Monitoring the quality control chain from vineyard to wine: An industrial case study

by

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it to any university for a degree.

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SUMMARY

The production of premium quality wine is dependant on excellent management of the total wine production value chain. To achieve this we need rapid and reliable analytical tools. Over the last decade Fourier transform infrared (FT-IR) spectroscopy has made a significant contribution to wine research and in the last five years South African institutions have also exploited the use of this technology not only for research, but also in industrial cellars.

The FT-IR apparatus is equipped with global calibrations and therefore we first investigated the validity of these for South African conditions. To achieve this new calibration sets for pH, titratable acidity and °Brix were made and compared to the global calibrations with statistical methods. Results obtained between the °Brix calibrations displayed high correlation and the global calibration can therefore be implemented. However, the new TA calibration was more accurate than the global calibration. Results were inconclusive for the new pH calibration sample set and therefore needs to be enlarged before it can be validated as the possibility of being more accurate exists. It was concluded that FT-IR spectroscopy in the simultaneous measurement for °Brix, pH and TA in grape must showed potential for accurate analysis and quality control purposes in an industrial cellar. Rapid analysis of these parameters will lead to higher throughput of grape must samples in the laboratory as well as adhering to good laboratory practices by validation.

The importance of correct sample preparation in the laboratory was illustrated when using FT-IR spectroscopy for one-step analysis and adjustments to global calibrations. Results obtained showed that grape parameters such as °Brix, nitrogen content were not influenced by the two sample preparation methods (hand pressed vs. homogenised), but pH, TA, colour index, anthocyanins and polyphenols were influenced.

Important key factors were identified in the quality control chain from vineyard to the cellar. Numerous grape loads had an increase in microbial populations after harvesting the vineyard and transport to the weighbridge. Transport is critical especially for the vineyards in the Lutzville area (had the highest yeast population), which are situated the furthest from the cellar. Sauvignon blanc had the highest acetic acid bacteria and lactic acid bacteria populations compared to the other cultivars. Gluconic acid, glycerol and arabitol was highly correlated to each other. High populations of acetic acid bacteria and lactic acid bacteria also had high levels of gluconic acid and 2,3-butanediol in the grape juice. Meso-inositol differed significantly between the vineyard and weighbridge and it had a high standard deviation compared to the mean value of all the samples between the vineyard and weighbridge. Temperature of grape loads delivered to the cellar ranged from 14 to 36ºC, which had a major impact on the grape quality and the resultant wine.

It can be concluded for this study that management of the total value chain is of critical importance to ensure that A-grade grapes results in good quality wine that merits the effort of the grape producer.

OPSOMMING

Die produksie van premiumgehalte wyn is afhanklik van ‘n uitnemende bestuurstelsel van die totale waardeketting van wynproduksie. Om hierdie doel te bereik is analitiese hulpbronne nodig wat vinnige, akkurate en herhaalbare resultate lewer. Fourier transformasie infrarooi (FT-IR)-spektroskopie het oor die laaste dekade ‘n aansienlik bedrae tot wynnavorsing gelewer. In Suid-Afrika het wynlaboratoriums en industriële kelders al hoe meer van hierdie tegnologie vir navorsing en die roetine analises van druif- en wynkomponente begin gebruik maak.

Die FT-IR-spektrometer wat gebruik is in hierdie studie word aangekoop met globale kalibrasies en daarom is die validasie van hierdie kalibrasies heel eerste ondersoek. Nuwe FT-IR kalibrasiemodelle vir °Brix, pH en titreerbare suur (TS) is gemaak en vergelyk met die globale kalibrasies met behulp van statistiese metodes. Baie goeie korrelasies is tussen die °Brix-kalibrasies verkry en die globale kalibrasie is geïmplementeer. Die nuwe TS-kalibrasie was egter meer akkuraat as die globale kalibrasie. Die nuwe pH-kalibrasie dui op die moontlikheid dat dit meer akkuuraat as die globale kalibrasie is, maar die aantal monsters moet vermeerder word, sodat validasie eers gedoen kan word alvorens ‘n besluit geneem kan word. Daar is gevind dat die gelyktydige meting van °Brix, pH en TS in druiwe m.b.v. FT-IR-spektroskopie potensiaal toon vir akkurate analises en kwaliteitsbeheer in ‘n industriële kelder. Vinniger analise van hierdie komponente sal aanleiding gee tot ‘n hoër deurset van monsters in die laboratorium.

Die belangrikheid van korrekte monstervoorbereidingsprosedures vir druifanalise is in hierdie studie geïllustreer. Die onderskeie waardes vir °Brix en stikstofinhoud (soos gemeet met FT-IR-spektroskopie) het geen verskil getoon wanneer die druiwe met twee monstervoorbereidingsmetodes, handgemaal en homogenisering onderskeidelik, voorberei is nie. Die resultate vir pH, titreerbare suur, kleurindeks, antosianiene en polifenole het wel beduidend verskil in monsters wat met dié twee metodes voorberei is.

Belangrike sleutelfaktore is geïdentifiseer in die waardeketting vanaf die wingerd tot by die kelder. Verskeie druifvragte het ‘n styging in mikrobiologieselading getoon ná oes in die wingerd en ná vervoer by die weegbrug. Vervoer is ‘n kritiese faktor vir al die wingerde in die Lutzville-area (hoogste gispopulasie) wat die verste van die kelder gelëe is. Sauvignon blanc bevat die hoogste asynsuur- en melksuurbakterieladings in vergelyking met die ander kultivars. Glukoonsuur, gliserol en arabitol was hoogs korreleerbaar met mekaar. Hoë populasies van asynsuur- en melksuurbakterieë het gepaard gegaan met hoë vlakke glukoonsuur en 2,3-butaandiol in die druiwesap. Meso-inositol het beduidend verskil tussen die wingerd en weegbrug, en het ‘n hoë standaardafwyking getoon in vergelyking met die gemiddelde waardes van al die druifvragte tussen die wingerd en weegbrug. Die temperatuur van die druifvragte met ontvangs by die kelder was tussen 14 en 36°C. Dit het ‘n beduidende impak op die druifkwaliteit en uiteindelike wynkwaliteit gehad.

Uit hierdie studie is dit dus duidelik dat die bestuur van die totale waardeketting krities belangrik is om te verseker dat A-graad druiwe die gewenste goeie kwaliteit wyn lewer en dat druifprodusente se wingerdkundige insette reflekteer in die gehalte van die wyn.

BIOGRAPHICAL SKETCH

Marinda Swanepoel was born in Port Elizabeth, South Africa on 7 January 1966. She attended Rowallan park Primary School and matriculated at Vredenburg High School in 1983. Marinda obtained a BSc degree in Home Economics in 1988 at the University of Stellenbosch.

Marinda joined Westcorp International as laboratory manager in 1999 later to become winemaker in the experimental cellar at Spruitdrift Winery. In 2002 she was promoted to Group Manager: Quality and Research and enrolled for an MSc degree in Wine Biotechnology at the Institute for Wine Biotechnology, Stellenbosch University.

This thesis is dedicated to my family for their continuous support and enthusiasm.

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PREFACE

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

Chapter 1 **General Introduction and Project Aims**

Chapter 2 **Literature Review**

Microbial metabolites as markers in wine grapes and their relationship to wine quality

Chapter 3 **Research Results**

The evaluation of Fourier transform infrared spectroscopy for the quantification of °Brix, pH and titratable acidity in grape must

Chapter 4 **Research Results**

The influence of sample preparation methodology on the analysis of wine grape samples, with Fourier transform infrared spectroscopy

Chapter 5 **Research Results**

Identification of key factors affecting the value chain of wine production: An industrial case study

Chapter 6 **General Discussion and Conclusions**

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION AND PROJECT AIMS

1. GENERAL INTRODUCTION AND PROJECT AIMS

1.1 INTRODUCTION

It has been said that if the outcome of a process can not be measured, then the process can not be controlled, and if the process can not be controlled it can not be improved (Gishen *et al*., 2001). Worldwide, producing cellars are implementing quality control measures to meet increasing consumer demands of wines of consistent and high quality (Poni *et al.*, 1995). For the producing wine cellar, a holistic approach is clearly necessary to identify the key factors that distract from wine quality and at the same time, to implement quality control strategies that will result in cost effective production of quality wine. One of the major obstacles in achieving this is the question what parameters should be monitored in the vineyard to ensure that optimal grape quality can be achieved. Grape quality is a complex, multi-facetted issue in wine production. In large industrial cellars the mere logistics of handling large volumes of heterogeneous and physically and chemically unstable raw grape material, are challenging and make the setting of appropriate quality control measures difficult (Linton & Wall, 1996; Riley, 1996).

The grape maturity level is one of the main criteria used to assess potential wine quality. The use of total soluble solids (TSS), ºBrix, pH and titratable acidity are well established criteria for the evaluation of grape maturity (Iland *et al*., 2000; Zoecklein, 2001). Several studies have shown that these traditional measurements may not be sufficient indicators of potential wine quality, particularly in warm climates. Grape soundness, juice aroma assessment and berry colour for red varieties are also important according to Iland *et al.* (2000).

One of the best methods to assess ripening and vineyard health is with systematic and representative sampling and establishing of trends that facilitate the setting of an optimal date for harvesting and also facilitate the early detection and monitoring of damage, disease and spoilage of grapes. Monitoring maturation in the vineyard does pose problems related to the large variability of berry composition within a vineyard. Precise data needs to be compared during maturity monitoring from one week to another within the same vineyard and over consecutive vintages.

Wineries carry out load assessment to reduce financial loss (Riley, 1996). From a quality system perspective, this means, for example, that different classified grapes such as ripe and unripe grapes, or premium and commercial grade grapes can be

grouped together for processing, to ensure that grapes are used in the highest potential end use. Grapes received at wineries are visually inspected for mould and severe microbial contamination. This leads to variability between inspectors at grape-receiving areas. Machine harvested grapes loses its structural integrity and makes visual inspection difficult (Kupina, 1984). Also, visual inspection only takes external contamination development into account. The degree of mould and rot contamination has always been hard to quantify in harvested grapes. *Botrytis cinerea* has already altered the grape composition before emerging to the surface of the grape. Furthermore, it is difficult to see infection on the surface of red grapes. To improve the assessment of the hygienic status of grape berries, a more reliable and objective strategy is needed.

Grape microbial flora has a strong impact on wine composition and it is therefore important to develop rapid and reliable tools for the assessment of berry flora (Bisson, 2001). The producing of premium wines relies on several factors such as excellent quality grapes, well controlled fermentation and ageing process, excellent bottling facilities, high standard corks and in general a hygienic cellar environment.

Quantification of mould, yeast and bacterial metabolites in collected juice samples can be used to help evaluate fruit quality. The nature and concentration of microbial metabolites differ as a function of biological and a-biotic factors (Zoecklein *et al.*, 1995). Key indicators of fruit rot, such as the presence and concentration of ethanol, glycerol, gluconic acid, galacturonic acid, citric acid, laccase, acetic acid and lactic acid should be rapidly and easy to quantify at winery grape intake.

When grape samples are measured for payment or quality control, analysis time, accuracy and precision are key parameters (Andersen *et al.*, 2002). Recently, however, focus has moved towards FT-IR technology utilizing the mid-infrared region, since it offers accurate and simultaneous determination of chemical components in a short period of time (Patz *et al.*, 2004). Calibrations were developed for grape analyses consisting of the maturity parameters that included sugar, ºBrix, total acid, potential degree alcohol, tartaric acid, malic acid, polyphenol index, colour intensity, alfa-amino nitrogen and ammonia as well as measuring metabolites produced by different microorganisms responsible for grape diseases (Dubernet *et al.*, 2001). The use of these calibrations has facilitated the rapid accumulation of chemical data necessary for establishing quality control strategies in the wine analytical laboratory.

1.2 PROJECT AIMS

The over-riding goal of this study was to evaluate the use of FT-IR spectroscopy as a rapid and reliable tool to assess quality of the total value chain in wine production that is, from vineyard to final wine product. This is the first study of its kind in an industrial cellar in South Africa and therefore of utmost importance for other cellars since the use of the Winescan instrument is widespread in wine analytical laboratories in the South African wine Industry.

The specific aims of the work were:

a) to validate the commercially available Winescan calibrations for ºBrix, pH and TA and to compare the prediction errors to those of new calibrations for these parameters, using data from grapes grown in the Olifants River Valley;
b) to determine the influence of two sample preparation methods on the results of the analysis of grape must using the Winescan FT 120;
c) to determine which sample preparation method correlates to cellar production activities;
d) to establish a data basis for maturity profiles of grapes from specific vineyards;
e) to determine the microbial population from vineyards in the Olifants River Valley;
f) to compare microbial population to microbial metabolites measured in the grape must using FT-IR;
g) to compare the microbial population and metabolites in each vineyard to the microbial population and metabolites when the grapes are delivered to the cellar;
h) to compare maturity parameters measured in the vineyard to measurements at the weighbridge;
i) to monitor the temperature of grape intake;
j) to monitor changes in grapes in maturity parameters and microbial metabolites, between grape loads from the same vineyard delivered at different times to the weighbridge;
k) to evaluate the outcome of specific vineyards graded as being of premium quality in relation to wine quality; and

l) to correlate sensorial data from wines made from specific vineyards to chemical analyses of the grapes.

1.3 LITERATURE CITED

Andersen, S.K., Hansen, P.W. & Andersen, H.V., 2002. Vibrational spectroscopy in the analysis of diary products and wine. In: Chalmers, J.M. & Griffiths, P.R. (eds.). Handbook of Vibrational Spectroscopy. John Wiley & Sons Ltd, Chicester.

Bisson, L., 2001. In search of optimal maturity. Practical winery and vineyard July/August, 32-43.

Dubernet, M., Dubernet, M., Dubernet, V., Coulomb, S., Lerch, M. & Traineau, I., 2001. Objective analysis of harvest quality by Fourier transform infrared spectroscopy (FTIR) and neuron networks. Bulletin de L'O.I.V.74,15-24.

Fleet, G.H., 2003. Yeast interactions and wine flavour. Int. J. Food Microbiol. 86, 11-23.

Gishen, M. Iland, P.G., Dambergs, R.G., Esler, M.B., Francis, I.L., Kambouris, A., Johnstone, R.S. & Hoj, P.B. (eds.), 2001. Objective measures of grape and wine quality. Eds. Blair, R., Williams, P. and Pretorius, S. In *Proceedings of the eleventh Australian wine industry technical conference*; 7-11 October 2001; Adelaide, South Australia. pp. 188-194.

Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. Techniques for accurate chemical analyses and quality monitoring during winemaking. Wine Promotions, Campbell Town, Australia.

Kupina, S.A.,1984. Simultaneous quantification of glycerol, acetic acid and ethanol in grape juice by high performance liquid chromatography. Am. J. Enol. Vitic. 35, 59-62.

Linton, G.R. & Wall, P.J., 1996. Customer quality issues and international wine markets. In: Hamilton, R. & Hayes, P. (eds.). ,Quality Management in Viticulture, ASVO Viticulture seminar. 3-4.

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

Poni, S., Peterlunger, E., Jacono, C., Intrieri, C. & Possingham, J., 1995. Opening comments to the workshop “Strategies to optimize wine grape quality”. ISHS Acta Horticultureae 427.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B. & Lonvaud, A., 2000. (1st Ed). Handbook of Enology, Volume 1: The Microbiology of Wine and Vinifications. John Wiley & Sons Ltd., England.

Riley, L., 1996. Load parameters at the winery weighbridge. In: Hamilton, R. & Hayes, P. (eds.). ,Quality Management in Viticulture, ASVO Viticulture seminar. 3-4.

Zoecklein, B.W., Fugelsang, K.C., Gump, B.H. & Nury, F.S., 1995. Wine Analysis and Production. Chapman & Hall Enology Library.

Zoecklein, B.W., 2001. Grape sampling and maturity evaluation for growers. Vitner's corner. 16, No. 1.

CHAPTER 2

LITERATURE REVIEW

Microbial metabolites as markers in wine grapes and their relationship to wine quality

2. LITERATURE REVIEW

2.1 INTRODUCTION

During grape maturity the microbial population increases as the sugar concentration accumulates (Suárez *et al.,* 1994). Grape microbial flora can have a strong impact on wine composition and is therefore important to develop rapid and reliable tools for the assessment of berry flora (Bisson, 2001). Damaged grapes caused by insects, rapid water flux or skin degeneration, due to over ripeness, favours the multiplication of oxidative yeasts and bacteria. Grapes heavily infected have altered chemical composition and lack organoleptic quality and chemical stability. Moulds, such as *Botrytis cinerea*, and Sour Rot is a complex problem in South African vineyards which lowers potential grape quality.

The excessive growth of moulds, acetic acid bacteria, lactic acid bacteria and other bacteria on grapes before harvest has the potential to produce substances that may inhibit or retard yeast growth during alcoholic fermentation (Drysdale & Fleet, 1989b; Donèche, 1989). With modern day mechanical harvesting, berries are exposed to oxidation and further microbial attack. Transport of the mechanically harvested grapes needs to be controlled especially with regard to temperature (Ough & Berg, 1971). The oenological consequences are serious in wine made from grapes which have been exposed to high temperatures. Oxidation, degradation of colour and aromas and fermentation difficulties can occur, therefore the objective measurement of the sanitary state of the harvest is necessary (Ribéreau-Gayon *et al*., 2000a).

This literature study will focus on the occurrence of yeasts, acetic acid bacteria, lactic acid bacteria and moulds on grapes and in grape must, the main metabolites they produce, that will have an influence on wine quality. It is therefore important to have rapid quantification methods for these metabolites and this is discussed in the last section.

2.2 MICROBIAL ECOLOGY

Wine production, in particular, the fermentation of grape must is a complex ecological and biochemical process. It involves the interaction of many microbial species, represented by fungi, yeasts, lactic acid bacteria and acetic acid bacteria (Fleet, 2003). These micro organisms play a prominent role in determining the chemical composition of the wine.

Grape soundness is the level of metabolic activity of fungi, yeasts and bacteria on grapes. The parameters usually measured for assessing grape quality include, pH, sugar and total acidity, however, these parameters are not indicators of the microbial load of grapes.

Many factors have an influence on the total microbial population diversity of grapes, such as temperature, rainfall and other climatic conditions, grape variety, degree of maturity at harvest, physical damage due to insect, bird and mould attack, and application of fungicides and insecticides (**Table 2.1**). All these factors can also act synergistically to enhance the influence on the microbial population associated with grapes.

Table 2.1. Factors influencing the microflora of vineyards, grapes, wineries and must.

A. Microflora of vineyards and grapes
1. **Climatic influences**
Temperature Rainfall Wind Microclimate as affected by viticultural practices such as canopy management
2. **Soil and viticultural practices**
Soil type Fertilization Irrigation Application of fungicides
3. **Grape**
Variety Physical damage by mould, insect or bird attack
B. Microflora of grape must
4. **Method of grape harvest**
Handpicked or mechanical Grape temperature
C. Microflora of harvesting equipment
5. **Cleaning and sanitation**
6. **Transport from vineyard to cellar**
Time Initial grape temperature Air temperature Sulfite addition
7. **Condition of grapes**
Temperature Sulfite addition
8. **Must treatment**
Cellar hygiene Aeration Sulfite addition Clarification method Temperature Inoculation with yeast starter cultures
D. Microflora of surfaces of winery equipment
9. **Nature of surfaces**
Irregular, unpolished surfaces Cracks and welds
10. **Cleaning and sanitation**

(Adapted from Pretorius *et al.,* 1999)

Mature grapes of sound physical integrity harbour a microbial population of about 10^3 to 10^5 cfu/g. Damaged grapes quickly develop microbial populations of 10^6 to 10^8 cfu/g, with a high proportion of moulds and acetic acid bacteria (Fleet, 1993;

Suárez *et al*., 1994). Damaged grapes may result from different causes: (1) increase of berry volume due to rapid rainwater absorption by the vines, especially when the bunches are rather tight and the berry skin is thin; (2) other meteorological events like hail and heavy rain; (3) attack by *Drosophila* spp., honey bees, wasps, moths and birds; and (4) attacks of phytopathogenic moulds (e.g., downy and powdery mildews, noble or grey rot) (Loureiro & Malfeito-Ferreira, 2003).

2.2.1 Yeasts

The microflora of grapes are highly variable, with a predominance of non-*Saccharomyces* fermentative yeasts. *Saccharomyces* fermentative species (e.g., *S. cerevisiae*) are rarely detected on sound, undamaged grapes (Martini *et al*., 1996; Van der Westhuizen *et al*., 2000). If present, their number never exceeded 10 cfu/cm^2. Mortimer & Polsinelle (1999) confirmed the above, however, they did find that grape berries that are damaged (i.e. the skin is broken) are rich depositories of microorganisms, *Hanseniaspora* (*Kloeckera*), *Zygosaccharomyces*, *Candida* and *Metschnikowia* species, including *S. cerevisiae*, and that one in four such berries are *S. cerevisiae*-positive (Bisiach *et al*., 1986; Guerzoni & Marchetti, 1987; Blancard *et al*., 1999; Fleet, 2003).

Gadoury *et al*. (2002) detected significant amounts of *Dekkera* and *Kloeckera*, which are probably disseminated by insects attracted to the infected grapes by the volatiles given off by ripening berries.

The majority of ecological surveys performed in vineyards used less optimal sampling, pre-isolation techniques, enrichment methods (Martini *et al*., 1996), isolation culture media, and incubation times, leading to insufficient knowledge of grape microbial ecology (Loureiro & Malfeito-Ferreira, 2003).

Suárez *et al*. (1994) found yeast population to be neglible at veraison but growing considerably during maturation and peaking at levels higher than 10^6 cfu/mL at harvest time. These populations can vary depending on the geographical situation of the vineyard, climatic conditions during maturation, the microbial load of the harvest and pesticide treatments applied to the vine. It is known that warm climatic conditions have the most abundant yeast populations (Ribéreau-Gayon *et al*., 2000a).

Strictly oxidative metabolism yeasts and alcohol sensitive species are the non-*Saccharomyces*, which are essentially found on grapes. Predominant non-*Saccharomyces* species found in South African vineyards were *Candida stellata, Kloeckera apiculata, Candida pulcherrima* and *Candida* colliculosa (Jolly *et al.,* 2003). The apiculate yeasts, (*Kloeckera apiculata*) and its sporogenous form *Hanseniaspora uvarum* account for 50-75% of the total yeast population. Species of *Candida* (*C. stellata* and *C. pulcherrima*), *Hansenula anomala, Pichia menbranaefaciens, Pichia fermentans, Rhodotorula minuta, Brettanomyces, Cryptococcus* and *Kluyveromyces* are found at lesser numbers on grapes. In a study by Shinohara *et al*. (2000), these yeasts were tested for the production of phenolic-off

flavours (POF). Ferulic and p-coumaric acids are always present in small quantities in grape must and a small amount of volatile phenols may be produced during grape must fermentation by phenolic yeasts. *Rhodotorula glutinis, R. rubra, C. guilliermondii, C. mycoderma, C. stellata, Cryptococcus albidus* and *K. apiculata*, had high to moderate POF productivity. *Brettanomyces bruxellensis* and *P. membranaefaciens*, which are considered wine spoilage yeasts had high to moderate POF productivity.

As grape sugars become available, released from microlesions in zones situated around the stomatal apparatus on the grape, *Saccharomyces* and non-*Saccharomyces* populations increase. Harvesting equipment, including mechanical harvesters, picking baskets, infrequently cleaned/sanitized gondolas and other delivery equipment, may present sites for microbiological activity before grapes enter the winery premises (Osborne *et al*., 1991). Lack of sanitation becomes an even more important consequence as travel time to the winery increases (Fugelsang, 1996).

2.2.2 Acetic Acid Bacteria

Acetic acid bacteria found on grapes belong to the family *Acetobacteraceae* are aerobic, Gram-negative, catalase-positive rods (De Ley *et al*., 1984; Holt *et al.*, 1994). Three genera are recognised within this family, *Acetobacter*, *Gluconacetobacter* and *Gluconobacter* (Yamada *et al*., 1997; Ruiz *et al*., 2000).

On healthy grapes, populations are low (10^2-10^3 cfu/mL), *Gluconobacter oxydans* are the species most represented. Rotten or damaged grapes can be very contaminated and populations can reach up to 10^5 - 10^6 cfu/ml and are mixed, comprising of *Gluconobacter* and *Acetobacter*, mainly *Acetobacter aceti* and *Acetobacter pasteurianus* (Barbe *et al.*, 2001).

Gluconobacter oxydans prefers a sugar-rich environment and usually dies-off during alcoholic fermentations. *Acetobacter* species (*Acetobacter aceti, Acetobacter pasteurianus*) and *Gluconacetobacter liquefaciens* and *Gluconacetobacter hansenii* prefer ethanol as carbon source (De Ley *et al.*, 1984; Drysdale & Fleet, 1988). Infection of yeasts and acetic acid bacteria occur when grapes are damaged. Glucose is oxidized to acetic acid by *Gluconobacter* and yeast metabolizes glucose to ethanol which is then also oxidised to acetic acid by *Acetobacter* (Matsushita *et al.*, 1994).

During cold soaking, in red wine making, acetic acid bacteria populations can increase exponentially. Acetic acid bacteria can survive and even grow in grape must during alcoholic fermentation. This can result in high levels of acetic acid in the resultant wine because of the oxidation of ethanol, stated previously (Couasnon, 1999). Du Toit & Lambrechts (2001), found varying cell numbers in grape must and its fermentations over two harvest seasons, from 10^6 – 10^7 to 10^4 – 10^5 cfu/mL for 1998 and 1999, respectively. In musts with a low pH (< 3.6) the numbers decreased and musts with a high pH (> 3.7) increased during fermentation. They also reported

the presence of *Gl. liquefaciens* and *Gl. hansenii* for the first time in must under fermentation.

2.2.3 Lactic Acid Bacteria

Lactic acid bacteria are Gram-positive, catalase negative, non-motile, non-sporeforming, rod- and coccus shaped (du Toit & Pretorius, 2000). These bacteria represents varies species of *Lactobacillus, Pediococcus, Leuconostoc* and *Oenococcus*.

Lactic acid bacteria, essentially *O. oeni*, are well known for the very important role they play during malolactic fermentation in wine. They are usually present in low numbers on grapes and in must, not exceeding 10^3 cfu/mL. In damaged or infected grapes substantial populations may develop. Population is also mainly correlated with pH; the higher the pH, the higher the total lactic acid bacteria population. Lactic acid bacteria is not well adapted to grow in grape must, which are very high in sugar concentrations (>210 g/L) and have a low pH 3.0 – 3.3 (Lonvaud-Funel, 1999). Higher pH must, in warm climates and red grape must, are more vulnerable for lactic acid bacteria growth. Red wine is a macerated wine. Grape quality directly influences grape skin maceration quality in red wine. Sanitary state of the grapes is therefore very important (Ribéreau-Gayon *et al*., 2000a).

It has been reported that acetic acid bacteria activity and *Botrytis cinerea*-stimulated growth of lactic acid bacteria in must (Fugelsang, 1996). Lactic acid bacteria survive at low, almost non-detectable, populations during alcoholic fermentation in the wine, waiting for a suitable opportunity to grow.

2.2.4 Mould

Grey mold caused by *Botrytis cinerea* is one of the most important diseases of grapes. Between flowering and veraison, grape berries are resistant to *B. cinerea*, although they can harbour the pathogen without any visible signs of disease development. After veraison, *B. cinerea*, can produce disease in susceptible grapes. In rainy weather, the infected grapes do not lose water and the percentage of sugar remains nearly the same or may decrease. Secondary infection by other microbes may follow. Under cold and wet conditions *Penicillium, Mucor* and *Aspergillus* spp., as well as other fungi and yeast may overgrow *Botrytis*, producing the condition known as grey rot. Breakdown of the grape integument provides a substrate for the growth of native (“wild”) yeasts and acetic acid bacteria and may produce a condition called sour rot. Elevated levels of acetic acid and lactic acid are frequently seen in wines made from *Botrytis*-infected grapes. These spoilage acids arise from growth of yeast and bacteria associated with the mould. Populations of acetic acid bacteria have been reported to reach levels of near 106 cells/g (Joyeux *et al.* 1984a, b).

The cause of grape cluster rot, appearing three to four weeks before ripening, is the combined activity of three groups of factors: a) primary factors (insects, birds, diseases such as mildews, mechanical and physiological injuries) which damage the

skin; b) secondary microorganisms (bacteria, yeasts and other fungi) that penetrate the broken skins at which stage berries injured by the primary factors begin to rot; and c) secondary insects (fermentation flies and beetles) quickly attracted by the rotting and fermenting grapes, enhancing the process and accelerating its spread throughout the entire cluster (Loinger *et al.*, 1977).

2.3 METABOLITES AS MARKERS FOR MICROBIAL ACTIVITY

Quanitification of mould, yeast and bacterial metabolites in collected juice samples can be used to help evaluate berry quality. The nature and concentration of microbial metabolites differ as a function of biotic and abiotic factors (Zoecklein, 1996). Key indicators of berry rot, such as the presence and concentration of ethanol, glycerol, gluconic acid, galacturonic acid, citric acid, laccase, acetic acid and lactic acid should be rapidly and easy to quantify at winery grape intake.

Corison *et al*. (1979) investigated ethyl acetate and acetic acid as possible indicators of mould and rot. Fermentation studies were set up to determine the relationship between must levels of ethyl acetate and acetic acid and levels present after fermentation. The must rejection level found for ethyl acetate in white and red grapes were 60 and 115 mg/L respectively. For acetic acid in white and red musts the rejection levels were 1190 and 900 mg/L respectively.

Aspergillus, Botrytis and *Penicillium* spp. oxidise glucose to produce gluconic acid. Since gluconic acid is not utilized by yeast or bacteria it may be used as an indicator of grape deterioration. Gluconic acid levels in “clean” fruit and in wines made from healthy grapes are near 0.5 g/L, whereas in wines produced from vines infected with *B. cinerea* levels range from 1 to 5 g/L. In the case of sour rot, where bacterial growth occurs along with mould growth, levels may also reach 5g/L. Sponholz & Dittrich (1985) showed that substances such as gluconic acid, seen as produced by *Botrytis*, are mainly produced by the co-infecting acetic acid bacteria.

Metabolic studies in vitro have shown that young mycelium of *B. cinerea* possesses the enzymes of the Embden-Meyerhof pathway, the hexose monophosphate shunt and the tricarboxylic acid cycle (Donèche, 1989). It directly oxidizes glucose into gluconic acid. This acid, which accumulates in the grape, is a characteristic secondary product of significant sugar degradation.

Botrytis cinerea also produces significant amounts of polyols of which glycerol is quantitavely the most important. The initial fungal development under the grape skin is marked by considerable glycerol accumulation. Quantities produced may be as high as 20 g/L (Ravji *et al.*, 1988). Glycerol may be metabolised by bacteria before harvest. Infected grapes then develops high levels of acetic acid and gluconic acid.

Ribereau-Gayon (1988) suggested that the ratio of glycerol to gluconic acid indicates the “quality” of the rot. Higher ratios indicated the growth of true noble rot, whereas lower ratios suggest sour rot. Sour rot owes it name to the characteristic ethyl acetate odour.

The most frequent yeast species found to be actively proliferating with grape sour rot were *Candida krusei*, *Kloeckera apiculata, Hanseniaspora, Torulopsis, Endomycopsella* and *Metschnikowa pulcherrima* (Zoecklein *et al.*, 2000). These species as well as *P. membranaefaciens* can be indicated as possibly being responsible for the biosynthesis of disease chemical markers and probably for the grape tissue disaggregation due to internal CO_2 produced, as indicated by the elevated alcohol content and pressure. Ethyl acetate and acetic acid can be considered a disease indicator ranging in these grapes from 0.3 to 12.5 mg/mL (Guerzoni & Marchetti, 1986).

Ethanol and CO_2 is produced by yeasts from glucose and fructose (alcoholic fermentation) (**Fig. 2.1**). Lactic acid bacteria can also ferment these sugars to form ethanol, lactic acid and CO_2 (heterolactic fermentation). Uncontrolled premature fermentation of sugars can occur before grape processing which can lead to a decline in wine quality (Ough & Berg, 1971). Measuring ethanol at grape intake can give an indication of the sanitary state.

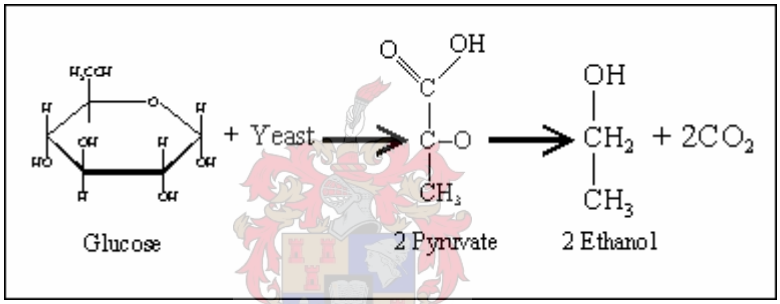


Figure 2.1 Chemical formula of the fermentation of sugars by yeasts.

Acetoin as disease marker in grapes at winery intake is important due to its aroma influence (Romano *et al.*, 2003). Yeasts use pyruvic acid to form acetoin, diacetyl and 2,3-butanediol. (Romano & Suzzi, 1993; Ribéreau-Gayon *et al.,* 2000). Acetoin is produced by yeasts by condensation of "active acetaldehyde": 1) with pyruvate giving α-acetolactate, which is decarboxylated to acetoin; 2) with acetaldehyde forming acetoin directly; and 3) with acetyl-CoA giving diacetyl, which is reduced to acetoin. Quantitive studies reporting the amounts of acetoin produced by individual species during fermentation are few. Romano & Suzzi (1993) reported four strains of *Saccharomyces cerevisiae* to produce high quantities of acetoin, up to 194,6 mg/L in grape must.

Aspergillus spp. have esterases to hydrolyze phenolic acids bound to tartaric acid, this can increase the free phenolic acid content in grape must (Okamara & Watanube, 1982). POF production during fermentation may increase. This supports the importance of sanitary conditions for wine grapes.

2.4 MICROBIAL METABOLITES AND WINE QUALITY

Indigenous or non-*Saccharomyces* yeasts are capable of anaerobic as well as aerobic growth and may persist during fermentation, competing with inoculated *Saccharomyces* species for nutrients, and may produce fatty acid esters and other compounds affecting the fermentation bouquet of the wine. The persistence of these non-*Saccharomyces* during fermentation depends upon many factors, such as temperature of fermentation, nutrient availability, inoculum strength of *Saccharomyces*, use and level of sulphur dioxide, and the quantity and identity of organisms initially present on the grapes.

Sluggish fermentations appear to be associated with insufficient availability of vitamins. Alexandre & Charmentier (1998), found that non-Saccharomyces yeasts were responsible for rapid assimilation of thiamine levels in must which could lead to depleting the medium in a few hours

Plata *et al.* (2002) demonstrated that *Kloeckera apiculata* and *Torulaspora delbrueckii* in comparison to *Pichia membranaefaciens*, *Candida guilliermondii*, *Hansenula subpelliculosa* and *Kluveromyces marxianus* were typical fermentative yeasts. They produced more than 5% v/v ethanol from glucose and the last four produced less than 1% v/v ethanol which testifies its scarce ability to ferment.

In the presence of oxygen, *Brettanomyces*, ferments glucose to large quantities of acetic acid, known as Custers effect (Carrascosa *et al.*, 1981). Aerobic yeasts (*Candida, Pichia, Hansenula*) produce acetic acid and ethyl acetate (Ribéreau-Gayon et al, 2000a). *Hansenula anomala* has limited fermentative capabilities producing 0.2% to 4.5% (vol/vol) alcohol as well as large amounts of acetic acid (1 -2g/L), ethyl acetate (2150 mg/L) and isoamyl acetate. *Hanseniaspora uvarum* and *Kloeckera apiculata* which represents the dominant yeast species at harvest are also capable of producing acetic acid and its esters in high concentrations before and during the early stages of fermentation. These non-*Saccharomyces* yeast are sensitive to ethanol which inhibits further growth however when fermentation temperatures are low (10 - 15°C), ethanol tolerance is extended. Mixed inoculum, consisting of *Kloeckera apiculata*, *Hanseniaspora uvarum*, *Saccharomyces bayanus* (T73), *Saccharomyces cerevisiae* (L2226), *Saccharomyces cerevisiae* (A48), were used by Gil *et al.* in 1996, to demonstrate the influence of apiculate yeast on the volatile aroma compounds in wine compared to pure cultures inoculum. The early dominance of apiculate yeasts proofed favourable in producing higher amounts of higher alcohols and volatile acids in total.

Gluconobacter are more present on the grape and in the must than *Acetobacter*. *Acetobacter* prefer, and are more commonly found, in partially fermented must and wine (Ribéreau-Gayon *et al.*, 2000a). Both carry out the oxidation of ethanol and glucose to acetic acid, however glucose oxidation activity is low in *Acetobacter*. *Gluconobacter* lacks key enzymes of the tricarboxylic acid cycle for further oxidation of acetic acid and lactic acid. *Acetobacter* can oxidize these two acids to form CO_2

and H_2O via the tricarboxylic acid cycle. This is the most important taxonomic difference between the two organisms.

During glucose metabolism the pentose phosphate pathway is used and acetic acid and lactic acid is formed. Glucose oxidase catalyze the reaction where glucose is oxidized to gluconic acid, as the first step in the metabolism. Gluconobacter forms keto-gluconic acids from gluconic acid (**Fig 2.2**). Large amounts of oxidated products can be accumulated in the must, depending on environmental conditions and must chemistry (pH and sugar concentration). Gluconic acid may accumulate to concentrations of 60-70 g/L.

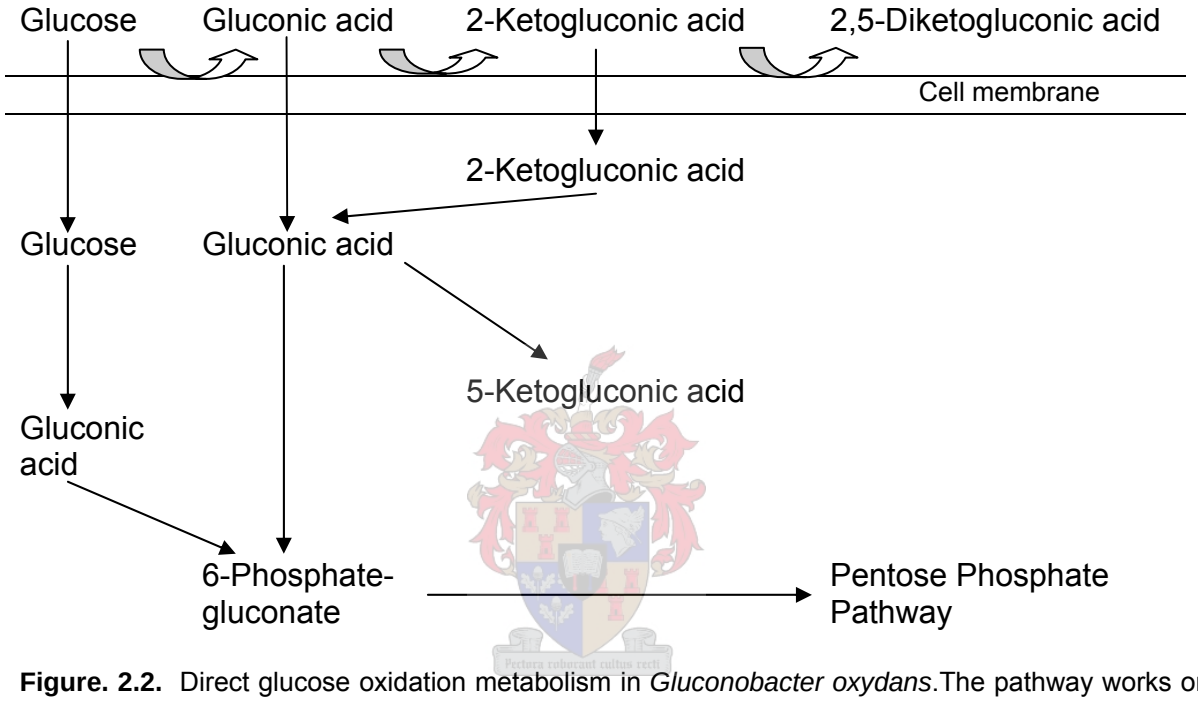


Figure. 2.2. Direct glucose oxidation metabolism in *Gluconobacter oxydans*.The pathway works only in the presence of 0.9-2.7 g/L glucose. (Adapted from Gupta *et al.,* 2001)

Results from Couto *et al.* (2003), shows that detected levels of gluconic acid up to 1-2 g/L were not accompanied by the presence of acetic acid, suggesting that gluconic acid formed in these grapes is of fungal origin or that it is produced from the attack of acetic acid bacteria to sugars but in the absence of ethanol. Higher levels of gluconic acid (from 2-3 g/L) were, however found to be correlated with detected levels of acetic acid, suggesting that it may have arisen from the metabolism of sugars by acetic acid bacteria and not by the growth of the fungus *B. cinerea*.

Spoiled grapes do not only have high acetic acid levels but also high amounts of ethanol, glycerol and low amounts of ethyl acetate. Ethanol and glycerol are products formed by yeast metabolism. *Acetobacter* oxidizes ethanol to form acetaldehyde and further to acetic acid. Glycerol is oxidized to dihydroxyacetone and gluconic acid.

Grape juice composition can be significantly altered by acetic acid bacteria infection (**Table 2.2**). Glucose preferentially, but also fructose, malic and citric acids are degraded with formation of gluconic, lactic, succinic acids, acetaldehyde and

ketonic substances (Joyeux *et al.*, 1984). This can lead to altered sensory quality of the wine, high levels of acetic acid (volatile acidity) in the wine and fermentation difficulties.

Table 2.2. The concentration (mg/L) of components in grape juice as a function of acetic acid bacteria infection (Sponholz & Dittrich, 1979).

Infected grapes (%)	Ethanol	Acetic acid	Glycerol	Dihidroxy-acetone	Gluconic acid
0	103	23	0	4	41
5	142	126	500	23	156
10	259	200	1100	41	252
15	257	210	900	56	410
20	494	463	2000	71	520
30	401	490	1100	107	1099
40	790	610	1500	143	859
50	577	1040	1900	184	1581
75	419	1690	1400	259	2586

Development of acetic acid bacteria, such as *Gluconobacter oxydans*, in the must inhibits growth and metabolism of *S. cerevisiae* (Joyeux *et al.*, 1984). During cold soaking growth of acetic acid bacteria occur (du Toit & Lambrechts, 2002). Acetic acid bacteria may survive through fermentation and therefore influence the growth of yeasts during alcoholic fermentation. In slowly fermenting must or stuck fermentations, acetic acid bacteria growth may be stimulated. Acetic acid bacteria directly oxidize glucose, fructose is only utilized to a limited extent. Oxidation of ethanol leads to formation of acetic acid. The resultant wine or stuck fermentation will exhibit a “sweet-sour” character (because of the relative sweetness of fructose).

High must pH favours proliferation of acetic acid bacteria. Advantages of having a low pH must with high SO_2 concentrations have been proven by du Toit and Lambrechts (2002).

Gluconic acid and keto-gluconic acid bind with sulphur dioxide, lowering its antimicrobial activity. Dihidroxyacetone and acetaldehyde affects the sensory quality and sulphur dioxide binding properties in wine. Grape must with a high activity of acetic acid bacteria which may survive through alcoholic fermentation can produce a wine with high amounts of acetic acid.

Legal limit for volatile acidity in wine for export purposes are 0.8 g/L expressed as acetic acid, above 1.2 g/L it becomes objectionable. Corison *et al.* (1979) found acetic acid rejection levels of 0.9 and 1.19 g/L in red and white musts. They also found a linear relationship between the amount of acetic acid and ethyl acetate present in must and the amount present after fermentation. This allows the prediction of acetic acid and ethyl acetate in wine levels from that in must levels.

Lactic acid bacteria degrade must and wine sugars with different affinity depending on the species and perhaps even the strain (Ribéreau-Gayon *et al.*, 2000a). Homofermentative lactic acid bacteria is the major type present on grapes

(Moreno-Arribas, 2002). Glucose and fructose are fermented into L- or D-lactic acid, or a mixture of the two forms, depending on the species. It has been said that these lactic acid bacteria disappear quickly after the start of alcoholic fermentation.

Edwards *et al.* (1998), isolated *Lactobacillus kunkeei*, a new species, from commercial grape wine undergoing sluggish/stuck alcoholic fermentation and producing high levels of acetic acid. Also, Gao *et al*. (2002), inoculated Chardonnay must with four spoilage lactic acid bacteria cultures. *Lactobacillus kunkeei*, being one, decreased malic acid concentration from 40 – 60% and produced acetic acid levels of 1 g/L. Edwards *et al*. (2002), also isolated *Lactobacillus nagelii* from a grape wine undergoing sluggish / stuck alcoholic fermentation.

B. cinerea development is detrimental to grape nitrogen compounds. Musts obtained from these infected grapes contain less ammonium and more complex forms of nitrogen than musts from healthy grapes.

Loinger *et al.* (1977), made wine from grapes at various stages of rot injury. It was found that an increase in percentage grape rot was paralleled by a decrease in wine quality. There was also a clear trend of increase in alcohol concentration with an increase in rot.

Fermentation difficulties of must from *Botrytis* infected grapes may occur because of reduced nitrogen levels, vitamin B6 and B10 which are used as important yeast nutrients.

Laccase, a soluble enzyme, are formed which catalyses phenolic oxidation. Because of its solubility it is difficult to remove. Laccase is very stable, resistant to SO_2 and alcohol and remains in the wine for months. An increased rate of oxidation takes place in the wine resulting in wine with a bitter taste and off-odours. Phenolic substances, tannins are oxidized to quinones. The wine consequently begins to brown as a result of this increased rate of oxidation.

Polysaccharides are also formed by *Botrytis* and may give problems with the filtration and fining of wine.

Wine made from rotten or botrytized grapes exhibit SO_2 binding phenomena. Up to 80% of the SO_2 binds with products, such as gluconic acid an dihydroxyacetone, of *Gluconobacter*, which easily grow on these grapes. Making these wines difficult to stabilize microbiologically (Eschenbruch & Dittrich, 1986).

2.5 HARVESTING CONSIDERATIONS

Wineries carry out load assessment to reduce loss (Riley, 1996). This means from a quality system perspective, for example, the streaming of different value fruit such as sweet and sour grapes or premium and commercial grapes can be carried out to ensure that grapes are used in the highest potential end product. Representative grape samples of each load are examined for material-other-than-grape (Material Other than Grapes; e.g., leaves, cane fragments), rot, fruit chemistry, juice aroma and flavour (Zoecklein, 1999). Grapes needs to be free of disease,

insect and bird damage, microbial contamination and oxidation as these can lower the quality of the resulting wine (Iland, 2000).

High temperatures accelerate oxidation reactions and could promote the onset of fermentation which can lead to lower wine quality. The temperature of a load of grapes should be lower than 20°C. Machine harvesting at night and limiting the time between picking and crushing can help to keep load temperature low (Clary *et al.*, 1990). Any delay between mechanical harvesting and delivery of the grapes to the winery can result in increased enzymatic activity and browning, oxidation (loss of colour), development of off-flavours and microbial growth (Ough *et al.*, 1971; Morris, 2000). Temperatures above 30°C are likely to cause deterioration in grape quality (Iland, 2000). Negative flavour changes in grapes within a short time period (less than 4 hours) at high temperatures (25ºC to 35ºC and above) occurred (Morris, 2000).

Grapes received at wineries are visually inspected for mould and severe microbial contamination. This leads to variability between inspectors at grape-receiving. Machine-harvested grapes loses its structural integrity and makes visual inspection difficult (Kupina, 1984). Also, visual inspection only takes external contamination development into account.

The extend of mould and rot contamination has always been hard to quantify in harvested grapes. *Botrytis cinerea* has already altered the grape composition before emerging to the surface of the grape. Further, it is difficult to see infection on the surface of red grapes. To improve this inspection procedure, a more reliable and objective method is needed.

Grapes that are machine harvested are damaged and these broken berries encourage massive growth of yeasts, causing to have high initial populations of wild yeasts in the beginning of fermentations, which could influence wine quality for example ester taint.

Improper transport of mouldy grapes from vineyards to wineries and allowing premature commencement of alcoholic fermentation of the released juices, especially mechanically harvested grapes, can lead to the formation of high concentration of acetic acid (Alexandre & Charpentier, 1998).

2.5.1 *Preservation solutions*

2.5.1.1 Sulphur Dioxide: Addition of sulphur dioxide (SO_2) to machine-harvested grapes can also decrease quality loss during holding (Ough *et al.,* 1971; Morris & Flemming, 1972). Sulphur dioxide discourages microbial spoilage and serves as an antioxidant to prevent juice browning. However, most winemakers want control of SO_2 at the winery since some wine styles may require no addition of SO_2 until after fermentation.

2.5.1.2. Lysozyme: Lysozyme is a natural antimicrobial protein. It causes cell lysis of Gram-positive bacteria cell walls, by cleaving the β(1-4) glycosidic linkage between *N*-acetylmuramic acid and *N*-acetylglucosamine in the peptidoglycan cell wall layer (Du Toit & Pretorius, 2000; Gao *et al.,* 2002; Bartowsky, 2003).

Thus, lysozyme is active against lactic acid bacteria (*Lactobacillus, Pediococcus* and *Oenococcus*) in grape juice and wine. Adding lysozyme to grape must reduces the risk of *Lactobacillus* spoilage and can also control lactic acid bacteria growth during stuck or sluggish alcoholic fermentations (Gao *et al.,* 2002). The elimination of an indigenous population of lactic acid bacteria may be desirable because of the increased risk of biogenic amine formation of uncharacterised lactic acid bacteria strains (Bartowsky, 2003).

2.6 ANALYTICAL TECHNIQUES FOR THE QUANTIFICATION OF MICROBIAL METABOLITES IN GRAPE JUICE

Past research focused more on the rapid determination of maturity of wine grapes than grape soundness. When grape samples are measured for payment or quality control, analysis time, accuracy and precision are key parameters (Andersen *et al.,* 2002). Recent research, showed the positive and negative impact of microbial metabolites in grape must and the importance to measure the extent of grape soundness rapidly, at winery intake (Dubernet *et al.,* 2001). Methods currently used for the rapid quantification of microbial metabolites as an indication of grape soundness include high performance liquid chromatography (HPLC), gas chromatography (GC) and spectroscopic methods which include enzymatic methods.

2.6.1 Chromatographic Techniques

Miklósy & Kerényi (2004) compared the volatile aroma compounds in noble rotted grape berries, determined by GC-MS. More than 80 compounds were identified of which are some of the following: alcohols (2.3-butanediol, ethanol), acids (acetic acid), aldehydes, esters (ethyl ester), acetals, epoxides, nitrils, furan derivatives and lactones. HS-SPME coupled to GC-MS was developed to study grape volatile compounds (Sánchez-Palomo *et al.,* 2005).

A method for the simultaneous quantification of glycerol, acetic acid and ethanol in grape juice by high performance liquid chromatography (HPLC) in 11 minutes was developed by Kupina (1984). Under South African conditions, this method was used by Baumgarten *et al.,* (1987). HPLC was performed on grape must samples obtained by a sampling drill used at grape intake in wineries. The HPLC method performed satisfactorily but centrifugation and filtration of the samples was too costly and a one step operation was needed. Also, all the infested berries were not broken by the sampling drill so that all the glycerol that the berries obtained could be set free in the juice.

The principal capability of gaining qualitative and quantitative information was presented by Vonach *et al.* (1998) through coupling HPLC and Fourier transform Infrared Spectroscopy (FT-IR) for the direct determination of glucose, fructose, glycerol, ethanol, acetic , citric, lactic, malic, succinic and tartaric acid in wine. The development of a molecular specific detection system for HPLC is an ongoing research field in analytical chemistry. Since these compounds absorb in the infrared region FT-IR can provide qualitative information about the compounds. The comparatively low sensitivity of aqueous phase HPLC-FT-IR compensates for the high speed of multi-component analysis, the low cost per analysis and the high degree of automation. With the application of multivariate data-processing techniques will lead to improvements of real time HPLC-FT-IR (Vonach *et al.,* 1998).

2.6.2 Infrared Spectroscopy

Spectroscopic analyses are well suited for process control and routine analyses, since measurements can be done in-line or on-line without or with minimal sample preparation.

Infrared spectroscopy is one of the most important analytical techniques available to today's chemists. Infrared spectrometers have been commercially available since the 1940's. Infrared spectroscopy is a technique based on the interaction of infrared radiation with the vibrations and rotations of the atoms of a molecule. An infrared absorption spectrum can be obtained by passing radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a chemical bond of a sample molecule. Different molecules absorb infrared radiation at different wavelengths. Thus, infrared spectrum contains both qualitative and quantitative information of the sample material.

Mid-Infrared (MIR) spectroscopy has been the most widespread method used for compositional analysis in the dairy industry. As early as in 1961 a patent application for a MIR method determining fat, protein and lactose in milk was filed (Andersen *et al.,* 2002). The use of vibrational spectroscopy for routine analyses of wine began much later with near-infrared (NIR) being the preferred method in the early years (Baumgarten, 1987). Recently, however, focus has moved towards FT-IR technology in the MIR region, since it offers a more accurate determination of more constituents and properties than the NIR method (Patz, *et al.,* 2004; Nieuwoudt, 2004).

2.6.3 Fourier Transform Infrared Spectrometry for Grape Analyses

Application of FT-IR spectroscopy is of special interest due to the presence of sharp and specific absorption bands. For the analysis of complex matrixes, such as grape must and wine, multivariate data analysis, for instance partial least squares regression (PLSR) have to be applied in order to resolve overlapping spectral features (Mortensen, 2004).

The first purpose built FT-IR spectrometer for wine analyses was marketed in 1998 by FOSS A/S and was called Winescan FT120. Multivariate prediction models are used for predicting wine constituents from the FT-IR absorbance spectrum (Andersen *et al.,* 2002). During, 1999, calibrations were developed for grape analyses consisting of the maturity evaluation which included sugar, ºBrix, total acid, potential degree, alcohol, tartaric acid, malic acid, polyphenol index, colour intensity, alfa-amino nitrogen and ammonia as well as measuring metabolites produced by different micro organisms responsible for grape diseases (Dubernet *et al,* 2001). Calibration developed by Dubernet, makes use of neural networks for the quantification of the extent of grape diseases.

Examples of FT-IR spectra of sugar, grape must, wine and an anthocyanin extract are shown in **Figure 2.4**. Wine and grape must are complex mixtures and therefore the full assignment of the spectral bands is a challenging task which will not be attempted.

The region between 1670 and 1530 cm^{-1} is excluded in the plot, since this part is very noisy due to strong vibrations from water. Generally in the region 1680-900 cm^{-1} numerous bands originating from wine phenols can be found. The band at 1520 cm^{-1} can be assigned to C=C bond vibrations which are typical for aromatic systems. A strong contribution of OH deformation vibrations can be found in the region 1410-1260 cm^{-1}. Strong C-O valence vibrations between 1150 and 1040 cm^{-1} overlap with aromatic fingerprint bands at 1225-950cm^{-1}. CH_3 symmetric deformation vibrations occur in the region 1190-1370 cm^{-1} (Edelman *et al.,* 2001). The sharp peak of wine around 1040 cm^{-1} represent ethanol (Andersen *et al.,* 2002).

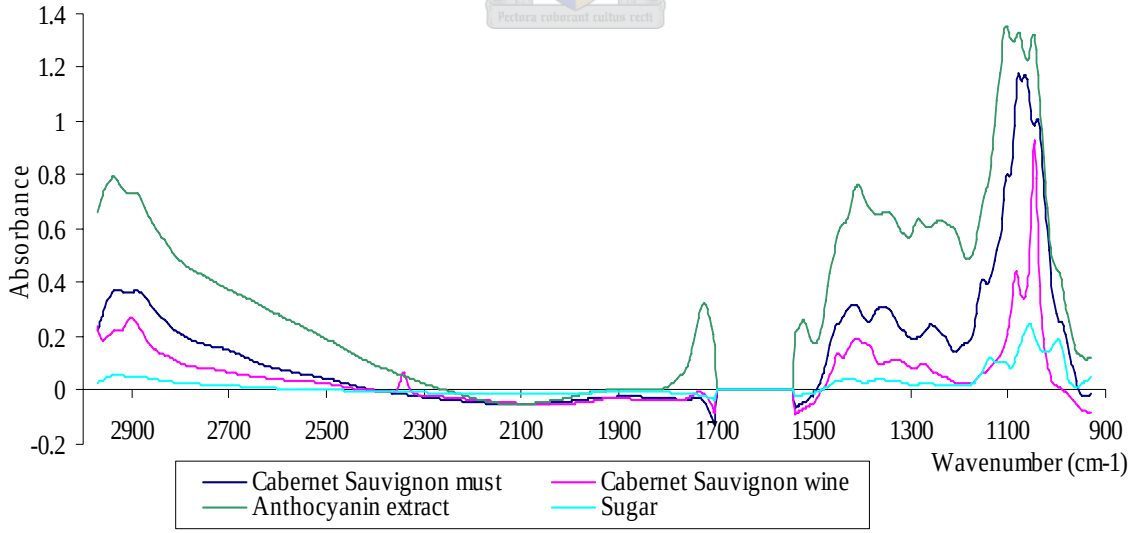


Figure 2.4. FT-IR spectra from 3000-900 cm^{-1}. The region between 1670 and 1530 cm^{-1} is excluded since this part is very noisy (Mortensen, R.R., 2004)

The need for authenticity, classification and differentiation of grape varieties and wines has lead to considerable advances in recent years in the application of spectroscopic techniques (Arvanitoyannis *et al.,* 1999; Palma & Barroso, 2002). FT-

IR spectrometry was also found as a suitable technique for rapid screening for the classification of white grape must (must before fermentation) in comparison to aroma sensor “electronic noses” and UV spectrometers (Roussel *et al.,* 2003).

FT-IR “fingerprinting” spectral analysis and discriminant analysis tools can be used for the confirmation of sample identification, wine style, spoilage, cultivar identity, origin and adulteration.

2.7 CONCLUSION

Wine quality is not just dependent on winemaking practices but very much dependant on the chemical characteristics of the grapes, in essential the metabolites produced by micro organisms in the grape must. Chemical changes in grapes occur in the vineyard but to a great extent between the vineyard and winery before grape processing. Mechanical harvesting made it possible to deliver grapes much faster to the winery but certain precautions are necessary due to grape berry exposure. To evaluate grape berry microbial flora is time consuming. For rapid determination of the sanitary state of the harvest it seems evident to measure microbial metabolites. The measurement of ethanol, acetic acid, gluconic acid, glycerol and ethyl acetate are the most important metabolites to measure. Acetoin and lactic acid could also be used.

2.8 LITERATURE CITED

Alexandre, H., Charpentier, C., 1998. Biochemical aspects of stuck and sluggish fermentation in grape must. J. Inl Microbiol Biotech, 20, 20-27.

Andersen, S.K., Hansen, P.W., Andersen, H.V., 2002. Vibrational Spectroscopy in the analysis of diary products and wine. In: Chalmers, J.M. and Griffiths, P.R., Handbook of vibrational spectroscopy. John Wiley & sons Ltd, Chicester.

Arena, M.E., Manca de Nadra, M.C., 2001. Biogenic amine production by *Lactobacillus.* J. Applied Microbiology, 90, 158-162.

Arvanitoyannis, I.S., Katsota, M.N., Psarra, E.P., Soufleros, E.H., Kallithara, S., 1999. Application of quality control methods for wine authenticity: Use of multivariate analysis (chemometrics). Trends in Food Science & Technology 10, 321-336.

Barbe, J.C., de Revel, G., Joyeux, A., Bertrand, A., Lonvaud-Funel, A., 2001. Role of botrytized micro-organisms in SO binding phenomena. J. Applied Microbiology, 90, 34-42.

Bartowsky, E., 2003. Lysozyme and winemaking. The Australian and New Zealand Grapegrower and Winemaker, 31st Annual Technical Issue, 101- 104.

Baumgarten, G.F., Burger, M., Kirschbaum, H., 1987. The evaluation of wine grapes for mould defects under South African conditions using high pressure liquid chromatography. Proc. 9th S.A. Association for Food Science and Technology, Sept. 1987, Cape Town, S.A. In: Food Review Feb-March 1988, 14-15.

Bisiach, M., Minervini, G., Zerbeto, F., 1986. Possible integrated control of grapevine sour rot. Vitis 25, 118-128.

Carrascosa, J.M., Viguera, M.D., Nunez de Castro, I., Scheffers, W.A., 1981. Metabolism of acetaldehyde and Custers effect in the yeast *Brettanomyces abstinens.* Antonie van Leewenhoek 47, 209-215.

Clary, C.D., Steinhauer, R.E., Frisinger, J.E., Peffer, T.E., 1990. Evaluation of machine- vs hand-harvested Chardonnay. Am. J. Enol. Vitic. 41,.2, 176-181.

Corison, C.A., Ough, C.S., Berg, H.W., Nelson, K.E., 1979. Must acetic acid and ethyl acetate as mold and rot indicators in grapes. Am. J. Enol. Vitic. 30,. 2, 130-134.

Couason, M.B., 1999. Une nouvelle technique: La maceration préfermentaire à froid-Extraction à la neige carbonique 1re partie: Résultats oenologiques. Revue-des Oenologues-et-des Techniques-Vitivinicoles-et-Oenolgiques, 92. 26-30.

Couto, J.A., graca, A.R., Soares-franco, J.M., Hogg, T., 2003. The use of gluconic acid level as an indicator of the activity of acetic acid bacteria in grapes. VII Symposium Intrnational d'Enologie, June 2003, Bordeaux.

De Ley, J., Gosselé, F., Swings, J., 1984. Genus I Acetobacter. In: Bergey's Manual of Systematic Bacteriology. Vol. 1, Williams & Wilkens, Maryland, U.S.A., 268-274.

Donèche, B., 1989. Carbohydrate metabolism and gluconic acid synthesis by *Botrytis cinerea.* Can. J. Bot. 67, 2888-2893.

Drysdale, G.S., Fleet, G.H., 1988. Acetic acid bacteria in winemaking: A review. Am. J. Enol. Vitic., 39: 143-154.

Drysdale,, G.S., Fleet, G.H., 1989b. The effect of acetic acid bacteria upon the growth and metabolism of yeast during the fermentation of grape juice. J. Appl. Bacteriol., 67. 471-481.

du Toit, M., Pretorius, I.S., 2000. Microbial spoilage and preservation of wine: Using weapons from nature's own arsenal - a Review. S. Afr. J. Enol. Vitic. 21, 74-96

du Toit,. W.J., Lambrechts, M.G., 2002. The enumeration and identification of acetic acid bacteria from South African red wine fermentations. International J Food Microbiology, 74, 57-64.

Dubernet, M., Dubernet, M., Dubernet, V., Coulomb, S., Lerch, M., Traineau, I., 2001. Objective analysis of harvest quality by Fourier transform infrared spectroscopy (FTIR) and neuron networks. Bulletin de L'O.I.V., 839-840.

Edelmann, A., Diewok, J., Schuster, K.C., Lendl, B. (2001). Rapid method or the discrimination of red wine cultivars based on mid-infrared spectroscopy of phenolic wine extracts. J. Agric. Food Chem., 49, 1139-1145.

Eschenbruch, B., Dittrich, H.H., 1986. Metabolism of acetic acid bacteria in relation to their importance to wine quality. Zentralbl. Mikrobiol., 141: 279-289.

Edwards, C.G., Haag, K.M., Collins, M.D., 1998. Identification and characterization of two lactic acid bacteria associated with stuck/sluggish fermentation. Am. J. Enol. Vitic. 49: 445-448.

Fleet, G.H., 2003. Yeast interactions and wine flavour. International J. Food Microbiology, 86, 11-23.

Fugelsang, K.C., 1996. Wine Microbology. Chapman and Hall, New York.

Gao, C. Y., Zhang, G., Krentz, S., Darius, S., power, J., Lagarde, G., 2002. Inhibition of spoilage lactic acid bacteria by lysozyme during wine alcoholic fermentation. Australian Journal of Grape and Wine Research, 8, 76-83.

Gil, J.V., Mateo, J.J., Jiménez, M., Pastor, A., Huerta, T., 1996. Aroma compounds in wine as influenced by Apiculate yeasts. Journal of Food Science,. 61, 1247-1249.

Guerzoni, E., Marchetti, R., 1987. Analysis of yeast flora associated with grape Sour Rot and of the chemical disease markers. Applied and Environmental Microbiology, 571-576.

Guerzoni, M.E., Piva, M., Gardini, F., 1985. Proposal of a rapid HS-GLC method for microbiological control of foods. Lebensm.-Wiss. u. -Technol., 18, 128-132.

Gupta, A., Singh, V.K., Qazi, G.N., Kumar, A., 2001. *Gluconobacter oxydans:* Its biotechnological applications. J. Mol. Biotechnol. 3(3): 445-456.

Heresztyn, T., 1986. Metabolism of volatile phenolic compounds from hydroxycinnamic acids by *Brettanomyces* yeast. Arch Microbiol 146: 96-98.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T., 1994. Genus *Acetobacter* and *Gluconobacter.* In: Bergey's Manual of Determinative Bacteriology. 9th ed., Williams & Wilkens, Maryland, U.S.A., 71-84.

Jolly, N.P., Augustyn, O.P.H., Pretorius, I.S., 2003. The occurrence of non-*Saccharomyces cerevisae* yeast species over three vintages in four vineyards and grape musts from four production regions of the Western Cape, South Africa. S.A. J. Enol. Vitic. 24, 35-42.

Joyeux, A., Lafon-Lafourcade, S., Ribereau-Gayon, P., 1984b. Metabolism of acetic acid bacteria in grape must: consequences on alcoholic and malolactic fermentation. Sci. Aliments 4, 247-255.

Kupina, S.A., Shrikhande, A.J., 2003. Evaluation of a Fourier transform infrared instrument for rapid quality-control wine analyses. Am. J. Enol. Vitic. 54:2, 131-134.

Kupina, S.A., 1984. Simultaneous quantification of glycerol, acetic acid and ethanol in grape juice by high performance liquid chromatography. Am.J. Enol. Vitic., Vol. 35, No.2, 59-62.

Loinger, C., Cohen, S., Dror, N., Berlinger, M.J., 1977. Effect of grape cluster rot on wine quality. Am. J. Enol. Vitic. 28, No.4, 196-199.

Lonvaud-Funel, A., 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie van Leeuwenhoek 76: 317-331.

Lonvaud-Funel, A., 2001. Biogenic amines in wines: role of lactic acid bacteria. FEMS Microbiology letter 199, 9-13.

Loureiro, V., Malfeito-Ferreira, M., 2003. Spoilage yeasts in the wine industry. Internation J. Food Microbiology, 86, 23-50.

Manley, M., van Zyl, A., Wolf, E.E.H., 2001. The evaluation of the applicability of Fourier transform near-infrared (FT-NIR) spectroscopy in the measurement of analytical parameters in must and wine. S. Afr. J. Enol. Vitic. 22, 93-100.

Martini, A., Ciani, M., Scorzetti, G., 1996. Direct enumeration and isolation of wine yeasts from grape surfaces. Am. J. Enol. Vitic. 47, 435-440.

Matsushita, K., Toyama, H., Adachi, O., 1994. Respiration chains and bioenergetics of acetic acid bacteria. Adv. Microb. Physiol., 36: 247-301.

Miklósy, E., Kerényi, Z., 2004. Comparison of the volatile aroma components in noble rotted grape berries from two different locations of the Tokaj wine district in Hungary. Anal. Chim. Acta. 513, 177-181.

Moreno-Arribas, M.V., Polo, M.C., Jorganes, F., Munoz, R., 2003. Screening of biogenic amine production by lactic acid bacteria isolated from grape must and wine. International J. Food Microbiology 84, 117-123.

Morris, J.R., 2000. Past, Present and Future of vineyard mechanization. Proc. ASEV. 50th Seattle, 155-164.

Morris, J.R., Flemming, J.W., 1972. Effects of SO_2 on postharvest quality of mechanically harvested grapes. Ark. Farm Res. 21, 5.

Mortensen, R.R., 2004. Prediction of wine colour based on rapid analyses on red grapes. Thesis, The Technical University of Denmark, Lyngby, Denmark and The Royal Veterinary and –Agricultural University, Copenhagen, Denmark.

Mortimer, R., Polsinelle, M., 1999. On the origins of wine yeast. Res Microbiol., 150, 3, 199-204.

Nieuwoudt, N., 2004. Glycerol and wine. Thesis, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.

Okamura, S., Watanabe, M., 1982. Purification and properties of hyroxycinnamic acid ester hydrolase from *Aspergillus japonicus.* Agric. Biol. Chem., 46. 1839-1848.

Ough, C.S., Berg, H.W., 1971. Simulated mechanical harvest and gondola transport. II. Effect of temprature, atmosphere, and skin contact on chemical and sensory qualties of white wines. Proc. 22nd American Society of Enologiste, annual meeting, June 1971, California, 24-26.

Ough,C.S., Berg, H.W., Coffelt, R.J., Cooke, G.M., 1971. The effect on wine quality of simulated mechanical harvest and gondola transport of grapes. Am. J. Enol. Vitic. 22, 65-70

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

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

Plata, C., Millan, C., Mauricio, J.C., Ortega, J.M., 2003. Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts. Food Microbiology 20, 217-224.

Pretorius, I.S., van der Westhuizen, T.J., Augustyn, Q.P.H., 1999. Yeast biodiversity in vineyards and wineries and its importance to the South African wine industry. A Review. S.Afr. J. Enol. Vitic. 20, No. 2, 61-74.

Ravji, R.G., Rodriguez, S.B., Thornton, R.J., 1988. Glycerol production by four common grape molds. Am.J. Enol. Vitic. 39, 77-82.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B. and Lonvaud, A. (eds). 2000a. 1st Ed. Handbook of Enology. Volume 1. The Microbiology of Wine and Vinifications. John Wiley & Sons, Ltd., West Sussex, England.

Ribéreau-Gayon, P., Dubourdieu, D.,Glories, Y.& Maujean, A., (eds). 2000b. 1st Ed. Handbook of Enology. Volume 2. The Chemistry of Wine Stabilization and Treatments. John Wiley & Sons, Ltd., West Sussex, England.

Riley, L., 1996. Quality management in viticulture. In: Hamilton, R., Hayes, P. (ed). Proc. Seminar Aust. Soc. Vitic. Oenol., August 1996, Mildura, Victoria, 5 - 6.

Romano, P., Suzzi, G., 1993. Acetion production in *Saccharomyces cerevisae* wine yeasts. FEMS Micro. Letters 108, 23-26.

Romano, P., Granchi, L., Caruso, M., Borra, G., palla, G., Fiore, C., Ganucci, D., Caligiani, A., Brandolini, V., 2003. The species-specific ratio of 2,3-butanediol and acetoin isomers as a tool to evaluate wine teast performance. Int J Food Microbiol. 86. 163-168.

Roussel, S., Bellon-Maurel, V., Roger, J.-M., Grenier, P., 2003. Authenticating white grapes must variety with classification models based on aroma snsors, FT-IR and UV spectrometry. J. Food Engineering, 60, 407-419.

Ruiz, A., Poblet, M., Mas, A., Guillamon, J.M., 2000. Identification of acetic acid bacteria by RFLP of PCR-amplified 16S rDNA and 16S-23S rDNA intergenic spacer. Int. J. Syst. Micrbiol., 50: 1981-1987.

Sánchez-Palomo, e., Díaz-Marot, M.C., Pérez-Coello, M.S., 2005. Rapid determination of olatiel compound in grapes by HS-SPME coupled with GC-MS. Talanta 66, 1152-1157.

Shinohara, T., Kubodera, S., Yanagida, F., 2000. Distribution of Phenolic yeasts and production of phenolic off-flavors in wine fermentation. J. Bioscience Bioengin. 90, 1, 90-97.

Sponholz, W.R. and Dittrich, H.H., 1979. Analytische Vergleiche von Mosten und Weinen aus gesunden und essigstichigen Traubenbeeren. Wein-Wissenschaft. 34, 270-292.

Sponholz, W.R. and Dittrich, H.H., 1985. Über die Herkunft von Gluconsäure, 2- und 5-Oxo-Gluconsäure sowie Glucurron- und Galacturonsäure in Mosten und Weinen. Vitis 24, 51-58.

Suárez, J.A., González, M.C., Callejo, M.J., Colomo, B., González, A., 1994. Contribution to the study of varietal wines from Rioja and Navarra. I. Microbial growth trends during grape maturation. Bulletin de L'O.I.V., 759-760.

van der Westhuizen, T.J., Augustyn, O.P.H., Pretorius, I.S., 2000. Geographical distribution of indigenous *Saccharomyces cerevisae* strains isolated from vineyards in the coastal regions of the Western Cape in South Africa. S. A. J. Enol. Vitic. 21, 3-9.

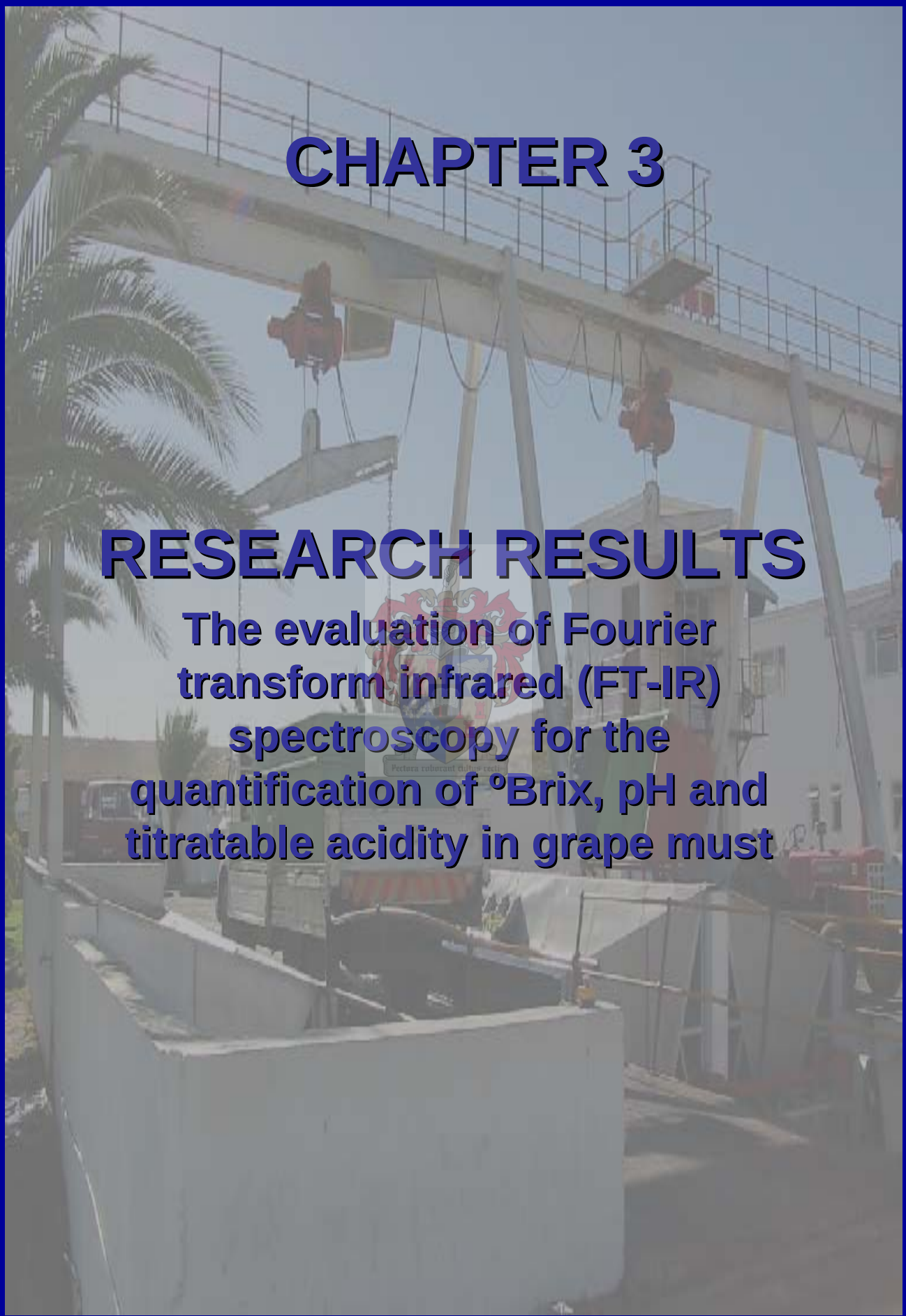
Vonach, R., Lendl, B., Kellner, R., 1998. High-performance liquid chromatography with real-time Fourier-transform infrared detection for the determination of carbohydrates, alcohols and organic acids in wines. J. Chromatography A, 824, 159-167.

Yamada, Y., Hoshino, K., Ishikawa, T., 1997. The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA: The elevation of the subgenus *Gluconobacter* to the generic level. Biosci. Biotech. Biochem., 61: 1244-1251.

Zoecklein, B.W., 1996. *Botrytis cinerea* and Sour rot. Vitner's corner. 11, No. 6.

Zoecklein, B.W., Williams, J.M., Duncan, S.E., 2000. Effect of Sour rot on the composition of White Riesling (*Vitis vinifera*L.) grapes. Small fruits review, 1(1), 63-77.

Zoecklein, B.W., Wolf, T.K., Duncan, N.W., Judge, J.M., Cook, M.K., 1992. Effects of fruit zone leaf removal on yield, fruit composition, and fruit rot incidence of Chardonnay and White Riesling (*Vitis vinifera* L.) grapes. Am. J. Enol. Vitic., 43, No. 2.



CHAPTER 3

RESEARCH RESULTS

The evaluation of Fourier transform infrared (FT-IR) spectroscopy for the quantification of ºBrix, pH and titratable acidity in grape must

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ABSTRACT

Principal component analysis (PCA) and Partial Least Square-Regression were used to construct the calibration models for °Brix, pH and TA and the validation of global FOSS calibrations. Fourier transform infrared (FT-IR) spectra of 677 grape must samples from 2003 to 2005 harvest were collected. The main source of variation between the grape samples were the sugar to acid content in the grape must samples. PCA helped to identify the deviating samples as well as the wavenumbers with highest loadings for each parameter. SEP of 0.35 and 0.074 and r^2 of 0.98 and 0.84 are reported for the validation of the °Brix and pH calibrations. A lower SEP of 0.24 g/L was obtained for a new TA calibration when all the samples were used. The mean bias of the validation of the global calibration was -3.04, indicating high residual values between the reference and predicted TA values. The new TA calibration could present better results. SEP for the global calibration was 0.25 g/L and RPD ratio 9.3. These results confirmed the successful implementation of FT-IR in the analysis of grape must samples.

3.1 INTRODUCTION

Wine quality has been correlated to grape and wine composition. Extensive research has been done to identify the physical and chemical characteristics of grapes that are associated with quality (Du Plessis, 1984; Callao *et al.,* 1991; Gishen *et al.,* 2002; Dambergs *et al.,* 2003; Tardaguila and Martinez de Toda, 2004).

Although it is considered not sufficient in the determination of grape quality, total soluble solids (predominantly sugars, measured as °Brix/Balling) and acidity (measured as pH and titratable acidity) is a widely accepted maturity gauge (Zoecklein, 2001). Initial sugar levels in grape must prior to fermentation were

proven to be important in the formation of esters in Chardonnay wine (Lee *et al.,* 2004). It is recognized that other components such as colour, phenolics, nitrogenous compounds, flavour and the sanitary state of grapes are equally important but are difficult to determine and quantify (du Plessis, 1984). Recent developments in rapid analytical techniques, for example Fourier transform infrared (FT-IR) spectroscopy, offer the prospect of providing the ability to measure these parameters in a cost effective manner more rapidly and more frequently than ever before (Skoog *et al.,* 1997).

FT-IR utilizes the interference of two radiation beams to yield an inteferogram. An inteferogram is a signal produced by two radiation beams. Inteferogram is the interference intensity as a function of the change of optical path difference. The two domains of distance and frequency are inter-convertible by the mathematical Fourier transformation. Although the basic optical component of FT-IR spectrometers, the Michelson interferometer has been known for almost a century, it was not until advantages in data acquisition and computing in late 70's and early 80's that the technique could be successfully and widely applied (Skoog *et al.,* 1997). Organic compounds are unique in the way that they have inter-atomic bonds, which are optically active and therefore vibrate in mid infrared radiation with characteristic frequencies. Covalent bonds in the functional groups are C-C, C-H, O-H, C=O and N-H. The technology is based on the measurement of the frequencies of the vibrations of the chemical bonds. The characteristic wavenumbers at which absorbance will depend on the bond itself and also upon its molecular environment. Thus a given molecule will absorb at wavenumbers characteristics within the infrared spectrum (Smith, 1999). Absorption intensity is directly proportional to the concentration of the molecule being examined. The FT-IR interferometer scans the full infrared spectrum (FOSS, 2001). Only in recent years Fourier transform spectroscopy combined with advances in chemometrics (multivariate data analysis) has found increasing use in industrial applications. The number of applications of FT-IR spectrometers is increasing continuously. The Winescan FT120 (FOSS Electric, Denmark) is a FT-IR spectrometer, with a purpose built interferometer, developed for oenological applications (Dubernet & Dubernet, 2000). Global calibrations consist of data to which a model has been fitted, so that it describes the data as good as possible (Esbensen, 2002).

The calibration process utilizes multivariate statistical procedures such as principal component analysis (PCA) and partial least squares (PLS) regression

(Esbensen, 2002). The successful validation of calibration models depend on accurate data for the reference method, the detection of extreme deviating (outlier) samples, identifying poorly predicted samples and including sample types in the validation set in the same range as the calibration model (Nieuwoudt, *et al.,* 2004).

Quantification of total soluble solids (°Brix) in must has been tested using Fourier transform near infrared and a standard error of prediction (SEP) value of 0.31 for °Brix was reported (Manley *et al.,* 2001). NIR spectroscopy calibration obtained a standard error of prediction of 0.05 – 0.08 units for pH (Dambergs *et al.,* 2003). The reference method for total soluble solids, pH and titratable acidity are routine and easily performed, but the ability of FT-IR spectroscopy to generate predictions for multiple measurements from one scan of filtered grape must gives it an advantage in terms of speed and convenience.

Sugar accumulation and acid reduction are the main reactions in grapes during ripening (Marais, 2004). These two parameters has also been positively correlated to wine quality and other grape constituents such as nitrogen and total phenols (Sinton *et al.,* 1978). Therefore they are commonly used as quality parameters. °Brix, pH and TA remain the quality parameters that are used by all wineries and grape growers. These parameters are important for structural characteristics of the wine and are the backbone of quality grading. Alcohol, which can be pre-determined using °Brix, has important fermentation and legal implications. pH and TA are important for stability and longevity of the end product. Therefore, the objective of this study was to establish a FT-IR calibration for °Brix, pH and TA, respectively.

3.2 MATERIALS AND METHODS

3.2.1 Grape samples: Sample sets for °Brix, pH and TA consisted of 647, 162 and 271 grape must samples, respectively. The samples represented 8 different cultivars (Sauvignon Blanc, Chardonnay, Colombar, Chenin Blanc, Merlot, Pinotage, Cabernet Sauvignon and Shiraz) and were collected over 3 consecutive vintages (2003 to 2005). Grape samples of various vineyards were collected in the Olifants River Valley. **Table 3.1** shows the descriptive statistics (average, standard deviation and range) of °Brix, pH and TA of the grape must samples.

Table 3.1. The component range, measured by reference method, of the grape must samples used in the design and validation of the °Brix, pH and TA calibrations.

	°Brix	pH	TA (g/L)
Samples (n)	647	162	271
Average	19.80	3.23	8.52
Minimum	11.0	2.77	4.26
Maximum	25.60	3.78	14.90
Standard Deviation	2.66	0.19	2.33

Upon arrival at the winery laboratory, the grape samples were immediately, pressed manually using a kitchen masher (**Fig.3.1**).

Figure 3.1. Grapes being pressed by hand on receipt at the laboratory.

The must were filtered with a Filtration Unit (type 79500, FOSS Analytical, Denmark) connected to a vacuum pump. The filter unit uses filter paper circles graded at 20 – 25 µm with diameter 185 mm (Schleicher & Schuell, reference number 10312714). The filtered must were mixed and divided for FT-IR spectral measurements and reference analysis to ensure homogeneity.

3.2.2 Reference analysis

The accuracy of the reference method was expressed as the standard error of laboratory (*SEL*) and calculated as:

$$SEL = \sqrt{\frac{(y_1 - y_2)^2}{2n}}$$

where y_1 and y_2 are the results of duplicate determinations and n is the number of samples.

3.2.2.1 Soluble solids determinations: Soluble solids (°Brix represent sucrose in gram per 100 g of solution) were assayed by refractometry (Zoecklein *et al.,* 1999). Using, an automated digital refractometer (Atago Palette model PR-32α, cat. No. 3405, Japan) with temperature compensation and an accuracy of 0.1% °Brix, calibrated against a 20 °Brix sucrose solution.

3.2.2.2 pH: The hydrogen ion concentration (pH) of each must sample, was determined using a pH meter as part of an automatic titrator equipped with a combination electrode (Crison, ref no. 4473624, LASEC) and temperature probe. Certified buffers (pH 7.00 and 4.00, LASEC, Cape Town, South Africa) were used for the calibration of the electrode (Zoecklein *et al.,* 1999).

3.2.2.3 Titratable acidity analysis: Titratable acidity (expressed as g/L Tartaric acid) of each 50 mL grape must sample was measured by potentiometric titration (Zoecklein *et al.,* 1999) using standardized 0.33 N Sodium hydroxide (LASEC, Cape Town, South Africa) to the end point of pH 7.00 (Crison Compact Titrator D, SN 01714, software version 5.6).

3.2.3 FT-IR spectral measurements: Duplicate scans were obtained of each must sample immediately after filtration using a Winescan FT120, equipped with a purpose built Michelson interferometer was used to generate the FT-IR spectra (FOSS Electric A/S, Hillerød, Denmark). The instrument settings include cell path length of 37 µm, sample temperature set to 40°C, and sample volume of 7 – 8 ml. The sample is pumped through the heat exchanger and the CaF_2-lined cuvette. Samples are scanned from 5011 to 926 cm^{-1} at 4 cm^{-1} intervals. These settings can not be changed. The frequencies of the infrared beam transmitted by a sample are recorded at the detector and used to generate an interferogram that is calculated from a total of 20 scans. The interferogram undergoes a Fourier transformation and converts it into a sample single-beam transmittance spectrum. Division of the sample single-beam spectrum by the zero-beam spectrum, obtained by doing a zero setting with a Foss Zero liquid S-6060 prior to the must samples, in order to correct for the background absorbance of water, gives the transmittance spectrum. A series of mathematical procedures allows the transmittance spectrum to be converted into a linearized absorbance spectrum (Winescan FT 120 Type 77110 and 77310 Reference manual, Foss Electric, Denmark, 2001).

***3.2.3.1 Wavenumber selection*:** To exclude noise being introduced in the spectral data, the Advanced Performance Module software allows only the following regions to be available for wavenumber selection, it is set by the manufacturer: 964 – 1562 cm^{-1}, 1716 – 1813 cm^{-1} and 2700 – 2970 cm^{-1}. The regions 1543 – 1716 cm^{-1} and 2970 – 3626 cm^{-1} are water absorption areas (Nieuwoudt *et al.,* 2004). The region from 3626 – 5011 cm^{-1} contains very little information (Winescan FT 120 Type 77110 and 77310 Reference manual, Foss Electric, Denmark, 2001). Fifteen "Filters", wavenumbers or groups thereof, are selected (by default) which best describes the correlation between the reference value and measured absorbance of the spectra.

3.2.3.2 Global calibrations: Calibrations are made using samples of specific component ranges (**Table 3.2**).

Table 3.2. Component range of the data sets used in the global calibrations for °Brix, pH and TA.

Component	Average	Min	Max	Reference
°Brix	15.88	8.34	23.10	Appl. Note 178, 2001
pH	3.27	2.64	4.07	Appl. Note 175, 2001
TA	5.59	1.60	12.99	Appl. Note 177, 2001

Global calibrations needs to be validated before implemented, in order to observe the rules of Good Laboratory Practise (GLP). Results from the global calibration can be adjusted, if necessary, to a final calibrated result by slope and intercept adjustment.

3.2.4 Chemometrics and data analysis

3.2.4.1 Principal Component Analysis (PCA): FT-IR spectra of each individual data set of °Brix, pH and titratable acidity were exported to the Unscrambler Software (version 9.1.2, Camo Process ASA, Oslo, Norway) for the purpose of PCA. Duplicate spectra were averaged. Mean-centering (subtracting the average value from a variable, for each data point) by column of the whole data matrix, defined by the variables (1056 wavenumbers) in columns and the samples in rows, was done. The complete data matrix were scaled or weighted by using the inverse of the standard deviation (1) in order to make all variable variances more comparable (Esbensen, 2002).

$$x^{scaled} = x * \frac{1}{SDev} \quad \text{.................................(1)}$$

With PCA the maximum directions of variation in a data set is modelled by projecting the objects (in this study, the FT-IR spectra) as a swarm of data points in space defined by principal components (PC's). PC's contain in decreasing order the main structured information in the data. A PC is the same as a score vector, and also called a latent (underlying) variable. PC's are calculated to be orthogonal to one another and therefore can be interpreted independently. This permits an overview of the data structure by revealing relationships between the objects as well as the detection of deviating objects. In order to find these sources of variation, the original data matrix, defines by X(*n,m*), is decomposed into the object space, the variable space and the error matrix. The latter represents the variation not explained by the extracted PC's and is dependent on the problem definition. The algorithm describing the decomposition is presented as:

$$X(n,m) = T(n,k)P(k,m)^{T} + E(n,m)$$

Where X is the independent variable matrix, T the scores matrix, P the loadings matrix, E the error matrix, *n* the number of objects, *m* the number of variables and *k* the number of PC's used (Eriksson *et al.,* 1999; Esbensen, 2002).

3.2.4.2 Partial Least Squares Regression (PLS-R): The strategy for the development and validation of a calibration in **Fig. 3.2** was followed. The validation of the calibration was done by an independent test set. The was preferred to full cross validation, the reason being that the sub-models are too identical to the calibration set and the cross-validation error is not based exactly on the full model. The criteria for the samples of the test set were the following: a) in the same range as the calibration set; b) samples representing the same cultivars; c) samples also from early, mid and late ripening season; d) sample set size a third to halve of that of the calibration set.

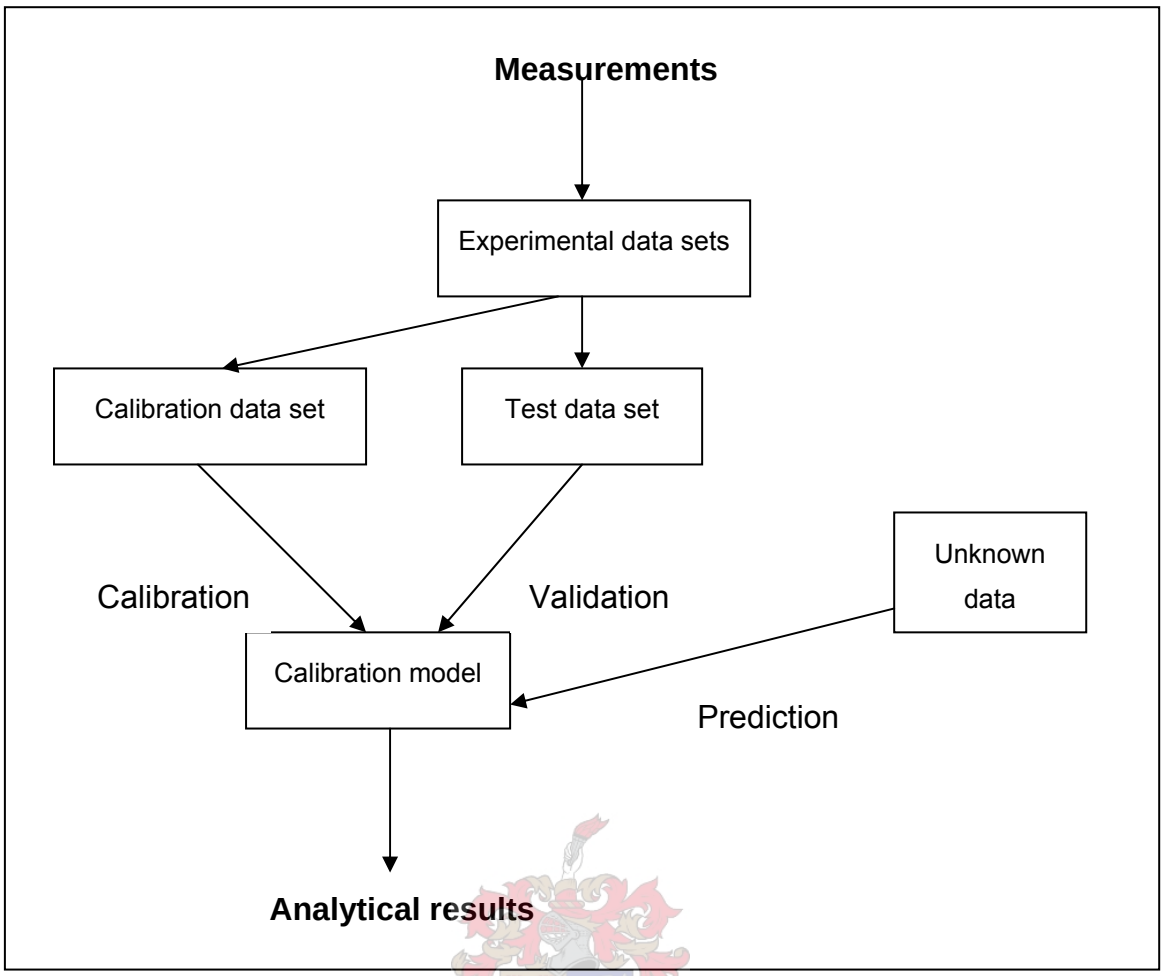


Figure 3.2. Flow diagram of the strategy to develop a calibration model.

The global calibrations for °Brix, pH and TA, of the Winescan FT120 Grapescan software, are made using Partial Least Squares (PLS), a bilinear modelling method. This mathematical technique is used to generate the calibration equation that best fits the reference value in the data set, for each individual parameter. PLS calibrations of these parameters were also made using the Unscrambler Software as well as the Advanced Performance Module version 2.2.2 which is part of the software of the Winescan FT120 instrument. The filters selected by the Winescan FT120 software for ºBrix, pH and titratable acidity calibrations were used as selected variables in the PLS regression calibration model for calibrations in the Unscrambler software. For the confirmation of outlier identification in PCA, the X-Y relation outlier plot (T vs. U scores) in PLS-R was investigated of each data set. Scores are information of several variables concentrated into a few underlying variables. Each sample has a score along each model component. Scores can be used to detect sample patterns, groupings, similarities or differences. T-scores are found in the X-matrix and U-scores in the Y-matrix. T scores vs. U scores plot shows directly how the regression works and gives a good overview of the relationship between X and Y

for one particular PC. All the samples form a straight regression line if the regression works well. Outliers "stick out" orthogonally from this line and extreme values lie at the ends. The loading weights (W), which are the effective loadings directly connected to building the regression relationship between X (data matrix-variables) and Y (reference value), for each PC were also investigated (Høskuldsson, 1996).

***3.2.4.3 Statistical indicators for evaluation of the performance of the calibration sets*:** Statistical indicators calculated by the Advanced Performance Module included bias, SECV (accuracy of the predictive ability of the calibration model in relation to the reference data) and SEP (accuracy in relation to the independent validation set). Bias gives an indication of a systematic error in the predictive values and it was calculated as the average of the residuals (residuals are the difference between the reference values and predicted values) (Esbensen, 2001).

$$SEP = \sqrt{\frac{\sum_{i=1} (\hat{y}_i - y_i - Bias)^2}{n - 1}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Equation for SEP, the Standard error of performance, corrected for the *Bias*.

The residual predictive deviation (RPD) was calculated for each calibration to evaluate how well the model could predict. The RPD ratio is defined as the standard deviation (SD) of the population's reference values divided by the standard error in prediction (SEP). If the RPD value is less than 3 than the calibration model is not robust and unsuitable for quantification purposes. A value above 3 can be considered good for prediction purposes (Williams, 1995).

3.3 RESULTS AND DISCUSSION

3.3.1 FT-IR Spectra

A FT-IR spectrum of grape must provides the collective absorbance of all IR active components present in the sample. The main features in the spectra are due to absorbance of water in the wavenumber regions, 1543 – 1716 cm^{-1} and 2970 – 3626 cm^{-1} (Winescan FT 120 Type 77110 and 77310 Reference manual, Foss Analytical, Denmark, 2001). **Fig. 3.5a** shows spectra of Chardonnay grape must (23.6 °B). Distinct variation between the FT-IR spectra of grape must at different % sugar levels

and wine was observed in the region 930 – 1600 cm^{-1} (**Fig. 3.5b**). The region 930 – 1600 cm^{-1}, referred to as the "fingerprint region" (similar molecules give different absorption patterns at these wavenumbers) shows prominent peaks. The most prominent filters for ºBrix, pH and TA were at wavenumbers 1720 – 1751 cm^{-1}, which corresponds to the – COOH (carboxylic acids) and C=O (carbonyl compounds). More than 83% of the variance between samples were explained by PC1 and correlates with variance of acid to sugar content in the grape must.

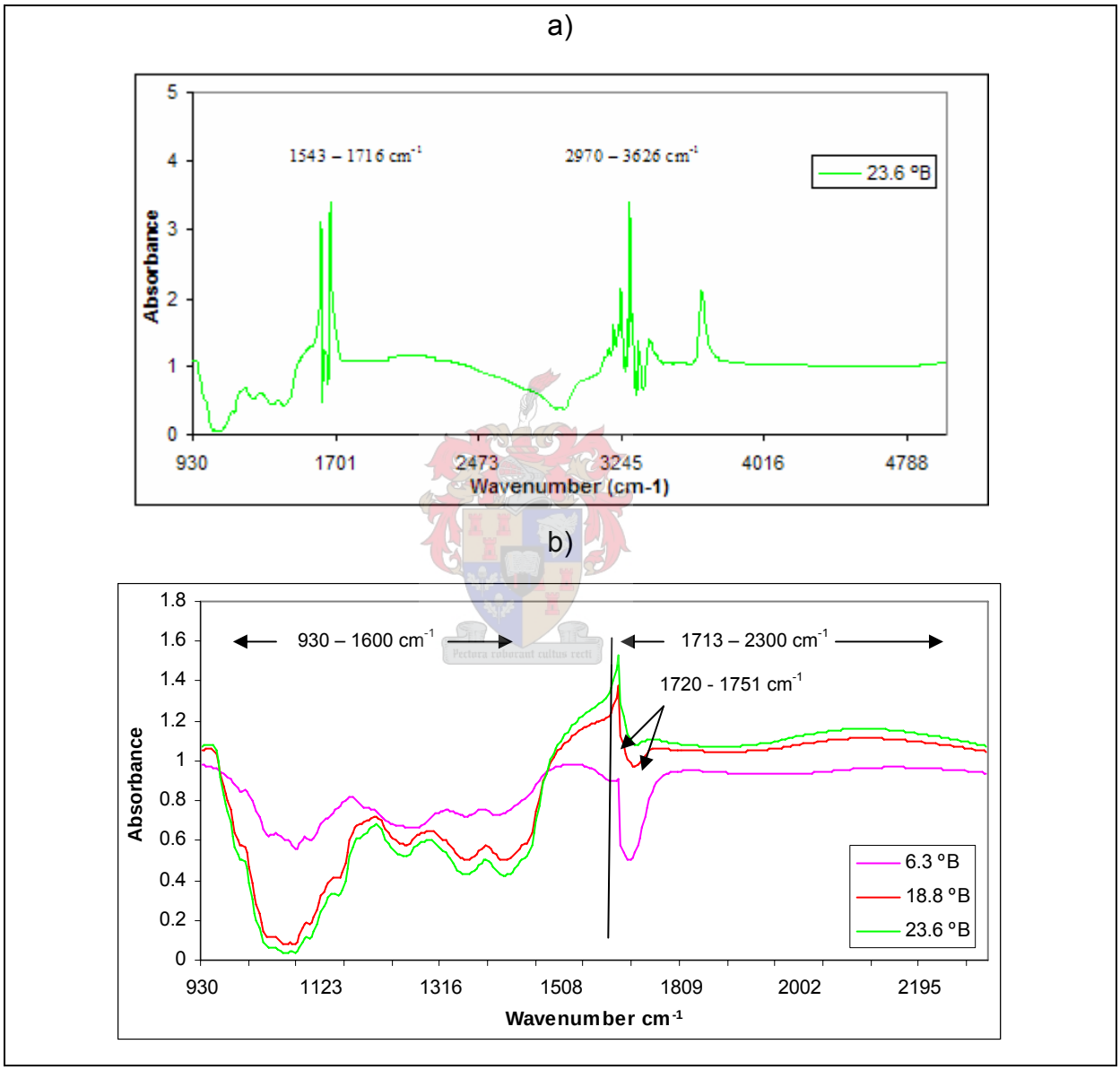


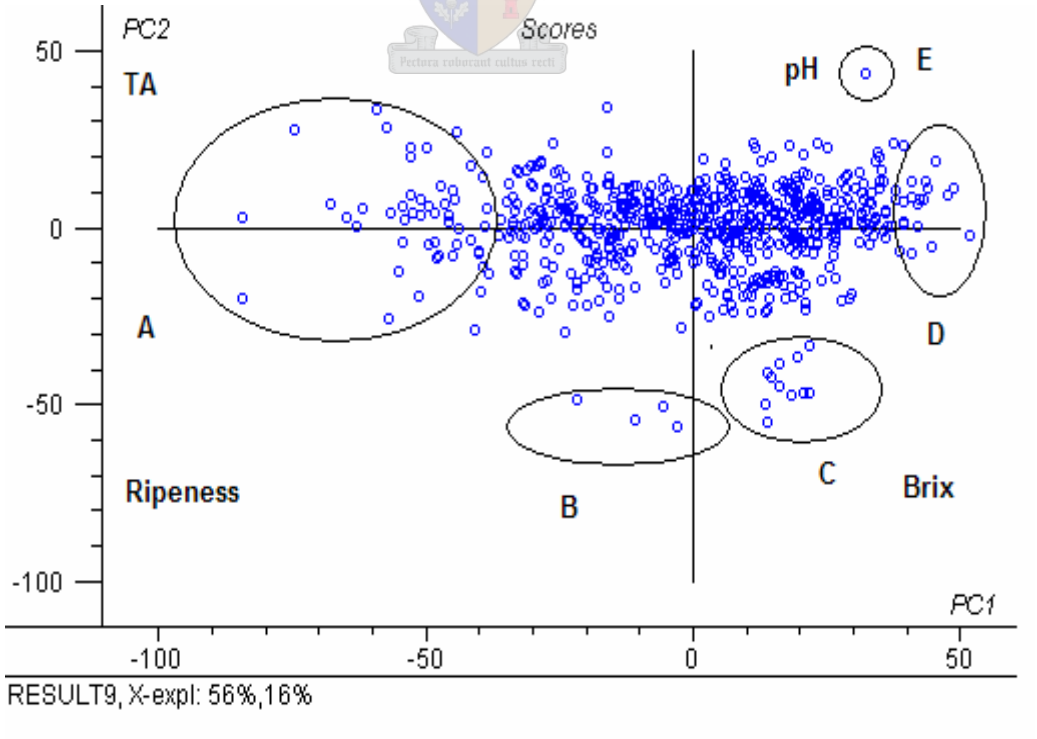
Figure 3.5. (a) FT-IR spectra of Chardonnay grape must in the region 930 – 5011 cm^{-1}, indicating the 2 water absorbance regions, 1543 – 1716 cm^{-1} and 2970 – 3626 cm^{-1}. (b) "Finger print" (930 – 1600 cm^{-1}) and 1713 – 2300 cm^{-1} region which include the peak at 1720 - 1751 cm^{-1} where 83% of the variance for acid to sugar content between grape must samples, were explained.

3.3.2 PCA Modelling

Principal Component Analysis was done on the complete data matrix, which included all the samples (n=667) and wavenumbers (**Fig. 3.6a**). Samples marked A in the

score plot, are low in °Brix and high in TA. Samples from B and C has the same °Brix but B has higher TA than the samples in C. The samples marked D has the highest °Brix (25 - 26°B). The one sample E has the same °Brix as the samples in the clusters below (±23°B) but the pH of this sample is low (pH = 3.2) in comparison to the others with the same °Brix. The loadings plot (**Fig. 3.6b**) shows the high loadings of wavenumbers in the water absorbance regions (B = 1543 – 1716 cm^{-1} and D = 2970 – 3626 cm^{-1}). The wavenumbers 1720 – 1751 cm^{-1} marked C is the filter which explained the samples the most. PC1 explained 56% of the variance between the samples. In order to evaluate the effective loadings of other wavenumbers, it was necessary to exclude the water absorbance regions from the PCA model. PC1 now explained 83% of the variance between samples (**Fig. 3.7a**). The wavenumbers with the highest loadings 1474 – 2685 cm^{-1}, corresponds to the range that were selected as filters for the calibration of °Brix, pH and TA in the Calibration Master of the Winescan FT 120 (**Fig. 3.7b**). Possible extreme deviating samples with a high negative score was grape must of lower than 10°Brix (far left bottom corner in **Fig. 3.7a**). These samples (n=4) was excluded from the sample sets, as they were far from the centre of the model.

a)



b)

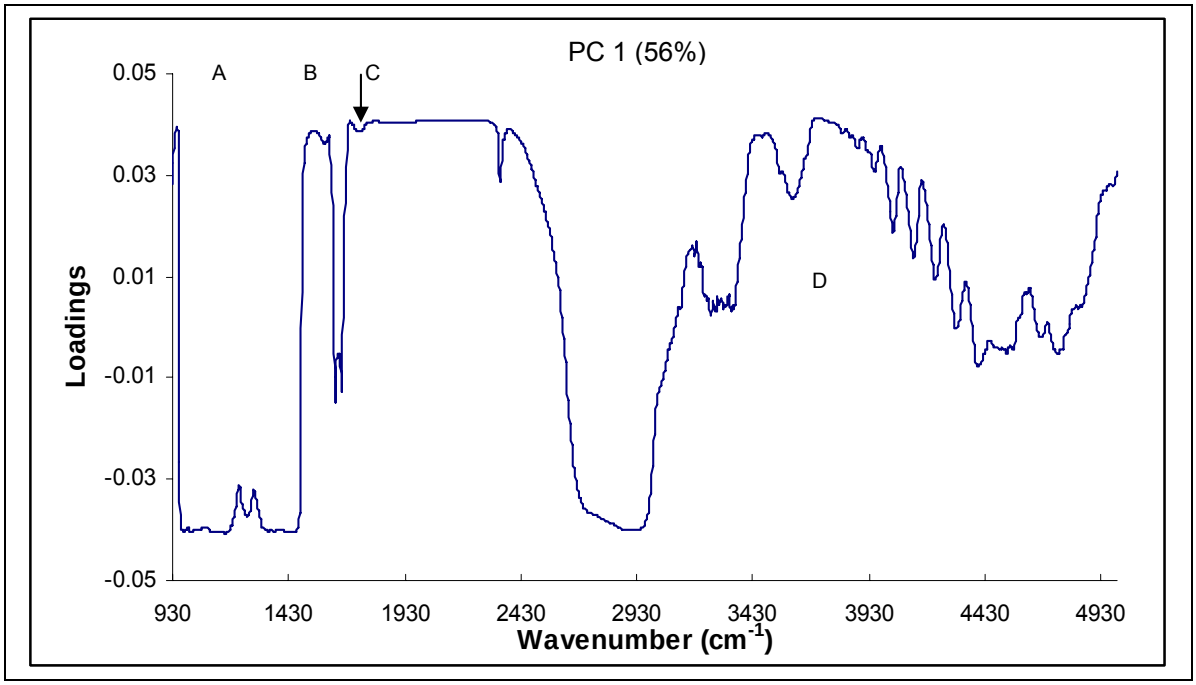
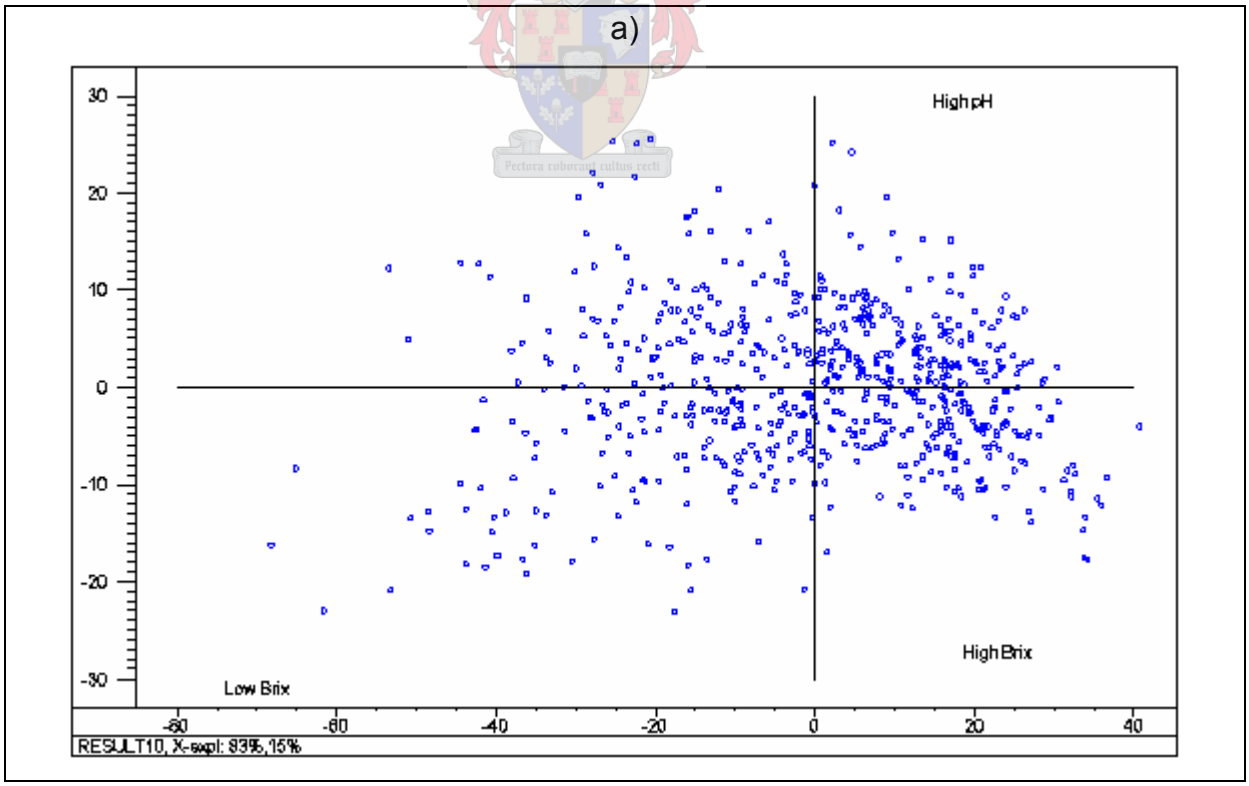


Figure 3.6: a) PCA score plot of all the grape must samples, PC1 versus PC2. PC1 explains 56% of variance between the grape must samples. b) Loadings plot of PC1, A is the loadings of the "finger print region" (930 – 1600 cm^{-1}), B is the loadings of water absorbance (1543 – 1716 cm^{-1}), C is the loadings of 1720 -1751 cm^{-1} and D is the loadings of water absorbance (2970 – 3626 cm^{-1}).

a)



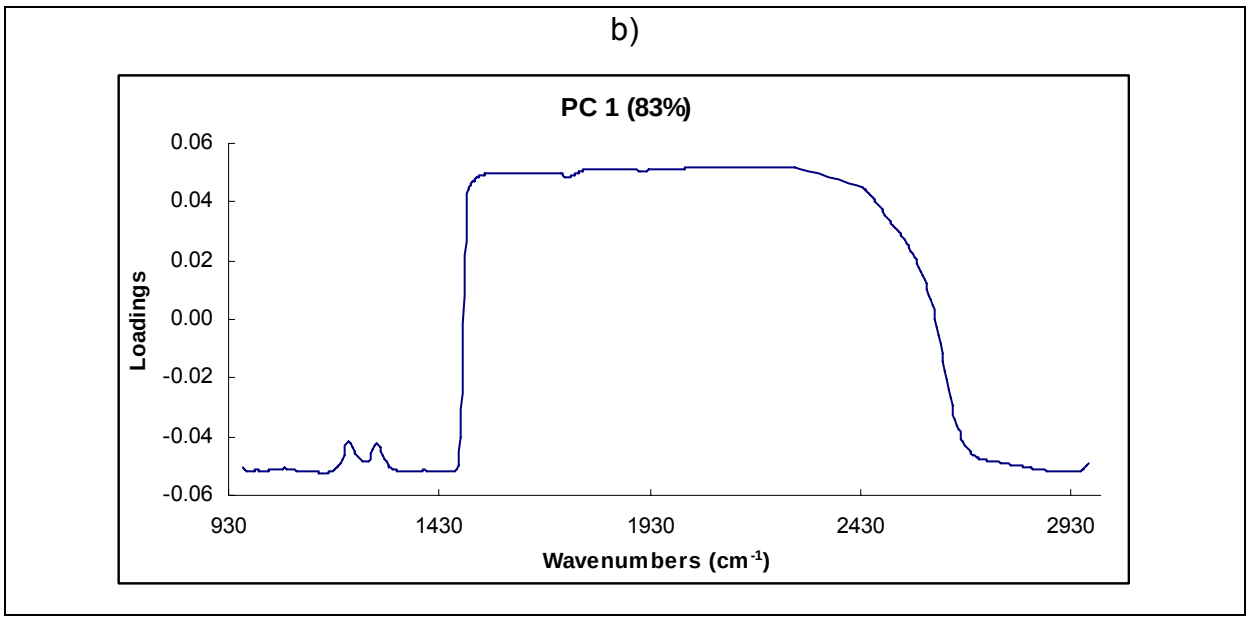


Figure 3.7. (a) PCA score plot on all the grape must samples, without the water absorbance regions (1543 – 1716 cm-1 and 2970 – 3626 cm-1). PC1 explains 83% of variance between the samples. (b) Loadings plot for PC1, 1474 – 2685 cm-1 has the highest loadings.

Subsequent PCA was done to model the relationship between the grape samples in regard to their sugar and acidity level. PCA was done on the data set without the extreme deviating samples, using only the selected wavenumbers based on 15 filters that explained more than 97% and 93.5% of the variation in the sugar content and acidity level, respectively, between the samples. Category variables were included in both models. There was a more defined separation of samples in the model based on the sugar content than acidity (**Fig. 3.8a)**. Sugar content had the largest variation in chemical composition between the grape samples based on the standard deviation. PC1 explained 91.4% of the variance between the samples. Wavenumber 1724 cm^{-1} had the highest loading (contributing to PC1) and explained 82.6% of the variance between the samples based on the sugar content of the grape must samples (**Fig. 3.8b**). This correlates with what was observed in the FT-IR spectra in the explorative stages.

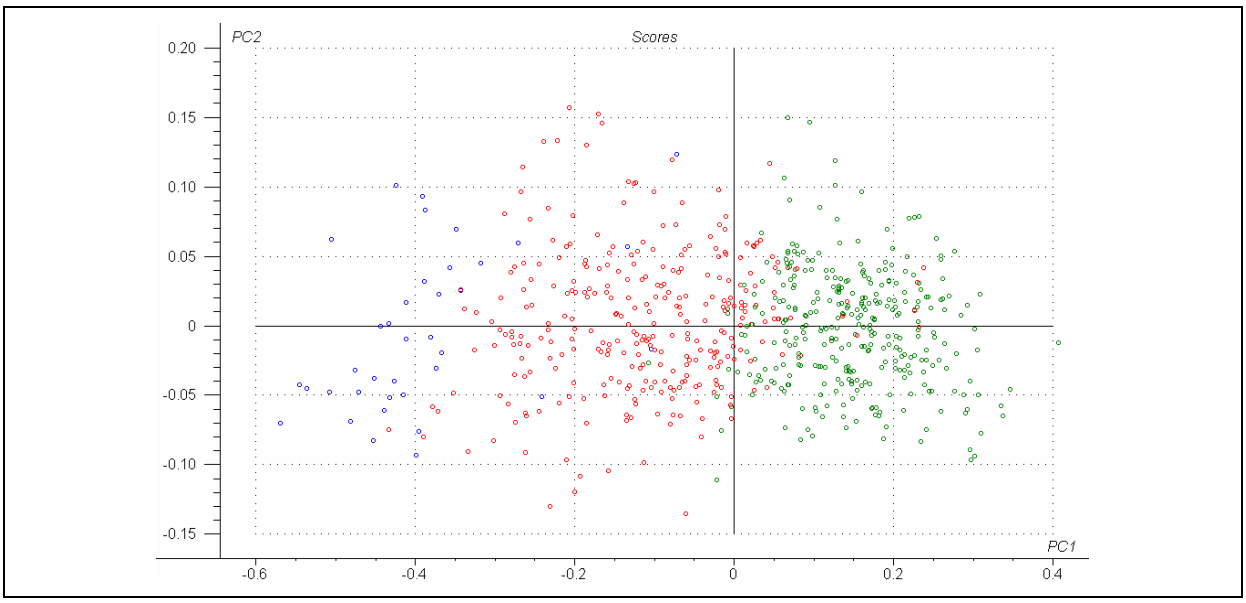


Figure 3.8a. PCA score plot without outliers and selected wavenumbers, with category variable differentiating between sugar levels (blue < 14.9°Brix, red 15 – 20°Brix, green > 20°Brix). PC1 explained 91.4% of the variance between the samples.

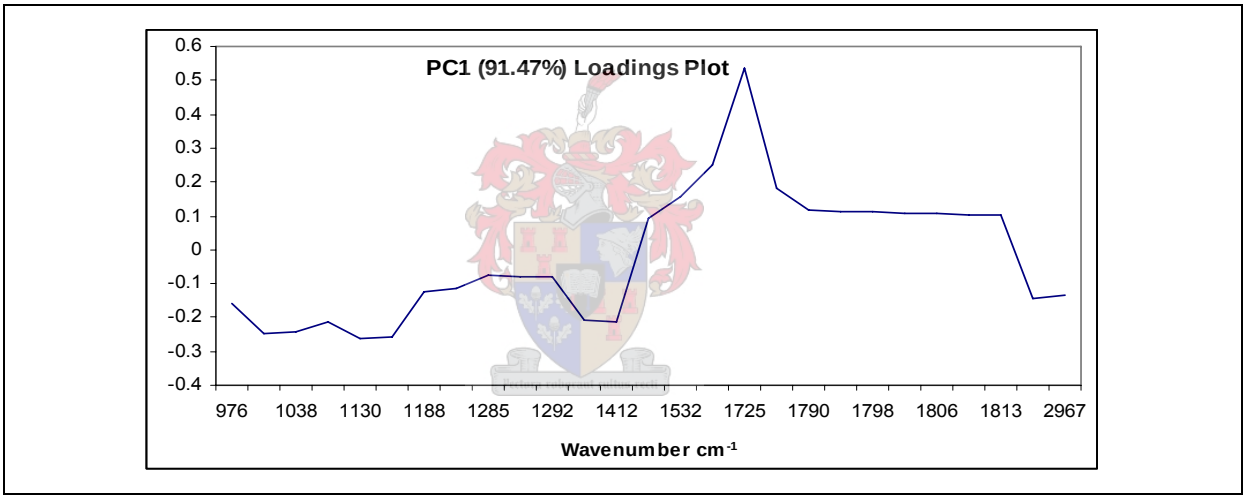


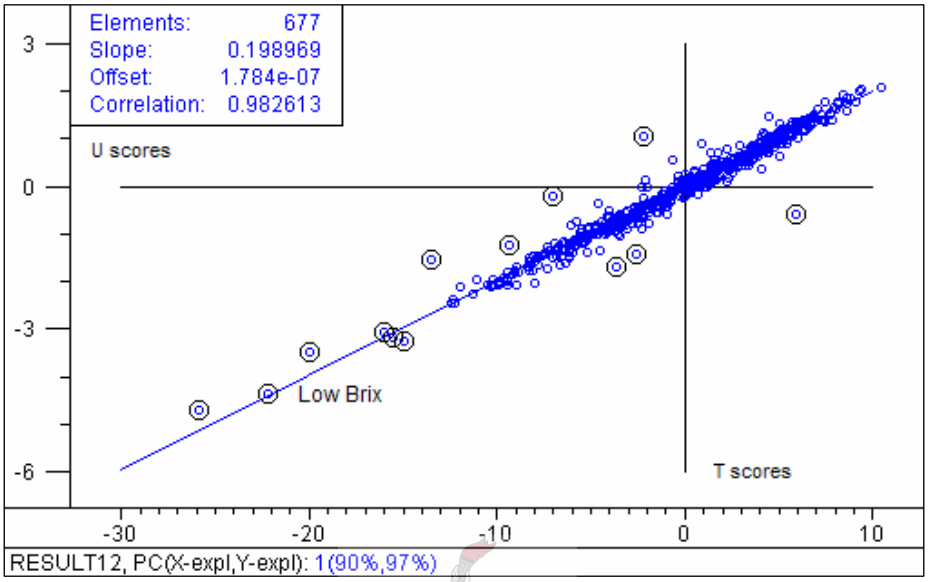
Figure 3.8b. PC1 (91.47%) loadings plot with peak area 1724 cm^{-1}.

3.3.3 Design and validation of calibration sets for °Brix, pH and Titratable acidity (TA) in grape must

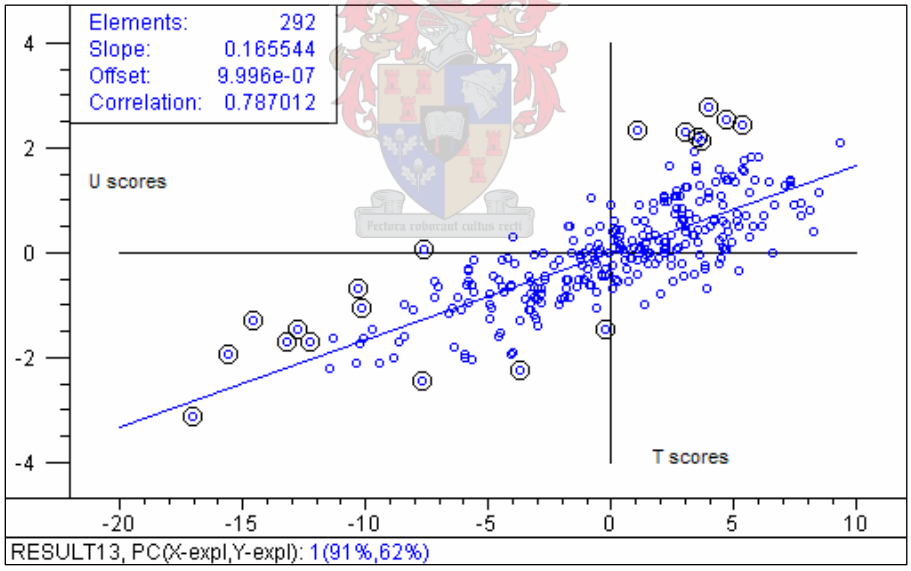
New calibration models for °Brix, pH and TA for grape must samples were first made and validated with an independent test set before validation of existing global Winescan FT120 calibration was attempted. A comparison could be made, using SEP values, between the calibrations of each parameter. Filters were deselected to ensure lowest possible SECV (indicating the accuracy of the predictive ability of the calibration model, based on the calibration samples) values. SEP values were used to express the accuracy of the calibration models based on validation samples. A comparison could be made, using SEP values, between the calibrations of each parameter to determine which calibration, new or global, was more accurate.

Extreme deviating samples detected by PCA and confirmed by the X-Y relation outlier plots (T vs. U scores) in PLS-R were not included in the models as well as samples that were poorly predicted or with high residual values. Outlier samples are marked in the T vs. U plots in **Fig 3.9**. These samples had high residual values.

a)



b)



c)

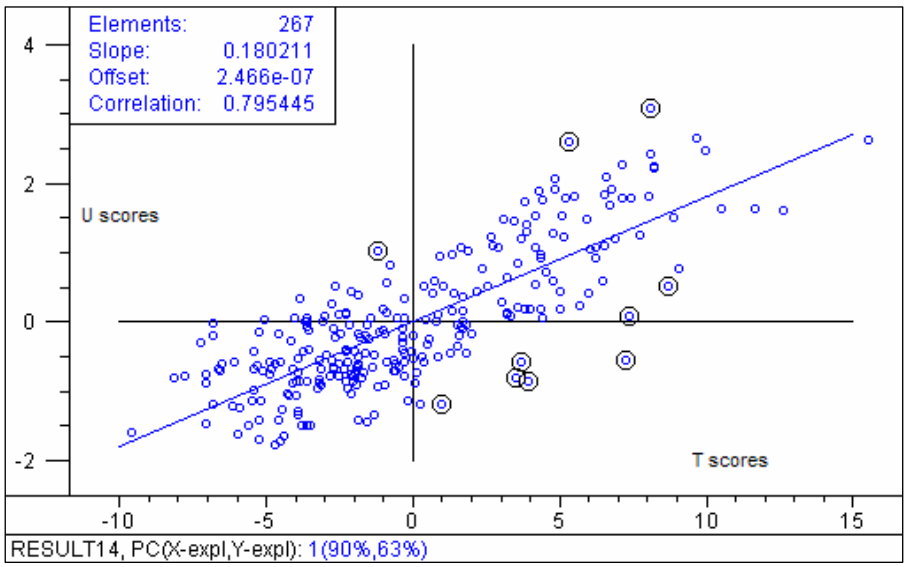


Figure 3.9. X-Y Relation outlier plots (T vs. U scores) of PC1 for a) °Brix, b) pH and c) TA with extreme deviating samples marked.

3.3.3.1 *°Brix:* Wavenumber 1724 cm^{-1} was selected as the first filter that explained 83% of the variance between the samples for sugar content. This correlated with the FT-IR spectra and loadings plot, in section 3.3.1. For the new °Brix calibration model, the sample set (n = 594) was divided in a calibration set (n = 379) and a validation set (n = 215) (**Fig. 3.10**). 15 Factors had the lowest SEC and absolute repeatability of 0.472. The bias of the validation of the new calibration was 0.07ºBrix and the coefficient of correlation 0.99. The SEC and SEP for this new calibration model was 0.34 and 0.32ºBrix, respectively. A RPD value of 7.5 was calculated by dividing the standard deviation of the reference data by the SEP. To obtain lowest possible SEC, filters were deselected. The lowest possible SEC was 0.33 and absolute repeatability of 0.045 with 8 PLS filters and factors. An independent validation set for this calibration had a SEC and SEP was 0.32 and 0.31ºBrix, respectively. The bias (0.068ºBrix) was much lower. The RPD value (8.5) was higher (**Table 3.3**).

In the residual plot of the PLS-R for °Brix, the residual values demonstrate a slight skewness (**Fig. 3.11**) with a small bias which correlates with the above. The bias is the difference between the average of the reference value minus predicted value. 92% of the samples had a prediction error smaller than ± 0.5 °Brix. 98.6% of the samples had a prediction error smaller than ± 1.0°Brix. 1.4% of the samples were predicted larger than ± 1.0°Brix. These results indicated an excellent calibration for quantification purposes.

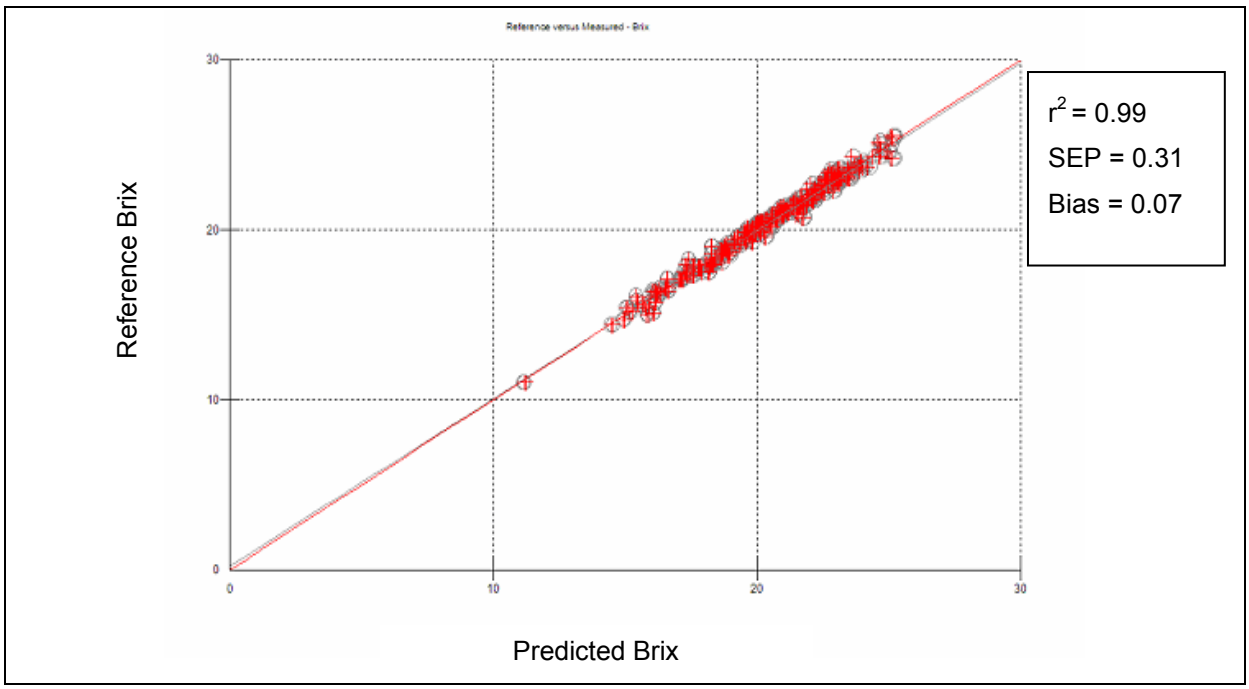


Figure 3.10. Reference (grey) vs. Predicted (red) ºBrix plot of the validation sample set for the New Calibration model for ºBrix (n=215).

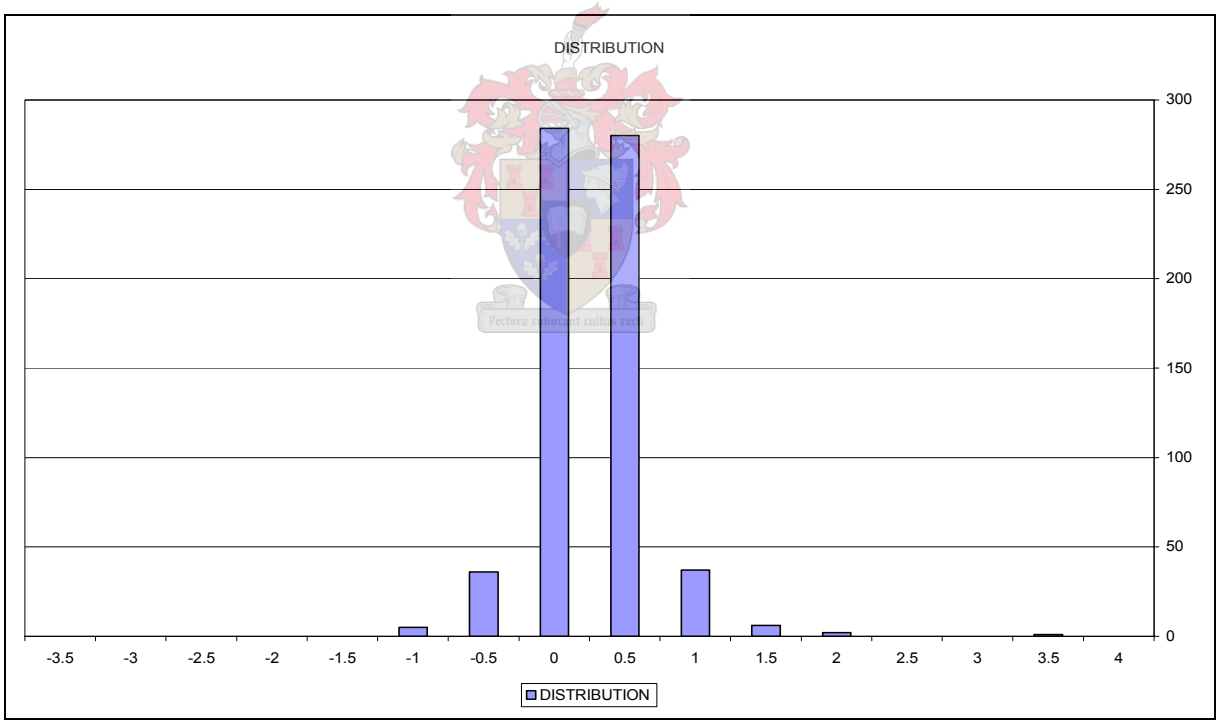


Figure 3.11. Residual plot of PLS-R for ºBrix of grape must samples. The x-values designate the means of intervals (± 0.5ºBrix).

The complete data set (n = 594) was used for the validation of the global FOSS calibration. The SEC of the calibration, was 0.42°Brix and bias was 0.21°Brix. The bias exceeds the repeatability of the reference method, which is 0.1°Brix. A slope and intercept adjustment was proposed which are calculated automatically by the Winescan FT 120 software. This adjustment can be described as:

Final Result = (Result from global calibration) * C_1 + I_1

where C_1 is the slope and I_1 is the intercept. After the above adjustment the SEP is 0.35ºBrix. The coefficient of correlation (r^2) was 0.98. A good correlation exists between the reference value and predicted value by the calibration model (**Fig. 3.12**). This corresponds to the T vs U plot, earlier (**Fig. 3.9a**) which explained X - 90% and Y- 97% relation.

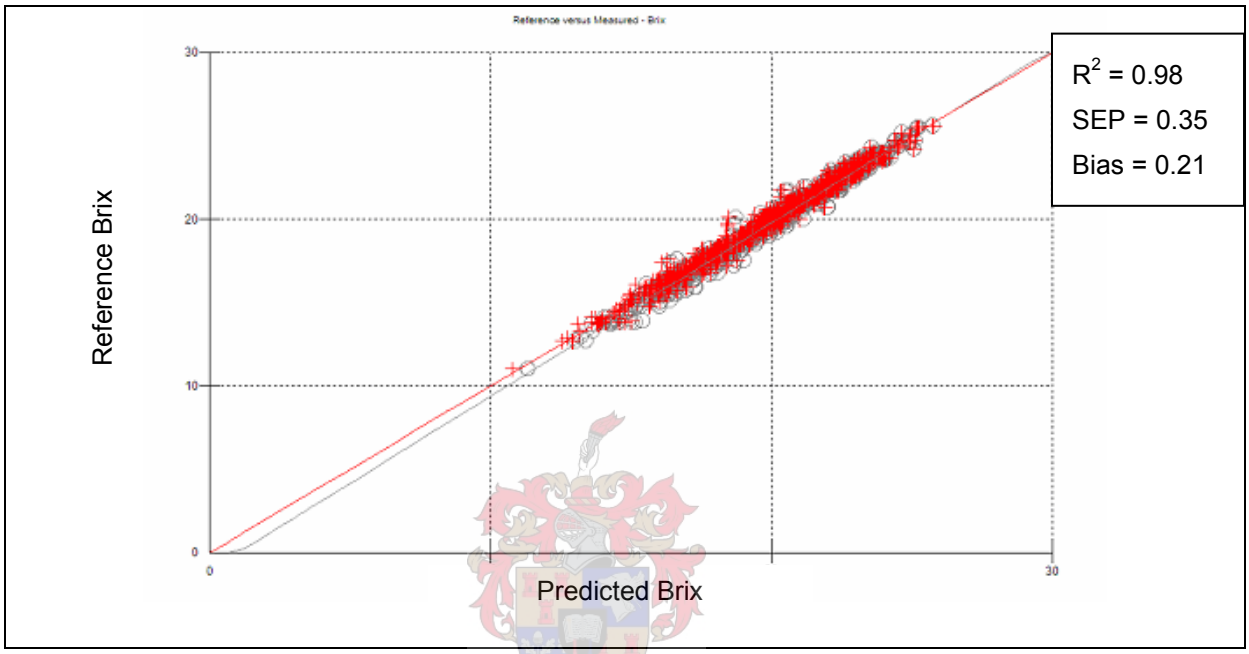


Figure 3.12. Reference (grey) vs. Predicted (red) ºBrix plot of the validation sample set for the Global ºBrix calibration (n=592).

3.3.3.2 pH: Wavenumber 1752 cm^{-1} explained 78% of the variance between samples for the pH of grape must. 7.6% Accumulated explained variance was described by wavenumber 1562 cm^{-1}. A total of 94% variance between samples were explained by 15 PLS filters. The SEC and SEP for the validation of the global commercial calibration was 0.094 and 0.074, respectively. The bias was -0.06 (**Table 3.3**). The correlation between the reference and predicted pH-values (r^2) was 0.84 (**Fig. 3.13**). This lower value was expected due to the X-Y relation observed in the T vs. U plot (**Fig. 3.9b**). Only 68% of Y (reference value) was explained. Samples with high residual values were not eliminated from the sample set at this stage to achieve a better prediction model because no valid reason could be found as to why some samples were predicted with higher residuals (**Fig. 3.14**).

Table 3.3. PLS-R validation statistics for the calibration of ºBrix, pH and TA.

ºBrix	New FT 120 Calibration	Global Calibration
SEC[a]	0.33	0.42
SEP[b]	0.31	0.35
r^{2} [c]	0.99	0.98
RPD[d]	8.5	7.5
Bias	0.07	0.21
Filters	15	
PLS factors	15	
pH		
SEC	0.04	0.094
SEP	0.04	0.074
r^{2}	0.95	0.84
RPD	4.8	2.66
Bias	0.004	-0.06
Filters	15	
PLS factors	15	
TA		
SEC	0.24	3.2
SEP	0.28	0.25
r^{2}	0.99	0.99
RPD	8.3	9.3
Bias	-0.5	-3.04
Filters	11	
PLS factors	8	

a: standard error of calibration

b: standard error of prediction

c: Correlation coefficient

d: Residual predictive deviation

A new calibration model using 15 filters and PLS factors consisting of 162 calibration samples and 81 validation samples had a SEC of 0.04. The absolute repeatability was 0.0118. For the validation of the new pH calibration the SEP was 0.04, bias 0.004 and the coefficient of correlation 0.95 (**Fig.3.14**).

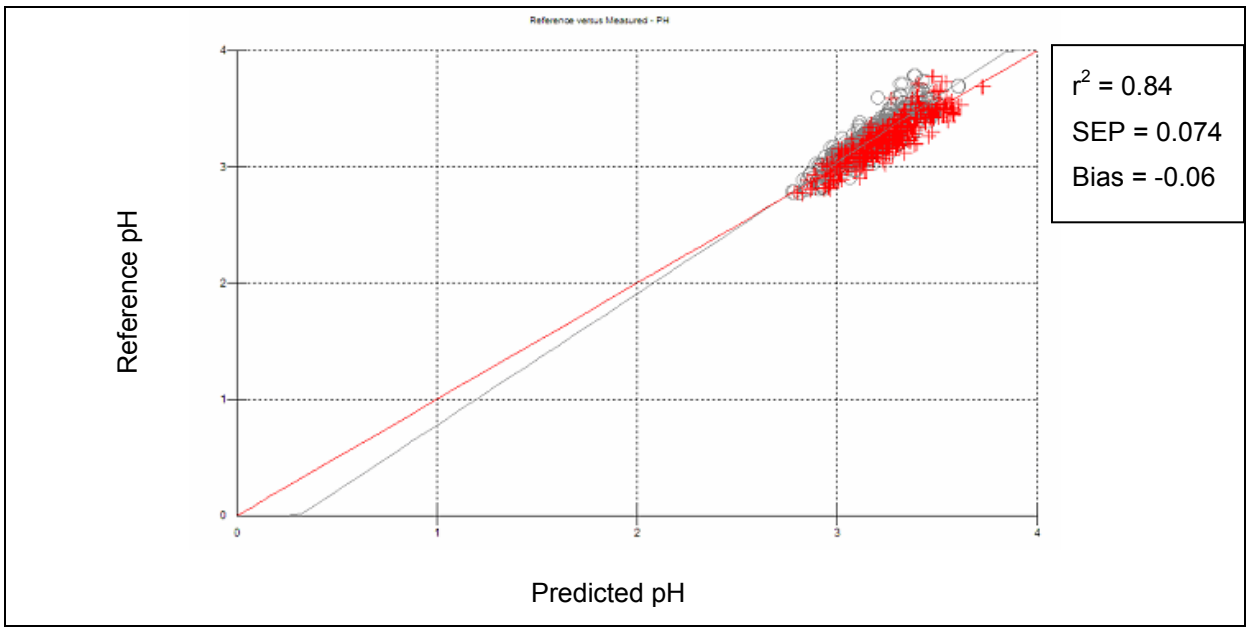


Figure 3.13. Reference (grey) vs Predicted (red) pH plot for the validation of the global pH calibration.

77% of the samples had a prediction error lower than ± 0.05 and 98% of the samples had a prediction error lower than ± 0.1 for pH (**Fig. 3.15**). Only 3% of the samples had a prediction error higher than ± 0.1. This model was more accurate than the validated commercial calibration but the reason for this could be the smaller sample set used to make the model. More samples will be added to the sample set before a decision of obtaining a better calibration can be made. At this stage a new calibration for pH looks promising.

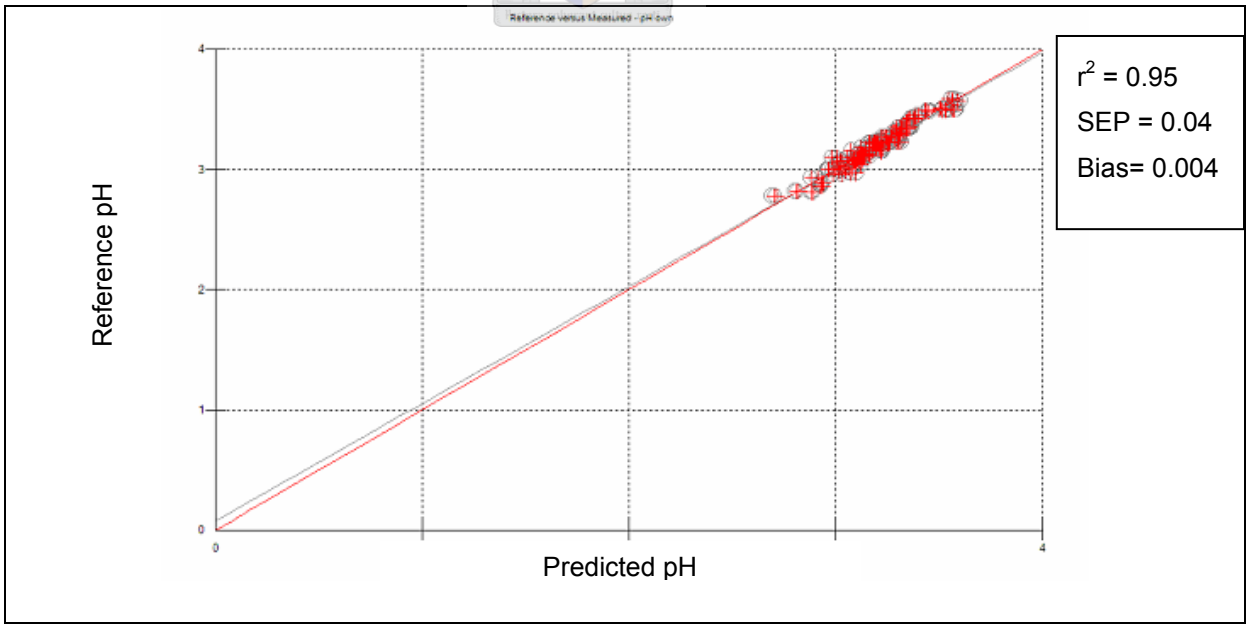


Figure 3.14. Reference (grey) vs predicted (red) pH plot of the validation of the new pH calibration model.

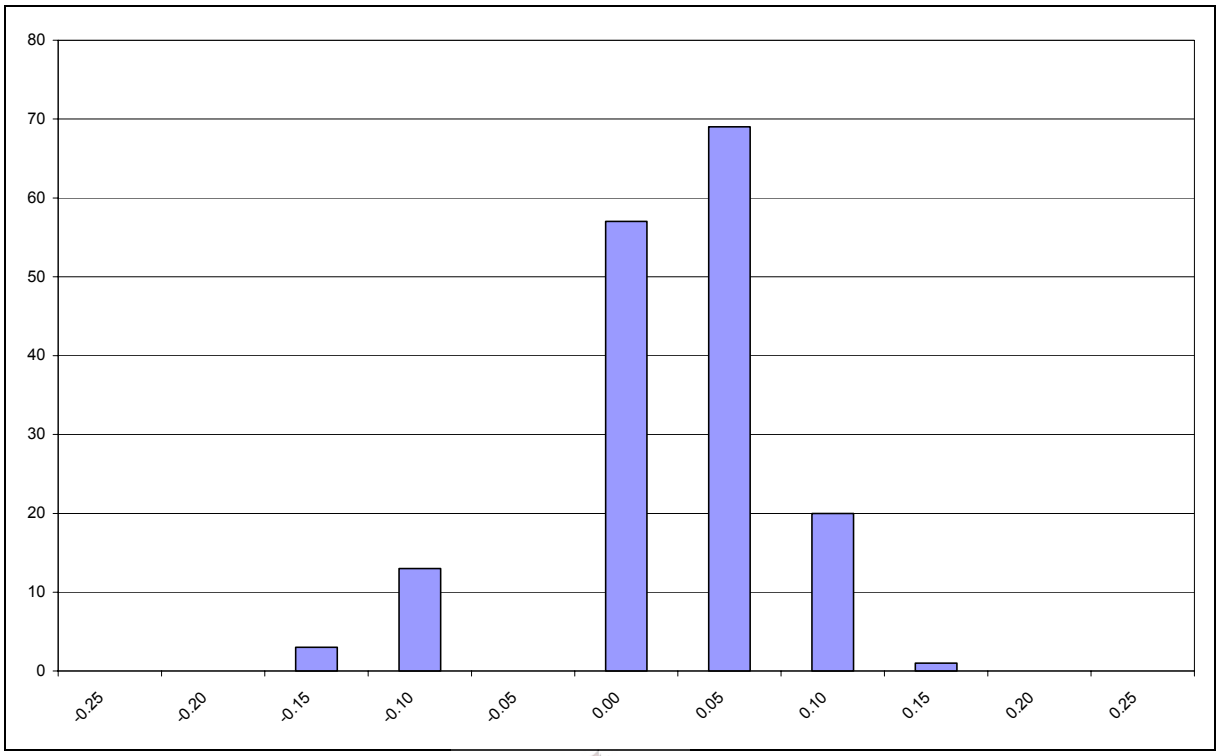


Figure 3.15. Residual plot of PLS-R for pH of grape must samples. The x-values designate the means of intervals (± 0.05).

3.3.3.3 TA: Wavenumber 1743 to 1747 cm^{-1} explained 86% of the variance for TA between the samples. The SEC for the new calibration on Winescan FT120 with n=142 with 15 filters and factors was 0.41 g/L. If samples were deselected with high residual and 7 PLS filters and 6 PLS factors were chosen a SEC of 0.34 was reported. The lowest SEC (0.24 g/L) for the calibration model (11 PLS filters and 8 factors) was achieved when all the samples were used (**Fig. 3.16**). Using 15 filters and factors a SEC of 0.25 g/L is obtained. The bias and correlation coefficient were -0.5 and 0.99, respectively. SEP was 0.28 g/L for the validation of the new TA calibration (**Table 3.3**).

The SEC for the validation of the global calibration was 3.2 g/L. The mean bias for the validation of the global calibration was -3.04, indicating that the average of the residuals (difference between reference and predicted values) of the samples were high (**Fig**. **3.17**). A point of concern is, that the regression statistics although giving excellent correlation coefficient, gives high systematic error as evident by the bias. After a slope and an intercept adjustment the SEP was 0.26 g/L and the coefficient of correlation 0.99. Due to the lower SEC achieved when a new calibration for TA was made with the FOSS Calibration Master software, it seems that a more accurate prediction will be obtained.

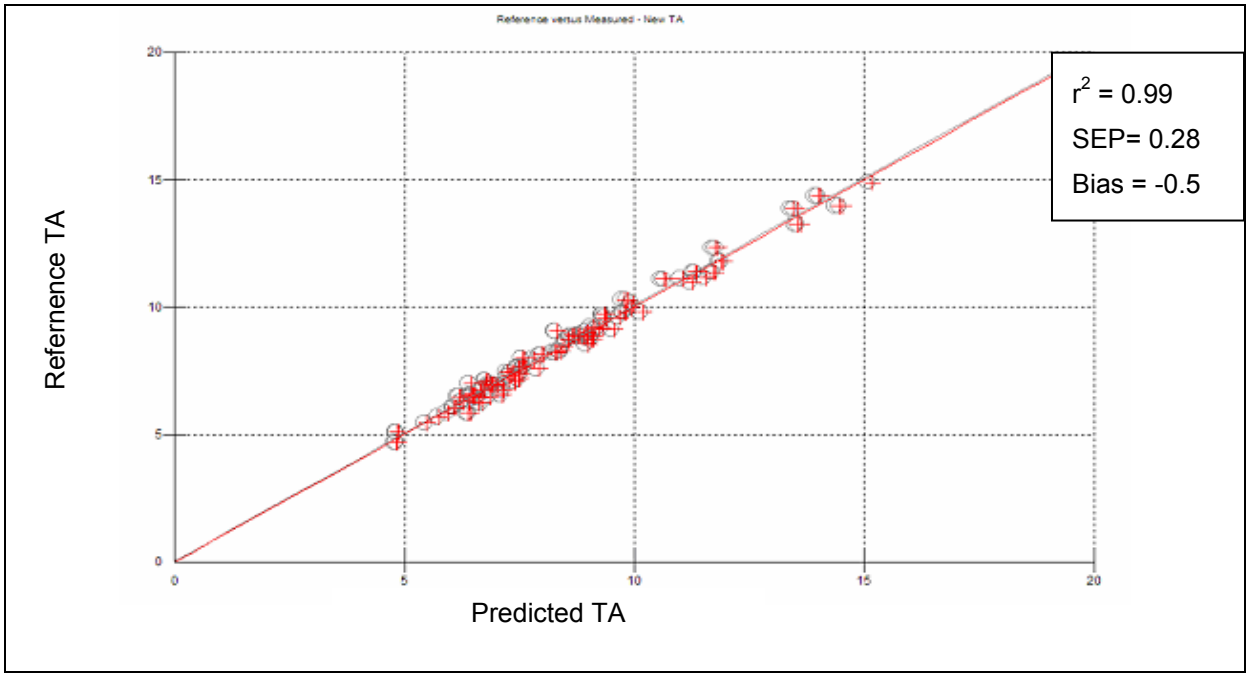


Figure 3.16. Predicted (red) vs Reference (grey) TA plot for the validation of a new TA calibration (n=67).

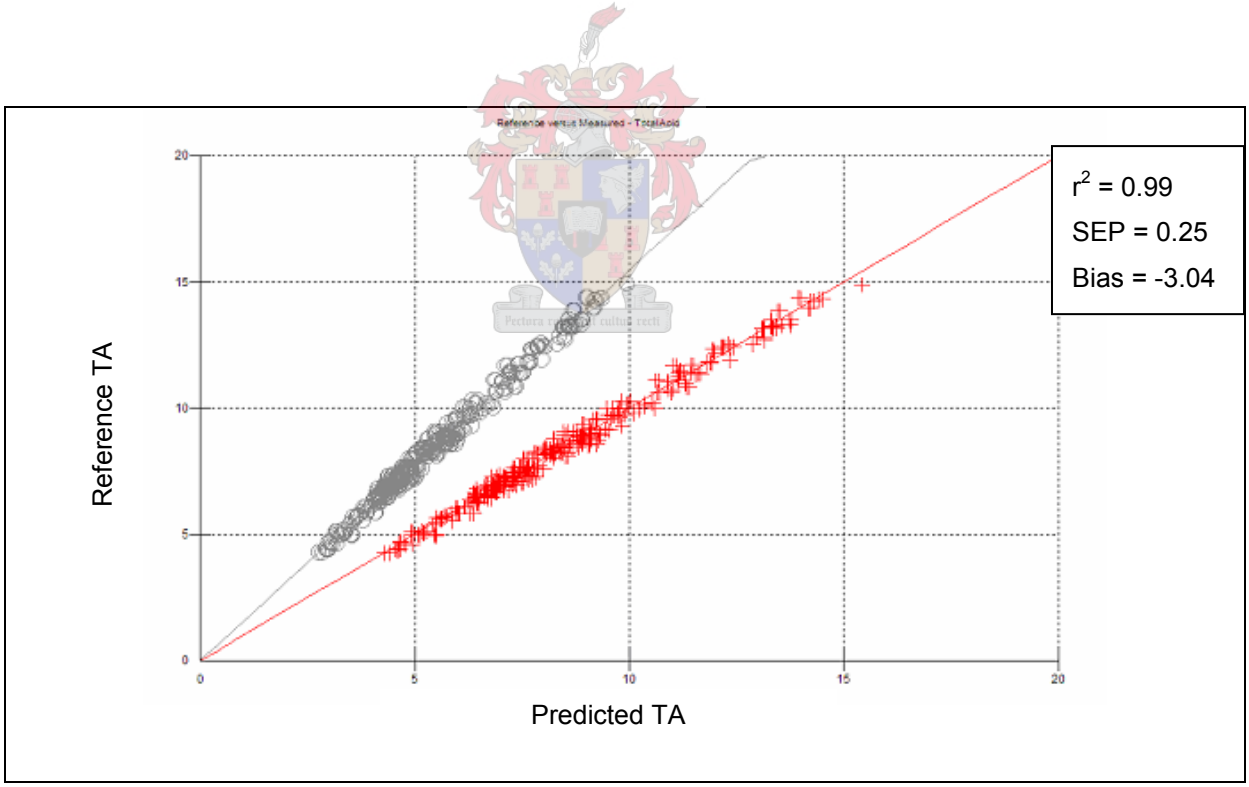


Figure 3.17. Reference (grey) vs. Predicted (red) TA in g/L of grape must samples for the validation of the global TA calibration.

3.4 CONCLUSIONS

The simultaneous measurement of °Brix, pH and TA in grape must using FT-IR spectroscopy shows potential for accurate analysis and quality control purposes in an

industrial cellar. The RPD values obtained for all calibrations proofed it fit for prediction of samples. Because a lower SEP was achieved for the TA calibration using all the samples, validation of this calibration will be done using samples of the next harvest. If this produces a better SEP then this calibration will be used instead of the global calibration. More accurate prediction results were obtained for pH using the new calibration than validation of the global calibration. Further improvements to calibrations will be made for other grape components such as FAN, alfa amino nitrogen, colour and polyphenols. Rapid analysis of these parameters will lead to higher throughput of grape must samples in the laboratory as well as adhering to good laboratory practices by validation.

3.5 LITERATURE CITED

Application Note 175, 2001. Issue 3GB, P/N 1025388, Foss Analytical, Denmark, GrapeScan Calibration Must – pH.

Application Note 177, 2001. Issue 3GB, P/N 1025390, Foss Analytical, Denmark, GrapeScan Calibration Must – Total acid.

Application Note 178, 2001. Issue 3GB, P/N 1025391, Foss Analytical, Denmark, Grapescan Calibaration Must – Brix.

Callao, M.P., Borras, J.M., Lopez, A. & Rius, F.X., 1991. Influence of the state of ripeness of Chardonnay grapes on wine composition. I. Physicochemical characteristics, higher alcohols, polyols and esters. Acta Alimen. 20, 47-54.

Dambergs, R.G., Cozzolino, D., Esler, M.B., Cynkar, W.U., Kambouris, A., Francis, I.L., Høj, P.B. & Gishen, M., 2003. The use of near infrared spectroscopy for grape quality measurement. Aust. Grapegrower and Winemaker 473a, 69-76.

Du Plessis, C.S., 1984. Optimum maturity and quality parameters in grapes: A Review. S. Afr. J. Enol. Vitic. 5, 35-42.

Dubernet, M., Dubernet, M., Dubernet, V., Coulomb, S., Lerch, M. & Traineau, I., 2001. Objective analysis of harvest quality by Fourier transform infrared spectroscopy (FTIR) and neuron networks. Bulletin de L'O.I.V. 74, 839-840.

Eriksson, L., Johansson, E., Kettaneh-Wold, N. & Wold, S., 1991. Introduction to Multi- and Megavariate data analysis using projection methods (PCA & PLS). 1st Ed. Umetrics AB, Umeå, Sweden.

Esbensen, K.H., 2002. (5th ed). Multivariate Data Analysis - In Practice. Camo ASA, Oslo, Norway.

Foss Analytical, Denmark. http://www.foss.dk.

Gishen, M., Iland, P.G., Dambergs, R.G., Esler, M.B., Francis, I.L., Kambouris, A., Johnstone, R.S. & Høj, P.B., 2002. Objective measures of grape and wine quality. In: Blair, R.J., Williams, P. & Høj, P.B. (eds). Proc. 11th Aust. Wine Ind. Tech. Conf. Oct. 2001, Adelaide, Australia. pp. 188 - 194.

Høskuldsson, A., 1996. Prediction models in Science and Technology, 1, Basic Theory. Thor Publishing, Denmark.

Lee, S-J., Rathbone, D., Asimont, S., Adden, R. & Ebeler, S.E., 2004. Dynamic changes in ester formation during Chardonnay juice fermentations with different yeast inoculation and initial ºBrix conditions. Am. J. Enol. Vitic. 55, 346-354.

Manley, M., van Zyl, A. & Wolf E.E.H., 2001. The evaluation of the applicability of Fourier transform Near-Infrared (FT-NIR) spectroscopy in the measurement of analytical parameters in must and wine. S. Afr. J. Enol. Vitic. 22, 93-100.

Marais, J., 2004. Optimum ripeness: Crucial for quality. http//www.newworldwinemaker.com/article.

Nieuwoudt, H.H., Prior, B.A., Pretorius, I.S., Manley, M. & Bauer, F.F., 2004. Principal Component Analysis applied to Fourier transform infrared spectroscopy for the design of calibration sets for glycerol prediction models in wine and for the detection and classification of outlier samples. J. Agric. Food Chem. 52, 3726-3735.

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

Skoog, D.A., Holler, F.J. & Nieman, T.A., 1997. Principles of Instrumental analysis. 5th ed. Harcourt Brace College Publishers, USA. pp. 380-403.

Sinton, T.H., Ough, C.S., Kissler, J.J. & Kasimatis, A.N., 1978. Grape juice indicators for prediction of potential wine quality. 1. Realationship between crop level, juice and wine composition, and wine sensory ratings and scores. Am. J. Enol. Vitic. 29, 267-271.

Smith, B., 1999. Infrared spectral interpretation: A systematic approach. 1st Ed. CRC Press LLC, Florida, USA.

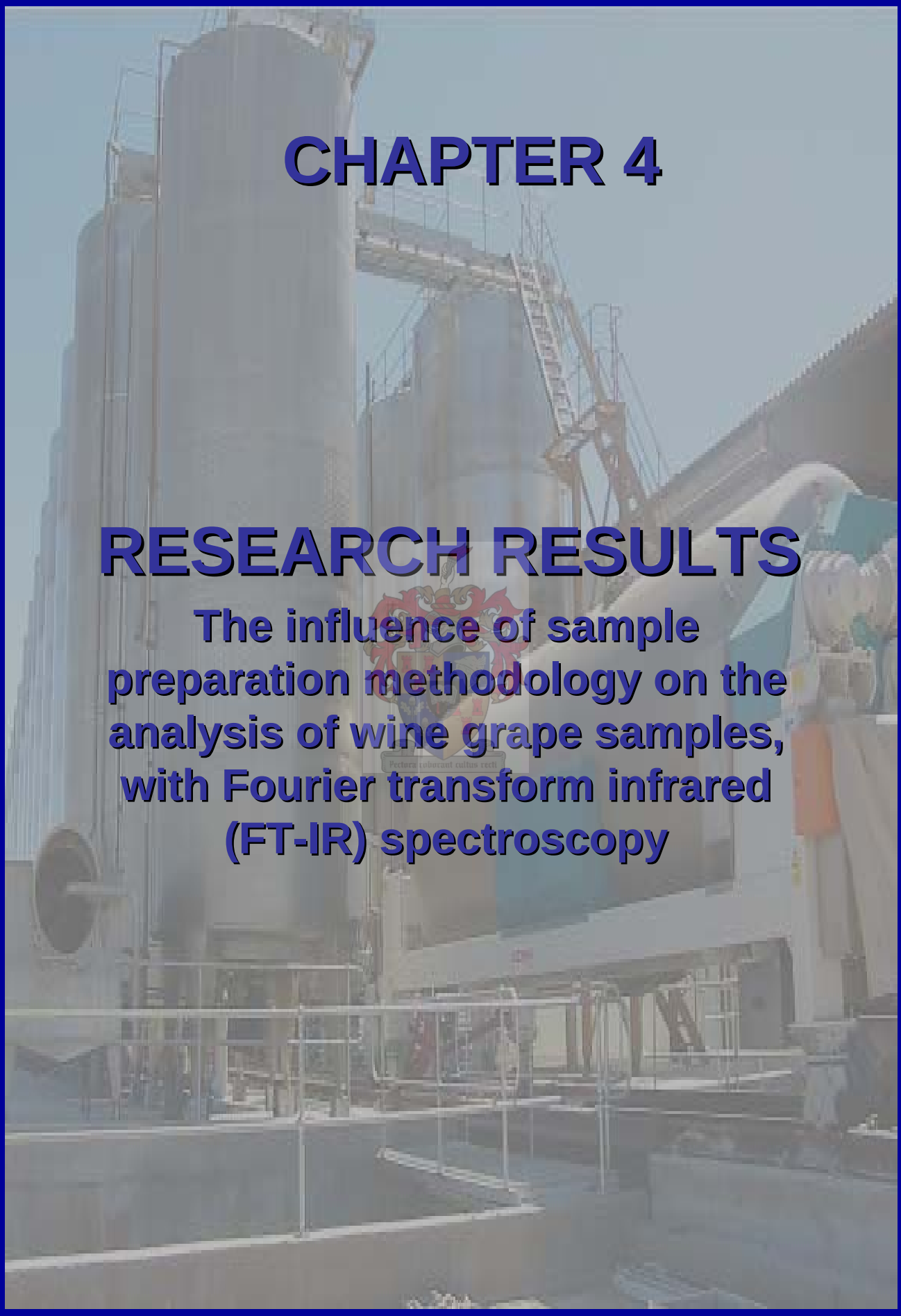
Tardaguila, J. & Martinez de Toda, F., 2004. Assessment of Wine quality in the vineyard. In: de Sequeira, Ó.A., Sequeira, J.C. (eds). Proc. 1st IS on Grapevine., Acta Hort. 652, ISHS 2004.

Williams, P., 1995. Near-infrard technology implementation. Canadian Grain Commission, Grain Research Laboratory: Winnipeg, MB.

Winescan FT 120 Type 77110 and 77310 reference manual, Issue 4 GB Foss Electric, Denmark, 2001. http://www.foss.dk

Zoecklein, B.W., 2001. Grape sampling and maturity evaluation for growers. Vitner's corner, 16.

Zoecklein, B.W., Fugelsang, K.C., Gump, B.H. & Nury, F.S., 1999. Wine Analysis and Production. Chapman & Hall, Aspen Publishers, Inc., Gaithersburg.



CHAPTER 4

RESEARCH RESULTS

The influence of sample preparation methodology on the analysis of wine grape samples, with Fourier transform infrared (FT-IR) spectroscopy

4. RESEARCH RESULTS

The influence of sample preparation methodology on the analysis of wine grape samples, with Fourier transform infrared (FT-IR) spectroscopy

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ABSTRACT

Two grape sample processing methods, pressing by hand and homogenization respectively, were used to evaluate the effect of these preparation methods on the accuracy of the quantification of the chemical components using the Winescan FT120 instrument and to evaluate the possibility of using a one-step sample preparation method for all FT-IR grape analysis. One hundred and twenty two samples of homogenized and hand pressed red grape berries were analyzed using Fourier transform infrared spectroscopy. The FT-IR spectra were subjected to multivariate data analysis using principal component analysis and a distinct clustering of samples based on sample preparation method could be seen. The chemical values obtained using the two sample preparation methods (and FT-IR analysis) were also compared to samples collected from the corresponding tanks after 24 hours of cold maceration. For this purpose 11 vineyards were sampled by hand just before harvest and the mechanically harvested grapes 24 hours after destemming and crushing. These grapes were therefore at optimal maturity. The same sample preparation methods were applied and the must collected after 24 hours in the tank in the cellar. ANNOVA was used to compare the 2 sample preparation methods and the corresponding tank sample. The sugar content, ammonium, alfa- amino nitrogen and malic acid showed no significant difference between the 2 sample preparation methods and the tank samples. There was a significant difference ($p<0.01$) between the samples for the analysis of polyphenols, anthocyanin and colour index. The homogenized sample preparation method had the highest variance in comparison to the hand press sample preparation method and the tank sample.

4.1 INTRODUCTION

The implementation of rapid and objective measures to asses the quality of grape loads delivered at the winery weighbridge has received increasing attention (Kennedy, 2001, Watson, 2003). Measures used are visual inspection, chemical analysis such as °Brix, colour, acidity. These measures are necessary for quality control purposes and to establish grape maturation trends during one harvest season and between consecutive vintages of the same vineyards. The ultimate aim of these measures is to predict wine quality.

Regardless of which parameters are measured to evaluate grape quality, the reliability of the data (assuming good accuracy of the laboratory method) greatly depends on the quality of the sampling process, proper sample storage and processing techniques prior to chemical analysis. Poor and unrepresentative sampling methods might introduce a greater degree of uncertainty and error in the data than the analytical procedures (Leamon, 2000) and strict control of these process variables is therefore necessary. The Total Sampling Error (TSE), defined by the combination of sample properties such as heterogeneity and the sampling process itself, is by far the dominating contributor to uncertainty of analytical results (Petersen *et al.,* 2004). Representative sampling in vineyards is particularly challenging and the sampling error tends to increase as heterogeneity and volume of sampled material increase.

Sample storage and processing are common procedures in laboratory analysis and aim to preserve the condition of samples before analysis by standard chemical and physical methods (Pomeranz & Meloan, 1987). Samples might undergo changes in their composition due to various chemical or physical reactions and one objective of sample preparation is to convert the original sample to a stable and homogeneous form that will facilitate the taking of representative sub-samples (Kratochvil & Taylor, 1981; Pomeranz & Meloan, 1987; Horwitz, 1988). The effects of sample preparation and storage on the determination of quality parameters in berries of *Vitis vinifera* have been reported (Cozzolino *et al*., 2004; Cynkar *et al*., 2004).

At Westcorp International, Vredendal South Africa, grape loads (6 ton capacity) are sampled at the weighbridge (using a sampling auger) prior to processing (destemming and crushing). These samples are processed in the routine chemical laboratory to extract juice and analysed by the Winescan FT120 instrument (FOSS Analytical, Denmark). The instrument utilizes Fourier transform infrared spectroscopy

(FTIR) and the quantification of chemical components is done using calibrations developed for each specific component of interest. Individual calibrations are grouped into a Product designed specifically for a particular matrix such as grape juice, fermenting must or wine (Winescan FT 120 Type 77110 and 77310 Reference manual, Foss Analytical, Denmark, 2001). After cold maceration (24 hours) in tanks (10 000L capacity) a must sample is then collected from the tank tap and again analysed by the Winescan using the same calibration. The accuracy of the analytical data generated at weighbridge and after cold maceration is very important and has financial implications for all parties. Grape growers are remunerated on the basis of the chemical analysis of their grapes at the weighbridge, while winemakers make decisions regarding must additions, such as tartaric acid to correct for acidity and pH and enzymes for colour extraction, on the tank samples after cold maceration. Ideally, one single processing method should be used to prepare grapes for FT-IR analysis and one calibration product for FT-IR analysis of must.

The aims of this study were: (1) to investigate the effect of two grape sample processing methods on the accuracy and robustness of the Winescan calibrations for the determination of the major chemical components in grape juice and must; (2) to compare the analytical data obtained from samples taken at the weighbridge with those obtained from corresponding maceration tanks; and (3) to use the information to evaluate the robustness of the FTIR calibrations.

4.2 MATERIALS AND METHODS

4.2.1 Grape samples used for comparison of sample processing methods:

122 Red grape samples (3 to 4 kg grape bunches per vineyard) were collected during 2003 and 2004 in the Olifants River Valley, South Africa during different stages of ripening. Grape bunches were picked randomly from different vines in a vineyard. Vines at the beginning and end of rows were not used. Samples were placed in sterile plastic bags that were subsequently sealed and transported at 4^0C to the wine chemical laboratory where bunches per sample were destemmed by hand and the berries from each sample divided equally by weight (**Fig.4.1**).

Two methods were used to process the samples for FTIR analysis. One half of grapes were manually pressed using a kitchen masher (**Fig. 4.2a**) and the other half homogenized for 30 seconds, using a Braun stick blender (**Fig.4.2b**), (400 watt MR4050CA, Spain). Processed grape samples were immediately filtered, using a

filtration unit (type 79500, FOSS Analytical, Denmark) connected to a vacuum pump and filter paper circles graded at 20 – 25 µm with diameter 185 mm (Schleicher & Schuell, reference number 10312714, Lasec, Cape Town, South Africa) and then scanned with the Winescan as described in section 4.2.3.

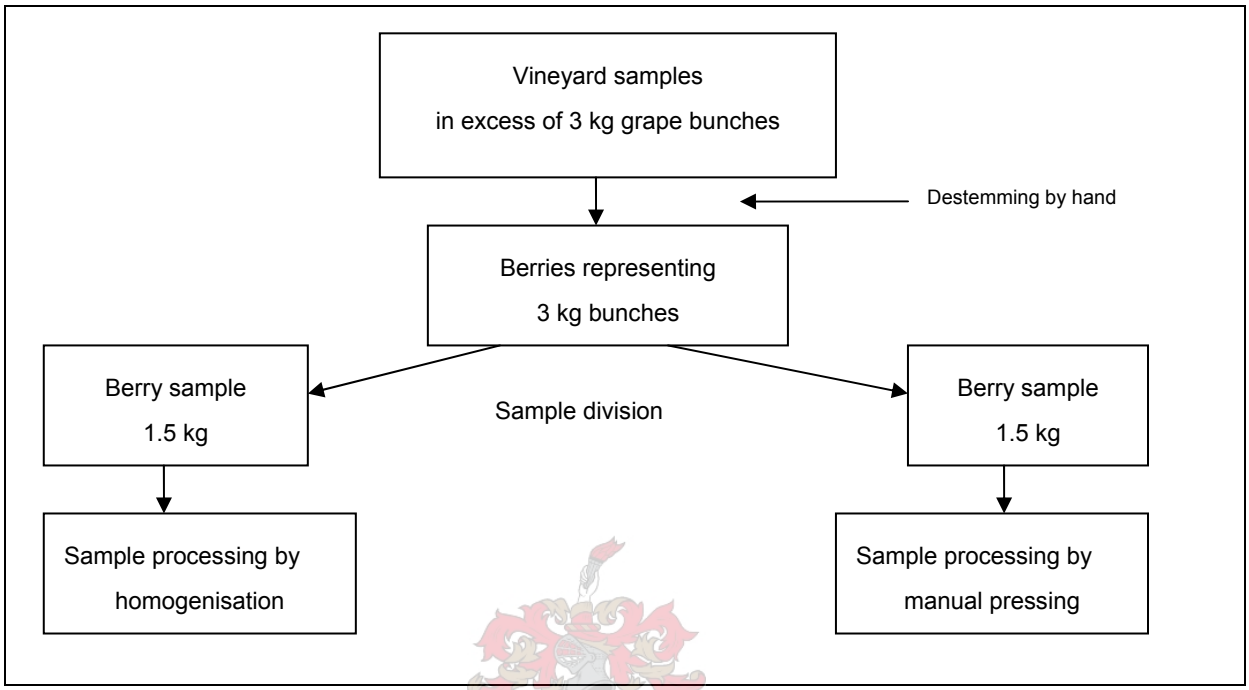


Figure 4.1. Diagram of grape sample preparation and processing.

4.2.2 Grape samples used for comparison of vineyard samples and grape must tank samples: Shiraz, Merlot and Cabernet Sauvignon grape bunch samples were also collected from 11 vineyards just before mechanical harvesting. The grapes from these vineyards were taken to the laboratory and processed for FT-IR analysis as described in section 4.2.1. The mechanically harvested grape loads were transported to the cellar and after destemming and crushing, the must was pumped into 15 ton capacity fermentation tanks (10 000L) in the cellar. Each vineyard was represented in an individual tank. After receipt of grapes in the tanks, the temperature was adjusted at 10ºC for 24 hours for cold maceration. Grape must samples obtained from the tanks at this stage are referred to as tank samples in the text.

a)

b)

Figure 4.2. a) Grapes pressed manually and b) homogenized with a stick blender.

4.2.3 FT-IR spectral analysis: The filtered must samples were immediately scanned after filtration in duplicate. A Winescan FT120 instrument (software version 2.2.2) equipped with a purpose built Michelson interferometer was used to generate the FT-IR spectra (FOSS Electric A/S, Hillerød, Denmark). Instrument settings included: cell path length of 37 µm, sample temperature set to 40°C, and sample

volume of 7 – 8 ml. Samples are pumped through the heat exchanger and the CaF_2-lined cuvette and scanned from 5011 to 926 cm^{-1} at 4 cm^{-1} interval (Winescan FT 120 Type 77110 and 77310 Reference manual, Foss Electric, Denmark, 2001). Global calibrations (which refer to the commercial ready-to-use-calibrations provided with the instrument) convert the linearised absorbance spectra into quantifiable results. GrapescanA 2003 version 3.0 software was used. Global calibrations for Brix, pH and TA were validated using independent validation sets generated in the chemical laboratory of the cellar and the calibrations were adjusted as described in Chapter 3 of this thesis. The adjusted calibrations were used. The global calibrations for glucose-fructose, polyphenol content, colour index, anthocyanins, potassium and nitrogen content were not validated prior to use due to restrictions on available analytical infrastructure, and predicted results from these calibrations were used for comparative purposes only. Metabolites formed mainly trough microbial activity that were obtained using the global calibrations included ethanol, gluconic acid, glycerol, lactic acid, volatile acidity, ethyl acetate, mannitol, sorbitol, acetaldehyde, methyl-3-butanol, 2,3-butanediol, arabitol, meso-inositol and isoamyl acetate. Negative values were converted to zero.

4.2.4 Data analysis

4.2.4.1 ***Principal component analysis (PCA):*** PCA was used to examine the relevant and interpretable structure in the data and to identify extreme deviating samples. For this purpose FT-IR spectra were exported to the Unscrambler Software (version 9.1.2, Camo Process AS, Oslo, Norway). The complete data table, defined by the variables (1056 wavenumbers) in columns and the samples in rows, set was mean-centered (subtracting the average value from a variable, for each data point) by column. Duplicate spectra were averaged. The complete data matrix was scaled or weighted by using the following equation:

$$x^{scaled} = x * 1 / SDev$$

This was done to make the contribution of all variable variances more comparable (Esbensen, 2002). With PCA the maximum directions of variation in a data set is modelled by projecting the objects (in this study, the FT-IR spectra) as a swarm of data points in space defined by principal components (PC's). PC's contain in decreasing order the main structured information in the data. A PC is the same as a

score vector, and is also referred to as a latent (underlying) variable (Esbensen, 2002). PC's are calculated to be orthogonal to one another and therefore can be interpreted independently.

4.2.4.2 ***Statistical analysis:*** Statistica version 7 (Microsoft Corporation, USA) was used for the analysis of variance (ANOVA) of the chemical data obtained for the 2 sample preparation methods, as well as for a comparison between the respective data obtained for the grape samples (both preparation methods) and the tank sample. Significance was expressed using a 99% confidence interval ($p<0.01$). The decision to use a high confidence interval was based on the huge financial implications of the decision-making at various stages of the winemaking process. The sample size was relatively small and therefore these statistical methods are not dependant on the estimation of parameters (such as standard deviation and means) describing the distribution of the variables of the population. The Bonferroni test was used to compare the means of more than 2 groups and determining which group are more significant different to the others. Kruskal-Wallis, a nonparametric method, was also used to determine the significant difference between the groups. This method is based on ranks and not means of the sample groups. The sample size was relatively small and therefore these statistical methods that are not dependant on the estimation of parameters such as standard deviation and means normally used to describe the distribution of the variables of the population.

4.3 RESULTS AND DISCUSSION

4.3.1 PCA Modeling

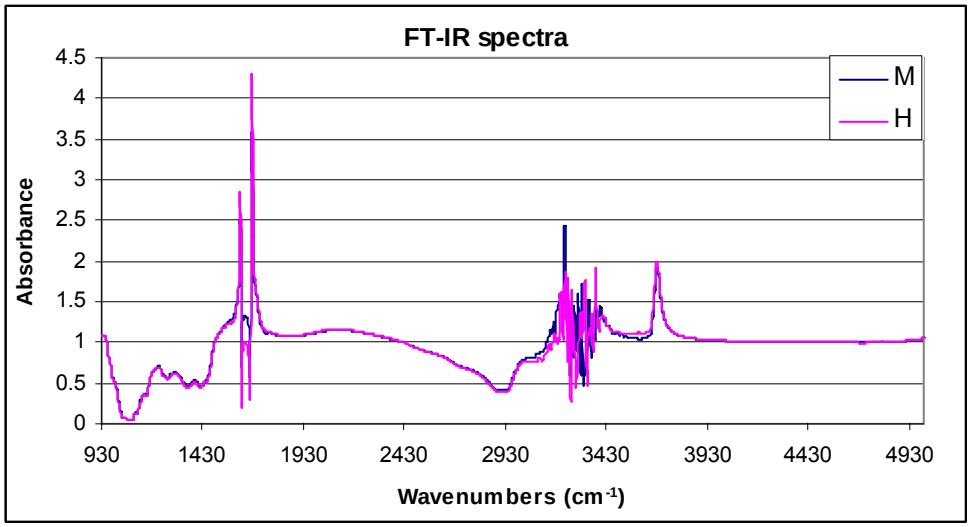
The FT-IR spectra of the two sample preparation methods showed the typical absorbance of grape must (**Fig. 4.4a**). The most prominent features of the spectra are due to absorbance of water in the wavenumber regions, 1543 – 1716 cm^{-1} and 2970 – 3626 cm^{-1} respectively (Winescan FT 120 Type 77110 and 77310 Reference manual, Foss Electric, Denmark, 2001). The region 930 – 1600 cm^{-1}, referred to as the "fingerprint region" by some (Smith, 199) captures a significant amount of information related to the chemical composition of the samples, and prominent peaks were also seen in this region. Obvious differences in the spectra between the two sample preparation methods could not be seen by mere visual inspection (**Fig. 4.4b**). Since it was expected that the different sampling procedures would alter the chemical composition of the samples, principal component analysis (PCA) was done on the

complete data matrix which included 244 grape must samples and all the wavenumbers excluding the water absorbance regions (1543 – 1716 cm^{-1} and 2970 – 3626 cm^{-1}). PC1 and PC2 explained 98% of the variance between the samples. In the initial analysis 16 grape must samples (all Shiraz and Cabernet Sauvignon) were identified as extreme samples since these located far from the centre of the PCA model and from the other samples. The sugar content of these samples was much higher and their acidity much lower than those of the rest of the samples (results not shown). These samples were deleted from the sample set. No obvious separation of the samples related to the sample preparation method could be seen in the score plot PC1 versus PC2, and a clear separation that could be interpreted in terms of sample preparation was found for the third principal component PC3 that explained 2% of the variance in the sample set (**Fig. 4.5**). It could therefore be concluded that sample preparation had a minor, but noticeable effect on the spectral properties of the samples. The wavenumbers which had the highest loadings for PC3 (2%) were 1462 - 1736 cm^{-1} (**Fig. 4.6**).

4.3.2 Analysis of variance between the 2 sample preparation methods and the tank sample

To determine which parameters differed between the 2 sample preparation methods as well as comparing the tank sample results, analysis of variance (ANOVA) was performed. These statistics are summarised in **Table 4.1.** There was no significant statistical difference between the 2 sample preparation methods and tank sample for the measurement of sugar content (which included glucose-fructose, ºBrix and density), ammonium, alfa-amino nitrogen, TA and malic acid measured in grape must. For the parameters titratable acidity, pH, tartaric acid, polyphenols, colour index, anthocyanins, potassium, 2,3-butanediol, arabitol, sorbitol, meso-inositol and isoamyl acetate, the homogenized sample preparation method resulted in significantly differences between the hand pressed sample and the homogenized sample **(Table 4.1.).** The results of statistical analysis obtained for the differences in pH levels of the samples prepared by the two methods are shown in **Fig. 4.6a.**

a)



b)

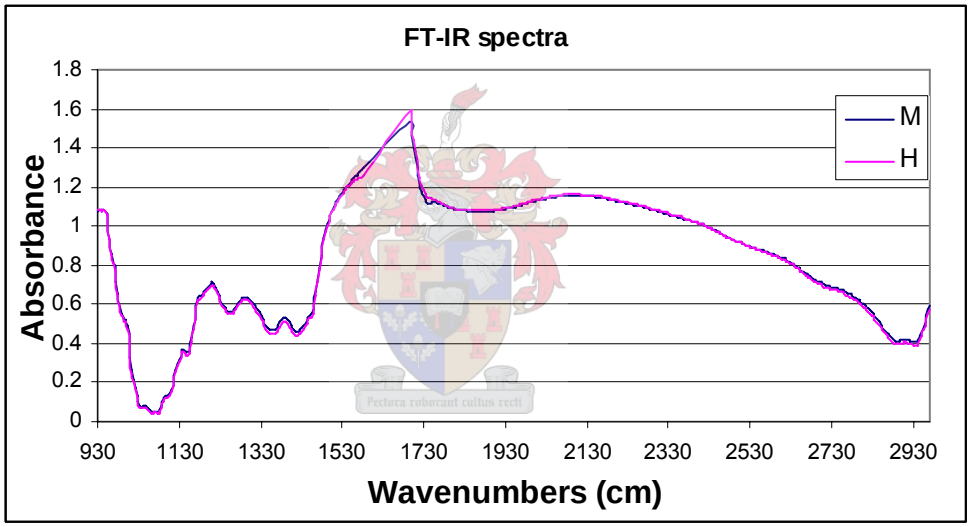


Figure 4.4. a) FT-IR spectra of Shiraz grapes samples prepared by hand pressing or alternatively homoginization in the absorbance range 930-4930 cm^{-1} (N = manually pressed and H = homogenized). b) FT-IR spectra of Shiraz grape samples with the water absorbance regions (1543 – 1716 cm^{-1} and 2970 – 3626 cm^{-1} deselected. The area 930-2930 is enlarged to show the absorbance in the fingerprint region (930 – 1600 cm^{-1}).

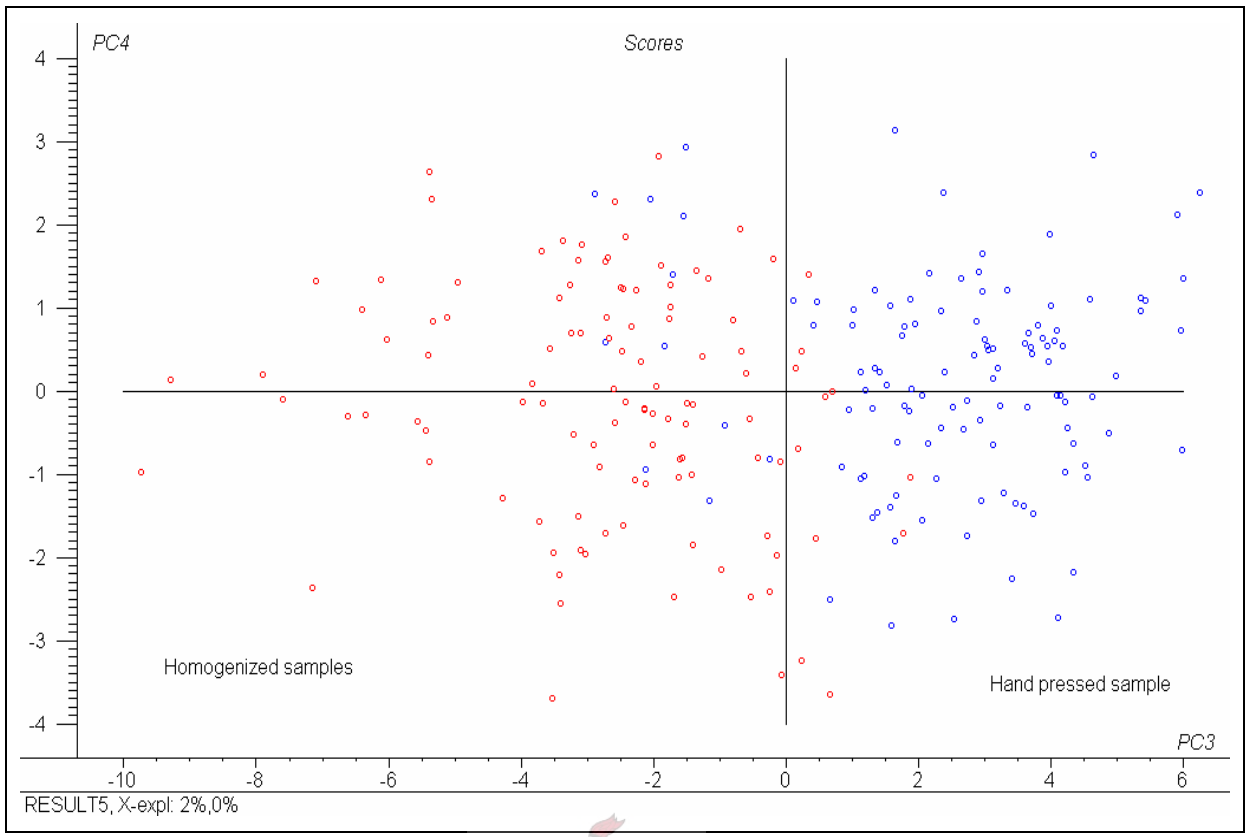


Figure 4.5. PCA score plot of PC3 which explains 2% of the variance between the samples. The water absorbance regions were excluded (1543 – 1716 cm^{-1} and 2970 – 3626 cm^{-1}). n=122

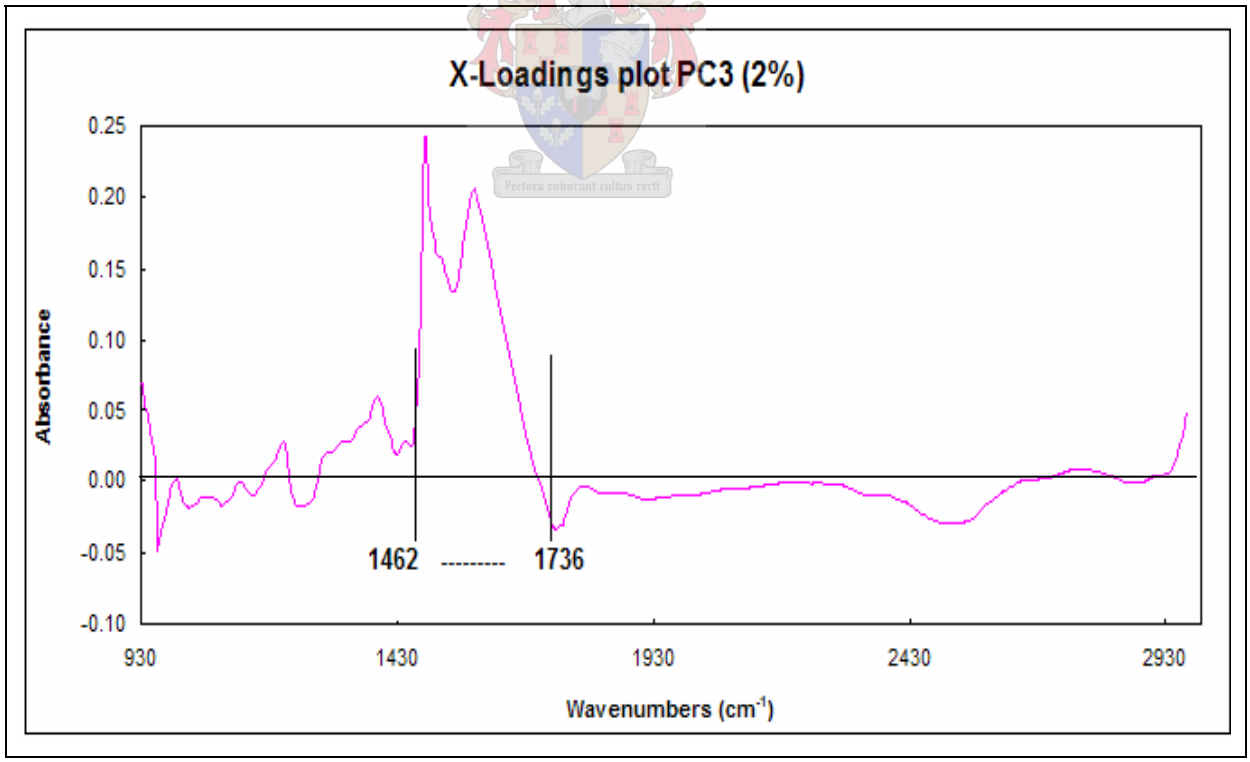


Figure 4.6. X-Loadings plot of PC3 (2%) with 1462 – 1736 cm^{-1} having the highest loadings for the PCA model.

Table 4.1. Summary of the statistical analysis between the 2 sample preparation methods and tank sample of grape must (n=11).

Quality Parameters	Bonferroni test p - values	Statistical Analysis – Sample Type
Glucose-Fructose	0.35	N = H = T
Soluble Solids ºB	0.02	N = H = T
Density	0.15	N = H = T
Ammonium	0.34	N = H = T
Alfa-amino Nitrogen	0.24	N = H = T
Malic acid	0.14	N = H = T
Titratable acidity	0.04	N > H = T
pH	< 0.01	N = T < H
Tartaric acid	< 0.01	N = H > T
Polyphenols	< 0.01	N = T < H
Colour Index	< 0.01	N = T < H
Anthocyannins	< 0.01	N = T < H
Potassium	< 0.01	N < H = T
Gluconic acid	0.17	N = H = T
Ethanol	0.05	N = H = T
Methyl-3-butanol	0.12	N = H = T
Acetaldehyde	0.14	N = H = T
Ethyl acetate	< 0.01	N = H > T
Mannitol	< 0.01	N = H > T
2,3-Butanediol	0.01	N = T < H
Arabitol	< 0.01	N = T < H
Sorbitol	< 0.01	N = T < H
Meso-inositol	< 0.01	N = T < H
Isoamyl acetate	< 0.01	N = T < H

= no significant difference; < significant difference, value smaller than; > significant difference, value larger than; N hand pressed sample preparation method; H homogenized sample preparation method; T tank sample

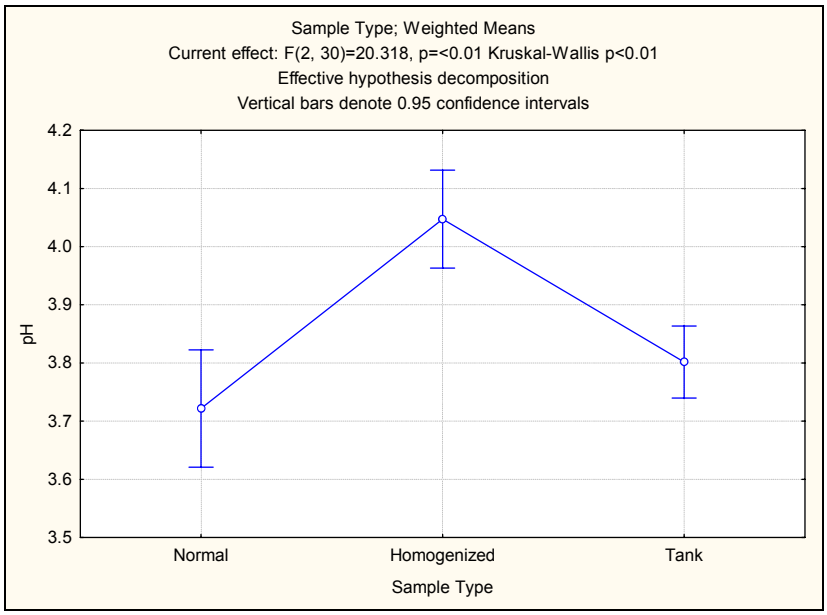
Tartaric acid content in the tank sample ($p<0.01$) was much lower than the values obtain in the hand pressed and homogenized sample preparation methods (**Fig. 4.6b**). The potassium level of the hand pressed sample (1934 mg/L) was much lower than that of the tank sample (2586 mg/L) and homogenized sample (2409 mg/L) (**Fig. 4.6c**). This was to be expected as more potassium is released when the grape sample is homogenized which will have an influence on the acidity and pH of the must. During cold maceration more potassium is released from the grape skins and forms tartrate bonds which lowers the acidity and increases the pH (Possner & Kliewer, 1985). If the results from the hand pressed sample preparation were to be used it could mean that the amount of tartaric acid addition will not be sufficient as

well as incorrect pH correction of the grape must. Microbes, yeast and bacteria, will have time to proliferate at higher pH.

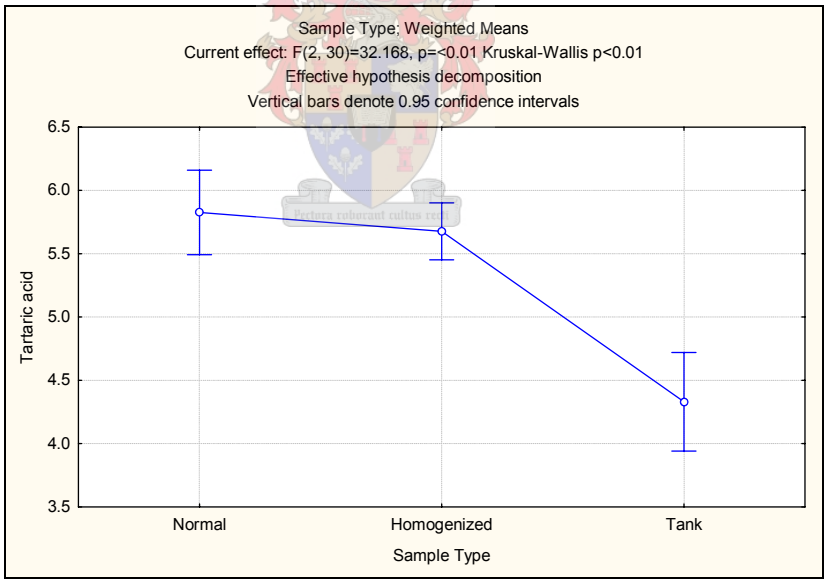
Anthocyanins and polyphenols are located in grape skin and it was demonstrated that there was a significant difference ($p<0.005$) between the homogenized sample preparation method and the hand pressed sample preparation method and tank sample (**Figs. 4.6d & e**). This result was expected. For colour index, anthocyannins and polyphenols there were no significant difference between the hand pressed sample and tank sample. The homogenized sample preparation has a higher extraction effect on colour and polyphenols. This is because colour pigments and polyphenols are located in the grape skins. During cold maceration the extraction of these components were not as high as with homogenization of the grape berry sample. During alcoholic fermentation more extraction of these components might occur. Keeping in mind that not all polyphenols are extractable this result was important. Because the hand pressed sample preparation method corresponded more to the tank sample this method might be sufficient for qualitative analysis to asses grape quality.

Gluconic acid, ethanol, methyl-3-butanol and acetaldehyde showed no significant difference between the 2 sample preparation methods and the tank sample. Ethyl acetate differed significantly in the tank sample to the hand pressed and homogenized sample preparation methods. The mean value of the sample was lower in the tank. Mannitol in the homogenized sample was higher and differed significantly to the hand pressed sample and tank sample. The mean value of mannitol was also lower in the tank. In the homogenized sample, 2,3-butanediol, arabitol, sorbitol, meso-inositol and isoamyl acetate, the mean values were higher and differed significantly to that of the hand pressed sample and tank sample. There were no differences between the last two sample types.

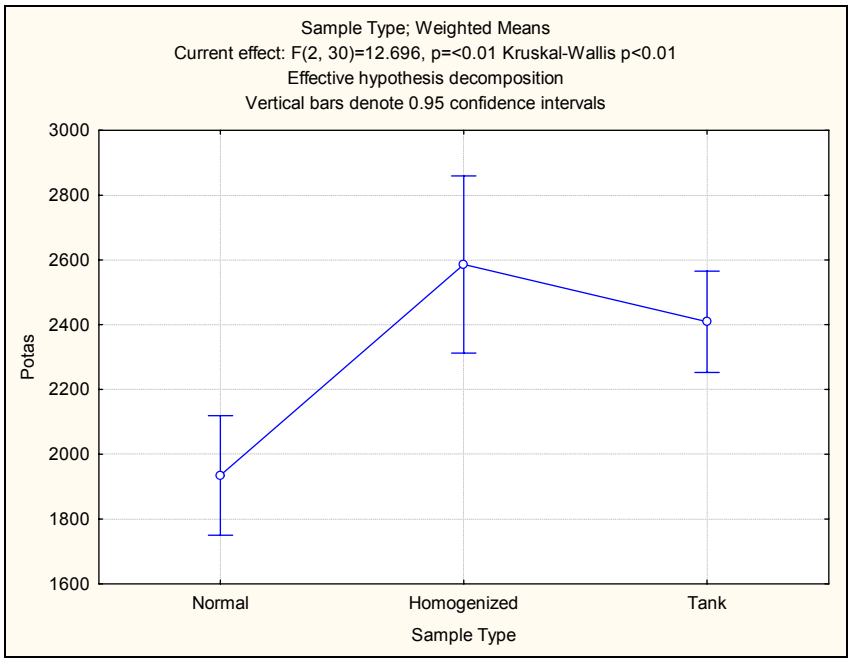
a) pH



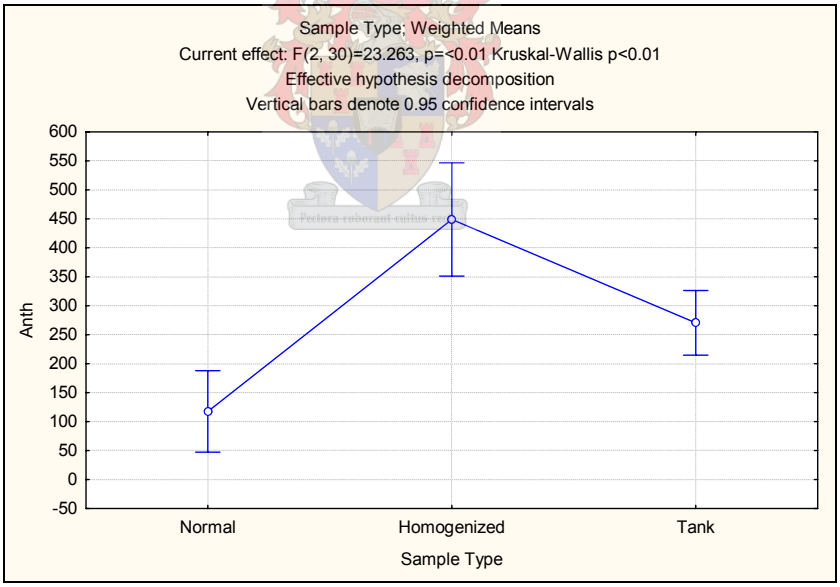
b) Tartaric Acid



c) Potassium



d) Anthocyannins



e) Polyphenols

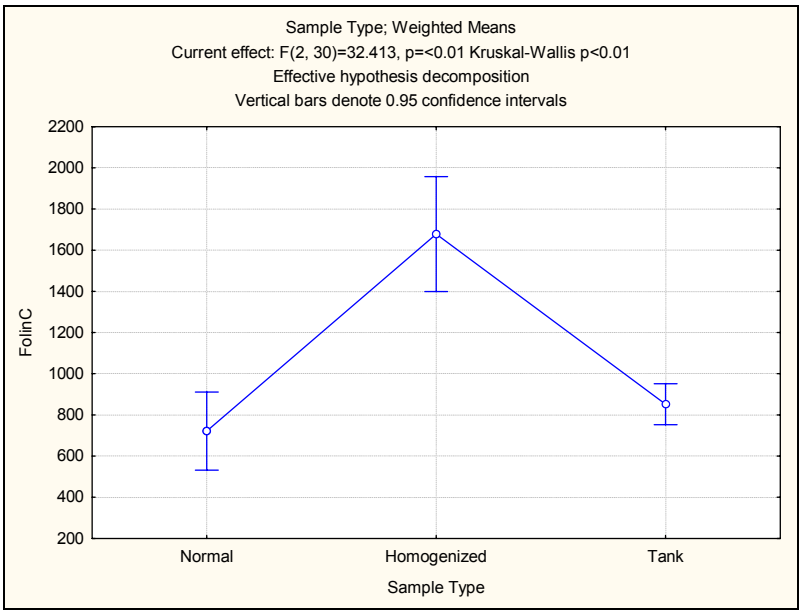


Figure 4.6. Box-and-whisker plots of a) pH, b) tartaric acid, c) potassium, d) anthocyanins and e) polyphenols illustrating significant differences between the 3 sample types (n=11).

4.4 CONCLUSIONS

When comparing the analytical data obtained on grape samples using the Winescan instrument and the available calibrations, the hand pressed sample preparation method and the tank sample yielded more similar values as compared to the homogenized sample for most of the chemical components measured. For the analyses of colour and polyphenols, the hand pressed sample compared well with the tank sample while the homogenized samples consistently had higher average values. This was to be expected since homogenization releases more of these components from the berry skins. An interesting finding was that 2.3-butanediol, arabitol, sorbitol, meso-inositol and isoamyl acetate (metabolites usually associated with microbial activity) were significantly higher in the homogenized samples as opposed to the hand pressed or tank samples. The validity of this trend will be investigated in future research. At this stage of investigation results indicate that it will be feasible to use a one step sample preparation method and the hand pressed method for grape processing was implemented in the wine analytical laboratory at WestCorp International. For the quantification of colour and polyphenols the homogenized samples should be used for reference analysis. For this industrial cellar, colour quantification is not an immediate priority. Currently, colour analysis is only used for trend evaluation during grape maturation. Preliminary industrial trends at this cellar show that microbial spoilage is a key factor that affects wine quality at

this cellar and therefore the optimization of the predictive abilities of the Grapescan calibrations is a high priority. Future efforts will be directed towards large-scale evaluation of the global calibrations and where necessary, adjustments dictated by the process technology or stage of fermentation, will be made.

4.5 LITERATURE CITED

Bowman, F. & Renmmenga, E.E., 1965. A sampling plan for determining quality characteristics of green vegetables. Food Tech. 19, 617-619.

Foss Electric, Denmark. http://www.foss.dk.

Grier, J.D., 1966. Preparation of plant material for analysis. J. Assoc. Off. Anal. Chem. 49, 291-298.

Horwitz, W., 1988. Sampling and preparation of samples for chemical examination. J. Assoc. Off. Anal. Chem. 71, 241-245.

Kratochil, B. & Taylor, J.K., 1981. Sampling for chemical analyses. Anal. Chem. 53, 924A-938A.

Leamon, K., 2000. Defining wine grape quality. http://www.nre.vic.gov.au.

Petersen, L., Minkkinen, P. & Esbensen, K.H., 2004. Representative sampling for reliable data analysis: Theory of sampling. Chemom. Intel. Lab. Sys. 77, 261-277.

Pomeranz, Y. & Meloan, C.E., 1987. Food Analysis. Theory and Practice. 2nd ed. Van Nostrand Reinhold, New York.

Possner, D.R.E. & Kliewer, W.M., 1985. The localization of acids, sugars, potassium and calcium in developing grape berries. Vitis 24, 229-240.

Smith, B. (ed). Infrared Spectral Interpretation: A Systematic Approach. 1st Ed. CRC Press LLC, Florida, USA, 1999.

StatSoft, Inc. (2004). STATISTICA (data analysis software system), version 7. www.statsoft.com.

Watson, B., 2003. Evaluation of winegrape maturity. In: E.W. Hellman (ed), Oregan Viticulture, Oregan State University Press, Corvallis, Oregan. pp. 235–245.

Winescan FT 120 Type 77110 and 77310 reference manual, Issue 4 GB Foss Electric, Denmark, 2001. http://www.foss.dk.

Zoecklein, B.W., 2001. Grape sampling and maturity evaluation for growers. Vitners' corner 16.

CHAPTER 5

RESEARCH RESULTS

Identification of key factors affecting the value chain of wine production: An industrial case study

5. RESEARCH RESULTS

Identification of key factors affecting the value chain of wine production: An industrial case study

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ABSTRACT

Wine quality depends upon many factors and one of the major challenges is to ensure the quality control from the vineyard to the winery. It was found that microbial population and metabolites do not necessarily correlate. An increase in metabolic activity was however evident between the vineyard and weighbridge. Malic acid and the microbial metabolites such as meso-inositol (p=0.0028) differed significantly between the 2 sampling points. PCA of the grape loads showed that ethanol, gluconic acid and butanediol was significant between grape loads from the same vineyard delivered to the cellar at different temperatures. The areas Lutzville, Spruitdrift and Vredendal were compared using ANOVA for variance in microbial population. Lactic acid bacteria and acetic acid bacteria populations did not differ between the areas. Lutzville (p=0.047) had a higher yeast population than Spruitdrift and Vredendal. The yeast populations between the cultivars did not differ significantly. However, Sauvignon Blanc had a significant higher acetic acid bacteria and lactic acid bacteria populations than Chardonnay, Cabernet Sauvignon, Shiraz and Pinotage from all the areas. The individual wine scores were compared to esters, higher alcohols and volatile acids of the wines, using General Regression Model (GRM). Acetoin, methanol, and 2-phenyl ethanol had the most influence on tha wine score. Mallow's CP indicated pH, volatile acidity, and ethyl acetate as grape parameters that have an influence on white wine quality and malic acid, mannitol, ethanol, sorbitol and isoamyl acetate on red wine quality.

5.1 INTRODUCTION

In order to gain a competitive edge in the wine industry it is imperative to add value and not cost to the supply chain. A greater knowledge on the management of each process in the value chain (**Fig.5.1**) and how it affects wine quality is essential.

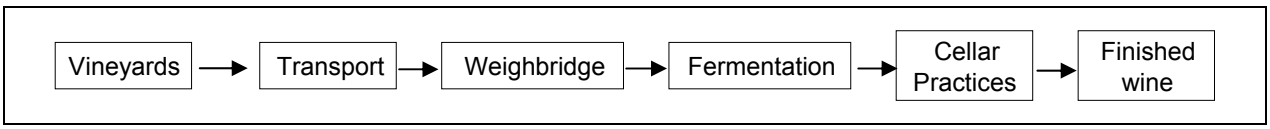


Figure 5.1. Critical steps in the value chain of wine production.

The quality of wine is largely dependant on the composition of the berries as well as the microorganisms associated with the winemaking (du Plessis, 1984; Fleet, 2002) but external factors can also influence wine quality such as grape temperature (Marais, 2001).

The ability to measure the presence and population of microorganisms on grapes is fundamental in the understanding of the science of winemaking and therefore the implementation of quality management programs (Fleet, 2002). Yeasts associated with grapes are mainly non-*Saccharomyces* species (*Candida stellata, Kloeckera apiculata, Candida pulcherrima* and *Candida colliculosa*) (Jolly *et al.,* 2003) and it has been shown that the metabolites they produce influence wine quality. Some have a positive influence due to ester production but others i.e. *Kloeckera apiculata* is associated with acetic acid production.

Lactic acid bacteria are mainly associated with the malolactic fermentation in red wine. They are usually present in low numbers on healthy grapes and in the concomitant must. Lactic acid bacteria survive at low, almost non-detectable populations during alcoholic fermentation in the wine, waiting for a suitable opportunity to grow and have been associated with stuck or sluggish alcoholic fermentation (Edwards *et al.,* 1998).

Acetic acid is produced by acetic acid bacteria through the oxidation of ethanol which is formed through the metabolism of grape sugars by yeasts. Acetic acid in grapes is therefore always accompanied by high amounts of ethanol. Infection of acetic acid bacteria can alter grape juice constituents considerably. This affects the sensory quality of wine but can also cause stuck or sluggish alcoholic fermentation, as well influence the growth of lactic acid bacteria (Fleet, 2002).

Guidelines are set by wine producers for grape growers in the form of prescriptions and specifications which are part of a cellar's grading system. Maturity parameters such as °Brix, must be repeatable and is the most important parameter to measure at winery intake (Berg & Ough, 1977; Riley, 1996). It has been suggested already in the early 1980's that the measurement of °Brix, pH and acidity is not sufficient for determining grape quality (du Plessis, 1984). Wineries especially in Australia have been using grape colour in their grading systems (Johnstone, 1995).

The grading system, used in this industrial cellar, is based on two principles firstly, viticultural practices applied in the vineyards and secondly, maturity parameters (°Brix, pH and acidity) measured at the weighbridge. A vineyard score card is used to class vineyards with respect to viticultural practices. Strict viticultural practice guidelines need to be adhered to in order to receive an A-grade for a particular vineyard. These vineyards receive the highest financial remuneration. Wines made from these vineyards are expected to reach premium quality status. Quality ratings of wines from the A-grade vineyards are done by an in-house tasting panel of winemakers. The cellar has 250 grower members and harvests 100 000 tons of grapes annually. Mechanically harvested grapes are delivered in open bins (6 ton capacity) to the winery. Grapes from A-grade vineyards do receive priority at grape intake but queuing is a reality within our system of grape intake at the weighbridge. While day temperatures can range from 30 to 45ºC during harvest, this is a logistical problem that needs to be addressed, if necessary.

The main objective of this study was to identify the critical process key factors in our industrial cellar that will influence the value chain from the vineyard to the wine. Forty-four vineyards from the Olifants River Valley were selected as sample plots. Grape bunches were analyzed for chemical parameters using FT-IR, microbial populations and wines were made according to standard winemaking practices from the 44 different vineyards and then sensorially evaluated. All data generated were subjected to statistical analysis.

5.2 MATERIALS AND METHODS

5.2.1 Demographics of sampling area: In the Olifants River Valley, South Africa, 44 vineyards were selected as premium vineyards according to viticultural practices, during the 2003 and 2004 harvest seasons. The vineyards are located in different areas (Lutzville, Vredendal and Spruitdrift) within the Olifants River Valley region (**Fig. 5.2**). Lutzville area is situated furthest from the cellar (30 to 50 km) but closer to the cold Atlantic Ocean (5 km). The vineyards in the Lutzville area are on the banks of the Olifants River while the Vredendal and Spruitdrift are not. The average day temperature in the Olifants River Valley is 22 to 24°C. Day temperatures can be as high as 45°C at mid-harvest during February and night temperatures as low as 10 to 15°C. The reason for this difference in day and night temperatures is the cool Atlantic breezes, starting late afternoons. The Lutzville area is slightly cooler than

the other 2 areas in the Olifants River Valley Sauvignon Blanc grapes are the first to ripen, from middle to the end of January. Chardonnay, Pinotage and Merlot are harvested from the beginning to end of February. Shiraz and Cabernet Sauvignon reaches optimal maturity middle-of March.

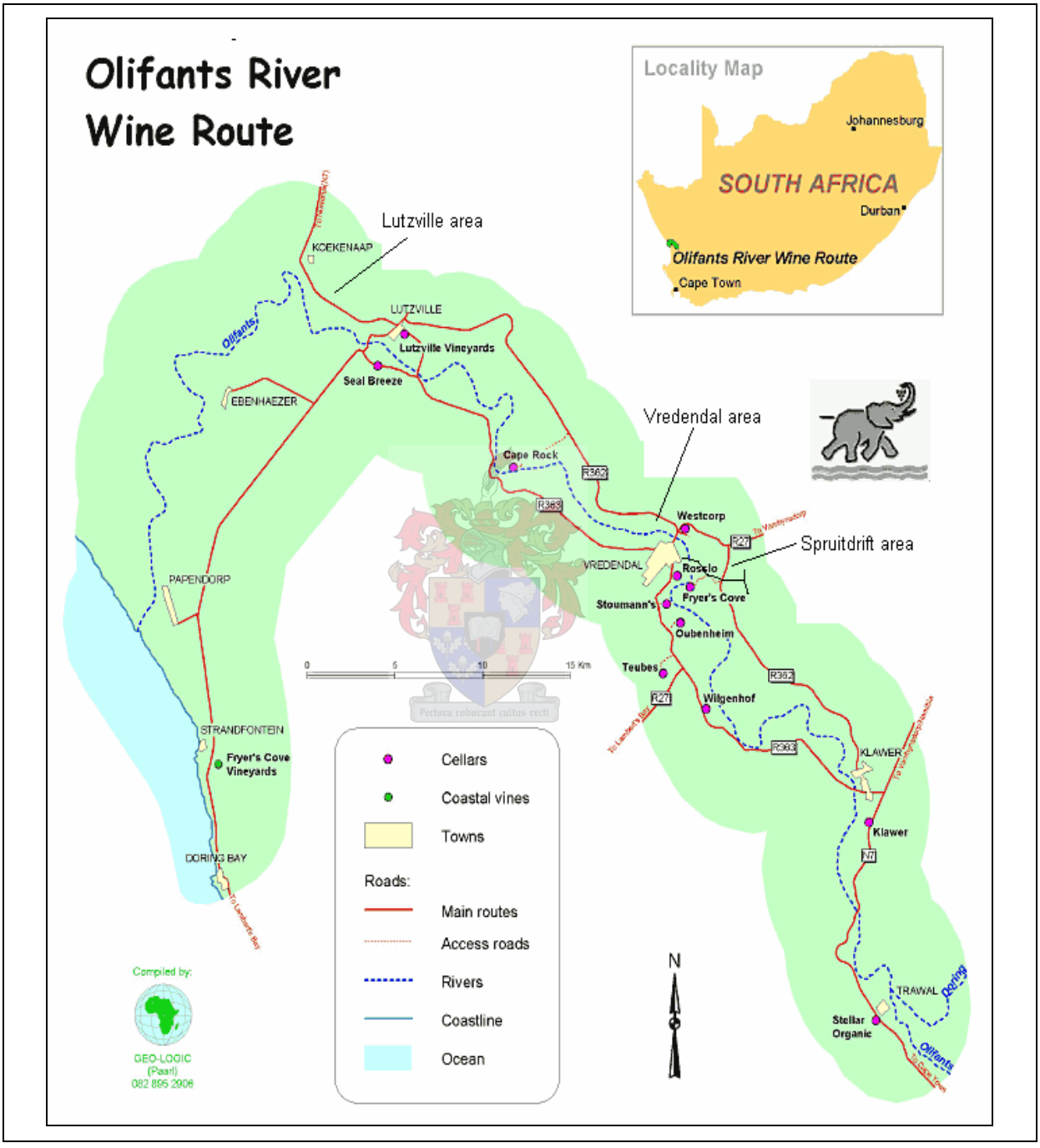


Figure 5.2. Map of the Olifants River Valley which includes the areas Spruitdrift, Vredendal and Lutzville (Olifants River wine route, 2005).

5.2.2 Grape samples: Forty-four vineyards classified as A-grade vineyards according to the WestCorp grading system were selected as sample plots. All

vineyards are mechanically harvested. The cultivars from the respective vineyards are Cabernet Sauvignon (n=6), Merlot (n=5), Shiraz (n=6), Pinotage (n=10), Chardonnay (n=7) and Sauvignon blanc (n=10). Three to four kg grape bunches from different vines per vineyard at optimum maturity (according to °Brix and acidity analyses) on the day of harvesting, were collected aseptically and placed into sterile plastic bags. Sampling of each vineyard was repeated at the winery weighbridge. After collection the sealed plastic bags were immediately taken to the laboratory in cool bags. At the laboratory the grapes were crushed by hand in the sealed plastic bags and the grape juice poured into 500 mL sterile bottles and sealed.

Eighteen of the A-grade vineyards were monitored for chemical differences (°Brix, pH, TA, malic acid, tartaric acid, polyphenols, colour, potassium, nitrogen, volatile acidity, ethanol, gluconic acid, glycerol, ethyl acetate, mannitol, lactic acid, methyl-3-butanol, isoamyl acetate, arabitol, 2,3-butanediol, acetaldehyde) in grape loads at the weighbridge, impact of day temperatures and logistics of grape delivery were investigated. At the weighbridge, grape samples were taken with the grape bore from every grape load from the different vineyards. Grape loads varied from 3 to 6 loads per vineyard. These different grape loads were monitored for any changes in the chemical composition of the grapes.

5.2.3 Chemical analysis: Grape must samples were filtered with a filtration Unit (type 79500, FOSS Electric, Denmark) connected to a vacuum pump and filter paper circles graded at 20 – 25 µm with a diameter of 185 mm (Schleicher & Schnell, reference number 10312714). Grape parameters measured were °Brix, pH, total acidity, tartaric acid, malic acid, volatile acidity (expressed as g/L acetic acid), gluconic acid, ethanol, glycerol, ethyl acetate, lactic acid, mannitol, methyl-3-butanol, 2,3-butanediol, arabitol, sorbitol, meso-inositol, acetaldehyde and isoamyl acetate. A FT-IR spectrometer, Grapescan FT120 (FOSS, Denmark) equipped with global calibrations, was used for analysis.

5.2.4 Microbial enumeration: Yeasts, lactic acid bacteria and acetic acid bacteria were enumerated by plating 200 µL of a dilution series of juice, in duplicate, on selective culture media. Colonies were counted and expressed as colony forming units (cfu) per mL.

5.2.4.1 Yeasts: Yeasts were counted using YPD agar (1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose) (Merck). The growth of lactic acid bacteria and acetic acid bacteria were eliminated by adding 20 mg/L chloramfenicol. YPD plates were incubated at 30ºC for 3 days.

5.2.4.2 Acetic acid bacteria: GYC agar (5% w/v glucose, 1% w/v yeast extract, 3% w/v $CaCO_3$ and 2% w/v agar) (Merck) and Mannitol agar (2.5% w/v mannitol, 0.5% w/v yeast extract, 0.3% w/v peptone and 1.5% w/v agar) (Merck) were used for the isolation of acetic acid bacteria. As an inhibitor of yeast growth pimaricin (Danisco, Denmark) was added (50 mg/mL made up in water and 1 ml added to 1 L medium) and lysozyme (Warrenchem, South Africa) (300 mg/L made up in water and 1 ml added to 1 L medium) as an inhibitor of lactic acid bacterial growth. The plates were incubated at 30ºC for 5 to 7 days.

5.2.4.3 Lactic acid bacteria: MRS agar (Merck) was used for the isolation and enumeration of Lactobacilli, Pediococci and Leuconostocs. Spread plates were incubated, anaerobically (Anaerocult system, Merck) at 30ºC for 10 days. Apple juice agar (MRS (Merck) supplemented with 20% apple juice, and 15 g/L agar, (pH adjusted to 5.2) was used for the detection of *Oenococcus*. To both media Pimaricin (Danisco, Denmark) (50 mg/mL made up in water and 1 mL added to 1 L of medium) was added as an inhibitor for yeasts and kanamycin (Merck) (25 mg/mL made up in water and 1 mL added to 1 L medium) was added as an inhibitor for acetic acid bacteria. The spread plates were incubated, anaerobically at 30ºC for 14 days.

5.2.5 Winemaking and sensorial evaluation: Wine from each selected A-grade vineyard (section 5.2.2) was made according to standard winemaking practices in an industrial cellar. No skin contact was applied to the white winemaking but for red wine, the grapes were kept on the skins for 24 hours at 10°C before inoculation with commercial yeasts and fermented to dryness (**Table 5.1**).

Table 5.1. Commercial yeast used and fermentation temperature applied during alcoholic fermentation of the 6 different cultivars.

Cultivar	Commercial Yeast	Fermentation temperature (°C)
Cabernet Sauvignon (n=6)	RG12	25 - 27
Merlot (n=5)	RG12	25 - 27
Pinotage (n=10)	RG12	25 - 27
Shiraz (n=6)	RG12	25 - 27
Chardonnay (n=7)	Maurivin R2	14 - 15
Sauvignon Blanc (n=10)	K7	13 - 14

Wine samples from each selected vineyard were evaluated by a panel of 5 wine judges. Each wine was scored out of 20 according to colour, aroma and taste. The average of all individual scores was used for classification of the wines into the different categories (**Table 5.2**).

Table 5.2. Classification of wines according to final score received out of 20

Class Description	Score
Premium*	>15
Typical	12 -14
Neutral	10 - 11
Atypical	< 10

* (in-house quality rating)

5.2.6 Data analysis: Analysis of variance (ANOVA) was used as statistical technique to determine significant differences ($p<0.05$) between variables. PLS2 regression and General Regression Model (GRM) was also employed as statistical techniques. Data was exported to the Unscrambler Software (CAMO, Norway) to detect extreme outlier samples as well as to determine which grape parameters had the highest loadings in Principal Component Analysis (PCA).

5.3 RESULTS AND DISCUSSION

5.3.1 Microbial populations

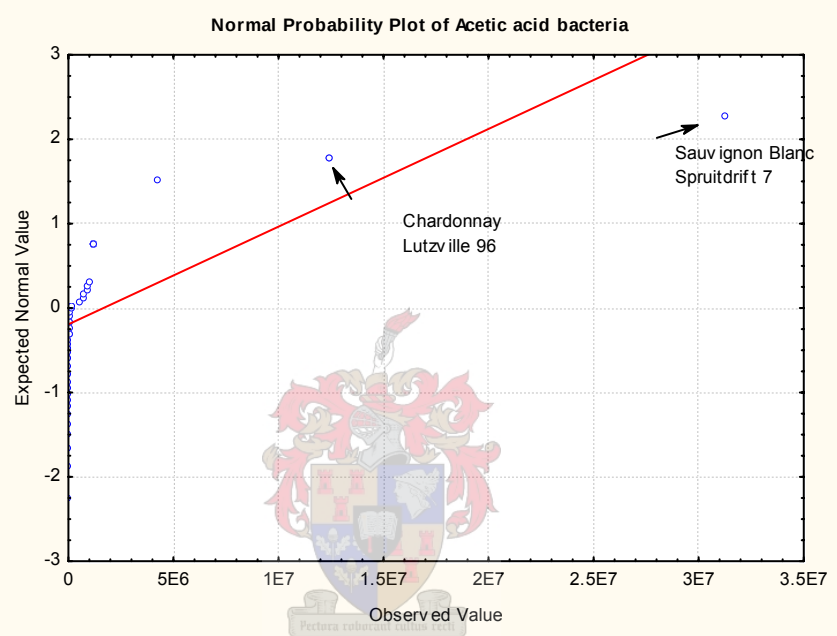
5.3.1.1 Vineyard and Weighbridge: 22 of the 44 vineyards were monitored in the vineyard and at the weighbridge for the selected microbial populations present.

Grape samples collected from selected vineyards (n=22) before harvest and again at the weighbridge showed no significant difference in the total average microbial population between the grape samples from the vineyard and the grape samples at the weighbridge ($p<0.05$). The microbial population measured in the vineyards and at the weighbridge does not follow a normal distribution, statistically (**Fig. 5.3**). The minimum and maximum cfu/mL for the microbial populations varied from zero to 10^7 (**Table 5.3**). The extreme deviating samples with respect to acetic acid bacteria in **Fig. 5.3a** is a Sauvignon Blanc vineyard in the Spruitdrift area (3.13×10^7 cfu/mL) and a Chardonnay vineyard in the Lutzville area (1.25×10^7 cfu/mL). This Chardonnay vineyard in Lutzville also had high population levels of yeasts (1×10^7 cfu/mL) (**Fig. 5.3b**) and lactic acid bacteria (9×10^6 cfu/mL) (**Fig. 5.3c**). The same Sauvignon Blanc vineyard in the Spruitdrift area also had high lactic acid bacterial numbers (6.6×10^6 cfu/mL) as well (**Fig. 5.3c**). 14 Vineyards had an increase in yeast population from the vineyard to the weighbridge. Vineyards 5, 67 and 141 displayed the biggest increase from vineyard to weighbridge of 4 to 5 log units (**Fig. 5.4a**). Acetic acid bacteria populations increased slightly in 9 vineyards and 5 vineyards had the same cfu/mL in the vineyard and at the weighbridge (**Fig. 5.4b**). The biggest difference between vineyard and weighbridge for acetic acid bacteria was approximately 2 log units (decrease from vineyard to weighbridge) (**Fig. 5.4b**). For the lactic acid bacteria nine vineyards increased from 0 to between 3 and 5 log units (**Fig. 5.4c**). This was due to long waiting periods at the winery weighbridge (varied from 1 to 3 hours). In contrast, SO_2 was added to the grape loads of the Chardonnay vineyard in the Lutzville area which had high microbial populations for yeasts, acetic acid bacteria and lactic acid bacteria, as shown in **Fig. 5.3**. In **Fig. 5.4a, b & c** this vineyard (96) clearly showed a decrease in microbial populations. The Levene test of Homogeneity of variance was applied. The hypothesis of equal variance within each group, that is, yeast ($p=0.049$), acetic acid bacteria ($p=0.00008$) and lactic acid bacteria ($p=0.0048$) are therefore rejected. This is expected as microbial activities are governed by many factors such as disease status, climate, state of ripeness and terrior. There were no correlation between the total average microbial numbers and metabolites measured. Concluding, that high microbial numbers do not necessarily mean high metabolite concentrations in grape must.

Malic acid demonstrated a slight variance between vineyard and weighbridge samples ($p=0.05$) of the same vineyard. Of the metabolites measured, meso-inositol

(p=0.0001), sorbitol (p=0.011), lactic acid (p=0.032) and acetaldehyde (p=0.013) showed significant variance within each group. Meso-inositol (p=0.0028) was the only metabolite that differed significantly between the vineyard and weighbridge measuring site. This metabolite is formed as a result of the conversion of glucose and fructose during the growth of moulds, yeasts and bacteria present on grapes (Zoecklein *et al., 2000*).

a)



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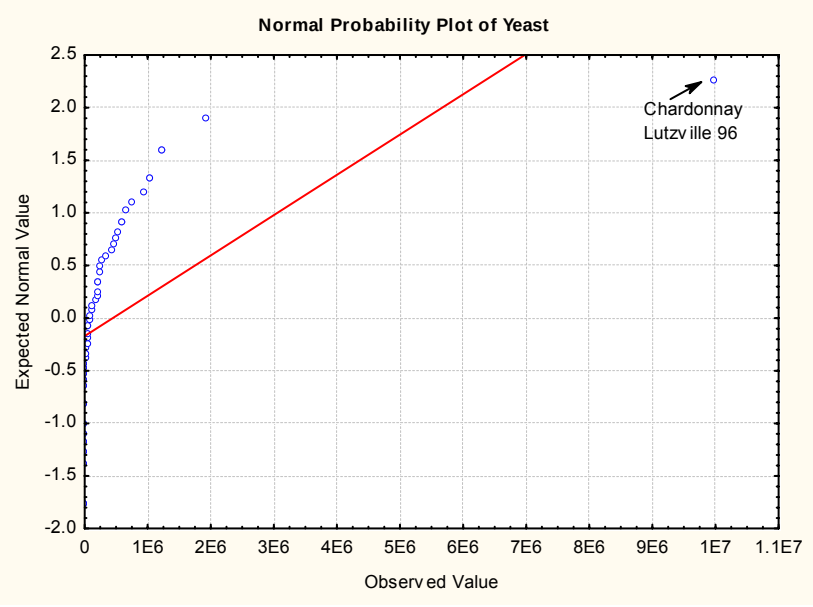


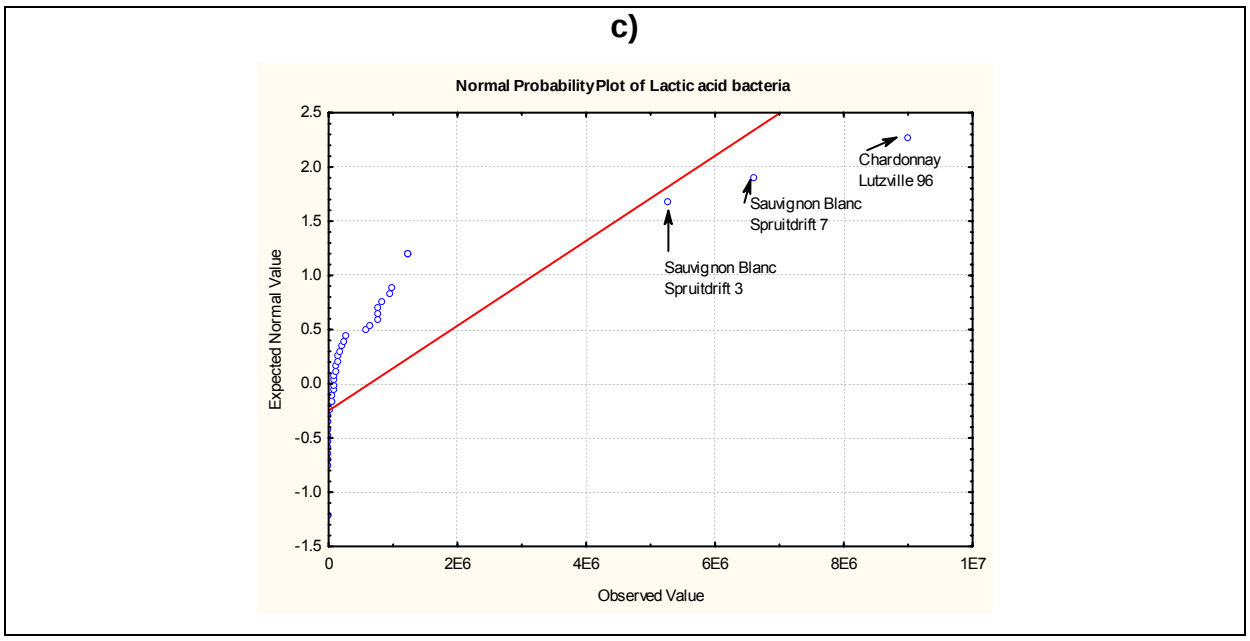
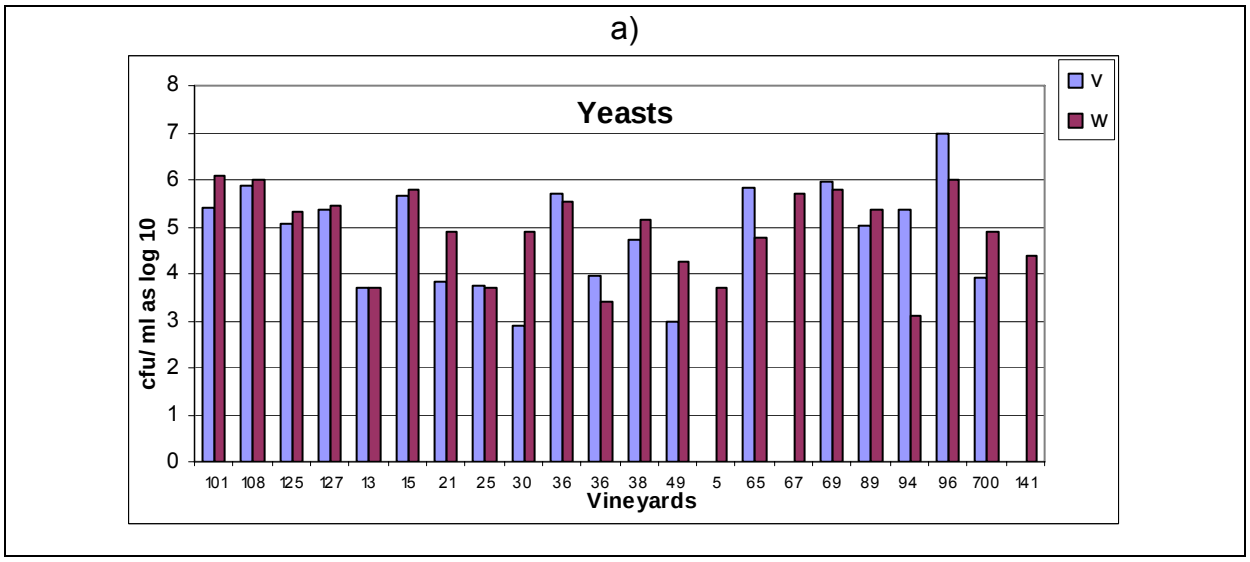


Figure 5.3. Normal probability plots of a) Acetic acid bacteria, b) Yeasts and c) Lactic acid bacteria populations, illustrating the distribution of the samples.

Table 5.3. Minimum and maximum colony forming units per mL (cfu/mL) in grape samples, for the different microbial groups tested.

	Acetic acid Bacteria	**Yeasts**	**Lactic acid Bacteria**
min.	1.0×10^2	0	0
max.	1.25×10^7	1.00×10^7	9.00×10^6



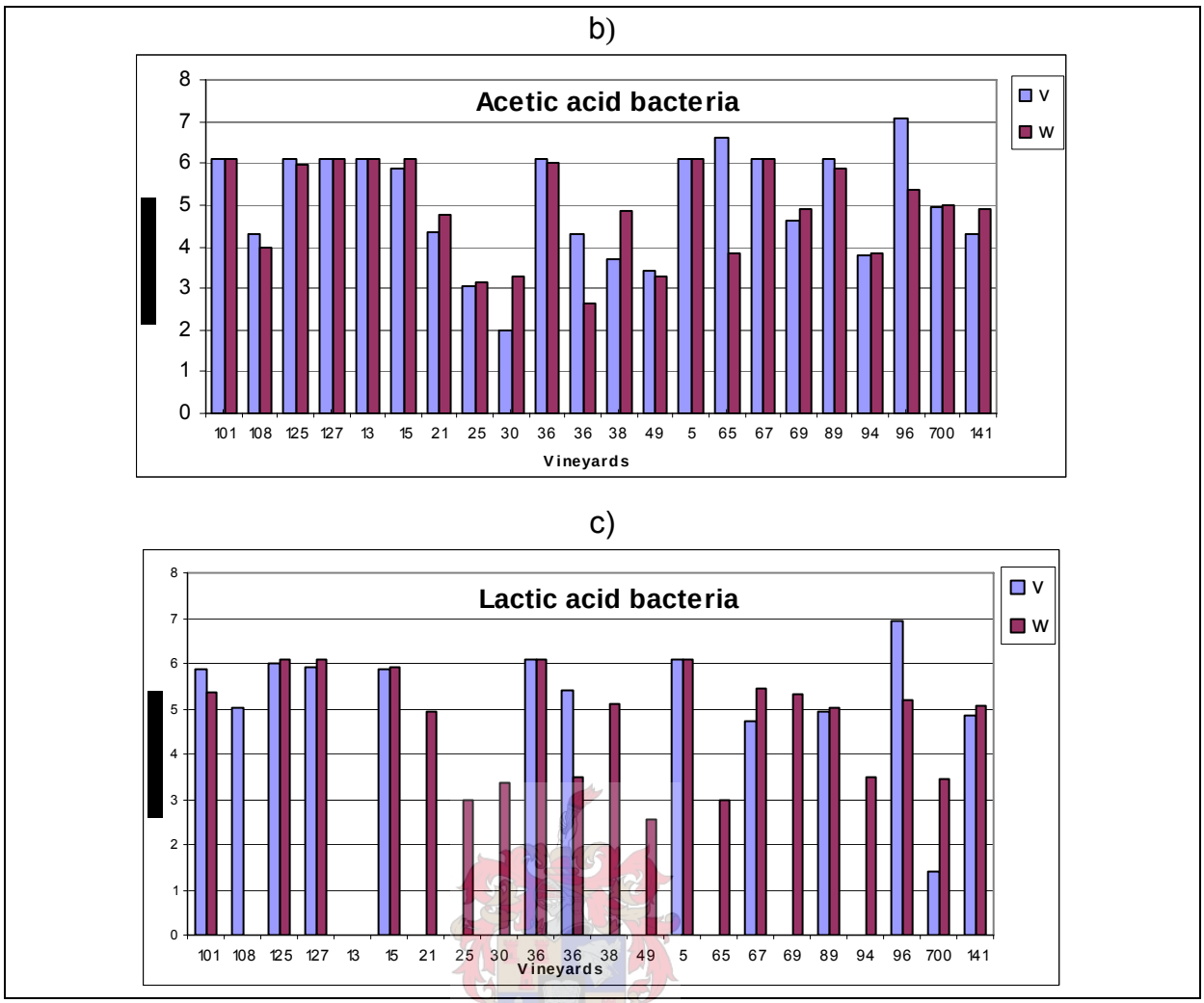


Figure 5.4. Microbial population of grape samples (n=22) in the Olifants River Valley, measured in the vineyard (v) and at the weighbridge (w). a) Yeasts, b) Acetic acid bacteria and c) Lactic acid bacteria.

The chemical data, which included the maturity parameters and metabolites measured of each sample, were exported to the Unscrambler software version 9.2 (Camo, Norway) for the purpose of PCA. Seven extreme deviating samples were noted (**Fig. 5.5**). Four of these grape samples (15w, 15v, 96w & 96v) had higher than average levels of gluconic acid, glycerol and arabitol than the sample set (**Tables 5.4 & 5.5**). This correlates with the PCA score plot of PC1 and PC2 where samples A are grouped together (**Fig. 5.5**). Sample 96v had higher concentrations of methyl-3-butanol and 2,3-butanediol (Group B) (**Fig. 5.5**). The group B samples has higher concentrations of ethanol, methyl-3-butanol and 2,3-butanediol. Glycerol and gluconic acid are highly correlated. Arabitol is also correlated to these two metabolites (**Fig. 5.6**). The loadings in **Fig. 5.6** of methyl-3-butanol and butanediol correlates to the scores for the samples in group B in **Fig. 5.5**. The Chardonnay sample which was identified as an extreme deviating sample in the normal probability plots for microbial populations, especially for acetic acid bacteria and lactic acid

bacteria is the same sample identified by PCA as a extreme deviating sample i.e. sample 96.

Table 5.4. Mean and standard deviation of measured parameters of vineyard (n=22) samples and weighbridge samples (n=22).

Parameters	Mean	SD
°Brix	24.13	1.07
pH	3.50	0.19
Tartaric acid	5.22	1.04
Malic acid	2.97	0.91
Gluconic Acid	0.61	1.69
Ethanol	0.61	0.59
Glycerol	0.15	0.49
Volatile acid	0.17	0.22
Ethyl acetate	0.13	0.18
Lactic acid	0.10	0.23
Mannitol	0.04	0.02
Methyl-3-Butanol	0.12	0.18
2,3-Butanediol	0.92	0.75
Arabitol	0.13	0.31
Sorbitol	0.07	0.05
Meso-inositol	0.19	0.36
Acetaldehyde	0.02	0.05
Isoamyl acetate	0.01	0.03

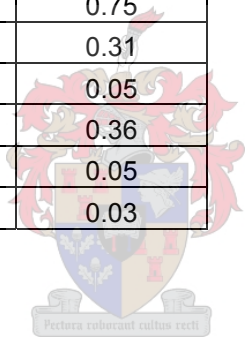


Table 5.5. Concentrations of metabolites in grape samples from the vineyard and weighbridge.

Sample code	Cultivar	Acetic acid bacteria	Yeast	Lactic acid bacteria	Gluconic Acid	Ethanol	Glycerol	Volatile acid
36v	Chardonnay	2.05E+04	9.25E+03	2.55E+05	0.611361	1.29	0.097809	0.047902
36w	Chardonnay	4.20E+02	2.60E+03	3.00E+03	0	1.29	0	0.011
65v	Chardonnay	4.31E+06	6.80E+05	0.00E+00	0.219952	1.257594	0	0.115116
65w	Chardonnay	6.88E+03	6.00E+04	9.25E+02	0.358377	1.062256	0	0.045245
30v	Cabernet Sauvignon	1.00E+02	7.50E+02	0.00E+00	0	0.148927	0	0.095707
30w	Cabernet Sauvignon	1.90E+03	7.50E+04	2.30E+03	0	0.72518	0	0.137408
30w	Cabernet Sauvignon	4.63E+03	1.50E+04	3.35E+03	0	0.493401	0	0.120419
25v	Cabernet Sauvignon	1.15E+03	5.50E+03	0.00E+00	0	0.356283	0	0.069569
25w	Cabernet Sauvignon	1.33E+03	5.00E+03	9.50E+02	0	0.729483	0	0.044517
49v	Shiraz	2.60E+03	9.50E+02	0.00E+00	0	0.313695	0	0.034654
49w	Shiraz	2.83E+03	2.00E+04	4.25E+02	0	0.941831	0	0.043394
49w	Shiraz	1.95E+03	1.75E+04	3.50E+02	0	0.773389	0	0.072629
94v	Cabernet Sauvignon	6.40E+03	2.35E+05	0.00E+00	0	0.200895	0	0.08018
94w	Cabernet Sauvignon	6.88E+03	1.25E+03	3.00E+03	0	0.211659	0	0.1430295
700v	Shiraz	8.78E+04	8.00E+03	2.50E+01	0.507822	0.443021	0.418089	0.138514
700w	Shiraz	9.75E+04	8.10E+04	2.93E+03	0	1.771664	0.085695	0.3290005
94v	Cabernet Sauvignon	1.25E+07	1.95E+06	6.50E+05	0	0	0	0.137788
21v	Cabernet Sauvignon	2.25E+04	7.00E+03	0.00E+00	0	0.921668	0	0.0840314
21w	Cabernet Sauvignon	5.50E+04	7.50E+04	8.50E+04	0	1.655685	0	0.1231901
108v	Cabernet Sauvignon	2.00E+04	7.50E+05	1.03E+05	0	1.229358	0	0.1065402
108w	Cabernet Sauvignon	5.50E+04	1.05E+06	0.00E+00	0	1.573756	0	0.0301348
115v	Cabernet Sauvignon	1.48E+05	2.35E+05	5.50E+04	0	0.145656	0	0.1498781
91v	Cabernet Sauvignon	5.70E+05	5.00E+04	4.00E+04	0	0	0	0.1648778
9v	Chardonnay	3.50E+04	5.00E+03	1.60E+05	0	0	0	0.265084

Sample code	Cultivar	Acetic acid bacteria	Yeast	Lactic acid bacteria	Gluconic Acid	Ethanol	Glycerol	Volatile acid
1v	Chardonnay	1.25E+06	1.25E+06	1.25E+06	0.181564	0	0	0.3308106
45w	Chardonnay	1.25E+06	5.50E+05	1.00E+06	0.4828324	0.01004	0	0.2323942
36v	Chardonnay	1.25E+06	4.85E+05	1.25E+06	0	0.18226	0	0.2973143
36w	Chardonnay	1.00E+06	3.40E+05	1.25E+06	0.0638918	0	0	0.2594014
15v	Chardonnay	7.25E+05	4.45E+05	7.63E+05	0	1.734858	0	0.1854505
15w	Chardonnay	1.25E+06	6.00E+05	7.88E+05	0	1.655727	0	0.1918841
65w	Chardonnay	9.38E+05	2.15E+05	0.00E+00	0.1024711	0.124628	0	0.2562441
96w	Chardonnay	2.38E+05	1.05E+06	1.55E+05	0.8372629	2.251776	1.419151	0.17974
96v	Chardonnay	1.25E+07	1.00E+07	9.00E+06	3.0928698	0.406962	0.545644	0.5675225
5v	Merlot	1.25E+06	0.00E+00	1.25E+06	0	0.250633	0	0.1347668
5w	Merlot	1.25E+06	5.00E+03	1.25E+06	0.5287457	0.210505	0	0.1934286
69v	Merlot	4.00E+04	9.45E+05	0.00E+00	0	1.788003	0	0.2288241
69w	Merlot	7.75E+04	6.00E+05	2.00E+05	0	1.871358	0	0.0872705
127v	Pinotage	1.25E+06	2.40E+05	8.38E+05	0	0	0	0.2744615
127w	Pinotage	1.25E+06	2.85E+05	1.25E+06	0.0499419	0	0	0.2272942
125v	Pinotage	1.25E+06	1.15E+05	9.53E+05	0	0	0	0.3037422
125w	Pinotage	9.43E+05	2.05E+05	1.25E+06	0	0	0	0.1618589
89v	Pinotage	1.25E+06	1.05E+05	8.75E+04	0	0.22805	0	0.1843995
89w	Pinotage	7.50E+05	2.25E+05	1.08E+05	0.4983727	0.635982	0	0.212422
13v	Pinotage	1.25E+06	5.00E+03	0.00E+00	0	1.289966	0	0.1973283
13w	Pinotage	1.25E+06	5.00E+03	0.00E+00	0	0.08719	0	0.066288
67v	Pinotage	1.25E+06	0.00E+00	5.50E+04	0	0.404644	0	0.2045057
67w	Pinotage	1.25E+06	5.05E+05	2.73E+05	0	1.160083	0	0.1129943
101v	Pinot Noir	1.25E+06	2.50E+05	7.65E+05	0.7422571	0.017923	0	0.1724189
101w	Pinot Noir	1.25E+06	1.25E+06	2.30E+05	0.9358488	0.07625	0	0.1557978
7v	Sauvignon blanc	3.13E+07	0.00E+00	5.28E+06	0	0	0	0.2239302
3v	Sauvignon blanc	7.50E+03	0.00E+00	6.63E+06	2.3490642	3.452688	0	0
32w	Sauvignon blanc	1.25E+06	2.35E+05	5.78E+05	0	0	0	0.2264715
38v	Shiraz	5.00E+03	5.50E+04	0.00E+00	0	0.709956	0	0.0573417
38w	Shiraz	7.50E+04	1.35E+05	1.23E+05	0	0.670417	0	0.0291767
141v	Shiraz	2.00E+04	2.00E+03	7.00E+04	0	0	0	0.1003473
141w	Shiraz	7.75E+04	2.50E+04	1.13E+05	0	0.24915	0	0.0490787

Sample code	Ethyl acetate	Lactic acid	Mannitol	Methyl3Butanol	butanediol	arabitol	sorbitol	Meso-inositol	Acetaldehyde	Isoamyl acetate
36v	0.650077	0.767836	0.057039	0.598731	3.653837	0	0.115135	0	0	0
36w	0.103	0.3	0.047	0.251349	0.810571	0.078552	0.066734	0	0	0
65v	0.533234	0.266947	0.037075	0.391977	1.493869	0.006445	0.15748	0	0	0
65w	0	0.019307	0.045838	0.245713	0.605727	0.605727	0.069795	0.080403	0	0
30v	0	0	0.022023	0.071734	1.049992	0	0.107024	0.015201	0	0
30w	0.485442	0.386087	0.046989	0.010738	1.246431	0.078288	0.057879	0	0	0
30w	0.198434	0.386087	0.044258	0.066278	1.089621	0.013382	0.04846	0	0	0
25v	0	0	0.021268	0.057657	0.796296	0	0.076933	0	0	0
25w	0.397244	0.097643	0.038513	0	0.418471	0	0.009035	0	0	0
49v	0.116704	0	0.032587	0	0.505949	0.00977	0.095365	0	0	0.000514
49w	0.22069	0	0.030015	0.142533	1.293059	0	0.065334	0	0	0
49w	0.31441	0	0.039659	0	1.092721	0.034036	0.064599	0	0	0
94v	0.056994	0.244673	0.023168	0.232569	0.998541	0.04597	0.009325	0.181583	0	0
94w	0.268672	0.4766875	0.0099655	0.271399	0.9269045	0.020846	0.058782	0	0	0
700v	0.068552	0	0.0539715	0.2653065	1.494318	0.24492	0.069903	1.4814595	0.2420565	0.047041
700w	0.51444	0	0.0198455	0.5464735	1.769512	0	0.106649	0	0	0
94v	0	2.5750235	0.0640714	0.1026633	1.642717	0.387136	0	2.7761874	0	0.0165608
21v	0.0607739	0	0.0534899	0	1.0954788	0.01065	0.073136	0	0	0
21w	0.2090793	0	0.0552761	0.0042576	1.5017884	0	0.064296	0	0	0
108v	0	0	0.0412812	0.3127096	1.7834632	0	0	0	0.0553274	0
108w	0	0	0.0560447	0.1963332	1.4482153	0	0	0	0.0627207	0
115v	0.3321647	0.3297262	0	0.1376524	0.8594324	0.055595	0	0.8612139	0	0.0030638
91v	0.3792488	0.207487	0	0.1224951	0.7732997	0.115884	0	0.8281231	0	0.0120311
9v	0.082208	0	0.0323837	0.0605011	0.8014136	0.009986	0.087306	0	0	0
1v	0	0	0.0373749	0	0.4679544	0	0.12955	0	0	0
45w	0	0	0.0316958	0.0846106	0.6288106	0.000952	0.078613	0	0	0
36v	0.0452963	0	0.0541174	0	0.6883992	0.049677	0.129492	0	0	0
36w	0.0383275	0	0.0460953	0	0.6464061	0	0.105828	0	0	0
15v	0.173012	0	0.0511456	0.0234401	0.8998524	0	0.050014	0	0	0
15w	0.3451582	0	0.0532859	0.0196562	0.9620095	0	0.033167	0	0	0
65w	0	0	0.0411293	0	0	0.008555	0.076394	0	0	0.0002828
96w	0.1058197	0	0.0830619	0.3404023	1.568131	0	0.117699	0	0.109709	0
96v	0	1.1170658	0.1262041	0.4090771	2.3206787	0.155845	0	1.1553858	0.1898509	0

Sample code	Ethyl acetate	Lactic acid	Mannitol	Methyl3Butanol	butanediol	arabitol	sorbitol	Meso-inositol	Acetaldehyde	Isoamyl acetate
5v	0.2255936	0	0.0390042	0.0759083	0.7363788	0	0.05669	0	0	0
5w	0	0	0.040553	0	0	0	0.016212	0	0	0
69v	0.4837763	0	0.0505046	0.6367996	2.3039864	0	0.201055	0	0	0
69w	0.0505799	0	0.0444285	0.3463602	1.2978511	0	0.08232	0	0	0
127v	0	0	0.0299662	0	0.385257	0.03378	0.081847	0.6571874	0	0.0357162
127w	0	0	0.0358283	0	0.3063561	0.061336	0.026905	0.6028281	0	0.0428733
125v	0	0	0.0273617	0	0.6597816	0.070752	0.139974	0.9066213	0	0.0575189
125w	0	0	0.0337508	0	0.0820697	0.043605	0.054281	0.4724745	0	0.0511968
89v	0	0	0.0387578	0	0.3688557	0.043137	0.062934	0.345583	0	0.0344896
89w	0	0	0.062044	0	0.3663759	0.037056	0.074399	0	0	0.0178647
13v	0	0	0.0573528	0	1.1736741	0.136369	0.09988	0.1574956	0.0365248	0.0169885
13w	0.0715921	0	0.0378487	0	0.0587185	0.08544	0	0.5479739	0	0.0614217
67v	0.0441751	0	0.0389674	0	0.4587605	0.095214	0.115796	0.2009124	0	0.0305589
67w	0	0	0.0367465	0	0.3304497	0.036309	0.035735	0	0	0.0036953
101v	0	0	0.0560939	0	0	0.016406	0.040173	0	0	0.013899
101w	0	0	0.0624716	0	0	0.116587	0.073265	0	0.0753988	0.0386833
7v	0	0	0.02137	0	0.2206433	0.038579	0.044298	0	0	0.0025984
3v	0	0	0.1860479	0	0.3522844	0.127358	0	0.5386064	0.3371235	0.0444185
32w	0	0	0.0282611	0	0	0.004291	0.012501	0	0	0.0003614
38v	0.1215635	0	0.0583478	0	1.1967689	0.094023	0.124198	0.3544039	0	0.0099388
38w	0.2267475	0	0.0617773	0	0.9934014	0.000272	0.109128	0	0	0
141v	0.2455463	0	0.0669883	0	1.9113197	0.195691	0.230964	1.1385695	0.0491836	0.0756092
141w	0	0	0.053467	0	0.9923251	0.035744	0.124014	0	0	0

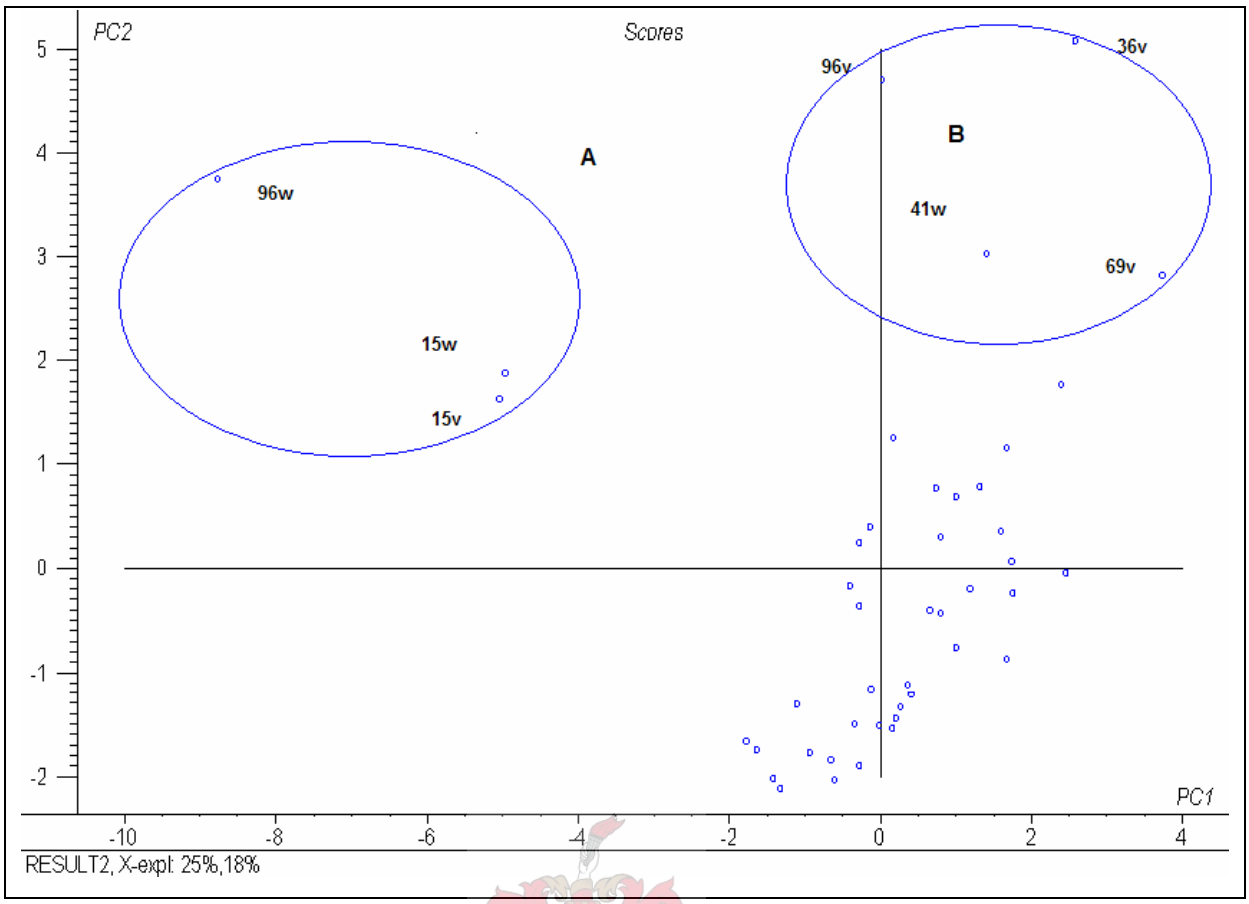


Figure 5.5. Score plot of PCA of all samples, vineyard (n=22) and weighbridge (n=22), PC1 and PC2 explains 25% and 18% respectively, of variances between samples.

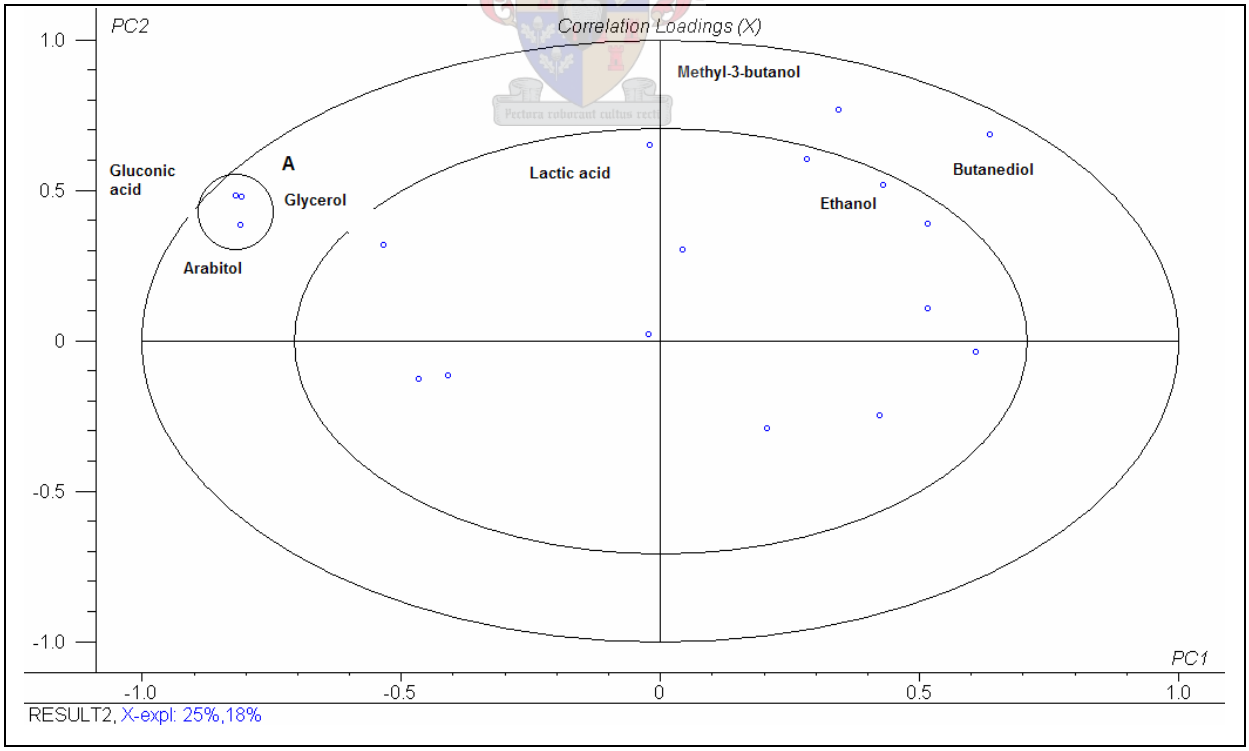
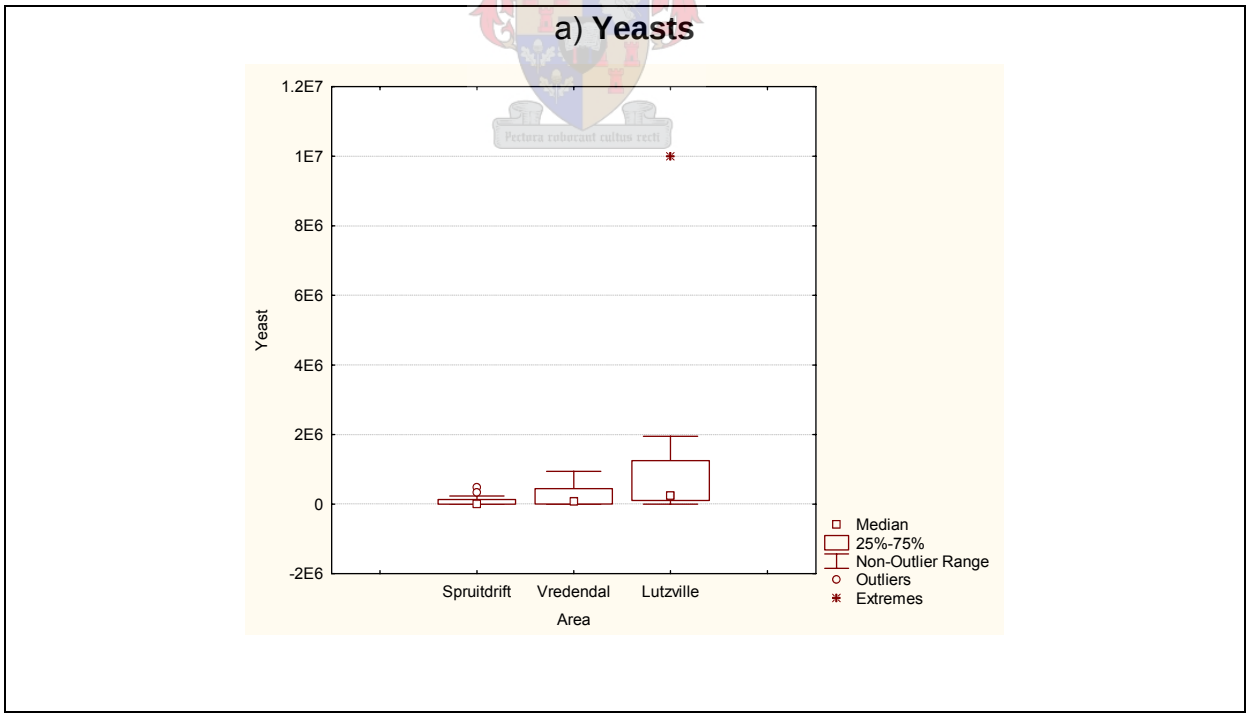


Figure 5.6. Loadings plot of PC1 of the metabolites of all the samples.

5.3.1.2 Microbial differences between areas: 56 Vineyards representative of the region were selected (the 44 A-grade vineyards, included) to investigate if there were a difference between the areas with regard to microbial population. The reason for adding other than A-grade vineyards were to increase the sample set. Microbial population between the 3 different areas (Lutzville n=15, Spruitdrift n=15 and Vredendal n=26) were compared (n=56). The variance for yeasts, acetic acid bacteria and lactic acid bacteria, within the different areas, were not homogenous. The yeast population differed significantly between the areas (p=0.047). Lutzville-area had the highest yeast population (**Fig. 5.7**). The vineyards in this area are close to the banks of the river which makes the vineyards prone to bird attacks. The grape berries are broken and microbial populations can increase due to the availability of growth substrates. Damaged grapes quickly develop microbial populations of 10^6 – 10^8 cfu/g, with a high proportion of acetic acid bacteria (Fleet, 1993; Suárez *et al*., 1994). Also, this area is the furthest from the cellar compared to the other 2 areas. The vineyards are mechanically harvested and exposure to higher temperature due to longer travel time makes it ideal for microbial activity. Lactic acid bacteria and acetic acid bacteria showed no significant difference between the areas.



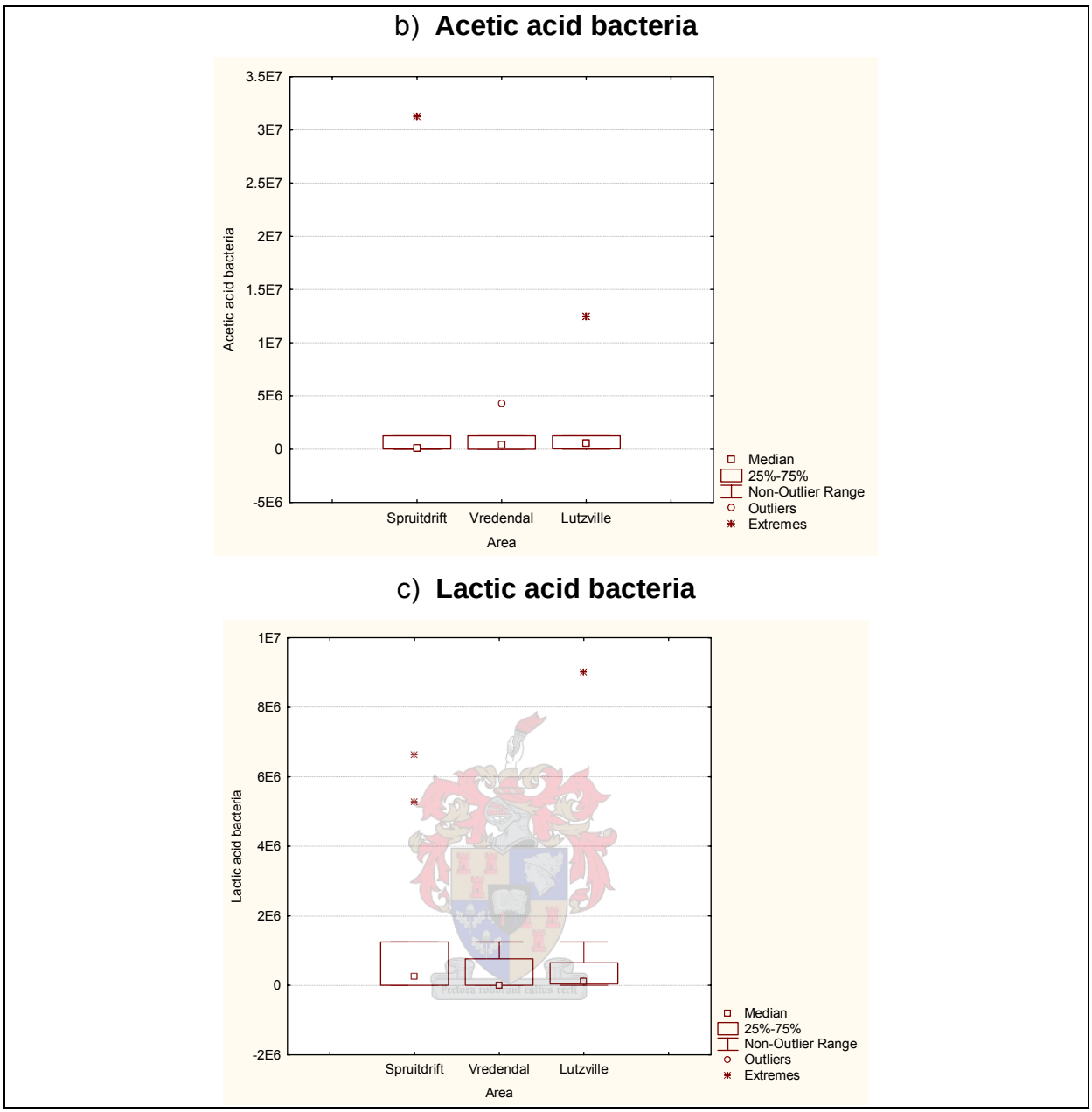


Figure 5.7. Boxplot of a) Yeast population, b) Acetic acid bacteria and c) Lactic acid bacteria in cfu/mL between 3 different areas in the Olifants River Valley.

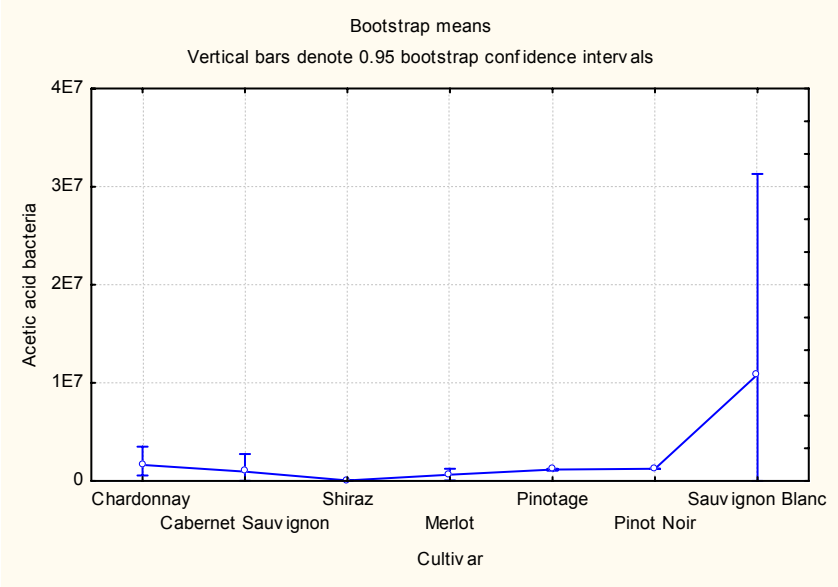
5.3.1.3 Cultivars: The hypothesis for variance for acetic acid bacteria, yeast and lactic acid bacteria were rejected ($p<0.05$) when the populations were compared within cultivar. Sauvignon blanc grape samples showed the highest significant difference for acetic acid bacteria and lactic acid bacteria populations between cultivars (**Figs. 5.8a & b**). The acetic acid bacteria population of the Pinot Noir and Merlot grape must did not differ significantly from that of Sauvignon blanc (**Table 5.6**). There were however a significant difference between Sauvignon blanc and the other cultivars (Chardonnay, Cabernet Sauvignon, Shiraz and Pinotage) (**Figs. 5.8a & b**). Yeast populations between the cultivars did not differ significantly (**Fig. 5.8c**). The lactic acid bacteria population between the cultivars did not differ significantly

except for Sauvignon blanc. Similar to acetic acid bacteria populations, Sauvignon blanc and Pinot Noir did not differ significantly in lactic acid bacteria populations (p=0.12). Sauvignon blanc is the cultivar which ripens earlier in the Olifants River Valley. These vineyards are prone to bird attacks and insects. The Olifants River Valley is a warm climate wine region therefore the pH of the grape must tends to be higher. This leads to a higher than average lactic acid bacteria population (**Table 5.6**). Lactic acid bacteria are normally not found on grapes or numbers very low (10^2 – 10^3 cfu/g). Lactic acid bacteria population are mainly correlated with pH; the higher the pH, the higher the total lactic acid bacteria population and diversity (Lonvaud-Funel, 1999).

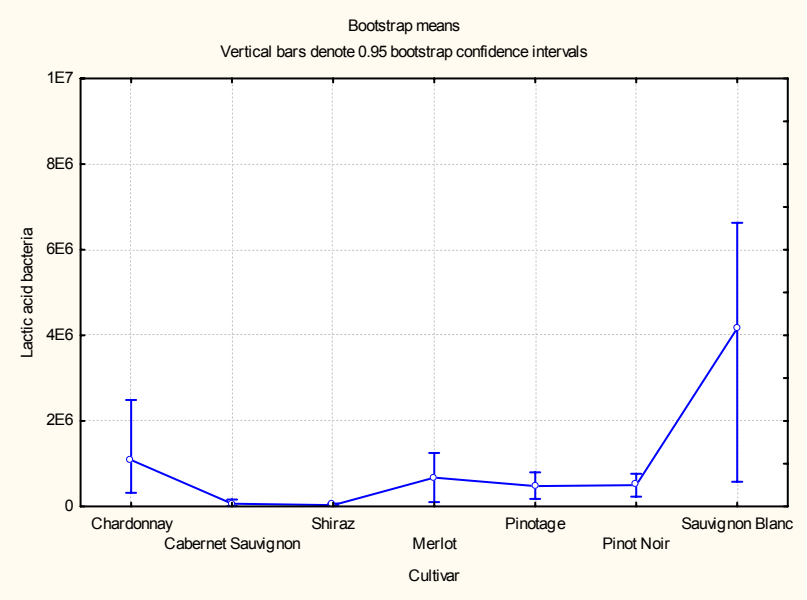
Table 5.6. Means of microbial population of acetic acid bacteria, yeasts and lactic acid bacteria in colony forming units per ml (cfu/mL) of different cultivars, prior to harvest.

Cultivar	Average pH	SD	Acetic acid bacteria	Yeast	Lactic acid bacteria
Chardonnay	3.47	0.13	1.77E+06	1.12E+06	1.13E+06
Cabernet Sauvignon	3.46	0.13	9.57E+05	3.18E+05	6.73E+04
Shiraz	3.74	0.17	4.11E+04	3.83E+04	3.44E+04
Merlot	3.56	0.20	6.54E+05	3.88E+05	6.75E+05
Pinotage	3.35	0.10	1.17E+06	1.69E+05	4.81E+05
Pinot Noir	3.16	0.01	1.25E+06	7.50E+05	4.98E+05
Sauvignon blanc	3.21	0.08	1.09E+07	7.83E+04	4.16E+06
Average of all cultivars			**1.57E+06**	**4.55E+05**	**6.81E+05**

a) **Acetic acid bactera**



b) **Lactic acid bacteria**



c) **Yeasts**

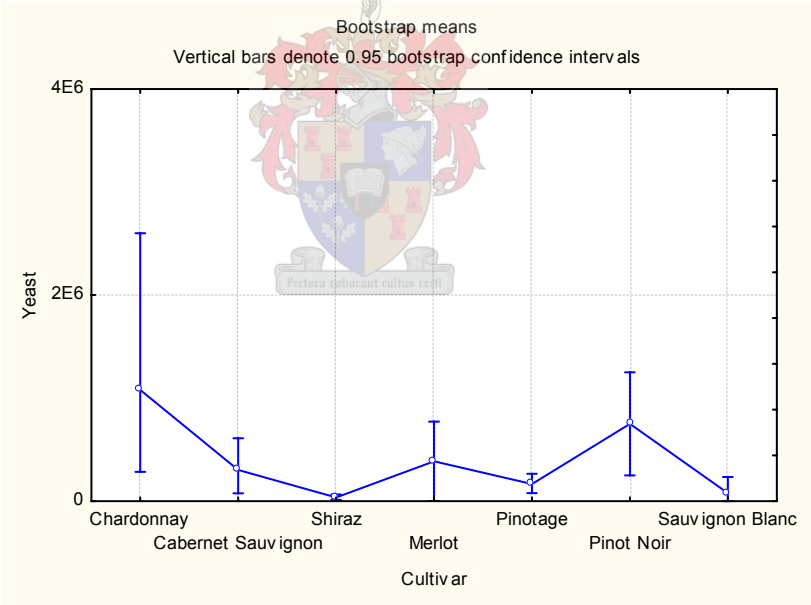


Figure 5.8. Comparison of means of a) acetic acid bacteria, b) lactic acid bacteria and c) yeasts in cfu/mL between cultivars (n=56).

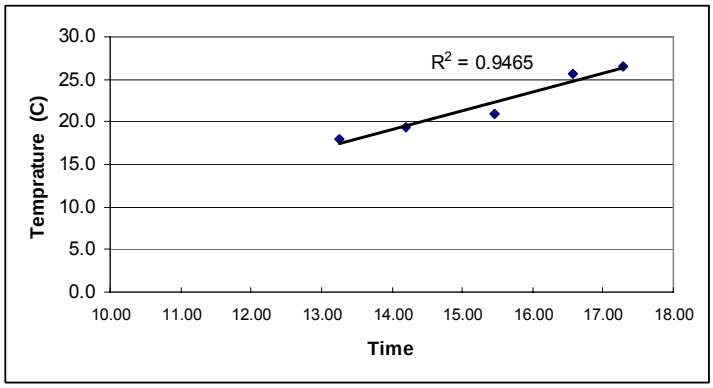
5.3.2 Grape load differences

Temperature varied significantly at the weighbridge, lowest being 14°C (11h00) to the highest 36°C (12h00). The time of day of grape intake is highly positive correlated to the temperature of the specific grape load at the winery weighbridge (**Fig. 5.9**).

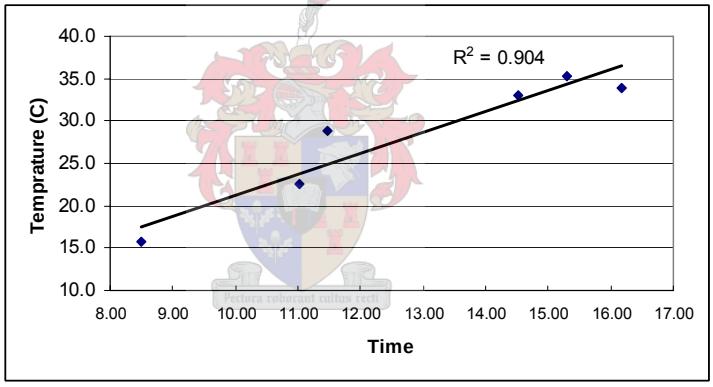
The time it takes to deliver the grape load from the vineyard to the weighbridge, as well as how long the grape load is exposed to the highest

temperature during the day are some of the factors that will have an influence on the quality of the grapes delivered (Clary *et al.,* 1990). Any delay between mechanical harvesting and delivery of the grapes to the winery can result in increased enzymatic activity, browning, oxidation (loss of colour), development of off-flavours and microbial growth (Ough *et al.,* 1971; Morris, 2000). Temperatures above 30°C are likely to cause a deterioration of grape quality, in general (Iland *et al.*, 2000).

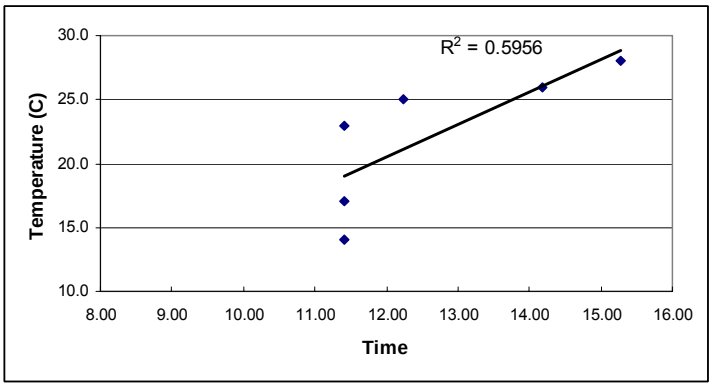
a)



b)



c)



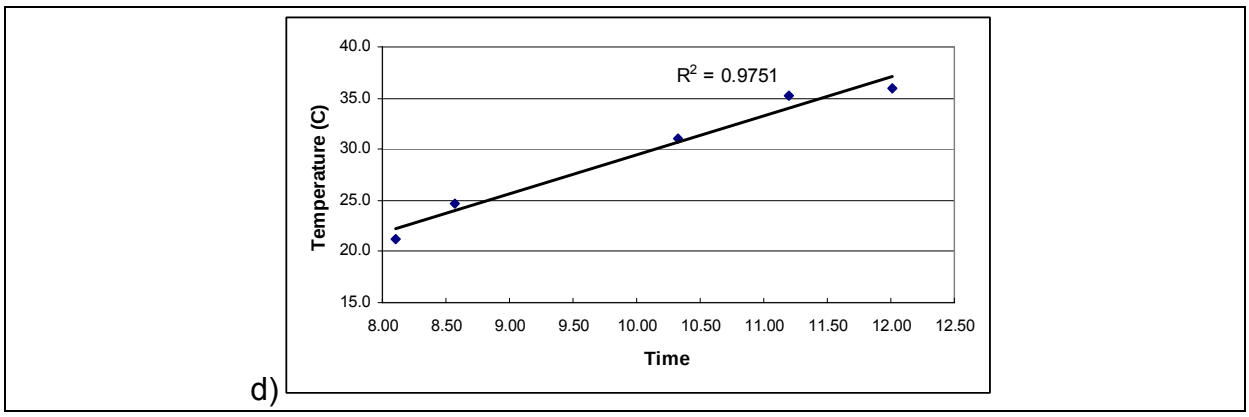


Figure 5.9. Temperature vs. time at the winery weighbridge of grape loads from different vineyards: a) Chardonnay (Vredendal), b) Chardonnay (Spruitdrift), c) Sauvignon blanc (Lutzville) and d) Pinotage (Spruitdrift) grape loads.

Upper and lower limits (UCL & LCL) with a 95% confidence level, were determined for each grape sample for the maturity parameters and metabolites measured, representative of each 18 vineyards and compared to the UCL and LCL of the average of the grape loads representative of that vineyard (**Table 5.7**).

Table 5.7. Upper and lower 95% confidence limits for tartaric acid measured at the weighbridge (mean of grape loads from the same vineyard) and compared to the tartaric acid measured in the vineyard before harvest.

WEIGHBRIDGE							VINEYARD		
	Vineyard Id	Tartaric acid Mean	Tartaric acid Std. Error	Tartaric acid -95.00%	Tartaric acid +95.00%	Grape loads		Vineyard Id	Tartaric acid Mean
1	1	4.40	0.12	4.10	4.70	6	1	1	6.59
2	5	4.14	0.31	2.83	5.45	3	2	5	4.78
3	9	5.40	0.11	5.09	5.70	5	3	9	6.02
4	15	4.00	0.46	2.01	5.98	3	4	15	6.64
5	23	6.88	0.23	6.14	7.62	4	5	23	8.29
6	28	3.74	0.21	3.08	4.40	4	6	28	7.28
7	30	4.44	0.26	3.33	5.56	3	7	30	6.37
8	32	6.56	0.14	6.16	6.96	5	8	32	5.32
9	34	5.28	0.12	4.94	5.61	5	9	34	5.39
10	41	5.28	0.36	3.74	6.83	3	10	41	5.83
11	45	5.43	0.25	4.38	6.49	3	11	45	5.72
12	61	4.73	0.60	3.06	6.40	5	12	61	6.77
13	73	6.45	0.28	5.57	7.34	4	13	73	6.48
14	77	6.76	0.33	5.93	7.60	6	14	77	7.31
15	119	5.40	0.31	4.60	6.20	6	15	119	6.35
16	125	4.25	0.57	2.79	5.71	6	16	125	5.08
17	127	4.80	0.18	4.31	5.30	5	17	127	5.13
18	139	4.45	0.57	2.62	6.27	4	18	139	5.60

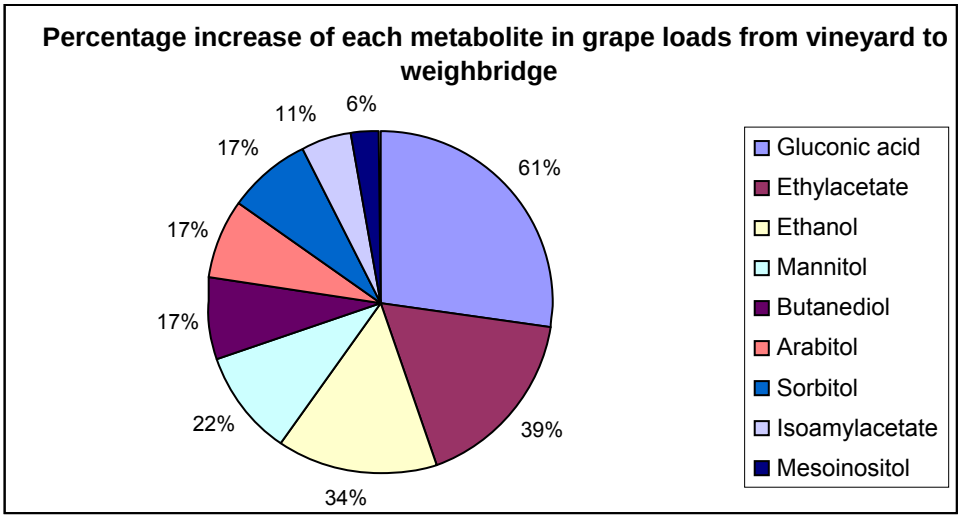
The amount of grape loads exceeding the UCL or under the LCL of the measured vineyard sample are expressed as a percentage of all the vineyards (**Fig. 5.10**). 61%

of the grape loads from the 18 vineyards had a higher gluconic acid content, exceeding the UCL at the weighbridge compared to that in the vineyard. Ethyl acetate and ethanol were also higher in 39% and 34%, respectively, of the grape loads. Other metabolites that were higher at the weighbridge were mannitol (22%), butanediol (17%), arabitol (17%), sorbitol (17%), isoamyl acetate (11%) and meso-inositol (6%) (**Fig. 5.11a**). Interestingly, the sugar concentration of 61% of the grape acid (44%), α-amino nitrogen (28%), ammonia (17%) and colour index (17%) was lower at the weighbridge than in the vineyard (**Fig. 5.10b**). Volatile acidity and acetaldehyde increased from vineyard to weighbridge of the different grape loads, but was within the 95% UCL and LCL. The increase in metabolites (**Fig. 5.10a**) from vineyard to weighbridge as well as the decrease in sugar concentration, nitrogen (α-amino nitrogen and ammonia) and colour index (**Fig**. **5.10b**) of the grape loads confirms microbial activity of yeasts, moulds and bacteria. This correlates with some of the findings in section 5.3.1.1 and **Figure 5.4**. During transport of the mechanically harvested grape loads from vineyard to weighbridge, potassium content in the grape must increased due to the release from grape skins. Tartrate salts could form when the potassium binds to tartaric acid. A decrease in tartaric acid therefore occurred. Due to the potassium-tartaric acid reaction the pH increased. Skin maceration also occurs during transport and it is expected that the colour indexes should increase. With white grapes an increase in colour indexes are an indication of the brown pigment forming due to oxidation of the grape must (Ribéreau-Gayon *et al.,* 2000a).

5.3.3 Wine Quality

To understand the impact of key operations in the process steps of the value chain of wine production it is necessary to evaluate wine quality. Mallows' CP (measures the quality of fit for a model) as criteria, in a General Regression Model (GRM), was used to establish the best subset of X-variables that fits the model for prediction of the wine score. GRM are used when there are many X-variables that might have an influence on a model simultaneously. Grape parameters which had the most influence on the final wine score according to Mallows' CP were pH (p=0.027), volatile acidity, ethyl acetate (p=0.04) as well as arabitol and sorbitol in white grapes. In red grapes malic acid (p=0.032) was found to influence wine quality score the most. Tartaric acid and pH also influenced the wine score. Of the metabolites measured in the grapes, mannitol (p=0.015) and ethanol influenced the final wine score the most with sorbitol and isoamyl acetate, also having an influence.

a)



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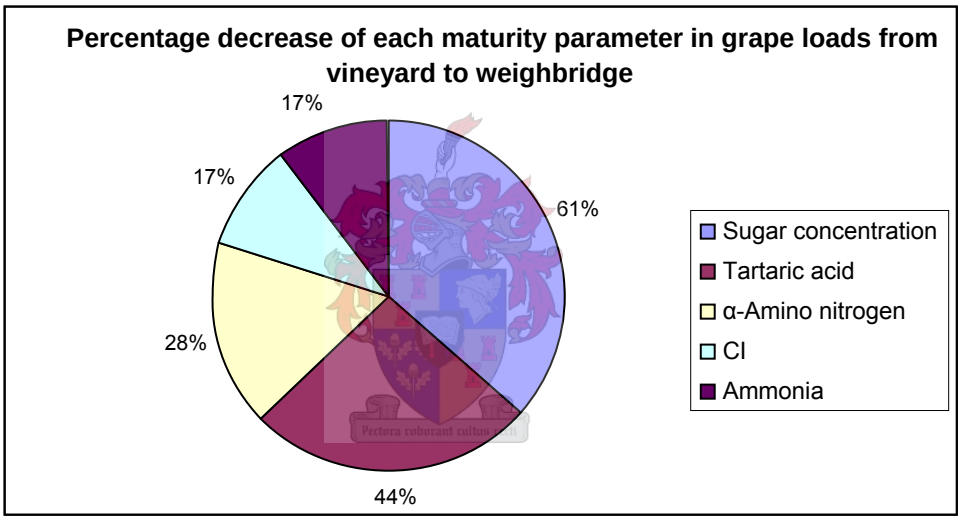

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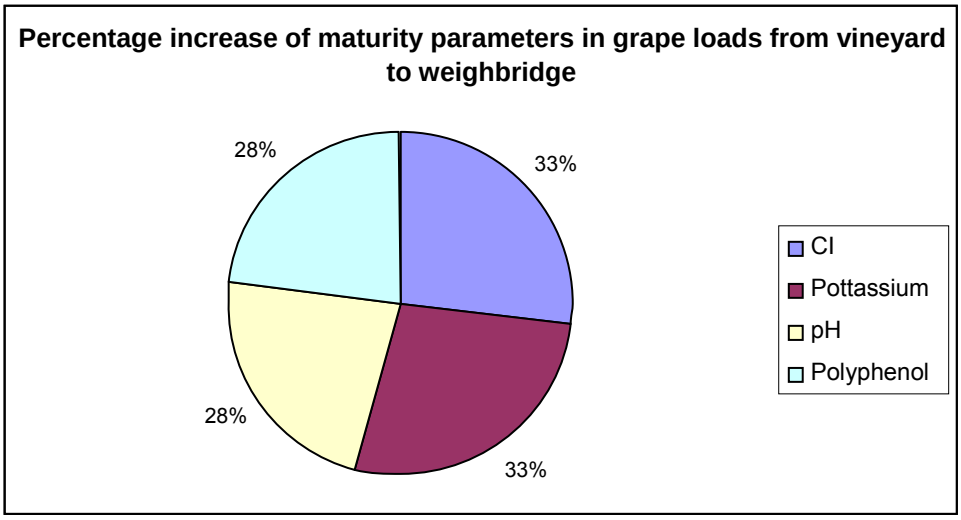


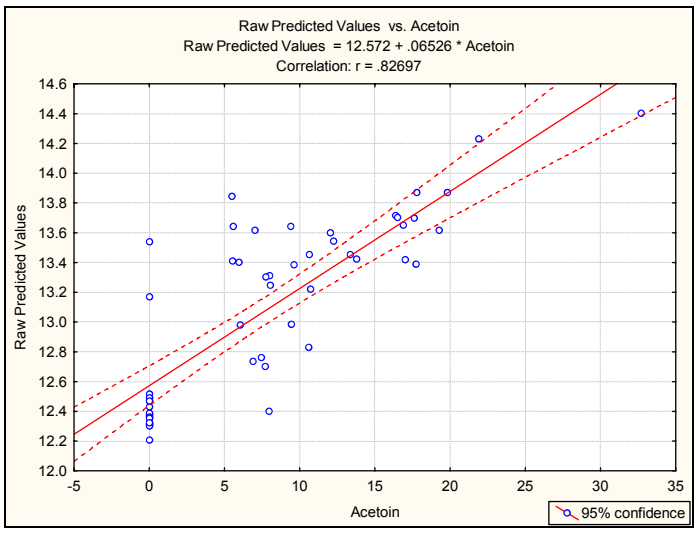
Figure 5.10. Percentage a) increase of each metabolite b) decrease of each maturity parameter and c) increase of each maturity parameter measured in grape must samples from 18 vineyards and at the weighbridge from each grape load.

The wine score were compared to esters, higher alcohols and volatile acids of the wine. Esters and higher alcohols contributed the most to the wine score ($p<0.05$). Acetoin, methanol and 2-phenyl ethanol were the best subset for the GRM (**Table 5.8**). The regression coefficient for the subset was 0.54. The individual regression coefficient for acetoin, methanol and 2-phenyl ethanol was 0.83, 0.72 and 0.38, respectively (**Fig. 5.11**). Acetoin and methanol are typically found in red wine. Acetoin are a secondary product formed during malolactic fermentation and methanol from fermentation on skins. Acetoin is also formed by yeast during fermentation of carbohydrates, it is then reduced to 2,3-butanediol. 2,3-Butanediol can affect wine quality because it is slightly bitter and wine body due to its viscosity (Romano, 1997). The total amounts of 2,3-butanediol and acetoin vary considerable as a function of the yeast species (Romano, 1997). *Kloeckera apiculata* and *C. stellata* produced the highest amounts of acetoin (Romano *et al.,* 2003). The higher alcohol, 2-phenyl ethanol, is an important sensorial compound that imparts rose characteristics and plays a role in the perception of wine fullness. It is often found in higher concentration in mixed culture yeast fermentations (Sponholz & Dittrich, 1974).

Table 5.8. Summary of best subset for X-variables. Mallow's CP and regression coefficient for each submodel.

	Mallow's CP	No. of effects	Total Esters	Total higher alcohols	Total volatile acids
1	2.621882	2	0.267955	0.278273	
2	4.000000	3	0.229656	0.225855	0.123452
3	4.357012	2	0.243269		0.232046
4	4.693313	2		0.241154	0.212616
5	4.790505	1		0.348789	
6	5.117755	1	0.341186		
7	5.391284	1			0.334698

a)



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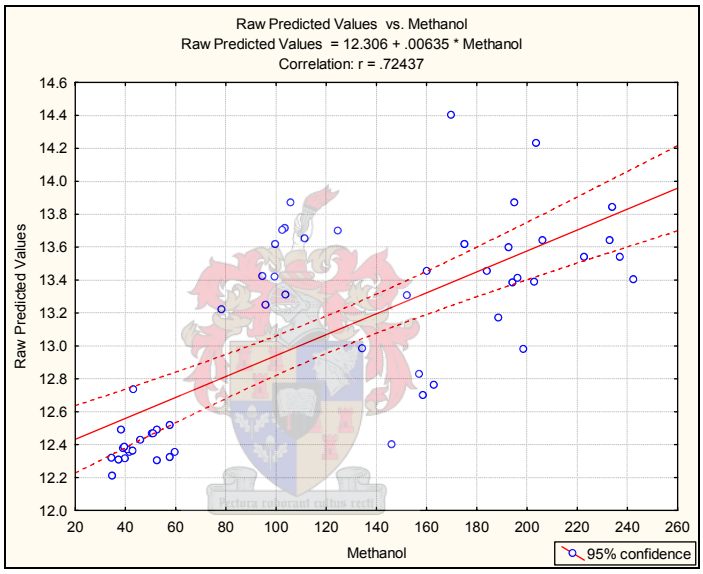

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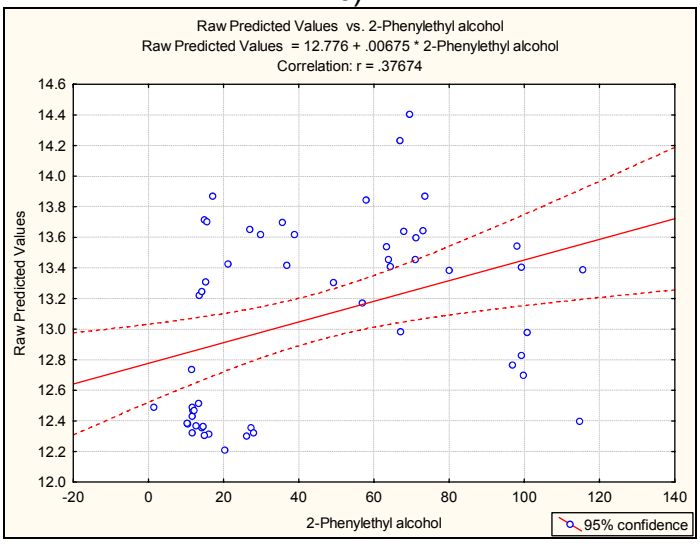

Figure 5.11. Wine score vs. a) Acetoin, b) Methanol and c) 2-phenyl ethanol were the best subset which fit the General Regression Model for the relation of wine score to higher alcohols and esters.

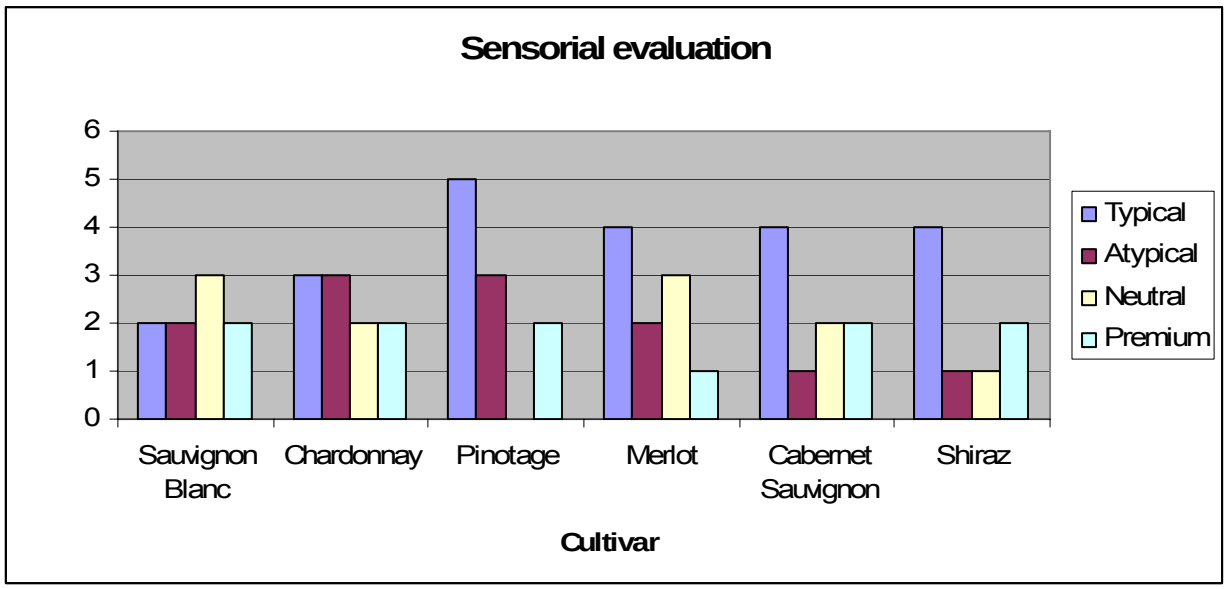

Figure 5.12. **C**lass descriptions of the sensorial analysis of the wines made from each selected A-grade vineyard.

Only a small percentage (25%) of the wines evaluated were classified as premium wine quality (**Fig. 5.12**) which do not correlate to the classification of the vineyards as premium. In the class description typical cultivar character, the red grape cultivars performed well. Sauvignon blanc and Chardonnay wines had the lowest wine score. The wines were either premium or neutral to atypical cultivar character. Taking the microbial metabolite increase of the grape loads in consideration, it is clear that it has an influence on the wine quality. Correlation between measured parameters and wine score validated this fact as stated earlier in the section. The wines from vineyards in the Lutzville area identified with high microbial population performed poor.

5.4 CONCLUSIONS

The metabolites measured, which are an indication of the sanitary state of grapes, are an indication of microbial activity although the microbial population did not differ significantly between the vineyard and weighbridge in most samples. Special attention needs to be given to vineyards in the Lutzville area, as this area had the highest yeast population and are furthest from the cellar. With the increase in temperature it was clear that the metabolites increased as well. In a warm area, like the Olifants River valley, Sauvignon blanc vineyards ripen early; this makes it prone to attack by birds and insects. Acetic acid bacteria were significantly present on this cultivar in comparison to the others. Metabolites had an influence on wine quality

such as ethyl acetate. pH was found to be the most important quality parameter for Sauvignon blanc and Chardonnay. Malic acid was an indicator of quality for red grapes. It is clear that quality control from vineyard to winery needs to be addressed in a quality protocol for harvest. Once it is known what adds cost to the value chain it can be monitored and managed.

5.5 LITERATURE CITED

Berg, H.W. & Ough, C.S., 1977. The relation of ºBalling to wine quality. Am. J. Enol. Vitic. 28, 235-238.

Clary, C.A., Steinhauer, R.E., Frisinger, J.E. & Peffer, T.E.,1990. Evaluation of Machine- *vs.* Hand-harvested Chardonnay. Am. J. Enol. Vitic. 41, 176-181.

Corison, C.A., Ough, C.S., Berg H.W. & Nelson, K.E., 1979. Must acetic acid and ethyl acetate as mold and rot indicators in grapes. Am. J. Enol. Vitic. 30, 130-134.

Couto, J.A., Graça, A.R., Soares-Franco J.M. & Hogg, T., 2003. The use of gluconic acid level as an indicator of the actvity of acetic aced bacteria in grapes. VII Symposium International d'Œnologie, Bordeaux.

Du Plessis, C.S., 1984. Optimum maturity and quality parameters in grapes: A Review. S. Afr. J. Enol. Vitic. 5, 35-42.

Edwards, C.G., Haag, K.M., Collins, M.D., 1998. Identification and characterization of two lactic acid bacteria associated with stuck/sluggish fermentation. Am. J. Enol. Vitic. 49, 445-448.

Fleet, G.H., 2002 (2nd ed.). Wine Microbiology and Biotechnology. Taylor & Francis, London.

Fleet, G.H., 2003. Yeast interactions and wine flavour. Int. J. Food Microbiol. 86, 11-23.

Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. Techniques for accurate chemical analyses and quality monitoring during winemaking. Wine Promotions, Campbell Town, Australia.

Jolly, N.P., Augustyn, O.P.H. & Pretorius, I.S., 2003. The occurrence of non-*Saccharomyces cerevisae* yeast species over three vintages in four vineyards and grape musts from four production regions of the Western Cape, South Africa. S. Afr. J. Enol. Vitic. 24, 35-42.

Johnstone, R., 1996. Specifying grape flavour. In: Hamilton, R. & Hayes, P. (eds). Proc. Seminar Aust. Soc. Vitic. Oenol., August 1996, Mildura, Victoria, 5-6.

Lonvaud-Funel, A., 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie van Leeuwenhoek 76, 317-331.

Marais, J., 2001. Effect of grape temperature and yeast strain in Sauvignon blanc wine quality. S. Afr. J. Enol. Vitic. 22, 47-51.

Ough, C.S., Berg, H.W., Coffelt, R.J. & Cooke, G.M., 1971. The effect on wine quality of simulated mechanical harvest and gondola transport of grapes. Am. J. Enol. Vitic. 22, 65-70.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B. and Lonvaud, A. (eds). 2000a. 1st Ed. Handbook of Enology. Volume 1. The Microbiology of Wine and Vinifications. John Wiley & Sons, Ltd., West Sussex, England.

Riley, L., 1996. Quality management in viticulture. In: Hamilton & R., Hayes, P. (eds). Proc. Seminar Aust. Soc. Vitic. Oenol., August 1996, Mildura, Victoria, 5-6.

Sponholz, W.R. & Dittrich, H.H., 1974. Die Bildung von SO_2–bindenden garungs-nebenprodukten, hoheren alkoholen und estern bein einigen reinzuchthefestammen und einigen fur die weinbereitung wichtigen “wilden” hefen. Wein Wiss. 29, 301-314.

Suárez, J.A., González, M.C., Callejo, M.J., Colomo, B. & González, A., 1994. Contribution to the study of varietal wines from Rioja and Navarra. I. Microbial growth trends during grape maturation. Bulletin de L'O.I.V. 67, 759-760.

Romano, P., 1997. Metabolic characterization of wine yeast strains during spontaneous and inoculated fermentation. Food Technol. Biotechnol. 35, 255-260.

Romano, P., Granchi, L., Caruso, M., Borra, G., Palla, G., Fiore, C., Ganucci, D., Caligiani, A. & Brandolini, V., 2003. The species-specific ratios of 2,3-butanediol and acetoin isomers as a tool to evaluate wine yeast performance. Int. J. Food Microbiol. 86, 163-168.

Zoecklein, B.M., Williams, J.M. & Duncan, S.E., 2000. Effetc of sour rot on the composition of white Riesling (*Vitis Vinifera L*.) grapes. Small Fruits Review 1, 63-77.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

6. GENERAL DISCUSSION AND CONCLUSIONS

WestCorp International, Vredendal, South Africa, is a “100 000 ton cellar”. This relative young company is today successfully competing in the global wine market. As wine export wine markets have expanded and become increasingly competitive, more emphasis has been placed the past three years in this industrial cellar on quality control.

The overall aim of this study was to obtain a broad overview of the status of the grape quality evaluation system at the cellar, with particular reference to the key factors from the vineyard to the weighbridge at the cellar, and which might have an influence on the quality of the wine produced at this cellar.

Quality control in the value chain of wine production involves many processes. A quality management system for an industrial cellar should include sound vineyard practices, a representative grape sampling plan, objective harvesting criteria, specifications for grape quality, good production practices in the cellar and at the bottling plant, and criteria for effective logistics during grape intake, as well as distribution of the final product. At the industrial cellar under question, grape growers are paid according to grape quality. To date, grape quality is measured by °Brix, pH and titratable acidity (TA), as by most cellars world-wide. Visual inspection of grapes in the vineyards and grape loads at the weighbridge is done to evaluate the health state of the grapes, but this system has already been proven not reliable by Baumgarten *et al.,* in 1987. Since then the search for rapid and reliable analytical methods has received tremendous attention.

One of the techniques with great potential as a rapid quality control tool is Fourier transform infrared (FT-IR) spectroscopy. World-wide this analytical technique has successfully established itself in wine analytical laboratories and the technology is also in widespread use in South Africa. One of the advantages of the technology is the simultaneous quantification of chemical components, usually requiring minimal sample preparation. In recent years a substantial number of publications have been published on applications of the technology (Gishen & Holdstock, 2000; Kupina & Shrikhande, 2003; Patz *et al*., 2004). The FT-IR commercial spectrometers designed specifically for grape and wine analysis, like the Winescan FT 120 instrument, is sold with global ready to use calibration products, but these calibrations must first be validated within a

specific environment to ensure that all variation in the local samples is included in the design of calibration models.

A specific aim of this study was to ensure that the global calibrations for °Brix, pH and TA, which is crucial at grape intake for determining grape quality and on which the winemaker makes decisions regarding handling of the grape must in the cellar, were validated. Results obtained showed that the global calibration for °Brix is of excellent prediction accuracy, but newly designed calibrations using grape samples from the Vredendal area, for the quantification of pH and TA were better suited for the local conditions experienced in the Vredendal area where high pH and low TA levels in must are realities. Samples from the 2006 harvest season will be used for further optimisation and validation of these calibrations. These calibration sets will also be made available to the broader wine industry and users of the FT-IR technology. In this way the preliminary work carried out at the cellar in question can make an important contribution to the analytical arsenal of wine laboratories in South Africa.

Sample preparation forms an integral part of analytical methods and can have a large influence on results. For quality control purposes, sample preparation in the laboratory must reflect the procedures of grape processing in the cellar. The second goal of this project was to evaluate the feasibility of using a one-step sample preparation method for grapes. The two methods that were compared were pressing the samples manually by hand to extract the juice and homogenization of whole berries. Results showed that FT-IR analysis (using the Winescan instrument) of manually pressed grape samples yielded results that correlated well to the results obtained by cellar processing of grapes. For some parameters such as colour, processing of grape samples by homogenization is preferable.

The third objective of this study was to identify key factors negatively affecting the value chain of wine production at this industrial cellar. For this purpose selected vineyards graded according to viticultural practices as premium wine-producing vineyards, were monitored from vineyard to weighbridge with respect to grape chemical composition, microbial populations on the grapes, grape load differences and wine quality achieved. This study showed that transport of grapes from the vineyard to the receiving cellar is one of the most crucial key factors that need to be addressed in the current quality control chain. Grapes need to be delivered earlier in the day to the cellar so

that grape load temperatures remain below 25 - 30ºC. The temperature of the grape loads delivered to the weighbridge was higher than reported by previous research (Ough *et al.,* 1971; Marais, 2000; Iland, 2000). The vineyards in the Lutzville area, South Africa need attention with regard to transport time and grape temperature at delivery to the weighbridge. The vineyards of this area had higher yeast populations than the other vineyards and uncontrolled, premature fermentation occurred that affected the chemical composition of the grape must. Results showed that Sauvignon blanc vineyards had high acetic acid bacteria activity, which is not surprising as most of these vineyards are situated very close to the Olifants River, which makes it prone to bird attack (birds nest in the reeds on the banks of the river). It was also found that pH is crucial for wine quality for Sauvignon blanc and Chardonnay. In red grapes, the malic acid content was correlated strongly wine quality. As Sauvignon blanc wine is part of the top-end of the wine market, a the frequent shortage of high-quality grapes supply justify investigating bird netting of these vineyards. Due to the presence of acetic acid bacteria on this cultivar, acetate and its ester ethyl acetate also had a negative influence on the resulting wine quality. Evaluating the chemical parameters and microbial metabolites from vineyard to weighbridge, fluctuations (increases and decreases) were seen for some of these parameters and therefore it was difficult to statistically correlate microbial populations present on the grapes with concentrations of metabolites in the must. Consequently, the wine quality was of a lower standard than what was expected or predicted according to viticultural practices. It is clear that the grapes in the vineyard do not always have the same chemical profile when delivered to the cellar. It is important that before optimising viticultural practices for better wine quality, quality control of transport of grapes to the cellar needs to be address seriously.

Further, vineyard quality control systems, needs to be develop in the context of the final product. Definition of the appropriate characteristics of a vineyard at each stage of the growing season is needed, measurement techniques must be developed or refined, and an integrated approach to quality control established. Quality control systems monitor and recommend adjustment in management techniques in response to specific objectives and conditions. With the appropriate systems, wine and grape juice quality could be improved and consistently predictable high quality products produced.

6.1 LITERATURE CITED

Baumgarten, G.F., Burger, M. & Kirschbaum, H., 1987. The evaluation of wine grapes for mould defects under South African conditions using high pressure liquid chromatography. Proc. 9th S.A. Association for Food Science and Technology, Sept. 1987, Cape Town, S.A. In: Food Review Feb-March 1988, pp 14-15.

Gishen, M. & Holdstock, M., 2000. Preliminary evaluation of the performance of the Foss WineScan FT 120 instrument for the simultaneous determination of several wine analyses. *Aust. Grapegrower Winemaker Ann. Tech. Issue.* pp 75-81.

Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. Techniques for accurate chemical analyses and quality monitoring during winemaking. Wine Promotions, Campbell Town, Australia.

Kupina, S.A. & Shrikhande, A.J., 2003. Evaluation of a Fourier transform infrared instrument for rapid quality-control wine analyses. *Am. J. Enol. Vitic*. **54**, 131-134.

Marais, J., 2001. Effect of temperature and yeast strain in Sauvignon Blanc wine quality. S. Afr. J. Enol. Vitic. 22, 47-51.

Ough, C.S. & Berg, H.W., 1971. Simulated mechanical harvest and gondola transport. II. Effect of temperature, atmosphere, and skin contact on chemical and sensory qualities of white wines. Proc. 22nd American Society of Enologist, annual meeting, June 1971, California. pp. 24-26.

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