent and was determined by the type of brandy desired for each individual company (Steger and Lambrechts, 2000).

During the production of cognac the most used yeast strain is *Saccharomyces cerevisiae*, However, Moreira *et al.,* (2005) showed that indigenous yeast strains can produce desirable sensory characteristics. There is however some controversy regarding the use of indigenous yeast strains on the organoleptic quality of wine. Studies by Gil *et al.,* (1996) observed that wines made from mixed yeast cultures resulted in higher concentrations of higher alcohols and acids, in contrast to those fermented only with *Saccharomyces cerevisiae*. Conversely, experiments conducted by Herraiz *et al.,* (1990) indicate that the production of higher alcohols increases with the use of *Saccharomyces cerevisiae*. Studies by Carnacini *et al.,* (1993) also show that the indigenous yeast strain *Hanseniaspora guilliermondii* increases the levels of 2-phenyethyl acetate and 2-phenylethanol, which are found to contribute positively towards the organoleptic quality of spirits. Therefore it can be concluded that the synthesis of secondary products is an individual and reproducible strain characteristic.

The final contribution of yeast will also depend on the winemaking processes followed as well as the fermentation conditions. Many authors have commented on the influence of fermentation temperature on the volatile compounds and have found that the amount of higher alcohols and aldehydes increase with an increase of temperature and that the esters and volatile organic acids increase with a decrease in temperature (Steger and Lambrechts, 2000).

It is recommended that the evaluation of the yeast strain for the production of distillates should only be analysed after the distillation procedure as distillation can influence the final composition of the distillate (Carnacini *et al.,* 1993).

Increasing fractions of yeast lees present during distillation lead to an increase in the concentration of ethyl caprate, ethyl caprylate and ethyl laurate. The ethyl esters of ethyl caproate, myristate and palmitate also increase in concentration but to a lesser extent (Guymon, 1974b; Carnacini and Di Stefano, 1989; Litchev, 1989; Watts *et al.,* 2003). No other effects on any other compounds were detected although this is probably due to the techniques utilized. The amount of lees used is an important factor to consider as even-numbered fatty acids have a large impact on the organoleptic properties of the distillate. Therefore the yeast strain used during the fermentation will ultimately influence the quality of the wine or distillate (Carnacini *et al.,* 1993; Riponi *et al.,* 1996; Steger and Lambrechts, 2000).

### 2.3.3 Malolactic fermentation

Malolactic fermentation is the fermentation caused by lactic acid bacteria, whereby malic acid is converted into lactic acid (du Plessis *et al.,* 2002). Lactic acid bacteria occur naturally on grapes and are able to flourish in grape juice and wine (Wibowo *et al.,* 1985). The genera *Lactobacillus*, *Leuconostoc*, *Oenococcus* and *Pediococcus* are the main lactic acid bacteria that are associated with the winemaking process.

Lactic acid bacteria can either be beneficial or detrimental to the quality of wine, and this depends on the species, strain and the time at which they occur in the winemaking practices (Lonvaud-Funel, 1999). Malolactic fermentation can contribute positively towards the flavour and aroma of the wine by increasing the "buttery aroma" flavour whilst decreasing the "green" characteristic in the wine. However if there is a large amount of lactic acid in the wine, this acid can combine with ethanol present to produce ethyl lactate thus making the wine undesirable. This reaction is accentuated if the wine is stored for a long time.

Malolactic fermentation is spontaneous and the percentage of malolactic fermentation that the base wine has undergone (partial or complete) will ultimately influence the final organoleptic profile of the distillate. Research by du Plessis *et al.,* (2002) show that *Oenococcus* is the most desirable genera of lactic acid bacteria as it has a more favourable influence on the brandy base wine and distillate quality.

Du Plessis *et al.,* (2002) found that spontaneous malolactic fermentation during prolonged storage of the base wine leads to an increase in ethyl lactate and diethyl succinate, which can impart a negative aroma and flavour into the resulting distillate. Compounds such as methanol and 2-butanol can also play a role, and be detrimental to the quality of the distillate (Dieguez *et al.,* 2005).

## 2.4 DISTILLATION

### 2.4.1 Techniques and apparatus

Distillation is the most important separation process in the chemical industry and entails a heating of a solution and condensing the resulting vapour into a different vessel (Léaute, 1990). The way in which volatile compounds will distill is governed by the distillation method and their volatility characteristics. This in turn is dependent entirely on the laws of vapour-liquid equilibrium thermo dynamics (Saco, 2006). Therefore distillation is a means of partial separation of the volatile components of the mixture.

There are many techniques used for the process of distillation, the main ones being batch distillation (discontinuous distillation) and column distillation (continuous distillation) (Carnacini, 1989). Most brandy is produced by batch distillation as this apparatus enhances the flavour and aroma of the resultant distillate. Studies conducted by Carnacini (1989) and Bougas (2009) show that batch or pot still distillation enhances the aromatic quality of the original wine, while continuous distillation results in a less aromatic end product.

**2.4.2 Pot stills**

Pot stills are used for what is known as double or batch distillation. Batch distillation is a term used for a distillation that entails distilling a mixture to obtain different component fractions. This is done before the distillation still is charged again with more mixture and the process is repeated (Bernot *et al.,* 1990). These stills are composed of copper in order to reduce the levels of off-flavour sulphur compounds. Copper reacts with the $SO_2$ in the wine during distillation and forms $CuSO_4$ (copper sulphate) thus reducing the levels of off-flavour sulphur compounds in the resultant distillate (Léaute, 1990).

There are two stages in batch distillation. The first entails taking wine and distilling it until the alcohol strength is 28-30% v/v. The latter is known as low wine and can be stored for a long period of time, as it is protected against microbial spoilage by the high alcohol content. The second stage envolves distilling the low wine and collecting it in three fractions. These are known as the heads, hearts and the tails. Each of these fractions contains different amounts and types of compounds (Léaute, 1990).

However, it is the heart fraction that is of importance as this is the fraction that is matured. The alcohol strength of the heart fraction ranges from 65 to 75% abv. The heads and the tails are carried back into another batch of low wine and redistilled to ensure that all the alcohol is recovered (Gold, 1972).

Not all the heads and tails are carried back to be redistilled. This will ultimately depend on the distiller as too much of these fractions can lead to a build-up of undesirable aromas associated with cereal-like flavours (Cantagrel, 1988). It has been found that an excess in tail fraction in the heart leads to an increase in ethyl lactate and 2-phenyl ethanol, and increase in the head section leads to increased short chain ethyl esters, aldehydes and higher alcohols (Cantagrel, 1988).

Figure 2.3 is a diagram representing a pot still (Alambic Charentaise) which is used to produce Cognac in the distillation technique known as "*Methode Charentaise*" (Léaute, 1990).
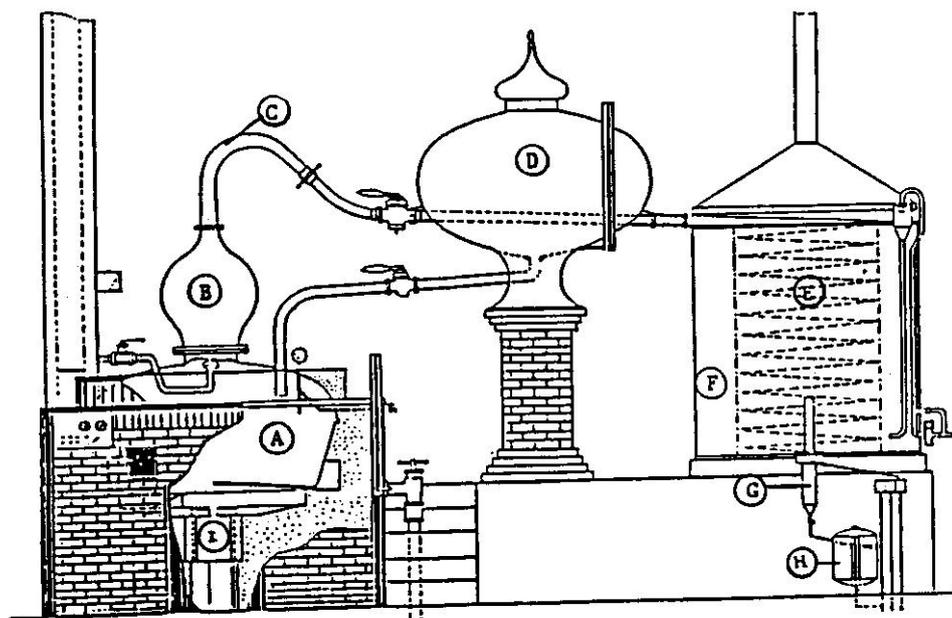
**Figure 2.3 Alambic Charentaise style Pot still. A (Boiler); B (Pot still head); C (Swan's neck); D (Reboiler); E (Copper coils); F (Condenser); G (Collector) and H (Distillation safe) (Léaute 1990).**

The heat intensity together with the distillation time throughout the distillation has a decisive influence on the formation and degradation of compounds. Reactions that occur during distillation of wine:

Esterfication/hydrolysis

Acid + Alcohol ⟷ Ester + Water

Acetal formation

Aldehyde + Alcohol ⟷ Acetal + Water

Maillard reaction

Sugar + Amino acid ⟶ Pyrazine, Furans

Strecker degradation

α-Amino acid ⟶ Aldehydes ⟶ Acetals
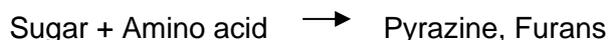
The choice of the distillation technique using either pot still or column still distillation is dependent of the style of final product (Guymon, 1974a; Léaute, 1990; Postel and Adam, 1990a). Changes of the distillation system greatly alter the volatile compounds found in distilled beverages. Therefore it is important to have a good understanding of the production process, as the distillation technique is of fundamental importance in influencing the organoleptic properties of the end product.

Differences in shapes of the pot still head and swan's neck will also alter the composition of the final distillate (Léaute, 1990; Carnacini, 1989). Studies by Léaute (1990) and Bougas (2009) found that the original "onion" shaped pot still head produced a more aromatic

distillate. The pot still that is used in South Africa is more cylindrical than the bulbous shaped one used in the "*Methode Charentaise*" method (Toerien, 2008).

### 2.4.3 Reflux

Reflux is a distillation technique involving the condensation of vapours and the return of this condensate to the system from which it originated. If the still head has a surface area that is either too large or long, then the vapours will cool and condense and run back down into the original liquid inside the pot still (Kister, 1992).

This is important as the vapours that have condensed and run back down will be boiled again. This reflux in the system ultimately influences the amount and types of compounds that will distil over into the distillate or un-matured Pot still brandy (Hampel and Hawley, 1982).

Studies by Léaute (1990) show that the shape and the volume of the pot still heads that are used will influence the separation, selection and concentration of the different volatile compounds found in the final product. A brandy that is made from a pot still that has a longer still head will be less flavoursome and contain less of the more full-bodied compounds, such as the longer chain fatty acids. The height of the swan's neck and the larger the still head in relation to the boiler will inevitably increase the rectification and therefore contribute to a smoother brandy with less character (Faith, 1992).
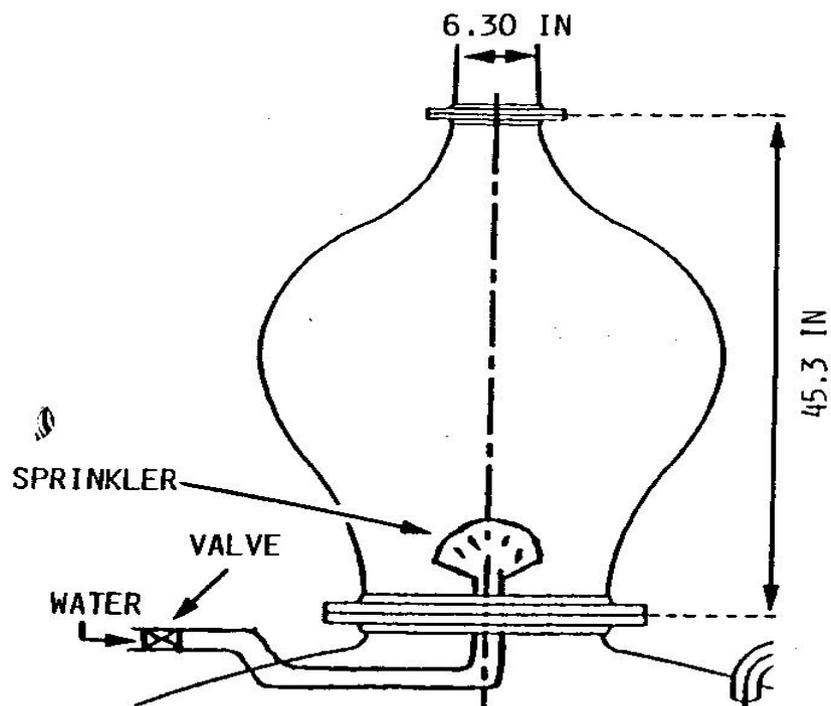
**Figure 2.4 Pot still head of Prulho pot still head (Léaute, 1990).**

Figure 2.4 represents the original Pot still head of the Prulho Pot still, which is used to produce Cognac. This Pot still is "onion" shaped and has a smaller surface area and therefore has less reflux. Brandies produced by this Still head are more aromatic and contain larger amounts of flavour compounds (Léaute, 1990).

Further studies by Madrera (2003) show that pot stills with a small vapour surface area generate poorer reflux during the distillation process as they do not allow for the recondensation of water into the pot and therefore the enrichment of volatile fraction in ethanol. This results in the distillate having an alcoholic content that was not as high as that obtained with other distillation systems namely discontinuous distillation.

### 2.4.4 Volatility of compounds

Wine is made up of mainly water and alcohol along with certain volatile compounds (Léaute, 1990). It is not only their vapour-phase equilibrium that will determine the way in which these volatile compounds will ultimately distill but also their boiling point, their relationship with alcohol or water, and lastly, the variation of alcohol content in the vapour during the distillation.

These volatile compounds are mainly polar in nature and therefore they are more soluble in water. There are a number of possibilities with regards to the relationship that the volatile compound has with alcohol or water namely:

Classification no 1, the compound is completely or partly soluble in alcohol and will distill when the vapour is rich in alcohol.

Classification no 2, the compound is soluble in water and will distill over when the vapour is low in alcohol.

Classification no 3, the compound is soluble in both and will distill over the entire distillation.

Classification no 4, the compound is not soluble in water, but the water vapour will carry it through to the final distillate (Léaute, 1990).

Compounds that are completely or partially soluble in alcohol have low boiling points and will be the first to distill over as the concentration of alcohol is high at the beginning of the process. As the distillation continues, the compounds that are more soluble in water will start to be recovered as they have a higher boiling point and are more polar in nature. The mixture contains less and less alcohol as the distillation continues and as time goes on more of the alcohol is recovered (Faundez, 2004). The boiling point of a certain compound together with the solubility in both water and alcohol has a significant effect on the way in which these compounds distill over into the final distillate, which will influence the sensory outcome and profile of the unmatured Pot still brandy.

Knowledge of how each compound reacts in the distillation process is valuable as this ensures the correct timing involved in the separation of unwanted compounds in the final product, thereby enabling the distiller to have control over the process and to ensure the production of optimum quality brandy (Saco, 2006).

## 2.5 Maturation

Maturation of distilled beverages is one of the most important and also costly factors that influence the quality of the desired product. Both the duration of the maturation period together with the type of oak cask used have a profound effect on the outcome of the spirit beverage (Mosedale and Puech, 1998).

For the production of wine and brandy, a different type of oak is selected depending on the desired characteristics needed by the producer (Bayly, 2002).

Most of the brandies produced in Europe are aged in oak barrels made from *Quercus robur* (Guichard *et al.,* 1995). In South Africa as well as Australia, Germany and Mexico, *Quercus robur* from the Limousin region in France seems to be most popular. For the production of Cognac, oak from the Limousin and Tronçias areas is preferred (Guymon and Crowell, 1972). The oak imparts aromatic elements to the brandy in greater or lesser amounts according to the choice of oak used, as coarse grain oak imparts tannins more easily than fine grain. The amount of time the staves are allowed to dry and their thickness is also a contributing factor.

Barrels undergo toasting of the inner surface. There are different degrees of toasting that are determined by the desired characteristics of the final brandy product. Toasting of the barrel leaves the inner surface toasted which releases important wood complexes into the spirit therefore altering the flavour, aroma and increasing its colour (Guymon and Crowell, 1972).

Hydrolysable tannins that are released into the spirit undergo different reactions such as the formation of esters from alcohols and acids. Lignin is a strengthening material of wood which releases aromatic aldehydes such as vanillin, conferaldehyde and syringaldehyde. Vanillin is a characteristic flavour of Cognacs and bourbons and is found in very high concentrations in new barrels after toasting (Bertrand, 2003).

Certain unpleasant compounds are extracted in the barrel; however the active toasted inner surface of the barrel contains carbon which can adsorb the unpleasant flavours such as methional, methional acetate, and dimethyl sulphide as well as dimethyl disulphide. Other compounds such as oak lactones, more specifically cis and trans-$\beta$-methyl-$\gamma$-octalactones, are also important as they have very low threshold levels and are responsible for the coconut, oaky characteristics that are typically associated with matured spirits (Perez-Coello *et al.,* 1999). When the brandy is stored in barrels, it is important to moderate the temperature, as this influences the maturation rate. An increase in temperature leads to an increase in the loss of ethanol from the barrel (Frederick, 2000). During the maturation period, pleasant compounds are released into the spirit, as well as different compounds such as esters which make an important contribution to the flavour of the spirit.

The process of aging and maturation is characterized by changes in flavour, aroma and colour of the distilled beverage. There is also a decline in both the volume and the alcoholic content throughout the maturation process. These changes can be attributed to a number of reactions, namely, the interaction between the wood and the constituents of the raw material, between only the distillate components, reactions between the wood extractives, direct extractions of wood compounds and the evaporation of volatile compounds (Mosedale and Puech, 1998).

The alcoholic strength of the spirit at the time of aging as well as the age of the oak barrel will play a role in the extraction of compounds that will ultimately influence the quality and characteristics of the brandy (Bertrand, 2003).

The length of the maturation period will also influence the amount and type of extraction of compounds. Table 2.2 shows the levels of these compounds in brandies and their evolution over time. It can be seen that the longer the spirit is matured, the higher the levels of aromatic compounds such as vanillin and coniferaldehyde. These compounds along with others are found to add positive characteristics to Cognac and brandies. Therefore, the type and duration of maturation is crucial in the outcome of the desired product (Bertrand, 2003).

**Table 2.2 Evolution of compounds during the maturation period in Cognac (Bertrand, 2003).**

| Type of compound (mg/L) | 0.7 yrs | 5 yrs | 13 yrs |
|---|---|---|---|
| Gallic acid | 4.6 | 9.0 | 15.3 |
| Vanillic acid | 0.3 | 1.4 | 2.8 |
| Syringic acid | 0.6 | 2.6 | 7.0 |
| 5-Hydroxymethylfurfural | 4.3 | 4.2 | 6.3 |
| Furfural | 26.8 | 24.7 | 21.3 |
| 5-Methylfurfural | 1.5 | 1.4 | 1.6 |
| Vanillin | 0.9 | 4.4 | 8.8 |
| Syringaldehyde | 2.25 | 8.9 | 17.6 |
| Coniferaldehyde | 3.65 | 5.9 | 6.7 |
| Sinapaldehyde | 9.45 | 17.8 | 17.0 |

Barrel maturation clearly has a significant effect to produce a soft and eloquent final product. As brandy is matured for anything from 6 months to 3 years, depending on the country in which it is produced, the quality of brandies will differ significantly. The balance is a delicate one: the newer the wood in which the spirit is stored and the longer it remains in the wood, the greater the influence (Robinson, 1994).

## 2.6 VOLATILE COMPOUNDS IN DISITLLATES AND FINAL BRANDY PRODUCTS

### 2.6.1 Higher alcohols

Higher alcohols, commonly known as fusel oils are alcohols that contain more than two carbon atoms and therefore have a higher molecular weight and higher boiling point than ethanol. They have an important aromatic effect in wines and especially distillates (Steger and Lambrechts, 2000).

The most abundant and important higher alcohols produced during the fermentation by the yeast are isoamyl alcohol, active isoamyl alcohol, isobutanol and n-propanol (Jounela-Eriksson, 1981). A number of investigations show clearly that the strongly smelling aliphatic alcohols such as 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol (isoamyl alcohol) together with phenethyl alcohol are produced *via* the anabolic pathways through which amino acids are synthesized. The production of higher alcohols is somewhat repressed with an increase of assimilable nitrogen in the grape must.

The amount of higher alcohols in brandy can range from 600 to 1200 mg/L. Brandies found to have a concentration of 600 to 750 mg/L are considered light brandies, those from 750 to 900 mg/L to be medium bodied and those brandies containing amounts higher than 900 mg/L are classified as full or heavy-bodied. The optimum level of fusel alcohols is approximately 800 mg/L (Warentin, 1952). Studies by Hough (1985) found that ratios of different higher alcohols can be used as criteria for the evaluation of brandy. The ratio of isoamyl alcohol to isobutanol must be at least 3:1 and that of isoamyl alcohol to propanol must be between 4:1 and 8:1 while the ratio of isobutanol to propanol should be between 1:1 and 2:1.

The ideal amount in brandies is still subject to controversy and depends on personal preferences and experience. The amount of higher alcohols found in brandy is determined by the final style required by the producer. The odour and taste of isoamyl and active amyl alcohols are found not to be pleasant therefore arguing that these values should be as low as possible. However, even though these compounds may exhibit unpleasant characteristics on their own, they can contribute towards the complexity of the matrix when found in small amounts and when mixed with other compounds (Postel and Adam, 1980). Furthermore, some of the higher alcohols will be esterfied during ageing, and these esters are more aromatically pleasant than their equivalent fusel alcohols (Guymon, 1972).

## 2.6.2 Esters

Esters are abundant constituents in foods and beverages, and their presence strongly influences the bouquet of wine and distilled beverages. One can expect to find at least 400 to 500 mg/L of total volatile esters in finished brandies. Even though the total ester concentration comprises only 9-14% of the total volatile compounds present they still have a large influence on the overall organoleptic properties of the spirit product (Postel and Adam, 1990a).

The total ester content in distilled beverages varies greatly. The content can be used as a criterion to classify quality in rum, whisky and cognac. Studies by Von Adam *et al.,* (1996) found that French and Italian distillates or brandies possess slightly higher ester concentrations (550 and 580 mg/L respectively), whilst German brandies possessed the lowest at 10%. Of the esters present in brandy, ethyl acetate and ethyl lactate are found to be the highest in concentration. The production of acetic and lactic acids, from which the esters are derived by reaction with alcohol, is due to microbial spoilage, and impart negative characteristics into the distillate.

In the "*Methode Charentaise*" of pot still distillation, ethyl acetate appears mainly in the heads fraction. By increasing the amount of head section that is removed during the distillation process the ethyl acetate concentration can be reduced in the heart fraction. During column still distillation rectification takes place which drastically reduces the amount of ethyl acetate and other volatile compounds found in the distillate when compared to pot still distillation. Different distillation techniques can account for the varying levels of ethyl acetate in distillates as studies by Postel and Adam (1990) show that German distillates contain 50 to 460 mg/L, Italian distillates concentrations are approximately between 86 and 1150 mg/L and Spanish

distillates contain 150 to 410 mg/L of ethyl acetate. It is recommended that there be a minimum of 250 mg/L and a maximum of 850 mg/L of ethyl acetate in all wine distillates.

Ethyl lactate is considered to be a tails fraction component and therefore levels can be reduced by cutting off the heart fraction early enough as the amount of ethyl lactate decreases dramatically after an alcoholic cut-off point of 85% (Von Adam *et al.,* 1996). If however the concentrations of these acids are low they can contribute positively to the overall aroma (Salo *et al.,* 1972). It is thus important to consider the origin and factors that may influence the production of these two compounds in further detail (Von Adam *et al.,* 1996).

The ethyl esters of the long-chained fatty acids are found to also contribute to the overall aroma and flavour of brandy (Bayly, 2002). Studies by Salo *et al.,* (1972) indicate that the ethyl esters of even carbons between 6 and 12 to be major contributing factors to the flavour of distilled beverages. Levels of these esters are found to range between 3 to 100 mg/L in wine distillates and studies by Von Adam *et al.,* (1996) mention that the levels of these compounds can be used to distinguish between Cognac and other brandies. The levels of these compounds were found to be at an average of 72 mg/L in French distillates, 39 mg/L in Armagnacs and in the case of Spanish brandies 47 mg/L.

The amount of these compounds are influenced by the lees content, where the more lees used during the distillation process results in a higher amount of the esters (Cantagrel *et al.,* 1992). Despite high boiling points, fatty acids/esters appear early on in the distillate obtained and contribute immensely to the aroma and flavour of the distillate (Simpson, 1971). These ethyl esters of caproic ($C_6$), caprylic ($C_8$), capric ($C_{10}$) and lauric acid exhibit characteristic fruity and flowery odour notes (Ferrari *et al.,* 2004, Watts and Butzke, 2003). Of particular interest is the ratio of ethyl caprate to ethyl caprylate. With increasing fractions of added lees, the concentration of ethyl caprylate increases significantly above that of ethyl caprate (Cantagrel *et al.,* 1992). Thus the ratio of ethyl caprate to ethyl caprylate can serve as valuable indicator for the amount of yeast lees used in the distillation (Von Adam *et al.,* 1996).

According to Von Adam *et al.,* (1996), the $C_6$ to $C_{16}$ esters behave differently in terms of their volatility and time of distilling over during pot still distillation. When the heads section was set at 1% of total distilling volume, and the heart fraction was swung to tails at 60% volume, the heart of the distillate showed a ±34% decrease in ethyl caproate concentration, approximately 40% for ethyl caprylate and 50% for ethyl caprate. However, during column still distillation, the levels of ethyl caproate, caprylate and caprate remain constant. Under normal distillation conditions, when a portion of the yeast lees is distilled with the wine, a ratio above 0.3 can be expected for ethyl caprate: ethyl caprylate (Cantagrel *et al.,* 1992).Caproate is fragrant and has an odour similar to that of banana oil; caprylate is more pungent and less fragrant and resembles crude grape fusel oil; caprate is less intense and milder with fatty tones and finally laurate is the least aromatic and has a waxy candle-like odour (Guymon, 1969).

Ethyl propionate, diethyl succinate and ethyl butyrate are all so-called spoilage indicators which can be used to evaluate the distillate raw material. The concentration of ethyl propionate should not exceed 150 mg/L. Higher values are generally found in distillates made from microbially spoiled wines (Postel and Adam, 1990a).

### 2.6.3 Acids

In wine distillates, the amount of volatile fatty acids should not vary greatly from the base wine used for the distillation as fatty acids with carbon atoms between 1 and 10 are volatile enough to be distilled over. It is common practice to distill the base wine with a portion of yeast lees for the production of brandy thus contributing to the amount of fatty acids present in wine distillates. The main sources of fatty acids are from autolysis and thermal degradation of the yeast cells during distillation (Sponholz *et al.,* 1990).

Of the fatty acids, acetic acid is found to be the dominant acid in spirit products and constitutes 40-95% of the total volatile acids in whisky, 50-75 % in Cognac and brandy, and for rum 75-90 %. If one disregards acetic acid then capric acid is the largest component, with octanoic and decanoic acid comprising up 30% of the total volatile acids and hexanoic acid (8%) and propionic acid, isobutyric acid, isovaleric acid making up the difference (Nykänen, 1983).

Studies conducted by Sponholz *et al.,* (1990) were able to use the levels of fatty acids as a way in which to distinguish different brandies. French brandies were found to have higher levels of even chain fatty acids (predominantly yeast derived) due to the distillation technique employed where the second distillation results in a separation of the head, heart and tail sections. It is known that the long-chain fatty acids are of higher importance than the short-chain fatty acids when considering the overall sensory perception of distilled beverages (Nykänen, 1986).

### 2.6.4 Aldehydes

Among the volatile compounds present in distilled beverages, aldehydes are found to be of importance as they are present in large amounts. Of the carbonyl compounds, acetaldehyde is the major component and constitutes approximately 90% of the total aldehyde content in alcoholic beverages (Guymon, 1969; Ferrari *et al.,* 2004).

The amounts of acetaldehyde vary greatly and relatively large concentrations are found in whisky, cognac, brandy and rum. Together with acetaldehyde, 2,3-butanedione (diacetyl) should also be considered as they are a ubiquitous flavour in distilled beverages. These compounds have very low sensory threshold values. 2,3-Butanedione exhibits a butter-like odour (threshold value of 0.2 mg/L) and is found to be higher in rum than in brandy or whisky (Ferrari *et al.,* 2002).

## 2.7 QUALITY INDICATORS IN BRANDY

Wines infected with *Botrytis cinerea* can lead to a defect in the organoleptic properties of the distillate. These infected wines (normally amongst other things) display an increase in ethyl lactate, acetic acid and the fungus *Botrytis cinerea* (Cantagrel, 1988; Panosyan *et al.,* 2002).

Infected wines will exhibit characteristics such as a loss of fruitiness, appearance of lactone, sotolon and oxidation flavours such as prune and maderized.

Gas chromatographic analysis can also ascertain defects such as sourness due to the ethyl acetate and pungency which correlates with the sum of acetal and acetaldehyde (50-200 mg/L). Stagnant flavours and odours can be associated with ethyl butyrate and 1-butanol. Compounds such as acrolein can present a plastic characteristic in the distillate (Panosyan *et al.,* 2002).

Studies conducted by Cantagrel (1988) show that there are certain limits to the amount that a compound can be within a distillate before it will be considered a defect. There are threshold values that will affect the sensory perception of the distillate.  The amount of ethyl acetate must not exceed 600 mg/L, whereas compounds such as 1-butanol and 2-butanol can range from 6-7 mg/L before they render the product unsatisfactory. Acetaldehyde is limited to 60 mg/L, ethyl butyrate between 4-5 mg/L and 2, 3-butanediol an average of 8 mg/L.

Positive indicators for brandy quality include compounds such as isoamyl acetate (0.3-10 mg/L) which affords fruity notes to the distillate. The fatty acid ethyl esters are responsible for the floral aroma found in distillates and the value ranges from 10-30 mg/L. Herbaceous characteristics are associated with the compound 3-hexanol, and buttery flavours and aromas are produced from diacetyl, though a concentration above 4 mg/L is considered negative.

Freshly distilled cognac can contain certain compounds that are assigned to specific odour notes that arise from the distillation process and grapes; however their aromatic quality depends on the interaction of these compounds in the mixture (Ferrari *et al.,* 2004).

## 2.8 CONCLUSIONS AND FUTURE TRENDS

Future trends in the brandy industry will most probably be largely shaped by consumer needs and expectations as well as legislation with regards to driving alcohol limits, advertisements etc. Consumer needs will ultimately drive for unique differentiation of brandy products thereby promoting innovation, new product development and versatility due to increased global competition.

The cognac for example recently experienced a significant decline in sales due to cognac in France being perceived as too old fashioned and the younger generation was also less inclined to drink cognac neat- it was not made to be mixable (Peskett, 2006). The industry responded with new products and ideas to add Cognac to mixers and in cocktails. Another way in which the sale of brandy could increase would be flavour extensions to the product. Many of these products have been released with Cognac infused with vanilla, jasmine and apple flavours (Davies, 2003). This had led to an increase in consumption of Cognac by younger or new consumers.

Brandy is competing with many other alcoholic and non-alcoholic beverages and therefore the brandy industry will have to keep on innovating and to maintain and grow its consumer base. This innovation can be in many areas such as marketing, packaging, concepts, the

product itself. The future trends for brandies, Cognac and Armagnac seem to be around versatility.

Cognac is sold according to its classification system namely VS (Very Special), VSOP (Very Special Old Pale) and XO. This classification system can be confusing, especially in countries outside of France as it does not indicate what the age of the Cognac is. Consumers are hesitant to purchase a product that they know little about and therefore, to enable the product to be more user friendly and to encourage sales of Cognac, if the age were to be indicated on the label, it would provide the consumer with more information, therefore resulting in a better understanding of the product.

The brandy industry can also learn from whisky as there are many specific vintage whiskies available, however for brandy there are no brandies that are made up of a single vintage. If a brandy was classified according to the year it was made and this was indicated on the label, one could perhaps then compare the different vintages with each other.

In the wine industry many yeast strains are used and much research has been conducted regarding what yeast strains are ideal for a certain type of wine style. However, with the production of brandy, Cognac and Armagnac there are only a few types of yeast strains used. More research on the effect of different yeast strains on the style and quality of brandy could result in the production of new products which would be beneficial to the brandy industry.

Additionally, investigation into individual grape cultivars and the production of a brandy that is cultivar specific could result in the exploitation of the characteristics that are unique to that cultivar to drive the sale of the product. For example in the case of Sauvignon blanc, methoxypyrazines (green pepper aroma and flavour) are favourable amongst consumers.

Unfortunately there is limited research available regarding the distillation process, with many companies choosing to keep information obtained in-house. Together with the limited amount of information pertaining to distillation techniques available, there is also a gap linking the spirit production process together with an ever developing field of sensory science.

An area of research that could deliver interesting results, but which does not appear to be actively pursued, is the linking of sensory research to consumer preferences. In contrast numerous articles have been published establishing the drivers of liking of consumers for non-distilled wine products. These results are actively used to optimize wine styles and to develop new styles to fill market gaps.

In conclusion, although the forecasted growth of spirits, including brandy, is predicted to be positive for the short to medium term, especially due to the opportunities that exists in the developing countries, the brandy industry will need to be proactive to maintain and grow their share of consumer spend and their relevance in the minds of the consumer. South African brandy's big opportunity is to continuously build on its global quality credentials if it wishes to expand into global markets.

## 2.9 LITERATURE CITED

Bayly, J.C. 2002. Manipulating the levels of ethyl acetate and isoamyl acetate production during the formation of wine and brandy. Dissertation. Department of Wine biotechnology. Stellenbosch University.

Berberoglu, H. 2010. A survey of brandy producing countries of the world. Professor Emeritus of Hospitality and Tourism Management specializing in Food and Beverage.

Bernot, C.M., M. Doherty., M.F. Malone. 1990. Patterns of composition of change in multicomponent batch distillation. Chem. Eng. Sci. 45: 1207.

Bertrand, A. 2003. Brandy and Cognac: Armagnac, Brandy,  Cognac, and their manufacture. Encyclopedia of Food Sciences and Nutrition. 584-601.

BNIA (Bureau National Interprofessionel de l'Armagnac). 2010.

BNIC (Bureau National Interprofessionel du Cognac). Decree 1986.

BNIC (Bureau National Interprofessionel du Cognac). 2010.

BTI (Beverage Testing Institute). 2010.

Cantagrel, R. 1988. A scientific approach to quality control for Cognac spirits. Distilled Beverage Flavour: Recent developments. Eills Horword. Chichester.

Cantagrel, R.; Mazerolles, G.; Vidal, J.P.; Galy, B.; Boulesteix, J.M. 1992. First International Symposium in Cognac. Cognac.

Carnacini, A; Di Stefano, R. 1989. Effect of winemaking practices on the volatile composition of distillates. Ital. J. Food. Sci. 1, 4: 13-22.

Davies, C. 31 October 2003. Armagnac. Harpers. 24-26.

Diequez, S.C., M. Luisa., L.G. de la Pena., E.F.Gomez. 2005. Volatile composition and sensory characters of commercial Galician orujo spirits. J. Agric. Food. Chem. 53: 6759-6765.

du Plessis, H.W., C.L.C Steger., M. du Toit., M. Lambrechts. 2002. The occurrence of malolactic fermentation in brandy base wine and its influence on quality. J. Appl. Micro. 92: 1005-1013.

Electronic code of Federal regulations. 2010. Title 27: Alcohol, Tobacco and Firearms. Section (d): Class 4: Brandy.

Faith, N. 1992. Nicholas Faith's guide to Cognac and other  brandies. Mitchell Beazley International Ltd. New York.

Faundez, C. 2004. Phase equilibrium modeling in binary mixtures found in wine and must distillation. J. Food. Eng. 65:577-583.

Ferrari, G., O. Lablanquie., R. Cantagrel., T. Payot., N. Fournier., E. Guichard. 2004. Determination of key odourant compounds in freshly distilled Cognac using GC-O, GC-MS and sensory evaluation. J. Agric. Food Chem. 52: 5670-5676.

Frederick, J.F. 2000. Wiley encyclopedia of food science and technology. (2nd Ed). 1: 509-539.

Gargett, K. 2007. Brandy power. National Liquor News. 32-34.

Gil, J.V., J.J Mateo., M. Jimenez., A. Pastor., T. Huerta. 1996. Aroma compounds in wines as influenced by Apiculate yeasts. J. Food. Sci. 61, 6: 1247-1250.

Gold, A.H. 1972. (2nd Ed). Wines and spirits of the world. Virtue & Co Ltd, London.

Grossman, H.J. 1964. Grossman's guide to wines, spirits and beers-fourth revised edition. New York.

Guichard, E., N. Fournier., G. Masson., J-L. Puech. 1995. Stereoisomers of β-Methyl-γ-Octalactone. I. Quantification in brandies as a function of origin and treatment of barrels. Am. J. Enol. Vitic. 46, 4: 419-423.

Guymon, J.F. 1969. GC determination of ethyl esters in brandy or wine distillates. Am. J. Enol. Vitic. 20, 2: 76-85.

Guymon, J.F and E.A Crowell. 1972. GC-separated brandy components derived from French and American oaks. Am. J. Enol. Vitic. 23, 3: 114-120.

Guymon, J.F. 1972a. Higher alcohol production in brandy beverage production. Wines and Vines. 49, 1: 25-28.

Guymon, J.F. 1974. Chemical aspects of distilling wine into brandy. Adv. Chem. 137: 232-253.

Hampel, C.D., and G.G Hawley. 1982. Glossary of chemical terms. (2nd Ed). Academic press. New York.

Heckle, H. February 2003. Spain: Brandy de Jerez. Wine and Spirit International. 36-37.

Herraiz, T., G. Reglero., M. Herraiz., P.J. Martin-Alvarez., M. Dolores Cabezudo. 1990. Influence of the yeast and the type of culture on the volatile composition of wines fermented wit sulphur dioxide. Am. J. Enol. Vitic. 41, 4: 313-318.

Hilmen, E. 2000. Separation of Azeotropic mixtures: Tools for analysis and studies on batch distillation operation. Dissertation. Norwegian University of Science and technology.

Holtzkamp, E. 2012. Liquor consumption patterns in South Africa. South African Wine Industry Information and Systems.

Hough, B.J. 1985. The effect of cultivar, region, and vintage on higher alcohol concentration of unmatured South African brandies (Unpublished results from S.A Brandy Foundation Research).

Jolly, N.P., and S. Hattingh, S. 2000. A brandy wheel for South African brandy. S. Afr. J. Enol. Vitic. 22, 1: 98-115.

Jounela-Eriksson, P. 1981. Predictive value of sensory and analytical data for distilled beverages. In Flavour'81. Schreier, P. (1st Ed). Berlin.

Kister, H.Z. 1992. Distillation design. (1st Ed). McGraw-Hill. Columbus.

Léaute, R. 1990. Distillation in Alambic. American journal of Enology and Viticulture. 41, 1: 90-103.

Ledauphin, J. 2006. Gas chromatographic quantification of aliphatic aldehydes in freshly distilled Calvados and Cognac using 3-methylbenzothiazolin2-one. Journal of chromatography A. 1115: 225-232.

Lem, W. 2004. Anthropologia: Commodities, Capitalism and Globalization. 46, 2. Canadian Anthropology Society. Editor: Winnie Lem. Trent University.

Lenoir, J. 2009. Cognac Aroma Wheel. Second International Cognac Summit. Bureau Interprofesionel du Cognac.

Le Roux, J. 1997. Van Ryn's advanced brandy course. The Van Ryn Wine an Spirit Company.

Litchev, V. 1989. Influence of oxidation processes on the development of the taste and flavour of wine distillates. Am. J. Enol. Vitic. 40. 247-253.

Lonvaud-Funel, A. 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Biomedical and life sciences. 76, 1-4: 317-331.

Lurton, L; Snakkers, G; Roulland, C; Galy, B; Versavaud, A. 1995. Influence of the fermentation yeast strain on the composition of wine spirits. J. Sci. Food. Agric.67:4. pp 485-491.

Madrera, R. 2003. Influence of the distillation system, oak wood type, and aging time on the volatile compounds of cider brandy. J. Agric. Food. Chem. 51: 5709-5714.

Marťinez Montero, C., M. Del Carmen Rodríguez Dodero., D. Antonio Guillén Sánchez., and C. García Barroso. 2005. Sugar contents of Brady de Jerez during its Aging. J. Agric. Food. Chem. 54: 1058-1064.

Moore, W.J. 1962. Physical chemistry. (3rd Ed). Prentice hall. New Jersey, 140-142.

Moreira, N., F. Mendes., T. Hogg., I. Vasconcelos. 2005. Alcohols, esters and heavy sulphur compounds produced by pure and mixed apiculate wine yeasts. Int. J. Food. Micro. 103, 3: 285-294.

Mosedale, J.R and J-L. Puech. 1998. Wood maturation of distilled beverages. Trends in Food Science and technology. 9, 3: 95-101.

Nykänen, L. 1983. Aroma of beer, wine, and distilled alcoholic beverages. (1st Ed). D. Riedel Publishing Company. Dordrecht, Holland.

Nykänen, L.H. 1986. Formation and occurrence of flavour compounds in wine and distilled beverages. Am. J. Enol. Vitic. 28, 3: 152-158.

Perez-Coello, M.S., J. Sanz., M.D. Cabezudo. 1999. Determination of volatile compounds in hydroalcoholic extracts of French and American oak wood A. J. Enol. Vitic. 50, 2: 162-165.

Peskett, S. 2006. Cognac: Business is booming. Harpers supplement: Cognac. 3-5.

Postel, W and Adam, L. 1990a. Fluechtige Gaschromatograpische Karakterisierung von Cognac und Armagnac-Gehalte von fleuchtigen Verbindungen. Branntweinwirtschaft. 130: 208-213.

Postel, W.; Adam, L. 1980. Gaschromatographisch charakterisierung von Weinbrand, Cognac und Armagnac. Branntweinwirtschaft. 120: 154-163.

Quady, A.K., and J.F. Guymon. 1973. Relation for maturity, acidity, and growing region of Thompson seedless and French Colombard grapes to wine aroma and the quality of a brandy distillate. Am. J. Enol. Vitic. 24, 4: 166-175.

Rose, A. 1977. Alcoholic beverages. London; New York: Academic Press.

Riponi, C; Carnacini, A; Castellari, L; Zambonelli, C. 1996. Influence of yeast strain on the composition of wines for the production of brandy. J. Wine. Res. 8: 41-50.

Saco, P. 2006. Rule-based intelligent monitoring and control of marc brandy stills. Computers and chemical engineering. 30: 1132-1140.

SAWIS (South African Wine Industry Information and Systems). 2009. Euromonitor international: country sector briefing.

Serjeantson, H. 2010. Armagnac. 700 years young. Category report. IWSR spirits review.

Sponholz, W.R.; Dittrich, H.H.L.; Bausch, H. 1990. Fluechtige Fettsauren in Weindestillaten, Cognacs und Deutschen Weinbranden. Deutsche Lebensmittel Rundschau. 4:  12-15.

Steger, C.L.C and M. Lambrechts. 2000. The selection of yeast strains for the production of premium quality South African brandy base products. J. Ind. Micro. Biotech. 24, 6: 431-440.

Snyman, C.L.C. 2005. Alcohol distillation. Oenology 444. Course work. Stellenbosch University.

Snyman, C. 2010. Personal communication.

South African Liquor Products. 1989. Act No.

South African Brandy Foundation.

Spencer, H. 1983. Cognac Country: The Hennessey book of people and their spirit. Quiller Press Ltd. London.

Toerien, W. 2008. Firewater. Quivertree publications. Cape Town. South Africa.

Tritton, S.N. 1975. Spirits, aperitifs and liqueurs: their production. London. Faber and Faber limited.

Venter, B. September 1996. Armagnac: Die Franse Stiefkind. Wynboer Tegnies. T3-T5.

Von Adam, L.; Haug, M; Kolb, E.; Schwartz, C.H. 1996. Contribution to the knowledge of volatile compounds present in wine distillates from the years 1990 to 1993. Die Branntweinwirtsschaft. 6: 82-90.

Watts, V.A., and C.E. Butzke. 2003. Analysis of microvolatiles in brandy: relationship between methylketones concentration and Cognac age. J. Sci. Food. Agric. 83: 1143-1149.

Warkentin, H. 1952. Analytical evidence of improvement of brandy. Wines and Vines. 33, 5:175-184.

Wibowo, D., R. Eschenbruch., C.R. Davis., G.H. Fleet., T.H. Lee. 1985. Occurrence and growth of lactic acid bacteria in wine: a review. A. J. Enol. Vitic. 36, 4: 302-313.

# Chapter 3

## Research results

## IDENTIFYING THE CHEMICAL PROFILES OF DIFFERENT STYLES OF THREE-YEAR BARREL MATURED BRANDY SAMPLES

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# 3. Identifying the chemical profiles of different styles of three-year barrel matured brandy samples

## ABSTRACT

Three-year barrel old matured brandy samples from a large distillery were classified into five different styles (class one until class five) by an internal sensory panel.  Class one and class five were the most differentiating styles from each other, and therefore the purpose of this study was to determine the chemical profiles of these two extreme styles of three-year barrel old matured brandy samples and identify those compounds/groups of compounds that were found to differentiate between the two sensory classes. The samples were analysed using GC-FID (Gas flame ionization detection) and HPLC (High performance liquid chromatography) to identify the chemical profiles of the respective styles. The class one three-year barrel old matured brandy samples were found to contain overall less total esters, less acids, a higher concentration of total higher alcohols, total phenols and higher ratio of higher alcohols vs. esters, higher alcohols vs. acids and aldehydes vs. esters and were perceived to be less "fruity" in aroma and flavour when compared to the class five samples The class five three-year barrel old matured brandy samples were found to be correlated to higher concentration of total esters, total acids, total furans, total lactones and a lower ratio of higher alcohols vs. esters, higher alcohols vs. acids, but however a higher ratio of aldehydes vs. acids. These samples were also found to be more "fruity" in aroma and flavour. The differences in concentration of these compounds (esters, higher alcohols, acids and the ratios) were found to be the differentiating factor between the sensory style classification of a class one and a class five three-year barrel old matured brandy sample.

## 3.1 INTRODUCTION

There are many different styles of brandy available on the market today, each vary with regards to aroma, flavour and overall organoleptic perception (Gold, 1972). Brandy producers in South Africa cater for diverse market needs, and thus have divergent opinions as to what comprises a good base wine and distillate for their individual end-product style in mind (Toerien, 2008).

The main categories of brandy in South Africa include blended, vintage, and pot still brandy. Each of these brandies varies greatly with regards to their sensory profile and is firstly dependent on legal classification. In the case of blended brandy, it consists of a minimum of 30% pot still brandy which has been matured for three years together with a maximum of 70% un-matured wine spirit (le Roux, 1997). Vintage brandy has a distinct wood character when compared to pot still and blended brandy (Toerien, 2003). It consists of a minimum of 30% pot still brandy matured for 8 years, 60% column still brandy matured for at least 8 years and a maximum of 10% unmatured wine spirits The alcohol concentration of this brandy is minimum 38% v/v. Pot still brandy consists of 100% Pot still brandy with an alcohol concentration of 38% v/v (Wine and Spirits Control Act No 47 of 1970).

In earlier studies, it was believed that the flavours of alcoholic beverages were only made up of small amounts of compounds. However over 1300 different volatile compounds have been identified and if the non-volatile components are also included, then the amount would probably double (Nykänen, 1986). There are many factors that will influence the production of the volatile and non-volatile compounds and therefore the style of brandy. These include the type of vintage, geographical origin, cultivar, vinification techniques, the extent of malo-lactic fermentation, distillation and maturation (Bayly, 2002; Watts and Butzke, 2002; Panosyan, 2002). The way in which factors influence the aroma and characteristics of the brandy, either as a whole or individually are important to understand and being able to manipulate them to such an extent could lead to an overall better control of the production process and therefore better quality products (Léaute, 1990).

Both the major and minor components found in brandy are responsible and are essential for the total brandy aroma. Esters, acids, carbonyls and higher alcohols are the dominant compounds and play an important role in the profile of the spirit product and it is not necessarily the type of compound but the quantity of it and how the compounds interact with each other that will ultimately influence the sensory perception (Steger and Lambrechts, 2000).

Higher alcohols have an important aromatic effect in wines and especially distillates (Steger and Lambrechts, 2000). The most abundant higher alcohols found in distillates are i-amyl alcohol, active i-amyl alcohol, i-butanol and n-propanol (Jounela-Eriksson, 1981). The amount of higher alcohols in brandy can range from 600 to 1200 mg/L. Brandies found to have a concentration of 600 to 750 mg/L are considered light brandies, those from 750 to 900 mg/L to be medium bodied and those brandies containing amounts higher than 900 mg/L are classified as full or heavy-bodied. The optimum level of fusel alcohols is approximately 800 mg/L (Jounela-Eriksson, 1981).

Esters are abundant constituents in foods and beverages, and their presence strongly influences the bouquet of wine and distilled beverages. One can expect to find at least 400 to 500 mg/L of total volatile esters in finished brandies. Even though the total ester concentration comprises only 9-14% of the total volatile compounds present they still have a large influence on the overall organoleptic properties of the spirit product (Postel and Adam, 1990a). Of the esters present in brandy, ethyl acetate and ethyl lactate are found to be the highest in concentration. The ethyl esters of the long-chained fatty acids are found to also contribute to the overall aroma and flavour of brandy. Studies by Salo *et al.,* (1972) indicate that the ethyl esters of even carbons between 6 and 12 to be major contributing factors to the flavour of distilled beverages.

Of the fatty acids, acetic acid is found to be the dominant acid in spirit products and constitutes 40-95% of the total volatile acids in whisky, 50-75% in Cognac and brandy, and for rum 75-90%. If one disregards acetic acid then capric acid is the largest component, with octanoic and decanoic acid comprising up 30% of the total volatile acids and hexanoic acid (8%) and propionic acid, i-butyric acid, i-valeric acid making up the difference (Nykänen, 1983). It is known that the long-chain fatty acids are of higher importance than the short-chain fatty acids when considering the overall sensory perception of distilled beverages (Nykänen, 1986).

Among the volatile compounds present in distilled beverages, aliphatic carbonyls are found to be of importance as they are present in large amounts. Of the carbonyl compounds, acetaldehyde is the major component and constitutes approximately 90% of the total aldehyde content in alcoholic beverages (Guymon, 1972). Together with acetaldehyde, acrolein and 2,3-butanedione (diacetyl) should also be considered as they are a ubiquitous flavour in distilled beverages (Panosyan, 2002)..

In the industry, spirit products are evaluated using descriptive testing such as profiling or Quantitative Descriptive Analysis (QDA), where the product is assessed in order to gauge their aroma, flavour and mouth-feel and accordingly their style classification. A trained panel is used to evaluate the spirit products and to produce attributes that best describe the product. Past studies indicate that sensory evaluation should be used together with the chemical analysis of the product so that a correlation can be made between the sensory data and the compounds that play a role in the profile of the product (Jack, 2003).

However the purpose of this study was to identify the chemical profiles of known style class one and class five classified three-year barrel old matured brandy samples as well as to determine correlations between the chemical profiles and the desired style. Thus determining what compounds/groups of compounds are found to differentiate the class one and class five three-year barrel old matured brandy samples from each other.

## 3.2 MATERIALS AND METHODS

Previous findings by Bougas (2009) indicated that the sensory classification of un-matured pot still brandy shifted when the ratio of esters, higher alcohols, acids and aldehydes were altered. Therefore, three-year barrel old matured brandy samples were analysed for both the

volatile and non-volatile compounds present in the samples in order to determine their chemical composition.  Three-year barrel old matured brandy samples are routinely drawn and undergo a sensory style classification by an internal brandy panel. These three-year barrel old matured brandy samples are classified using a sensory style scale ranging from class one to class five and evaluated according to aroma, flavour and overall mouth-feel. Both class one and class five are found to be on the extreme ends of the sensory style scale and are considered to be vastly different from each other.

Over a three year period a total of 104 three-year barrel old matured brandy samples were collected. An internal sensory panel classified these samples into a class one to class five styles. It is important to note that there was no consensus obtained throughout the panel when tasting as each panellist tasted alone and at different times. Of the 104 samples drawn, 7 were class one, 11 were class two, 18 a class three, 41 a class four and 23 were classified as a class five three-year barrel old matured brandy sample. Class one three-year barrel old matured brandy samples were perceived to be less "fruity" on aroma and flavour, and were considered to have a smoother mouth-feel than class five three-year barrel old matured brandy samples. The inverse was found to be true for class five three-year barrel old matured brandy samples.

### 3.2.1. Chemical analyses of the class one and five three-year barrel old matured brandy samples

The three-year barrel old matured brandy samples were analysed  using GC-FID (Gas flame ionization detection) and HPLC (High performance liquid chromatography) to quantify the volatile compounds: (Ethyl acetate, ethyl butyrate, i-amyl acetate, ethyl hexanoate, hexyl acetate, ethyl lactate, ethyl octanoate, ethyl decanoate, di-ethyl succinate, 2-phenyl ethyl acetate, n-propanol, i-butanol, n-butanol, i-amyl alcohol, hexanol, 2-phenyl ethanol, acetic acid, n-butyric acid, i-butyric acid, hexanoic acid, octanoic acid, decanoic acid, acetaldehyde, acetoin, 5-hydroxymethyl furfural, furfural and 5-methyl furfural, cis-lactone, trans-lactone, vanillin, guaiacol, ethyl guaiacol and eugenol) and non-volatile compounds: (Gallic acid, protocatechuic acid, p-hydroxy benzoic acid, vanllic acid, caffeic acid, syringic acid, ferulic acid, ellagic acid, p-coumaric acid, syringaldeyde, coniferaldehyde, sinapaldehyde, catechin, epicatechin, o-cresol, phenol, p-cresol and 2,6-dimethoxy phenol) compounds found in the samples. Samples were measured in triplicates and the standard deviation was ≤ 5%.

A volume of 5 mL of each sample ca. 70% (a/v) three-year barrel matured brandy sample was spiked with 0.25 mL of the internal standard solution (4-methyl-2-pentanol at a concentration of 2 g/L in absolute ethanol) and transferred into 2 mL crimp-top autosampler vials. The vials were sealed and a volume of 2 µL was injected into the GC inlet. The operating conditions were used as described in Steger and Lambrechts (2000).

Neutral wood phenols were analysed by capillary gas chromatography. Six mL of the distillate solution was added to 4 mL of deionized water, 5 mL of di-ethyl ether and 0.4 mL of a 2-phenethyl acetate alcohol solution at 100 mg/L. Samples underwent continuous liquid-liquid extraction in a rotary mixer at 60 rpm (no vacuum) for 30 minutes before removing 1 mL of the upper di-ethyl ether layer for subsequent analysis. Samples were eluted on an HP

5890 series II gas chromatograph with an HP 7673 injector couples to an HP 3396A integrator. Column:  LabAlliance polyethylene glycol (capillary column); Dimensions: 60 m x 0.32 mm ID x 0.5 µm. Injector temperature: $200^0$C; Detector (FID) temperature: $250^0$C; Gas flows: $H_2$ (column) 3 – 5 ml/min, $N_2$ (make-up) 30 ml/min; synthetic air 300 mL/min; Oven program: $80^0$C increasing to $210^0$C at $3^0$C/min and thereafter up to $230^0$C at $10^0$C/min; Split flow: 10 mL/min; Column head pressure: 14 psi; Injection volume: 5 µL (Steger and Lambrechts, 2000).

Phenolic acid and related aldehydes were determined using an Agilent 1100 high pressure liquid chromatograph. Column: Phenomenex Luna 5 µm C18(2); Dimensions: 150 m x 4.60 mm x 5 µm; Oven temperature: $40^0$C; Column flow: 1 mL/min; Stop time: 36 minutes; Post time: 9 minutes; Solvents: A = 100% acetonitrile; B= 0.1% perchloric acid; Solvent A = 100 – (solvent B); Wavelenght: 280nm ((Steger and Lambrechts, 2000).

The pH and TA (total acidity) for each sample was also measured. The pH was determined using an automatic titrator equipped with a combination electrode (Crison, catalogue no. 4473624, LASEC, SA) and a temperature probe. Certified buffers (pH 7.00 and pH 4.00, LASEC, SA) were used to calibrate the electrode. TA was expressed as g/L tartaric acid and measured by potentiometric titration (Crison Compact Titrator D, SN 01714, Spain, software version 5.6) using standardised 0.33 N sodium hydroxide (LASEC, Cape Town, SA) to the end point of pH 7.00, as described by Zoecklein *et al.* (1999).

### 3.2.2 Statistical analyses

The results obtained from the chemical analysis for the class one and class five three-year barrel old matured brandy samples were subjected to statistical analysis by conducting a one-way analysis of variance (ANOVA) and a principal component analysis (PCA) to determine those compounds that were found to be significantly different amongst the class one and class five three-year barrel old matured brandy samples. Together with the above mentioned statistical analysis, an Agglomerative Hierarchical Clustering (AHC) was also carried out on the individual classes (class one and class five) as well as the both classes simultaneously in order to determine whether there are groupings within the specific class. Only correlation coefficients greater than r=0.650 were reported. Standard deviation was carried out on the triplicates for each sample and was found to be ≤ 5% (data not shown).

### 3.3 RESULTS AND DISCUSSION

### 3.3.1 Chemical analyses of the class one and class five three-year barrel old matured brandy samples

Table 3.1 shows the results of the one-way ANOVA of the chemical compounds of the class one and class five three-year barrel old matured brandy samples. In total there were 7 class

one three-year barrel old matured brandy samples and 23 class five three-year barrel matured brandy samples.

**TABLE 3.1 ANOVA of the chemical analyses (average) for the class one and class five three-year barrel old matured brandy samples.**

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Class one (Average of 7 samples) | Class five (Average of 23 samples) |
|---|---|---|---|
| Ethyl acetate | 0.028 | 351.720  b | 402.486 a |
| Ethyl butyrate | 0.000 | 2.109 b | 2.555 a |
| i-Amyl acetate | 0.006 | 12.727 b | 15.615 a |
| Ethyl hexanoate | 0.000 | 6.753 b | 9.641 a |
| Hexyl acetate | 0.042 | 1.681 a | 1.461 b |
| Ethyl lactate | 0.050 | 37.094 b | 46.829 a |
| Ethyl octanoate | 0.015 | 18.243 b | 23.710 a |
| Ethyl decanoate | 0.000 | 28.786 b | 36.861 a |
| Di-ethyl succinate | 0.063 | 3.942 a | 5.372 a |
| 2-Phenyl ethyl acetate | 0.241 | 0.878 a | 0.942 a |
| **Total esters** | **0.002** | **463.965 b** | **545.473 a** |
| n-Propanol | 0.970 | 249.525 a | 249.867 a |
| i-Butanol | 0.012 | 181.031 a | 156.014 b |
| n-Butanol | 0.348 | 4.759 a | 5.025 a |
| i-Amyl alcohol | 0.002 | 929.860 a | 873.387 b |
| Hexanol | 0.000 | 15.354 a | 12.925 b |
| 2-Phenyl ethanol | 0.254 | 8.087 a | 9.057 a |
| **Total higher alcohols** | **0.003** | **1388.616 a** | **1306.274 b** |
| Acetic acid | 0.002 | 157.979 b | 185.523 a |
| Gallic acid | 0.002 | 3.835 b | 5.574 a |
| i-Butyric acid | 0.037 | 1.024 b | 1.263 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Class one (Average of 7 samples) | Class five (Average of 23 samples) |
|---|---|---|---|
| n-Butyric acid | 0.002 | 0.710 b | 1.037 a |
| Protocatechuic acid | 0.262 | 0.155 a | 0.244 a |
| p-Hydroxy benzoic acid | 0.214 | 0.079 a | 0.867 a |
| Vanillic acid | 0.029 | 0.478 b | 0.790 a |
| Caffeic acid | 0.976 | 0.716 a | 0.721 a |
| Syringic acid | 0.093 | 0.533 a | 1.257 a |
| Ferulic acid | 0.185 | 0.022 a | 0.069 a |
| p-Coumaric acid | 0.006 | 0.170 b | 0.389 a |
| Hexanoic acid | 0.301 | 28.365 a | 23.532 a |
| Octanoic acid | 0.008 | 27.321 b | 35.364 a |
| Decanoic acid | 0.044 | 25.548 b | 30.414 a |
| **Total acids** | **0.001** | **247.067 b** | **287.042 a** |
| Acetaldehyde | 0.224 | 84.348 a | 91.398 a |
| Acetoin | 0.036 | 1.436 b | 1.906 a |
| Vanillin | 0.059 | 1.342 a | 2.196 a |
| Syringaldehyde | 0.012 | 0.320 b | 1.760 a |
| Coniferaldehyde | 0.320 | 0.387 a | 0.470 a |
| Sinapaldehyde | 0.010 | 0.951 b | 1.634 a |
| **Total aldehydes** | **0.140** | **90.542 a** | **99.364 a** |
| Furfural | 0.003 | 12.293 b | 16.946 a |
| 5-Methyl furfural | 0.167 | 0.603 a | 0.727 a |
| **Total furans** | **0.003** | **12.896 b** | **17.673 a** |
| Cis-Lactone | 0.006 | 0.379 b | 0.521 a |
| Trans-Lactone | 0.002 | 0.133 b | 0.202 a |
| **Total lactones** | **0.002** | **0.512 b** | **0.723 a** |
| Catechin | 0.218 | 0.057 a | 0.588 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Class one (Average of 7 samples) | Class five (Average of 23 samples) |
|---|---|---|---|
| Epicatechin | 0.118 | 0.966 a | 1.931 a |
| o-Cresol | 0.039 | 0.060 a | 0.010 b |
| p-Cresol | 0.138 | 0.034 a | 0.009 a |
| Phenol | 0.421 | 0.130 a | 0.094 a |
| Ethyl guaiacol | 0.768 | 2.187 a | 1.992 a |
| Eugenol | 0.041 | 0.039 b | 0.116 a |
| 2,6-Dimethoxy phenol | 0.056 | 1.960 a | 2.393 a |
| **Total phenols** | **0.204** | **5.434 a** | **7.132 a** |
| HA[a] vs. esters | <0.0001 | 3.008 a | 2.419 b |
| HA vs. acids | <0.0001 | 5.687 a | 4.584 b |
| Esters vs. acids | 0.996 | 1.908 a | 1.907 a |
| Aldehydes vs. esters | 0.255 | 0.196 a | 0.183 a |
| Aldehydes vs. acids | 0.235 | 0.372 a | 0.346 a |
| Aldehydes vs. HA | 0.055 | 0.065 a | 0.076 a |
| Methanol | 0.935 | 272.176 a | 271.509 a |
| pH | 0.054 | 4.758 a | 4.975 a |
| TA | 0.010 | 0.251 b | 0.295 a |

**Compounds/groups of compounds with different letters in the same row are found to be significantly different (p≤0.05). HA[a] (Higher alcohols).**

**3.3.1.1 Esters**

Overall the total ester concentration was found to be higher in (≤0.05) the class five three-year barrel old matured brandy samples. The only esters not found to follow this trend was 2-phenyl ethyl acetate and di-ethyl succinate where no significant difference was found. The inverse was however found to be true for the compound hexyl acetate, where levels were found to be higher in the class one three-year barrel old matured brandy samples.

Esters are responsible for the fruity aromas found in wine and spirit beverages (Nykänen, 1986), therefore class five three-year barrel old matured brandy samples were perceived to display a more "fruity" aroma and flavour when compared to the class one samples by the sensory panel.

**3.3.1.2 Higher alcohols**

The levels of i-butanol, i-amyl alcohol, hexanol as well as the overall total higher alcohol concentration were found to be highest (≤0.05) in the class one three-year barrel old matured brandy samples when compared to the class five three-year barrel old matured brandy samples.

Higher alcohols have an important aromatic effect in wines and especially distillates as they are found in higher concentrations in distillates (Steger and Lambrechts, 2000). The production of higher alcohols depends on the raw material and the yeast employed, and during the distillation processes the low molecular-weight alcohols increase and the high-molecular-weight alcohols decrease due to the effects of differing volatility during distillation (Piggott, 1983). The major higher alcohols found in wine in order of amounts produced are, i-amyl alcohol, i-butanol and n-propanol (Jounela-Eriksson, 1981).

Studies conducted by Boscolo *et al.*, (2000) indicate that the higher alcohols in wine and spirit, the most important one to consider is i-amyl alcohol as this higher alcohol if present in large concentrations can render the product unpleasant. Due to the fact that the distillation technique enhances the amount of higher alcohols found in the distillate, it is important to monitor these levels.

**3.3.1.3 Acids**

The total acid concentrations as well as the level of acetic acid were highest (≤0.05) in the class five three-year barrel old matured brandy samples when compared to the class one three-year barrel old matured brandy samples. The main volatile acid found in distilled beverages is acetic acid. This acid constitutes 40- 95% of the total volatile acids in whisky, 50-75% in Cognac and brandy, and for rum 75-90% (Nykänen, 1986). Octanoic and decanoic acids are also prominent and make up 30% of the total acids, followed by hexanoic acid.

The increased level of acetic acid present in the class five three-year barrel old matured brandy samples could be due to the hydrolysis of the ethyl acetate which is found in increased levels in these samples. Acetic acid can be removed during the recovery of the head fraction in the distillation process. It could also be theorized that perhaps the head fraction of these samples were collected in a different manner, thereby leading to an increase in acetic acid concentration (Léaute, 1990; Kister, 2000). However, this cannot be confirmed as falls out of the scope of the study.

The level of octanoic, decanoic, i-butyric and n-butyric acid are also found to be higher in the class five three-year barrel old matured brandy samples when compared to the class one three-year samples. The levels of gallic, vanillic and p-coumaric acid (acids derived from wood and grape) are also higher in concentration in the class five three-year barrel old matured brandy samples when compared to the class one three-year barrel old matured samples.

Increases in concentrations of fatty acids in distillates are dependent on the yeast strain used during the fermentation process as well as the amount of yeast lees present during the distillation procedure as those fatty acids with even carbon atoms, (C2-C10) are products of biochemical metabolism (Von Adam *et al.,* 1996). Therefore perhaps the class five barrel old matured brandy samples were distilled with a higher percentage of yeast lees or with different yeast when compared to the class one samples. Unfortunately this cannot be confirmed as this information was not provided and falls out of the scope of this study.

### 3.3.1.4 Aldehydes

The only aldehyde compounds found to be significantly different between the class one and class five three-year barrel old matured brandy samples are acetoin, syringaldehyde and sinapaldehyde with levels being higher (≤0.05) in the class five samples. However, of the aldehyde compounds, acetaldehyde is the major component and constitutes approximately 90% of the total aldehyde content in alcoholic beverages (Guymon, 1972). This is found to be the case for this study. However there are also aldehydes that are derived during the wood maturation process as a result of degradation of lignin (namely vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde) and these compounds also play a significant role in the flavour and aroma of distilled beverages (Chatonnet *et al.,* 1993).

### 3.3.1.5 Total phenols, furans and lactones

Total furans and more specifically furfural were found to be highest (≤0.05) in concentration in the class five three-year barrel old matured brandy samples. Total lactones are also found to be highest in the class five three-year barrel old matured brandy samples. The overall total phenol concentration was found not to be significantly different (≤0.05) between the class one and class five three-year barrel old matured brandy samples. However the compound eugenol was found to be higher in the class five three-year barrel old matured brandy

samples and o-cresol was found to highest in the class one three-year barrel old matured brandy samples.

Therefore the class five three-year barrel old matured brandy samples are found to contain higher levels of the compounds that originate from the wood when compared to the class one samples. This could reinforce the theory that it is not the type of compound that distinguishes one style of brandy from another but rather the amount of compounds present (Steger and Lambrechts, 2000; Ibern-Gomez, 2001).

### 3.3.1.6 Ratios and TA

The ratio of higher alcohols vs. esters and higher alcohols vs. acids were found to be significantly higher (≤0.05) in the class one three-year barrel old matured brandy samples when compared that of the class five three-year barrel old matured brandy samples. The TA value is also found to be higher in the class five three-year barrel old matured brandy samples.

### 3.3.1.7 Principal Component Analysis (PCA) of the class one and class five three-year old barrel matured brandy samples and their chemical analysis

Figure 3.1 shows the chemical compounds amongst the entire class one and class five three-year barrel old matured brandy samples displayed in a PCA bi-plot. The first principal component (F1) accounted for 22.79% of the total variation and the second principal component (F2) accounted for 12.34% of the total variation.
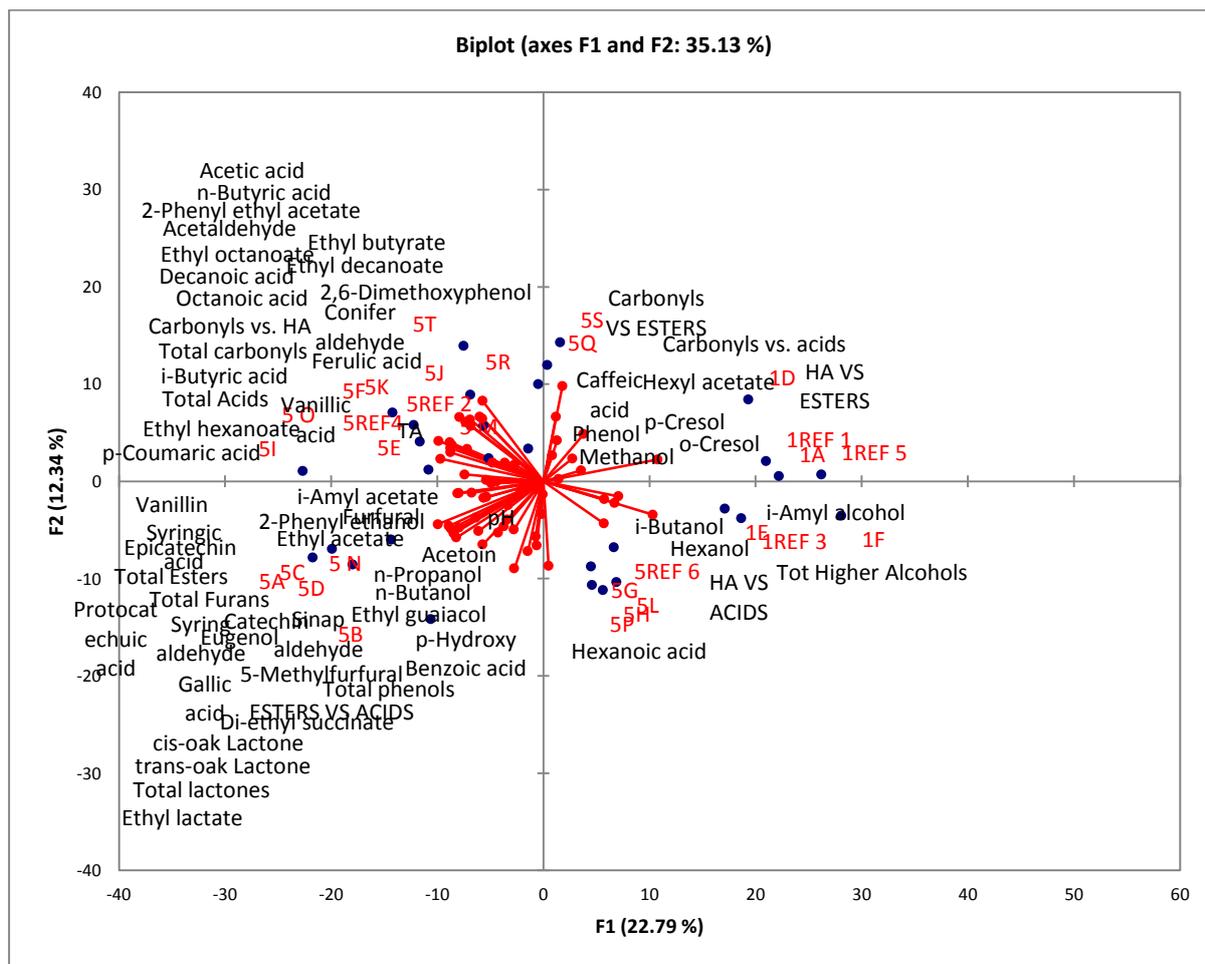
**FGIURE 3.1 PCA correlation loadings plot showing the chemical compounds for the entire class one and class five three-year barrel** old **matured brandy samples. 1 (class one three-year barrel old matured brandy samples); 5 (class five three-year barrel old matured brandy samples);**

F1 indicated that the samples 1A (factor score=5.818), 1D (factor score=5.062), 1E (factor score=4.480), 1F (factor score=7.360), 1REF1 (factor score=5.502), 1REF3 (factor score=4.885), 1REF5 (factor score=6.865), 5G (factor score=1.178), 5H (factor score=1.476), 5L (factor score=1.806), 5REF6 (factor score=1.744), 5P (factor score=1.208) were found to be positively associated to the F1 axis and was found to contrast the most with 5E (factor score=-2.830), 5F (factor score=-3.717), 5I (factor score=-5.935), 5J (factor score=-1.794), 5K (factor score=-3.195), 5REF4 (factor score=-1.344), 5REF2 (factor score=-1.472), 5T (factor score=-1.961), 5A (factor score=-5.688), 5B (factor score=-2.770), 5C (factor score=-5.209), 5D (factor score=-4.705), 5N (factor score=-3.750) and 5O (factor score=-3.044).

The compounds/groups of compounds namely the ratios of higher alcohols vs. esters (r=0.839) and higher alcohols vs. acids (r=0.803) were found to be positively correlated to the F1 axis and therefore positively associated to the samples 1A, , 1D, 1E, 1F, 1REF1, 1REF3, 1REF5, 5G, 5H, 5L, 5REF6, 5P and negatively associated to 5E, 5F, 5I, 5J, 5K, 5REF4, 5REF2, 5T, 5A, 5B, 5C, 5D, 5N and 5O.

Inversely the compounds/groups of compounds TA (r=-0.681), ethyl acetate (r=-0.651), ethyl hexanoate (r=-0.684), acetic acid (r=-0.680), n-butyric acid (r=-0.668), ethyl decanoate (r=-0.768), total esters (r=-0.772), total acids (r=-0.752), total lactones (r=-0.657), gallic acid (r=-0.676) and sinapaldehyde (r=-0.692) were found to be negatively correlated to the F1 axis and therefore negatively associated to the samples 1A, 1D, 1E, 1F, 1REF1, 1REF3, 1REF5, 5G, 5H, 5L, 5REF6, 5P but however positively associated to the samples 5E, 5F, 5I, 5J, 5K, 5REF4, 5REF2, 5T, 5A, 5B, 5C, 5D, 5N and 5O.

F2 indicated that 1D (factor score=2.993), 1REF1 (factor score=0.786), 5F (factor score=2.515), 5J (factor score=3.169), 5K (factor score=2.063), 5REF4 (factor score=0.847), 5REF2 (factor score=2.034), 5Q (factor score=4.252), 5R (factor score=3.559), 5S (factor score=5.085), 5T (factor score=4.957), 5M (factor score=1.191) and 5O (factor score=1.448) was found to be positively associated to the F2 axis and contrasted the most with 1E (factor score=-1.006), 1F (factor score=-1.260), 1REF3 (factor score=-1.353), 5A (factor score=-2.789), 5B (factor score=-5.041), 5C (factor score=-2.476), 5D (factor score=-3.043), 5G (factor score=-3.115), 5H (factor score=-3.985), 5L (factor score=-3.684), 5REF6 (factor score=-2.420), 5P (factor score=-3.788) and 5N (factor score=-2.129).

The compounds/groups of compounds namely decanoic acid (r=0.644) and the ratio of carbonyls vs. esters (r=0.762) was found to be positively correlated to the F2 axis and therefore positively associated to 1D, 5F, 5J, 5K, 5REF4, 5REF2, 5Q, 5R, 5S, 5T, 5M  and 5O and negatively associated to 1E, 1F, 1REF3, 5A, 5B, 5C, 5D, 5G, 5H, 5L, 5REF6, 5P and 5N.

Conversely the compounds/groups of compounds n-butanol (r=-0.696) and hexanoic acid (r=-0.675) was found to be negatively correlated to the F2 axis and therefore negatively associated to 1D, 5F, 5J, 5K, 5REF4, 5REF2, 5Q, 5R, 5S, 5T, 5M  and 5O and positively associated to 1E, 1F, 1REF 3, 5A, 5B, 5C, 5D, 5G, 5H, 5L, 5REF6, 5P and 5N.

From the results shown in figure 3.1 it is evident that there is variation within the specific classes. Some of the class five three-year barrel old matured brandy samples are found to be associated with the class one three-year barrel old matured brandy samples and were perhaps incorrectly classified by internal brandy panel. These samples include 5G, 5H, 5L, 5P and ref 6. According the chemical analysis of these individual samples (data not shown) as well as the results obtained from the AHC, they were found to contain on average a lower concentration of total esters and total acids when compared to the other class five samples, as well as a higher concentration of total higher alcohols and a higher ratio of higher alcohols vs. esters and higher alcohols vs. acids which is more representative of the chemical profile of the class one three-year barrel old matured brandy samples. Although these samples do not contain the same concentrations of the compounds as the class one three-year barrel old matured brandy samples, they do however more closely resemble the same chemical composition/profile of the class one three-year barrel matured brandy samples rather than the class five three-year barrel old matured brandy samples, and perhaps if reclassified would score a class two or class three in the sensory classification system employed. However, only a limited amount of compounds were analysed, and perhaps the classification might be explained by other chemicals not determined in this study.

### 3.3.2 Chemical analyses of class one three-year barrel old matured brandy samples

The class one three-year barrel old matured brandy samples were subjected to an Agglomerative Hierarchical Clustering (AHC) in order to determine if there are any variances/groupings within the class one samples. According the AHC, the samples can be grouped into three groups, namely group 1A (samples 1E, 1A, 1REF5 and 1REF1), group 1B (1D) and group 1C (samples 1F and 1REF3). Table 3.2 shows the results of the one-way ANOVA of the averages for the chemical compounds of the three groups within the class one three-year barrel matured brandy samples.

**TABLE 3.2 ANOVA of the chemical analyses of the three groups within the class one three-year barrel old matured brandy samples.**

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Group 1A | Group 1B | Group 1C |
|---|---|---|---|---|
| Ethyl acetate | 0.407 | 358.201 a | 299.321 a | 364.958 a |
| Ethyl butyrate | 0.425 | 2.144 a | 2.032 a | 2.079 a |
| i-Amyl acetate | 0.492 | 13.992 a | 9.661 a | 11.731 a |
| Ethyl hexanoate | 0.684 | 6.724 a | 7.261 a | 6.558 a |
| Hexyl acetate | 0.963 | 1.690 a | 1.694 a | 1.657 a |
| Ethyl lactate | 0.014 | 32.166 b | 29.028 b | 50.989 a |
| Ethyl octanoate | 0.481 | 17.186 a | 20.998 a | 18.890 a |
| Ethyl decanoate | 0.853 | 28.012 a | 29.109 a | 30.172 a |
| Di-ethyl succinate | 0.142 | 3.307 a | 2.974 a | 5.696 a |
| 2-Phenyl      ethyl acetate | 0.503 | 0.877 a | 0.937 a | 0.852 a |
| **Total esters** | **0.209** | **464.297 a** | **403.015 a** | **493.669 a** |
| n-Propanol | 0.742 | 243.646 a | 247.539 a | 262.278 a |
| i-Butanol | 0.132 | 167.473 a | 140.999 a | 228.162 a |
| n-Butanol | 0.186 | 4.623 a | 4.684 a | 5.071 a |
| i-Amyl alcohol | 0.135 | 920.163 a | 901.005 a | 963.681 a |
| Hexanol | 0.002 | 13.381 b | 16.966 a | 17.595 a |
| 2-Phenyl ethanol | 0.426 | 7.585 a | 6.999 a | 9.635 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Group 1A | Group 1B | Group 1C |
|---|---|---|---|---|
| **Total higher alcohols** | **0.002** | **1357.321 b** | **1318.192 b** | **1486.421 a** |
| Acetic acid | 0.004 | 141.740 c | 209.734 a | 164.580 b |
| Gallic acid | 0.636 | 3.990 a | 3.690 a | 3.599 a |
| i-Butyric acid | 0.011 | 0.920 b | 1.289 a | 1.101 a |
| n-Butyric acid | 0.301 | 0.703 a | 0.821 a | 0.670 a |
| Protocatechuic acid | 0.205 | 0.271 a | 0.000 a | 0.000 a |
| p-Hydroxy benzoic acid | 0.636 | 0.091 a | 0.189 a | 0.000 a |
| Vanillic acid | 0.133 | 0.564 a | 0.334 a | 0.379 a |
| Caffeic acid | 0.112 | 0.796 a | 0.751 a | 0.540 a |
| Syringic acid | 0.804 | 0.458 a | 0.999 a | 0.451 a |
| Ferulic acid | 0.246 | 0.039 a | 0.000 a | 0.000 a |
| p-Coumaric acid | 0.293 | 0.194 a | 0.134 a | 0.141 a |
| Hexanoic acid | 0.528 | 27.693 a | 26.587 a | 30.998 a |
| Octanoic acid | 0.662 | 28.058 a | 23.734 a | 27.641 a |
| Decanoic acid | 0.016 | 22.835 b | 34.912 a | 26.293 b |
| **Total acids** | **0.030** | **228.579 b** | **303.194 a** | **255.991 ab** |
| Acetaldehyde | 0.583 | 83.214 a | 85.678 a | 85.951 a |
| Acetoin | 0.166 | 1.408 a | 1.275 a | 1.573 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Group 1A | Group 1B | Group 1C |
|---|---|---|---|---|
| Vanillin | 0.238 | 1.731 a | 0.699 a | 0.886 a |
| Syringaldehyde | 0.764 | 0.310 a | 0.250 a | 0.377 a |
| Coniferaldehyde | 0.048 | 0.415 a | 0.366 ab | 0.341 a |
| Sinapaldehyde | 0.654 | 0.976 a | 0.876 a | 0.939 a |
| **Total aldehydes** | **0.964** | **91.130 a** | **89.144 a** | **90.066 a** |
| Furfural | 0.452 | 12.385 a | 9.766 a | 13.373 a |
| 5-Methyl furfural | 0.370 | 0.621 a | 0.721 a | 0.508 a |
| **Total furans** | **0.492** | **13.066 a** | **10.487 a** | **13.881 a** |
| Cis-Lactone | 0.525 | 0.382 a | 0.321 a | 0.404 a |
| Trans-Lactone | 0.615 | 0.139 a | 0.138 a | 0.118 a |
| **Total lactones** | **0.770** | **0.521 a** | **0.459 a** | **0.522 a** |
| Catechin | 0.545 | 0.080 a | 0.079 a | 0.000 a |
| Epicatechin | 0.403 | 0.994 a | 0.922 a | 0.934 a |
| o-Cresol | 0.346 | 0.034 a | 0.000 a | 0.142 a |
| p-Cresol | 0.148 | 0.017 a | 0.000 a | 0.086 a |
| Phenol | 0.870 | 0.132 a | 0.167 a | 0.107 a |
| Ethyl guaiacol | 0.209 | 2.008 a | 1.542 a | 2.869 a |
| Eugenol | 0.035 | 0.021 b | 0.000 b | 0.096 a |
| 2,6-Dimethoxy phenol | 0.304 | 1.986 a | 1.488 a | 2.147 a |
| **Total phenols** | **0.170** | **5.270 a** | **4.198 a** | **6.379 a** |
| HA[a] vs. esters | 0.550 | 2.939 a | 3.271 a | 3.015 a |
| HA vs. acids | 0.033 | 5.961 a | 4.348 b | 5.807 a |
| Esters vs. acids | 0.097 | 2.042 a | 1.329 a | 1.928 a |
| Aldehydes vs. esters | 0.299 | 0.197 a | 0.221 a | 0.182 a |
| Aldehydes vs. acids | 0.245 | 0.402 a | 0.294 a | 0.352 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Group 1A | Group 1B | Group 1C |
|---|---|---|---|---|
| Aldehydes vs. HA | 0.408 | 0.067 a | 0.068 a | 0.061 a |
| Methanol | 0.004 | 258.728 b | 264.990 b | 302.665 a |
| pH | 0.767 | 4.795 a | 4.845 a | 4.640 a |
| TA | 0.641 | 0.245 a | 0.270 a | 0.255 a |

**Compounds/groups of compounds with different letters in the same row are found to be significantly different (p≤0.05). HA[a] (Higher alcohols).**

### 3.3.2.1 Ethyl lactate

The level of ethyl lactate was found to be significantly different (≤0.05) between the three groups within the class one three-year barrel old matured brandy samples, with group 1C containing the highest concentration. Ethyl lactate is formed when lactic acid combines with ethanol in the matrix, and lactic acid in-turn is produced as by-product through malo-lactic fermentation (du Toit *et al.,* 2011). This could indicate that the group 1C samples were produced by wine that perhaps had undergone malolactic fermentation prior to the distillation process (du Plessis *et al.,* 2004). However due to the fact that the three-year barrel old matured brandy samples are blended and not kept in individual lots, it is difficult to confirm this.

### 3.3.2.2 Higher alcohols

The level of hexanol and overall total higher alcohols were found to be significantly different (≤0.05) between the different groups within the class one samples. It was found that group 1C contained the highest concentration of total higher alcohols, and group 1A contains the highest level of hexanol when compared to the other groups. The production of higher alcohols depends on the raw material and the yeast employed, and during the distillation processes the low molecular-weight alcohols increase and the high-molecular-weight alcohols decrease due to the effects of differing volatility during distillation (Nykänen, 1968; Carnacini, 1989; Piggott, 1983;). It could be theorized that the samples in group 1C (when compared to those samples of groups 1A and 1B) were fermented with different base wines and/or yeast strains. However again due to the fact that three-year barrel old matured brandy samples are blended and not kept separately, it is difficult to confirm this theory.

### 3.3.2.3 Acids

The concentration of acetic acid was found to be highest (≤0.05) in the samples of group 1B. If one looks at the level of ethyl acetate in these samples, it can be seen that even though it is not found to be significantly different between the different groups it does seem to contain a lower concentration of ethyl acetate. The reason for the elevated level of acetic acid in group 1 B could be attributed to the hydrolysis of the ester ethyl acetate (Nykänen, 1968). Hydrolysis is promoted with an increase in temperature, however further investigation into the storage temperature and humidity would be needed to conclude this, and does not fall in the scope of this study.

The level of i-butyric acid was found to be significantly different (≤0.05) within the class one samples with the group 1A containing the lowest concentration. Butyric acid and other short chain volatile organic acids are the compounds responsible for the rancid odours emitted by the spirit when stored in less desirable circumstances (Silva and Malcata, 1998).

The concentration of decanoic acid was also found to be significantly different (≤0.05) with group 1B samples containing the highest level. Levels of total acids are also found to be highest in concentration in group 1B when compared to group 1A, but however not when compared to those samples of group 1C.

### 3.3.2.4 Lactones and phenols

No significant differences (≤0.05) were found between the different groups with regards to their overall total lactone concentration. The same was found for the total furans and total phenol concentration. However the level of eugenol was found to be highest in concentration of samples found in group 1C when compared to the other groups. Oak lactones are extracted directly from oak barrels during the maturation period and are said to impart a woody aroma and flavour into the spirit product (Ibern-Gomez *et al.,* 2001).

### 3.3.2.5 Ratios and methanol

The ratio of higher alcohols vs. acids was found to be lowest (≤0.05) in the samples of group 1B. The concentration of methanol was significantly higher in the samples or group 1C when compared to the other groups within the class one samples. Methanol is found in the head fraction of the distillate and levels can be either increased or decreased depending on the cut-off point during the distillation process (Léaute, 1990). Perhaps the samples in group 1C were not subjected to the same cut-off time during the distillation.

### 3.3.2.6 Principal Component Analysis (PCA) of the different groups of class one three-year barrel old matured brandy samples and their chemical analysis

Figure 3.2 shows the PCA bi-plot (Principal Component Analysis) for the chemical analysis of the class one three-year barrel old matured samples. The first principal component (F1) accounted for 32.15% of the total variation and the second principal component (F2) accounted for 20.76% of the total variation.
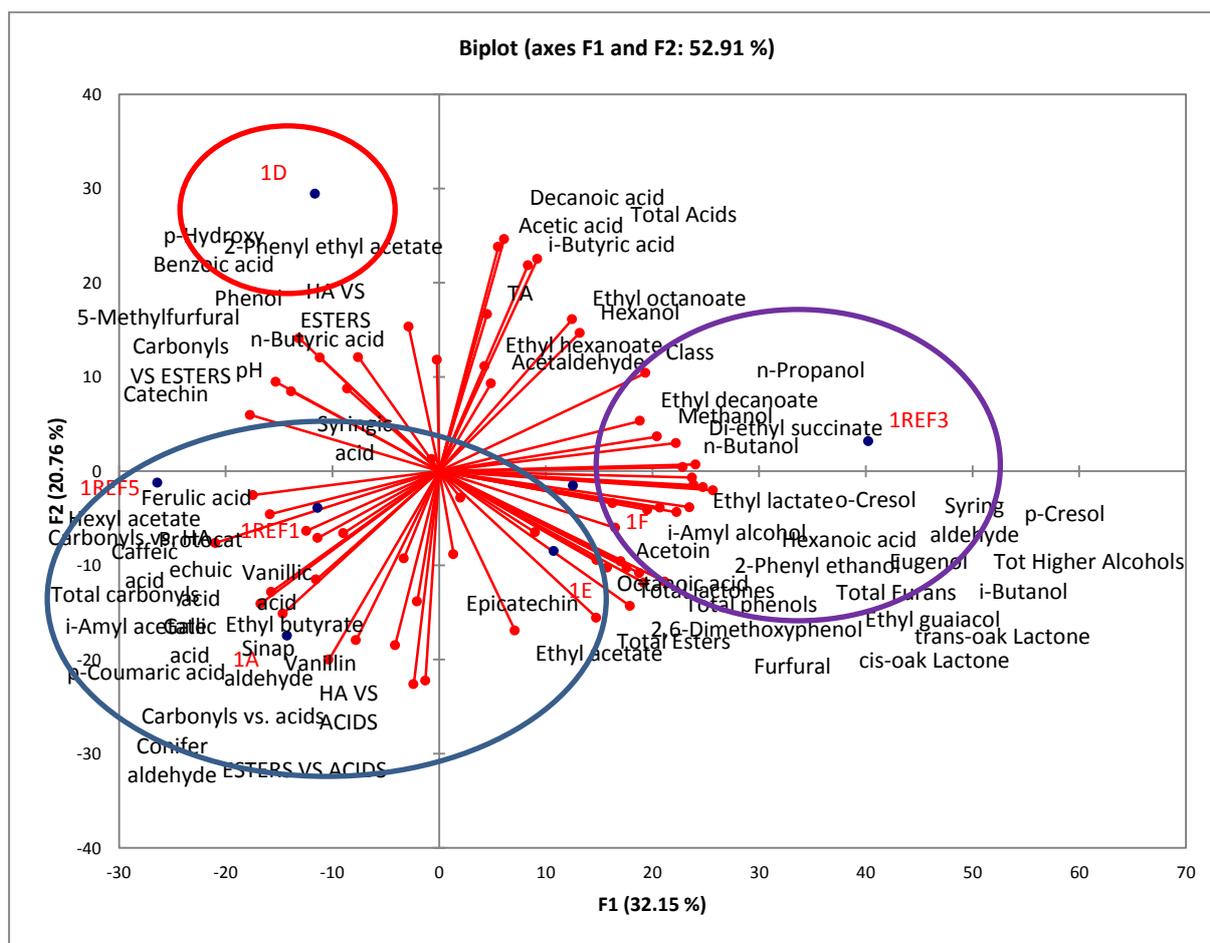


**FIGURE 3.2 PCA bi-plot showing the relationship between the class one three-year barrel old matured brandy samples and the compounds/groups of compounds on F1 and F2.**

From figure 3.2 it can be seem that within the class one three-year barrel matured brandy samples there are three distinct groups. Group 1A (1E, 1A, 1REF5 and 1REF1), Group 1B (1D) and group 1C (samples 1REF3 and 1F).

F1 indicated that the samples Group 1A [1E (factor score=2.357)] and group 1C [samples 1F (factor score=2.749) and 1REF3 (factor score=8.805)] were found to be positively associated to the F1 axis and contrasted the most with group 1A [samples 1A (factor score=-3.115), 1REF1 (factor score=-2.446) and 1REF5 (factor score=-5.771)] and group 1B [1D (factor score=-2.539)],

The compounds/groups of compounds methanol (r=0.771), n-propanol (r=0.838), n-butanol (r=0.862), i-amyl alcohol (r=0.736), ethyl lactate (r=0.895), ethyl decanoate (r=0.711), di-ethyl succinate (r=0.908), hexanoic acid (r=0.901), total higher alcohols (r=0.841), total phenols (r=0.798), furfural (r=0.664), cis-oak lactone (r=0.724), o-cresol (r=0.934), ethyl guaiacol (r=0.709), p-cresol (r=0.969), eugenol (r=0.886), 2,6-dimethoxyphenol (r=0.676) and syringaldehyde (r=0.781) were found to be positively correlated to the F1 axis and therefore positively associated to the samples group 1A (1E), groups 1C (1F and 1REF3), but negatively associated to group 1A (1A, 1REF1 and 1REF5) and group 1B (1D).

Conversely the compounds/group of compounds hexyl acetate (r=-0.657), catechin (r=-0.668) and caffeic acid (r=-0.791) were found to be negatively correlated to the F1 axis and therefore negatively associated to group 1A (1E), groups 1C (1F and 1REF3), but positively associated to group 1A (1A, 1REF1 and 1REF5) and group 1B (1D).

F2 indicated that the samples Group 1B [1D (factor score=8.020)] and group 1C [1REF3 (factor score=0.862)] and were found to be positively associated to the F2 axis and contrasted the most with samples group 1A [samples 1A (factor score=-4.758), 1E (factor score=-2.312) and 1REF1 (factor score=-1.061)].

The compounds/groups of compounds acetic acid (r=0.899), i-butyric acid (r=0.825), decanoic acid (r=0.929) and total acids (r=0.849) were found to be positively correlated to the F2 axis and therefore positively associated to group 1B (sample 1D) and group 1C (sample 1REF3), but however negatively associated to group 1A (samples 1A, 1E, 1REF1).

Conversely the compounds/groups of compounds higher alcohols vs. acids (r=-0.853), esters vs. acids (r=-0.839), aldehydes vs. acids (r=-0.755), vanillin (r=-0.697) and sinapaldehyde (r=-0.676) were found to be negatively correlated to the F2 axis and therefore negatively associated to group 1B (sample 1D) and group 1C (sample 1REF3), but however positively associated to group 1A (samples 1A, 1E, 1REF1).

Even though the results of Table 3.2 do not show any differences between the different class one three-year barrel old matured brandy groups with regards to their total furans, total phenols and total lactones, Figure 3.2 shows that group 1C (samples 1F and 1REF3) of the class one three-year barrel old matured brandy samples are found to be more associated to these above mentioned chemical groups. Figure 3.2 also shows that group 1A (samples 1A, 1E, 1REF1 and 1REF5) of the class one three-year barrel old matured brandy samples are found to be more associated to total aldehydes and total esters. Group 1B (sample 1D) was found to be more associated to the ratios of higher alcohols vs. esters and aldehydes vs. esters.

Therefore one can see that within the class one three-year barrel old matured samples there are three distinct groups. Even though these samples were classified as class one three-year barrel old matured brandy samples there seems to be variations within this class. This is expected as each of these samples (even though they are classified as class one three-barrel old matured brandy samples) come from a different "lot". Each "lot" of three-year barrel matured brandy samples are made up of a blend of different brandy samples coming from barrels that differ with regards to their storage temperature, storage humidity, seasoning and age. The only treatments that are found to be the same for the barrels used during the maturation are the degree of toasting and origin.

The reason for the small sample set of class one three-year barrel old matured brandy and the class five three-year barrel old matured brandy samples is due to the fact that these samples are classified by the panel after the maturation process and of the 104 samples analysed, only a small portion were classified as either a class one or class five three-year barrel old matured brandy sample.

Table 3.1 shows that one of the main differentiating factor between the class one and class five three-year old barrel matured brandy samples are the levels of higher alcohols. Even though the class one three-year barrel old matured brandy samples are found to contain higher concentrations of total higher alcohols when compared to the class five three-year barrel old brandy samples, they vary within their own class with regards to the amount of total higher alcohols present. This reinforces findings by Jack (2003) whereby sensory analysis together with chemical analysis is crucial in the evaluation of spirit products.

### 3.3.3 Chemical analyses of class five three-year barrel old matured brandy samples

The class five three-year barrel old matured brandy samples were subjected to an Agglomerative Hierarchical Clustering (AHC) in order to determine if there are any variances/groupings within the class five samples. According the AHC, the samples can be grouped into three groups, namely group 5A (samples 5E, 5I, 5REF2, 5B, 5C, 5D, 5O, 5N), group 5B (samples 5F and 5P) and group 5C (samples 5J, 5K, 5REF4, 5Q, 5R, 5S, 5T, 5A, 5G, 5H, 5L, 5REF6, 5M). Table 3.3 show the results of the one-way ANOVA of the averages for the chemical compounds of the three groups within the entire class five three-year barrel old matured brandy samples.

**TABLE 3.3 ANOVA of the chemical analyses of the three groups within the class five three-year barrel old matured brandy samples.**

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Group 5A | Group 5B | Group 5C |
|---|---|---|---|---|
| Ethyl acetate | <0.0001 | 464.852 a | 362.420 b | 370.270 a |
| Ethyl butyrate | 0.101 | 2.524 a | 2.968 a | 2.511 a |
| i-Amyl acetate | 0.301 | 16.248 a | 16.620 a | 15.071 a |
| Ethyl hexanoate | 0.120 | 9.351 a | 12.126 a | 9.438 a |
| Hexyl acetate | 0.603 | 1.521 a | 1.313 a | 1.448 a |
| Ethyl lactate | 0.675 | 49.755 a | 44.396 a | 45.403 a |
| Ethyl octanoate | 0.283 | 24.049 a | 29.153 a | 22.665 a |
| Ethyl decanoate | 0.520 | 38.373 a | 36.221 a | 36.029 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Group 5A | Group 5B | Group 5C |
|---|---|---|---|---|
| Di-ethyl succinate | 0.432 | 6.028 a | 5.305 a | 4.979 a |
| 2-Phenyl ethyl acetate | 0.814 | 0.933 a | 1.003 a | 0.939 a |
| **Total esters** | **<0.0001** | **613.633 a** | **511.523 b** | **508.752 b** |
| n-Propanol | 0.101 | 259.503 a | 262.090 a | 242.058 a |
| i-Butanol | 0.171 | 152.249 a | 167.881 a | 156.505 a |
| n-Butanol | 0.368 | 5.299 a | 5.126 a | 4.841 a |
| i-Amyl alcohol | 0.000 | 880.308 b | 963.240 a | 855.304 b |
| Hexanol | 0.123 | 13.165 a | 14.111 a | 12.594 a |
| 2-Phenyl ethanol | 0.489 | 9.723 a | 9.082 a | 8.643 a |
| **Total higher alcohols** | **0.000** | **1320.247 b** | **1421.528 a** | **1279.944 c** |
| Acetic acid | 0.459 | 189.968 a | 192.490 a | 181.717 a |
| Gallic acid | 0.065 | 6.375 a | 5.713 a | 5.060 a |
| i-Butyric acid | 0.032 | 1.130 b | 1.672 a | 1.281 b |
| n-Butyric acid | 0.019 | 1.023 b | 1.470 a | 0.980 b |
| Protocatechuic acid | 0.577 | 0.297 a | 0.197 a | 0.218 a |
| p-Hydroxy benzoic acid | 0.505 | 0.382 a | 0.498 a | 1.222 a |
| Vanillic acid | 0.699 | 0.773 a | 0.996 a | 0.769 a |
| Caffeic acid | 0.645 | 0.661 a | 0.965 a | 0.720 a |
| Syringic acid | 0.687 | 1.520 a | 1.194 a | 1.104 a |
| Ferulic acid | 0.527 | 0.072 a | 0.000 a | 0.077 a |
| p-Coumaric acid | 0.210 | 0.485 a | 0.331 a | 0.339 a |
| Hexanoic acid | 0.172 | 29.460 a | 25.898 a | 19.520 a |
| Octanoic acid | 0.322 | 36.147 a | 28.065 a | 36.005 a |
| Decanoic acid | 0.557 | 29.788 a | 26.963 a | 31.330 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Group 5A | Group 5B | Group 5C |
|---|---|---|---|---|
| **Total acids** | **0.286** | **298.079 a** | **286.448 a** | **280.342 a** |
| Acetaldehyde | 0.064 | 100.436 a | 78.527 a | 87.817 a |
| Acetoin | 0.216 | 2.177 a | 1.902 a | 1.739 a |
| Vanillin | 0.631 | 2.501 a | 2.078 a | 2.026 a |
| Syringaldehyde | 0.843 | 1.976 a | 1.901 a | 1.606 a |
| Coniferaldehyde | 0.789 | 0.499 a | 0.528 a | 0.442 a |
| Sinapaldehyde | 0.137 | 1.995 a | 1.258 a | 1.471 a |
| **Total aldehydes** | **0.032** | **109.584 a** | **86.192 b** | **95.101 b** |
| Furfural | 0.384 | 18.177 a | 18.007 a | 16.026 a |
| 5-Methyl furfural | 0.905 | 0.755 a | 0.694 a | 0.715 a |
| **Total furans** | **0.392** | **18.932 a** | **18.700 a** | **16.741 a** |
| Cis-Lactone | 0.227 | 0.568 a | 0.584 a | 0.482 a |
| Trans-Lactone | 0.075 | 0.225 a | 0.243 a | 0.182 a |
| **Total lactones** | **0.126** | **0.793 a** | **0.826 a** | **0.664 a** |
| Catechin | 0.578 | 0.418 a | 0.000 a | 0.783 a |
| Epicatechin | 0.290 | 1.241 a | 1.865 a | 2.366 a |
| o-Cresol | 0.556 | 0.000 a | 0.000 a | 0.018 a |
| p-Cresol | 0.603 | 0.000 a | 0.000 a | 0.016 a |
| Phenol | 0.947 | 0.088 a | 0.075 a | 0.100 a |
| Ethyl guaiacol | 0.327 | 2.715 a | 1.400 a | 1.639 a |
| Eugenol | 0.821 | 0.119 a | 0.153 a | 0.109 a |
| 2,6-Dimethoxy phenol | 0.711 | 2.479 a | 2.114 a | 2.383 a |
| **Total phenols** | **0.794** | **7.059 a** | **5.606 a** | **7.412 a** |
| HA[a] vs. esters | <0.0001 | 2.162 c | 2.783 a | 2.521 b |
| HA vs. acids | 0.285 | 4.440 a | 5.007 a | 4.608 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Group 5A | Group 5B | Group 5C |
|---|---|---|---|---|
| Esters vs. acids | 0.017 | 2.066 a | 1.764 b | 1.827 b |
| Aldehydes vs. esters | 0.601 | 0.179 a | 0.168 a | 0.188 a |
| Aldehydes vs. acids | 0.126 | 0.368 a | 0.299 a | 0.341 a |
| Aldehydes vs. HA | 0.029 | 0.083 a | 0.061 b | 0.074 ab |
| Methanol | 0.064 | 266.720 a | 292.656 a | 271.204 a |
| pH | 0.023 | 4.786 b | 5.005 ab | 5.087 a |
| TA | 0.760 | 0.303 a | 0.300 a | 0.289 a |

**Compounds/groups of compounds with different letters in the same row are found to be significantly different (p≤0.05). HA[a] (Higher alcohols).**

### 3.3.3.1 Esters

The amount of ethyl acetate and overall total esters were found to be significantly different (≤0.05) between the different groupings within the class five three-year barrel old matured brandy samples. The group 5A was found to contain a higher concentration of ethyl acetate and total esters. The amount of ethyl acetate present in distilled beverages is determined by amongst many things the cut-off point in the head fraction during the distillation process (Léaute, 1990). Alternatively the production of esters is also influenced by the yeast strain used during the fermentation process (Salo *et al.,* 1972). Either the samples in group 5A were fermented with a different yeast strain, or alternatively theses samples were stored at different temperatures which could affect the levels of ethyl acetate by either promoting esterification or hydrolysis. However, due to the blending of the three-year barrel old matured samples these factors cannot be further investigated.

### 3.3.3.2 Higher alcohols

The level of i-amyl alcohol and overall total higher alcohol concentration was found to be highest (≤0.05) in the samples of group 5B, but lowest in those of group 5C. The production of higher alcohols depends on the raw material and the yeast employed, and during the distillation processes the low molecular-weight alcohols increase and the high-molecular-weight alcohols decrease due to the effects of differing volatility during distillation (Piggott, 1983).

Therefore the samples in group 5B and 5C could have been fermented with different base wines and/or yeast strains. However again due to the fact that three-year barrel matured brandy samples are blended and not kept separately, it is difficult to confirm this theory.

### 3.3.3.3 Acids

The acids n-butyric and i-butyric were found to be significantly higher in the samples of group 5B. Butyric acid and other short chain volatile organic acids are the compounds responsible for the rancid odours emitted by the spirit when stored in less desirable circumstances (Silva and Malcata, 1998).

### 3.3.3.4 Aldehydes

The samples in group 5A were found to contain a higher concentration ($\leq 0.05$) of overall all total aldehyde compounds. Even though the levels of vanillin, sinapaldehyde and syringaldehyde were not found to be significantly different ($\leq 0.05$) between the different groups, levels were found to be slightly elevated in the samples of group 5A. It would seem that the group 5A contains on average a higher level of aldehyde compounds derived specifically from wood maturation (Ibern-Gomez *et al.,* 2001). Of the carbonyl compounds, acetaldehyde is the major component and constitutes approximately 90% of the total aldehyde content in alcoholic beverages (Guymon, 1972). These findings were consistent with study.

### 3.3.3.5 Ratios and pH

The ratio of higher alcohols vs. esters was found to be significantly higher ($\leq 0.05$) in the samples of group 5B. Both the ratios of esters vs. acids and carbonyls vs. higher alcohols were found to be higher ($\leq 0.05$) in the samples of group 5A. However, the ratio of carbonyls vs. higher alcohols was not found to be significantly different between the groups 5A and 5C. The pH was significantly different ($\leq 0.05$) between groups 5A and 5C, but however not between group 5B and 5C.

### 3.3.3.6 Principal Component Analysis (PCA) of the different groups of class five three-year barrel old matured brandy samples and their chemical analysis

Figure 3.3 shows the PCA bi-plot (Principal Component Analysis) for the chemical analysis of the class five three-year barrel old matured samples. The first principal component (F1) accounted for 16.26% of the total variation and the second principal component (F2) accounted for 14.85% of the total variation.
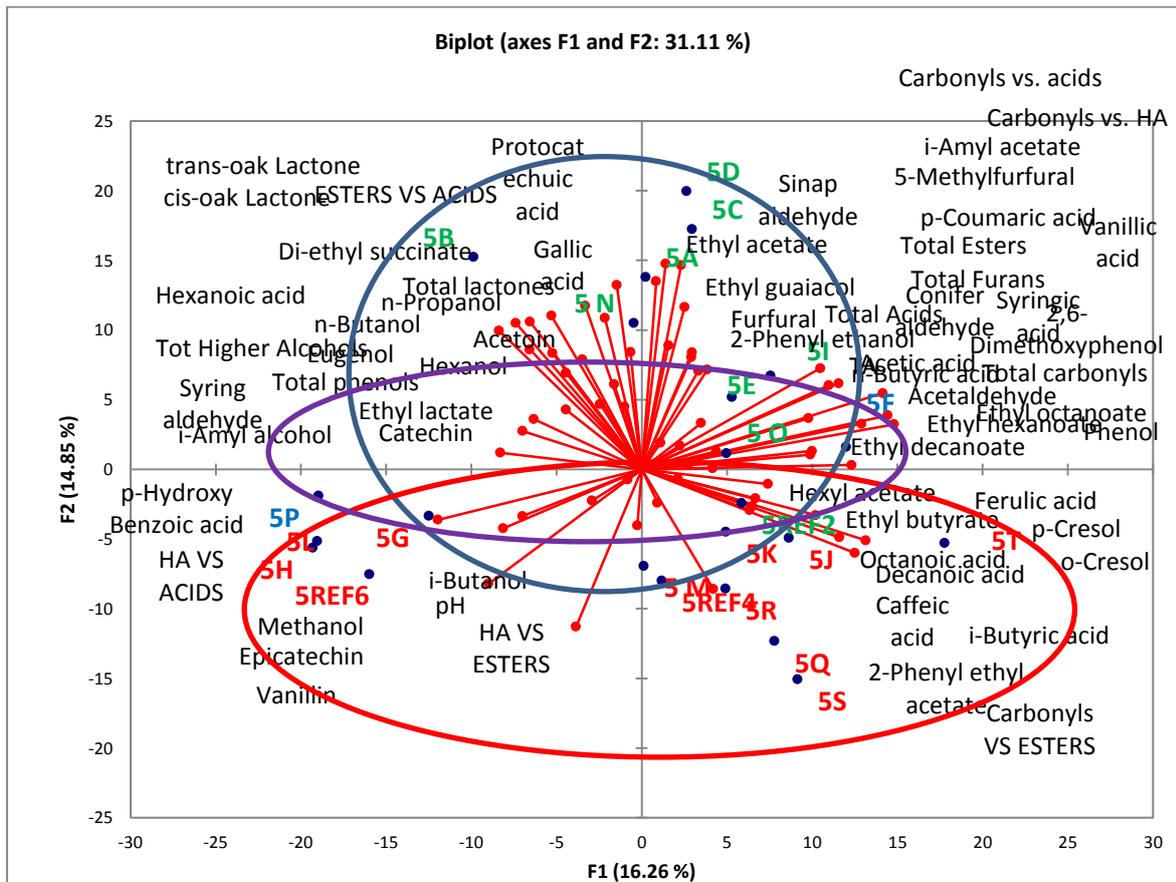
**FIGURE 3.3 PCA bi-plot showing the relationship between the class five three-year barrel age matured brandy samples and the compounds/groups of compounds on F1 and F2.**

From figure 3.3 it can be seem that within the class five three-year barrel old matured brandy samples there are three distinct groups. Group 5A (5E, 5I, 5REF2, 5B, 5C, 5D, 5O, 5N), group 5B (5F and 5P) and group 5C (5J, 5K, 5REF4, 5Q, 5R, 5S, 5T, 5A, 5G, 5H, 5L, 5REF6, 5M).

F1 indicated that group 5A [5E (factor score=1.642), 5I (factor score=2.346), 5C (factor score=0.914), 5D (factor score=0.817), 5REF2 (factor score=1.819), 5O (factor score=1.545)], group 5B  [5F (factor score=3.717)]  and group 5C [5A (factor score=0.071), 5J (factor score=2.677), 5K (factor score=1.532), 5T (factor score=5.510), 5Q (factor score=2.416), 5R (factor score=1.525), 5S (factor score=2.837), 5M (factor score=0.041) and 5REF4 (factor score=0.369)] were found to be positively associated to the F1 axis and contrasted the most with group 5A [5B (factor score=-3.056) and 5N (factor score=-0.142)], group 5B [5P (factor score=-5.876)] and group 5C [5G (factor score=-3.869), 5H (factor score=-5.982), 5L (factor score=-5.900), 5REF6 (factor score=-4.953)].

The compounds/groups of compounds acetaldehyde (r=0.803), ethyl decanoate (r=0.717), ethyl octanoate (r=0.685), decanoic acid (r=0.696), total aldehydes (r=0.787), aldehydes vs. esters (r=0.731) and aldehydes vs. HA (r=0.824) were found to be positively correlated group 5A (5E, 5I, 5C, 5D, 5REF2, 5O), group 5B  (5F) and group 5C (5A, 5J, 5K, 5T, 5Q, 5R, 5S,

5M  and 5REF4) to the F1 axis and therefore positively associated to, but however negatively associated to group 5A (5B and 5N), group 5B (5P) and group 5C (5G, 5H, 5L, 5REF6).

Conversely the compounds/groups of compounds HA vs. acids (r=-0.664) was found to be negatively correlated to the F1 axis and therefore negatively associated to  group 5A (5E, 5I, 5C, 5D, 5REF2, 5O), group 5B  (5F) and group 5C (5A, 5J, 5K, 5T, 5Q, 5R, 5S, 5M  and 5REF4), but however positively associated to group 5A (5B and 5N), group 5B (5P) and group 5C (5G, 5H, 5L, 5REF6).

F2 indicated that the samples group 5A [5E (factor score=1.685), 5I (factor score=2.182), 5B (factor  score=4.947),  5C  (factor  score=5.594),  5D  (factor  score=6.482),  5N  (factor score=3.406)] and group 5B [5A (factor score=4.476)] were found to be positively associated to the F2 axis and contrasted the most with group 5C [5J (factor score=-1.598), 5K (factor score=-1.456, 5T (factor score=-1.710), 5M (factor score=-2.248), 5Q (factor score=-3.996), 5R (factor score=-2.778), 5REF4 (factor score=-2.587)], group 5A [5REF2 (factor score=-0.787),  5G (factor score=-1.083), 5H (factor score=-1.828), 5L (factor score=-1.665) and 5REF6 (factor score=-2.440)].

The compounds/groups of compounds ethyl acetate (r=0.821), total esters (r=0.815), total lactones (r=0.653), gallic acid (r=0.736) and sinapaldehyde (r=0.751) were found to be positively correlated to the F2 axis and therefore positively associated to group 5A (5E, 5I, 5B, 5C, 5D, 5N) and group 5B (5A), but however negatively associated to group 5C (5J, 5K, 5T, 5M, 5Q, 5R, 5REF 4), group 5A (5REF2,  5G, 5H, 5L  and 5REF6).

Therefore one can see that the within the class five three-year barrel old matured samples there are three distinct groups. Even though these samples were classified as class five three-year barrel old matured brandy samples there seems to be variations within this class.

Table 3.1 shows that one of the main differentiating factors between the class one and class five three-year barrel old matured brandy samples are the levels of esters. Even though the class five three-year barrel old matured brandy samples are found to contain higher concentrations of total esters when compared to the class one three-year barrel old matured brandy samples, they vary within their own class with regards to the amount of total esters present. They also however differ with their amount of furanic aldehydes, where some groups contain a larger amount of these barrel maturation compounds. It can be seen that one of the differentiating factors within the class five three-year barrel old matured brandy samples is amount of wood associated compounds present. Even though the results obtained in Table 3.3 show that there are no significant differences between the 3 groups of the class five three-year barrel old matured brandy samples, Figure 3.3 shows that the compounds *cis*-lactone and *trans*-lactone are found to be associated to the samples 5E, 5I, 5B, 5C, 5D, 5N and 5A.

The level of total lactones present in matured spirit beverages are found to be attributed to the degree of barrel toasting and previous studies by Ibern-Gomez *et al.,* (2001) show that levels are found to increase with an increase in barrel toasting. Due to the fact that these barrels underwent the same degree of toasting at the beginning of their life span, it could be theorized that perhaps the above mentioned samples were matured in barrels of different ages, origin, or perhaps stored under different temperature and humidity conditions.

Even though the ANOVA in Table 3.3 shows no significant differences between these compounds and the respective samples, Figure 3.3 shows that the amount of total acids, total furans and total esters are found to be associated to samples 5A, 5C, 5D, 5E, 5I, 5O and 5F. The amount of acids and more specifically phenolic acids as well as total furans are compounds derived from oak barrels during maturation and the type and amount found in spirit products is dependent on the type of oak, seasoning, aging and origin (Ibern-Gomez *et al.,* 2001). An increase in concentration of these compounds are said to be found in distillates produced with new wood.

From Figure 3.3 the above mentioned compounds are also found to be associated to vanillic acid when compared to those samples namely 5L, 5H, 5G, 5P and 5REF4 who are found to be associated to the compound vanillin. Studies by Bloem *et al.,* (2007) found that lactic acid bacteria were found to reduce vanillic acid into the by-product namely vanillin. Therefore perhaps these samples were produced by wines that previously had undergone malolactic fermentation prior to the distillation process or alternatively in barrels of different ages, origin or seasoning.

It is important to note that one would expect variations within a class of three-year barrel matured brandy samples as barrels as storage conditions (humidity, temperature), barrel age and seasoning greatly influence the extraction of compounds found in the resultant product. Certain compounds can either enhance or supress certain quantities of the chemicals found in the beverage matrix, thus making further studies of barrel maturation and the influence on spirit products of fundamental importance.

## 3.4 CONCLUSIONS

The class one three-year barrel old matured brandy samples contained a higher amount of total higher alcohols, a higher ratio of higher alcohols vs. esters and higher alcohols vs. acids and a lower amount of total esters, total acids, total lactones and total furans and were perceived to be less "fruity" in aroma and flavour when compared to the class five samples. Inversely the class five three-year barrel old matured brandy samples contained a higher concentration of total esters, total acids, total furans, total lactones and a lower level of the ratios higher alcohols vs. esters and higher alcohols vs. acids and were found to be more "fruity" in aroma and flavour.

Figure 3.1 shows that some of the class five three-year there are large variations within the specific classes, and the range in which a sample barrel old matured brandy samples could have been incorrectly classified and if re-classified would perhaps obtain a sensory score of class two or three. Or alternatively can score is larger. The class one three-year barrel old matured brandy samples were all found to contain a higher concentration of total higher alcohols when compared to the class five three-year barrel old matured brandy samples, however within class one the samples differed with regards to their higher alcohol content. Similarly the class five three-year barrel old matured brandy samples contained a higher concentration of total esters and acids than the class one three-year barrel old matured brandy samples.

As the samples came from lots whereby different barrel age, seasoning, storage temperature and humidity differed one would expect to find variations within a specific class as barrel maturation and complex reactions taking place during the maturation process greatly influence the outcome of the chemical composition of spirit product.

It is therefore crucial that one does not solely rely on the sensory classification as a sole way of identifying and classifying three-year barrel matured brandy samples. A less subjective method of chemical analyses using GC-FID and HPLC to quantify and qualify the volatile and non-volatile compounds present in the three-year barrel old matured brandy samples are a greater tool in classifying the samples into specific styles.

## 3.5 LITERATURE CITED

Bloem, A., Bertrand, A., Lonvaud-Funel, A., De Revel, G. 2007. Vanillin production from simple phenols by wine-associated lactic acid bacteria. Appl. Micro. 44, 1: 62-67.

Bougas, N.V., 2009. Evaluating the effect of pot still design on the resultant distillate. Dissertation. Stellenbosch University. South Africa.

Boulton, R. 1980. The relationship between total acidity, titratable acidity and pH in wine. Am. J. Enol. Vitic. 81, 1: 76-80.

Boscolo, M., C.W.B. Bezerra., D.R. Cardoso., B.S. Limo Neto., D.W. Franco. 2000. Identification and dosage by HRCG of minor alcohols and esters in Brazilian sugar-cane spirit. Braz. J. Chem. Soc. 11, 1:86-90.

Chatonnet, P., D. Dubordieu., J. Boidron. 1993. Effect of fermentation in oak barrels on the composition and quality of white wines. Wine Industry Journal. 2: 73-84.

H.W. du Plessis¨ L.M.T. Dicks., I.S. Pretorius., M.G. Lambrechts., M. du Toit. 2004. Identification of lactic acid bacteria isolated from South African brandy base wines. Int. J. Food. Micro. 91, 1:19-29.

Du Toit, M., Engelbrecht, L., Lerm, E., Krieger-Weber, S. 2011. Lactobacillus: The next generation of malo-lactic fermentation starter cultures-An overview. Food. Bioprocess. Tech. 4,6: 876-906.

Gold, A.H. 1972. (2nd Ed). Wines and spirits of the world. Virtue & Co Ltd, London.

Guymon, J.F. 1972. Higher alcohols in beverage brandy. Wines and Vines. January, 37-40.

Ibern-Gomez, M., Andrés-Lacueva., Lamuela-Raventós, R.M., Lao-Luque, C., Buxaderas, S., De la Torre-Boronat, M.C. 2001. Differences in phenolic profile between Oak Wood and Stainless Steel fermentation in White Wines. Am. J. Enol. Vitic. 52,2: 159-164.

Jack, F. 2003. Development of guidelines for the preparation and handling of sensory samples in the Scotch Whiskey industry. J. Inst. Brew.109, 2: 114-119.

Jounela-Eriksson, P. 1981. Predictive value of sensory and analytical data for distilled beverages. *In* Flavour'81. Schreier, P. (1st Ed). Berlin.

66

Léaute, R. 1990. Distillation in Alambic. Am. J. En. Vitic. 41, 1: 90-108.

Lawless, H.T., and H. Heymann. 1995. Sensory evaluation of food: Principles and Practices. Chapman and Hall, New York.

Le Roux, J. 1997. Van Ryn advanced brandy course. The Van Ryn wine and spirit company.

Nykanen, L.H. 1986. Formation and occurrence of flavour compounds in wine and distilled beverages. Am. J. Enol. Vitic. 37, 1: 84-94.

Piggott, J.R. 1983. Flavour of distilled beverages: Origin and development. Ellis Horwood Ltd,

Chichester.

Postel, W and Adam, L. 1990a. Fluechtige Gaschromatograpische Karakterisierung von Cognac und Armagnac-Gehalte von fleuchtigen Verbindungen. Branntweinwirtschaft. 130: 208-213.

Salo, P., Nykänen, L Suomalainen, H. 1972. Odour thresholds and relative intensities of volatile aroma components in an artificial beverage imitating whiskey. J. Food. Sci. 37, 3: 394-398.

Silva, M.L., and F.X. Malcata. 1998. Relationships between storage conditions of grape pomace and volatile composition of spirits obtained therefrom. Am. J. Enol. Vitic. 49, 1:56-64.

Steger, C.L.C., and M. Lambrechts. 2000. The selection of yeast strains for the production of premium quality South African brandy base products. J. Ind. Micro. Biotech. 24, 6: 431-440.

Toerien, W. 2008. Firewater. Quivertree publications. Cape Town. South Africa.

Von Adam, L., M. Haug., E. Kolb., C.H. Schwartz. 1996. Contribution of the knowledge of volatile compounds present in wine distillates from the years 1990-1993. Branntweinwirtschaft. 6: 82-90.

# Chapter 4

# Research results

## CHEMICAL PROFILES OF BASE WINES PRIOR TO DISTILLATION

This manuscript is prepared according to the style of the **South African Journal of Enology and Viticulture**

# 4. Chemical profiles of base wines prior to distillation

## ABSTRACT

Wines used for the production of brandy (base wines) are sensorially evaluated and scored on a ranking scale from one to five that described the perceived fruity aroma and taste of the wine. In this project wines that scored on the opposite ends of the sensory scale (one and five) were chemically analysed using GC-FID (Gas flame ionization detection). It was then determined whether the chemical profiles of the base wine could be correlated to different brandy styles. Chemically, it was found that the base wine samples with a sensory score 1 were found to contain less total esters (due to lower levels of ethyl acetate, ethyl butyrate, i-amyl acetate, ethyl hexanoate and hexyl acetate), more total higher alcohols (due to higher levels of i-amyl alcohol and i-butanol) and total acids (due to higher levels of acetic acid) and also a higher ratio of higher alcohols vs. esters when compared to those base wine samples which scored a five. Base wine samples with a sensory score of 1 were found to be less fruity in overall aroma and flavour. Conversely the base wine samples with a sensory score of five were found to contain more total esters (due to the higher levels of ethyl acetate, ethyl butyrate, i-amyl acetate, ethyl hexanoate and hexyl acetate), less total acids (acetic acid) and total higher alcohols and a lower ratio of higher alcohols vs. esters. These base wine samples were found to be fruitier in overall aroma and flavour.  It was found that the base wine samples with a sensory score of five were considered fruitier due to the higher level of esters present; the inverse was true for the base wine samples with a sensory score of one.

## 4.1 INTRODUCTION

Brandy production is a multi-stage process involving viticulture, winemaking, distillation and maturation. During these processes many compounds are produced that play a key role in the aroma and flavour of distilled beverages and more specifically brandy (Nykänen, 1983). Of these compounds it is mainly the esters, acids as well as the higher alcohols that contribute the most to the overall organoleptic quality, style and profile of the product (Marais, 1978).

The most abundant esters found in alcoholic beverages are ethyl acetate and the medium-chain fatty acid ethyl esters. Studies by Guymon (1969) reported that the threshold value of ethyl acetate is 160 mg/L in wine and it can be an indicator of microbial spoilage (Ferrari *et al.,* 2004). Due to the fact that ethanol and acetic acid are the dominating alcohol and fatty acid present in wine, ethyl acetate is produced in large amounts due to the reaction between these two compounds. Other than ethyl acetate, the ethyl esters namely ethyl hexanoate, ethyl octanoate and ethyl decanoate are the most quantitatively dominant and are generated through fermentation and exhibit characteristic fruity and flowery odour notes (Guymon, 1969).

Of the higher alcohols present in wine, the most important one to consider is iso-amyl alcohol as this higher alcohol, if found in to high concentration, can render the product unpleasant (Boscolo *et al.,* 2000). Fatty acids are generated in the fermentation process as well as due to the thermal degradation and autolysis of yeast cells during the distillation process into the resultant spirit product (Nykänen, 1983). The most abundant volatile acid found in wine is acetic acid and constitutes between 45 and 90% of the total volatile acid concentration. If acetic acid is disregarded then the volatile acids octanoic, decanoic and hexanoic acid are the largest components in descending order (Von Adam *et al.,* 1996).

Research shows that the production of the above mentioned compounds are produced mainly during the fermentation process and more specifically by the yeast strain used as they are not found in their active state in the grape berry (Suomalainen and Lethonen, 1978). Yeast has been used for the production of alcoholic beverages, including wine since ancient times. The task of choosing a yeast strain is important and significant as they are responsible for the production of volatile compounds and thus the aromatic quality of wine (Steger and Lambrechts, 2000).

However, the distillation process also plays an important role as certain compounds namely ethyl acetate, methanol and acetic acid are removed in the head fraction and the compounds i-propanol and i-amyl alcohol are found in higher concentrations in the tail fractions, thus decreasing the concentration of total esters, acids and some higher alcohols in the distillate and in-turn the three-year brandy samples (Claus and Berglund, 2004). During the maturation process, depending on the pH of the alcoholic beverage, the level of esters and acids can either decrease or increase depending on if hydrolysis or esterification is promoted (Makhotkina and Kilmartin, 2012), thus making this process also an important factor to consider.

Studies by Bandion (1972) show that when evaluating the chemical composition of brandies and base wine, it is not an individual compound but rather the overall matrix that is found to be correlated to the brandy quality and aroma.  He concluded that the sensory evaluation of brandies by expertly trained panel members is the most accurate way to asses brandy quality. Research conducted by Goranov (1983) show that brandies and base wine with significantly higher concentrations of higher alcohols (greater than 900 mg/L) lead to a perception of "roughness" on the palate and makes an un-balanced wine. Goranov (1983) also found that wine with decreased levels of esters to be of poorer quality (50 to 460 mg/L).

The aim of this study was to determine a chemical profile of the base wines prior to distillation and to identify compounds or groups of compounds that differentiate between base wines.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Sensory analysis

One hundred and twenty two base wine samples were obtained from commercial wine farms for the purpose of distilling. Samples were evaluated by an internal panel and classified using a sensory classification scale ranging from a tasting score of one until five. Figure 4.1 shows the line-scale used for the purpose of the sensory evaluation. Focus will only be given on tasting samples that scored a one and five, as these are the two extreme tasting average scores and they differ greatly from each other. It is important to note that the differences between the one and five base wine samples (according to the internal panel) were scored on their overall "fruity" aroma and flavour. Base wines that scored a sensory score of five were considered to be more "fruity" with regards to aroma and flavour than those that scored a one, whereas base wine samples that scored a sensory score of one were found to have a less "fruity" aroma and flavour and more neutral bouquet and were also perceived to have a higher acidity in the overall mouth-feel.

It is important to note that the base wine samples that received a sensory score of one were not considered to be of negative quality per se, (these samples passed all the quality parameters needed for a base wine as stipulated in the South African Liquor Products Act No. 60 of 1989), but were found to be less "fruity" overall. The term "quality" is loosely defined and it needs to be stressed that the base wines were evaluated based on their "fruity" aroma and overall sensory perception.
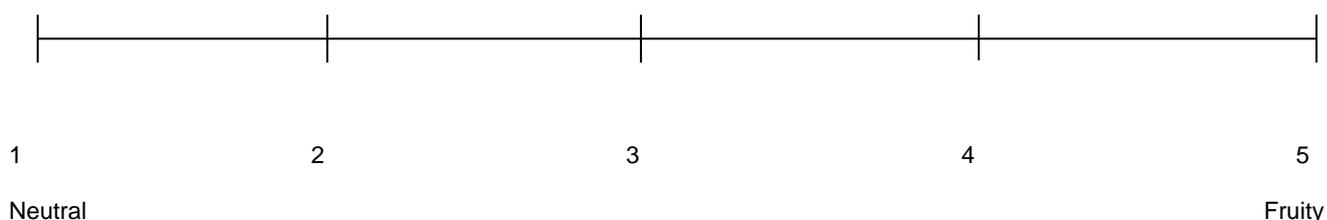
**FIGURE 4.1 Line-scale used for the purpose of base wine classification.**

### 4.2.2 Chemical analysis

Each base wine sample was subjected to gas chromatography flame-ionization detection (GC-FID) to quantify the volatile compounds and therefore produce chemical profiles of base wine samples that scored a one and five.

A volume of 10 mL for the analysis of the base wine ca. 10% (a/v) was pipetted into a screwcapped glass test tube and spiked with 0.4 mL internal standard (4-methyl-2-pentanol at a concentration of 1.2 g/L in absolute ethanol). A volume of 6.5 mL diethyl ether was added to each test tube. The test tubes were capped and placed in a rotary mixer, where after the samples were extracted for 30 minutes at a speed of 60 rpm. The upper diethyl ether layers were transferred into 2 mL crimp-top autosampler vials, the vials were sealed and a volume of 3 µL of each extract was injected into the GC inlet. Each base wine was extracted in triplicates to ensure reproducibility and the standard deviation obtained was ≤ 5%.

The prepared samples/extracts were analysed by GC-FID using Agilent HP6890 series gas chromatograph, equipped with a 60 m x 0.32 mm ID x 0.5 µm film thickness, HP-Innowax fused silica capillary column, connected to a flame ionization detector (FID). The split/splitless injector was set at 200°C. Split injectors were used for the 10% (a/v) samples viz. split ratios of 10:1. The initial oven temperature was set to 30°C (for 17 min), then increased to 240°C (for 5 min) at a heating rate of 8°C/min. The carrier gas was hydrogen 5.0 (Air Products) with a nominal initial pressure of 83.5 kPa with an average linear velocity of 48 cm/s for the 10% (a/v) samples. A constant flow mode was employed. The detector temperature was set at 250°C, with an air (medical air, Air Products) flow of 400 mL/min, a hydrogen flow of 30 mL/min and nitrogen 5.0 (Air Products) make up gas flow of 30 mL/min.

### 4.2.3 Statistical analysis

Statistical analyses were performed using Microsoft XLStat version 2012. A one-way ANOVA (analysis of variance) which tests for interactions between one categorical independent variable (namely the base wine and the chemical composition) was carried out. A PCA (principal component analysis) displaying the interrelationships between the base wine and the chemical composition were also carried out. The standard deviation obtained was ≤ 5%.

## 4.3 RESULTS AND DISCUSSION

### 4.3.1 Chemical composition of the two extreme base wine samples

Table 4.1 shows the mean values for the one-way ANOVA for the different compounds found in the base wines that scored a one or five. A total of 11 base wine samples that scored a sensory score of one and 27 base wine samples that scored a sensory score of five were analysed respectively.

**TABLE 4.1 ANOVA of the chemical composition of the averages for the two extreme base wines (sensory score one and five)**

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Sensory score 1 | Sensory score 5 |
|---|---|---|---|
| Ethyl acetate | <0.0001 | 56.053 b | 88.306 a |
| Ethyl butyrate | 0.024 | 0.427 b | 0.526 a |
| i-Amyl acetate | <0.0001 | 5.232 b | 11.113 a |
| Ethyl hexanoate | 0.009 | 1.125 b | 1.515 a |
| Hexyl acetate | <0.0001 | 0.635 b | 1.300 a |
| Ethyl lactate | 0.024 | 4.602 a | 0.938 b |
| Ethyl octanoate | 0.137 | 1.351 a | 1.518 a |
| Ethyl decanoate | 0.662 | 1.122 a | 1.071 a |
| Di-ethyl succinate | 0.944 | 0.568 a | 0.563 a |
| 2-Phenyl ethyl acetate | 0.275 | 0.338 a | 0.399 a |
| **Total esters** | **<0.0001** | **73.653 b** | **104.330 a** |
| n-Propanol | 0.216 | 36.516 a | 41.337 a |
| i-Butanol | 0.011 | 24.223 a | 15.326 b |
| n-Butanol | 0.044 | 0.659 b | 0.922 a |
| i-Amyl alcohol | 0.033 | 144.121 a | 126.037 b |
| Hexanol | 0.096 | 1.744 a | 1.454 a |
| 2-Phenyl ethanol | 0.076 | 12.982 a | 9.681 a |
| **Total higher alcohols** | **0.015** | **224.383 a** | **195.620 b** |
| Acetic acid | 0.016 | 168.637 a | 115.972 b |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | Sensory score 1 | Sensory score 5 |
|---|---|---|---|
| Propionic acid | 0.284 | 7.934 a | 4.783 a |
| i-Butyric acid | 0.095 | 0.775 a | 0.602 a |
| n-Butyric acid | 0.764 | 1.377 a | 1.366 a |
| i-Valeric acid | 0.210 | 0.141 a | 0.066 a |
| n-Valeric acid | 0.479 | 0.662 a | 0.732 a |
| Hexanoic acid | 0.465 | 4.877 a | 5.228 a |
| Octanoic acid | 0.101 | 7.953 a | 9.239 a |
| Decanoic acid | 0.140 | 3.608 a | 4.140 a |
| **Total acids** | **0.044** | **186.365 a** | **147.535 b** |
| Acetaldehyde | 0.098 | 9.907 a | 6.998 b |
| Acetoin | 0.032 | 1.644 a | 0.754 b |
| **Total aldehydes** | **0.377** | **9.653 a** | **8.587 a** |
| HA[b] vs. esters | 0.034 | 2.931 a | 2.047 b |
| HA vs. acids | 0.383 | 1.360 a | 1.550 a |
| Esters vs. acids | 0.015 | 0.480 b | 1.023 a |
| Aldehydes vs. esters | 0.052 | 0.138 a | 0.093 a |
| Aldehydes vs. acids | 0.094 | 0.061 a | 0.096 a |
| Aldehydes vs. HA | 0.619 | 0.051 a | 0.045 a |
| Methanol | 0.001 | 48.389 a | 38.418 b |
| pH | <0.0001 | 3.570 a | 3.280 b |
| TA | <0.0001 | 6.880 a | 7.123 b |

**HA[b] (Higher alcohols). Compounds that have different letters assigned to them in the same row are found to be significantly different (≤0.05).**

**4.3.1.1 Chemical analysis of esters**

**4.3.1.1.1 Ethyl lactate**

Base wine samples that scored a one contained higher concentrations of ethyl lactate and were also perceived to be less "fruity" when evaluated by the internal brandy panel (Table 4.1). Ethyl lactate is a by-product formed during malolactic fermentation and increased levels of ethyl lactate in the base wines are due to partial or spontaneous malolactic fermentation. Studies by Du Plessis *et al.*, (2002) found that in wine and unaged distillate samples where malolactic fermentation had occurred there was a loss in "fruitness" and intensity of aroma. Higher levels of ethyl lactate were also found to be correlated to poorer quality base wines. The pH of base wines with a sensory score of 1 were higher (Table 4.1) when compared to the base wine samples that scored a sensory score of 5. Wines with a higher pH value are found to have increased levels of ethyl lactate as a higher pH value promotes the growth of malo-lactic bacteria (du Plessis *et al.,* 2002).

**4.3.1.1.2 Ethyl acetate**

It was found that base wine samples that received a tasting score of five contained higher levels of ethyl acetate when compared to those of the base wine samples that scored a one. Ethyl acetate is the most abundant ester found in wine as a result of the dominant alcohol and acid present in wine being ethanol and acetic acid. Esters are formed either through chemical or enzymatic esterification, and is dependent on many factors such as fermentation temperature, yeast strain, nitrogen compounds and must solids (Nykänen, 1986) A low pH promotes (chemical) esterification and therefore the production of esters in the medium; however it is a slow process that takes place over a long period of time. The increased levels of esters found in the above mentioned wine were a result of enzymatic esterification produced by the yeast strain present (Sumby *et al.,* 2010).

The base wines that scored a sensory score of 5 contained on average a higher concentration of ethyl acetate and had a lower pH (TABLE 4.1). The reasons for the elevated levels of ethyl acetate present could be due to a higher fermentation temperature or a certain yeast strain employed during the fermentation process. However, the fermentation conditions of the base wines are not known and this cannot be confirmed.

**4.3.1.1.3 Ethyl butyrate**

Levels of ethyl butyrate were found to be higher in those base wine samples with a score of five. Ethyl butyrate is formed when butyric acid combines with ethanol to produce ethyl butyrate. The aroma associated with ethyl butyrate is reminiscent of apple (Diequez *et al.*, 2005). This reinforces the correlation between the base wine samples that scored a sensory

score of 5 and an increase in "fruitness". Both of the base wine samples (Sensory score 1 and 5) are found to be above the threshold level found in wines.

### 4.3.1.1.4. i-Amyl acetate

Levels of i-amyl acetate are found to be significantly higher in base wines that scored a sensory score of 5. Increased levels of i-amyl acetate are said to be linked with an increase in wine quality, therefore possibly indicating that these base wine samples are of better "quality" (Snyman, 2004). The aroma associated with i-amyl acetate are said to resemble that of banana's (Lilly *et al.*, 2000; Bayly, 2002; du Plessis *et al.,* 2002; Diequez *et al.*, 2005). These samples are also said to display more "fruity" characteristics which could be attributed to the increased level of i-amyl acetate. Both of the base wine samples (Sensory score 1 and 5) are found to be above the threshold level found in wine for i-amyl acetate concentrations.

### 4.3.1.1.5 Ethyl hexanoate and hexyl acetate

Base wine samples that received a sensory score five contained higher concentrations of ethyl hexanoate and hexyl acetate. Decreased levels of ethyl hexanoate and hexyl acetate are said to be linked with a decrease in quality (Snyman, 2004). Ethyl hexanoate and hexyl acetate are associated with "fruity" notes. This was confirmed in the findings of this study.

### 4.3.1.1.6 Total esters

A higher concentration of esters was found in those base wine samples that received a tasting score of five. Esters are responsible for the fruity aromas found in wine and spirit beverages (Nykänen, 1986). An increase in levels of esters are said to be correlated to an increase in quality of the product (Snyman, 2004). Esters are formed during the fermentation process and certain factors such as an increase in fermentation temperature, certain yeast strain employed, and a decrease in YAN (Yeast Assimilable Nitrogen) could lead to a decrease in the formation of esters (Vilanova *et al.,* 2007). However, the fermentation conditions of the base wines in this study were not known and this correlation can therefore not be confirmed.

### 4.3.2 Chemical analysis of higher alcohols

### 4.3.2.1 i-Amyl alcohol

The base wines that scored a sensory score of five were considered to more "fruity" than those that scored a one and interestingly they were found to contain lower amounts of i-amyl

alcohol, which to contradicts previous findings. Studies by Snyman (2004) found that the levels of i-amyl alcohol decreases with a decrease in quality.

Studies by Guymon (1969) classified i-amyl alcohol as one of the main fermentation higher alcohols and is known to contribute positively to aromatic complexity of wine when present in concentrations below 300 mg/L. The level of i-amyl alcohol in both the base wines that scored a sensory score of one and five were found to contain levels below the recommended 300 mg/L.

### 4.3.2.2 Total higher alcohols

Base wines that scored a sensory score of one were found to contain higher amounts of total higher alcohols. The concentration of total higher alcohols found in wine is generally dependent on the fermentation conditions and more specifically the yeast strain that is used (Guymon, 1969; Lambrechts and Pretorius, 2000). An increase in fermentation temperature as well as lower levels of available nitrogen will lead to an increase in higher alcohol formation (Valdés *et al.,* 2011).

### 4.3.3 Chemical analysis of acids

### 4.3.3.1 Total acids

Levels of acetic acid are higher in the base wines that scored a sensory score one when compared to those that scored a sensory score of five. Threshold levels for acetic acid in wine is between 700-1000 mg/L and in brandy the legal limit for base wine is below 0.7 mg/L (Lambrechts and Pretorius, 2000). Acetic acid levels are considered a by-product of spoilage whereby acetic acid bacteria convert acetaldehyde and ethanol into acetic acid (Bartowsky and Henschke, 2008). It can also be produced by lactic acid bacteria and other yeasts. However, both base wine groups (one and five) were found to be well below the threshold value, thus indicating that neither group were considered to have increased levels due to spoilage (Snyman, 2004).

The medium chained fatty acids such as hexanoic, octanoic and decanoic acid are determined by the fermentation conditions (Blanco-Gomis *et al.*, 2001), and during the distillation process their concentration will also be dependent on the amount of yeast biomass found in the product (Bertrand, 2003). Of the acids found in the respective base wines only acetic acid is found to be significantly different.

### 4.3.4 Principal Component Analysis (PCA) of the base wines

Figure 4.2 displays the interrelationships of the chemical compounds/groups of compounds and the two different base wine groups, namely sensory score one and five. The first

principal component (F1) accounted for 77.69% of the total variation, whereas the second principal component (F2) accounted for 22.31% of the total variation.
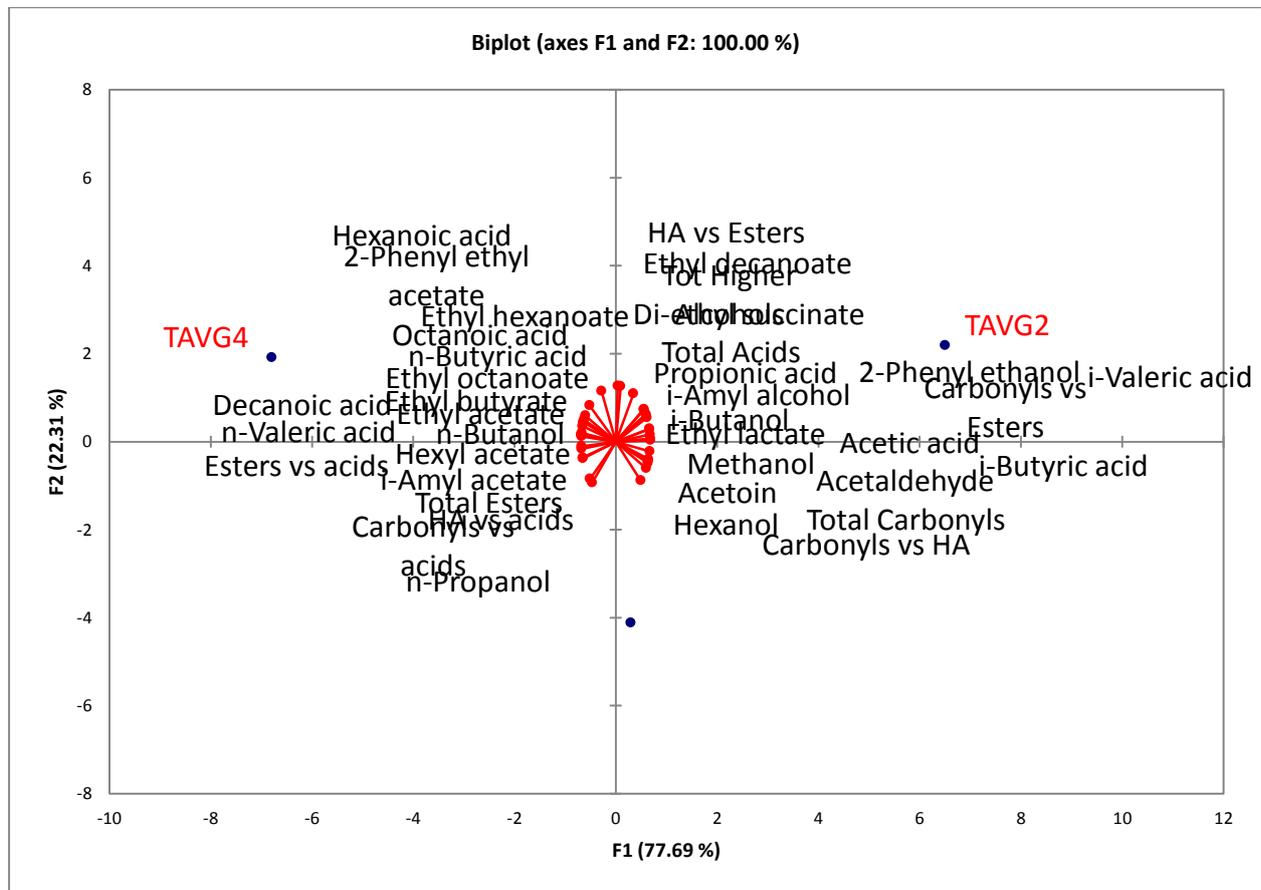


**FIGURE 4.2 PCA bi-plot showing the relationship between the base wine samples and the chemical compounds/groups of compounds on F1 and F2.**

F1 indicated that base wines with a sensory score of one (factor score=6.501) were found to be positively associated to the F1 axis and contrasted the most with base wines that received a sensory score of five (factor score=-6.798).

The chemical compounds/groups of compounds that were found to be positively correlated to the F1 axis and therefore positively associated to the base wine samples with a sensory score of one and negatively associated to the base wine samples that scored a sensory score of five were acetaldehyde (r=0.950), methanol (r=0.985), i-butanol (r=1.000), i-amyl alcohol (r=0.875), acetoin (r=0.924), ethyl lactate (r=0.999), hexanol (r=0.722), acetic acid (r=0.973), i-butyric acid (r=0.999), i-valeric acid (r=0.869), 2-phenyl ethanol (r=0.817), total higher alcohols (r=0.902), total acids (r=0.812), total carbonyls (r=0.880), higher alcohols vs. esters (r=0.974), carbonyls vs. esters (r=0.992) and carbonyls vs. higher alcohols (r=0.937).

Conversely the following chemical compounds/groups of compounds were found to be negatively correlated to the F1 axis and therefore negatively associated to base wines with a sensory score of one, but however found to be positively associated to base wine samples that received a sensory score of five were ethyl acetate (r=-0.990), ethyl butyrate (r=-0.932), n-propanol (r=-0.987), i-amyl acetate (r=-0.959), n-butanol (r=-0.996), ethyl hexanoate (r=-

0.991), hexyl acetate (r=-0.997), ethyl octanoate (r=-0.884), n-valeric acid (r=-0.761), 2-phenyl ethyl acetate (r=-0.994), hexanoic acid (r=-0.937), octanoic acid (r=-0.920), total esters (r=-0.954), higher alcohols vs. acids (r=-0.750), esters vs. acids (r=-0.992) and carbonyls vs. acids (r=-0.685).

From figure 4.2 one can see that the base wine samples that scored a sensory score of one were found to be positively associated with total higher alcohols, total acids, and total carbonyls, higher alcohols vs. esters, carbonyls vs. esters and carbonyls vs. higher alcohols. It was also found that the base wine samples that scored a sensory score of five were positively associated with total esters, higher alcohols vs. acids, esters vs. acids and carbonyls vs. acids.

## 4.4 CONCLUSIONS

There are significant differences between the chemical compositions of the base wine groups used in this study. Those base wine samples that received a sensory score of one contained less total esters, but contained higher concentrations of total higher alcohols, total acids, total aldehydes, higher alcohols vs. esters, aldehydes vs. esters and aldehydes vs. higher alcohols when compared to those base wine samples that scored a sensory score of five. These base wine samples were perceived to be less "fruity". Base wine samples that scored a sensory score of five were found to contain more total esters, but less total higher alcohols, total acids, higher alcohols vs. acids, esters vs. acids and carbonyls vs. acids. These samples were perceived as being more "fruity".

Previous findings conclusively state that esters are responsible for the fruity aroma in alcoholic beverages. Given that base wine samples with a sensory score of one contain a lower concentration of esters, and therefore are less "fruity" in their aroma and flavour, it can be assumed that three-year brandy samples produced from these base wines will contain a lower concentration of total esters and therefore be less "fruity" overall. The inverse is found to be true for the base wine samples that scored a sensory score five.

The results obtained in chapter 3 show that the class one three-year barrel matured brandy samples were found to contain a higher amount of total higher alcohols, a higher ratio of higher alcohols vs. esters and higher alcohols vs. acids and a lower amount of total esters, total acids, total lactones, total furans and were found have a less "fruity" aroma and flavour as well as a smoother mouth-feel. Inversely the class five three-year barrel matured brandy samples contained a higher concentration of total esters, total acids, total furans, total lactones and a lower level of the ratios higher alcohols vs. esters and higher alcohols vs. acids. These samples were also perceived to more "fruity" in aroma and flavour.

Therefore if a base wine with a sensory score of one was to be distilled, it could yield a class one three-year barrel matured brandy sample, and inversely if a base wine with a sensory score of five were to be distilled it in-turn would yield a class five three-year barrel matured brandy sample. Each company has their own idea of what comprises a good base wine and decisions regarding what type is needed will be dependent on the type and style of brandy desired.

## 4.5 LITERATURE CITED

Bandion, F. 1972. Zue analytischen untersuchung von Brennweinen und Spirituosen II. Eigene Untersuchung. Mitt Hoeheren Bundesler Versuchsanst. Wein-Obstbau, Klosterneuburg. 22: 253-278.

Bartowsky, E.J and Henschke, P.A. 2008. Acetic acid bacteria spoilage of bottled red wine- A review. Int. J. Food. Micro. 125, 1: 60-70.

Bayly, J.C. 2002. Manipulating the levels of ethyl acetate and isoamyl acetate production during the formation of wine and brandy. Dissertation. Department of Wine biotechnology. Stellenbosch University.

Bertrand, A. 2003. Brandy and Cognac: Armagnac, Brandy, Cognac, and their manufacture. Encyclopedia of Food Sciences and Nutrition. 584-601.

Blanco-Gomis, D., Mangas Alonso, J.J., Margolles Cabrales, I., Arias Abrodo, P. 2001. Gas chromatographic analysis of total fatty acids in cider. J. Agirc. Food. Chem. 49, 3: 1260-1263.

Boscolo, M., Bezerra, C.W.B., Cardoso, D.R., Lima Neto, B.S., Franco, D.W. 2000. Identification and dosage by HRGC of minor alcohols and esters in Brazilian sugar-cane spirit. J. Braz. Chem. Soc. 11, 1: 17-25.

Claus, M.J and Berglund, K.A. 2004. Fruit brandy production by batch column distillation with reflux. J. Food. Pro. Eng. 28, 1: 53-67.

Diequez, S.C., M. Luisa., L.G. de la Pena., E.F.Gomez. 2005. Volatile composition and sensory characters of commercial Galician orujo spirits. J. Agric. Food. Chem. 53: 6759-6765.

Du Plessis, H.W., C.L.C Steger., M. du Toit., M. Lambrechts. 2002. The occurrence of malolactic fermentation in brandy base wine and its influence on quality. J. Appl. Micro. 92: 1005-1013.

Ferrari, G., O. Lablanquie., R. Cantagrel., T. Payot., N. Fournier., E. Guichard. 2004. Determination of key odourant compounds in freshly distilled Cognac using GC-O, GC-MS and sensory evaluation. J. Agric. Food Chem. 52: 5670-5676.

Goranov, N. 1983. Der Einfluss von Aromastoffen auf die Qualitat von Weinen und Spirituosen. Nahrung. 27, 5: 497-503.

Guymon, J.F. 1969. GC determination of ethyl esters in brandy or wine distillates. Am. J. Enol. Vitic. 20, 2: 76-85.

Lambrechts, M.G and Pretorius, I.S. 2000. Yeast and its importance to wine aroma- A review. SA. J. Vitic. Enol. 21: 97-129.

Léaute, R. 1990. Distillation in Alambic. American journal of Enology and Viticulture. 41, 1: 90-103.

Lilly, M., Lambrechts, M.G., Pretorius, I.S. 2000. Effect of yeast alcohol acetyltransferase activity on flavour profiles of wine and distillates. Appl. Environ. Micro. 66, 2: 744-753.

Marais, J. 1978. The effect of pH on esters and quality of Colombard wine during maturation. Vitis-Berichte ueber Rebenforschung mit Dokumentation der Weinbauforschung. 17, 4: 396-403.

Makhotkina, O and Kilmartin, P.A. 2012. Hydrolysis and formation of volatile esters in New Zealand Sauvignon blanc wine. Food Chem. 135, 2:486-493.

Nykänen, L. 1983. Aroma of beer, wine, and distilled alcoholic beverages. (1st Ed). D. Riedel Publishing Company. Dordrecht, Holland.

Nykänen, L.H. 1986. Formation and occurrence of flavour compounds in wine and distilled beverages. Am. J. Enol. Vitic. 28, 3: 152-158.

Patel, S and Shibamoto, T. 2003. Effect of 20 different yeast strains on the production of volatile components in Symphony wine. J. Food. Comp. Anal. 16, 4: 469-476.

Pino, J.A. 2007. Characterization of rum using solid-phase microextraction with gas chromatography-mass spectrometry. Food. Chem. 104, 1: 421-428.

Ribereau-Gaymon, P., Y. Glories., A. Maujean., D. Dubordieu. 2000. The chemistry of wine, stabilization and treatments. Vol 2. Handbook of Enology. Wiley. New York.

Shinohara, T and Wantanabe, M. 1976. Gas chromatographic analysis of higher alcohols and ethyl acetate in table wines. Agric. Bio. Chem. 40, 2: 2475-2477.

Silva, M.L and Malcata, F.X. 1998. Relationships between storage conditions of grape pomace and volatile composition of spirits obtained therefrom. Am. J. Vitic. Enol. 49, 1: 56-64.

Snyman, C.L.C. 2004. The influence of base wine composition and wood maturation on the quality of South African brandy. Dissertation. Stellenbosch University.

Steger, C.L.C and Lambrechts, M.G. 2000. The selection of yeast strains for the production of premium quality South African brandy base products.  J. Ind. Micro. Biotech. 24, 6: 431-440.

Sumby, K.M., Grbin, P.R., Jiranek, V. 2010. Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. Food. Chem. 121, 1: 1-16.

Suomalainen, H and Lehtonen, M. 1978. The production of aroma compounds by yeasts. J. Inst. Brew. 85: 149-156.

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I.S., Henschke, P.A. 2007. Assimiable nitrogen utilization and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeast. Appl. Micro. Biotech. 77, 1: 145-157.

Valdés, E., Vilanova, M., Sabio, E., Benalte, M.J. 2011. Clarifying agents effect on the nitrogen composition in must and wine during fermentation. Food Chem. 125, 2: 430-437.

Von Adam, L.; Haug, M; Kolb, E.; Schwartz, C.H. 1996. Contribution to the knowledge of volatile compounds present in wine distillates from the years 1990 to 1993. Die Branntweinwirtsschaft. 6: 82-90.

# Chapter 5

# Research results

# INVESTIGATING THE EFFECT OF YEAST STRAIN AND FERMENTATION TEMPERATURE ON THE VOLATILE COMPOSITION OF BRANDY BASE WINES

# 5. Investigating the effect of yeast strain and fermentation temperature on the volatile composition of brandy base wines

## ABSTRACT

Previous studies have concluded that diverse styles of brandies are defined by the amount and concentration of compounds (both the volatile and non-volatile) namely, esters, higher alcohols, acids, carbonyls as well as the ratios thereof. This study focussed on the effect of yeasts (228, Alchemy 1, VIN13 and D254B) and fermentation temperature (15ºC and 24ºC) in altering the chemical composition of base wines and, in-turn, the style of brandy. Four grape musts were obtained from commercial distilleries and underwent alcoholic fermentation after which the resultant base wine was analysed using GC-FID to identify the volatile compounds present. The results obtained showed that the level of ethyl acetate and overall total ester concentration was found to be significantly higher in the yeast Alchemy 1 when compared to the control yeast 228. The higher alcohol i-amyl alcohol was found to decrease when the yeast VIN13 and Alchemy 1 were used when compared to the control yeast 228. The overall total acid concentration was found to be highest in concentration when the yeast Alchemy 1 was employed in comparison to all the other yeasts and more specifically the control yeast 228. The ratio of higher alcohols vs. esters decreased when the yeast Alchemy 1 was used in comparison to the control yeast 228. The higher alcohols namely i-butanol, i-amyl alcohol, 2-phenyl ethanol and overall total higher alcohols, total esters and total acids were all found to be significantly higher in the 24ºC fermentation temperature treatment when compared to the 15ºC fermentation treatment. The ratios higher alcohols vs. acids, esters vs. acids, carbonyls vs. esters, carbonyl vs. acids and carbonyls vs. higher alcohols were all found to be significantly higher in the 15ºC fermentation treatment. Therefore by using the 24ºC fermentation temperature treatment, it will result in a base wine with an increased level of higher alcohols and total acids (more specifically acetic acid) as well as a higher ratio of higher alcohols vs. esters. Similarly by using the yeast Alchemy 1 will result in a base wine with elevated concentrations of total esters and more specifically ethyl acetate.

## 5.1 INTRODUCTION

The aim of this study was to investigate the effect of yeast strains and fermentation temperature on the volatile composition of the resultant base wine and therefore

manipulation of these fermentation conditions could produce a base wine with a desired chemical profile and in-turn a brandy with a specific style.

Yeast has been used for the production of alcoholic beverages, including wine since ancient times. The task of choosing a yeast strain is important and significant as they are responsible for the production of volatile compounds and thus the aromatic quality of wine (Patel and Shibamoto, 2003). The use of *S. cerevisiae* yeast strains are most widely used in the wine industry as they allow for rapid and reliable fermentation, reducing the risk of stuck or sluggish fermentation and microbial contamination. *S. cerevisiae* yeast strains are known to produce different volatile profiles which in turn determine the final aroma and flavour of the wine (Lambrechts and Steger, 2000; Beltran *et al.,* 2008). It is important to know the potential differences in volatile biosynthesis between the various different strains of yeast to select the best strain that will produce a good quality wine. The use of selected yeast strains can affect the wine composition and sensory profile and consequently can affect the wine quality (Girard *et al.* 2001).Therefore the yeast strain used during the fermentation process will ultimately influence the quality and style of the wine and spirit product.

Steger and Lambrechts (2000) indicated that the yeast strain 228 is specifically used for the production of base wine for brandy in South Africa and results in a wine with low sulphur and volatile acidity content. Previous findings by Swiegers *et al.,* (2009) and Molina *et al.,* (2009) found that the yeast strain VIN13 produced a higher concentration of the short chain ethyl esters and displayed banana, fruity and yeasty attributes. The yeast alchemy 1  was formulated to increase the floral fruity characteristics of a wine by increasing the ester concentration but however limiting the level of volatile thiol production. Wines made by D254B are said to have sensory descriptors pertaining to that of butterscotch, hazelnut and almond aromas (Swiegers *et al.,* 2009).

Temperature during alcoholic fermentation is one of the most important factors as it affects the rate of fermentation as well the production of volatile compounds (Fleet and Heard, 1993). Temperature influences the fermentation process by either speeding up or decreasing the yeast growth and enzyme action (doubling approximately with every 10ºC increase) (Torija *et al.,* 2002). Studies by Beltran *et al.,* (2008) found that the lower the temperature, the higher the production on unsaturated fatty acids.

Winemakers are favouring alcoholic fermentation at lower temperatures (10-15ºC) as it leads to an increase in ethyl and acetate esters, medium-chain fatty acids and a decrease in higher alcohol production and produces overall a wine with a more pronounced aromatic profile (Feuillat *et al.*, 1997). It has been well documented that an increase in ester concentration and a decrease in higher alcohol levels are found to be beneficial towards the aromatic quality and profile in table wines. However, these low temperatures can cause sluggish or stuck fermentation (Feuillat *et al.*, 1997).

Many studies have been conducted on the effect of low fermentation temperature on the volatile composition of table wine; however there is limited research as what constitutes a desirable aroma profile for base wines. The decision as what style and quality of base wine is used for the production of brandy is determined by each individual company and their desire as to what comprises a good base wine and therefore ultimately a good brandy (Guymon, 1969). Base wine differs from table wine as it contains little to no sulphur (≤ 20 mg/L total

sulphur content), has high acidity and a low alcohol concentration (9 to 11% a/v) (Léaute, 1990).

Volatile compounds play an important role in wine as they are largely responsible for quality of the final product. Previous studies thought that the aroma of wine was determined only by a small amount of compounds, but research shows that there are over 1300 volatile compounds present (Nykänen, 1986; Dieguez *et al.,* 2005). Of these volatile compounds the most important groups are higher alcohols, esters, fatty acids and carbonyls and they are responsible for the overall aroma profile of the final product. These compounds are produced during the fermentation process and are influenced by variables such as fermentation temperature, yeast strain, nitrogen source and soluble solids (Jounela-Eriksson, 1981). Other groups of compounds namely methoxypyrazines and thiols are also of great importance and influence the outcome of the final product (Nykänen, 1986).

The composition and concentration of these compounds can vary from a few ng/L to hundreds of mg/L and research shows that it is not the qualitative, but rather the quantitative composition of flavour and aroma that influences the outcome of the product (Plutowska and Wardencki, 2008). Although a number of the volatile compounds are found in the grape must, the majority of the compounds are formed during yeast fermentation (Margalith and Schwartz, 1970; Torija *et al.,* 2003).

Higher alcohols are those alcohols that contain more than two carbon atoms and therefore have a higher molecular weight and boiling point than ethanol (Steger and Lambrechts, 2000) and are found in high concentrations in wine and spirits and thus have an important aromatic effect. The production of higher alcohols is a result of the metabolism of sugars and amino acids by yeast during the fermentation process and is determined by raw material and yeast strain employed (Lurton *et al.,* 1995; Riponi *et al.,* 1996). Of the higher alcohols present in wine, the most important one to consider is i-amyl alcohol as this higher alcohol when found in to high concentration can render the product unpleasant (Boscolo *et al.,* 2000).

Esters are the most abundant volatile constituents found in wine and are formed through the esterification of alcohol and free acids in the fermentation medium and the main source of ester formation is yeast growth (Torija *et al.,* 2003). Their presence strongly influences the bouquet of the wine. The main ester found in wines and spirits is ethyl acetate, and studies by Ribereau-Gayon *et al.,* (2000) reported that the threshold value of ethyl acetate is 160 mg/L in wine. If found in small concentrations it can impart a floral aroma, however levels of 150 to 200 mg/L can be an indicator microbial spoilage (Ferrari *et al.,* 2004). Other than ethyl acetate, the ethyl esters namely ethyl hexanoate, ethyl octanoate and ethyl decanoate as well as i-amyl acetate are the most quantitatively dominant and are generated through fermentation and exhibit characteristic fruity and flowery odour notes (Guymon, 1969).

Fatty acids are generated in the fermentation process as well as due to the thermal degradation and autolysis of yeast cells during the distillation process into the resultant spirit product (Nykänen, 1983). The most abundant volatile acid found in wine is acetic acid and constitutes between 45 and 90% of the total volatile acid concentration. If acetic acid is disregarded then the volatile acids octanoic, decanoic and hexanoic acid are the largest components in descending order (Von Adam *et al.,* 1996).

Aldehyde compounds are the most volatile compounds found in wine and spirit beverages and are formed during the fermentation process. The major carbonyl compound is acetaldehyde and constitutes 90% of total carbonyl content in alcoholic beverages (Dieguez *et al.,* 2005).

## 5.2 MATERIALS AND METHODS

### 5.2.1 Fermentations

Two different types of grape musts from the 2011(A and B) vintage, and two from the 2012 (C and D) vintage were obtained from a commercial winery. All grape musts were analyzed for pH, TA, balling, YAN, malic and tartaric acid (Table 5.1) and consisted of a blend of Chenin blanc and Colombard cultivars. Each juice was decanted into 10 L sterile containers and wine fermentations carried out in triplicates. Two variables were applied to each of the grape musts which included a) the effect of different yeast and b) the effect of different fermentation temperatures on the volatile composition of the base wines. The resultant difference on the volatile composition of base wines was then analysed.

### 5.2.1.1 Yeast  treatments

The effect of different yeasts on the volatile composition of the base wine was investigated. The 10 L containers were inoculated in triplicate for all four grape musts namely A, B, C and D with the *S. cerevisiae* yeasts 228 (control), VIN13, Alchemy 1 (yeasts VIN13 and VIN7) and D254B respectively. Steger and Lambrechts (2000) indicated that the yeast 228 (Anchor yeast) was widely used for brandy in South Africa. Therefore this was the most obvious choice for the control yeast in this experiment. The yeast D254B (Lallemand) was found to produce elevated levels of higher alcohols and similarly the yeasts VIN13 and Alchemy 1 (both Anchor yeasts) were found to be correlated with a higher concentration of total esters as well as acetate esters (Steger and Lambrechts, 2000; King *et al.,* 2008).

The yeast was rehydrated according to packet instructions. DAP (di-ammonium phosphate) was added to the fermentation containers (0.75 g/L) on day two of the fermentation. The containers (in triplicates) were placed in 15ºC fermentation rooms. Each container was measured daily for the temperature within the container and the temperature noted.

### 5.2.1.2 Fermentation temperature treatments

The effects of fermentation temperature on the volatile composition of base wine were investigated. The 10 L (in triplicates) sterile containers for all four grape must namely A, B, C and D were inoculated with the *S. cerevisiae* yeast 228 and the yeast was rehydrated

according to packet instructions. DAP was added to the fermentation containers (0.75 g/L) on day two of the fermentation to ensure there was sufficient nitrogen available to complete fermentation. The yeast 228 was selected as the control yeast, as this is standard yeast used in the industry for the production of brandy base wine (Steger and Lambrechts, 2000).The containers (in triplicates) were placed in 15ºC and at 24ºC fermentation rooms respectively. Each container was measured daily for the temperature within the container and the temperature noted.

### 5.2.2 Chemical analyses

The juice prior to alcoholic fermentation was analysed for pH, TA (titrateable acidity), balling, VA (volatile acidity), YAN (yeast assimilable nitrogen), malic and tartaric acid. All above parameters (besides YAN) were measured using the WineScan™. The YAN was analysed using the method as described in Zoecklein *et al.,* (1997).

After alcoholic fermentation the wine for each container was analysed for pH, TA, RS (residual sugar), free and total sulphur dioxide, alcohol concentration, VA, malic, lactic and tartaric acid. The wine fermentations (in triplicates) were also subjected to GC-FID (as stipulated in section 4.2.2) in order to identify the volatile compounds. The GC-FID analyses were also conducted in triplicates. The standard deviation was found to be ≤ 5%.

Each 10 L container was subjected to PCR (Polymerase chain reaction) whereby the yeast that completed the respective fermentation could be identified (data not shown). The PCR analyses were carried out according to the procedure as stipulated in studies conducted by Lopez *et al.* (2003).

### 5.2.3 Statistical analysis

Statistical analyses were performed using Statistica version 10. A one-way ANOVA (analysis of variance) which tests for interactions between one categorical independent variable (e.g. yeast strain and wine) and a PCA (principal component analysis) were carried out.

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Chemical analyses of the juice prior to alcoholic fermentation

The results of table 5.1 show the routine chemical analyses carried out on each juice prior to alcoholic fermentation.

**TABLE 5.1 Chemical analyses of each juice prior to alcoholic fermentation.**

| Juice | pH | TA | Balling (ºB) | YAN[a] | Malic acid (g/L) | Tartaric acid (g/L) |
|-------|------|------|------|------|------|------|
| A (2011) | 3.55 | 6.68 | 19.6 | 390 | 3.93 | 5.0 |
| B (2011) | 3.42 | 8.54 | 20.1 | 321 | 3.43 | 6.5 |
| C (2012) | 3.21 | 7.74 | 17.9 | 127 | 3.70 | 6.2 |
| D (2012) | 3.62 | 6.17 | 19.5 | 390 | 4.71 | 6.4 |

**YAN[a] (yeast assimilable nitrogen).**

From table 5.1 it is clear that the juices prior to alcoholic fermentation differ from each other with regards to their chemical composition. According to literature the amount of YAN needed to ensure a complete fermentation is dependent on the balling of the juice. The minimum recommended amount of YAN needed is 140 mg/L (Bell and Henschke, 2005). The results of table 5.1 indicate that there is indeed sufficient YAN available in order to complete alcoholic fermentation for those grape musts of A, B and D, however grape must C is below the recommended amount and could struggle to complete fermentation. The level of TA was found to be highest in that of juice B and lowest in juice D.

**5.3.2 Chemical analyses of the mean values of the wines (A, B, C and D) produced from the individual yeasts (228, VIN13, alchemy 1 and D254B) and the different fermentation temperatures (15ºC and 24ºC)**

**5.3.2.1 The effect of the different yeasts on the concentration of esters, higher alcohols and acids as well as the ratios of the mean values for the wines (A, B, C and D)**

The table below (5.2) shows the ANOVA averages for the chemical analyses of combined wines produced from the different yeasts namely 228 (control), VIN13, alchemy 1 and D254B.

**TABLE 5.2 ANOVA for the chemical compounds found in the individual yeasts produced from the combined wines (A, B, C and D).**

| Compound/groups of compounds (mg/L) | p-value (≤0.05) | 228 (Control) (15ºC) | VIN13 (15ºC) | Alchemy 1 (15ºC) | D254B (15ºC) |
|---|---|---|---|---|---|
| Ethyl acetate | 0.004 | 76.132 bc | 86.256 b | 103.770 a | 68.245 c |
| Ethyl butyrate | 0.000 | 0.409 b | 0.400 b | 0.355 b | 0.597 a |
| i-Amyl acetate | 0.334 | 6.095 a | 7.676 a | 9.463 a | 8.329 a |
| Ethyl hexanoate | 0.707 | 1.424 a | 1.167 a | 1.282 a | 1.289 a |
| Hexyl acetate | 0.647 | 0.770 a | 0.946 a | 1.202 a | 1.056 a |
| Ethyl lactate | 0.757 | 0.770 a | 0.908 a | 0.720 a | 1.350 a |
| Ethyl octanoate | 0.778 | 1.536 a | 1.421 a | 1.279 a | 1.665 a |
| Ethyl decanoate | 0.443 | 1.553 a | 1.442 a | 1.082 a | 1.743 a |
| Di-ethyl succinate | 0.003 | 0.591 b | 0.629 b | 0.511 b | 0.871 a |
| 2-Phenyl ethyl acetate | 0.233 | 0.331 a | 0.274 a | 0.485 a | 0.336 a |
| **Total esters** | **0.000** | **89.592 bc** | **101.119 b** | **120.150 a** | **85.482 c** |
| **Total esters (-Ethyl Acetate)** | **0.625** | **13.459 a** | **14.863 a** | **16.380 a** | **17.237 a** |
| n-Propanol | <0.0001 | 40.116 c | 73.044 b | 83.406 a | 37.231 c |
| n-Butanol | 0.001 | 1.001 ab | 0.856 bc | 1.164 a | 0.697 c |
| i-Amyl alcohol | 0.001 | 139.374 a | 123.461 b | 126.208 b | 145.888 a |
| i-Butanol | 0.008 | 25.651 a | 16.037 b | 17.010 b | 26.063 a |
| 2-Phenyl ethanol | 0.745 | 9.629 a | 8.019 a | 8.280 a | 8.560 a |
| Hexanol | 0.292 | 2.251 a | 1.735 a | 1.599 a | 4.356 a |
| **Total higher alcohols** | **0.336** | **218.002 a** | **223.154 a** | **237.666 a** | **222.795 a** |
| Acetoin | 0.641 | 1.037 a | 0.783 a | 0.888 a | 0.569 a |
| Acetaldehyde | 0.561 | 12.533 a | 14.509 a | 10.001 a | 8.363 a |
| **Total aldehydes** | **0.580** | **13.570 a** | **15.292 a** | **10.889 a** | **8.932 a** |

| Compound/groups of compounds (mg/L) | p-value (≤0.05) | 228 (Control) (15ºC) | VIN13 (15ºC) | Alchemy 1 (15ºC) | D254B (15ºC) |
|---|---|---|---|---|---|
| Acetic acid | 0.001 | 84.206 bc | 48.402 c | 184.674 a | 104.167 b |
| Propionic acid | 0.077 | 4.136 a | 5.956 a | 8.864 a | 6.652 a |
| i-Butyric acid | <0.0001 | 1.305 b | 0.927 c | 0.905 c | 2.088 a |
| n-Butyric acid | 0.038 | 1.219 b | 1.193 b | 1.130 b | 1.514 a |
| n-Valeric acid | <0.0001 | 0.635 b | 1.199 a | 1.228 a | 0.608 b |
| Hexanoic acid | 0.238 | 4.295 a | 4.729 a | 4.099 a | 5.466 a |
| Octanoic acid | 0.529 | 7.527 a | 7.804 a | 6.507 a | 8.936 a |
| Decanoic acid | 0.590 | 4.924 a | 4.849 a | 3.783 a | 4.904 a |
| **Total acids** | **<0.0001** | **104.272 bc** | **69.127 c** | **209.451 a** | **127.948 b** |
| HA[a] vs. esters | <0.0001 | 2.434 b | 2.220 c | 1.983 d | 2.616 a |
| HA vs. acids | 0.000 | 2.265 b | 3.330 a | 1.149 c | 1.909 bc |
| Esters vs. acids | 0.000 | 0.935 b | 1.513 a | 0.581 c | 0.724 bc |
| Aldehydes vs. esters | 0.440 | 0.150 a | 0.148 a | 0.091 a | 0.104 a |
| Aldehydes vs. acids | 0.043 | 0.133 ab | 0.213 a | 0.052 b | 0.075 b |
| Aldehydes vs. HA | 0.516 | 0.062 a | 0.068 a | 0.046 a | 0.040 a |
| Methanol | 0.965 | 38.361 a | 40.271 a | 40.262 a | 40.341 a |

**5.3.2.1.1 Esters**

The level of ethyl acetate and overall total ester concentration was found to be significantly higher (≤0.05) in the yeast alchemy 1. Ethyl butyrate was found to be highest in concentration in the D254B yeast. The level of total esters when compared to the control yeast 228 was found to increase when the yeast alchemy 1 was used. Figure 5.5 shows the increase or decrease of ethyl acetate and total esters in the other yeasts when compared to the control yeast 228.
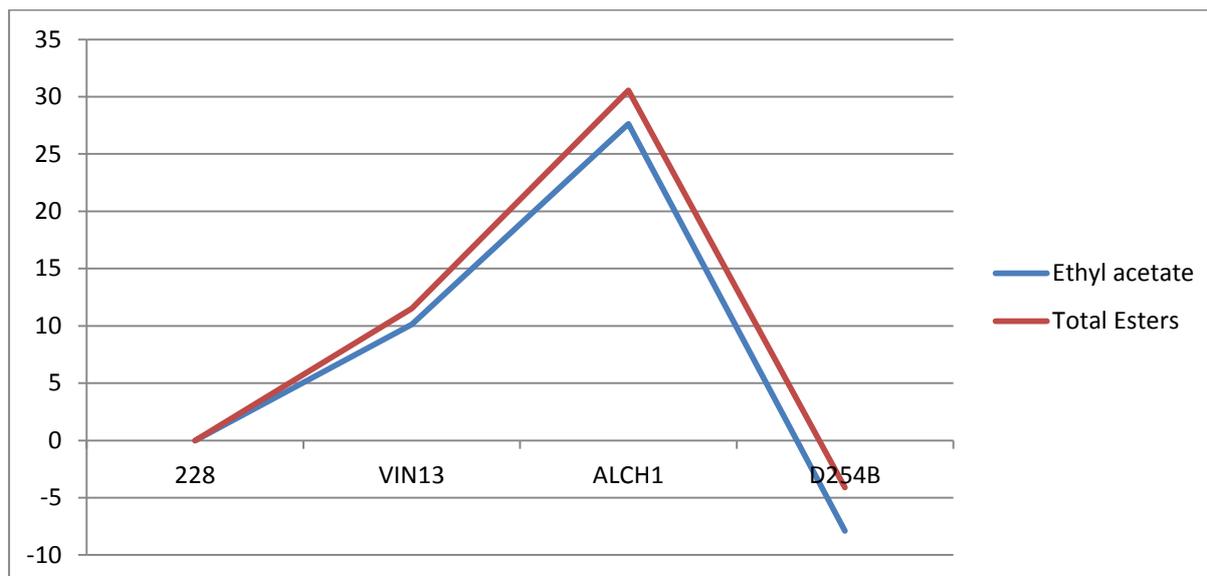
**FIGURE 5.1 Increase/decrease of ethyl acetate and total esters in the other yeasts when compared to the control yeast 228 (mg/L).**

## 5.3.2.1.2 Higher alcohols

Of the higher alcohols quantified, only n-propanol, n-butanol and i-amyl alcohol were found to be significantly different (≤0.05) between the different yeast. The level of n-propanol was found to be highest in the yeast alchemy 1. However n-butanol was found to be highest in concentration when compared to the yeasts VIN13 and D254B. i-Amyl alcohol was found to be highest in concentration in the yeast D254B when compared to the yeasts VIN13 and alchemy 1, but however not compared to the control yeast 228. When the yeast alchemy 1 was used (compared to the control yeast 228) levels of n-propanol where found to be higher in concentration. The higher alcohol i-amyl alcohol was found to decrease when the yeasts VIN13 and alchemy 1 were used when compared to the control yeast 228. Figure 5.2 shows the increase or decrease of n-propanol and i-amyl alcohol in the other yeasts when compared to the control yeast 228.
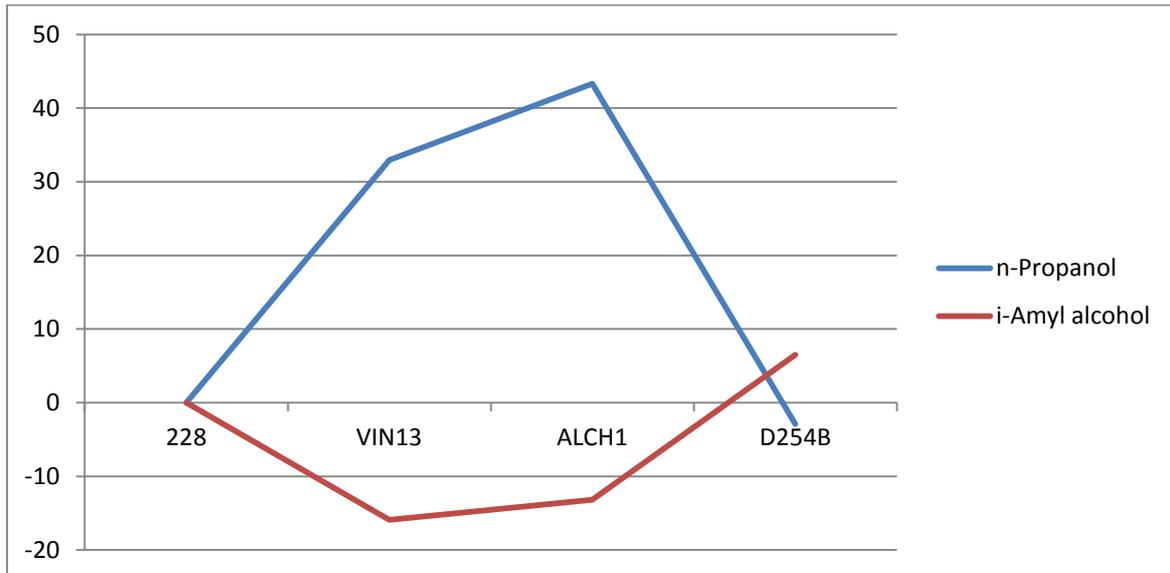
**FIGURE 5.2 Increase/decrease of n-propanol and i-amyl alcohol in the other yeasts when compared to the control yeast 228 (mg/L).**

**5.3.2.1.3 Acids**

The level of acetic acid was found to be highest ($\leq0.05$) in the yeast alchemy 1 when compared to all the other yeast and more specifically the control yeast 228. N-Butyric and i-butyric acid concentrations were also found to increase ($\leq0.05$) when the yeast D254B was used in comparison to the other yeast and again more specifically the control yeast 228. When the yeasts VIN13 and alchemy 1 were used for the alcoholic fermentation, levels of i-butyric acid were found to decrease ($\leq0.05$) when compared to the control yeast 228. The overall total acid concentration was found to be highest ($\leq0.05$) in concentration when the yeast alchemy 1 was employed in comparison to all the other yeast and more specifically the control yeast 228. Figure 5.3 shows the increase or decrease of acetic acid and total acids in the other yeasts when compared to the control yeast 228.
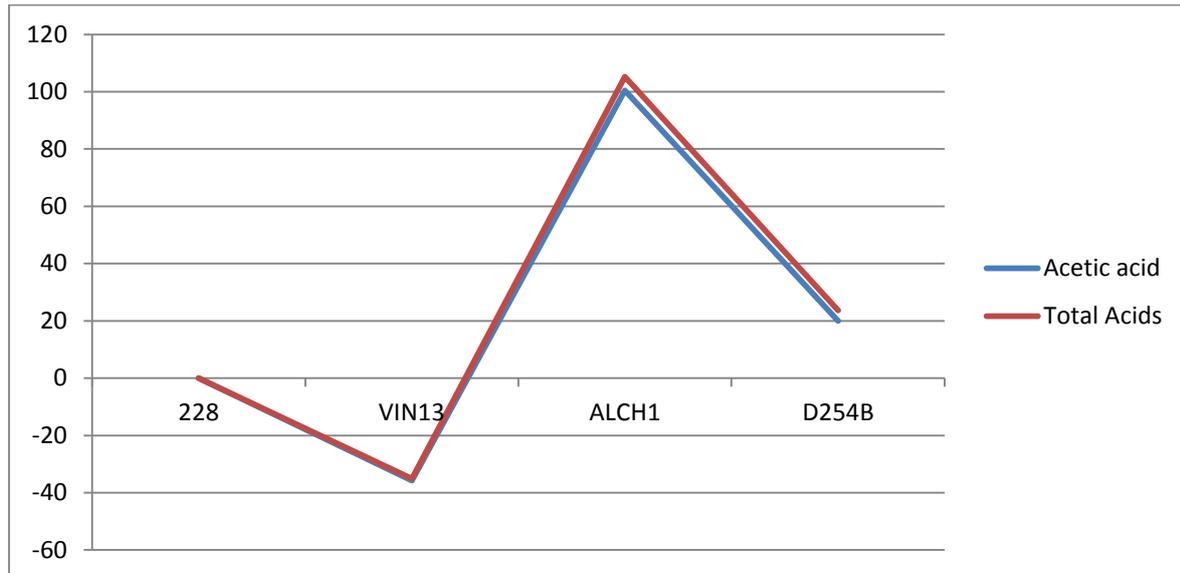
**FIGURE 5.3 Increase/decrease of acetic acid and total acids in the other yeasts when compared to the control yeast 228 (mg/L).**

## 5.3.2.1.4 Ratios

The ratios of higher alcohols vs. acids and esters vs. acids were found to be highest when the yeast VIN13 was used when compared to all the other yeast employed and more specifically in comparison to the control yeast 228. However for the ratio of higher alcohols vs. esters, the ratio was found to be increase when the yeast D254B was employed and decreased when the yeast alchemy 1 was used in comparison to the control yeast 228. Figure 5.4 shows the increase or decrease of the ratios higher alcohols vs. esters, higher alcohols vs. acids and esters vs. acids in the other yeasts when compared to the control yeast 228.
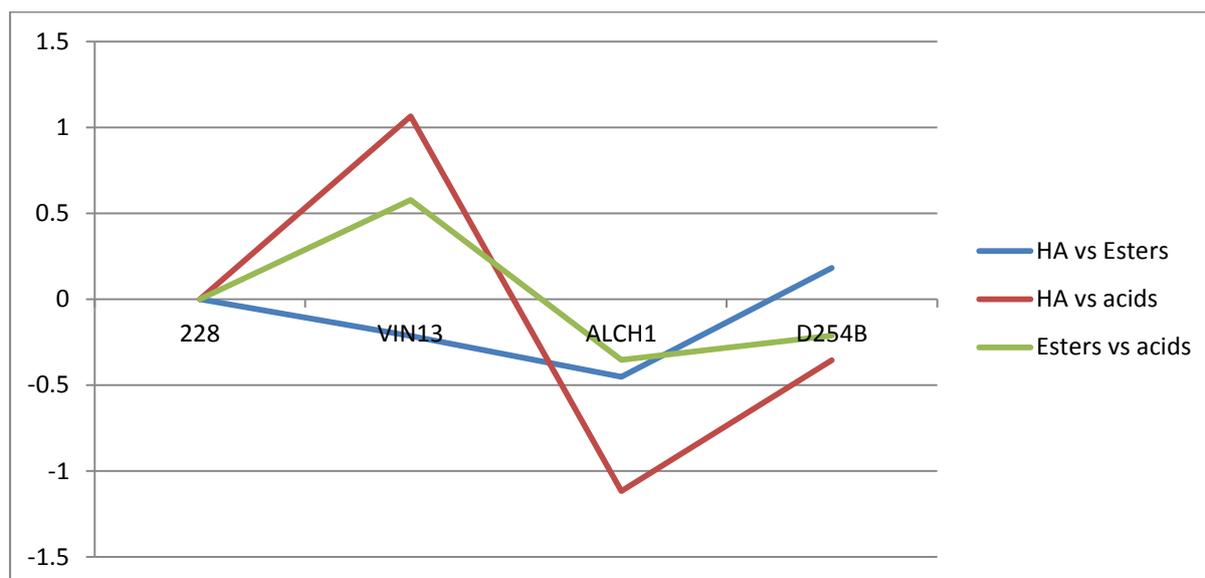
**Figure 5.4 Increase/decrease of the ratios higher alcohols vs. esters, higher alcohols vs. acids and esters vs. acids in the other yeasts when compared to the control yeast 228.**

**5.3.2.2 The effect of the different fermentation temperatures on the concentration of esters, higher alcohols, acids and aldehydes as well as the ratios of the mean values for the wines (A, B, C and D)**

The mean values for the chemical compounds found in all four wines (A, B, C and D) for the 15°C and 24°C fermentation temperatures were investigated. The table below (5.3) shows the ANOVA for the chemical analysis of combined wines produced from the different fermentation temperatures.

**TABLE 5.3 ANOVA for the chemical compounds found in the individual fermentation temperatures produced from the combined wines (A, B, C and D).**

| Compound/groups of compounds (mg/L) | p-value (≤0.05) | 24°C | 15°C |
|---|---|---|---|
| Ethyl acetate | 0.000 | 85.710 a | 76.681 b |
| Ethyl butyrate | 0.000 | 0.273 b | 0.385 a |
| i-Amyl acetate | 0.229 | 7.714 a | 6.323 a |
| Ethyl hexanoate | <0.0001 | 0.638 b | 1.244 a |
| Hexyl acetate | 0.520 | 0.640 a | 0.727 a |
| Ethyl lactate | <0.0001 | 1.770 a | 0.824  b |
| Ethyl octanoate | 0.075 | 0.992 a | 1.406 a |

| Compound/groups of compounds (mg/L) | p-value (≤0.05) | 24ºC | 15ºC |
|---|---|---|---|
| Ethyl decanoate | 0.306 | 1.034 a | 1.247 a |
| Di-ethyl succinate | 0.007 | 0.852 a | 0.656 b |
| 2-Phenyl ethyl acetate | 0.030 | 0.536 a | 0.334 b |
| **Total esters** | **<0.0001** | **100.159 a** | **89.855 b** |
| **Total esters (-Ethyl Acetate)** | **0.454** | **14.449 a** | **13.146 a** |
| n-Propanol | 0.559 | 41.534 a | 42.875 a |
| n-Butanol | 0.194 | 0.985 a | 1.063 a |
| i-Amyl alcohol | 0.004 | 158.690 a | 144.163 b |
| i-Butanol | <0.0001 | 46.521 a | 26.648 b |
| 2-Phenyl ethanol | 0.004 | 12.868 a | 10.191 b |
| Hexanol | 0.308 | 1.970 a | 2.203 a |
| **Total higher alcohols** | **0.000** | **262.567 a** | **227.113 b** |
| Acetoin | 0.257 | 0.691 a | 0.918 a |
| Acetaldehyde | 0.034 | 9.483 b | 14.809 a |
| **Total aldehydes** | **0.037** | **10.147 b** | **15.760 a** |
| Acetic acid | <0.0001 | 178.389 a | 77.650 b |
| Propionic acid | 0.015 | 12.950 a | 2.946 b |
| i-Butyric acid | <0.0001 | 2.645 a | 1.385 b |
| n-Butyric acid | 0.004 | 1.006 b | 1.328 a |
| i-valeric acid | <0.0001 | 0.101 a | 0.004 b |
| n-Valeric acid | 0.132 | 0.632 a | 0.685 a |
| Hexanoic acid | 0.002 | 3.064 b | 4.466 a |
| Octanoic acid | 0.033 | 5.302 b | 7.678 a |
| Decanoic acid | 0.074 | 3.586 a | 4.617 a |

| Compound/groups of compounds (mg/L) | p-value (≤0.05) | 24ºC | 15ºC |
|---|---|---|---|
| **Total acids** | **<0.0001** | **199.845 a** | **97.940 b** |
| HA[a] vs. esters | 0.358 | 2.625 a | 2.537 a |
| HA vs. acids | <0.0001 | 1.321 b | 2.413 a |
| Esters vs. acids | <0.0001 | 0.502 b | 0.965 a |
| Aldehydes vs. esters | 0.013 | 0.103 b | 0.175 a |
| Aldehydes vs. acids | <0.0001 | 0.051 b | 0.157 a |
| Aldehydes vs. HA | 0.006 | 0.039 b | 0.068 a |
| Methanol | 0.787 | 39.595 a | 40.330 a |

**Compounds/groups of compounds with different letters in the same row are found to be significantly different (p≤0.05). HA[a] (Higher alcohols)**

### 5.3.2.2.1 Esters

Of the esters quantified the concentrations of ethyl acetate, ethyl lactate, di-ethyl succinate, 2-phenyl ethyl acetate and overall total esters were all found to be significantly higher (≤0.05) in the 24ºC fermentation temperature when compared to the 15ºC fermentation temperature. However the inverse was found to be true for the levels of ethyl butyrate and ethyl hexanoate. Figure 5.5 shows the increase or decrease of ethyl acetate and total esters in the different fermentation temperatures when compared to the control yeast 228.
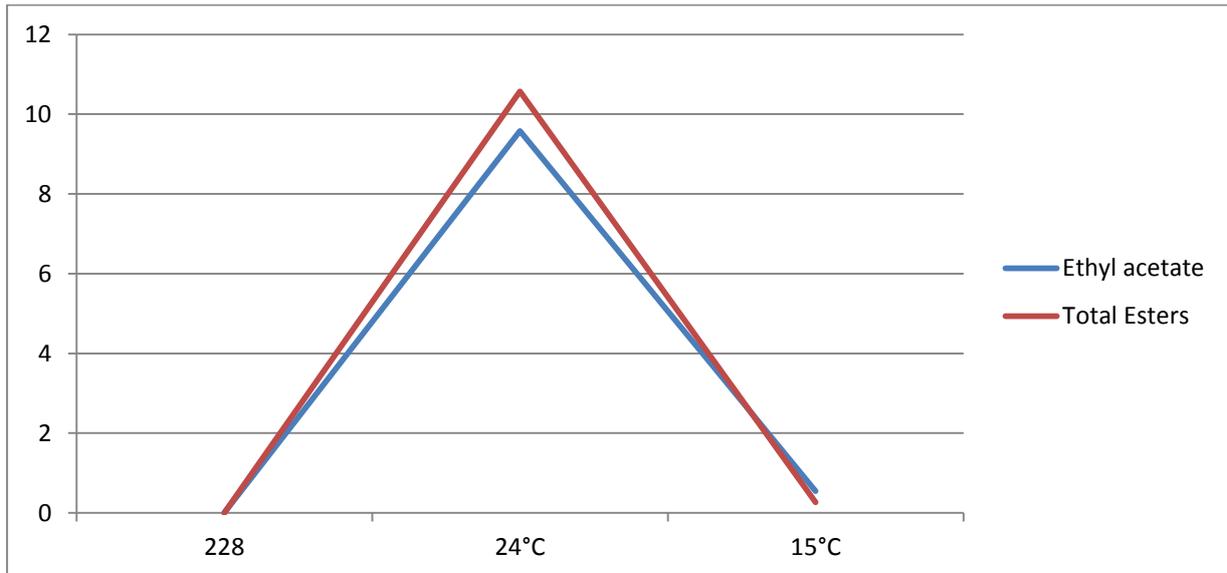
**Figure 5.5 Increase/decrease of ethyl acetate and total esters in the different fermentation temperatures (24°C and 15°C) when compared to the control yeast 228 (mg/L).**

## 5.3.2.2.2 Higher alcohols

The higher alcohols namely i-butanol, i-amyl alcohol, 2-phenyl ethanol and overall total higher alcohols were all found to be significantly higher (≤0.05) in the 24ºC fermentation temperature treatment when compared to the 15ºC fermentation treatment. Figure 5.6 shows the increase or decrease of i-amyl alcohol and total higher alcohols in the different fermentation temperatures when compared to the control yeast 228.
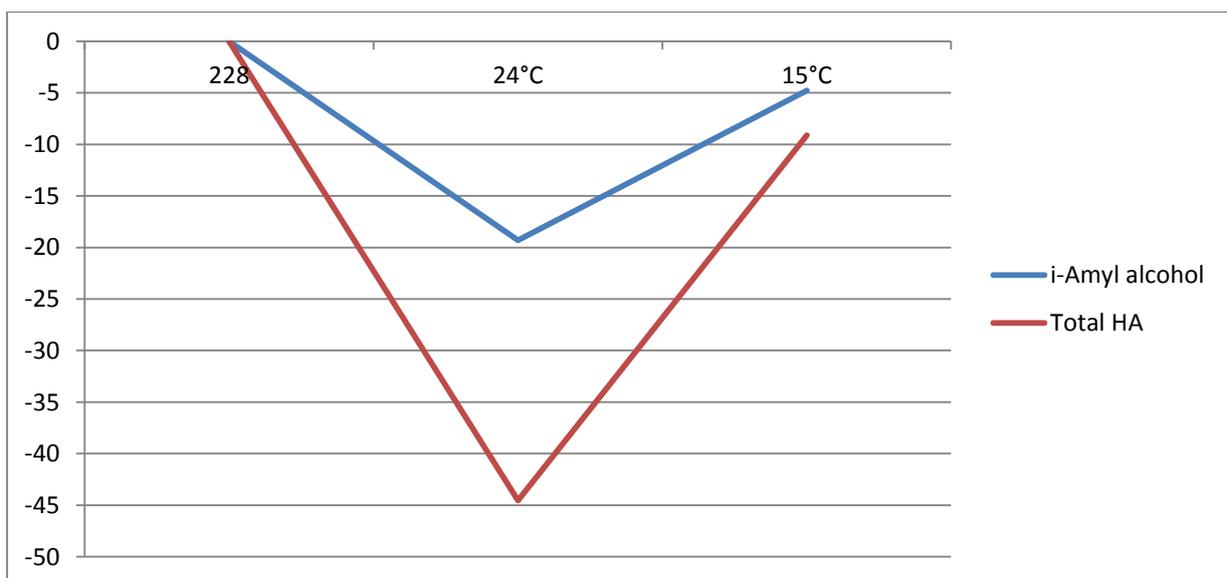


**Figure 5.6 Increase/decrease of i-amyl alcohol and total higher alcohols in the different fermentation temperatures (24°C and 15°C) when compared to the control yeast 228 (mg/L).**

### 5.3.2.2.3 Acids

The concentration of acetic acid, i-butyric acid, i-valeric acid and overall total acids were found to be significantly higher (≤0.05) in the 24ºC fermentation treatment when compared to that of the 15ºC fermentation treatment. The inverse was found for the compounds n-butyric acid, hexanoic acid and octanoic acid. Figure 5.7 shows the increase or decrease of acetic acid and total acids in the different fermentation temperatures when compared to the control yeast 228.
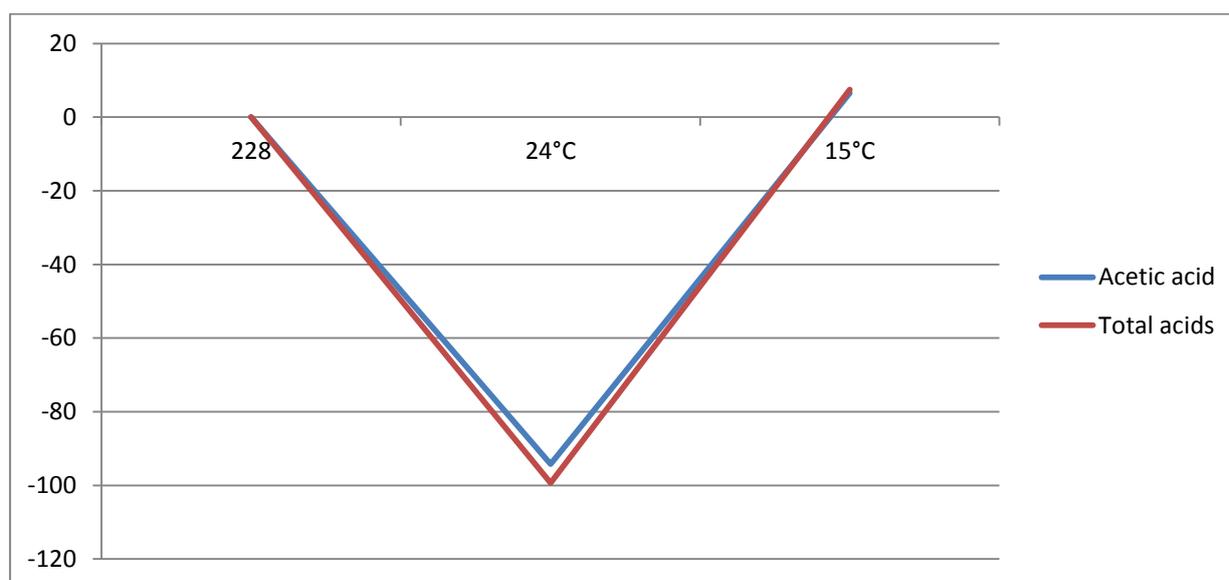


**Figure 5.7 Increase/decrease of acetic acid and total acids in the different fermentation temperatures (24°C and 15°C) when compared to the control yeast 228 (mg/L).**

### 5.3.2.2.4 Aldehydes and ratios

The only aldehyde compound found to differ between the respective fermentation temperature treatments was acetaldehyde, with levels significantly higher (≤0.05) in the 15ºC fermentation treatment. The ratios higher alcohols vs. acids, esters vs. acids, carbonyls vs. esters, carbonyl vs. acids and carbonyls vs. higher alcohols were all found to be significantly higher (≤0.05) in the 15ºC fermentation treatment when compared to the 24ºC fermentation treatment.

## 5.4 CONCLUSIONS

If one looks at the results of each wine individually there are general trends observed that are found to be consistent throughout all four wines. Ethyl acetate, i-amyl acetate and total esters were found to be highest in all of the wines (A, B, C and D) fermented with the yeast

Alchemy 1. The compound ethyl butyrate was found to be highest in concentration in the yeast D254B in all of the wines; perhaps indicating that irrespective of the original juice used prior to fermentation, the use of this yeast will produce a wine with elevated levels of this compound.

The concentration of n-propanol is higher in the yeast Alchemy 1 in all of the wines A, B, C and D. The compounds i-butanol, 2-phenyl ethanol and total higher alcohols are found in higher concentrations in all of the wines (A, B, C and D) fermented with the 24ºC fermentation temperature treatment.

The total acid concentration in general was found to be highest in the 24ºC fermentation temperature treatment as well as the yeast alchemy 1. Levels of acetic acid were observed to be highest in all of the wines (A, B, C and D) fermented with the 24ºC fermentation temperature as well as the yeast Alchemy 1. Reinforcing the theory that fermenting at a higher temperature leads to an increase acetic acid levels.

The medium-chain fatty acids namely hexanoic, octanoic and decanoic acid do not seem to follow a general trend specific to a treatment and it would seem that overall the production of medium-chain fatty acids is determined by both the yeast used as well as the original juice composition prior to fermentation. The concentration of total aldehydes also did not seem to follow similar trends throughout the wines. The ratio of higher alcohols vs. acids and esters vs. acids was highest in that of VIN13 these results were found to be true for all four wines individually.

The ratio of higher alcohols vs. esters is highest in all of the wines (A, B, C and D) fermented with the 24°C fermentation temperature and the yeast D254B. The lowest of this ratio was found to be present in the yeast Alchemy 1. The ratio of higher alcohols vs. acids was found to be lowest in the yeast Alchemy 1, and the highest in the VIN13. For the ratio of esters vs. acids, overall the highest level of this ratio was found in the yeast VIN13 and the lowest in the 24ºC fermentation temperature fermentation treatment.

The results obtained indicate that if one was to use the yeast Alchemy 1 it would produce a wine with an elevated total ester concentration and more specifically ethyl acetate. Similarly if the wine was fermented at 24ºC it would result in the increased production of higher alcohols, total acids (more specifically acetic acid) and a higher ratio of higher alcohols vs. esters.

## 5.5 LITERATURE CITED

Bell, S-J and Henschke, P.A. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Aus. J. Grape. Wine. Res. 11, 3: 242-295.

Beltran, G., Novo, M., Guillamón.,J. M., Mas, A., Rozés. 2008. Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. Int. J. Food. Micro. 121: 169-177.

Boscolo, M., C.W.B. Bezerra., D.R. Cardoso., B.S. Limo Neto., D.W. Franco. 2000. Identification and dosage by HRCG of minor alcohols and esters in Brazilian sugar-cane spirit. Braz. J. Chem. Soc. 11, 1:86-90.

Diequez, S.C., M. Luisa., L.G. de la Pena., E.F.Gomez. 2005. Volatile composition and sensory characters of commercial Galician orujo spirits. J. Agric. Food. Chem. 53: 6759-6765.

Drysdale, G.S and Fleet, G.H. 1989. The growth and survival of acetic acid bacteria in wines at different concentrations of oxygen. Am. J. Enol. Vitic. 40, 2: 99-105.

Du Toit, M., Engelbrecht, L., Lerm, E., Krieger-Weber, S. 2011. Lactocbacillus: The next generation of malolactic fermentation starter cultures- A review. Food. Bio. Tech. 4, 6: 876-906.

Du Toit, M and Lambrechts, M. 2002. The enumeration and identification of acetic acid bacteria from South African red wine fermentations. Int. J. Food. Micro. 74: 57-64.

Fleet, G. H., and Heard, G.M. 1993. Yeasts: growth during fermentation. Wine. Micro. Biotech. 27-54.

Ferrari, G., O. Lablanquie., R. Cantagrel., T. Payot., N. Fournier., E. Guichard. 2004. Determination of key odourant compounds in freshly distilled Cognac using GC-O, GC-MS and sensory evaluation. J. Agric. Food Chem. 52: 5670-5676.

Feuillat, M., Charpentier, C., Massoutier, C., 1997. Intérêt oenologique des souches de levures Saccharomyces cryotolérantes. Revue des Oenologues 85: 18–21.

Girard, B; Yuksel, D; Cliff, M.A; Delaquis, P; Reynolds, A.G. 2001. Vinification effects on the sensory, colour and GC profiles of Pinot noir wines from British Columbia. Food. Res. Int. 34, 6: 483-499.

Guymon, J.F. 1969. GC determination of ethyl esters in brandy or wine distillates. Am. J. Enol. Vitic. 20, 2: 76-85.

Jounela-Eriksson, P. 1981. Predictive value of sensory and analytical data for distilled beverages. In Flavour'81. Schreier, P. (1st Ed). Berlin.

Karagiannis, S and Lanaridis, P. 2006. Insoluble grape material present in must affects the overall all fermentation aroma dry white wines made from three grape cultivars cultivated in Greece. J. F. Sci. 67, 1: 369-374.

King, E.S., Swiegers, J.H., Travis, B., Francis, I.L., Bastian, S.E.P., Pretorius, I.S. 2008. Coinoculated fermentations using *Saccharomyces* yeasts affect the volatile composition and sensory properties of *Vitis vinifera* L. cv. Sauvignon blanc wines. J. Agric. Food. Chem. 56, 22: 10829-10837.

Léaute, R. 1990. Distillation in Alambic. Am. J. Enol. Vitic. 41, 1: 90-103.

Lopez, V., Fernandez-Espinar, M.T., Barrio, E.,  Ramon, D., Querol, A. 2003. A new PCR-based method for monitoring inoculated wine fermentations. Int. J. Food. Micro. 81, 1: 63-71.

Lurton, L; Snakkers, G; Roulland, C; Galy, B; Versavaud, A. 1995. Influence of the fermentation yeast strain on the composition of wine spirits. J. Sci. Food. Agric.67:4. pp 485-491.

Margalith, P and Schwartz, Y. 1970. Flavor and microorganisms. Ad. Appl. Micro. 12: 35-88.

Molina, A.M., Guadalupe, V., Varela, C., Swiegers, J.H., Pretorius, I.S., Agosin, E. 2009. Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. Food. Chem. 117: 189-195.

Nykänen, L. 1983. Aroma of beer, wine, and distilled alcoholic beverages. (1st Ed). D. Riedel Publishing Company. Dordrecht, Holland.

Nykänen, L.H. 1986. Formation and occurrence of flavour compounds in wine and distilled beverages. Am. J. Enol. Vitic. 28, 3: 152-158.

Patel, S and Shibamoto, T. 2003. Effect of 20 different yeast strains on the production of volatile components in Symphony wine. J. Food. Comp. Anal. 16, 4: 469-476.

Plutowska, B and Wardencki, W. 2008. Application of gas chromatography-olafactory (GC-O) in analysis and quality assessement of alcoholic beverages- A review. Food.Chem. 107, 1: 449-463.

Ribereau-Gaymon, P., Y. Glories., A. Maujean., D. Dubordieu. 2000. The chemistry of wine, stabilization and treatments. Vol 2. Handbook of Enology. Wiley. New York.

Riponi, C; Carnacini, A; Castellari, L; Zambonelli, C. 1996. Influence of yeast strain on the composition of wines for the production of brandy. J. Wine. Res. 8: 41-50.

Steger, C.L.C and M. Lambrechts. 2000. The selection of yeast strains for the production of premium quality South African brandy base products. J. Ind. Micro. Biotech. 24, 6: 431-440.

Swiegers, J.H; Kievit, J.L; Siebert, T; Lattey, K.A; Bramley, B.R; Leigh Francis, I; King, E.S; Pretorius, I.S. 2009. The influence of yeast on the aroma of Sauvignon Blanc wine. Food. Micro. 26, 2: 204-211.

Torija, M.J., Roes, N., Pblet, M., Guillamon, M.J., Mas, A. 2002. Effects of fermentation temperature on the strain population of *Saccharomyces cerevisiae*. Int. J. Food Microbiol. 80: 47–53.

Torija, M.J., Beltran, G., Novo, M., Poblet, M., Guillamon, J.M., Mas, A., Rozes, N., 2003. Effects of fermentation temperature and Saccharomyces species on the cell fatty acid composition and presence of volatile compounds in wine. Int. J. Food. Micro. 85: 127–136.

Von Adam, L., M. Haug., E. Kolb., C.H. Schwartz. 1996. Contribution of the knowledge of volatile compounds present in wine distillates from the years 1990-1993. Branntweinwirtschaft. 6: 82-90.

Zoecklein BW*,* Marcy JE*,* Williams JM & Jasinski Y .1997*.* Effect of native yeasts and selected strains of *Saccharomyces cerevisiae* on glycosyl glucose, potential volatile terpenes, and selected aglycones of white Riesling (*Vitis vinifera* L.) wines*.* J Food Comp Anal 10*:* 55–65*.*

# Chapter 6

# Research results

# THE EFFECT OF CONDENSER WATER TEMPERATURE ON THE VOLATILE COMPOSITION OF THE RESULTANT DISTILLATE

This manuscript is prepared according to the style of the **South African Journal of Enology and Viticulture**

# 6. The effect of condenser water temperature on the volatile composition of the resultant distillate

## ABSTRACT

The effect of different condenser water temperatures during the distillation process on the volatile composition of the resultant distillate was investigated. Three different low wines (LW1, LW2 and LW3) were used and three different condenser water temperatures were applied namely 8ºC, 12ºC and 20ºC respectively. The low wines prior to distillation and the resultant distillates were chemically analyzed using GC-FID to identify the volatile compounds present. It was found that the different condenser water temperatures did not have an effect on the total ester and total higher alcohol concentration however, the total acid concentration was found to differ between the distillates produced by low wine one (LW1) and low wine three (LW3). The distillates produced by low wine one (LW1) were found to contain a higher concentration of total acids in the 8ºC treatment; however the distillates produced by low wine three (LW3) were found to contain a higher level of total acids in the 20ºC treatment. It was also found that throughout all of the distillates produced by low wine one (LW1), two (LW2) and three (LW3), the pH value was found to be highest in the 8ºC treatment and lowest in the 20ºC treatment. The TA value was also found to differ between the different condenser water temperatures, whereby the 8ºC treatment contains a higher TA value and the 20ºC treatment a lower TA value. However, further investigation into the evolution of total acids in the resultant distillate is needed in order to conclusively confirm the effect of the different condenser water temperatures.

## 6.1 INTRODUCTION

Distillation is one of the most important separation methods in the chemical industry (Leaute, 1990). It entails heating of a solution, condensing and collecting the fractions in separate containers (Saco, 2006). For the production of brandy, the choice of the preferred distillation system that is more commonly used is known as the pot still.

Pot stills are comprised mainly of copper and the distillation process is performed in two different stages (Carnacini, 1989). The first stage entails distilling wine until the alcohol concentration is approximately 29-30% abv and is known as low wine. The second stage involves heating the low wine and collecting it in three separate fractions, namely the heads, heart and tails. The head fraction consists mainly of acetaldehyde, ethyl acetate, acetic acid and methanol. The heart contains the majority of the higher alcohols, whereby the tail fraction constitutes mainly the medium chain fatty acids and those compounds with a high boiling point and low volatility (Léaute, 1990).

Each of these fractions contain different types and amounts of chemical compounds, however it is only the heart fraction that is of importance as this is the fraction that is matured. The heart fraction has an alcohol concentration of between 65 and 70% abv (Léaute, 1990).

In alcoholic distillation the mixture consists mainly of ethanol and water, but also includes small amounts of volatile and non-volatile compounds known as congeners. These congener compounds are an essential part of the aroma of distilled products and therefore their concentrations are important enological parameters (Faúndez and Valderrama, 2004).

The way in which these congener compounds distill over is dependent on their volatility characteristics together with the distillation method employed. There are many parts of the distillation system that influence the outcome of the chemical composition of the resultant distillate (Nykänen, 1986).

Studies by Léaute (1990) mention that the shape of the pot still has a profound effect on the way in which the compounds distill over. It was found that pot stills that had a longer still head produced a distillate that contained less long chain fatty acids and was considered to be overall less flavoursome (Bougas, 2009). The length of the swan's neck and the larger the still head in relation to the boiler will lead to an increase in rectification and ultimately lead to a smoother brandy with less character. The heating time throughout the distillation also influences the concentration of the congener compounds in the resultant distillate as there are many reactions that take place within the pot still. These reactions include among many, esterification, acetal formation, malliard reaction as well as the Strecker degradation. Changes in the distillation system greatly alter the chemical compounds found in the distilled beverages however the choice of the distillation technique is dependent on the final category of the product that is desired (Guymon, 1974a).

There is very limited research conducted on the effect of the distillation system and more specifically that of the condenser water temperature during the distillation process on the chemical composition of the resultant distillate. Therefore a need for such a study arose to investigate the effect of condenser water temperature on the chemical composition of the resultant distillate.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Distillations

One low wine from the 2011 (LW1) vintage and two from the 2012 (LW2 and LW3) vintage with alcohol percentages of approximately 30% v/v were obtained from a commercial distillery for the purpose of this experiment. Three different condenser water temperatures were applied (8ºC, 12ºC and 20ºC respectively) to each low wine to investigate the effect of different cooling temperatures on the volatile composition of the resultant distillate (the control temperature being 20ºC as this is the temperature used by Distell).

Distillations for each of the condenser temperatures were carried out in triplicates using a 140 L Prulho pot still and for each distillation 100 L of low wine was used. LP gas was used

as the heat source and the gas burner was set at constant setting to ensure a consistent heating temperature. Each distillation took 8 hours; boiler and condenser water temperatures were measured throughout each distillation and found to be consistent.   The distillate was collected in three fractions, namely the heads, hearts and tails. The head fraction was collected after the first fifteen minutes have elapsed (as stipulated by the industries guidelines), the heart fraction was collected directly thereafter up until a boiler temperature of 92ºC and the tail fraction was collected from temperatures between 92 and 101ºC. However, only the heart fraction was analysed as this is the fraction that is matured.

### 6.2.2 Chemical analyses

For the low wines prior to distillation, a volume of 10 mL ca. 30% (a/v) was pipetted into a screw capped glass test tube and spiked with 0.4 mL internal standard (4-methyl-2-pentanol at a concentration of 1.2 g/L in absolute ethanol). A volume of 6.5 mL diethyl ether was added to each test tube. The test tubes were capped and placed in a rotary mixer, where after the samples were extracted for 30 minutes at a speed of 60 rpm. The upper diethyl ether layers were transferred into 2 mL crimp-top autosampler vials, the vials were sealed and a volume of 3 µL of each extract was injected into the GC inlet. Each low wine was extracted in triplicates to ensure reproducibility. The low wine samples were analysed in triplicates using GC-FID methods (as stipulated in section 3.2.2).

The pH was determined using an automatic titrator equipped with a combination electrode (Crison, catalogue no. 4473624, LASEC, SA) and a temperature probe. Certified buffers (pH 7.00 and pH 4.00, LASEC, SA) were used to calibrate the electrode. TA was expressed as g/L tartaric acid and measured by potentiometric titration (Crison Compact Titrator D, SN 01714, Spain, software version 5.6) using standardised 0.33 N sodium hydroxide (LASEC, Cape Town, SA) to the end point of pH 7.00, as described by Zoecklein *et al.* (1999).

After the distillation process the heart fraction was also subjected to chemical analyses using GC-FID (as stipulated in section 3.2.2) to identify the chemical composition of the distillates. Again routine pH and TA of the distillates were also carried out. The standard deviation was found to be ≤5% for each of the respective triplicates.

### 6.2.3 Statistical analysis

Statistical analyses were performed using Microsoft XLStat version 2012. A one-way ANOVA (analysis of variance) which tests for interactions between one categorical independent variable and PCA (principal component analysis) were carried out. The average of the triplicates for each condenser water temperature was used and outliers were removed if the standard deviation was found to be ≤5%. Only those correlation coefficients ≤ 0.650 will be reported.

## 6.3 RESULTS AND DISCUSSION

### 6.3.1 Chemical analyses of the low wines prior to distillation

Table 6.1 shows the results of the one-way ANOVA of the chemical composition for low wine one (LW1), low wine two (LW2) and low wine three (LW3) prior to distillation. The compounds acetaldehyde, n-propanol, i-butanol, i-amyl acetate, n-butanol, i-amyl alcohol, ethyl hexanoate, hexyl acetate, hexanol, ethyl octanoate, ethyl decanoate, total esters, total higher alcohols and total aldehydes are found in significantly higher concentrations in LW1 when compared to LW2 and LW3. All three low wines are significantly different ($\leq 0.05$) to each other with regards to the above mentioned compounds, whereby LW1 contains the most and LW3 contains the lowest concentrations.

The level of ethyl acetate, methanol, ethyl butyrate and the ratio of higher alcohols vs. acids were all significantly higher ($\leq 0.05$) in LW1 and LW2 when compared to LW3. LW1 and LW2 in turn were not significantly different ($\leq 0.05$) from each other.

Compounds acetoin, n-butyric acid, 2-phenyl ethanol and decanoic acid were significantly lower ($\leq 0.05$) in concentrations in LW1 and LW2 when compared to LW3, however LW1 and LW2 were not significantly different ($\leq 0.05$) from each other with regards to these compounds.

Ethyl lactate, di-ethyl succinate, hexanoic acid, octanoic acid and the ratio of acids vs. carbonyls concentrations were significantly different ($\leq 0.05$) between all three low wines with LW3 containing the highest levels and LW1 the lowest.

The amount of total acids and the ratio of higher alcohols vs. esters were significantly different ($\leq 0.05$) between the low wines with LW3 containing the highest concentrations and LW2 the lowest. Acetic acid levels were highest in LW1 compared to LW2 and LW3, where the latter two did not differ significantly ($\leq 0.05$) from each other.

The compound 2-phenyl ethyl acetate is significantly different ($\leq 0.05$) between all three low wines, with LW1 containing the highest concentration and LW2 the lowest. The ratio of esters vs. acids is also found to be significantly different ($\leq 0.05$) between the low wines prior to distillation with LW2 containing the highest ratio and LW3 the lowest. When comparing the ratio of aldehydes vs. esters, LW1 and LW3 did not differ significantly ($\leq 0.05$) from each other but both of these low wines were found to differ significantly from LW2 whereby they contained the highest amount of this ratio. The ratio of aldehydes vs. higher alcohols where not significantly different ($\leq 0.05$) between LW2 and LW3 but was found to be significantly different when compared to LW1 where the ratio of aldehydes vs. higher alcohols was lower in LW1. The pH is found to be highest ($\leq 0.05$) in LW3 and lowest in LW2, and the TA value is highest ($\leq 0.05$) in LW1 and lowest in LW2.

It is clear that the chemical compositions of the low wines prior to distillation are very different from one another and as the low wine was bought, and it is not certain what fermentation parameters were employed for the production of the base wine.

**TABLE 6.1. ANOVA for the chemical composition of LW1, LW2 and LW3 prior to distillation.**

| Compounds/groups of Compounds (mg/L) | P-value (p≤0.05) | LW1 | LW2 | LW3 |
|---|---|---|---|---|
| Ethyl acetate | <0.0001 | 101.530 a | 102.137 a | 60.673 b |
| Ethyl butyrate | <0.0001 | 1.000 a | 0.923 a | 0.610 b |
| i-Amyl acetate | <0.0001 | 11.290 a | 9.473 b | 6.577 c |
| Ethyl hexanoate | <0.0001 | 2.557 a | 2.143 b | 1.803 c |
| Hexyl acetate | <0.0001 | 1.590 a | 1.133 b | 0.887 c |
| Ethyl lactate | <0.0001 | 11.863 c | 13.817 b | 17.333 a |
| Ethyl octanoate | <0.0001 | 5.130 a | 3.847 b | 3.290 c |
| Ethyl decanoate | <0.0001 | 10.777 a | 8.130 b | 6.203 c |
| Di-ethyl succinate | <0.0001 | 0.317 c | 0.477 b | 0.577 a |
| 2-Phenyl ethyl acetate | 0.000 | 0.730 a | 0.603 c | 0.670 b |
| **Total esters** | **<0.0001** | **146.783 a** | **142. 678 b** | **98.623 c** |
| n-Propanol | <0.0001 | 62.893 a | 56.853 b | 51.483 c |
| i-Butanol | <0.0001 | 154.200 a | 137.087 b | 119.153 c |
| n-Butanol | <0.0001 | 1.733 a | 1.540 b | 1.407 c |
| i-Amyl alcohol | <0.0001 | 339.670 a | 293.817 b | 266.500 c |
| Hexanol | <0.0001 | 4.730 a | 4.133 b | 3.837 c |
| 2-Phenyl ethanol | <0.0001 | 12.777 b | 12.833 b | 17.277 a |
| **Total higher alcohols** | **<0.0001** | **576.003 a** | **506.258 b** | **459.657 c** |
| Acetic acid | 0.000 | 69.197 a | 53.367 b | 56.213 b |
| i-Butyric acid | 0.000 | 0.923 b | 0.873 b | 1.140 a |
| n-Butyric acid | 0.069 | 1.217 a | 1.320 a | 1.313 a |
| Hexanoic acid | <0.0001 | 6.653 c | 8.410 b | 11.077 a |
| Octanoic acid | <0.0001 | 18.220 c | 20.617 b | 29.233 a |
| Decanoic acid | 0.000 | 12.257 b | 12.197 b | 15.307 a |
| **Total acids** | **<0.0001** | **108.467 b** | **96.781 c** | **114.283 a** |

| Compounds/groups of Compounds (mg/L) | P-value (p≤0.05) | LW1 | LW2 | LW3 |
|---|---|---|---|---|
| Acetaldehyde | <0.0001 | 13.833 a | 10.030 b | 8.673 c |
| Acetoin | 0.001 | 0.833 b | 0.847 b | 0.973 a |
| **Total aldehydes** | **<0.0001** | **14.667 a** | **10.877 b** | **9.647 c** |
| HA[a] vs. esters | <0.0001 | 3.924 b | 3.548 c | 4.661 a |
| HA vs. acids | <0.0001 | 5.311 a | 5.233 a | 4.022 b |
| Esters vs. acids | <0.0001 | 1.353 b | 1.475 a | 0.863 c |
| Aldehydes vs. esters | 0.000 | 0.100 a | 0.076 b | 0.098 a |
| Aldehydes vs. HA | 0.000 | 0.025 a | 0.021 b | 0.021 b |
| Aldehydes vs. acids | <0.0001 | 0.135 a | 0.112 b | 0.084 c |
| Methanol | 0.000 | 49.667 a | 49.983 a | 45.947 b |
| pH | <0.0001 | 4.500 b | 4.140 c | 4.930 a |
| TA | <0.0001 | 0.400 a | 0.300 c | 0.380 b |

**Compounds/groups of compounds with different letters in the same row are found to be significantly different (p≤0.05). HA[a] (Higher alcohols). TA[e] (Titrateable acidity (g/L).**

### 6.3.2 Chemical analyses of the distillates produced from low wine one

Table 6.2 shows the one-way ANOVA for the compounds/groups of compounds of the distillates produced from low wine one using the three different condenser temperatures namely 8, 12 and 20°C respectively.

### 6.3.2.1 Esters

Due to the fact that esters are volatile one would expect that a decrease in the condenser water temperature would result in an increase in their concentration in the resultant distillate (Nykänen, 1986). The esters did not seem to be influenced by the different condenser water temperatures. The only observation to be reported would be that the level of ethyl acetate and overall total esters concentration was found to increase during the distillation process when compared to the original ester concentration prior to the distillation process. Even though there were no significant differences found between the different condenser water temperature treatments with regards to their total ester concentration, the general trend noted was that the 8°C condenser water temperature did contain a higher amount of total esters.

**6.3.2.2 Higher alcohols**

The total higher alcohol concentration was found not to be influenced by the different condenser water temperatures, it can be reported that the overall total higher alcohol concentration is found to approximately triple in concentration during the distillation process.

**6.3.2.3 Total acids**

The results from Table 6.2 show that the distillates produced from the 8ºC condenser temperature were found to contain overall higher concentrations (≤0.05) of total acids, and more specifically decanoic acid. Literature states that the medium-chain fatty acids are easily entrained with the alcohols by the distillation vapour (Pino, 2007) thereby distilling over. There is limited literature that tests the effect of temperature on the concentration of acids found in the resultant distillate. It would seem that the cooler condenser temperature has a solidification effect on the medium-chain fatty acids thereby resulting in a form of precipitation and therefore increasing the concentration.

Perhaps the increase in total acids in the distillates produced from the 8ºC is a result in the decrease in volatility of these compounds due to the cooler temperature. However of the total acid concentration, the amount of acetic acid is drastically reduced to approximately half of the original concentration as found in the low wine prior to distillation. The reason being that acetic acid is removed during the collection of the head fraction during the distillation process (Léaute, 1990).

**6.3.2.4 Ratios**

The ratio of higher alcohols vs. acids, esters vs. acids and carbonyls vs. acids were found to be lowest (≤0.05) in the distillates of the 8ºC when compared to that of the 12ºC and 20ºC distillates.

**6.3.2.5 pH and TA**

No significant differences (≤0.05) between the pH were found for the distillates produced by low wine one. However, for the TA value it was be highest (≤0.05) in the 8LW1 treatment when compared to the other condenser water treatments.

**TABLE 6.2 ANOVA for the chemical composition of the distillates produced from low wine one.**

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | 8LW1[b] | 12LW1[c] | 20LW1[d] |
|---|---|---|---|---|
| Ethyl acetate | 0.785 | 156.702 a | 151.406 a | 148.116 a |
| i-Amyl acetate | 0.700 | 10.022 a | 9.127 a | 9.074 a |
| Ethyl hexanoate | 0.486 | 2.112 a | 1.912 a | 1.891 a |
| Hexyl acetate | 0.349 | 1.980 a | 1.769 a | 1.752 a |
| Ethyl lactate | 0.286 | 10.472 a | 9.723 a | 9.757 a |
| Ethyl octanoate | 0.544 | 5.052 a | 4.627 a | 4.534 a |
| Ethyl decanoate | 0.985 | 10.417 a | 10.228 a | 10.220 a |
| Di-ethyl succinate | 0.997 | 0.505 a | 0.508 a | 0.510 a |
| 2-Phenyl ethyl acetate | 0.508 | 1.037 a | 0.923 a | 0.909 a |
| **Total esters** | **0.769** | **197.792 a** | **189.661 a** | **186.304 a** |
| n-Propanol | 0.485 | 238.762 a | 235.524 a | 232.367 a |
| i-Butanol | 0.475 | 184.680 a | 185.543 a | 181.637 a |
| n-Butanol | 0.128 | 5.717 a | 5.568 a | 5.530 a |
| i-Amyl alcohol | 0.296 | 1198.255 a | 1193.505 a | 1171.565 a |
| Hexanol | 0.415 | 15.870 a | 15.982 a | 15.737 a |
| 2-Phenyl ethanol | 0.052 | 3.155 a | 1.881 a | 2.042 a |
| **Total higher alcohols** | **0.335** | **1646.438 a** | **1638.002 a** | **1608.877 a** |
| Acetic acid | 0.085 | 15.132 a | 12.349 a | 10.944 a |
| i-Butyric acid | 0.525 | 0.472 a | 0.432 a | 0.423 a |
| n-Butyric acid | 0.786 | 0.270 a | 0.308 a | 0.285 a |
| Hexanoic acid | 0.470 | 2.980 a | 3.114 a | 2.920 a |
| Octanoic acid | 0.106 | 16.432 a | 10.174 a | 11.205 a |
| Decanoic acid | 0.013 | 17.845 a | 9.482 b | 9.850 b |
| **Total acids** | **0.045** | **53.130 a** | **35.857 b** | **35.626 b** |
| Acetaldehyde | 0.128 | 70.242 a | 70.022 a | 80.983 a |
| Acetoin | 0.489 | 0.755 a | 0.703 a | 0.684 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | 8LW1[b] | 12LW1[c] | 20LW1[d] |
|---|---|---|---|---|
| **Total aldehydes** | **0.128** | **70.966 a** | **70.724 a** | **81.667 a** |
| HA[a] vs. esters | 0.879 | 8.382 a | 8.642 a | 8.643 a |
| HA vs. acids | 0.017 | 31.272 b | 45.696 a | 45.175 a |
| Esters vs. acids | 0.016 | 3.727 b | 5.293 a | 5.233 a |
| Aldehydes vs. esters | 0.223 | 0.361 a | 0.374 a | 0.439 a |
| Aldehydes vs. HA | 0.083 | 0.043 a | 0.043 a | 0.051 |
| Aldehydes vs. acids | 0.011 | 1.347 b | 1.970 a | 2.293 a |
| Methanol | 0.790 | 276.737 a | 273.934 a | 271.413 a |
| pH | 0.167 | 6.440 a | 7.065 a | 6.809 a |
| TA[e] | 0.000 | 0.260 a | 0.047 b | 0.093 b |

**Compounds/groups of compounds with different letters in the same row are found to be significantly different (p≤0.05). HA[a] (Higher alcohols). 8LW1[b] (8ºC distillates of low wine one). 12LW1[c] (12ºC distillates of low wine one). 20LW1[d] (20ºC distillates of low wine one). TA[e] (Titrateable acidity (g/L).**

### 6.3.2.6 Principal Component Analysis (PCA) of the distillates produced from low wine one

The interrelationships between the compounds/groups of compounds and the distillates produced from low wine one is shown in figure 6.2. The first principal component (F1) accounted for 80.27% of the total variation and the second principal component (F2) accounted for 19.73% of the total variation.
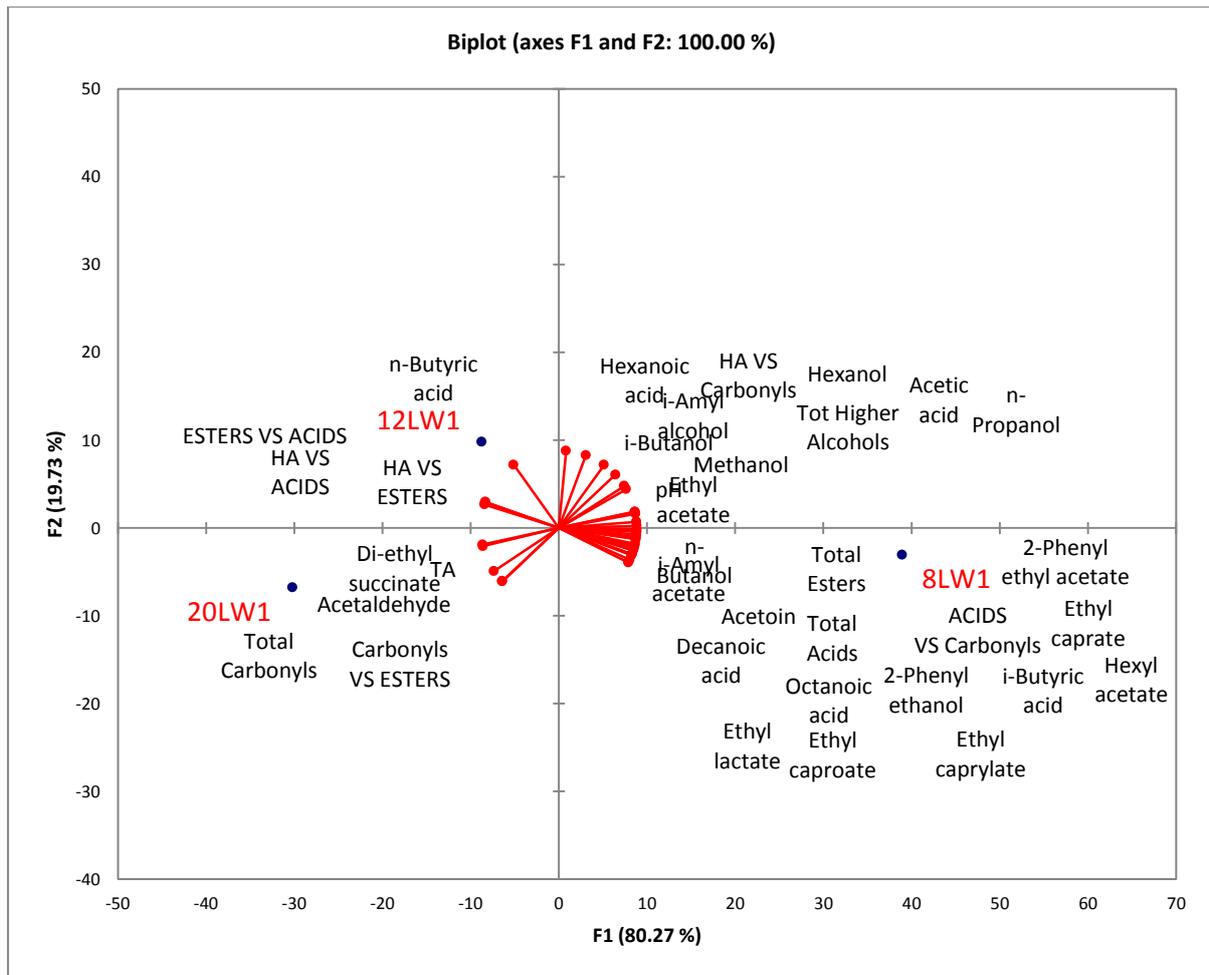
**FIGURE 6.2 PCA bi-plot showing the relationship between the distillates produced by low wine one and the compounds/groups of compounds on F1 and F2. 8LW1 (8°C distillates of low wine one). 12LW1 (12°C distillates of low wine one). 20LW1 (20°C distillates of low wine one).**

F1 indicated that 8LW1 (factor score=7.234) was found to be positively associated to the F1 axis and contrasted the most with 12LW1 (factor score=-1.624) and 20LW1 (factor score=-5.670). The compounds/groups of compounds namely pH (r=0.979), ethyl acetate (r=0.997), methanol (r=0.984), n-propanol (r=0.980), i-amyl acetate (r=0.935), n-butanol (r=0.993), i-amyl alcohol (r=0.844), ethyl hexanoate (r=0.974), hexyl acetate (r=0.969), acetoin (r=0.999), ethyl lactate (r=0.938), ethyl octanoate (r=0.990), acetic acid (r=1.000), i-butyric acid (r=0.989), ethyl decanoate (r=0.961), 2-phenyl ethyl acetate (r=0.978), 2-phenyl ethanol (r=0.909), octanoic acid (r=0.892), decanoic acid (r=0.938), total esters (r=1.000), total higher alcohols (r=0.869) and total acids (r=0.955) were found to be positively correlated to the F1 axis and therefore found to be positively associated to 8LW1 and negatively associated to 12LW1 and 20LW1.

Conversely the compounds/groups of compounds TA (r=-0.973), acetaldehyde (r=-0.731), di-ethyl succinate (r=-0.978), total aldehydes (r=-0.728), higher alcohols vs. esters (r=-0.952), higher alcohols vs. acids (r=-0.941), esters vs. acids (r=-0.940), carbonyls vs. esters (r=-0.834), aldehydes vs. higher alcohols (r=-0.747) and aldehydes vs. acids (r=-1.000) were found to be negatively correlated to the F1 axis and therefore negatively associated to 8LW1 and positively associated to 12LW1 and 20LW1.

F2 indicated that 12LW1 (factor score=6.789) was found to be positively associated to the F2 axis and found to contrast the most with 8LW1 (factor score=-1.161) and 20LW1 (factor score=-2.518). The compounds/groups of compounds namely i-butanol (r=0.811), hexanol (r=0.936), n-butyric acid (r=0.818) and hexanoic acid (r=0.995) were found to be positively correlated to the F2 axis and therefore positively associated to 12LW1 and negatively associated to 8LW1 and 20LW1.

Conversely the compounds/groups of compounds acetaldehyde (r=-0.842), total carbonyls (r=-0.686) and carbonyls vs. higher alcohols (r=-0.665) were found to be negatively correlated to the F2 axis and therefore negatively associated to 12LW1, but however positively associated to 8LW1 and 20LW1.

Table 6.2 shows that there are no significant differences between the different condenser temperatures in terms of their total esters, total higher alcohols and total aldehydes, but indeed between the total acid concentration.

However figure 6.2 shows that 8LW1 is found to be more associated with the total esters and total higher alcohols, as well as total acids and the distillates, namely 12LW1 and 20LW1, were found to be positively associated to ratios of higher alcohols vs. esters, higher alcohols vs. acids, esters vs. acids, aldehydes vs. esters, aldehydes vs. higher alcohols and aldehydes vs. acids.

### 6.3.3 Chemical analyses of the distillates produced from low wine two

Table 6.3 shows the one-way ANOVA for the compounds/groups of compounds of the distillates produced from low wine two using the three different condenser temperatures namely 8, 12 and 20ºC respectively.

### 6.3.3.1 Esters

No significant differences (≤0.05) between the respective condenser water temperatures were to be reported. These findings were found to be the same as in the case of the distillates produced from low wine one.

### 6.3.3.2 Higher alcohols

Of the higher alcohols found in the distillates only n-propanol and n-butanol were found to be significantly different (≤0.05) amongst the respective condenser water temperatures. These compounds were found to be lowest in 20LW2 when compared to the 8LW2 and 12LW2. The total higher alcohol concentration was found not to be influenced by the different condenser water temperatures, it can be reported that the overall total higher alcohol concentration is found to approximately triple in concentration during the distillation process. These findings were consistent with those distillates produced by low wine one.

### 6.3.3.3 Acids

No significant differences (≤0.05) between the different condenser water temperatures were reported. These findings were not consistent with the findings of the distillates produced from low wine one where the total acid concentration was found to be significantly higher in the 8ºC distillates.

### 6.3.3.4 Methanol

Methanol is not a higher alcohol but is actually a by-product formed during alcoholic fermentation as a result of the degradation of pectic substances in raw materials (Bindler *et al.,* 1988). The methanol content can be decreased by increasing the time of the collection of the heads fraction during the distillation process as this compound is very volatile and is distilled over into the heads fraction and is separated. Distillates that contain higher methanol content could be a due to an increase in the collection of methanol in the heart fraction. However even though the methanol concentration is significantly lower in the distillates of the 20ºC condenser water temperature, all heart fractions were collected for the respective condenser water temperatures were collected in the same manner.

### 6.3.3.5 pH and TA

Both the pH was not found to be significantly different (≤0.05) between the distillates produced by low wine two. These findings are consistent with those distillates produced by the low wine one. The TA value in the distillates of 8LW1 had a higher TA value; however no significant differences (≤0.05) were noted between the distillates produced by low wine two.

**TABLE 6.3 ANOVA for the chemical composition of the distillates produced from low wine two.**

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | 8LW2[b] | 12LW2[c] | 20LW2[d] |
|---|---|---|---|---|
| Ethyl acetate | 0.917 | 102.070 a | 97.975 a | 98.931 a |
| i-Amyl acetate | 0.992 | 4.852 a | 4.819 a | 4.922 a |
| Ethyl hexanoate | 0.970 | 1.252 a | 1.235 a | 1.274 a |
| Hexyl acetate | 0.408 | 1.032 a | 0.856 a | 0.962 a |
| Ethyl lactate | 0.297 | 17.333 a | 16.855 a | 15.910 a |
| Ethyl octanoate | 0.995 | 2.717 a | 2.693 a | 2.730 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | 8LW2[b] | 12LW2[c] | 20LW2[d] |
|---|---|---|---|---|
| Ethyl decanoate | 0.637 | 6.892 a | 6.520 a | 6.020 a |
| Di-ethyl succinate | 0.701 | 0.568 a | 0.544 a | 0.510 a |
| 2-Phenyl ethyl acetate | 0.421 | 1.933 a | 1.912 a | 1.825 a |
| **Total esters** | **0.872** | **138.647 a** | **133.406 a** | **133.083 a** |
| n-Propanol | 0.012 | 232.978 a | 230.282 a | 222.303 b |
| i-Butanol | 0.498 | 163.385 a | 162.510 a | 158.142 a |
| n-Butanol | 0.035 | 5.444 a | 5.379 a | 5.098 b |
| i-Amyl alcohol | 0.145 | 1106.429 a | 1101.646 a | 1064.537 a |
| Hexanol | 0.235 | 17.182 a | 17.297 a | 16.080 a |
| 2-Phenyl ethanol | 0.337 | 4.408 a | 4.213 a | 4.219 a |
| **Total higher alcohols** | **0.106** | **1529.825 a** | **1521.326 a** | **1470.378 a** |
| Acetic acid | 0.639 | 15.270 a | 16.038 a | 13.092 a |
| i-Butyric acid | 0.655 | 0.711 a | 0.713 a | 0.664 a |
| n-Butyric acid | 0.450 | 0.710 a | 0.671 a | 0.614 a |
| Hexanoic acid | 0.944 | 5.654 a | 5.533 a | 5.445 a |
| Octanoic acid | 0.317 | 18.960 a | 16.713 a | 17.572 a |
| Decanoic acid | 0.145 | 18.249 a | 15.306 a | 15.319 a |
| **Total acids** | **0.493** | **59.522 a** | **54.976 a** | **52.703 a** |
| Acetaldehyde | 0.668 | 23.937 a | 25.610 a | 28.155 a |
| Acetoin | 0.485 | 0.892 a | 0.860 a | 0.718 a |
| **Total aldehydes** | **0.700** | **24.829 a** | **26.470 a** | **28.873 a** |
| HA[a] vs. esters | 0.906 | 11.074 a | 11.413 a | 11.107 a |
| HA vs. acids | 0.736 | 25.946 a | 27.681 a | 27.994 a |
| Esters vs. acids | 0.912 | 2.364 a | 2.428 a | 2.543 a |
| Aldehydes vs. esters | 0.697 | 0.179 a | 0.199 a | 0.221 a |
| Aldehydes vs. HA | 0.597 | 0.016 a | 0.017 a | 0.020 a |
| Aldehydes vs. acids | 0.457 | 0.424 a | 0.481 a | 0.545 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | 8LW2[b] | 12LW2[c] | 20LW2[d] |
|---|---|---|---|---|
| Methanol | 0.013 | 299.379 a | 294.789 a | 284.824 b |
| pH | 0.568 | 7.099 a | 7.047 a | 6.795 a |
| TA[e] | 0.729 | 0.045 a | 0.042 a | 0.079 a |

**Compounds/groups of compounds with different letters in the same row are found to be significantly different (p≤0.05). HA[a] (Higher alcohols). 8LW2[b] (8ºC distillates of low wine two). 12LW2[c] (12ºC distillates of low wine two). 20LW2[d] (20ºC distillates of low wine two). TA[e] (Titrateable acidity (g/L).**

### 6.3.3.6 Principal Component Analysis (PCA) of the distillates produced from low wine two

The interrelationships between the compounds/groups of compounds and the distillates produced from low wine two is shown in figure 6.3. The first principal component (F1) accounted for 78.22% of the total variation and the second principal component (F2) accounted for 21.78% of the total variation.
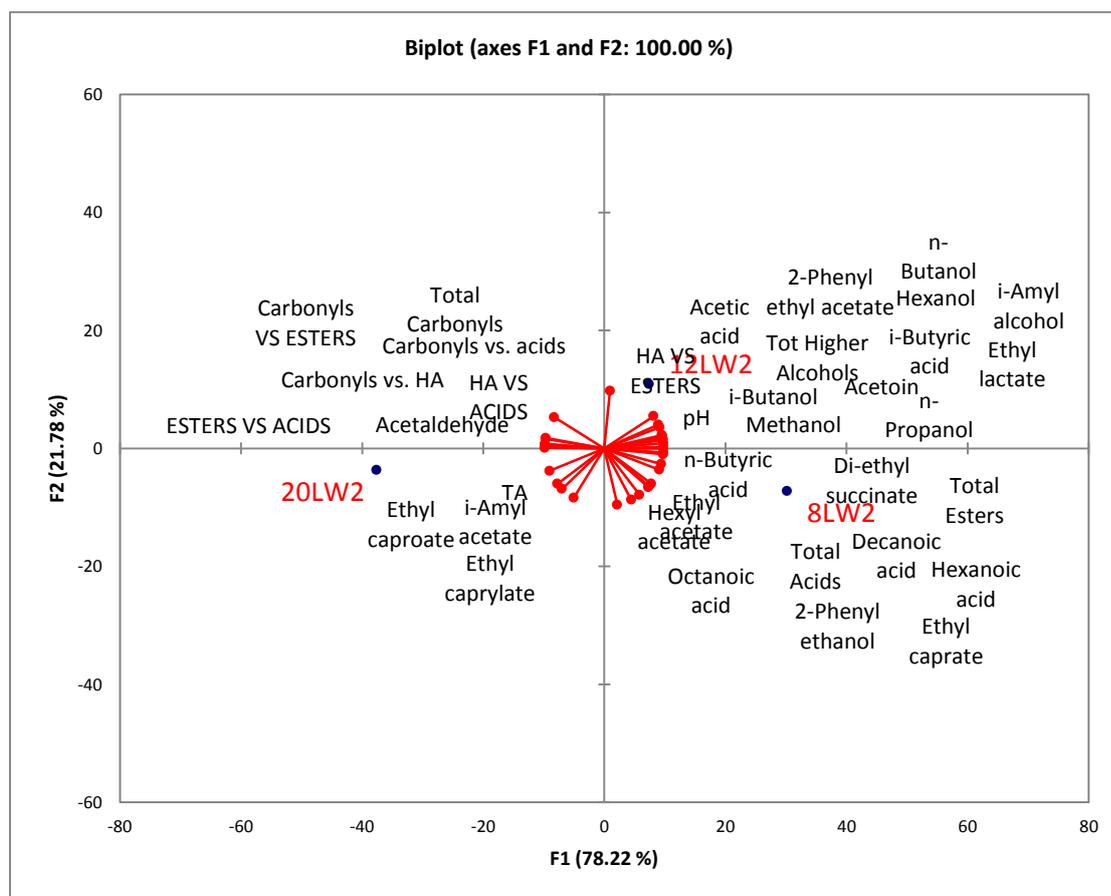


**FIGURE 6.3 PCA bi-plot showing the relationship between the distillates produced by low wine two and the compounds/groups of compounds on F1 and F2. 8LW2 (8ºC distillates of low wine two). 12LW2 (12ºC distillates of low wine two). 20LW2 (20ºC distillates of low wine two).**

F1 indicated that 8LW2 (factor score=5.688) and 12LW2 (factor score=1.396) were found to be positively associated to the F1 axis and contrasted the most with 20LW2 (factor score=-7.084). The compounds/groups of compounds namely pH (r=0.983), methanol (r=1.000), n-propanol (r=0.996), i-butanol (r=0.984), n-butanol (r=0.987), i-amyl alcohol (r=0.973), acetoin (r=0.987), ethyl lactate (r=1.000), hexanol (r=0.912), acetic acid (r=0.831), i-butyric acid (r=0.932), n-butyric acid (r=0.997), ethyl decanoate (r=0.995), di-ethyl succinate (r=0.997), 2-phenyl ethyl acetate (r=0.989), hexanoic acid (r=0.962), 2-phenyl ethanol (r=0.741), decanoic acid (r=0.756), total esters (r=0.791), total higher alcohols (r=0.979) and total acids (r=0.929) were found to be positively correlated to the F1 axis and therefore positively associated to 8LW2 and 12LW2 but however negatively associated to 20LW2.

Conversely the compounds/groups of compounds TA (r=-0.921), acetaldehyde (r=-0.998), i-amyl acetate (r=-0.789), ethyl hexanoate (r=-0.712), total aldehydes (r=-0.997), higher alcohols vs. acids (r=-0.843), esters vs. acids (r=-1.000), aldehydes vs. esters (r=-0.986), aldehydes vs. higher alcohols (r=-1.000) and aldehydes vs. acids (r=-0.984) were found to be negatively correlated to the F1 axis and therefore negatively associated to 8LW2, 12LW2 and positively associated to 20LW2.

F2 indicated that the compounds/groups of compounds 12LW2 (factor score=3.891) was found to be positively associated to the F2 axis and contrasted the most with 8LW2 (factor score=-2.583) and 20LW2 (factor score=-1.308). The compounds/groups of compounds namely the ratio higher alcohols vs. esters (r=0.995) was found to be positively correlated to the F2 axis and therefore positively associated to 12LW2, but however negatively associated to 8LW2 and 20LW2.

Conversely the compounds/groups of compounds ethyl acetate (r=-0.805), ethyl hexanoate (r=-0.702), hexyl acetate (r=-0.976), ethyl octanoate (r=-0.857), 2-phenyl ethanol (r=-0.671), octanoic acid (r=-0.891) and decanoic acid (r=-0.655) were found to be negatively correlated to the F2 axis and therefore negatively associated to 12LW2, but however positively associated to 8LW2 and 20LW2.

Even though the results of table 6.3 show that there are no differences between the condenser water temperatures in terms of their overall total ester, higher alcohol, total acids and total aldehyde concentrations. Figure 6.3 shows that 8LW2 and 12LW2 were found to be positively associated and 20LW2 negatively associated to the above mentioned chemical groups.

When comparing the findings of the distillates produced from low wine one with those of low wine two, results show that in both cases the 8ºC condenser water temperatures were found to be positively associated with total esters, total higher alcohols and total acids. However with regards to the distillates produced from low wine two, the 12ºC condenser water temperature distillates were also found to be positively associated with above mentioned chemical groups.

With regards to the ratios in the case of the distillates produced from low wine one, both the 12ºC and 20ºC distillates were found to be positively associated (figure 6.2) to the ratios of higher alcohols vs. esters, higher alcohols vs. acids, esters vs. acids, aldehydes vs. esters, aldehydes vs. higher alcohols and aldehydes vs. acids. However in the distillates produced from low wine two, only the 20ºC distillates were found to be positively associated with the above mentioned ratios.

### 6.3.4 Chemical analyses of distillates produced from low wine three

Table 6.4 shows the one-way ANOVA for the compounds/groups of compounds of the distillates produced from low wine three using the three different condenser temperatures namely 8, 12 and 20ºC respectively.

### 6.3.4.1 Esters

Due to the fact that esters are volatile one would expect that a decrease in the condenser water temperature would result in an increase in their concentration in the resultant distillate (Nykänen, 1986). Significant differences (≤0.05) were found between the different condenser water temperatures whereby levels of ethyl acetate and total ester concentration were lowest in 20LW3 when compared to 8LW3 and 12LW3. The levels of hexyl acetate and octanoate were also found to be significantly different (≤0.05) between 8LW3 and 20LW3 whereby levels were highest in the 8ºC condenser water temperature. No differences were however found between the 8LW3 and 12LW3 for the compounds hexyl acetate and ethyl octanoate. The results of the distillates produced by low wine three were consistent with these findings (Nykänen, 1986).

The results obtained from the distillates produced by low wine three were not consistent with the findings of the distillates produced by low wine one and two whereby no significant difference were found between the esters in the different condenser water temperatures. However, for all the distillates (LW1, LW2 and LW3) there are indeed trends that indicate that the total ester concentration is somewhat higher in the 8°C condenser water temperature treatment.

### 6.3.4.2 Higher alcohols

No significant differences (≤0.05) between the different condenser water temperatures with regards to the total higher alcohols were found. These findings are consistent with the results obtained from the distillates produced by low wine one, but however not for low wine two.

### 6.3.4.3 Acids

The concentrations of acetic acid, octanoic acid and total acids were found to be significantly highest (≤0.05) in the 20LW3 when compared to that of the 8LW3 and 12LW3 condenser water temperatures.

The results from table 6.2 show that the distillates produced from low wine one showed that the 8ºC condenser temperature were found to contain overall higher concentrations (≤0.05) of total acids, and more specifically decanoic acid. The inverse was found to be true for

those distillates produced by low wine three whereby the 20ºC condenser water temperature was found to contain the highest amount of total acids, acetic acid and octanoic acid.

Literature states that the medium-chain fatty acids are easily entrained with the alcohols by the distillation vapour (Pino, 1999) thereby distilling over. There is limited literature that tests the effect of temperature on the concentration of acids found in the resultant distillate.

Even though the total acid concentration was found to be highest in the 8ºC distillates produced by low wine one, the level of acetic acid was not found to differ between the different condenser water temperatures. Differences were found mainly between the medium chain fatty acids. However for the distillates produced by low wine three, the total acid concentration was composed mainly of acetic acid and this was the main reason for the elevated total acid concentration between the different condenser water temperatures.

The results obtained in table 6.1 shows that however the total acid concentration prior to distillation was found to highest in low wine three and this could be the reason for the elevated levels of acids found in the distillates. Even though acetic acid can be removed by collecting it in the head fraction, perhaps the elevated level found prior to distillation was too large in concentration and thus could not be totally removed (Léaute, 1990).

### 6.3.4.4 Aldehydes

The total aldehyde concentration prior to distillation was found to be highest in low wine one and lowest in low wine three. The results obtained from the distillates produced by low wine one and two found no significant differences (≤0.05) between the different condenser water temperatures. However for the distillates produced by low wine three levels were found to highest (≤0.05) in the 20ºC condenser water treatment, with the main aldehyde compound being acetaldehyde.

### 6.3.4.5 Ratios

The ratios of higher alcohols vs. acids and esters vs. acids were found to be lowest (≤0.05) in the 20LW3 when compared to the 8LW3 and 12LW3. However the results obtained in table 6.2 show that for the distillates produced by low wine one found that the ratios of higher alcohols vs. acids, esters vs. acids were found to be lowest (≤0.05) in the distillates of the 8ºC condenser water treatment.

The ratios of aldehydes vs. higher alcohols, aldehydes vs. esters and aldehydes vs. acids were found to be highest (≤0.05) in the 20LW3 when compared to the 8LW3 and 12LW3. Higher alcohols vs. esters were found to be significantly different (≤0.05) between the 8LW3 and the 20LW3 whereby 20LW3 has a higher ratio of higher alcohols vs. esters.

**6.3.4.6 pH and TA**

The pH value was significantly lower (≤0.05) in the 20LW3 when compared to the 8LW3 and 12LW3. However, the pH was found to be not significantly different (≤0.05) in the distillates produced by low wine one and two. The TA value was also only found to be significantly higher (≤0.05) in the distillates of low wine one, whereby the 8°C condenser water temperature had a higher TA value. With regards to the distillates produced by low wine three, the TA value was found to be significantly highest (≤0.05) in the 20LW3 when compared to the 8LW3 and 12LW3 distillates. These findings were not found to be consistent with the findings of the distillates produced by low wine one and two. However, there is a trend whereby a lower pH and higher TA value are found to be correlated to a slightly higher total acid concentration.

**TABLE 6.4 ANOVA for the chemical composition of the distillates produced from low wine three.**

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | 8LW3[b] | 12LW3[c] | 20LW3[d] |
|---|---|---|---|---|
| Ethyl acetate | 0.019 | 117.204 a | 110.154 a | 98.980 b |
| i-Amyl acetate | 0.053 | 6.395 a | 5.713 ab | 5.107 a |
| Ethyl hexanoate | 0.148 | 1.624 a | 1.489 a | 1.382 a |
| Hexyl acetate | 0.030 | 1.205 a | 1.137 ab | 1.037 b |
| Ethyl lactate | 0.286 | 17.789 a | 18.214 a | 18.517 a |
| Ethyl octanoate | 0.152 | 3.535 a | 3.247 a | 3.002 a |
| Ethyl decanoate | 0.020 | 7.479 a | 6.829 ab | 6.027 b |
| Di-ethyl succinate | 0.220 | 0.484 a | 0.509 a | 0.532 a |
| 2-Phenyl ethyl acetate | 0.385 | 1.629 a | 1.669 a | 1.712 |
| **Total esters** | **0.022** | **157.340 a** | **148.957 a** | **136.293 b** |
| n-Propanol | 0.602 | 213.579 a | 215.445 a | 211.385 a |
| i-Butanol | 0.180 | 153.417 a | 153.292 a | 148.690 a |

| Compounds/groups of compounds (mg/L) | P-value (≤0.05) | 8LW3[b] | 12LW3[c] | 20LW3[d] |
|---|---|---|---|---|
| n-Butanol | 0.369 | 4.986 a | 5.074 a | 4.888 a |
| i-Amyl alcohol | 0.774 | 1011.555 a | 1015.169 a | 1000.922 a |
| Hexanol | 0.924 | 14.279 a | 14.399 a | 14.385 a |
| 2-Phenyl ethanol | 0.144 | 4.112 a | 4.187 a | 4.333 a |
| **Total higher alcohols** | **0.689** | **1401.926 a** | **1407. 563 a** | **1384.603 a** |
| Acetic acid | 0.017 | 11.297 b | 10.237 b | 14.930 a |
| i-Butyric acid | 0.227 | 0.652 a | 0.697 a | 0.735 a |
| n-Butyric acid | 0.075 | 0.580 a | 0.519 a | 0.630 a |
| Hexanoic acid | 0.448 | 6.182 a | 6.315 a | 6.422 a |
| Octanoic acid | 0.034 | 22.305 b | 22.774 b | 24.638 a |
| Decanoic acid | 0.077 | 18.715 a | 18.370 a | 21.510 a |
| **Total acids** | **0.022** | **59.730 b** | **59.000 b** | **68.875 a** |
| Acetaldehyde | <0.0001 | 21.490 b | 20.389 b | 40.338 a |
| Acetoin | 0.175 | 0.750 a | 0.747 a | 0.837 a |
| **Total aldehydes** | **<0.0001** | **22.240 b** | **21.586 b** | **41.175 a** |
| HA[a] vs. esters | 0.031 | 8.916 b | 9.461 ab | 10.158 a |
| HA vs. acids | 0.019 | 23.481 a | 23.920 a | 20.105 b |
| Esters vs. acids | 0.018 | 2.634 a | 2.535 a | 1.980 b |
| Aldehydes vs. esters | <0.0001 | 0.142 b | 0.145 b | 0.302 a |
| Aldehydes vs. HA | <0.0001 | 0.016 b | 0.015 b | 0.030 a |
| Aldehydes vs. acids | 0.000 | 0.373 b | 0.366 b | 0.598 a |
| Methanol | 0.602 | 297.727 a | 301.540 a | 295.772 a |
| pH | <0.0001 | 7.212 a | 7.065 a | 6.971 b |
| TA[e] | <0.0001 | 0.046 b | 0.046 b | 0.092 a |

**Compounds/groups of compounds with different letters in the same row are found to be significantly different (p≤0.05). HA[a] (Higher alcohols). 8LW3[b] (8ºC distillates of low wine three). 12LW3[c] (12ºC distillates of low wine three). 20LW3[d] (20ºC distillates of low wine three). TA[e] (Titrateable acidity (g/L).**

### 6.3.4.7 Principal Component Analysis (PCA) of the distillates produced from low wine three

The interrelationships between the compounds/groups of compounds and the distillates produced from low wine three is shown in figure 6.4. The first principal component (F1) accounted for 85.55% of the total variation and the second principal component (F2) accounted for 14.45% of the total variation.



**FIGURE 6.4 PCA bi-plot showing the relationship between the distillates produced by low wine three and the compounds/groups of compounds on F1 and F2. 8LW3 (8ºC distillates of low wine three). 12LW3 (12ºC distillates of low wine three). 20LW3 (20ºC distillates of low wine three).**

F1 indicated that 20LW3 (factor score=7.740) was found to be positively associated to the F1 axis and were found to contrast the most with 8LW3 (factor score=-4.996) and 12LW3 (factor score=-2.744). The compounds/groups of compounds namely TA (r=0.986), acetaldehyde (r=0.981), acetoin (r=0.980), ethyl lactate (r=0.899), acetic acid (r=0.933), i-butyric acid (r=0.918),n-butyric acid (r=0.732), di-ethyl succinate (r=0.929), 2-pheynl ethyl acetate (r=0.944), hexanoic acid (r=0.912), 2-phenyl ethanol (0.985), octanoic acid (r=1.000), decanoic acid (r=0.965), total acids (r=0.973), total aldehydes (r=0.981), higher alcohols vs. esters (r=0.959), aldehydes vs. esters (r=0.989) and aldehydes vs. higher

alcohols (r=0.980) and aldehydes vs. acids (r=0.982) are found to be positively correlated to the F1 axis and therefore positively associated to 20LW3 and negatively associated to 12LW3 and 8LW3.

Conversely the compounds/groups of compounds pH (r=-0.881), ethyl acetate (r=-0.974), n-propanol (r=-0.800), i-butanol (r=-0.990), i-amyl acetate (r=-0.924), n-butanol (r=-0.789), i-amyl alcohol (r=-0.916), ethyl hexanoate (r=-0.911), hexyl acetate (r=-0.969), ethyl octanoate (r=-0.919), ethyl decanoate (r=-0.956), total esters (r=-0.971), total higher alcohols (r=-0.919), higher alcohols vs. acids (r=-0.963) and esters vs. acids (r=-1.000) are found to be negatively correlated to the F1 axis and therefore positively associated to 8LW3 and 12LW3, but however negatively associated to 20LW3.

F2 indicated that 12LW3 (factor score=3.022) was found to be positively associated to the F2 axis and contrasted the most with 8LW3 (factor score=-2.488) and 20LW3 (factor score=-0.534). The compound namely methanol (r=0.747) and hexanol (r=0.883) were found to be positively correlated to the F2 axis and therefore positively associated 12LW3 and therefore negatively associated to 8LW3 and 20LW3. Conversely the compound n-butyric acid (r=-0.681) was found to be negatively correlated to the F2 axis and therefore negatively associated to 12LW3 and therefore positively associated to 8LW3 and 20LW3.

From figure 6.4 and table 6.4 it can be seen that 8LW3 and 12LW3 are found to be positively associated total esters and total higher alcohols. These findings are consistent with those results obtained in the distillates produced by low wine one and low wine two. However for the distillates of low wine one, the 8ºC condenser water temperature treatment was also found to be associated to total acids, which is not the case for the distillates of low wine two and three.

The ratios of higher alcohols vs. acids and esters vs. acids were found to be lowest in the 20LW3 when compared to the 8LW3 and 12LW3. However, the results obtained in table 6.2 show that for the distillates produced by low wine one found that the ratios of higher alcohols vs. acids, esters vs. acids were found to be lowest in the distillates of the 8ºC condenser water treatment.

The 20LW3 treatment was found to be positively associated to total acids, total aldehydes, higher alcohols vs. esters, aldehydes vs. esters and aldehydes vs. higher alcohols and aldehydes vs. acids.

## 6.3.5 The overall effect of the distillation process on the chemical composition of the resultant distillates

Table 6.5 shows the chemical composition of the low wines prior to distillation and the effect of the distillation process on the resultant distillates produced by LW1, LW2 and LW3.

**6.3.5.1 The overall effect of the distillation process on esters**

Table 6.5 shows that the distillation process overall increases the concentration of total esters in the resultant distillates for LW1 by 1.2 fold and LW3 by 1.5 fold. However for the distillates of LW2 there is little to no change in the concentration of total esters. During the distillation process, the level of esters, more specifically ethyl acetate, is reduced as it is removed in the head fraction (Léaute, 1990).

**6.3.5.2 The overall effect of the distillation process on higher alcohols**

The total higher alcohol concentration was found to be greatly influenced by the distillation process, whereby the distillates produced by LW1, LW2 and LW3 were found to contain on average 2.9 times higher concentrations of total higher alcohols when compared to the low wine prior to distillation. Higher alcohols are collected in the heart fraction during the distillation process and studies by Léaute (1990) show that the level of higher alcohols are found to increase in concentration during the distillation process.

**6.3.5.3 The overall effect of the distillation process on acids**

The total acid concentration is found to decrease during the distillation procedure as levels in the resultant distillate are half the concentration found in the low wine prior to distillation. This is to be expected as the main acid found in the low wine prior to the distillation process is acetic acid and acetic acid is removed in the head fraction during the distillation process (Léaute, 1990 and Nykänen, 1986).

**6.3.5.4 The overall effect of the distillation process on aldehydes**

The total carbonyl concentration overall was found to increase during the distillation process, whereby the distillates of LW1 contained on average 4.7 times higher levels than the original low wine prior to distillation. The distillates of LW2 and LW3 were also found to contain on average 2.3 times higher concentrations than in the low wines prior to distillation. It was found that if the low wine prior contained a higher amount of carbonyl compounds, then the distillate produced would therefore also contain higher amounts of these compounds (as seen in LW1 where the level of carbonyl compounds prior to distillation is higher than in LW2 and LW3).

Even though the majority of acetaldehyde is removed in the head fraction of the distillation process, levels are found to increase overall throughout the distillation procedure (Léaute, 1990).

**TABLE 6.5 Overall effect of the distillation process on the chemical composition of the resultant distillates produced by LW1, LW2 and LW3.**

| LW[a]/distillate | Total esters (mg/L) | Ratio (esters) | Total HA[b] (mg/L) | Ratio (HA) | Total acids (mg/L) | Ratio (acids) | Total aldehydes (mg/L) | Ratio (aldehydes) |
|---|---|---|---|---|---|---|---|---|
| **LW1-prior** | **146.783** | | **576.003** | | **108.467** | | **14.667** | |
| 8[c]LW1 | 197.792 | 1.34 | 1646.438 | 2.858 | 53.13 | 0.48 | 70.966 | 4.83 |
| 12[d]LW1 | 189.661 | 1.29 | 1638.002 | 2.843 | 35.857 | 0.33 | 70.724 | 4.82 |
| 20[e]LW1 | 186.304 | 1.26 | 1608.877 | 2.793 | 35.626 | 0.32 | 81.667 | 5.56 |
| **LW2-prior** | **142.678** | | **506.258** | | **96.781** | | **10.877** | |
| 8LW2 | 138.647 | 0.97 | 1529.825 | 3.021 | 59.522 | 0.61 | 24.829 | 2.28 |
| 12LW2 | 133.406 | 0.93 | 1521.326 | 3.005 | 54.976 | 0.56 | 26.47 | 2.43 |
| 20LW2 | 133.083 | 0.932 | 1470.378 | 2.904 | 52.703 | 0.54 | 28.873 | 2.65 |
| **LW3-prior** | **98.623** | | **459.657** | | **114.283** | | **9.647** | |
| 8LW3 | 157.34 | 1.59 | 1401.926 | 3.04 | 59.730 | 0.52 | 22.24 | 2.30 |
| 12LW3 | 148.957 | 1.51 | 1407.563 | 3.062 | 59.000 | 0.51 | 21.586 | 2.23 |
| 20LW3 | 136.293 | 1.38 | 1384.603 | 3.01 | 68.875 | 0.60 | 41.175 | 4.26 |

LW[a] (Low wine). HA[b] (Higher alcohols). 8[c] (8°C condenser water temperature). 12[d] (12°C condenser water temperature). 20[e] (20°C condenser water temperature).

## 6.4 CONCLUSIONS

The results obtained in these experiments show that the use of different condenser water temperatures during the distillation process has little to no effect on the total ester or total higher alcohol concentration of the resultant distillate. It would seem that the chemical groups' esters and higher alcohols found in the distillates are predetermined by the chemical composition of the low wine prior to distillation rather than the condenser water temperature employed. The condenser water temperature does indeed influence the total acid concentration in the resultant distillate; however it would seem that further experiments would be needed in order to confirm these results.

The condenser water temperatures had no effect on the total aldehyde concentration for the distillates produced by low wine one and low wine two. However for the distillates produced by low wine three levels were found to highest in the 20ºC condenser water treatment, with the main aldehydes compound being acetaldehyde. Again, more experiments would need to confirm these findings.

The overall distillation process does indeed influence the concentration of total esters, total higher alcohols, total acids and total aldehydes whereby levels are found to increase in the resultant distillates; however this is not found to be true for the concentration of total acids. The levels of total acids are found to be lower in the resultant distillates. Even though the concentration of the above mentioned chemical groups are influenced if distilled using a lower condenser water temperature (8ºC) it is not a feasible option to employ as this would increase capital output of the company. Therefore it is recommended that more emphasis be placed on the low wine used prior to distillation rather than the condenser water temperature employed.

## 6.5 LITERATURE CITED

Bindler, F., Voges, E., Laugel, P. 1988. The problem of methanol concentration admissible in distilled fruit spirits. Food. Add. Con. 5, 3: 343-351.

Bougas, N.V., 2009. Evaluating the effect of Pot still design on the resultant distillate. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Carnacini, A., Di Stefano, R., Riponi, C., Antonelli, A., 1989. Effect of wine-making process on the volatile compounds of distillates. Ital. J. Food. Sci. 1(4), 13-22.

Desauziers, V., Avezac, M., Fanlo, J.L., 2000. Simple analysis of odorous fatty acids in distillery effluents by capillary electrophoresis. Analusis. 28(2), 163-167.

Faúndez, C.A and Valderrama, J.O., 2004. Phase equilibrium modelling in binary mixtures found in wine and must distillation. J. Food. Eng. 65(4), 577-583.

Guymon, J.F., 1974a. Chemical aspects of distilling wines into brandy. Advances in chemistry. 137(11), 232-253.

Lambrechts, M., Pretorius, I.S., 2000. Yeast and its importance to wine aroma - A review. SA. J. Enol.Vitic. 21, 97-129.

Leaute, R., 1990. Distillation in Alambic. Am. J. Enol.Vitic. 41(1), 90-103.

Madrera, R.R., Gomis, D.B., Alonso, J.J.M., 2003. Influence of distillation system, oak wood type, and aging time on volatile compounds of cider brandy. J. Agric. Food. Chem. 15(19), 5709-5714.

Mateos, J.A.R., Pĕrez-Nevado, F., Fernăndez., 2006. Influence of Saccharomyces cerevisiae strain on major volatile compounds of wine. Enz. Micro. Tech. 40(1), 151-157.

Nykänen, L., 1983. Aroma of beer, wine and distilled alcoholic beverages. Akademie-Verlag, Berlin.

Nykänen, L., 1986. Formation and occurrence of flavour compounds in wine and distilled alcoholic beverages.  Am. J. Enol.Vitic. 37(1), 84-96.

Pino, J.A. 2007. Characterization of rum using solid-phase microextraction with gas chromatography–mass spectrometry. Food. Chem. 104, 1: 421-428.

Saco, P., Flores, J., Taboada, J., Otero, A., Varela, J., 2006. Rule-based intelligent monitoring and control of marc brandy stills. Comp. Chem. Eng. 30(6-7), 1132-1140.

Snyman, C.L.C., 2004. The influence of base wine composition and  wood maturation on the quality of South African brandy. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Vilanova M, Ugliano M, Varela C, Siebert T, Pretorius IS and Henschke PA (2007) Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by Saccharomyces cerevisiae wineyeasts. Appl Microbiol Biotechnol 77: 145–157.

Zoecklein, B.W., Fugelsang, K.C., Gump, B.H. and Nury, F.S. 1999. (1 1 ed). Wine analysis and production. Chapman & Hall, Gaithersburg.

# Chapter 7

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# Research results

## THE EFFECT OF BARREL AGE ON THE VOLATILE COMPOSITION OF THREE-YEAR BARREL OLD MATURED BRANDY

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# 7. The effect of barrel age on the chemical composition of three-year barrel old matured brandy

## ABSTRACT

The effect of different barrel age on the chemical composition of three-year barrel matured brandy samples was investigated. Distillates were matured in different barrels (*new barrels*, barrels that were *18 years* of age and *red wine barrels).* Seventy-nine of these different barrel matured brandy samples were analyzed using GC-FID (Gas chromatography flame ionization), HPLC (High performance liquid chromatography). Routine analysis such as pH, TA (Total acidity) and TP (Total polyphenols) were also carried out on samples. The results obtained indicate that the use of *18 year barrels* produces a three-year barrel matured brandy with a lower amount of TA (titrateable acidity), TP (total polyphenols), total esters, acetaldehyde, gallic, vanillic, syringic, caffeic, p-coumaric and ellagic acids, acetic acid as well as a lower overall total acid concentration. The ratios of carbonyls vs. esters and carbonyls vs. HA are also found to be lowest in the *18 year barrels*, together with a lower concentration of total lactones, total furans and phenols (more specifically syringaldehyde, vanillin, coniferaldehyde sinapaldehyde, syringaldehyde, guaiacol and eugenol), as well as a higher ratio of HA vs. esters, HA vs. acids and esters vs. acids. The inverse was found to be true for *new barrels* for the above mentioned compounds and ratios. Therefore if a three-year barrel matured brandy with a lower concentration of esters, acids, furans, lactones, phenols and a higher ratio of HA vs. esters, HA vs. acids and esters vs. acids was desired, barrels that are *18 years in age* should be used for maturation. Inversely, if a three-year barrel matured brandy sample with a higher concentration of esters, a decreased ratio of HA vs. esters, HA vs. acids and esters vs. acids was desired, then the use of *new barrels* would be advised for maturation purposes.

## 7.1 INTRODUCTION

Barrels have been used for centuries to store spirit beverages and are fundamental in contributing to the final taste and aroma of the end product. During the barrel maturation process, the spirit product acquires a very characteristic flavour and aroma which is distinctly different to the initial distillate. The origin, heat treatment of barrel and maturation time are all fundamental in the influence on the sensory attributes of the spirit product (Caldiera *et al.,* 2010). The main constituents of oak wood are hemicellulose, cellulose, lignin and tannins and during the maturation process there are a great deal of reactions that take place that result in an increased or decreased concentration of certain compounds (Nykänen, 1986 and Puech, 1984). One of the main reactions includes the direct extraction of wood compounds (both volatile and non-volatile) (Mosedale and Puech, 1998).

The main volatile compounds extracted from the wood (lactones, furanic compounds, phenolic derivatives and vanillin derivatives) have an important sensory impact on the spirit beverage (Caldiera *et al.,* 2010). The way in which these compounds are extracted during the maturation period is dependent on many factors particularly the quantity of volatile compounds available and their precursors presented in the wood matrix (Caldeira *et al.,* 2006).

Of the oak wood extracted from the barrels, *cis*-oak lactone and *trans*-oak lactones are regarded as the most important to the sensory characteristics of spirit beverages and are found to impart a "coconut" aroma and flavour. Lactones are found to be associated to the quality of oak-aged spirits and are often used as a quality indicator (Pollnitz *et al.,* 1999).

Furanic aldehydes are another important group extracted from the lignin found in oak barrels, but they are found not to influence the final organoleptic characteristic of the spirit product (Batista de Aquino *et al.,* 2006). In general it is found that syringaldehyde and vanillin are the most predominant compounds in aged alcoholic beverages. However other compounds such as syringic acid, sinapic acid, vanillic acid and ferulic acid can also be found. These reactions and the amount of compounds extracted from the oak depends on the contact time between the spirit product and the wood, the amount of extractable compounds present and the initial spirit composition (Cerdán *et al.,* 2003). Together with these above mentioned compounds, esters, higher alcohols, acids and other carbonyls are also found in spirit beverages and are of great importance (Nykänen, 1986).

Esters are abundant constituents in foods and beverages, and their presence strongly influences the bouquet of wine and distilled beverages. One can expect to find at least 400 to 500 mg/L of total volatile esters in finished brandies (Nykänen, 1986). Of the esters present in brandy, ethyl acetate and ethyl esters of the medium-chain fatty acids are found to be the highest in concentration. The ethyl esters of the long-chained fatty acids are found to also contribute to the overall aroma and flavour of brandy. Studies by Salo *et al.,* (1972) indicate that the ethyl esters of even carbons between 6 and 12 to be major contributing factors to the flavour of distilled beverages.

Higher alcohols have an important aromatic effect in wines and especially distillates (Steger and Lambrechts, 2000). The most abundant higher alcohols found in distillates are isoamyl alcohol, active isoamyl alcohol, isobutanol and n-propanol (Jounela-Eriksson, 1981). The amount of higher alcohols in brandy can range from 600 to 1200 mg/L.

Of the fatty acids, acetic acid is found to be the dominant acid in spirit products and constitutes 40-95% of the total volatile acids in whisky, 50-75% in Cognac and brandy, and for rum 75-90% (Nykänen, 1986). Among the volatile compounds present in distilled beverages, aliphatic carbonyls are found to be of importance as they are present in large amounts. Of the carbonyl compounds, acetaldehyde is the major component and constitutes approximately 90% of the total aldehyde content in alcoholic beverages (Guymon, 1969).

The purpose of this experiment was to investigate the effect of barrel age on the chemical composition of the resultant three-year barrel matured brandy sample and to therefore be able to utilise barrels with a specific age to produce a three-year barrel matured brandy sample with a known sensory classification.

## 7.2 MATERIALS AND METHODS

In South Africa by law potstill brandies must be matured for a minimum period of three years in oak casks no bigger than 340 liters (South African Liquor Products Act No. 60 of 1989). Seventy-nine low wines (± 30% v/v) were distilled to produce distillates (± 70% v/v) and transferred into different barrels. Each barrel initially underwent a medium toast at the start of their lifetime as a brandy barrel after which they were stored in the same warehouse under identical storage conditions.

The three different types of barrels used for the purpose of this experiment were: *New barrels*: barrels that have not yet been used for the storage of potstill brandy (13 samples), *18 year old barrels*: barrels that have been filled five times with potstill brandy (39 samples) and *Red wine barrels*: barrels that have previously been used for the storage of red wine (27 samples).

### 7.2.1 Analyses of the volatile and non-volatile compounds

Samples were drawn after three-years of barrel aging and analyzed for volatile aroma compounds (using gas chromatography) and phenolic compounds [using GC and higher pressure liquid chromatography (HPLC)]. The method as described in chapter 3 (section 3.2.1) was used for the distillate volatile compound analyses. Samples were analyzed in triplicates and the standard deviation was found to be ≤ 5%.

### 7.2.2 Statistical analysis

The results obtained from the chemical analyses (7.1) was subjected to statistical analyses by conducting a one-way analysis of variance (ANOVA) and a principal component analysis

(PCA) to determine those compounds that were found to be significantly different amongst the respective three-year rebate samples and the barrels used. Only correlation coefficients greater than r=0.650 were investigated.

## 7.3 RESULTS AND DISCUSSION

### 7.3.1 Chemical analyses of the three-year barrel matured brandy samples after aging in barrels of different ages (*new barrels*, *18 year old barrels* and *red wine barrels)*

Table 7.1 shows the results of the one-way ANOVA for the totals of the chemical compounds/groups of compounds as well as the pH, TA and TP (total polyphenols) found in the different barrel treatments respectively.

### 7.3.1.1 pH, TA and TP

From the one-way ANOVA it is apparent that the pH is found to be significantly different (p≤0.05) amongst the different barrel treatments with the highest pH value being found in the barrels that are *18 years* in age. The *new barrels* and *red wine barrels* are both found to be significantly different to the *18 years barrels* (p≤0.05), but not when compared to each other. These two treatments are found to contain the lowest pH values.

When comparing the TA (total acidity), it is found that the highest TA value is found in the *new barrels* and is also found to be significantly different (p≤0.05) to all the other barrels. The lowest TA value amongst the different barrels is found in the *18 years barrels*. Total polyphenols (TP) were found to be highest in the *new barrels*. The *18 years barrels* are found to be significantly different (p≤0.05) to the other treatments by containing the lowest amount of TP.

### 7.3.1.2 Esters

The level of ethyl acetate and total ester concentration is found to be highest (p≤0.05) in *new barrels.* The reason for this could be due to the lower pH value found in the treatment using *new barrels*. A decrease in pH aids the production of esters by promoting esterification thereby increasing the concentration of esters (Perez-Prieto *et al.,* 2003). Studies by Ramey and Ough (1980) also show that the level of esters is found to be highest in the product at the beginning of maturation and are found to decrease as the maturation process continues. Again these findings are consistent with those of previous authors.

The compounds ethyl butyrate and the ethyl esters of the medium-chain fatty acids are lowest in concentration in the *red wine barrels.* However the levels of ethyl lactate and di-ethyl succinate are highest in the *red wine barrels*; thus indicating that malo-lactic fermentation had previously occurred in the *red wine barrels.* Due to the fact that red wine undergoes malo-lactic fermentation in barrels, the resultant compounds (ethyl lactate and di-ethyl succinate) are by-products of this secondary fermentation (Lonvaud-Funel, 1999) thus indicating that this could be the reason for the elevated levels of the above mentioned compounds.

### 7.3.1.3 Higher alcohols

The overall level of total higher alcohols and more specifically the concentration of n-propanol, i-butanol and i-amyl alcohol were all found to be lowest (p≤0.05) in the red wine barrels. Studies by Cerdán *et al.,* (2003) show that there is little increase in the concentration of higher alcohols during the ageing of alcoholic products and the levels of higher alcohols found in the product is determined by the amount of esterification and hydrolysis that takes place in the barrel during the ageing process. The pH was found to be highest in the *18 year*

*barrels*, but lowest in both the *new barrels* and *red wine barrels.* A lower pH aids esterification and therefore promotes the formation of esters. Esters are formed by the reaction of higher alcohols with acids; therefore if the reaction that promotes esters is favoured it results in a decrease of higher alcohols.  This trend can be seen for the treatment of *red wine barrels*, however this is not found to be the case for the treatment *new barrels*. Even though the pH is lower in the *new barrels*, perhaps the elevated level of esters is the reason for the increase in higher alcohol production.

### 7.3.1.4 Acids

The acids produced mainly during the fermentation process namely i-butyric, n-butyric, hexanoic, octanoic and decanoic acid were all found to be highest ($p \leq 0.05$) in concentration in the *red wine barrels.* Those acids that stem from the maturation and are supplied by the oak barrel namely gallic, vanillic, caffeic, syringic, p-coumaric and ellagic acid were all found to be lowest ($p \leq 0.05$) in concentration in the *18 year old barrel*, and inversely the acids gallic, syringic, p-coumaric and ellagic acid were highest ($p \leq 0.05$) in the *new barrels.*  The level of acetic acid and overall total acid concentration was also lowest ($p \leq 0.05$) in the *18 year barrels.* Studies by Caldeira *et al.*, (2002) show that the level of acetic acid was found to increase during the aging process irrespective of the maturation technique employed. The acetic acid level during the aging process was a result of oxidation reactions of ethanol and from wood extraction. Together with the acetic acid found in the distillate, acetic acid is also produced from the acetyl groups present in the wood xylans, which is an important group of hemicelluloses.

### 7.3.1.5 Aldehydes

The main aldehyde compound present in distilled beverages is acetaldehyde. Acetaldehyde levels are found to increase in the presence of oxygen as the production is a result of the oxidation of ethanol. The age of the barrel used for maturation will also influence the acetaldehyde concentration as older barrels have a slower diffusion rate as many of the pores are blocked with previous deposits (Nevares and Del-Alamo, 2008). The results shown in table 8.1 indicate that the level of acetaldehyde is higher in the *new barrels* and lower in the *18 year barrels*, therefore consistent with previous findings.

### 7.3.16 Ratios

The ratios of higher alcohols (HA) vs. esters, HA vs. acids and esters vs. acids were all found to be highest ($p \leq 0.05$) in the *18 year barrels*. The inverse was found to be true for *new barrels.* However for the ratio of aldehydes vs. esters and aldehydes vs. HA the level was found to be lowest ($p \leq 0.05$) in the *18 year barrels*. For the *new barrels*, the ratio of aldehydes

vs. HA was found to be highest (p≤0.05), and in the case of aldehydes vs. acids was lowest in the *red wine*.

## 7.3.1.7 Lactones

*Cis*-oak lactone and *trans*-oak lactone are products of the dehydration of 2-methyl-3-(3,4-dihydroxy-5-meth-oxybenzo)-octanoic acid (Onishi *et al.,* 1977). Studies by Chatonnet *et al.,* (1993) showed that these compounds were found to increase in concentrations with a higher degree of toasting. The results from table 8.1 show that the overall total lactone level is highest (p≤0.05) in the *new barrels* and lowest in the *18 year barrels*. Even though the barrels prior to storage underwent the same degree of toasting, the *18 year barrels* have stored 5 times more three-year rebate samples (3 years per storage session) and therefore have a decreased level of wood compounds as many of these compounds have already been extracted from previous storage times. Inversely the levels of lactones are highest in concentration in the *new barrels.* This is due to the fact that the new barrels have not previously been used for the storage of three-year rebate samples and therefore contain a higher concentration of these compounds that can ultimately be extracted into the final product.

## 7.3.1.8 Furanic compounds

The furanic compounds furfural and 5-methyl furfural are formed during the toasting of the wood through the degradation of carbohydrates and furfuryl alcohol is a result of enzymatic reduction of its analogous aldehyde during the aging process in the barrel and is dependent on temperature and pH (Boidron *et al.,* 1988). The amount of furfural and 5-methyl furfural found in the resultant product is not only determined by the degree of toasting but also the age of the barrel. The results found in table 7.1 show that the level of 5-hydroxy-methyl-2-furfural was found to be lowest (p≤0.05) in concentration in the *18 year barrels*. These findings are consistent with those of Cerdán *et al.,* (2003) whereby these compounds are found to be less in barrels that are older in age. Inversely the concentration of 5-hydroxy-methyl-2-furfural was found to be highest (p≤0.05) in concentration in the *new barrels.*

## 7.3.1.9 Volatile phenols

The compounds namely phenolic aldehydes, acetovaillone and phenolic alcohols are a result of thermal degradation of lignin. Lignins are branch-chained phenylpropanoid polymers and are found to be complex structures. The mechanism of extraction of phenolic aldehydes occurs in three different ways: through thermal degradation of lignin during toasting, through the extraction of free monomers present in lignin and through ethanolysis of lignin (Nishimura *et al.,* 1983). However, studies by Cerdán *et al.,* (2003) show that the concentrations of phenolic aldehydes found in the product is mainly determined by the type of oak (French or

American) rather than the age of the barrel. The results obtained in this study show that the level of vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde are all found to be highest (p≤0.05) in the *new barrels* and lowest in the *18 year barrels*. Thus indicating the barrels that are younger in age contain more of the above mentioned compounds when compared to those barrels that have previously been used for storage of three-year brandy samples. Even though these results are not consistent with previous findings, used barrels have shown to contain a smaller concentration of phenolic aldehydes, therefore resulting in a lower concentration of phenolic acids in the final brandy product.

Of the volatile phenols present, only guaiacol and eugenol are found to be significantly different between the different barrel treatments. The production of guaiacol is a result of thermal degradation of lignin and is formed from the second phase of heating after that of the phenolic aldehydes and consequently this compound is found in high quantities when toasting is more intense (Jackson, 1994). The concentration of guaiacol was found to be highest (p≤0.05) in the *new barrels* and lowest in the *18 year barrels*. *New barrels* contain a higher amount due to the fact that it has not previously been used for storage and the effect of toasting is more prominent. The inverse is true for the *18 year barrels*.

From the results seen in table 7.1 it is clear that the different barrel treatments have a significant effect on the chemical composition of the three-year barrel matured brandy samples.

**TABLE 7.1 ANOVA for the means of the totals of the chemical compounds for the different barrel treatments that are found to be significantly different (p≤ 0.05).**

| Compound (mg/L) | P-value | New barrels | 18 yrs barrels | Red wine barrels |
|---|---|---|---|---|
| pH | <0.0001 | 4.584 b | 5.132 a | 4.637 b |
| TA | <0.0001 | 0.565 a | 0.313 c | 0.485 b |
| TP | <0.0001 | 342.877 a | 102.074 c | 227.246 b |
| Ethyl acetate | 0.009 | 339.115 a | 275.203 b | 283.778 b |
| Ethyl butyrate | 0.018 | 2.482 a | 2.174 a | 1.796 b |
| i-Amyl acetate | 0.996 | 9.367 a | 9.469 a | 9.453 a |
| Ethyl lactate | <0.0001 | 61.185 b | 52.959 b | 74.494 a |
| Ethyl hexanoate | 0.010 | 9.018 a | 8.658 a | 7.909 b |
| Hexyl acetate | 0.439 | 1.128 a | 1.042 a | 1.118 a |
| Ethyl octanoate | 0.012 | 29.115 a | 27.124 a | 24.475 b |
| Ethyl decanoate | 0.005 | 41.035 a | 37.362 a | 34.027 b |
| Di-ethyl succinate | <0.0001 | 10.472 a | 5.338 b | 10.297 a |

| Compound (mg/L) | P-value | New barrels | 18 yrs barrels | Red wine barrels |
|---|---|---|---|---|
| 2-Phenyl ethyl acetate | <0.0001 | 0.793 b | 0.890 b | 1.044 a |
| **Total esters** | **0.001** | **503.710 a** | **420.219 b** | **448.389 b** |
| n-Propanol | 0.001 | 280.401 a | 264.794 b | 255.042 c |
| i-Butanol | 0.001 | 150.184 a | 143.868 a | 135.937 b |
| n-Butanol | 0.060 | 4.761 a | 4.573 a | 4.395 a |
| i-Amyl alcohol | 0.007 | 842.281 a | 818.146 a | 785.138 b |
| Hexanol | 0.022 | 12.933 a | 12.371 ab | 11.759 b |
| 2-Phenyl ethanol | <0.0001 | 12.751 b | 8.301 c | 15.213 a |
| **Total HA[a]** | **0.003** | **1303.310 a** | **1252.053 a** | **1207.483 b** |
| Acetaldehyde | <0.0001 | 107.903 a | 75.961 c | 86.773 b |
| Acetoin | <0.0001 | 2.311 b | 2.090 b | 3.393 a |
| Vanillin | <0.0001 | 3.370 a | 1.073 c | 2.654 b |
| Syringaldehyde | <0.0001 | 5.863 a | 1.704 c | 4.967 b |
| Coniferaldehyde | <0.0001 | 2.343 a | 0.113 c | 1.699 b |
| Sinapaldehyde | <0.0001 | 5.122 a | 0.412 c | 3.788 b |
| **Total aldehydes** | **<0.0001** | **126.901 a** | **81.353 c** | **103.267 b** |
| Acetic acid | <0.0001 | 234.798 a | 149.647 b | 246.428 a |
| Gallic acid | <0.0001 | 12.704 a | 2.666 c | 9.989 b |
| i-Butyric acid | 0.000 | 1.368 b | 1.422 b | 1.749 a |
| n-Butyric acid | 0.062 | 1.094 b | 1.138 b | 1.251 a |
| Hexanoic acid | 0.000 | 9.123 b | 10.484 b | 12.765 a |
| Octanoic acid | 0.001 | 42.044 c | 50.642 b | 58.185 a |
| Decanoic acid | 0.002 | 34.109 b | 39.948 b | 45.226 a |
| Protocatechuic acid | <0.0001 | 0.049 a | 0.000 b | 0.003 b |
| p-hydroxy-benzoic acid | 0.033 | 0.352 a | 0.000 b | 0.135 ab |

| Compound (mg/L) | P-value | New barrels | 18 yrs barrels | Red wine barrels |
|---|---|---|---|---|
| Vanillic acid | <0.0001 | 1.662 a | 0.286 b | 1.056 a |
| Caffeic acid | <0.0001 | 0.754 a | 0.004 b | 0.808 a |
| Syringic acid | <0.0001 | 2.671 a | 0.693 c | 1.955 b |
| p-Coumaric acid | <0.0001 | 0.546 a | 0.000 c | 0.527 b |
| Ferulic acid | 0.074 | 0.017 a | 0.000 a | 0.000 a |
| Ellagic acid | <0.0001 | 33.808 a | 12.804 c | 21.687 b |
| **Total acids** | **<0.0001** | **375.097 a** | **269.735 b** | **401.974 a** |
| 5-hydroxymethyl-2 furfural | <0.0001 | 1.583 a | 0.242 c | 1.163 b |
| Furfural | 0.007 | 18.998 b | 19.223 b | 21.091 a |
| 5-Methyl furfural | 0.000 | 0.844 a | 0.708 b | 0.895 a |
| **Total furans** | **0.000** | **21.425 ab** | **20.183 b** | **23.149 a** |
| Epicatechin | 0.400 | 0.000 a | 0.000 a | 0.019 a |
| Catechin | 0.000 | 1.160 a | 0.516 b | 0.050 c |
| Guaiacol | <0.0001 | 0.565 a | 0.265 c | 0.451 b |
| o-Cresol | 0.358 | 0.000 a | 0.011 a | 0.000 a |
| Phenol | 0.153 | 0.000 a | 0.014 a | 0.022 a |
| Ethyl guaiacol | 0.491 | 1.856 a | 1.743 a | 1.581 a |
| p-Cresol | 0.834 | 0.031 a | 0.016 a | 0.024 a |
| Eugenol | 0.022 | 0.985 a | 0.895 a | 0.211 b |
| 2,6-dimethoxy phenol | 0.450 | 1.187 a | 2.876 a | 1.432 a |
| **Total phenols** | **0.282** | **5.782 a** | **6.336 a** | **5.931 a** |
| t-Lactone | <0.0001 | 0.472 a | 0.011 c | 0.364 b |
| Cis-Lactone | <0.0001 | 1.006 a | 0.073 c | 0.658 b |
| **Total lactones** | **<0.0001** | **1.477 a** | **0.085 c** | **1.022 b** |
| Methanol | 0.519 | 308.962 a | 298.103 a | 306.704 a |
| HA vs. esters | 0.004 | 2.600 b | 3.042 a | 2.770 b |

| Compound (mg/L) | P-value | New barrels | 18 yrs barrels | Red wine barrels |
|---|---|---|---|---|
| HA vs. acids | <0.0001 | 3.517 b | 4.784 a | 3.167 b |
| Esters vs. acids | <0.0001 | 1.352 b | 1.606 a | 1.181 b |
| Aldehydes vs. esters | <0.0001 | 0.253 a | 0.195 b | 0.236 a |
| Aldehydes vs. HA | <0.0001 | 0.098 a | 0.065 c | 0.086 b |
| Aldehydes vs. acids | 0.002 | 0.340 a | 0.307 a | 0.271 b |

**Compounds/groups of compounds with different letters in the same row are found to be significantly different (p≤0.05). HA[a] (Higher alcohols).**

## 7.3.1.10 Principal Component Analysis (PCA) of the different barrels used and their chemical composition

The interrelationships between the compounds/groups of compounds and the different barrel types are shown in figure 7.1. The first principal component (F1) accounted for 58.04% of the total variation and the second principal component (F2) accounted for 41.96% of the total variation.
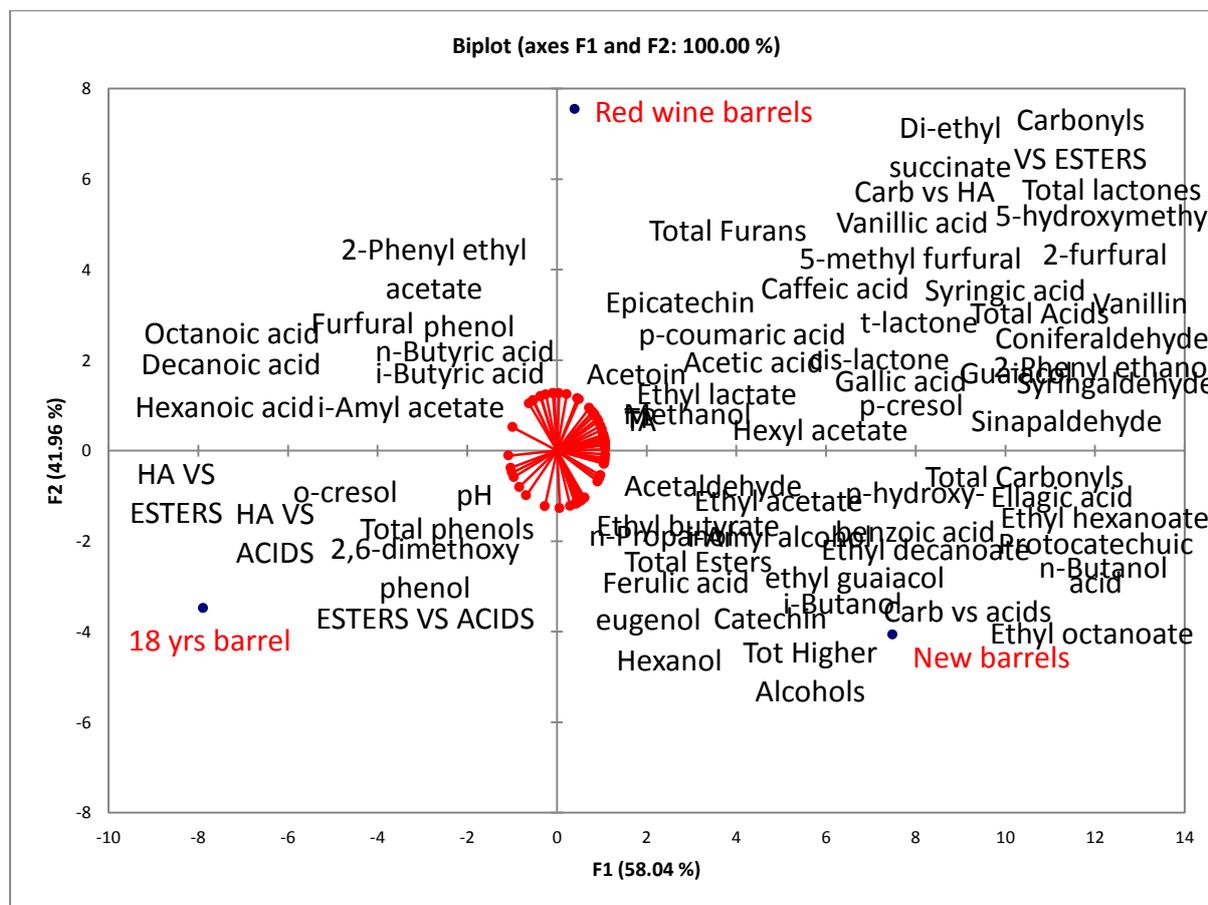


**FIGURE 7.1 PCA bi-plot showing the relationship between the different barrel types and the compounds/groups of compounds on F1 and F2.**

F1 indicated that the treatment *new barrels* (factor score=7.486) was found to be positively associated to the F1 axis and contrasted the most with *18 years barrels (*factor score=-7.887). The compounds/groups of compounds namely TA (r=986), TP(r=978), acetaldehyde (r=0.974), ethyl acetate (r=903), methanol (r=0.961), hexyl acetate (r=0.932), acetic acid (r=0.832), di-ethyl succinate (r=0.901), 2-phenyl ethanol (r=0.660), total esters (r=0.974), total acids (r=0.783), total carbonyls (r=0.998), the ratio of carbonyls vs. esters (r=0.981), carbonyls vs. HA (r=0.994), total lactones (r=0.988), gallic acid (r=0.977), 5-hydroxymethyl-2-furfural (r=0.986), protocatechuic acid (r=0.875), p-hydroxy benzoic acid (r=0.984), 5-methyl furfural (r=0.736), vanillic acid (r=1.000), syringic acid (r=0.994), syringaldehyde (r=963), p-coumaric acid (r=0.774), ferulic acid (r=0.843), ellagic acid (r=0.991), coniferaldehyde (r=0.981), sinapaldehyde (r=0.980), guaiacol (r=0.996), *trans*-lactone (r=968), *cis*-lactone (r=0.995), p-cresol (r=0.992) were found to be positively correlated to the F2 axis and therefore positively associated to *new barrels* and negatively associated to *18 year barrels*.

Inversely the compounds/groups of compounds pH (r=-0.925), i-amyl acetate (r=-0.913), the ratio of higher alcohols vs. esters (r=-0.996), higher alcohols vs. acids (r=-0.774), o-cresol (r=-0.888), 2,6-dimethyoxy phenol (r=-0.954) were found to be negatively correlated to the F1 axis and therefore negatively associated to *new barrels*, but however positively associated *18 year barrels.*

F2 indicated that the treatment red wine barrels (factor score=7.547) was found to be positively associated to the F2 axis and contrasted the most with 18 year barrels (factor score=-3.478) and new barrels (factor score=-4.068). The compounds/groups of compounds namely acetoin (r=0.979), ethyl lactate (r=0.908), i-butyric acid (r=0.996), n-butyric acid (r=0.974), 2-phenyl ethyl acetate (r=0.939), hexanoic acid (r=0.945), 2-phenyl ethanol (r=0.743), octanoic acid (r=0.870), decanoic acid (r=0.975), total furans (r=0.889), furfural (r=0.998), 5-methyl furfural (r=0.677), epicatechin (r=0.999), phenol (r=0.818) were found to be positively correlated to the F2 axis and therefore positively associated to *red wine barrels* and negatively associated to *18 year barrels* and *red wine barrels.*

Inversely the compounds/groups of compounds ethyl butyrate (r=-0.913), n-propanol (r=-0.819), i-butanol (r=-0.916), n-butanol (r=-0.880), i-amyl alcohol (r=-0.925), ethyl hexanoate (r=-0.962), hexanol (r=-0.899), ethyl octanoate (r=-0.922), ethyl decanoate (r=-0.875), total higher alcohols (r=-0.868), the ratio of esters vs. acids (r=-0.777), carbonyls vs. acids (r=-0.912), total phenols (r=-0.968), catechin (r=-0.841), ethyl guaiacol (r=-0.862) and eugenol (r=-0.998) were found to be negatively correlated to the F2 axis and therefore negatively associated to red wine barrels, but however positively associated to *18 year barrels* and *new barrels.*

## 7.4 CONCLUSIONS

The results obtained in this study indicate that barrels of different ages produce three-year brandy samples with specific chemical compositions. If a distillate was matured in a *18 year barrel*, it was found that the resultant three-year barrel old matured brandy contained overall a lower amount of TA (titrateable acidity), TP (total polyphenols), total esters, acetaldehyde,

gallic, vanillic, syringic, caffeic, p-coumaric and ellagic acids, acetic acid as well as a lower overall total acid concentration. The ratios of aldehydes vs. esters and aldehydes vs. HA are also found to be lowest in the *18 year barrels*, together with a lower concentration of total lactones, total furans and phenols (more specifically syringaldehyde, vanillin, coniferaldehyde sinapaldehyde, syringaldehyde, guaiacol and eugenol). The reason for the decreased level of the above mentioned compounds is due to the fact that the extraction of the compounds from the oak barrels is exhausted as a result of the repeated usage for the storage of three-year brandy samples therefore yielding a product that contains a significantly lower amount of wood and volatile compounds.

The inverse was found to be true for the *new barrels* for the above mentioned compounds and ratios. However, the pH and the ratios of HA vs. esters, HA vs. acids and esters vs. acids were found to be higher in the *18 year barrels.*

The production of higher alcohols cannot be conclusively decided as some results do display similar trends to previous studies. However further investigation is needed in this regard as the results obtained for *new barrels* is not what was expected in terms of the total higher alcohol concentration.

In the *red wine barrels* the levels of ethyl lactate and di-ethyl succinate were higher in concentration again this could be due to the malolactic fermentation that previously took place in the barrels prior to storage of the distillates. The ratio of aldehydes vs. acids was lowest in the *red wine barrels*, however the levels of the medium-chain fatty acids were found to be higher in concentration.

It would seem that by using *18 year barrels* one could produce a three-year barrel old matured brandy that was lower in esters, acids and a higher ratio of HA vs. esters, HA vs. acids and esters vs. acids. If *new barrels* were used then the three-year barrel matured brandy sample would contain a higher concentration of esters, with a decreased ratio of HA vs. esters, HA vs. acids and esters vs. acids.

## 7.5 LITERATURE CITED

Batista de Aquino, F.W., Rodrigues,S., Ferreira do Nascimento, R., Casimiro, A.R.S. 2006. Simultaneous determination of aging markers in sugar cane spirits. Food. Chem. 98: 569-574.

Boidron, J.N., Chantonnet, P., Pons, M. 1988. Influence du bois sur certaines substances odorantes des vins. Connaissance-de-la-Vigne-et-du-Vin. 22, 4: 275-294.

Caldeira, T.G., Belchior, A.P., Climaco, M.C., Bruno de Sousa, R. 2002. Aroma profile of Portuguese brandies aged in chestnut and oak woods . Analytica. Chimica.Acta. 458, 1: 55-62.

Caldeira, I., M.C. Clı´maco a, R. Bruno de Sousa b, A.P. Belchior. 2006. Volatile composition of oak and chestnut woods used in brandy ageing: Modification induced by heat treatment. J. Food. Eng. 76. 202-211.

Caldeira,I., Anjos, O., Portal, V., Belchior, A.P., Canas, S. 2010. Sensory and chemical modifications of wine-brandy aged with chestnut and oak wood fragments in comparison to wooden barrels. Anal.Chim.Acta. 660: 43-52.

Cerdán, T.G., Goni, D.T., Azpillicueta, C.A. 2003. Accumulation of volatile compounds during ageing of two red wines with different composition. Jounral of food engineering. 65, 3: 349-356.

Chatonnet, P., D. Dubordieu., J. Boidron. 1993. Effect of fermentation in oak barrels on the composition and quality of white wines. Wine. Ind. J. 2: 73-84.

Guymon, J.F. 1969. GC determination of ethyl esters in brandy or wine distillates. Am. J. Enol. Vitic. 20, 2: 76-85.

Jackson, R.S. 1994. Wine Science: Principles and applications. Academic Press. Oxford, UK.

Jounela-Eriksson, P. 1981. Predictive value of sensory and analytical data for distilled beverages. *In* Flavour'81. Schreier, P. (1st Ed). Berlin.

Lonvaud-Funel, A. 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Biomedical and life sciences. 76, 1-4: 317-331.

Mosedale, J.R and J-L. Puech. 1998. Wood maturation of distilled beverages. Trends in Food Science and technology. 9, 3: 95-101.

Nevares, I and Del-Alamo, M. 2008. Measurement of dissolved oxygen during red wines tank aging with chips and micro-oxygenation. . Anal. Chim. Act. 621, 1: 68-78.

Nishimura, K., M. Onishi., M. Masuda., K. Koga., R. Matsuyama. 1983. Reactions of wood components during maturation. *In* Flavour of distilled Beverages: Origin and development. JR Pigott (Ed). Ellis Horword, Chichester UK.  241-255.

Nykanen, L.H. 1986. Formation and occurrence of flavour compounds in wine and distilled beverages. Am. J. Enol. Vitic. 37, 1: 84-94.

Onishi, M., J.F. Guymon., E.A. Crowell. 1977. Changes in some volatile constituents of brandy during aging. Am. J. Enol. Vitic. 28, 3: 152-158.

Perez-Prieto, L.J., Lopez-Roca, J.M., Martinez-Cutillas, A., Pardo-Minquez, F., Gomez-Plaza, E. 2003. Extraction and Formation Dynamic of Oak-Related Volatile Compounds from Different Volume Barrels to Wine and Their Behavior during Bottle Storage. J. Agric. Food Chem. 51, 18: 5444–5449.

Puech, J-L. 1984. Characteristics of oak wood and biochemical aspects of Armagnac aging. Am. J. Enol. Vitic. 35, 2: 77-81.

Pollnitz, A.P.,  Jones, G.P., Sefton, M.A. 1999. Determination of oak lactones in barrel-aged wines and in oak extracts by stable isotope dilution analysis. J. Chrom. A. 857: 239-246.

Ramey, D. D., & Ough, C. S. 1980. Volatile ester hydrolysis or formation during storage of model solutions and wines. J. Agric. Food. Chem. 28, 928–934.

Salo, P., Nykänen, L Suomalainen, H. 1972. Odour thresholds and relative intensities of volatile aroma components in an artificial beverage imitating whiskey. J. Food. Sci. 37, 3: 394-398.

Steger, C.L.C., and M. Lambrechts. 2000. The selection of yeast strains for the production of premium quality South African brandy base products. J. Ind. Micro. Biotech. 24, 6: 431-440.

# Chapter 8

# DISCUSSION AND CONCLUSION

# 8. DISCUSSION AND CONCLUSION

## 8.1 CONCLUDING REMARKS AND OTHER PERSPECTIVES

South Africa is one of the largest brandy producing countries in the world and falls 8th in the global market with total sales of over forty eight million litres (Holtzkamp, 2012). Considering the total South African population and the fact that only a small percentage is exported makes brandy one of the most important spirits consumed in the country (South African Wine Industry Information and Systems 2013). Local statistics indicate that brandy is one of the most purchased spirit beverages, second to whisky and is therefore such an integral part of the South African landscape (Toerien, 2008).

The production of brandy involves a multitude of steps, namely the harvesting of grapes, alcoholic fermentation, distillation, barrel maturation and blending of the final product. Due to the fact that brandy is a multi-step process, there are many factors that can influence the outcome, composition and quality of the final product.

In South Africa there are three main categories of brandy: blended, vintage and pot still. Within each category there are many different styles which are ultimately dependent on the producing company's needs and desires (Snyman, 2010). Each of these styles of brandy varies greatly with regard to their organoleptic profile (Toerien, 2008). Depending on the category of brandy being made, the three year pot still brandy is either bottled as is (pure pot still brandy) or used as part of a blended or a vintage brandy (Snyman, 2005).

Blended brandy must contain at least 30% of pot still brandy matured for a minimum of three years in barrels. The remainder of the brandy is made up of neutral wine spirit, and this must not exceed 70% of the final blend. Blended brandy is diluted to minimum 43% v/v as it is intended to be enjoyed in a long drink with a mixer and ice or in cocktails (Kister, 1992).

Pot still brandy is by far the most complex brandy of the three types and is generally the richer, fruitier and smoother (Toerien, 2008; Léaute, 1990). By South African law, pot still brandy must contain at least 100% of pot still brandy matured for a minimum of three years. Many pot still brandies are matured for much longer periods. Pot still brandies are bottled at a minimum alcohol concentration of 38% v/v and are ideally enjoyed neat or over ice. Vintage brandy must have at least a minimum of 30% potstill brandy matured for at least 8 years, a maximum of 60% column still spirit matured for at least eight years and a maximum of 10% wine spirits (unmatured). Due to the blend composition of vintage brandies, these tend to typically have a more dominant wood maturation flavour profile and a drier finish on the palate. They are also bottled at a minimum alcohol concentration of 38% abv and are also enjoyed neat or over ice.

The matrix of distilled beverages are complex and past research has identified over 500 volatile compounds and if the non-volatile compounds were to be taken into account the amount would probably triple (Nykänen and Suomalainen, 1983). The flavour and aroma of distilled beverages are determined by many compounds, and there is not an individual

compound responsible for different nuances found within the product but rather the combination and the interaction of the compounds present.

There has been a great deal of research conducted over the years with regards to the aroma and flavour of alcoholic beverages. However the main focus was pertaining to the quality and not taking into account the style of the product. As a considerable amount of time and capital goes into the production of brandy, it is crucial that there is a thorough understanding of the complexities affecting the perception of not only quality but also style of the product. Therefore the aim of the study was to identify factors that influence the style of brandy and by doing so, being able to manipulate these factors in order to produce a brandy with a desired style characteristic.

The end goal of this study was to identify what differentiates all brandy styles, however due to the complexity of this hypothesis it was decided to focus mainly on the factors that differentiate between the two extreme styles (class one and class five) from each other with not only with regards to their aroma and flavour but also their chemical composition. Once these criteria are known, manipulation of certain factors can then be used in order to produce a brandy with a desired style and characteristic. This therefore limits the amount of error, time and capital needed to produce a brandy that does not meet the company's needs and desires.

The first part of the study involved identifying the chemical composition (both volatile and non-volatile) of the two extreme styles (class one and class five) of three-year barrel old matured brandy samples. The results obtained showed that the class five three-year barrel old matured brandy samples contained a lower ratio of higher alcohols vs. esters and higher alcohols vs. acids and higher concentration of total esters, total acids, total furans and total lactones and were found to be more "fruity" in aroma and flavour. The class one three-year barrel matured brandy samples were found to contain a higher concentration of the ratio of higher alcohols vs. esters and higher alcohols vs. acids and a higher concentration of total higher alcohols and total phenols and a lower amount of total esters, total acids, total lactones and total furans and were perceived to be less "fruity" in overall aroma and flavour when compared to the class five brandy samples. However results showed that there are indeed variations within a specific class and a larger database and further investigation is needed to conclusively determine this theory.

The second part of the study involved identifying the chemical profiles of base wines. The results in chapter 4 show that the base wine samples that received a sensory score of one contained less total esters, but however contained higher concentrations of total higher alcohols, total acids and a higher ratio of higher alcohols vs. esters when compared to those base wine samples that scored a sensory score of five. These base wine samples were also perceived to be less "fruity" and are found to more "neutral" in aroma and flavour. Base wine samples that scored a sensory score of five were found to contain more total esters, but less total higher alcohols and total acids and were considered to be fruitier in nature.

As mentioned in chapter 3, class one three-year barrel matured brandy samples are found to also contain lower amounts of total esters and total acids, but contain higher concentrations of total higher alcohols and a higher ratio of higher alcohols vs. esters and higher alcohols vs. acids. For the class five three-year barrel matured brandy samples they were found to contain higher amounts of total esters and total acids but lower amounts of total higher

alcohols, and ratios of higher alcohols vs. esters and higher alcohols vs. acids. It seems there is a correlation between the chemical composition of those base wines that received a sensory score of one and class one three-year barrel matured brandy samples, and those of base wine samples that scored a sensory score of five and the class five three-year barrel matured brandy samples.

Even though the amount of total acids is higher in the base wines with a sensory score of one when compared to the class one three-year barrel old matured brandy samples, studies indicate that the total acids (specifically acetic acid) decreases during the distillation process as it can be removed in the head fraction. The total acids are found to be higher in the class five three-year barrel old matured brandy samples when compared to the base wine samples that received a sensory score of five; however these acids could be attributed to the accumulation of acids derived from the maturation process.

Therefore theoretically one could deduce that if a base wine with a sensory score of one was to be distilled; it could possibly yield a class one three-year barrel matured brandy sample. The same trend could be observed for the base wine samples that receive a sensory score of five which in turn could yield a class five three-year barrel matured brandy sample.

The third part of the study involved investigating the effect of yeasts (228, VIN13, Alchemy 1 and D254B) and fermentation temperature (15ºC and 24ºC) on the chemical composition of the base wines.

The results obtained in chapter 5 show that with regards to the yeast used the level of ethyl acetate and overall total ester concentration was found to be significantly higher in the yeast alchemy 1 and was found to increase when compared to the control yeast strain 228. The higher alcohol i-amyl alcohol was found to decrease when the yeast VIN13 and Alchemy 1 were used when compared to the control yeast 228. The overall total acid concentration was found to be highest in concentration when the yeast alchemy 1 was employed in comparison to all the other yeast strains and more specifically the control yeast 228.

The higher alcohols namely i-butanol, i-amyl alcohol, 2-phenyl ethanol and overall total higher alcohols were all found to be significantly higher in the 24ºC fermentation temperature treatment when compared to the 15ºC fermentation treatment. The concentration of acetic acid, i-butyric acid, i-valeric acid and overall total acids were found to be significantly higher in the 24ºC fermentation treatment when compared to that of the 15ºC fermentation treatment.

Therefore by using the 24ºC fermentation temperature treatment, it will result in a base wine with an increased level of higher alcohols and total acids (more specifically acetic acid) as well as a higher ratio of higher alcohols vs. esters. Similarly by using the yeast Alchemy 1 will result in a base wine with elevated concentrations of total esters and more specifically ethyl acetate.

The fourth part of the study investigated the use of different condenser water temperatures on the chemical composition of the resultant distillate. The results obtained in these experiments (Chapter 6) show that the low wine prior to distillation has a larger influence on the production of the volatile compounds in the resultant distillate than the condenser water temperature employed during the distillation process.

The total ester concentration and more specifically ethyl acetate was not found to differ between the different condenser water temperatures employed for the distillates produced by low wine one and low wine two. However for the distillates produced by low wine three, levels of total esters and ethyl acetate were found to be significantly lower in the 20ºC treatment when compared to that of the 8ºC and 12ºC treatment. The total higher alcohol concentration did not seem to differ between the different condenser water temperatures in the distillates produced from all three low wines.

The results show that the distillates produced from low wine one showed that the 8ºC condenser temperature were found to contain overall higher concentrations of total acids, and more specifically decanoic acid. The inverse was found to be true for those distillates produced by low wine three whereby the 20ºC condenser water temperature was found to contain the highest amount of total acids, acetic acid and octanoic acid. No differences between the carbonyl concentrations were noted for the different condenser water temperatures.

The pH value was significantly lower (≤0.05) in the 20LW3 when compared to the 8LW3 and 12LW3. However, the pH was found to be not significantly different in the distillates produced by low wine one and two. However, it was found to be lower in the 20LW3 treatment when compared to the other condenser water temperatures. The TA value was also only found to be significantly higher in the 8LW1 and the 20LW3 treatment respectively.

The results obtained in these experiments show that the use of different condenser water temperatures during the distillation process has little to no effect on the total ester or total higher alcohol concentration of the resultant distillate. It would seem that the chemical groups' esters and higher alcohols found in the distillates are predetermined by the chemical composition of the low wine prior to distillation rather than the condenser water temperature employed.

The amount of total acids found in the resultant distillate could be influenced by the condenser water temperature. In the distillates produced by low wine one the 8ºC condenser water temperature treatment was found to produce a distillate with a higher total acid concentration, and in that of the distillates produced by low wine three the 20ºC condenser water temperature treatments was found to produce a distillate with a higher total acid level. It would seem that further experiments would be needed in order to confirm these results.

The final and sixth part of the study involved investigating the influence of different type of barrel age on the chemical composition of the two extreme three-year barrel old matured brandy samples (class five and class one). The results obtained in chapter 7 show that *18 year barrels* produce a three-year barrel matured brandy samples that was lower in esters, acids and a higher ratio of HA vs. esters, HA vs. acids and esters vs. acids. If *new barrels* were used then the three-year barrel old matured brandy samples would contain a higher concentration of esters, with a decreased ratio of HA vs. esters, HA vs. acids and esters vs. acids.

In summary it is concluded that the use of certain factors, namely the original makeup of the base wine (chemical composition), fermentation temperature (24ºC), yeast (Alchemy 1) and barrel maturation (barrels *18 years* in age and *new barrels*) do in fact influence the outcome of the style of three-year barrel old matured brandy samples (class one and class five) and can be manipulated to produce a brandy with a desired style. However, the use of condenser

water temperature has little to no effect on the chemical composition of the resultant distillate and in turn the three-year barrel old matured brandy samples and further investigation is needed to conclusively determine these findings.

It is recommended that analysing the base wine prior to distillation using GC-FID to identify the chemical composition is the most important step in determining the outcome of the style of brandy.

## 8.2 LITERATURE CITED

Holtzkamp, E. 2012. Liquor consumption patterns in South Africa. South African Wine Industry Information and Systems.

Kister, H.Z. 1992. Distillation design. (1st Ed). McGraw-Hill. Columbus.

Léaute, R. 1990. Distillation in Alambic. American journal of Enology and Viticulture. 41, 1: 90-103.

Nykanen, L and Soumalainen, H. 1983. Aroma of beer, wine and distilled alcoholic beverages. Akademie-Verlag. Berlin. Germany.

South African Wine Industry Information and Systems. 2013

Snyman, C. 2010. Personal communication.

Toerien, W. 2008. Firewater. Quivertree publications. Cape Town. South Africa.