The Colour and Phenolic content of Robertson Red Grape Cultivars: Distribution, correlation with wines and analyses

by

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Declaration

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Summary

South African red wine is often acknowledged world wide as being full bodied and deep in colour. This is often the result of high temperatures that is experienced during the important growth stages of grapes especially post *véraison*. In the Robertson area in South Africa however, temperatures often exceeds the range for optimal anthocyanin development during these growth stages. The distinction between grapes being technologically ripe and being ripe on a phenolic level is also accepted as an important determining factor for the perfect time to pick grapes. In co-operative wineries such as Robertson Winery (RW) where grapes are delivered from a large area and different producers, it is difficult to individualise grape blocks when it comes to ripeness level in terms of sugar or phenolic ripeness. In most circumstances a generalised set of parameters for deeming grapes ripe or acceptable for delivery is the best substitute. The levels of these parameters are based on research literature that is available for the area as well as data collected through years of maintaining the vineyards of that area. The grape parameters that are currently being used by RW for ripeness and quality are pH, titratable acidity (TA) and sugar level. In recent years RW in conjunction with the Department of Viticulture and Oenology, Stellenbosch University, decided to investigate more parameters to determine the quality of grapes at the time of harvest. Most importantly for the grape growers this quality is connected to a price point and therefore compensation. Two important quality parameters of red wine are the red colour and mouth feel of wine. Anthocyanin and tannins are respectively connected to these two quality attributes and are both widely accepted as quality indicators. Wine with high anthocyanin and tannin content often originates from grapes with a high colour and phenolic profile. The existence of a correlation between grape and wine anthocyanin and tannin content is therefore the basis of attempting to use these parameters in the grape to predict end wine's colour and phenolic quantity. Determination of anthocyanin and tannin content of grapes has already become part of some private owned wineries' standard set of determinations. However, sample preparations, extractions and consumables needed are all factors that need to be reduced to make the measurement and therefore the use of these parameters more viable in a co-operative cellar laboratory, where large volumes of grapes are received during harvest.

The first objective of this work was to determine the levels of anthocyanin and tannin in red grapes from different vineyard blocks from the producers of RW from three successive vintages. This would give insight as to what can be seen as a low and high anthocyanin and tannin content for grapes received at the cellar. For this purpose, blocks of the most important red wine cultivars for RW was selected and analysed for these compounds. The ranges and average levels of anthocyanin and tannin content were determined using measurement techniques that could be used by any winery. The average mono flavanol and total colour level of the grapes were found to be lower than those often reported in literature, with total grape

flavanols being higher. However, a wide range of values for these compounds were found that correlated with those found in other studies. The possible reasons for differences in levels of occurrence of these compounds were discussed and mostly pertain to differences in cultivar, micro climatic and season.

The second objective was to determine the correlation between levels of colour and phenolic compounds in grapes and their corresponding wines. Such correlations will form the foundation for the use of phenolic content to predict the colour and phenolic potential of the wine and possibly wine quality as well. When the grape and wine colour and phenolic data were correlated for all seasons and cultivars inclusive it was found that grape and wine colour showed better correlations than for instance total phenols and tannins. This was especially true for total colour pigments in red grapes, measured with HPLC, when correlated with certain spectrophotometric analysis of wine colour. Cultivar and season as well as the synergism between the two were further investigated for its role in affecting correlations. When these relationships were further differentiated by season and by cultivar the resulting correlations varied. This work contributed a great deal of information to support the use of grape colour and phenolic compounds for the prediction of end wine colour and phenolic composition.

The third objective was to investigate near infrared spectroscopy (FT-NIR) as a viable option to rapidly measured anthocyanins, tannins and total phenolics in red grapes. If proven successfully, this could be employed by a large cellar such as RW. FT-NIR has been used with success on grape extracts and in this instance the focus was to establish a calibration on the grape homogenate itself. Preliminary results showed that FT-NIR could be applied for the use of determination of anthocyanin and tannin levels in red grapes originating from RW. The prediction of total phenols was not found to be as accurate, but this could also be due to the reference method that was used.

This work brought some interesting, practical information not only of importance for RW, but all wineries that are concerned with improving the basis on which grape quality is determined. The use of aerial data mapping for indicating areas regarding important grape colour and phenolic parameters was used in this study and is a very visual way of showing the distribution of certain ripeness parameters over a large area. Correlations between the grape and wines of such a large amount of red grape blocks for a specific area have not also been reported in South Africa before. The use of FT-NIR to determine anthocyanins and tannin concentrations in grape homogenates is also novel for its use in South African wineries. This work may assist grape and wine producers as well as analysts on the phenolic and colour profile of grapes and wines from RW.

Opsomming

Suid-Afrikaanse rooiwyn word wêreld-wyd geken aan ‘n dieprooi kleur en vol struktuur. Die grootste rede vir hierdie verskynsel is hoë temperature wat ervaar word tydens rypwording en veral na *véraison*. In die Robertson wynstreek is temperature egter tydens rypwording dikwels vêr bo dit was as optimaal vir antosianien ontwikkeling beskou word. Die gepaste tyd om druiwe te pluk word nie net gedryf deur die tegnologiese rypheidsvlak nie, maar ook deur fenoliese rypheid. In ‘n koöperatiewe kelder omgewing soos Robertson Wynkelder (RW) word ‘n hoë lading druiwe elke dag ontvant vanaf verskillende produsente oor ‘n breë streek. Dit maak dit moeilik om te bepaal watter druiwe werklik beide tegnologies en fenolies ryp is. Die beste manier om hiervoor te vergoed is om ‘n standaard te stel vir ‘n reeks voorafbepaalde parameters. Die vlakke van die gekose parameters is, word bepaal deur navorsinguitsette sowel as die geskiedkunde data wat ingesamel is vanaf elkeen van die bepaalde blokke. Die parameters wat tans in gebruik is by RW om oesdatum en kwaliteit by inname te bepaal is pH, titreerbare suur (TA) en suiker vlak. Die tekortkoming hier is dat kwaliteit van druiwe beswaarlik met slegs hierdie informasie kan bepaal word, maar dat dit die betaling van die produsent by aflewering wesenlik kan beïnvloed. Dit het RW genoop om in samewerking met die Departement van Wingerd en Wynkunde, Universiteit van Stellenbosch nog parameters te ondersoek wat hierdie rypheid- en kwaliteitsbepaling by inname sou kon versterk. Twee belangrike faktore wat kwaliteit van rooiwyn bepaal is die kleur en struktuur. Antosianiene en tanniene is onderskeidelik verantwoordelik vir hierdie kwaliteits eienskappe van wyn. Wyn wat bestempel word as hoog in kleur en tannien inhoud word dikwels verbind met druiwe wat hoog is in hierdie faktore. Die moontlike korrelasie tussen die antosianien en tannien inhoud van druiwe en die wyn wat daarvan berei word is dus die basis waarop die potensiële toepassing van hierdie parameters berus. Die bepaling van antosianien en tannien vlakke word reeds in sommige laboratoriums gedoen. Die monster voorbereidings tyd, ekstraksies, toerusting en verbruikbare items nodig om hierdie tipe analieses te doen is egter hoog. Die analiese van hierdie komponente is meer lewensvatbaar in groot laboratoriums (soos in ‘n koöperatiewe kelder) waar groter volume druiwe ingeneem word gedurende parstyd.

Die eerste doelwit van hierdie studie was om te bepaal teen watter vlakke antosianiene en tanniene in druiwe voorkom, spesifiek van die Robertson area. Die het behels ‘n wye verskeidenheid van blokke, verspreid oor die hele streek wat oor 3 seisoene gemonitor is in terme van veral kleur en tanniene maar ook ander belangrike parameters. Die idee hier is om insig te kry rakende watter vlakke bestempel kan word as laag en hoog in terme van antosianien en tanniene vir die Robertson streek. Daarvoor is slegs die mees aangeplantste rooi kultivars gebruik. Die verspreiding en gemiddelde vlakke waarteen antosianien en tanniene voorkom was bepaal deur gebruik te maak van metodes wat as relatief algemeen in laboratoria gebruik word. Die gemiddelde mono-flavonoïed en totale kleur pigment inhoud van die druiwe

was laer as van die vlakke wat in die literatuur beskikbaar is, met totale flavanole wat hoër was. Die wyer verspreiding van die waardes het egter beter gekorreleer met die waardes soos beskryf in die literatuur. Die moontlike redes vir die verskillende vlakke word in die studie bespreek en word waarskynlik bepaal deur verskille in kultivar, mikro-klimaat en seisoen.

Die tweede doelwit was om te bepaal of daar ‘n korrelasie te vinde is tussen die kleur en tannien inhoud van die druiwe en ooreenstemmende wyne. Sulke tipe korrelasies sal die basis vorm om antosianien en tannien inhoud van wyn reeds in die druiwe fase te kan voorspel. Nadat die ingesamelde druif en wyn data as ‘n geheel beskou was, was dit sigbaar dat die wynkleur parameters beter korrelasies bied as meeste tannien en totale fenole. Dit was veral waar in die geval van totale kleur pigmente soos gemeet met die HPLC teenoor die wynkleur parameters gemeet met spektrofotometriese metodes. Verdere ondersoeke in terme van die impak wat die kultivar en seisoenale kan hê het tot variërende korrelasies gelei.. Hierdie werk het ‘n groot bydrae gelewer om voorspellings van wyn kleur en fenoliese inhoud reeds met sukses vanaf die druif te bepaal.

Derdens het die werk fourier transformasie naby infrarooi skandering (FT-NIR) ondersoek as ‘n lewensvatbare metode vir die bepaling van antosianien, tannien en totale fenoliese inhoud van druiwe en wyn. FT-NIR word reeds oor ‘n wye reeks wyne en druiwe ekstraksiemonsters toegepas en die doelwit hier was om druiwe homogenaat as matriks te kalibreer. Voorlopige resultate het bevind dat antosianien en tannien vlakke in druiwe van RW gemeet kan word met die FT-NIR, maar dat die kalibrasie vir totale fenole nog verbeter kan word.

Hierdie werk het ‘n wye reeks interessante en prakties bruikbare informasie na vore gebring wat van onskatbare belang is vir RW en ander kelders wat besorgd is oor die verbetering van algemene druifkwaliteit. Geografiese kaarte wat belangrike druifkleur en fenoliese parameters aandui is in hierdie studie gebruik en wys hoe data visueel voorgestel kan word om die geheelindruk van gekose parameters oor ‘n groot area te vergelyk. Korrelasies tussen druiwe en wyn van so ‘n groot hoeveelheid druiwe blokke is nog nooit voorheen in Suid-Afrika getoon nie. Dieselfde geld vir die gebruik van FT-NIR vir die meet van kleur en fenoliese parameters in druiwe homogenate. Hierdie werk kan druiwe- en wynproduseerders sowel as analiste assisteer in terme van die kleur en fenoliese profiel van druiwe en wyn van RW.

This dissertation is dedicated to my husband
Renier van der Merwe
and children
Rehan and Ilne van der Merwe
Thank you for teaching me patience, perseverance and loving me unconditionally.

Biographical sketch

Hanneli van der Merwe (neé Walters) was born in De Aar, South Africa. She was schooled at Ladismith High School in the little Karoo. She started her BScAgric (Viticulture and Oenology) in 2000 and followed that with an MScAgric (Oenology) starting in 2005. This PhD (Agric) in Oenology was started in 2007. Since July 2010 Hanneli has been employed by the Cork Supply Group as Technical Manager in South Africa.

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Wine is sunlight,

held together by water.

Galileo Galilei

Preface

This dissertation is presented as compilation of 6 chapters. Each chapter is introduced separately and is written according to the style of the South African Journal of Enology and Viticulture. Chapter 3 was submitted and accepted for publication..

Chapter 1 **General Introduction and project aims**

Chapter 2 **Literature review**
Red grapes and wines: Anthocyanin and tannin content for the use in quality prediction

Chapter 3 **Research results**
Comprehensive survey of the distribution of colour and phenolics of different red grape vineyard blocks from the Robertson area in South Africa

Chapter 4 **Research results**
Correlations between grapes and wines from the Robertson area of South Africa: Colour, phenolic content and sensorial contribution

Chapter 5 **Research results**
Rapid measurement of anthocyanin, total phenolic and tannin content in red grape homogenates using near-infrared spectroscopy and chemometric methods

Chapter 6 **General discussion and conclusions**

Table of Contents

Chapter 1

General Introduction and Project aims

1. General Introduction and Project Aims

1.1 Introduction

Robertson Winery (RW) is an important role player in the South African wine industry. This cellar produces various wines styles from a wide range of cultivars under different marketable labels. It is one of largest wine producers in South Africa and therefore also significantly contributes to the exporting sector. RW is constantly searching for novel ideas to improve their winemaking techniques and the production efficiency of their cellar in general.

Recently there has been an increasing demand for quality red wines in South Africa as well as for exporting purposes. This in turn enhanced the need for high quality red grapes. The Robertson area is home to various red cultivars of which Shiraz, Cabernet, Merlot and Pinotage are the most important. At co-operative wineries such as RW grapes are bought from adjoining farms in the area and payment for these grapes may often seem biased. Chemical parameters as well as the physical condition of the grapes and viticultural practices performed during the annual maintenance of vineyard blocks are used for quality determination. The traditional chemical parameters used for depicting grape quality were pH, titratable acidity (TA) and degrees Balling (ºB), but whether these are sufficient to determine grape quality is debatable. This prompted the need to investigate various avenues regarding the use of colour and phenolic composition of Robertson red grapes as an additional tool in predicting the potential colour, phenolic composition and possibly quality of the resulting wines.

Phenolic compounds have been found to be critically important to the quality of all wines (Peynaud, 1996). It is responsible for colour of wine, important for mouth feel, influences wine aroma and ageing ability of wines and therefore influences wine quality in general (Somers & Evans, 1974; Singleton, 1987; Du Toit *et al*., 2006; Rossi & Singleton, 1966; Robichaud & Noble, 1990).

Phenolic compounds in both white and red grapes can be divided into non-flavonoid phenols (which are present at the same levels in red and white wines, but are more important to white wines) and flavonoids. These flavonoids are normally present in much higher levels in red wines than in white wines. In a young wine, they are normally in a more un-polymerised state, but as wine matures they undergo polymerisation reactions. The most important flavonoids in wine are the anthocyanins and tannins (consisting of flavan-3-ols; flavan-3,4-dioles). Anthocyanins influence mainly the colour and flavanols the taste of red wine (Monagas, *et al*., 2005). These compounds are produced in grapes and therefore the level

and composition thereof contributes directly to the phenolic composition of its corresponding wine (Du Toit & Visagie, 2012).

The amounts to which these phenolic compounds are present in grapes are dependent on various factors of which viticultural practices and environmental impacts (Downey, *et al*., 2005) are some of the most important. During grape ripening the anthocyanins and tannins develop at different stages. The seed tannins were found to be at its maximum at véraison and slightly decreased towards ripeness, while the skin tannins increase from véraison to ripeness (Ribèreau-Gayon & Glories, 1986). Anthocyanins only start to accumulate from véraison and reach a maximum at full ripeness.

When grapes are used during vinification the phenolic compounds are extracted from the berries. Inherent extractability as well as various winemaking techniques influences the amount and type of phenolic compounds that will be extracted. Cultivar, fermentation temperatures, the addition of SO_2 and pectolytic enzymes and skin maceration time may all influence the extraction of phenolics (Romero-Cascales *et al*., 2005; Sacchi *et al*., 2005).

After the extraction of the phenolic compounds into the wine various reactions can occur that will constantly change the structures of the phenolic compounds and the effect it has on colour, mouth feel, taste and the aroma of wine. The main reactions will be polymerization and precipitation (Ribèrau-Gayon *et al*., 2001).

For the use of phenolic content as additional tools to predict wine quality from a specific vineyard block of a producer such as RW, there should be some critical information available. Firstly the contents of these phenolic compounds in the grapes and wine of such a producer should be known. These measurements will reveal an array of important information regarding grape and wine constitution.. The next important aspect is to determine the correlation between grape and wine phenolic composition for this producer to determine whether trends observed in the grapes are also seen in the wines. Various authors have found different levels of correlations between grapes and wines (Iland, 1987; Marais *et al*., 2001; Gonzalez-Neves *et al*., 2004; Marais & October, 2005; Romero-Cascales *et al*., 2005; Jensen *et al*., 2008; Cagnasso *et al*., 2008; Du Toit & Visagie, 2012). The existence of positive correlations will support the application of colour and tannin analyses in grapes.

The methods of colour and phenolic analyses should be fast and easy to apply in practical winery conditions. Some of the latest technologies available for such analyses are Fourier Transform near- and mid-infrared spectroscopy (FT-NIR and FT-MIR). Recent articles indicated that the possibility to use these methods for determination of phenolic compounds does exist, but should be further explored (Cozzolino *et al*., 2004; Cozzolino *et al*., 2008; Fragosa *et al*., 2011). In those cases where FT-NIR or FT-MIR technology is not available other methods could also be used.

1.2 Project aims

The main aim of this research was to obtain extensive data regarding the phenolic constitution of four red grape cultivars and their corresponding wines from RW over three successive vintages. This work was thus a full circle investigation from the vineyard through to important winemaking stages in terms of colour and phenolic analyses. Samples were taken at pivotal stages such as at grape harvest, post-alcoholic fermentation and post-malolactic fermentation. This data forms a foundation for using phenolic compounds as an additional grading tool for grapes already at intake. There should be a relationship between the levels of phenolic compounds between grapes and the wines. If this is the case the potential use of this type of data is viable for predicting the wine phenolic content from the grapes. Moreover, rapid and accurate measurement of the phenolic compounds in grapes is therefor is also important. This project formed an integral part in the on-going efforts of RW to produce red wine of better quality.

The specific aims of this study were:

- Evaluate the colour and phenolic composition of selected vineyard blocks from RW;
- Establish if certain tendencies observed in these blocks are also reflected in the corresponding wines;
- Development of a rapid anthocyanin and tannins analyses method for grapes using FT-NIR spectroscopy

1.3 References

Cagnasso, E., Rolle, L., Caudana, A. & Gerbi, V., 2008. Relationship between grape phenolic maturity and red wine phenolic composition. It. J. Food Sci. 20(3):365-380.

Cozzolino, D., Kwiatkowski, M.J., Parker, M., Cynkar, W.U., Dambergs, R.G., Gishen, M. & Herderich, M.J., 2004. Prediction of phenolic compounds in red wine fermentations by visible and near infrared spectroscopy. Anal. Chim. Acta. 513: 73-80.

Cozzolino, D., Cynkar, W.U., Dambergs, R.G., Mercurio, M.D. & Smith, P.A., 2008. Measurement of condensed tannins and dry matter in red grape homogenates using near infrared spectroscopy and partial least squares. J. Agric. Food Chem. 56: 7631-7636.

Du Toit, W.J. & Visagie, M., 2012. Correlations between South African red grape and wine colour and phenolic composition: Comparing the Glories, Iland and Bovine Serum Albumin Tannin Precipitation Methods. S. Afr. J. Enol. Vitic. 33: 33-41.

Du Toit, W.J., Lisjak, K., Marais, J. & du Toit, M., 2006. The effect of micro-oxygenation on the phenolic composition, quality and microbial composition of South African red wines. S. Afr. J. Enol. Vitic. 27: 57-67.

Downey, M.O., Dokoozlian, N.K., & Kristic, M.P., 2005. Cultural practice and environmental impacts on the flavonoid composition of grapes and wines: A Review of Recent Research. Am. J. Enol. Vitic. 57: 257-268.

Fragoso, S., Aceña, L., Guasch, J., Busto, O. & Mestres, M., 2011. Application of FT-MIR spectroscopy for fast control of red grape phenolic ripening. J. Agric. Food Chem. 59: 2175-2183.

Gonzalez-Neves, G., Charamelo, D., Balado, J., Barreiro, L., Bochicchio, R., Gatto, G., Gil, G., Tessore, A., Carbonneau, A., & Moutounet, M., 2004. Phenolic potential of Tannat, Cabernet Sauvignon and Merlot grapes and their correspondence with wine composition. Anal. Chim. Acta. 513: 191.

Iland, P., 1987. Predicting red wine colour from grape analysis. The Australian Grape grower and Winemaker, 285: 29.

Jensen, J.S., Werge, H.H.M., Egebo, M. & Meyer, A.S., 2008. Effect of wine dilution on the reliability of tannin analysis by protein precipitation. Am. J. Enol. Vitic. 59(1): 103-105.

Marais, J., Iland, P. & Swart, E., 2001. Exploring the relationships between Pinotage grape colour and wine quality–preliminary results. Wynboer, 2001. http://www.wineland.co.za/wynboer_archive/recentarticles/0201explore.html

Marais, J. & October, F., 2005. Relationship between grape colour and wine quality. Wynboer 191: 15-17. http://www.wineland.co.za/wynboer_archive/recentarticles/200507colour.php3

Monagas, M., Bartolome, B. & Gomez-Cordoves, C., 2005. Updated knowledge about the presence of phenolic compounds in wine. Crit. Rev. Food Sci. Nutr. 45: 85-118.

Peynaud, E., 1996. The Taste of Wine. The Art and Science of Wine Appreciation. 2nd ed. Wiley & Sons, New York.

Ribèreau-Gayon, P. & Glories, Y., 1986. Phenolics in grapes and wines. In: Proceedings of the 6th Australian Wine Industry Technical Conference. Ed. T. Lee (Australian Wine Industry Technical Conference Inc.: Adelaide). pp. 247–256.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. & Dubourdieu, D., 2001. Handbook of Enology, Volume 2, The Chemistry of Wine stabilization and Treatments, John Wiley & Sons, LTD.

Robichaud, J.L. & Noble, A.C., 1990. Astringency and bitterness of selected phenolics in wine. J. Sci. Food & Agric. 53: 343-353.

Romero-Cascales, I., Ortega-Regules, A, López-Roca, J.M., Fernández-Fernández, J.I. & Gómez-Plaza, E., 2005. Differences in anthocyanin extractability from grapes to wines according to variety. Am. J. Enol. Vitic. 56(3): 212-219.

Rossi, J.A., Jr. & Singleton, V.L., 1966. Flavor effects and adsorptive properties of purified fractions of grape seed phenols. Am. J. Enol. Vitic. 17: 240-246.

Sacchi, K.L., Bisson, L.F. & Douglas O.A., 2005. A Review of the effect of winemaking techniques on phenolic extraction in red wines. Am. J. Enol. Vitic. 56: 3.

Singleton, V.L., 1987. Oxygen with phenols and related reactions in musts, wine and model systems: Observations and practical implication. Am. J. Enol. Vitic. 38: 69-77.

Somers, T.C. & Evans, M.E., 1974. Wine Quality: Correlation with colour density and anthocyanin equilibria in a group of young red wines. J. Sci. Food Agric. 25: 1369-1379.

Chapter 2

Literature review

Red Grapes and Wines: Anthocyanin and Phenolic content for the use in quality prediction

2. Literature Review: Red Grapes and wines: Colour and Tannin content for the use of quality prediction

2.1 Introduction

The selection of high quality grapes is the first critical control point in the process of winemaking. Parameters traditionally used for the determination of optimally ripe grapes were total soluble solids, pH and TA. Recently colour and phenolic compounds have been evaluated by various authors as possible components to add diversity and value to the traditional data set. This would especially be beneficial in a cooperative cellar where grape growers are also remunerated on account of the chemical composition of the grapes at harvest. Colour and tannin concentrations were not actively measured in the past in vineyards for quality determination of grapes due to its time consuming and expensive nature. However, recent advances in infrared technology are making it possible to rapidly determine colour and tannins in grape homogenate. The biggest problem facing grape research is the amount of factors that influences the vineyard and therefore development of the grapes. Internal and external factors should be managed and evaluated to ensure high quality grapes. Internal factors refer to the various metabolisms involved in the development of the different components in the vine. External factors include most importantly climate, soils, trellis systems, site specifics, water status, cultivar/clone and viticultural practices. After grapes enter the cellar colour and tannins are extracted during winemaking and is again the subject of change and influenced by many factors.

This literature review aims to address the subjects of grape colour and phenols and its relationship to the wines made thereof. Firstly it shows the importance of truly mature grapes and what should be taken into account in this regard; secondly it explains the most important colour and phenolic compounds present in grapes and wines and lastly it also addresses the most popular methods employed for the determination of colour and phenolic compounds in grapes and wine.

2.2 Optimum Grape Maturity and Quality of Wine

In Oenology it is important to determine factors that could potentially influence the quality of the wines that will be produced. Grapes are the corner stone for this process and therefore emphasis falls on determining the point at which grapes are at their best to be harvested and used to produce the highest quality wine. Optimum grape maturity has been studied extensively and has been the subject of many scientific publications (Amerine & Winkler,

1941; Berg, 1958; Reutlinger, 1973; Kourakou, 1974; Sinton *et al*., 1978; Coombe *et al*., 1980).

The maturity stage of grapes can have a distinct effect on wine quality (Amerine & Roessler, 1958; Ough & Singleton, 1968; Ough & Alley, 1970; Du Plessis, 1975; Bisson, 2001, Kontoudakis *et al*., 2010) and the importance of work on this subject is still currently relevant. Grape maturity studies initially started out with the determination of the sugar content of the pulp/grape juice. The sugar level mostly determines the amount of alcohol and residual sugar in the resulting wine and was therefore (apart from cultivar/region) the deciding factor with regards to wine style. Sugar alone is not seen as adequate to describe optimum maturity and parameters such as acid concentration and pH should be included to make conclusion on quality. These 3 measurements were used in various combinations of which sugar/acid and Sugar X pH ratios have been used rather extensively. Phenolic maturity is also of the utmost importance when making the decision to harvest grapes. This influences the visual and mouth feel properties of a wine drastically and plays an important role in the overall quality perception of a wine.

Today the aim has shifted to add colour and phenolics as a quality attribute for the determination of the optimum point to harvest red grapes. Fair remuneration systems combining annual cultivation practices in the vineyard, yield, chemical and phenolic content of the grapes is essential for especially cooperative wineries. Methods to determine these compounds should be easy to execute, yield rapid results and must be linked to the quality of wine.

2.2.1 Optimum ripeness of grapes

The first parameters of interest for measuring grape maturity were sugar concentrations, acid concentrations and pH.

The earliest work on this subject dates back to 1941 when Amerine and Winkler looked at total dissolved solids (TSS), total acidity (TA), pH and ratios together with the site of the grapes to determine which cultivars should be used for table or dessert wines. There were 3 classes for cultivars A) having sugar/acid ratios of < 28.6, 31.4 and 34.3 at 20ºB, 22ºB and 24ºB; B) having sugar/acid ratios exceeding the abovementioned ones and C) cultivars which at 20ºB and 22ºB have < than these given above but at 24ºB an increased ratio than the abovementioned one. Depending on the group in which the grapes were classed, it dictated whether table or dessert wines would have should be produced from these grapes. They continued in 1944 by applying the sugar/acid ratio to classify American grape cultivars (Amerine & Winkler, 1944). Berg stated in 1958 that Balling ‘is particularly useless as a measure of the quality that the grape is capable of obtaining’. Balling/acid ratio should rather be used to determine the optimum time of picking (Tudosie *et al*. 1972, Fazinic *et al*., 1976;

Flora & Lane, 1979; Freeman *et al*., 1976; Du Plessis, 1977; Coombe *et al*., 1980; Failla *et al*., 2005). In 1960 Berg used sugar/acid data to classify 52 wines according to quality. In the end this system was more secondary to quality prediction and rather used to determine the amount of crop reduction that should be implemented to obtain a specific sugar/acid ratio. He continued his work in the 70's when he decided that more information was needed regarding the relationship of must composition to wine quality because of '1) the increasing failure to mature grapes properly; 2) the greatly increased production of table wines; and 3) the ever-increasing emphasis on quality by wineries'. Today, these points are still extremely relevant and are prompting new studies all around the winemaking world. In the abovementioned study white and red wines from five different regions in the state of California (USA) consisting of 12 prominent cultivars were used. The sugar (°B) data of the grapes were tabulated and compared with wine quality scores. The resulting data were used to compile sugar ripeness ranges for optimum wine quality for a number of table wine varieties. It seemed that higher sugar level ranges (20-25°B) were preferred and a significant difference was found between at least two of the five regions between the wines made of grapes of minimum and maximum sugar levels. They also stated that the sugar/acid ratio should rather be used (Berg's system; Berg, 1960). Furthermore and very importantly for South Africa it was pointed out that in warmer regions pH is perhaps a more important criterion than total acidity for the establishment of the grape maturity-wine quality relationship. Other work in the 1970's further classified grape maturity in terms of physiological, technological and industrial maturity. This refers respectively to when sugar production in the berry has generally ceased, when the grape has reached optimum quality and when grapes would give the most economical return (Kourakou, 1974; Marteau & Schaeffer, 1978).

Burger (1977) agreed with the previous authors when he pointed out the necessity of specifying parameters which could be used to classify grape quality. This also includes the aspect of grape maturity which is the most important facet of grape quality (Carrol *et al*., 1978) and regarded by some as being even more critical than viticultural practices (Slesinger, 1975).

Du Plessis and Van Rooyen (1982) wrote that total soluble solids (degrees balling (ºB)) has been used in South Africa for a considerable time as an index for optimum maturity in grapes, but without much success, because other equally important factors are not taken into account. It was important that more precise, reliable and applicable maturity parameters were found to improve or replace those being used (Du Plessis & Van Rooyen, 1982). This preludes their study of potential sugar/acid ratio and its connection to wine quality. Four South African cultivars were analysed over a 5-6 year period for titratable acidity (TA), °B and hydrogen ion concentration (pH). This data was used in 13 different ratios. Correlations

giving acceptable probability levels of the relationship between ratio and wine quality were obtained for 7 of the indices studied, ºBxpH, ºB/TA, ºB/pH, TA/pH, TAxpH, ºBxpH/TA, ºB/TAxpH, of which the first two listed showed promising results. A curvilinear fit of ºB/TA (ºBxpH) data to wine quality were obtained which were mostly due to environmental and therefore vintage influences. They also showed in this study that seasonal effects can be to such an extent that no clear maximum of wine quality may be found at a specific ºB/acid ratio. Cabernet Sauvignon and Pinotage wines were found to have more or less similar sugar to TA ratios at maturity (3.9 units) and ºBxpH of 85-95 units. In a consecutive study by Van Rooyen *et al*. (1984) the optimum range for Pinotage and Cabernet Sauvignon were approximately 2.9-4.4 (3.6 averages) and 3.1-5.2 (4.2 average) respectively for the ºB/TA parameter. For the ºBxpH the ranges were much smaller 78-106 and 79-95 respectively for Pinotage and Cabernet Sauvignon. Coombe *et al*. in 1980 showed that $ºBxpH^2$ is an even better indicator of optimum ripeness, their motivation for attaching a bigger weight to pH lies in the significant role it plays in fermentation as well as in wine stability. According to their measurement, the best wines are made at index values ranging from 200-270 units.

Around the same time authors Cootes, Wall and Nettlebeck (1981) came up with a system to predict the time of harvest and the quality of the grapes at harvest. It was called the 'Grape quality assessment scheme' and consisted of aroma and taste of the juice; altitude of the vineyard; ºB; TA; pH; physical conditions of the grapes and sulphur dioxide level of the juice (sulphur dioxide is supposed to be added directly after picking on the harvested grapes to protect it during transport to the cellar). Grapes were thus assessed and the total bonus percentage gained was determined. The total bonus percentage is the amount of points the grapes scores in these abovementioned categories. These points in turn were related to grape quality for which the producer is remunerated.

Through the decades of active research to find a model system for determining optimum grape quality clear tendencies came to the fore. Firstly that using the standard chemical analyses of the pulp (sugar, acid, pH) is of the utmost importance. Secondly that the other factors such as phenolics, turbidity of solids, nitrogenous compounds, physical aspects, aromatic compounds and polysaccharides (Du Plessis, 1982) also plays a role during this important physiological occurrence. The point of optimum grape maturity can also be extremely susceptible to seasonal climatic fluctuations.

Grapes in South Africa ripen while the temperatures are still increasing which is very much different from areas such as Chili, South Australia and the biggest parts of France. The grapes therefore reaches sugar ripeness before other components such as flavours and tannins have reached optimum maturity. This makes it very difficult to determine optimum ripeness in South Africa. The best method to establish optimum ripeness of grapes could be the use of indices such as sugar concentration multiplied by pH (ºBxpH), backed by

sensorial evaluation of grapes and accompanying observations (browning of the pip, colour of the brush, etc.) (Van Schalkwyk & Archer, 2000).

2.2.2 Phenolic maturity

As discussed in the previous section other compounds such as colour and phenolic compounds should also be taken into account to make a more accurate determination of ripeness (Saint-Criq *et al*., 1998ab; Celotti & Carcereri, 2000; Gonzales-Neves *et al*., 2004) (Table 2.1). The amounts to which phenolic compounds are present in grapes are dependent on various factors of which cultivation practices and environmental impacts (Downey *et al*., 2006) are most important. During grape ripening anthocyanins and tannins develop at different stages. Seed tannins were found to be at its maximum at *véraison* and slightly decreased towards ripeness, while skin tannins increase from *véraison* to ripeness. Anthocyanins only start to accumulate in the grapes from *véraison* and reach a maximum at full ripeness (Winkler *et al*., 1974; Wulf & Nagel, 1978; Roggero *et al*., 1986; Boss *et al*., 1996a; Boss *et al*., 1996b; Kennedy, 2002; Adams, 2006).

Table 2.1 Effect of environmental and viticultural practices on important wine grape composition parameter (table adapted from Jackson & Lombard, 1994).

Parameter	Fruit level	Mesoclimate	Soil conditions	Canopy management (at veraison)	Crop load (level)
Soluble solids (°B)	High	Mean Temp: 16-30°C throughout growth stages I-III[1]	Low soil moisture in stage III; or petiole N 1.5%-2.0%	Exposed canopy[2]	Moderate crop load[4]
	Low	Mean Temp: Above 30°C or below 9°C in stage III	Excessive/deficit soil moisture soil moisture (II and III) high or low N.	Shaded canopy[3]	High crop load[5]
Titratable acid (TA)	High	Night temp below 15°C in Stage III or cloudey in stage III	Excessive soil moisture in Stage III	Shaded clusters[6]	High crop load
	Low	Night temp above 15°C in Stage III or mean temp above 22°C in Stage I	Deficit soil moisture in Stages I-III	Shaded canopy	Low crop load
pH	High	Night temp above 15°C in Stage III	Excessive soil moisture or high K or excessive N application in Stage III	Shaded canopy	Low crop load
	Low	Night temperatures below 15°C in Stage III		Exposed canopy	High crop load
Phenols/ Anthocyanins	High	Night temp 5-15°C; mean temp 9-29°C or high sunlight in Stage III	Deficit soil moisture; petiole N 2.0-2.5% in Stage III.	Exposed clusters[7]	Moderate crop load
	Low	Night temp above 15°C; mean temp above 20°C; or cloudy in Stage III	Excessive soil moisture; petiole N above 2.0% in Stage III or high K must	Shaded clusters	High crop load

Table 2.1 (cont.)

Flavour/Aroma	High	Night temp 5-15°C or mean temp 9-20°C in Stage III	Deficit soil moisture in Stage III	Exposed canopy	Moderate crop load
	Low	Night temp above 15°C or mean temp above 20°C in Stage III	Petiole N above 2.5% or excessive soil moisture in Stage III	Shaded canopy	Low or high crop load
Herbaceousness	High		Excessive soil moisture	Shaded canopy	

[1]Stage I: Initial rapid berry growth stage immediately after bloom; Stage II: Lag growth phase of grape berry during which organic acid reaches a maximum; Stage III: Veraison and onwards;
[2]Exposed canopy: leaf layer 1.0-1.5 av., minimum shoot length 10-15 nodes, thinned to 5-16 shoots/meter row;
[3]Shaded canopy: leaf layers above 3.0 av, topped to less than 10 nodes/shoot, or dense canopy of more than 20 shoot/meter rows;
[4]Moderate crop load: 4-10 kg/kg yield to pruning weight;
[5]High crop load: more than 10 kg/kg yield to pruning weight;
[6]Shaded cluster: less than 40%-60% cluster exposure; un-topped shoots of more than 15 nodes;
[7]Exposed cluster: more than 60% exposed; more than 5000 vines/ha.

Celotti and Carcereri (2005) proposed an inline measurement of the grape colour and phenolic content using UV-VIS spectroscopy. The concentration of phenolic compounds, sugar, acid and pH can thus be determined after the rotary drilling system collects a sample of the entire vertical section of the grapes on the back of a trailer when it arrives at a cellar. They found that it is possible to objectively classify the phenolic quality of red grapes at the time of delivery using a global index related to phenolic compounds and therefore apply it as an indication of the quality of the grapes. FT-NIR and FT-MIR have also been proposed and studied for its use in prediction the colour and phenolic content of the grapes at ripeness (Cozzolino *et al*., 2004; Cozzolino *et al*., 2008; Edelmann *et al*., 2001; Jensen *et al*., 2008).

Other methods known for the measurement of phenolic maturity are: 'Glories method' otherwise known as the extractability index (Glories & Agustin, 1993; Saint-Cricq *et al*., 1998); ITV method (Institute Technique de la Vigne et du Vin) (Cayla *et al*., 2002); Australian Wine Research Institute (AWRI) method (Iland *et al*., 2004); Cromoenos method (Cromoenos, 2010); grape skin texture analysis (Segade *et al*., 2008); remote sensing to predict grape phenolics and colour at harvest (Lamb *et al*., 2004).

The first four methods use one of various extraction techniques before determination of the colour and total phenols of grape samples during ripening or at delivery. More importantly, methods should be used to determine the correct time of harvest with regards to the phenolic ripeness of the grapes, in other words to predict the harvest date during early ripening stages. Grape berry texture is such a method and during this study it was found that the extractability of the anthocyanins correlated well with the berry skin break force and thickness of the berry skin. Remote sensing is being studied as a potential tool to predict berry phenolics and colour at harvest. Correlations have been drawn between some berry parameters and these images, but are at this stage still very dependent on the resolution quality of the images (Lamb *et al*., 2004).

2.3 Phenolic Compounds of Grapes and Wines

In the grape berry the occurrence of phenolic compounds are divided into defined areas. The skin contains tannins, pigments and flavanols, the pulp contains juice with phenolic acids but normally no pigments and the seeds contain tannins. During red wine making phenolic compounds that is present in grape berries are released into the hydro alcoholic solution through extraction and maceration. Phenolic compounds can generally be divided into two main groups: flavonoids and non-flavonoids. The flavonoids are further divided into the flavonols, flavan-3-ols, flavan-3,4-diols, anthocyanins and tannins. Certain non-flavonoids in grapes are also known as phenolic acids, which consist of cinnamic acid derivatives (largest group of non-flavonoids), benzoic acid derivates, stilbenes and viniferens which play an important role in especially white wine production. Only flavonoids will be discussed in further detail due to their importance in this particular study.

2.3.1 Flavonols

The most common flavonols found in grapes and wines are kaempferol, quercetin and myricetin (Basic structure Figure 2.1). It is intense yellow in colour and found in the skins of red and white grapes, where it protects the berry from UV rays. In grapes these compounds occur as the corresponding glucosides, galactosides and glucuronides, esterified at position 3 on the C ring. In wines these compounds occur also without these esterifications. Concentrations differ from 100 mg/L in red wine to 3 mg/L in white wine and vary according to cultivar.

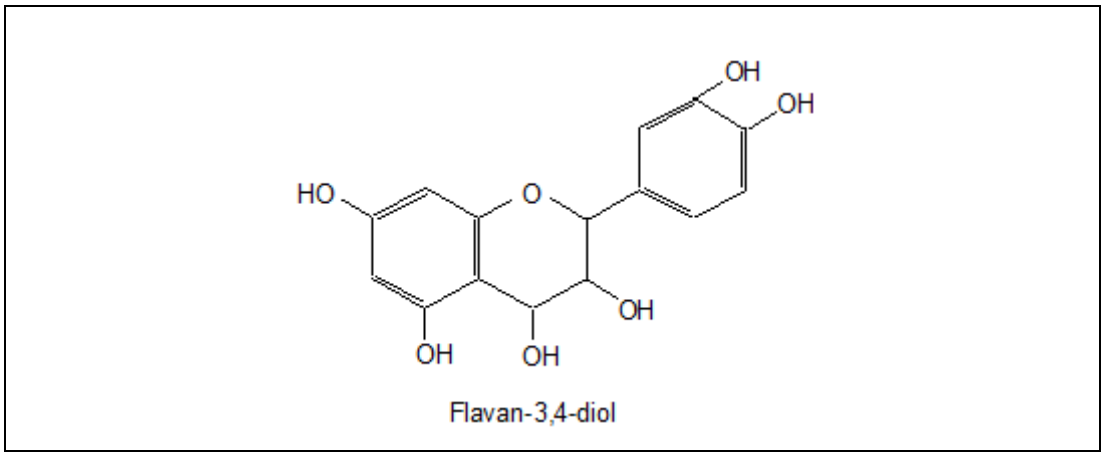

Figure 2.1 The basic structure of flavanols.

2.3.2 Anthocyanins

Anthocyanins are the second most abundant phenolic compounds and are normally found in the grape skins. These compounds are responsible for the red colour of grape skins and

wine (Zoeklein, 1995; Boulton *et al*., 1996; Ribéreau-Gayon *et al*., 2001). Some teinturier varieties also have coloured flesh (Cheynier *et al*., 2006). It is widely accepted that anthocyanins are of the utmost importance in red wine quality, affecting not only the wine colour but also its intensity in wines (Guidoni *et al*., 2002).

Anthocyanins are synthesised in the second growth period of berry development (Coombe & McCarthy, 2000), between *véraison* and full ripeness. During this period the berry doubles in volume and the sigmoidal synthesis of the anthocyanins reaches a plateau, which is sometimes followed by a decline in its concentration. However, when the berries starts to shrivel an increase could occur in these later stages of ripening that could compensate for the chemical degradation reaction of anthocyanin molecules. The enzyme controlling the synthesis of anthocyanins has been found to be flavonoid 3-glucosyl transferase (UFGT) (Boss *et al*., 1996a/b/c; Nakajima *et al*., 2001; Springob *et al*., 2003).

Studies on the structure of anthocyanin molecules have started more than a 100 years ago (Pasteur, 1866; Laborde, 1908; Trillat, 1908) and the determination of a general anthocyanin structure occurred in the early part of the twentieth century (Willstätter & Everest, 1913; Levy *et al*., 1931; Robinson & Robinson, 1933).

With the development of paper chromatography (Bate-Smith, 1948) grape phenolic research was better understood and intensified. This led to the determination of the general anthocyanin structure for *Vitis vinifera* grapes and wine in 1959 (Ribéreau-Gayon) (Figure 2.2), with malvidin-3-O-glucoside being found to be the major anthocyanin present along with its acylated forms. There are five main groups of anthocyanins that normally exist in the grapes skins namely cyanidin, delphinidin, peonidin, petunidin and malvidin. They could be present as a stable glucoside (anthocyanin) or be acylated with *p*-coumaric, caffeic and acetic acid when it is an unstable aglycone (anthocyanidin) (Wulf & Nagel, 1978; Roggero *et al*., 1986; Boss *et al*., 1996a).

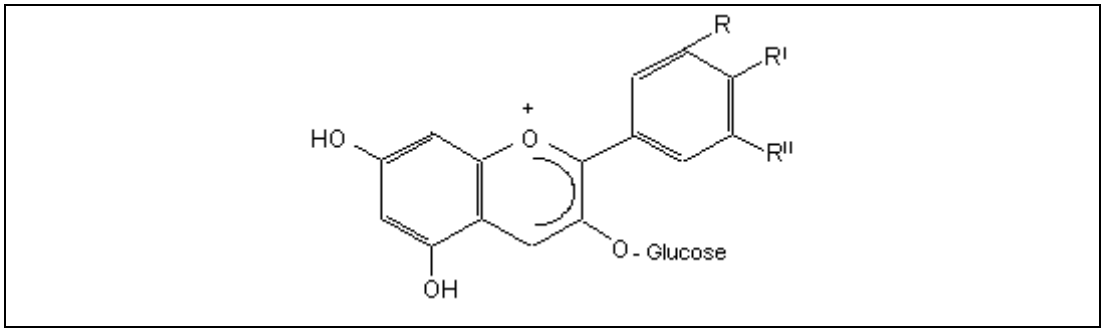

Figure 2.2 General structure of the anthocyanin molecule esterified to glucose. R, R' and R'' represent positions where different combinations of H, OH and OCH_3 can attach.

Of the phenolic compounds, anthocyanins are the most researched, with studies that included changes during berry ripening, influence of environmental and viticultural practices

on their production and also their extraction into wine (Ribéreau-Gayon, 1971, 1972; Pirie & Mullins, 1977; Kliewer & Torres 1972; Kliewer, 1977; Wicks & Kliewer, 1983; Downey *et al*., 2006). Colour of grapes is dependent on temperature, too cold or too warm temperatures are associated with poor colour development in grapes (Winkler *et al*., 1974). The optimum range for anthocyanin synthesis is approximately between 17 and 26ºC (Pirie & Mullins, 1977). In a study by Kliewer and Torres (1972) and Kliewer (1977) they amongst other things showed that temperatures above 30ºC could lead to no colour formation. Other important factors such as soil conditions, canopy management and crop load could also influence anthocyanin levels in grapes. It is also known that the amount of anthocyanins vary according to cultivar and this have even been used in the authentication of red cultivar wines (Burns *et al*., 2002). The relative levels have been used to determine the parentage of grape cultivars (Castia *et al*., 1992; Gonzales-Neves *et al*., 2004).

The form in which anthocyanins occur is highly pH dependent and is found to exist in equilibrium between a few distinct chemical forms. These are the quinoidal (violet) and carbinol (colourless) base (increase in pH), flavene sulphonate (due to SO_2 addition, colourless) and calcone (due to age, yellow) (Ribereau-Gayon *et al*., 2001). It was also recently shown in South African literature that berry size (and weight) plays an important role in the anthocyanin content and quality of Shiraz grapes. The smaller the size of the grape berry, the higher the quality thereof (Barbagallo *et al*., 2011).

2.3.3 Condensed Tannins

Condensed tannins also known as proanthocyanidins are the most occurring phenolic compounds found in grape berries and was started to be characterized in the 1920's (Freudenberg, 1924).

Tannins are found in the hypodermal layers of the skin and soft parenchyma of the seeds between the cuticle and the hard seed coat. They are defined as compounds that produce stable bonds with proteins and polysaccharides. When Proanthocyanidins are heated under acidic conditions, the corresponding anthocyanidin is formed. Tannins are related to the bitter and astringent properties of wines (Robichaud & Noble, 1990; Gawel, 1998).

Flavan-3-ol monomers (Figure 2.3) and hydroxycinnamic acids are formed during the first developmental cycle of the grape berry, between bloom and veraison (Romeyer *et al*., 1982; Kennedy *et al*., 2000a; 2001). Tannins are formed by polymerisation of either flavan-3-ol (catechins) and/or flavan-3,4-diols molecules, with molecular weights ranging from 600 – 3500 Da (Ribéreau-Gayon, 2001). Condensed tannins are made up of combinations of four general sub-units: catechin, epicatechin, epigallocatechin and epicatechin gallate, which are mostly linked by C_4-C_8 and C_4-C_6 interflavan bonds (Prieur *et al*., 1994). These polymers vary in size from dimers, trimers to oligomers with more than 30 sub-units. Catechin is

formed from leucocyanidin which is transformed by the enzyme leucoanthocyanidin reductase (LAR). Epicatechin originates from leucocyanidin that is first transformed to cyanidin by the enzyme leucoanthocyanidin dioxygenase (LDOX). Cyanidin in turn is transformed by anthocyanidin reductase (ANR) to the final epicatechin molecule (Robinson & Walker, 2006).

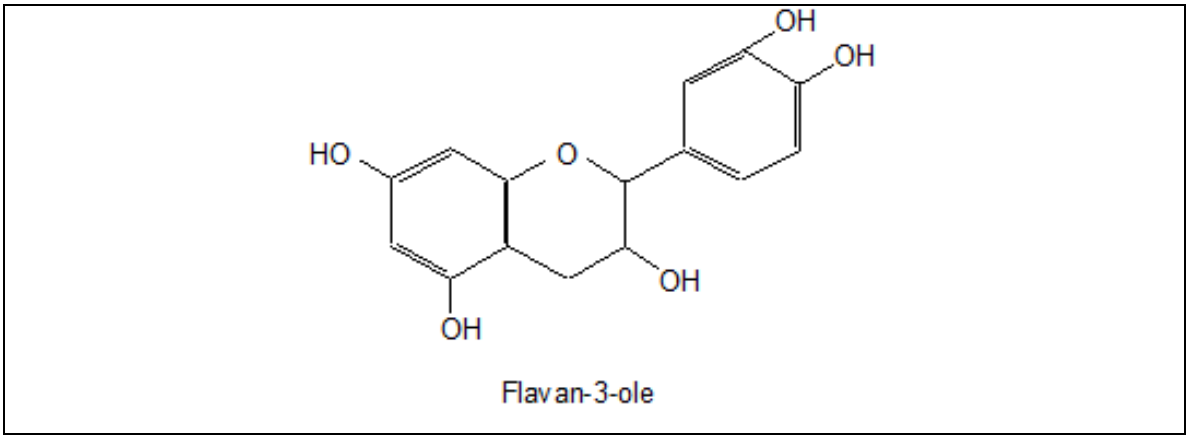

Figure 2.3 Flavan-3-ol structure.

Skin tannins are generally more polymerised and thus larger than seed tannins. Skin tannins also contain epigallocatechin subunits whereas seed tannins generally lack epigallocatechin. However, the smaller seed tannins usually have a higher proportion of their subunits as epicatechin gallate, whereas epicatechin gallate is usually not present in skin tannin (Cheynier, 2006). This difference in signature subunits has been used to estimate the relative proportions of skin tannins and seed tannins in some wines (Peyrot des Gachons & Kennedy, 2003; Adams, 2006).

Tannin/total phenols of grapes have been the focus of numerous studies through the years. During the 1990's the interest on determining phenolic maturity sparked a large amount of research on especially the evolution of the content of phenolic compounds during berry development. Recent publications focussed on the developmental changes of procyanidins in red grape seed and skins of a variety of red grape cultivars (de Freitas *et al*., 2000; Kennedy *et al*., 2000a; Kennedy *et al*., 2000b; Harbertson *et al*., 2002; Downey *et al*., 2003; Hanlin & Downey, 2009; Mattivi *et al*., 2009).

2.4 Correlations between Grape and Wine Phenolics

It has been known for some time that colour intensity of young red wines often correlates well with the overall wine quality as perceived by wine consumers (Jackson *et al*., 1978; Somers & Evans, 1974). The colour of red wine depends on the actual content of

anthocyanin and related compounds found in the grapes (Cheynier *et al*., 2006; Fulcrand *et al*., 2007; Somers, 1971).

As mentioned in a previous section anthocyanins and condensed tannins are the two most important groups of phenolic compounds found in grapes. The anthocyanins are basically situated in the outer layers of the grape skin and under acidic conditions (like wine), it is in the highly coloured flavillium cation form (Adams, 2006). Tannins are situated in grape seed and skins and are very important for mouth feel properties of red wine (Cheynier *et al*., 2006).

Phenolic compounds of grapes are extracted during winemaking by the process of maceration and normally last anything from 5-14 days. The extraction of these phenolic compounds rarely exceeds 50% of the phenolic material that could potentially be harvested from the grapes (Haslam, 2005). Anthocyanins and skin proanthocyanidins diffuse faster into red grape must than the proanthocyanidins from the seeds. In actual fact anthocyanin extraction reaches a maximum early in fermentation and the concentration may drop thereafter (Nagel & Wulf, 1979; Watson *et al*., 1995; Gao *et al*., 1997), while extraction of tannins increases with longer skin and seed contact times (Singleton & Draper, 1964; Ribéreau-Gayon, 1974; Ozmianski *et al*., 1986). Various factors, such as fermentation temperature, sulphur dioxide additions, cold soaking, must or grape freezing, thermo vinification, carbonic maceration, pre-fermentation juice runoff, pectolytic enzymes usage, method of mixing the skins with the juice, maceration time and yeast selection all influence the extraction of phenolic compounds (Sacchi, 2005).

However, studies regarding the direct correlation between grapes and wines are actually not that numerous, despite this known relationship between wine colour and its source. The earliest work done by Iland (1987) was based on an extensive extraction of anthocyanins from grapes and correlating the results with wine colour density. He found that the correlation coefficient (r^2) between the above mentioned parameters to be 0.82 and that a relationship therefore exists. In a South African preliminary study conducted by Marais *et al*. (2001) a good correlation were found between Pinotage grape and wine colour. This was followed by a study that stretched over three consecutive seasons on Pinotage, Shiraz and Cabernet Sauvignon grapes (Marais & October, 2005). The results showed good correlations for individual seasons or cultivars, but when all seasons and cultivars were correlated as a unit the grape and wine phenols showed poor correlations. They also showed that the correlation between grape and wine colour are influenced by the degree of ripeness of the grapes. Pinotage for instance had a correlation of r^2=0.47 between grape colour and modified colour density, when all the data was combined. However, at sugar levels of 23-25 ºB and 24-26 ºB this increased to 0.56 and 0.65 respectively. They further concluded that as far as the relationship between grape colour and overall wine quality is

concerned, Shiraz showed the most potential for prediction of quality. The most statistically significant correlations were obtained from this cultivar. Using the models in question it was possible to predict wine quality based on grape colour to a 66% level of accuracy in the case of all the wines from the Robertson region and a 71% level of accuracy in the case of the Graham Beck Robertson wines only. González-Neves *et al*. (2004) wanted to determine if the pH of the extraction solvent influenced this relationship. They found that a similar correlation exist between grape colour and wine colour intensity regardless of extraction in a solution at wine pH or pH 1. This was corroborated by Romero-Cascales *et al*. (2005) who found anthocyanins extracted at wine pH correlated well with the colour of the corresponding wines (r^2=0.69). It was also discovered by these authors that anthocyanin extractability influences the relationship between grape and wine colour and that grape seed tannins and wine tannins correlated very strongly (r^2=0.9). Using this information as a basis Jensen *et al*. (2008) tried a multivariate approach to predict wine quality from grape polyphenols. They found that individual wine phenolics in general correlated well with several grape phenolics, this further substantiated the value of using a multivariate approach. The prediction of wine polymeric pigments was improved using the multivariate system. Wine anthocyanins were predicted at the same level (r^2=0.91) as in the case when a direct correlation were made between that and grape anthocyanins (r^2=0.93). Another compound that was predicted well using this system was colour due to co-pigmentation, colour due to anthocyanins and colour intensity. However, there seems to be a lack of data in the literature on the correlation between colour and phenolics in grapes and their evolution during malolactic fermentation, as most studies only quantified these correlations after alcoholic fermentation in wine. In another publication where the relationships between flavonoid indexes and wine phenolic composition were investigated high positive correlations were found in various instances (Cagnasso *et al.,* 2008). The Glories method was performed on the grapes and HPLC determination of individual phenolic compound content, chromatic properties (Glories & Augustine, 1993) and CIELAB index values were determined for the wine. In the case of the experimental winemaking the results were really positive for potentially predicting the composition of the future wine. High positive correlations were found between the chromatic properties (absorbance at 420, 520 and 620 nm) of the wines and both extraction fractions obtained from the grape analyses (r^2=0.82-0.92) (Glories & Augustine, 1993). The same was true between the phenolic compounds of the wines determined by HPLC and the 280nm absorbance reading of the grapes (r^2=0.76-0.96). The authors also tried to correlate the parameters for grapes and the wines made on industrial scale. They again found high positive correlations between the chromatic properties of the wines and both extractions made from the grape samples (r^2=0.62-0.93) and only total anthocyanins (HPLC) and both grape extractions (r^2=0.85 and r^2=0.93). They feel that there exists a clear connection

between the phenolic composition indexes of wines and the grapes they were made of. Therefore phenolic parameters of the grapes considered can function as good prediction indexes of the future wine and are therefore of special technological interest. They also showed on experimental scale and through industrial winemaking that the modality and time of maceration can make the yield of the extraction process more uniform even for each variety studied. The correlations were also strongly influenced by vintage. Therefore more work is needed showing data from more vintages in the data. Most recently work was published on correlations between grape and wine colour and tannin (Du Toit & Visagie, 2012). They showed high positive correlations between the colour of grapes and wines as well as significant relationships in the case of tannin content. They also found that the colour and tannin content of Merlot grapes tended to associate more with seed tannins than was the case with Pinotage and Shiraz.

The determination of grape colour is very simple and can be done already in the vineyard. With recent developments in more rapid and precise methods of phenolic analyses, such as near infrared (NIR) or mid infrared (MIR) spectroscopy and even remote sensing, this goal is definitely more reachable than ever before. This knowledge of the relationship between grape and wine phenolics is important to the wine industry because it would enable prediction of the potential wine quality at a very early stage. It will also enable grape growers to identify problem blocks and what viticultural practices to apply in order to correct this. However, grape colour should not be solely used as a quality parameter, because there are a lot of other factors that play a role in establishing quality.

2.5 Analytical techniques for colour and phenolic compounds

2.5.1 UV-Vis spectroscopy

2.5.1.1 Iland method (grapes)

This method of colour analysis measures the amount of red coloured pigments in berries and can give an indication of the potential colour of wine made from those grapes. Patrick Iland and associates (2000) based this method on work that was started by Somers and Evans in 1974. The relationship between the measurement of grape colour and wine colour is based on the assumptions that all the anthocyanins are extracted from the skins, there is no loss of anthocyanins due to precipitation or polymer formation and all wines are made in a similar manner (Iland *et al*., 2000).

For this method 50 grape berries of a particular sample is weighed and crushed using a homogeniser until a smooth textured solution is obtained. Approximately 1 gram of this homogenate is taken for extraction using 50% (v/v) ethanol (pH 2). The extraction period is 1 hour and the choice of ethanol as extract is based on its similarity to the extraction process

during winemaking. After extraction the solution is centrifuged and 1 mL of the extract is diluted with hydrochloric acid and left to stand for 3 hours. The absorption of this solution is measured with a spectrophotometer at 280 and 520 nm. The calculation of the analysis is done according to the law of Beer-Lambert: $C = Abs/\varepsilon \times l$, which states that the concentration of a solution is the result of the absorbance of that compound in the solution divided by the extinction coefficient of the compound and the path length. In the case of the red pigments at 520nm the extinction coefficient of malvidin-3-glucoside of 500 is used, and the result is expressed as equivalents of this anthocyanin per berry or per gram berry. Any extinction coefficient can be taken as default for the phenolics; therefore the results are expressed in absorbance units per berry or per gram berry. The measurement at 280 nm provides an estimate of the concentration of total phenolics in this solution, expressed in absorbency units.

2.5.1.2. Chromatic measurements (grapes and wines)

Colour density (CD) can be described as the sum of the absorbance of a red wine at 420 nm, 520 nm and 620 nm (Ribéreau-Gayon, 1974; Somers & Evans, 1974, 1977; Ribéreau-Gayon *et al*., 2001). Red wine colour has its maximum absorbance at 520 nm (the red colour pigments) and lowest at 420 nm (yellow colour pigments). At 620 nm the purple/blue colours are measured and this is of special importance for young red wines such as was used in this study (Ribéreau-Gayon *et al*., 2001). To further classify wine pigments as co-pigments, free pigments and polymerised pigments a similar measurement can be performed to obtain the modified colour density (MCD), where the bleaching effect of sulphur dioxide is negated by the addition of acetaldehyde. The pH of the wines is also adjusted to 3.6 which give a better reflection of the differences in colour between a set of samples with different pH values. Another important colour measurement is to read the absorption of an acidified wine sample at 280 nm and 520 nm after 3 hours in a 1 M HCl solution. This acidification converts all the anthocyanins into the red flavillium cation form and gives the total phenols (Tphen) and total red pigments (TRP) of a wine sample respectively. Results from the abovementioned indices are reflected as absorbance units and can also be used to determine the amount of free, co-pigmented and polymeric anthocyanins in wine samples (Somers & Evans, 1974; Boulton *et al.,* 1996).

Another method that determines the anthocyanin content (mg/L) of a wine sample spectrophotometrically was developed by Ribéreau-Gayon and associates (2001).

Two sets of the same wine sample are prepared simultaneously. The wine in both test tubes is acidified to get all the anthocyanins in the flavillium cation form. Then the anthocyanin in one wine is bleached using sulphur dioxide. The difference in the 520 nm reading of these

two samples will then be the amount of anthocyanin in mg/L after multiplication using the extinction coefficient of malvidin-3-glucoside.

2.5.1.3 Bovine serum albumin (BSA) precipitation

The bovine serum albumin (BSA) method currently being used is based on the historical use of animal blood for the fining of wines. The method by Bate-Smith (1973) suggested the use of blood from a freshly pricked thumb to precipitate tannins. Using commercial preparations of hemoglobin were found to be unsatisfactory (Asquith & Butler, 1985). This hemoglobin method was initially not widely adopted for the use in wines and it was only after better precipitants were discovered that protein precipitation methods started to become one of the most used precipitation methods of our time. They experienced interference from plant pigments because the measurement was done at 578 nm and from saponins (Schultz *et al*., 1981).

Hagerman and Butler (1978) suggested BSA as replacement precipitant for tannin. Their method involved the formation of a protein-tannin complex between the tannin-containing solution and the protein BSA. The protein-tannin complex is dissolved in a detergent consisting of sodium dodecyl sulfate and triethanolamine in distilled water. The triethanolamine maintains high alkalinity and helps in dissolution of the complex. The tannin/phenolics present in the dissolved complex are measured at 510 nm after addition of ferric chloride. The results are expressed as A_{510nm}/ g of the material or relative to tannic acid equivalents. The data obtained are a function not only of the amount of tannins precipitated but also of their structure. The method was found satisfactory for comparison of tannins from the same sample types but not for samples from a different source (Martin & Martin, 1982). Non-specific binding of the phenols of the complex could introduce a large error in the method (Hagerman & Butler, 1978).

In 1980 they started experimenting with dye labeled BSA (idodine-125) (Hagerman & Butler, 1980). Asquith and Butler (1985) furthered this method by using dye-labeled BSA for precipitation, but this method was insensitive and form complexes that's more soluble. Radial diffusion was also tried and it was insensitive to acetone (extract) very simple but involved an element of subjectivity (Hagerman, 1987).

Various authors built on this method of Hagerman and Butler (1978) and indirectly measure the tannin by rather measuring the protein, but with low success rate. The Lowry or Bradford assays, Kjeldahl method, ninhydrin and alkaline phosphatase were tried by authors in combination with various chemical solutions for buffering and extracting with varying levels of success (Martin & Martin, 1982, 1983; Amory & Schubert, 1987; Verzele *et al*., 1986; Makkar *et al*., 1987b; Marks *et al*., 1987; Makkar *et al*., 1988b; Adams & Harbertson, 1999). Finally Harbertson *et al*. (2003) further expanded the method to also determine different

forms of anthocyanins. They employed bisulphate bleaching for attaining the monomeric anthocyanins and two fractions of polymeric anthocyanins, i.e. small polymeric pigments and large polymeric pigments as well as the tannin content.

The amounts of tannins in USA wines as determined using the BSA method ranged between 30 - 1895 mg/L, with an average of 544 mg/L (Harbertson *et al*., 2008). Averages of 672 mg/L, 559 mg/L and 455 mg/L were found for Cabernet Sauvignon, Merlot and Shiraz, respectively. Other data showed a range of 162 - 569 mg/L in 41 Australian wines with a range of 51 – 109 mg/L tannin in grape extracts obtained by macerating grape homogenate in 50% ethanol (Mercurio & Smith, 2008).

2.5.1.4 Methyl cellulose precipitation (MCP)

The discovery of polymers other than protein able to precipitate tannins started with the use of the polymer polyethyleneglycol (PEG) that was shown to bind strongly with tannins (Jones, 1965; Jones & Mangan, 1977; Makkar *et al*., 1995). This phenomenon was combined with radio labelling to allow the quantification of tannins (Silankove *et al*., 1996). The cross linked counterparts PVP and PVPP were also studied quite extensively due to its strong bond with tannins (Makkar *et al*., 1993). The biggest problem with polymers that strongly bind tannins is its lack of specificity, which means that it generally also remove other non-tannic structures (Molyneux & Frank, 1961; Clifford, 1974). Other avenues that were pursued were the use of copper acetate (Yebra *et al*., 1995) and ytterbium for the precipitation of tannins (Reed *et al*., 1985; Herderich & Smith, 1995). Montedoro and Fantozzi (1974) developed a precipitation assay using a hydrophobic, commercially available polymer, which has almost no absorbance at 280 nm. This method was effective in grape extract containing 50% ethanol, showed no interference from anthocyanins and correlated well with the sensory perception of red wine when increasing amounts of grape seed derived tannin were added (Smith, 2005).

The methyl cellulose precipitation (MCP) method was finally developed in 2006 by Australian scientists (Sarneckis *et al.* 2006). This method is based on the precipitation of tannins using methyl cellulose, a polysaccharide polymer. For grape samples an extraction must be done on the grapes prior to analyses. The extraction is based on the methodology of Iland *et al*. (2000). The actual precipitation method proposed two formats, the 10 mL and 1 mL version. Two samples are used, one is precipitated with methyl cellulose and the other is not (control). After contact time the sample is centrifuged and the absorbance of the supernatants is recorded. The tannin in mg/L epicatechin equivalents is the difference between the control and the precipitated sample. This difference is entered into standard curve linear equation and multiplied by the dilution factor. Tannin concentrations, measured

with the MCP method, in Australian grape extracts and wines range between 338 – 524 mg/L and 1450 – 2300 mg/L respectively (Mercurio & Smith, 2008).

2.5.2 High performance liquid chromatography (HPLC)

According to a review by Thorngate (2006) chromatography started in 1901 when Mikhail Tswett described his technique for the determination of chlorophyll. For the first 20 years or so this method was not widely applied. However, in the 1940's liquid-liquid and paper chromatography was developed by Martin and Synge (1941) and Consden *et al*. (1944). The use of these techniques and the development of gas chromatography (James & Martin, 1952) was a revolutionary event in analytical chemistry and biochemistry. The pioneers of using HPLC in oenology were Wulf and Nagel who published a series of articles that was among the first applications on HPLC related to phenolic analyses (Wulf & Nagel, 1976; 1878; Nagel & Wulf, 1979). At first studies underlined the speed and novelty of the method. Soon this changed and through the 1980's it was applied to investigate the development of phenolics during fermentation and ageing, as well as to characterize cultivars based on phenolics and changes of colour components during ripening. The development of a DAD (diode array detector) for HPLC in 1982 further improved the utility of the HPLC for phenolic analyses and was especially applied for anthocyanin research in the late 1980's and early 1990's. Structural characterization of procyanidins is one of the most important analyses that this technique is used for, whether using acid-catalyzed degradation in the presence of a nucleophilic trapping agent or by normal phase or gel permeation chromatography (Prieur *et al*., 1994; Kennedy *et al*., 2001).

Nowadays HPLC analyses are considered an effective and accurate technique for monomeric and some oligomeric phenol analyses of seed, grape as well as wine extracts. Peng *et al*., (2002) determined that the polymeric proanthocyanidins of grapes can be effectively distinguished from the other peaks at the 280 nm wavelength using reverse phase HPLC. They found a large degree of variability in the content of seed procyanidins for different grape varieties even at almost identical sugar concentrations. Normal phase HPLC was used to study the polymerisation of procyanidins in seeds during ripening and it was found that a reduction occured in the mean degree of polymerisation, but an increase in concentration of the greater molecular sized compounds (Kennedy *et al*., 2000a).

2.5.3. Fourier transform infrared spectroscopy

The current methods for determination of phenolic compounds in grapes and wines mostly entail wet chemistry as well as HPLC determinations. Although these methods have been verified and are widely applied, they can be time consuming. Anthocyanin as well as tannin concentrations are recognised as parameters that are important for the determination of

grape and hence wine quality. The need for more rapid analysis methods for these compounds therefore exists.

Infrared, especially near- and more recently mid-infrared spectroscopy represents a solution to this problem (Fernandez & Agosin, 2007; Versari *et al*., 2006). It has been employed in the wine industry in the past for the analysios of several other important components in wine, including ethanol, organic acids and sugar concentrations (Kupina & Shrikbande 2003; Patz *et al*., 2004.)

The methodology is based on using infrared light to determine the concentration of organic compounds in various solutions. Organic compounds are connected by inter-atomic links, which vibrate when infrared radiation is absorbed by such structures. This radiation comes from a broadband light source that contains the full spectrum of wavelengths that will be measured. The light shines into mirrors that is configured in a specific way, called the Michelson interferometer, this allows some wavelengths to pass through and some to be blocked. The beam is modified for each new data point by moving one of the mirrors, which then change the set of wavelengths that moves through. The organic compounds of wine will absorb this luminous energy at specific wavelengths in the infrared region. This absorption intensity is relative to the concentration of the molecule. The wavelengths are dependent on the type of link, for instance C-O, C-C, C-H linkages or the molecular environment it is present in. A spectrum (interferogram) generated by emitting an infrared source into a wine sample therefore contains an enormous amount of information (light absorbed at each mirror position). This information is extracted by data analyses using powerful software tools to determine the concentrations of compounds of interest (Dubernet & Dubernet, 2005). The first investigations were conducted on wine in its original state as well as purified wine/grape extracts. This set the corner stone for eventually successfully analysing grape homogenates without any form of sample preparation. This is the first truly rapid method for tannin or phenolic analyses ever established.

In 2001 a group of German authors (Edelmann *et al*. 2001) set out to determine the success of using mid-infrared spectroscopy to discriminate between red wine cultivars and phenolic wine extracts. They used attenuated total reflectance (ATR)-mid infrared (MIR) spectroscopy to discriminate both the authentic wines and phenolic extracts. They were not able to discriminate between the different cultivars when applying the technique on the wines, but success was achieved for the phenolic extracts. Interference due to varying concentrations of the main wine components such as sugar and organic acids caused the difficulty when analysing the wines. To use sample purification as mentioned above is not viable when trying to achieve rapid analyses for industrial use. Jensen et al. (2008) build on this knowledge by investigating four different variable selection tools to identify the most important spectral regions using mid-infrared spectroscopy (MIRS) for the determination of

tannins. These variable selection methods did not identify identical spectral regions but all of them included the regions 1458-1425 cm^{-1} and 1060-995 cm^{-1} as very important for tannin quantification. Other grape/must/wine components that have already been successfully calibrated using MIRS are TSS, pH and titratable acidity (Swanepoel *et al*., 2007).

The use of fourier transform near infrared spectroscopy (FT-NIR) (2500 nm-400 nm) was investigated to simultaneously predict the concentrations of malvidin-3-glucoside, pigmented polymers and tannin in red wines (Cozzolino *et al*., 2004). Commercial scale fermentations that were conducted at three different temperature ranges were sampled over two seasons. Calibration equations were developed from HPLC and NIR data using partial least squares regression with internal cross validation. The correlations for the prediction of the three components were $r^2 > 0.8$, with a standard error in cross validation (SECV) of 1.8-5.8. It was therefore concluded that FT-NIR could be very successfully used for the prediction of the concentration of certain phenolic compounds in red wine fermentations. The work was continued (Cozzolino *et al*., 2008) to establish a calibration for the determination of tannins in grape homogenates using FT-NIR and UV-VIS techniques. The coefficient of determination and cross-validation and the standard error of cross-validation were 0.92 and 0.83% (w/w) for dimers and 0.86 and 0.46 mg/g epicatechin equivalents for condensed tannins. The standard error in prediction was 1.34% w/w for dry matter and 0.89 mg/g epicatechin equivalents for condensed tannins respectively. This means that in-line measurement of tannin is possible in commercial wineries to handle the high-throughput of samples. This also includes other important components for instance pH and TSS (Dambergs *et al*., 2006). More recently the application of FT-MIR was demonstrated to be a fast and reliable technique for monitoring the phenolic ripening in red grapes during the harvest period (Fragoso *et al*., 2011). This study included 6 varieties, 192 samples and UV-vis spectroscopy was used as reference method. This work presents a preliminary attempt to apply the FT-MIR instrument to predict the phenolic composition of specific grape varieties, which is a starting point for the design of specific models according to the requirements of wineries if a greater number of samples were considered.

2.6 Conclusion

The importance of grape colour and phenolic compounds as a quality parameter is undisputed. Theory on anthocyanins and tannins and its occurrence in wine and connection to quality is ample. Measurement techniques are available to measure these components faster and efficiently to such an extent that the use thereof in a commercial winery is viable. This has been seen with recent advances in FT-NIR and FT-MIR techniques. The use of NIR and MIR scanning for grape colour and phenolics should be further confirmed for the

South African wine industry and demonstrated by using local samples. Connecting wine colour and phenolics to that of the grapes that it is produced from is the first important building block in the process of predicting wine quality at early stages. This will change the dynamics of the remuneration process in commercial wineries drastically and for the better.

2.7 References

Adams, D.O., 2006. Phenolics and ripening in grape berries. Am. J. Enol. Vitic. 57: 249-256.

Adams, D.O. & Harbertson, J.F., 1999. Use of alkaline phosphatase for the analysis of tannins in grapes and red wines. Am. J. Enol. Vitic. 50(3): 247-252.

Amory, A.M. & Schubert, C.L., 1987. A method to determine tannin concentration by the measurement and quantification of protein-tannin interactions. Oenologia 73: 420-424.

Amerine, M.A. & Roessler, E.B., 1958. Field testing grape maturity. Hilgardia 28: 93-114.

Amerine, M.A. & Winkler, A.F., 1941. Maturity studies with California grapes. The Balling-acid ratio of wine grapes. Proc. Am. Soc. Hort. Sci. 38: 379-387.

Amerine, M.A. & Winkler, A.F., 1944. Composition and quality of musts and wines of California grapes. Hilgardia 15: 493-673.

Asquith, T.N. & Butler, L.G., 1985. Use of dye-labeled protein as spectrophotometric assay for protein precipitants such as tannin. J. Chem. Ecol. 11: 1535-1544.

Barbagallo, M.G., Guidoni, S. & Hunter, J.J., 2011. Berry size and qualitative characteristics of *Vitis vinifera* L.cv. Syrah. S. Afr. J. Enol. Vitic. 32(1): 129-136.

Bate-Smith, E.C., 1948. Paper chromatography of anthocyanins and related substances in petal extracts. Nat. 161: 835-838.

Bate-Smith, E.C., 1973. Haemanolysis of tannins: The concept of relative astringency. Phytochem. 12: 907-912.

Berg, H.W., 1958. Better grapes for wine. Nature of the problem. Am. J. Enol. Vitic. 9: 203-204.

Berg, H.W., 1960. Grade classification by total soluble solids and total acidity. Wine Institute, San Francisco, Mimeo. pp. 53.

Bisson, L., 2001. In search of optimal grape maturity. Practical Winery and Vinyard. July/Aug. 32-43.

Boss, P.K., Davies, C. & Robinson, S.P., 1996a. Anthocyanin composition and anthocyanin pathway gene expression in grapevine sports differing in berry skin colour. Aus. J. Grape Wine Res. 2: 163-170.

Boss, P.K., Davies, C. & Robinson, S.P., 1996b. Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. Shiraz grape berries and the implications for pathway regulation. Plant Phys. 111: 1059-1066.

Boss, P.K., Davies, C. & Robinson, S.P., 1996c. Expression of anthocyanin biosynthesis pathway genes in red and white grapes. Plant Mol. Biol. 32: 565-569.

Boulton, R.B., Singleton, V.L., Bisson, L.F. & Kunkee, R.E., 1996. Principles and practices of Winemaking. Chapman and Hall, New York, NY.

Burger, J.D., 1977. Paiement des produits de la vigne salon des sytemes tenant compte de la qualite de la vendange. Presented at the XV Congres international de la vigne et du vin. OIV, Nyon, Switzerland, Jul. 24-30.

Burns, J., Mullen, W., Landrault, N., Teissedre, P., Lean, M.E.J. & Crozier, A., 2002. Variations in the profile and content of anthocyanins in wines made from Cabernet Sauvignon and hybrid grapes. J. Agric. Food Chem. 50: 4096-4102.

Cagnasso, E., Rolle, L., Caudana, A. & Gerbi, V., 2008. Relationship between grape phenolic maturity and red wine phenolic composition. It. J. Food Sci. 20(3): 365-380.

Carrol, D.E., Ballinger, W.E., McClure, W.F. & Nesbitt, W.B., 1978. Wine quality versus ripeness of light –sorted Carlos muscadine grapes. Am. J. Enol. Vitic. 29: 169-171.

Castia, T., Franco, M.A., Mattivi, F., Muggiolu, G., Sferlazzo, G. & Versini, G., 1992. Characteriazation of grapes cultivated in Sardinia : Chemometric methods applied to the anthocyanic fraction. Sci. Aliments. 12, 239-255.

Cayla, L., Cottereau, P. & Renard, R., 2002. Estimation de la baturite phenolique des raisins rouges par la methode I.T.V. standard. Rev. Fr. Oenol. 193: 10-16.

Celotti, E. & Carcereri, G., 2000. Studio della maturita fenolica delle uve rosse per valorizzare l'are viticola dei Colli Berici. L'Enotec. 36: 79-84.

Celotti, E. & Carcereri de Prati, G., 2005. The phenolic quality of red grapes at delivery: Objective evaluation with colour measurements. Am. J. Enol. Vitic. 26 (2): 75-82.

Cheynier, V., Dueñ-Paton, M., Salas, E., Maury, C., Souquet, Sarni-Manchado, P. & Fulcrand, H., 2006. Structure and properties of wine pigments and tannins. Am. J. Enol. Vitic. 57(3): 298-305.

Clifford, M.N., 1974. The use of poly-n-vinylpyrolidone as the adsorbent for the chromatographic separation of chlorogenic acids and other phenolic compounds. J. Chrom. 94: 261-266.

Coombe, B.G., Dundon, R.J. & Short, A.W.S., 1980. Indices of sugar-acidity as ripeness criteria for wine grapes. J. Sci. Food Agric. 31:495-502.

Coombe, B.G. & McCarthy, M.G., 2000. Dynamics of grape berry growth and physiology of ripening. Aus. J. Grape Wine Res. 6: 131-135.

Cootes R.L., Wall, P.J. & Nettlebeck, R.J., 1981. Grape quality assessment. Presented at seminar on grape quality: assessment from vineyard to juice preparation. Aus. Soc. Vitic. Oenol. Inc. Melbourne.

Cozzolino, D., Kwiatkowski, M.J., Parker, M., Cynkar, W.U., Dambergs, R.G., Gishen, M. & Herderich, M.J., 2004. Prediction of phenolic compounds in red wine fermentations by visible and near infrared spectroscopy. Anal. Chim. Acta. 513: 73-80.

Cozzolino, D., Cynkar, W.U., Dambergs, R.G., Mercurio, M.D. & Smith, P.A., 2008. Measurement of condensed tannins and dry matter in red grape homogenates using near infrared spectroscopy and partial least squares. J. Agric. Food Chem. 56: 7631-7636.

Cromoenos. http://bioenos.com/cromoenos/index.php.

Dambergs, R.G., Cozzolino, D., Cynkar, W.U., Janik L., & Gishen, M., 2006. The determination of red grape quality parameters using the LOCAL algorithm. Near infrared spectroscopy, 14: 71-79.

De Freitas, V.A.P., Glories, Y. & Monique, A., 2000. Developmental changes of procyanidins in grapes of red vitis vinifera varieties and their composition in respective wines. Am. J. Enol. Vitic. 51(4): 397-403.

Downey, M.O., Harvey, J.S. & Robinson, S.P., 2003. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aus. J. Grape Wine Res. 9:15-27.

Downey, M.O., Dokoozlian, N.K. & Krstic, M.P., 2006. Cultural practice and environmental impacts on the flavonoid compositions of grapes and wine: A review of recent research. Am. J. Enol. Vitic. 57: 257-268.

Dubernet, M. & Dubernet, M., 2005. Use of Fourier transform infrared analysis for routine analysis in winemaking, Laboratoire Dubernet, Narbonne.

Du Plessis, C.S., 1975. Fermentation formed composnents in relation to wine quality. Presented at the Fourth International Enological symposium, Valencia, Spain, May 26-29. pp. 374-390.

Du Plessis, C.S., 1977. The influence of juice clarifying techniques and juice clarity on wine quality. Wynboer. 554:67-70.

Du Plessis, CS & Van Rooyen, PC., 1982. Grape Maturity and Wine Quality. S. Afr. J. Enol. Vitic. 2:41-45.

Du Toit, W.J. & Visagie, M., 2012. Correlations between South African Red Grape and Wine Colour and Phenolic Composition: Comparing the Glories, Iland and Bovine Serum Albumin Tannin Precipitation Methods. S. Afr. J. Enol. Vitic. 33: 33-41.

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

Failla, O., Brancodoro, L. & Scienza, A., 2005. Maturazione, maturita e qualita dell'uva. L'informatore Agrario (Supplemento al n.1 al N, 14): 7-13.

Fazinic, N., Briza, K., Milosavljevic, M., Lazic, S. & Condric, P., 1976. Physiologie de la croissance et de la maturation des baie. Causes des variations des constituants principaux. Influence des operations en vert et des interventions chimiques. Bull. Off. Int. Vin. 49: 565-574.

Fernandez, K. & Agosin, E., 2007. Quantitative analysis of red wine tannins using Fourier-transform mid-infrared spectrometry. J. Agric. Food Chem. 55: 7294-7300.

Flora, L. & Lane, R.P., 1979. Effect of ripeness and harvest date on several physical and compositional factors of Conart muscadine grapes. Am. J. Enol. Vitic. 30:241-246.

Fragoso, S., Aceña, L., Guasch, J., Busto, O. & Mestres, M., 2011. Application of FT-MIR spectroscopy for fast control of red grape phenolic ripening. J. Agric. Food Chem. 59: 2175-2183.

Freeman, B.M., Turkington, C.R. & Kaye, G.R., 1976. Quality assessment of Hunter Valley and Griffith experimental varietal wines. Aust. Wine Brew. Spirit Rev. Mrch. pp. 35-36.

Freudenberg, K., 1924. Mitteilung über gerbstoffe und ähnliche verbindungen.16. raumisomere catechine, IV. Liebigs Ann. Chem., 437: 274-285.

Fulcrand, H., Duenas, M., Salas, E., & Cheynier, V., 2007. Phenolic reactions during winemaking and aging. Am. J. Enol. Vitic. 57(3): 289-305.

Gao, L., Girard, B., Mazza, G. & Reynolds, A.G., 1997. Changes in anthocyanins and color characteristics of Pinot noir wines during different vinification processes. J. Agric. Food Chem. 45: 2003-2008.

Gawel, R., 1998. Red wine astringency: a review. Aus. J. Grape Wine Res. 4: 74-95.

Glories, Y. & Agustin, M., 1993. Proceedings Colloque Journee Technique du CIVB, Bordeaux. pp. 56.

Gonzalez-Neves, G., Charamelo, D., Balado, J., Barreiro, L., Bochicchio, R., Gatto, G., Gil, G., Tessore, A., Carbonneau, A. & Moutounet, M., 2004. Phenolic potential of Tannat, Cabernet Sauvignon and Merlot grapes and their correspondence with wine composition. Anal.Chim. Acta, 513: 191.

Guidoni, S., Allara, P. & Schubert, A., 2002. Effect of cluster thinning on berry Anthocyanin composition of Vitis vinifera cv. Nebbiolo. Am. J. Enol. Vitic. 53:224-226.

Hagerman, A.E., 1987. Radial diffusion method for determining tannin in plant extracts. J. Chem. Ecol. 13: 437-449.

Hagerman, A.E. & Butler, LG., 1978. Protein precipitation method for the quantitative determination of tannins. J. Agric. Food Chem. 26: 809-812.

Hagerman, A.E. & Butler, L.G., 1980. Determination of protein in tannin-protein precipitates. J. Agric. Food Chem. 28: 944-977.

Hanlin, R.L. & Downey, M.O., 2009. Condensed tannin accumulation and composition in skin of Shiraz and Cabernet Sauvignon Grapes during berry development. Am. J. Enol. Vitic. 60(1): 13-23.

Harbertson, J.F., Kennedy, J.A. & Adams, D.O., 2002. Tannin in skins and seeds of Cabernet Sauvignon, Syrah and Pinot noir Berries during ripening. Am. J. Enol. Vitic. 53(1): 54-59.

Harbertson, J.F., Picciotto, E.A. & Adams, D.O., 2003. Measurement of polymeric pigments in Grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. Am. J. Enol. Vitic. 54(4): 301-306.

Harbertson, J.F., Hodgins, R.E., Thurston, L.N., Schaffer, L.J., Reid, M.S., Landon, J.L., Ross, C.F. & Adams, D.O., 2008. Variability of tannin concentration in red wines. Am. J. Enol. Vitic. 59: 210 - 214.

Haslam, E., 2005. Taste, bitterness and astringency. In Practical polyphenolics; Haslam, E., Ed.; Cambridge University Press; New York, 195.

Herderich, M.J. & Smith, P.A., 2005. Analysis of grape and wine tannins: Methods, applications and challenges. Aus. J. Grape Wine Res. 11: 205-214.

Iland, P., Bruer, N., Wilkes, E. & Edward, G., 2004. Anthocyanins (colour) and total phenolics of grape berries. In Chemical Analysis of Grapes and Wine: Techniques and Concepts, 1st ed.; Winetitles: Broadview, Australia, 2004. pp. 44.

Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. Techniques for chemical analysis and quality monitoring during winemaking. Patrick Iland Wine Promotions PTY LTD. Campbelltown, Australia.

Jackson, D., Timberlake, C.F., Bridle, P., Vallis, L., 1978. Red wine quality – correlations between colour, aroma and flavour and pigment and other parameters of young beaujolais. J. Sci. Food Agric. 29(8): 715-727.

Jensen, J.S., Egebo, M. & Meyer, A.S., 2008. Identification of spectral regions for the quantification of red wine tannins with Fourier transform mid-infrared spectroscopy. J.Agric. Food Chem. 56: 3493-3499.

Jensen, J.S., Werge, H.H.M., Egebo, M. & Meyer, A.S., 2008. Effect of wine dilution on the reliability of tannin analysis by protein precipitation. Am. J. Enol. Vitic. 59(1): 103-105.

Jones, D.E., 1965. Banana tannin and its reaction with polyethylene glycols. Nature, 206: 299-300.

Jones, W.T. & Mangan, J.L., 1977. Complexes of the condensed tannins of sainfoin (Onobrychis viciaefolia Scop) with fraction 1 leaf protein and with sub maxillary muco protein and their reversal by PEG and pH. J. Sci. Food Agric. 28(2): 126-136.

Kennedy, J.A., Matthews, M.A., & Waterhouse A.L., 2000a. Changes in grape seed polyphenols during fruit ripening. Phytochem. 55: 77-85.

Kennedy, J.A., Troup, G.J., Pilbrow, J.R., Hutton, D.R., Hewitt, D., Hunter, C.R., Ristic, R., Iland, P.G. & Jones, G.P., 2000b. Development of seed polyphenols in berries from *Vitis vinifera* L. cv. Shiraz. Aust. J. Grape Wine Res. 6: 244-254.

Kennedy, J.A., Hayasaka, Y., Vidal, S., Water, E.J. & Jones, G.P., 2001. Composition of grape skin procyanidins at different stages of berry development. J. Agric. Food Chem. 49: 5348-5355.

Kennedy, J., 2002. Understanding grape berry development. Australian grape- and winegrower. July/August 2002: 14-23.

Kliewer, W.M., 1977. Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. Am. J. Enol. Vitic. 28: 96-103.

Kliewer, W.M. & Torres, R.E., 1972. Effect of controlled day and night temperatures on grape coloration. Am. J. Enol. Vitic. 23: 71-77.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J.M. & Zamora, F., 2010. Comparison of methods for estimating phenolic maturity in grapes : Correlation between predicted and obtained parameters. Anal. Chim. Act. 660: 127-133.

Kourakou, S., 1974. Optimaler Reifegrad der Trauben in Bezug auf den Gewünschten Weintyp. Paper delivered XIV Congres International de la Vigne et de Vin. Bolzano-Trento, Italy.

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.

Laborde, J., 1908. Sur l'origine de la matiére chromogéne des raisins pendant la maturation. Comt. Rend., 147 :753-755.

Lamb, D.W., Weedon, M.M. & Bramley, R.G.V., 2004. Using remote sensing to predict grape phenolics and colour at harvest in a Cabernet Sauvignon vineyard: Timing observations against vine phenology and optimising image resolution. Aus. J. Grape Wine Res. 10: 46-54.

Levy, LF, Posternack, T & Robinson, R., 1931. Experiments on the synthesis of the anthocyanins. Part VIII. A synthesis of oenin chloride. J. Chem. Soc., 2701-2715.

Marais, J., Iland, P. & Swart, E., 2001. Exploring the relationships between Pinotage grape colour and wine quality–preliminary results. Wynboer, 2001. http://www.wineland.co.za/wynboer_archive/recentarticles/0201explore.html

Marais, J. & October, F., 2005. Relationship between grape colour and wine quality. Wynboer 191:15-17.http://www.wineland.co.za/wynboer_archive/recentarticles/200507colour.php3

Makkar, H.P.S., Dawra, R.K. & Singh, B., 1987b. Protein precipitation assay for quantitaion of tannins: determination of protein in tannin-protein complex. Ann. Biochem. 166: 435-439.

Makkar, H.P.S., Dawra, R.K. & Singh, B., 1988. Determination of both tannin and protein in a tannin-protein complex. J. Agric. Food Chem. 36(3): 523-525.

Makkar, H.P.S., Blummel, M., Borowy, N.K. & Becker, K., 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric. 61: 161-165.

Makkar, H.P.S., Blummel, M. & Becker, K., 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas-production and the true digestibility in in-vitro techniques. Brit. J. Nut. 73: 897-913.

Marks, D., Glyphis, J.,Leighton, M., 1987. Measurement of protein in tannin-protein precipitation using ninhydrin. J. Sci. Food Agric. 38: 255-261.

Marteau, G. & Schaeffer, A., 1978. Maturite optimale des raisins de cuve selon les types de vins a elaborer. Bull. Off. Int. Vin. 51 : 281-299.

Martin, J.S. & Martin, M.M., 1982. Tannin assay in ecological studies: Lack of correlations between phenolics, proanthocyanidins and protein-precipitating constituents, in mature foliage of sic oak species. Oecologia, 54: 205-211.

Martin, J.S. & Martin, M.M., 1983. Tannin assays in ecological studies: Precipitation of ribulose-1, 5,-biphosphate carboxylase/oxygenase by tannic acid, quebracho, and oak foliage extracts. J. Chem. Ecol. 9: 285-294.

Mattivi, F., Vrhovsek, U., Masuero, D. & Trainotti, D., 2009. Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. Aus. J. Grape Wine Res. 15: 27-35.

Mercurio, M.D. & Smith, P.A., 2008. Tannin quantification in red grapes and wine: comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency. J. Agric. Food Chem. 56 (14): 5528-5537.

Molyneux, P. & Frank, H.P., 1961. The interaction of plyvinylpyrrolidone with aromatic compounds in aqueous solution. Part I. J. Am. Chem. Soc. 83: 3169-3174.

Montedore, G. & Fantozzi, P., 1974. Dosage des tannins dans les mouts et les vins a l'aide de la methylcellulose et evalution d'autres fractions phenoliques. Lebensmittel-Wissenschaft und – Technology, 7 : 155-161.

Nagel, C.W. & Wulf, L.W., 1979. Changes in the anthocyanins, flavonoids and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet Sauvignon. Am. J. Enol. Vitic. 30:111-116.

Nakajima, J., Tanaka, Y., Yamazaki, M. & Saito, K., 2001. Reaction mechanism from leucoanthocyanidins to anthocyanidin 3–glucoside, a key reaction for colouring in anthocyanin biosynthesis. J. Biol. Chem. 276(28): 25797-25803.

Ough, C.S. & Alley, C.J., 1970. Effect of 'Thompson Seedless' grape maturity on winecomposition and quality. Am. J. Enol. Vitic. 21:78-84.

Ough, C.S. & Singleton, V.L., 1968. Wine quality prediction from juice Brix/acid ratio and associated compositional changes for White Riesling and Cabernet Sauvignon. Am. J. Enol. Vitic. 19:129-138.

Ozmianski, J., F.M., Romeyer, J.C. Sapis, & J.J. Macheix.,1986. Grape seed phenolics: Extraction as affected by some conditions occurring during wine processing. Am. J. Enol. Vitic. 37: 7-12.

Patz, C.D., Blieke, A., Ristow, R. & Dietrich, H., 2004. Application of FT-MIR spectrometry in wine analysis. Anal. Chim. Act. 513(1): 81-89.

Pasteur, L., 1866. Etudes sur le vin, ses maladies, causes qui les pro voquent, procédés nouveaux pourle conserver pour et le vieillir. Impériale, Paris.

Peng, Z., Iland, PG., Oberholster, A., Sefton, MA., & Waters, EJ., 2002. Analysis of pigmented polymers in red wine by reverse phase HPLC. Aust. J. Grape Wine Res. 8: 70-75.

Peyrot, des Gachons, C. & Kennedy, J.A., 2003. Direct method for determining seed and skin proanthocyanidins extraction into red wine. J. Agric. Food Chem. 51: 5877-5881.

Pirie, A. & Mullins, M.G., 1977. Interrelationships of sugars, anthocyanins, total phenols and dry weight in the skin of grape berries during ripening. Am. J. Enol. Vitic. 28: 204-209.

Prieur, C., Rigaud, J., Cheynier, V. & Moutounet, M., 1994. Oligomeric and polymeric procyanidins from grape seeds. Phytochem. 36: 781-784.

Reed, J.D., Horvath, P.J., Allen, M.S. & Vansoest, P.J., 1985. Gravimetric–determination of soluble phenolics including tannins from leaves by precipitation with trivalent ytterbium. J.Sci. Food Agric. 36: 255-261.

Reutlinger, H., 1973. Wine making with the object of producing fruity wines. Paper delivered at the: Third International Enological Symposium, Cape of Good Hope, South Africa, 6-10 March.

Ribéreau-Gayon, P., 1959. Recherches sur les anthocyannes des végétaux. Application au genre Vitis. Librairie general de l'enseignement, Paris.

Ribéreau-Gayon, P., 1971. Évolution des composés phénoliques au cours de la maturation du raisin I. Conn Vigne Vin. 5 : 247-261.

Ribéreau-Gayon, P., 1972. Évolution des composés phénoliques au cours de la maturation du raisin II. Discussion des résultats obtenus en 1969, 1970, et 1971. Conn Vigne Vin. 6: 161-175.

Ribéreau-Gayon, P., 1974. The chemistry of red wine color. In Chemistry of Winemaking. A.D. Webb (Ed.), American Chemical Society, Washington, DC. pp. 81

Ribéreau-Gayon, P., Glories, Y., Maujean, A. & Dubourdieu, D., 2001. Handbook of Enology, Volume 2, The Chemistry of Wine stabilization and Treatments, John Wiley & Sons, LTD.

Robichaud, J.L. & Noble, A.C., 1990. Astringency and bitterness of selected phenolics in wine. J. Sci. Food Agric. 53: 343-353.

Robinson, G.M. & Robinson R., 1933. A survey of anthocyanins III. Notes on the distribution of leuco-anthocyanins. J. Biochem. 27: 206-212.

Robinson, S. & Walker, M., 2006. When do grapes make tannins? The Australian & New Zealand Grapegrower & Winemaker. Ann. Tech. Iss. pp. 97-105.

Roggero, J.P., Coen, S. & Ragonnet, B., 1986. High performance liquid chromatography survey of changes in pigment content in ripening grape of Syrah. An approach to anthocyanin metabolism. Am. J. Enol. Vitic. 37: 77-83.

Romero-Cascales, I., Ortega-Regules, A., López-Roca, J.M., Fernández-Fernández, J.I. & Gómez- Plaza, E., 2005. Differences in Anthocyanin Extractability from grapes to wines according to variety. Am. J. Enol. Vitic. 56(3): 212-219.

Romeyer, F.M., Macheix, J.J., Goiffon, J.P., Reminiac, C.C. & Sapis, J.C., 1982. The browning capacity of grapes III. Changes and importance of hydroxycinnamic acid-tartaric acid esters during development and maturation of the fruit. J. Agric. Food Chem. 31:346-349.

Sacchi, K.L., Bisson, L.F. & Douglas O.A., 2005. A review of the effect of winemaking techniques on phenolic extraction in red wines. Am. J. Enol. Vitic. 56: 3.

Saint-Criq, N, Vivas, N & Glories, Y., 1998a. Apports récents a l'interprétation des critéres de la qualité des vins rouges. Rev. Fr. Oenol. 169: 19.

Saint-Criq, N., Vivas, N. & Glories, Y., 1998b. Maturation phénolique des raisins rouges relation avec la qualité des vins. Comparaison des cépges Merlot et Tempranillo. Prog. Agric. Vitic. 115: 306.

Sarneckis, C.J., Dambergs, R.G., Jones, P., Mercuric, M., Herderich, M.F. & Smith, R.A., 2006. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. Aus. J. Grape Wine Res. 12: 39-49.

Segade, S.R., Rolle, L., Gerbi, V. & Orriols, I., 2008. Phenolic ripeness assessment of grape skin by texture analysis. J. Food Comp. Anal., 21: 644-649.

Silanikove, N., Shinder, D., Gilboa, N., Eyal, M. & Nitsan, Z., 1996. Binding of poly ethylene glycol to samples of forage plants as an assay of tannins and their negative effects on rumenal degradation. J. Agric. Food Chem. 44: 3230-3234.

Singleton, V.L., & Draper, D.E., 1964. The transfer of polyphenolic compounds from grape seeds into wines. Am. J. Enol. Vitic. 15: 34-40.

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

Slesinger, Z., 1975. Untersuchungen über den einfluss der erziehungsnorm auf de trabenstruktuur bei deur sorte veltliner frührot. Mitt Rebe, Wein, Obstbau und fruchtevertung. 25: 271-278.

Somers, T.C., 1971. Polymeric nature of wine pigments. Phytochemistry, 10: 289-305.

Somers, T.C., & Evans, M.E., 1974. Wine Quality: Correlation with colour density and anthocyanin equilibria in a group of young red wines. J. Sci. Food Agric. 25:1369-1379.

Springob, K., Nakajima, J., Yamazaki, M., & Saito, K., 2003. Recent advances in the biosynthesis and accumulation of anthocyanins. Nat. Prod. Rep. 20(3): 288-303.

Swanepoel, M., Du Toit, M. & Nieuwoudt, H.H., 2007. Optimisation of the quantification of total soluble solids, pH and titratable acidity in South African grape must using Fourier Transform Mid-Infrared spectroscopy. S. Afr. J. Enol. Vitic. 28(2): 140-145.

Thorngate, J.H., 2006. Methods for analysing phenolics in research. Am. J. Enol. Vitic. 57(3): 269-279.

Tudosie, A.D., Moleavin, V. & Chirosca, J., 1972. Busuioaca de Bohotin, a high quality grape variety ofb the Husi vineyards. Indust. Alim. 23: 324-325

Trillat, A., 1908. L'aldehyde acétique dans le vin, son origine at ses effets. Ann. Instit. Pasteur. 22: 704-719; 753-762; 876-895.

Van Schalkwyk, H. & Archer, E., 2000. Die Wynboer, May 2000.

Verzele, M., Delahaya, P. & Damme F.V. Determination of the tanning capacity of tannic acids by high performance liquid chromatography. J. Chroma. 362: 363-374.

Watson, B.T., Price, S.F. & VAlladao, M., 1995. Effect of fermentationpractices on anthocyanin and phenolic composition of Pinot noir wines. Abstr. Am. J. Enol. Vitic. 46: 404.

Willstätter, R. & Everest, A.E., 1913. Untersuchungen über die anthocyane I. Über den Farbstoff der Kornblume. Liebigs Ann. Chem. 401: 189-222.

Wicks, A.S. & Kliewer, W.M., 1983. Further investigations into the relationship between anthocyanins,phenolics and soluble carbohydrates in grape berry skins. Am. J. Enol. Vitic. 34: 114-116.

Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lider, L.A., 1974. General Viticulture. University of California Press, Berkeley.

Wulf, L.W. & Nagel, C.W., 1978. High-pressure liquid chromatography of anthocyanins of *Vitis vinifera*. Am. J. Enol. Vitic. 5:29-33.

Yebra, M.C., Gallego, M. & Valcarcel, M., 1995. Indirect flow-injection determination of tannins in wines and tea by atomic absorption spectrometry. Anal. Chim. Act. 308: 357-363.

Zoeklein, B.W., Fugelsang, K.C., Gump, B.H. & Nury, F.S., 1995. Wine analysis and production. Chapman and Hall, 115th Avenue, New York, NY, 10003.

Chapter 3

Research results

Comprehensive survey of the distribution of colour and phenolics of different red grape wine vineyard blocks from the Robertson area in South Africa

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3. Comprehensive survey of the distribution of colour and phenolics of different red grape wine vineyard blocks from the Robertson area in South Africa

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3.1 Abstract

Colour and phenolic content of red grapes are two of the most important constituents to produce a quality red wine. In the Robertson grape growing area difficulty with colour development of grapes are sometimes experienced. This development is especially connected to site and most probably greatly influenced by a particular season. Forty four vineyard blocks consisting of the cultivars Pinotage, Merlot, Cabernet Sauvignon and Shiraz, were studied over 3 seasons with regards to mainly colour and phenolic content, but also total soluble solids, titratable acidity and pH of the grapes. High performance liquid chromatography and spectrophotometric methods were used to determine various colour and phenolic parameters present at harvest. This data was used to show how the colour and phenolic constitution of a part of the Robertson grape growing area was distributed due to various factors, such as cultivar and seasonal influence. GPS points were also used to map this data for the blocks in a more visual way. Results showed variable colour and phenolic content of these grapes in terms of blocks and certain phenolic compounds investigated. In terms of cultivar Shiraz showed a wider distribution of certain phenolic compounds over the three seasons than the other 3 cultivars. Season did have a great influence on these results as well, with certain outlier blocks being identified.

3.2 Introduction

Robertson is one of the warmer viticultural regions in the South Africa. Grape growers in the Robertson area are faced with average maximum temperatures that exceed 27ºC during the ripening phase of the grapes each harvest season. Grapes from certain vineyards from the Robertson area have been found to have a different colour concentration than those cultivated in so-called cooler areas. Already in 1974 Winkler and associates found that temperatures that are too hot or too cold have been associated with poor colour development in berries. They also classified the climates of winegrowing regions, an according to this system Robertson falls in the IV category (Categories I-V, Low to high temperatures) (Hunter & Bonnardot, 2011).

The optimum temperature range for anthocyanin synthesis was found to be around 17ºC to 26ºC (Prie, 1997). It has also been indicated that average maximum temperatures of 30ºC to 35ºC could lead to no colour development irrespective of the night temperatures in those periods (Kliewer, 1970; Kliewer & Torres, 1972). Some authors have stated that 'grapes do not produce quality fruit when grown in extreme heat conditions' (Turner, 2009) which is exactly what must be overcome by finding the balance between sugar and phenolic ripeness of the grapes to obtain the highest quality. Numerous factors influences flavonoid biosynthesis in plants, including light, temperature, altitude, soil type, water availability, nutrition, microbial interactions, pathogenesis, wounding, defoliation, plant growth regulators and various developmental processes. Despite the influence from all the abovementioned parameters the greatest influence on the flavonoid content of any cultivar are thought to be site and season and in particular water and temperature (Bakker *et al*., 1986; Gonzalez-San Jose *et al*., 1990; Revilla *et al*., 1997; McDonald *et al*., 1998; de Freitas & Glories, 1999; Guidoni *et al*., 2002; Ojeda *et al*., 2002). If, for a specific site, the soil and viticultural practices remains the same and nutrition is adequate, it could be postulated that primary seasonal differences will be due to climatic factors and such as sunlight and temperature (Downey *et al*., 2005).

The importance of phenolic compounds for the quality of wines has been shown many times over in literature (Peynaud, 1996). Firstly phenolic compounds are responsible for the colour of wine. The colour of wine could influence the preference of wine consumers (Somers & Evans, 1974) and it is an indication of the amount of oxygen exposure of the wine (Singleton, 1987). Colour has also been directly linked to wine quality (Du Toit *et al*., 2006). Secondly phenolic compounds play an important role in the taste and mouth feel properties of wine. For instance bitterness and astringency are linked to flavan-3-ols (otherwise known as catechins). The lower molecular weight flavan-3-ols relate to bitterness, whilst the higher molecular weight, protein

bound flavan-3-ols relates to astringency in wine (Rossi & Singleton, 1966; Robichaud & Noble, 1990). Thirdly it influences the odour of wines directly through compounds such as vanillin, ethyl phenols and vinyl phenols. The contribution of volatile phenols to wine could be positive or negative, dependent on compounds and the concentration thereof. Fourthly, phenolic compounds determine the oxidative ability of a wine and therefore also the ageing potential.

Studies concentrating on the amount of colour in Robertson grapes and wines up to date are not comprehensive enough to reflect the variation due to seasons and geographical position. A preliminary study in 2005 on the relationship between grape and wine colour in the Robertson region only involved three cultivars over one season (Marais & October, 2005). Therefore more information on this topic will not only aid the scientific world but could also be practically contributing to the industry.

The main aim of this study was thus to evaluate the colour and phenolic composition of a large number of vineyard blocks over time. Forty four vineyard blocks consisting of Pinotage, Merlot, Cabernet Sauvignon and Shiraz were studied over three seasons with regards to mainly colour and phenolic content, but also total soluble solids, titratable acidity and pH. A wide array of high performance liquid chromatography (HPLC) and spectrophotometric (spec) analyses for colour and phenolic characteristics were used to characterise grapes originating from these blocks. GPS points were used to map the blocks with regards to colour and phenolic content and how this was distributed over the three seasons.

3.3 Materials and methods

3.3.1 Vineyards

Pinotage (P1-9), Merlot (M1-12), Cabernet Sauvignon (C1-12) and Shiraz (S1-11) blocks from the Robertson grape growing area in South Africa were selected for this survey (Figure 3.1, Table 3.1). While the cultivars (cv.) and blocks remained the same between the seasons the sample the block numbers increased from 2007 to 2008 and 2009. The blocks varied in size, orientation, age, rootstocks being crafter on, degree of visual virus infection and soil characteristics (Table 3.1).

Weather stations located in this area were utilized to obtain temperature and rainfall data. Real coordinates were used to display the orientation of the stations with regards to the blocks on a topographical map containing information of the towns, roads, rivers and mountain ranges of the area as well (Figure 3.1). The data were taken for the periods 2007 -2009 and for the months of January to April from where averages of temperature and rainfall were statistically determined.

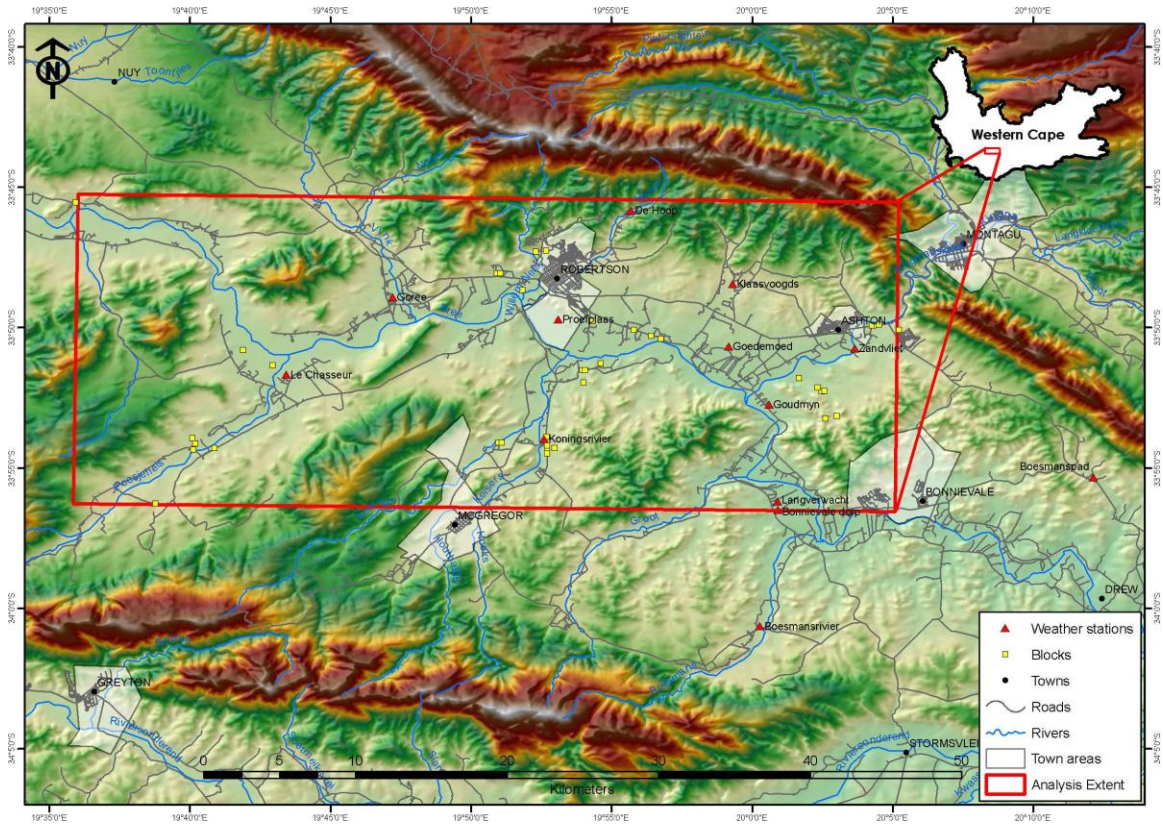


Figure 3.1 A topographical Robertson area indicating the coordinates of the weather stations and vineyard blocks evaluated during this survey (red square).

Table 3.1 The Pinotage (P1-9), Merlot (M1-12), Cabernet Sauvignon (C1-12) and Shiraz (S1-11) blocks chosen for this survey (2007-2009), their location and cultivation specifics.

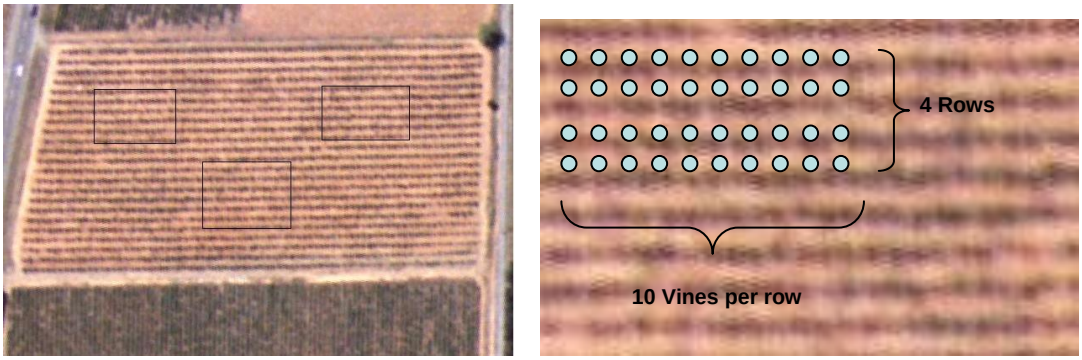
Sample code:	Size Ha[1]	Orientation[2]	Age (2007)[3]	Rootstock[4]	Virus infection (Leaf roll)	Soil	Trellis system[5]
P1	0.76	SE, Med slope	10	R110	Clear	Karoo	4WEP
P2	2.55	Flat	11	R99	Clear	Karoo	5WEP
P3	1.70	Flat	11	R110	Clear	Karoo	5WEP
P4	2.40	NW, Med slope	10	101-14 MGT	Clear	Karoo/Shale	5WEP
P5	3.00	SW, Med slope	11	R110	Low	Karoo/Shale	5WEP
P6	7.50	SE, Med slope	12	Varia	Clear	Karoo/Shale	4WEP
P7[6]	1.18	Flat	10	101-14 MGT	Clear	Karoo	4WEP
P8[6]	3.37	Flat	11	R110	Clear	Karoo	4WEP
P9[6]	2.58	Flat	12	R110	Medium	Karoo	4WEP
M1	12.20	Flat	8	R110	Clear	Soft Karoo/Alluvial	6WEP
M2	6.70	Flat	11	R110	Clear	Karoo	5WEP
M3	3.36	Flat	12	R99	Medium	Rock Terrace	5WEP
M4	6.40	Flat	8	R110	Low	Soft Karoo/Alluvial	4WEP
M5	3.50	Flat	8	R110	Low	Soft Karoo/Alluvial	4WEP
M6	8.20	Flat	8	US 8-7	Low	Karoo	5WEP
M7[6]	1.76	Flat	11	R110	Low	Alluvial	4WEP
M8[6]	1.93	Flat	13	R99	Low	Rock/Karoo	4WEP
M9[6]	3.05	Flat	12	R99	Low	Rock/Karoo	4WEP
M10[6]	3.57	Flat	12	R110	Medium	Karoo	4WEP
M11[6]	1.15	Flat	10	R110	100% infected	Alluvial	4WEP
M12[6]	4.90	Flat	10	R110	Medium	Karoo	5WEP
C1	3.49	Flat	9	US 8-7	Clear	Karoo/Shale	2 W H
C2	4.14	Flat	9	R110	Clear	Karoo	4WEP
C3	4.52	NE, Med slope	17	101-14 MGT	100% infected	Karoo/Shale	5WEP
C4	5.04	Flat	11	R110	Clear	Karoo/Shale	4WEP
C5	2.24	Flat	11	R110	Clear	Karoo	5WEP
C6	3.80	Flat	10	US 8-7	Clear	Karoo/Shale	4WEP
C7	2.40	NW, Med slope	10	101-14 MGT	Clear	Karoo/Shale	5WEP
C8	11.87	Flat	10	Varia	Medium	Karoo/Shale	5WEP
C9	2.16	Flat	8	R110	Clear	Karoo	4WEP
C10	13.36	S, Steep slope	12	101-14 MGT	Medium	Karoo/Shale	5WEP
C11	3.10	Flat	9	R110	Medium	Karoo/Shale	2 WH
C12	17.00	NE, Med slope	11	R110	Medium	Karoo/Shale	5WEP
S1	1.80	Flat	10	R110	Low	Karoo	4WEP
S2	5.00	Flat	12	R110	Clear	Karoo/Shale	4WEP
S3	3.57	Flat	8	R110	Clear	Soft Karoo/Alluvial	5WEP

Table 3.1 (cont.)

Sample code:	Size Ha[1]	Orientation[2]	Age (2007)[3]	Rootstock[4]	Virus infection (Leaf roll)	Soil	Trellis system[5]
S4	5.99	Flat	8	R110	Clear	Karoo/Shale	6WEP
S5	3.40	Flat	11	R110	Clear	Karoo	5WEP
S6	7.20	S, Med slope	9	R110	Clear	Karoo/Shale	4WEP
S7	7.32	Flat	10	Ramsey	Medium	Karoo/Shale	5WEP
S8	1.02	Flat	9	R110	100% infected	Karoo	2 WH
S9	3.74	S, Steep slope	11	R110	Low	Karoo	5WEP
S10	3.63	S, Steep slope	7	R110	Clear	Karoo	7WEP
S11	3.31	Flat	10	R110	Clear	Karoo	4WEP

[1]Ha: Hectare;
[2]Orientations as follows, SE: South Eastern; NW: North Western; SW: South Western; NE: North Eastern; S: Southern; Med: Medium;
[3]Age from planting date until 2007;
[4]Rootstock abbreviations as follows, R99: Richter 99; R110: Richter 110; 101-14 MGT: 101-14 Millardet Et De Grasset ; US 8-7: USVIT 8-7;
[5]Trellis systems abbreviated as follows, 4-7 WEP: 4-7 wire extended Perold system; 2 WH: double wire Hedge system;
Not included in 2007;

Grapes were sampled at commercial harvest from demarcated vines in selected blocks. Each vineyard block used during this survey was subdivided into two to three sites, depending on the size of the vineyard (Figure 3.2). Each site was placed at least five rows from the outside of the block and a minimum of two sections inside the block from the start of each row. The sites consisted of 40 vines from which five berries were picked which resulted in a sample size of 200 berries per site, thus 600 berries per block (Figure 3.2). The berries were removed from the top, bottom, furthest left, furthest right and from the backside of the growth zone from each vine. Of the 200 berries collected from each site 50 berries were weighed and homogenised (IKA®-Werke GMBH & CO.KG, Staufen, Germany) at 20 000 rpm for four minutes. A mini trial was initially done with regards to the time needed for complete homogenisation. A sub sample were immediately analysed for pH, ºB, TA and the rest of the berries were stored as homogenates at -20ºC.



A **B**

Figure 3.2 Each vineyard block was divided into 3 sites for sampling (A). Each of these sites was divided into 4 rows containing 10 vines, therefore 40 vines per site (B).

3.3.2 Analyses of grape samples

An ethanol extraction (50% v/v) was made using 1 g homogenate and 10 mL solution. This was left to extract for 1 hour during which the samples were shaken every ten minutes. After centrifugation 10 mL 1 M HCl was added to 1 mL of the supernatant and left for 3 hours after which the samples were analysed at 520 and 280 nm on the spectrophotometer (Analytic Jena Specord 50 UV-VIS Spectrophotometer; Jena, Germany). The resulting answers of anthocyanin and total phenol content were used to validate the quality of the homogenate. The coefficient of variation for anthocyanins concentration (mg/g) decreased from 10% to below 5% with an increased from three to four minutes homogenisation (data not shown).

Anthocyanin and total phenol concentrations of the grapes were analysed using Iland's method of grape extraction and acidification followed by measuring the absorbance at 280 nm and 520 nm (Iland *et al*., 2000). Chemicals used were 1M HCL (32% Hydrochloric acid, Merck Chemicals PTY, Ltd) and 50% ethanol (Absolute Ethanol, Merck).

The grape tannin analyses were done by using methyl cellulose precipitation (MCP Tannins, mg/L, Sarneckis *et al*., 2006) in 2007 and bovine serum albumin precipitation (BSA Tannins, mg/L, Harbertson *et al*., 2003) in 2008/2009. We chose the BSA method for further analyses because we found the method more reliable and repeatable than the MCP method. For the MCP method methyl cellulose (Sigma Aldrich Chemie, Steinheim, Germany) dissolved in distilled water, ammonium sulphate (Merck) and epicatechin (Sigma) for the standard curve were used. For the protein precipitation method only the tannin portion of the method were performed; the colour measurements were therefore excluded. The chemicals used in this precipitation method were glacial acetic acid (Saarchem, Merck), sodium chloride (Saarchem, Merck), potassium tartrate (Fluka, Sigma Aldrich), ethanol (Absolute Ethanol, Merck Chemicals

PTY, Ltd), triethanolamine (Fluka, Sigma Aldrich), Sodium dodecyl sulphate (Fluka, Sigma), iron chloride (Radchem), sodium sulphate (Merck), bovine serum albumin (Sigma) and catechin (Sigma) for the standard curve. All pH adjustments of the buffers for the BSA method were done using either 1M HCL (32% Hydrochloric acid, Merck) or 1M sodium hydroxide (Saarchem, Merck).

HPLC analyses for the determination of monomeric flavanols (Gallocatechin, Catechin, Proanthocyanin B1, Epicatechin, Proanthocyanin B2, Epicatechingallate), polymeric flavanols (large peak at 280 nm), total grape flavanols (sum of monomeric flavanols and polymeric flavanols), free anthocyanins (Delphinidin, Cyanidin, Petunidin, Peonidin and Malvidin as glucosides, acetoglucosides and coumaroyl glucosides), polymeric pigments (large peak at 520 nm) and the total colour pigments (sum of free anthocyanins and polymeric pigments) were done using ethanol (50%v/v) extracted grape samples after pH adjustment (Table 3.2). It was analysed using an Agilent 1100 series RP-HPLC system (PLRPS-S 100Á 3µm, 150 x 4.6mm column) with a diode array detector (Peng *et al*., 2002).

Table 3.2 Description of parameters measured with the HPLC for the grape and wine samples.

Parameter	Range
Monomeric flavanols	Sum of phenolic compounds at 280 nm, excluding the polymer
Polymeric flavanols	Large peak at 280 nm
Total grape flavanols	Sum of phenolic compounds at 280 nm
Free anthocyanins	Sum of pigments measured at 520 nm, excluding the polymer
Polymeric pigments	Large peak at 520 nm
Total colour pigments	Sum of colour pigments at 520 nm

Routine grape juice analyses were done on the juice after crushing of the grapes by hand. Sugar (ºBrix) were measured using a refractometer (Atago, Pocket PAL-1), while the pH and TA were measured using a Metrohm titration system (Metrohm, Titrino 702 SM).

Topographical and data maps were created using ArcGIS 9.3.1 (ESRI). The maps were made by using the GPS points of each block and connecting that wit the actual colour or phenolic data of that particular block. The concentration of a specific parameter was shown by different colours and the areas between the blocks were extrapolations between these GPS point data.

All statistical calculations were done using Statistica software version 9 (Microsoft).

3.4 Results and Discussion

3.4.1 Distribution of colour, phenolic content, ºB, TA and pH for all seasons and cultivars

The ºB at which the grapes were picked ranged between 18.7-27.0ºB (Table 3.3). This variability could influence the levels of phenolic compounds in the grapes, especially when samples of the lower ripeness ranges are compared to those in the higher sugar ranges. Of the 108 samples compared in this study only two samples were below 20.8ºB, both in 2007, while 13 were above 25ºB (2 blocks in 2007, 3 blocks in 2008 and 8 blocks in 2009). When these outlier samples (removed for all data reported) was removed the average sugar level in the grapes during this study was 23.67ºB, with a range of 20.8-25.0ºB. The titratable acidity (TA) and pH were found to have a negative correlation of 0.45 (r^2 value) indicating a higher pH led to lower TA values as expected. The TA ranged between 3.45 and 8.38 g/L for 2007 and 2009, while the pH ranged within 1.2 units for all the samples analysed in this study. Differences such as these are normally a function of different cultivars and time of the season that maturity is reached (Bramley, 2005).

The monomeric flavanol concentrations including all 3 seasons and 4 cultivars ranged between 0.01-0.60 mg/g berry weights (Table 3.3). These results correlated with those of Mattivi *et al*., (2009), whom found levels ranging between 0.06-0.32 mg/g berry for Merlot, Cabernet Sauvignon and Shiraz grapes. In the study by Mattivi *et al*., (2009) study the average monomeric flavanols levels were approximately 0.20 mg/g. When comparing the monomeric flavanols in our study to this average it was found that 73% of the Robertson data were below this average, despite the wider range.

Strong correlations were founds between polymeric flavanols (HPLC) and the total grape flavanols (HPLC) of the grape extracts (r^2=1.00) hence only the latter will be discussed (Table 3.3). The total grape flavanols from the Robertson area ranged between 1.12-7.14 mg/g berry weight for the 2007-2009 harvest period with about 33% of the blocks having values below 2 mg/g. In the East of Australia Hanlin *et al*. (2009) found tannin concentrations (Total grape flavanols (HPLC)) to range between 0.7 mg/g berry when ripe to 7.1 mg/g berry when phenolically unripe. Ripe values were more or less between 0.7 mg/g and 1 mg/g for this Cabernet and Shiraz grapes. Another author (Jensen *et al*., 2008) indicated levels of 0-4.5 mg/g berry, while Mattivi *et al*. (2009) showed average levels of about 0.6-3.3 mg/g berry. The total flavanols measured spectrophotometrically (total flavanols) had a much smaller range 0.45-1.88 mg/g for the same samples from 2007-2009 and did not correlate with the HPLC readings (r = 0.20 (neg)). According to a study by Bramley (Bramley, 2005) on Australian red cultivars the range was 0.45-2.48 mg/g, using the same measurement technique.

Therefore the total colour pigments and free anthocyanins are closely correlated and only the free anthocyanins will be discussed (r= 0.99). The levels for 2007-2009 were between 0.12-1.75 mg/g berry weight, which is in the same range as the 0.6-0.9 mg/g berry weight (average 0.75 mg/g) found by Romero-Cascales *et al*. (2005) in Cabernet, Shiraz and Merlot from South Eastern Spain. Another author (Segade *et al*., 2008) showed the anthocyanin content to be between 0.16 and 1.45 mg/g berry weight (average 0.81 mg/g) in their quest to understand the assessment of phenolic ripeness. The average for our study was 0.67 mg/g which was less that those found by the above-mentioned authors. The anthocyanin concentration as measured using the Iland method (Iland, 2000) did not correlate well the HPLC values (r=0.27), but was found to be in a similar range than the HPLC values (0.39-1.95 mg/g berry weight). In another study on red grape varieties the anthocyanin concentration measured by the same technique varied between 0.58-3.71 mg/g berry weight (Bramley, 2005), clearly indicating the higher colour found in these grapes.

Table 3.3 Ranges, averages and standard errors of Colour and phenolic compounds, sugar concentration (ºB) and total acidity (TA) of all the vineyards selected for this survey of Robertson grapes for all three seasons.

Parameter (n=108)[1]	Range	Mean ± SD[6]
Monomeric flavanols	0.01-0.60	0.17±0.07
Polymeric flavanols	1.04-7.03	2.96±0.12
Total grape flavanols	1.12-7.14	3.13±0.12
Free anthocyanins	0.12-1.75	0.67±0.03
Polymeric pigments	0.00-0.33	0.08±0.00
Total colour pigments	0.19-1.99	0.75±0.03
Anthocyanin[2]	0.39-1.95	0.85 ±0.05
Total phenols[3]	0.45-1.88	1.20 ±0.08
Tannin	0.22-8.18	2.24±0.09
º B[4]	18.7-27.0	23.8 ±0.21
pH	3.23-4.40	3.69 ±0.02
TA[5]	3.45-8.38	5.21 ±0.45

[1]All data are expressed in mg/g berry weight except for Anthocyanin, Total phenols, ºB, pH and TA;
[2]Anthocyanin in mg/g malvidin-3-glucoside units;
[3]Total phenols in mg/g absorbance units;
[4]Degrees Balling;
[5]g/L;
[6]standard deviation.

3.4.2 Seasonal differences in the distribution of colour, phenolic compounds, ºB, TA and pH.

The distribution of the colour and phenolic content of the selected vineyards were greatly influenced by season. After multivariate analyses of all the parameters measured, it was found that during 2007 the data were spread over a larger range than what was found in the 2008 and 2009 seasons (Figure 3.3). This variation was driven by polymeric pigments, polymeric flavanols and total flavanols (all by HPLC analyses). To illustrate this point the 2007 polymeric flavanol range were 1.23-7.03 while the ranges were 1.31-5.05 and 1.04-3.47 in 2008 and 2009 respectively (Table 3.4). Similar trends were seen with regards to the total grape flavanols and the polymeric pigments.

Parameters that were correlated in viewing the data from the perspective of season were free anthocyanins and total grape pigments, polymeric and total grape flavanols and a reverse correlation were found between pH and total acidity.

Significant differences were found for each parameter due to seasonal influences. The polymeric flavanols, total grape flavanols, free anthocyanins and total colour pigments differed significantly between the three seasons, with 2007 being the highest in concentration. In general polymeric and total flavanols were higher in 2008 than 2009, while free anthocyanins and total grape pigments showed the opposite tendency.

Polymeric pigments and pH were significantly higher in 2007 compared to the other two years, while the total phenols (Iland method) were significantly lower in 2007. Anthocyanins (determined by the Iland method) and sugar content showed a significant difference between 2007 and 2009, with 2008 falling somewhere in between. The anthocyanin level in 2007 showed an average of 0.74 mg/g berry weight versus the 0.97 mg/g observed in 2009. In 2007 the sugar concentration was significantly lower (23.4 ºB) than in 2009 (24.3 ºB). The removal of three outlier blocks sampled in 2007 (Blocks P6, S1 and S8) shifted this average to 23.7 ºB, which was no longer significantly different from the other seasons. Degree balling is connected to the sugar per berry and the volume of the berry, without which seasonal influence on anthocyanin level can only be, speculated (Wang *et al*., 3003). In another study on production norms of Robertson grape cultivars the grapes were picked each year at 23 ºB, but the amount of days from veraison to that point were also depicted. They found that for some seasons a specific block reached 23 ºB faster than others due to the climatic influence (van Schalkwyk & De Villiers, 1999), therefore at an increased ripening rate. Some authors have found that faster ripening rate is in most cases connected to a decrease in berry volume (Deloire, 2011).

Monomeric flavanols and total acidity did not differ significantly for 2007 and 2009, but differing significantly from 2008 levels. The free anthocyanins were lower during 2008 (0.14 mg/g) than for 2007 and 2009 (both 0.20 mg/g average). In the case of total acidity the highest acidity levels were found during 2008 (average 5.56 g/L) in comparison with the 5.28 g/L of 2007 and the 4.95 g/L of 2009.

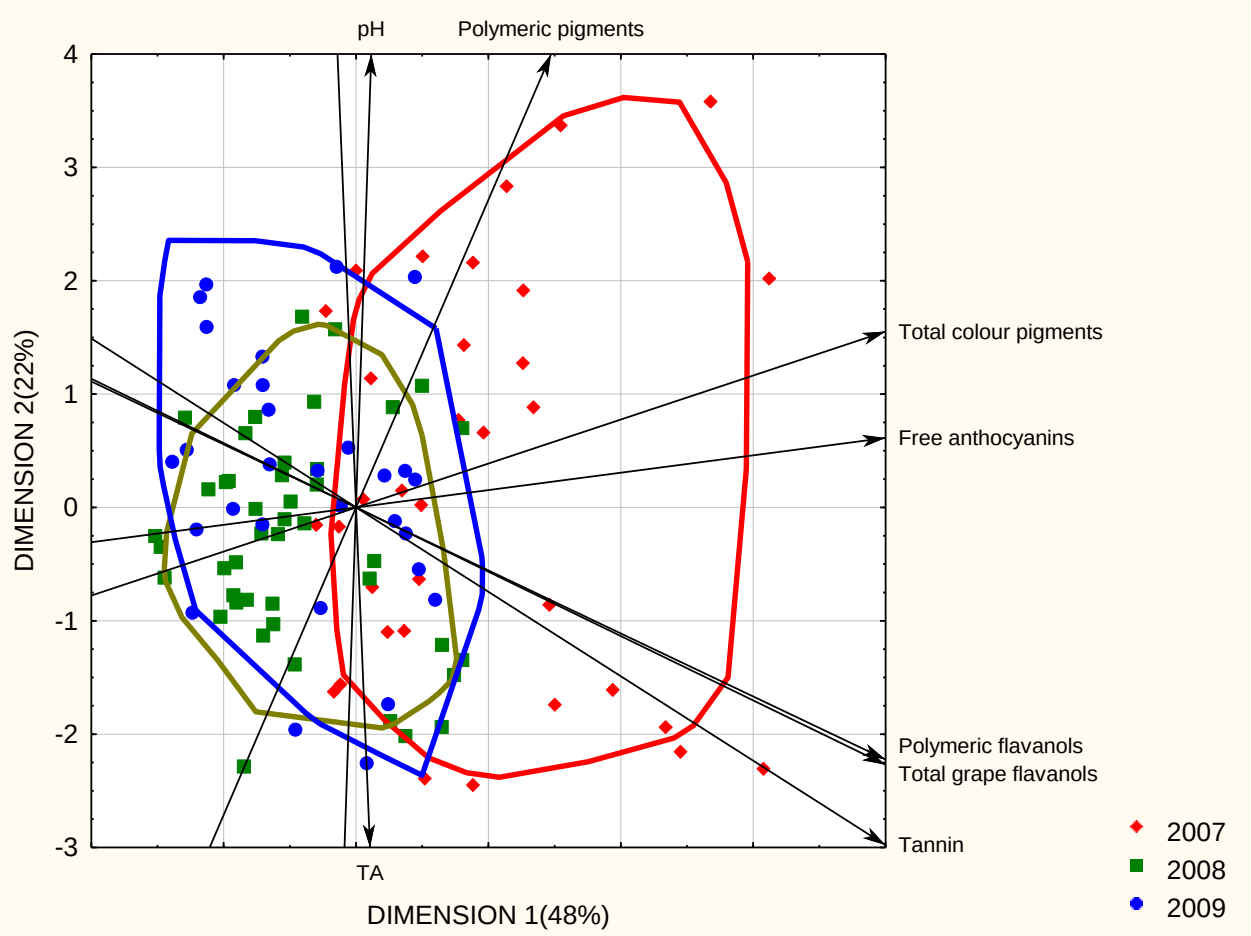


Figure 3.3 Distribution of grape berry parameters during the 2007-2009 harvest seasons. Lines drawn around data points indicate a 95% confidence level, points outside these lines are deemed outliers for the specific season.

Table 3.4 Ranges, averages and standard errors of colour and phenolic compounds, sugar concentration (ºB), pH and total acidity (TA) of Pinotage, Merlot Cabernet Sauvignon and Shiraz grapes during the 2007, 2008 and 2009 harvests in the Robertson wine region.

Parameter[1]	2007		2008		2009	
	Range	Means ± SD[6]	Range	Means ± SD	Range	Means ± SD
Monomeric flavanols	0.03-0.40	0.20[a]±0.02	0.01-0.32	0.14[b]±0.01	0.02-0.60	0.20[a]±0.02
Polymeric flavanols	1.23-7.03	4.08[a]±0.16	1.31-5.05	2.84[b]±0.14	1.04-3.47	2.11[c]±0.17
Total grape flavanols	1.33-7.14	4.28[a]±0.17	1.44-5.24	2.98[b]±0.14	1.12-3.99	2.30[c]±0.18
Free anthocyanins	0.41-1.75	0.87[a]±0.04	0.12-1.15	0.46[b]±0.04	0.33-1.25	0.75[c]±0.04
Polymeric pigments	0.00-0.33	0.09[b]±0.01	0.04-0.17	0.08[a]±0.01	0.04-0.12	0.07[a]±0.01
Total colour pigments	0.48-1.99	0.96[a]±0.05	0.19-1.23	0.54[b]±0.04	0.40-1.33	0.82[c]±0.05
Anthocyanin[2]	0.40 -1.22	0.74[a] ±0.05	0.39 - 1.44	0.86[ab] ±0.04	0.50 – 1.95	0.97[b] ±0.05
Total phenols[3]	0.45 -1.35	0.98[b] ±0.08	0.77 - 1.81	1.31[a] ±0.07	0.48 -1.88	1.28[a] ±0.09
Tannin	1.78-8.88	4.57±0.14	0.22-2.03	1.20±0.12	0.50-2.97	1.44±0.14
ºB[4]	18.7 - 26.7	23.4[a] ±0.22	21.8 - 26.3	23.8[ab] ±0.18	20.8 – 27.0	24.3[b] ±0.22
pH	3.30 - 4.40	3.72[b] ±0.02	3.37 - 4.11	3.64[a] ±0.02	3.23 - 4.05	3.63[a] ±0.03
TA[5]	3.45 - 6.71	5.28[a] ±0.16	3.48 - 8.38	5.56[b] ±0.13	3.60 - 7.27	4.95[a] ±0.16

[1]All data are expressed in mg/g berry weight except for Anthocyanin, Total phenols, ºB, pH and TA;
[2]Anthocyanin in mg/g malvidin-3-glucoside units;
[3]Total phenols in mg/g absorbance units;
[4]Degrees Balling;
[5]g/L;
[6]Standard deviation.

Table 3.5 Geographical maps of the Robertson region overlaid with the actual total colour pigment (A) and total grape flavanols (B) data as found for the 2007, 2008 and 2009 harvests.

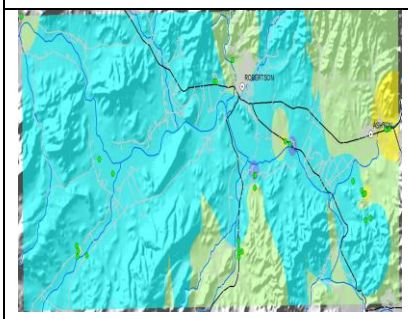
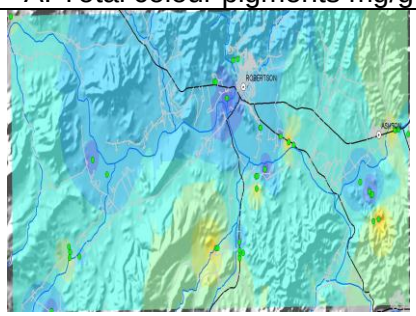
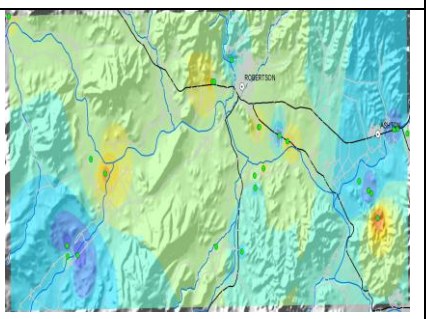
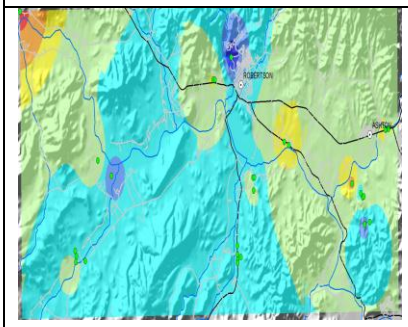
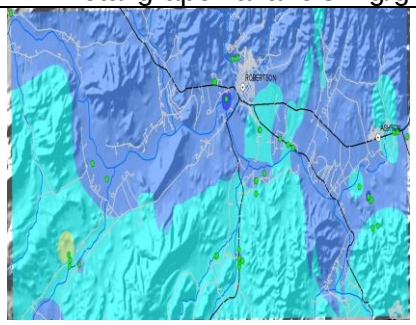
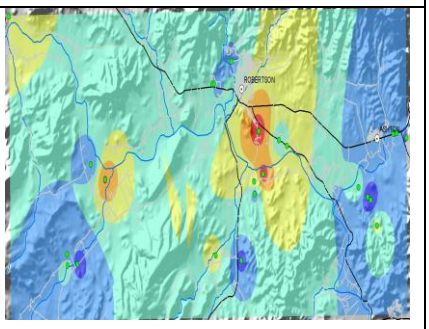
2007	2008	2009
Low  ***High***		
A: Total colour pigments mg/g		
		

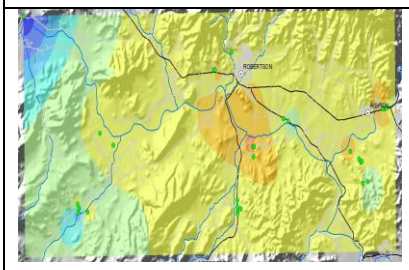
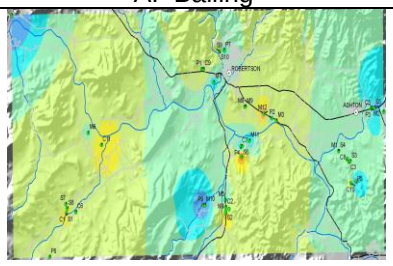
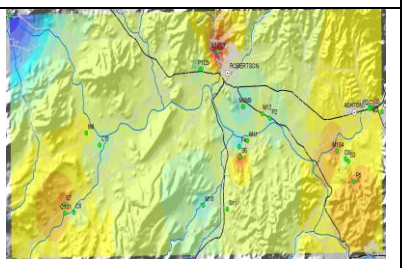
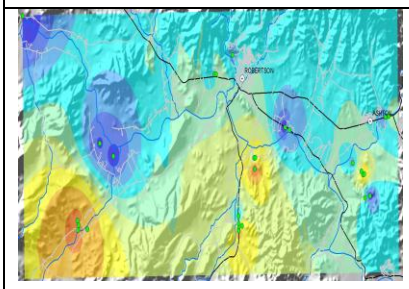
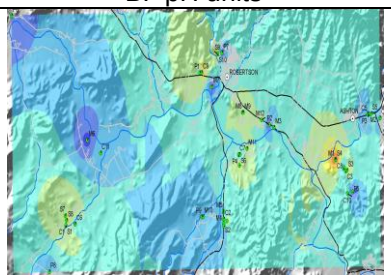
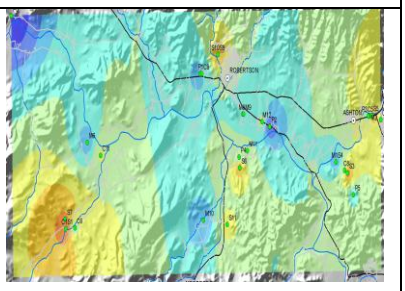
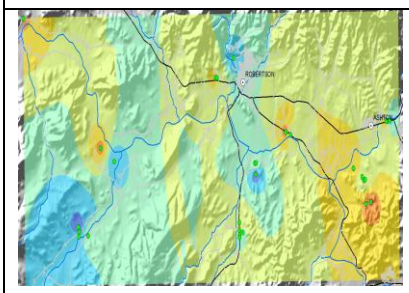
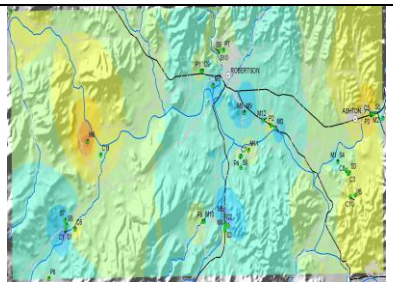
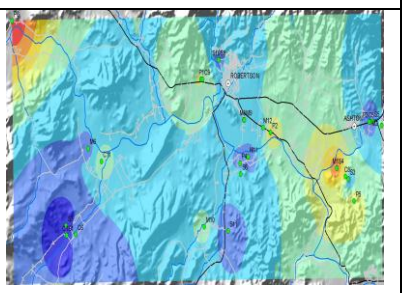
Table 3.5 (cont.)

2007	2008	2009
Low  ***High***		
B: Total grape flavanols mg/g		
		

The use of data mapping presents results in a more visual fashion on a large scale (Table 3.5). Due to the fact that the data were not collected in a grid-like manner covering the whole Robertson grape growing area there are some limitation in the interpretation of the data. The coloured areas in between the data points (blocks) are just a function of the average values of the components measured at the actual geographical positions of the blocks evaluated, therefore extrapolated data. As mentioned above the free anthocyanins and the total colour pigments correlated for the seasonal data, the same is true for the polymeric and total grape flavanols. Only one of each of these correlating parameters was therefore used to be illustrated using the GPS points. The total colour pigments and total grape flavanols were found to be significantly different in all three seasons (according to Table 3.5). This is then also visible from the data maps overlaying the topographical maps of the Robertson area. During 2007 and 2009 the average total colour pigments (0.96 and 0.82 mg/g berries respectively) were higher than in 2008 (0.54 mg/g). Small zones of high colour pigment levels are visible from the 2007 and 2009 maps, whilst a more moderated spread of total colour pigments can be seen in 2007.

The average total grape flavanols were higher in 2007 (4.28 mg/g berry) than in 2008 (2.98 mg/g) and 2009 (2.30 mg/g). The latter two seasons were both lower on average than 2007 but still statistically significantly different. The data ranges were from widest to closer 2007>2008>2009. Lower values of total grape flavanols tend to be linked to phenolically riper grapes (Hanlin *et al*., 2009). From the GPS maps 2007 had less dark blue areas (low total grape flavanols) than the other seasons, and all the statistically significant difference between the seasons are also completely visible.

Table 3.6 Geographical maps of the Robertson region overlaid with the actual Brix (A) pH (B) and titratable acidity (TA)(C) data as found for the 2007, 2008 and 2009 harvests.

2007	2008	2009
Low  ***High***		
A: Balling		
		
B: pH units		
		
C: TA		
		

The areas where high sugar levels were experienced can clearly be defined from the lower balling regions. For instance it was found that the balling in the 2007 season was significantly different from that of the 2009 harvest season (Table 3.6). It is clear from the visual data that in 2007 higher balling were reached in the centre (close to town) areas of the region that was covered by this survey, while in 2009 the higher balling levels were found on the outskirts of the region. With regards to pH the levels was found to be different between 2007 and the other two seasons. It seems from the spatial data mapping that in 2007 and 2009 is approximately similar and different in 2008, but from the statistical data 2008 and 2009 us different and lower on average than 2007. The average TA in 2008 was significantly different from the other seasons.

In 2008 the TA were at moderate levels throughout the region, while in the other two seasons there were more specific zones with high TA or low TA values.

Mapping of data over a region like Robertson would have been more viable when more data points would have been selected in the region. This should be done by establishing a virtual grid over the area and sampling vineyards in every square kilometre to get the complete picture. This would provide the area with valuable information like for instance, pin pointing certain niche areas of high colour or tannin, or identifying problem areas, or special pH, TA zones.

3.4.3 Cultivar differences in the distribution of colour and phenolic compounds

The distribution of colour and phenolic compounds as well as acidity (pH and TA) of the different cultivars showed that great variability did exist (Figure 3.4). A positive correlation were found between the levels of polymeric flavanols, total grape flavanols and tannins as was the case in the seasonal data. The positive correlation between the polymeric and total grape flavanols were due to the fact that very little monomeric flavanols were measured and therefore the total grape flavanols is almost a reflection of the polymeric flavanols in the grapes. The tannins which were precipitated by BSA (BSA, 2007 data not included in subsequent calculations) correlated with the HPLC results since more or less the same size order of flavanols were measured with these two methods. In other work positive correlations between BSA grape tannins and total grape flavanols as measured by the HPLC (50% ethanol grape extracts) have also been shown (Mercurio & Smith, 2008). The same was found for the free anthocyanins and total grape pigments, while pH and TA showed a high negative correlation with regards to each other. The Polymeric pigments did not correlate with any of the other colour measurements and the levels of these compounds present in grapes are normally extremely low. No significant differences were found between the average levels of anthocyanins and total phenols as measured using Iland's method (Iland *et al*., 2000) and would therefore not be further mentioned in discussion regarding cultivar differences. Shiraz had a much wider distribution of data points than the other cultivars for the combined data of the three seasons. This distribution was mostly driven by polymeric pigments and in some instances pH. Apart from inherent cultivar differences this could also be a function of the later ripening stages of Shiraz grapes versus the other cultivars. A wider distribution in the Pinotage was driven by tannins, polymeric flavanols, total grape flavanols and TA being higher. In this case when disregarding inherent cultivar characteristics it could be related to the fact that Pinotage is basically the first red grapes that are picked in a winemaking season. The Merlot, Cabernet Sauvignon and Pinotage blocks

expressed a much tighter data range than Shiraz. The data points (blocks) that are pulling the data outwards are all from the 2007 season and therefore also related to seasonal variance.

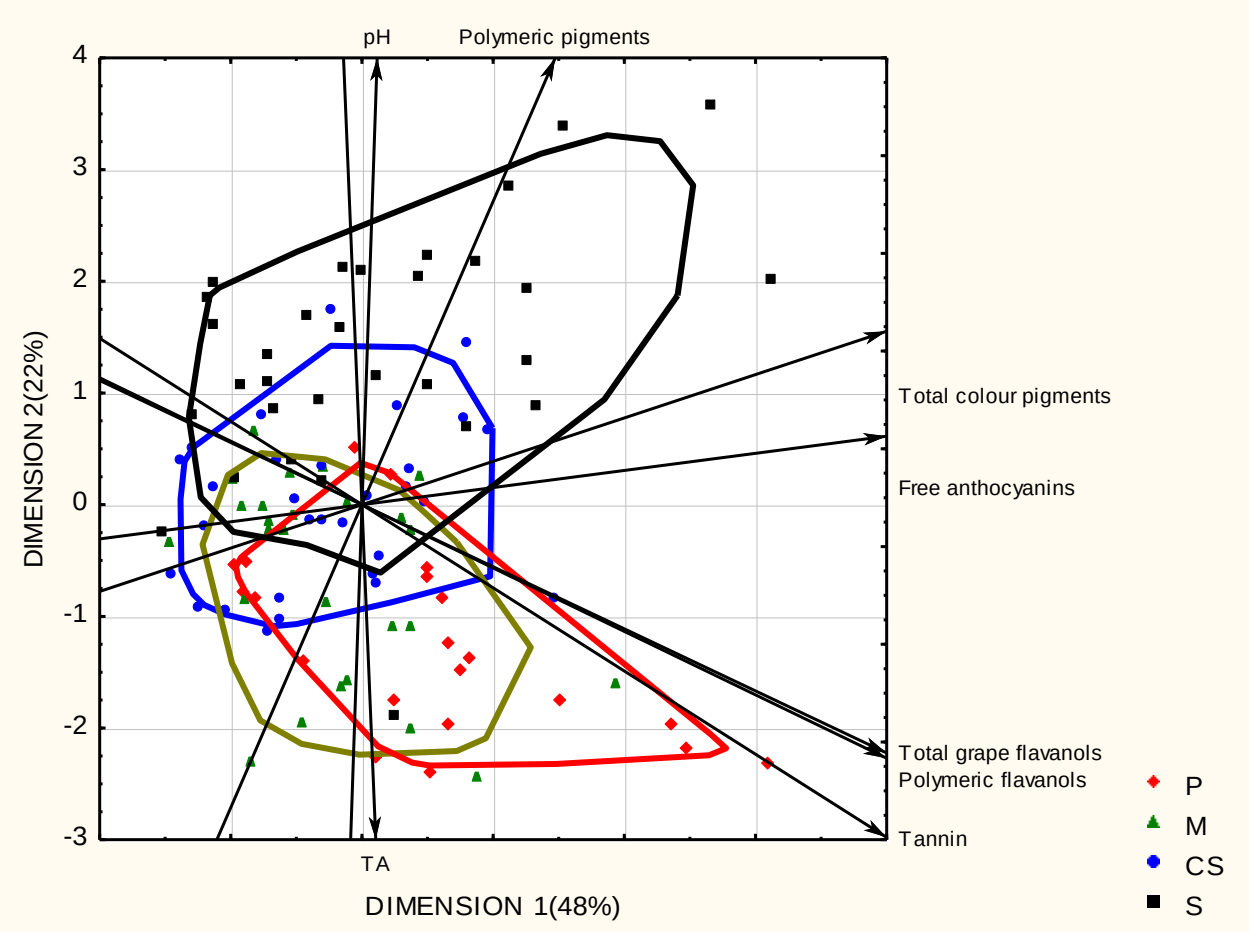


Figure 3.4 The distribution of grape berry measurements during the 2007-2009 harvest seasons, separated for each cultivar. P: Pinotage; M: Merlot; CS: Cabernet Sauvignon; S: Shiraz. Lines drawn around data points indicate a 95% confidence level, points outside these lines are deemed outliers for the specific cultivars. 2007 Tannin data was not included in calculations.

Table 3.7 The ranges, average levels and standard error of colour and phenolic compounds due to cultivar difference measured over 3 harvest seasons (2007-2009) in Pinotage, Merlot, Cabernet Sauvignon and Shiraz grapes from the Robertson wine region.

	Pinotage		Merlot		Cabernet Sauvignon		Shiraz	
Compound/ Parameter[1]	**Range**	**Average/ SD[6]***	**Range**	**Average/ SD***	**Range**	**Average/ SD***	**Range**	**Average/ SD***
Monomeric flavanols	0.01-0.33	0.16^{ab} ±0.02	0.08-0.60	0.24^{c} ±0.02	0.05-0.56	0.20^{ac} ±0.02	0.02-0.35	0.12^{b} ±0.02
Polymeric flavanols	1.78-7.03	3.68^{a} ±0.20	1.43-6.28	3.14^{ab} ±0.19	1.04-4.02	2.43^{b} ±0.17	1.19-5.05	2.79^{b} ±0.17
Total grape flavanols	1.95-7.14	3.83^{a} ±0.21	1.60-6.52	3.39^{ab} ±0.19	1.12-4.26	2.63^{c} ±0.18	1.21-5.24	2.91^{bc} ±0.17
Free anthocyanins	0.31-1.52	0.88^{c} ±0.05	0.15-0.90	0.57^{a} ±0.05	0.18-1.04	0.62^{ab} ±0.05	0.12-1.35	0.69^{b} ±0.04
Polymeric pigments	0.05-0.14	0.08^{a} ±0.01	0.00-0.11	0.05^{b} ±0.01	0.03-0.14	0.07^{ab} ±0.01	0.06-0.20	0.11^{c} ±0.01
Total colour pigments	0.36-1.63	0.96^{a} ±0.06	0.20-0.94	0.62^{b} ±0.05	0.22-1.13	0.69^{b} ±0.05	0.19-1.42	0.81^{a} ±0.05
Anthocyanins[2]	0.61-1.73	0.89 ±0.06	0.39-1.95	0.83 ±0.06	0.60-1.90	0.87 ±0.05	0.44-1.93	0.84 ±0.05
Total phenols[2]	0.48-1.88	1.23 ±0.10	0.56-1.55	1.20 ±0.10	0.75-1.77	1.26 ±0.09	0.45-1.81	1.31 ±0.08
Tannin	0.77-8.18	2.98^{a} ±0.17	0.62-6.89	2.76^{ab} ±0.16	0.50-5.32	1.99^{b} ±0.14	0.22-4.82	1.89^{b} ±0.14
ºB[3]	18.7-26.7	23.2^{a} ±0.27	21.9-26.1	23.8^{ab} ±0.25	21.2-25.6	24.0^{b} ±0.23	20.9-27.0	24.3^{b} ±0.22
pH[4]	3.23-3.68	3.44^{a} ±0.03	3.38-3.88	3.56^{b} ±0.03	3.36-4.11	3.70^{c} ±0.03	3.53-4.40	3.95^{d} ±0.03
TA[5]	4.18-7.27	6.04^{b} ±0.20	4.00-8.38	5.25^{a} ±0.18	3.52-7.00	5.16^{a} ±0.17	3.45-7.63	4.60^{c} ±0.16

[1]Data shown in mg/g berry weight except for Anthocyanins, Total phenols, ºB, pH and TA;
[2]Anthocyanin in mg malvidin-3-glucoside/g and Total phenols as absorbancy units (A280)/g;
[3]Degrees Balling;
[4]pH units;
[5]g/L;
[6]Standard deviation significant at a 5% level ($p<0.05$).

Pinotage had the highest average polymeric phenol (3.68 mg/g), total grape flavanols (3.83 mg/g), free anthocyanin (0.88 mg/g), total colour pigments (0.96 mg/g), tannin (2.98 mg/g) and total acidity (6.04 g/L) content of all four cultivars investigated (Table 3.7). In a study that compared the malvidin-3-glucoside and procyanidin (B1) content of Cabernet Sauvignon, Shiraz and Pinotage it was found that the latter had much higher levels of these components (Rossouw & Marais, 2003). Pinotage were harvested at the lowest average sugar levels (23.2ºB) and pH of 3.44 of all four cultivars.

Merlot was found to be lowest in colour components between the four cultivars. It had average levels of 0.57, 0.05 and 0.62 mg/g berry weight for free anthocyanins, polymeric pigments and total colour pigments respectively. This was also found in a study that compared the free anthocyanin levels between other red cultivars and Merlot, where the latter was found to have the lowest content at 0.58 mg/g (Romero-Cascales *et al*., 2005). However, the Merlot blocks had the highest average monomeric flavanol content at 0.24 mg/g berry weight compared the other three cultivars with average levels ranging between 0.12-0.20 mg/g (Table 3.7). For the other components Merlot had intermediate levels in comparison with the other cultivars. Cabernet Sauvignon were found to have intermediate levels of all the components measured during this study in comparison with the other cultivars.

Shiraz had the highest average levels of pH (3.95), ºB (24.3) and polymeric pigments (0.11). For the pH and Balling these results were the inverse of those found for Pinotage (Table 3.7). The lowest tannin (1.89 mg/g) and titratable acidity (4.60 g/L) were also found in the Shiraz blocks. In another study that compared the tannin content of Cabernet Sauvignon, Shiraz and Merlot it was found that Shiraz and Merlot had low tannin content (1.3 mg/g and 1.0 mg/g respectively), while the Cabernet Sauvignon had the highest tannin level at 2.2 mg/g (Romero-Cascales *et al*., 2005). Shiraz ripened at the end of the harvesting season and were marked by high pH's and lower acidity.

3.4.4 Blocks that was found to be outliers from the data set due to seasonal or cultivar influence

After statistical analyses of the data set, including all parameters over all three seasons and for all the cultivars, blocks that were found to be outliers from the data set were identified (95% confidence level) (Table 3.8). When the data was analysed from the perspective of seasonal influence there were 11 blocks that did not fit the data set at a 95% confidence level in 2007, 9 blocks in 2008 and 4 blocks in 2009. Most likely the environmental factors could have varied more in 2007 when compared with 2008 and 2009. This was indeed visible in the temperature

data (Figure 6, next section) especially during the crucial ripening months of January to April. Site specifics could also play a major role especially in cases where the same block was found to be an outlier for more than one season. For instance Pinotage 2 and 5, Merlot 6 and Shiraz 4 and 5 were such examples, which were identified over more than two seasons as outliers. The two Pinotage blocks (2 and 5) seem to fit the average site specifics of all 9 Pinotages selected for this survey: Average size, one flat and one sloped, same age, rootstocks differed, clear and low virus infection and Karoo soils. According to the parameters analysed these Pinotage blocks stood out due to extreme tannin and acid levels in comparison with the other blocks in 2007. Merlot 6 was the only Merlot planted on a USVIT 8-7 rootstock, one of the youngest and largest blocks of Merlot analysed during the study. These distinguishing site specifics are possible factors that could have led to higher total acidity and tannin content compared to other blocks. The effects of rootstocks on grape composition are not well known but are probably related to rootstock vigor and therefore it influences the growth and canopy exposure (Jackson & Lombard, 1993). Rootstocks promoting more vigorous growth yielded fruit with higher nitrogen, acidity, tannin and K levels and lower pH (Ough *et al*., 1968), while the devigorating rootstocks produced higher wine scores (McCarthy & Cirami, 1990). The rootstock is mostly influenced by the soil water status and therefore vine water status. This ultimately influences the vigour or rather berry growth, ripening and composition (Ollat *et al*., 2002; Ojeda *et al*., 2001). Shiraz 4 and 5 were higher in polymeric pigments and pH than the rest of the groups. They differed in size, age and soil type but were similar in orientation, rootstock and virus infection level. High polymeric pigments and pH could indicate that the grapes were harvested last or very late in the season and was overripe. For both blocks the degrees balling ranged between 22 and 27 over the three years, while the average sugar level for Shiraz throughout the study was approximately 24.

Table 3.8 A: Blocks that was found to be outliers with respect to the data set, from the aspect of seasons (Refer figure 3). B: Blocks that were outliers from the 95% confidence level due to cultivar differences (Refer figure 5).

	2007	2008	2009
A	**Blocks outlying due to seasonal differences**		
Pinotage[1]	P2, P3, P5	P2 AND P5	P6
Merlot[2]	M4, M5, M6	M2, M6, M7	M1
Cabernet Sauvignon[3]	C7 AND C11	NONE	C8
Shiraz[4]	S3, S4, S5	S1, S5, S6, S7	S4
B	**Blocks outlying due to cultivar differences**		
Pinotage	P3, P5	P4	P3, P4, P6
Merlot	M1, M6	M3, M6, M7	M11
Cabernet Sauvignon	C1, C7, C9	C5, C8	C6, C8
Shiraz	S3, S5, S6	S5, S2	S3, S5

[1]Pinotage blocks: P1-6, 6 Pinotage blocks from different farms in the Robertson area;
[2]Merlot blocks: M1-11, 11 Merlot blocks from different farms in the Robertson area;
[3]Cabernet Sauvignon blocks: C1-12, 12 Cabernet Sauvignon blocks from different farms in the Robertson area;
[4]Shiraz blocks: S1-12, 12 Shiraz blocks from different farms in the Robertson area.

When the outliers were determined from the perspective of the cultivar it was found that 4/9, 5/12, 5/12 and 4/12 of the Pinotage, Merlot, Cabernet Sauvignon and Shiraz blocks respectively fell outside the 95% confidence interval for all parameters measured in this study.

More specifically Pinotage 3 and 4, Merlot 6, Cabernet 5 and 8 as well as Shiraz 3 and 5 fell outside the 95% confidence level more than once. The Pinotage blocks again showed this tendency due to its high tannins and acidity, while Merlot 6 had a unique rootstock. Cabernet Sauvignon 8 was the second largest block sampled during this survey and was one of two blocks sampled planted on a steep slope and had a medium virus infection. Cabernet 8 was also the only Cabernet Sauvignon planted on a Varia rootstock, further distinguishing it from the rest. This block stood out due to very low levels of total colour pigments and free anthocyanins both in 2008 and 2009. Vines freed from major viruses are healthier and give higher yields and improved wine quality (Becker, 1985), therefore infected vines could be connected to restricted sugar and colour development in grape berries.

Other unique blocks not featuring as outliers were also found in this data set. With regards to block size Cabernet Sauvignon 10 and 12 were also > 10 ha in size and did not appear as outliers in any instance. Age plays an important role in the overall yield and quality of grapes. Block C3 was 17 years old versus the other blocks which were between 8-13 years and in no instance it was reported as an outlier. Blocks M11, C3 and S8 were 100% visually infected with leaf roll virus, which impacts the colour developments of such blocks negatively. M11 stood out in 2009 due to higher free anthocyanins and total colour pigments than the other blocks,

completely the inverse of what should be expected. Trellis systems are important for aeration and ensure proper bud and bunch exposure (May & Antcliff, 1963). Of the selected blocks for this study 3 blocks were 2 wired hedge systems for lower vigour vines they were in no case found to be outliers. The same goes for S10 on a 7 wire extended Perold trellis system, S7 that were the only Shiraz on a Ramsey and P6 were the only Pinotage on a Varia rootstock. The vigour of the vines is mostly controlled by irrigation practices which seem to be the case in these vineyards. Vine water status and especially the creation of water deficit periods at pivotal times in the ripening stage is key to colour and phenolic development of grape berries as well as overall grape and wine quality (Myburgh, 2006; Bindon *et al*., 2011).

3.4.5 Influence of environment and site on colour and phenolic content

To discuss the colour and phenolic content and the distribution thereof during the 2007-2009 harvesting period properly, the influence of the environment and site specific conditions should also be taken into account (Table 3.9).

Table 3.9 Average values of grape parameter data when statistically classified into main groups of orientation, soil and trellis systems of the 47 selected vineyards of the Robertson area.

	Orientation		Soil		Trellis system	
Grape parameter[1]	Flat	Slope	Karoo	Karoo/Shale	4WEP	5WEP
Polymeric flavanols	2.44	2.31	**2.31**	**2.47**	2.46	2.35
Total grape flavanols	2.58	2.45	**2.43**	**2.62**	2.60	2.48
Free Anthocyanins	0.51	0.56	0.55	0.56	0.51	0.53
Total colour pigments	0.57	0.63	0.61	0.63	0.57	0.59
Polymeric pigments	0.06	0.07	0.07	0.07	0.06	0.07
Anthocyanins[2]	0.86	0.84	**0.84**	**0.89**	0.85	0.82
Total phenols[3]	1.23	1.25	1.20	1.21	1.16	1.34
Tannin	1.71	1.69	**1.59**	**1.83**	1.59	1.78
^{o}B[4]	23.9	23.8	23.8	23.9	23.6	23.9
pH[5]	3.70	3.68	3.69	3.71	3.69	3.69
TA[6]	5.14	5.39	5.25	5.25	5.15	5.38
RW Grading[7]	4.24	4.45	**4.43**	**4.55**	4.12	4.40

[1]All data expressed in mg/g berry weight except Anthocyanins, total phenols, brix, pH, TA and RW grading;
[2]Anthocyanins: mg/g malvidin-3-glucoside;
[3]Total phenols: mg/g absorbancy units;
[4]^{o}B: Degrees Balling measured with balling meter;
[5]pH in pH units;
[6]TA: in g/L;
[7]RW Grading: Robertson Winery grading numerical value between 1 and 6, 1 pertaining to low quality and 6 to high quality.

Various environmental factors could influence the biosynthesis of flavonoids in grapes, such as light, temperature, altitude, soil type, water, nutritional status, microbial interactions, pathogenesis, wounding, defoliation, plant growth regulators as well as various developmental processes (Downey *et al*., 2006). Site/Vine specific data were obtained from the Robertson vineyards that were selected for this study and statistically analysed to determine if any of these factors correlate in some way with the levels of the parameters measured. It was found that there were a significant negative correlation between the level of virus infection (leaf roll) versus the colour of grapes (Free anthocyanins, $p< 0.04$; Total colour pigments, $p<0.03$). This means that with an increased intensity of virus infection the actual colour of the grapes at harvest decreased. The orientation, soil and trellis systems were divided into 2 main groups each to create large enough groups for improved statistical analyses (Table 3.9). The results showed that no significant differences exist between any of these groups (flat vs slope; karoo vs karoo/shale; 4WEP vs 5WEP), but rather that trends could be observed in some instances. For example a higher average level of all parameters measured was detected in the Karoo/Shale soils when compared to the levels found in the Karoo soils. Other authors have found that soil characteristics can affect grape quality dramatically; especially fertile soils that can cause higher yields (Winkler *et al*., 1974). Other authors that tested the effect of different soils on anthocyanin content of grapes could not find significant infliuences (Yokotsuka *et al*., 1999). However, it must also be kept in mind that these results are also impacted by the small datasets analysed.

The temperature for the Robertson area was constantly monitored using permanent weather stations throughout the area. The data showed that during 2008 the average maximum temperature (28.4 ºC) was lower than during 2007 and 2009 (29.4 and 29.1 ºC) (Figure 3.5). Further the variability in maximum temperature during 2007 and 2008 (standard deviation (SD) of ±2.2 and ±1.9 ºC respectively) was higher than what was experienced during 2009 (±1.6 ºC SD). Another important observation was that the data from the Le Chasseur and Zandtvliet weather stations were found to be on the higher end of the average maximum temperature range (29.1-30.2 ºC), while the De Hoop and Boesmanspad stations represents the lower end of the maximum temperature range for all three seasons (27.0-28.8 ºC) (Figure 5). The Le Chasseur and Zandtvliet stations are close to blocks P8, M6,C1, C6, C11, S1, S7, S8 and P3, M2, C5, C12, S5 respectively and are therefore exposed to the most extreme maximum temperatures every season, while the De Hoop station are close to blocks P7, S9, S10 and represents the lower spectrum of max temperature each season. In some instances the blocks in the higher temperature regions were associated with lower colour and visa versa.

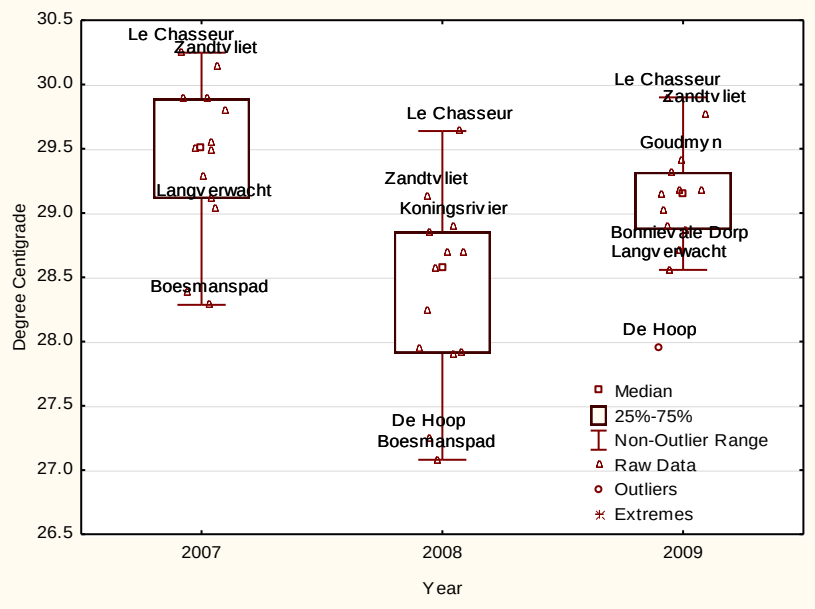


Figure 3.5 The average maximum temperature distribution for Jan to April 2007, 2008 and 2009 from 13 weather stations in the Robertson area.

Another important factor to consider in the development of the grapes during 2008 was the greater rainfall range that was experienced (Figure 3.6). Literature indicates that an area with an annual precipitation < 700 mm is good for grape/wine quality (which Robertson clearly falls under) and that high rainfall and excessive irrigation lowers quality (Jackson & Schuster, 1987). In the case of this region the annual rainfall is so low that irrigation is compulsory and this should be managed correctly during the growing season (Myburg, 2006; Laget *et al*., 2008). It has been shown in literature that with increased water availability, the pH and potassium level of must and wine can increase and that anthocyanin and therefore colour can be reduced (Bravdo *et al*., 1985; Freeman, 1983; Matthews & Anderson, 1986 and Morris & Cawthon, 1982). In another article irrigation had very little effect on the total anthocyanin concentration of grapes (Sipiora & Gutierriez Granda, 1998).

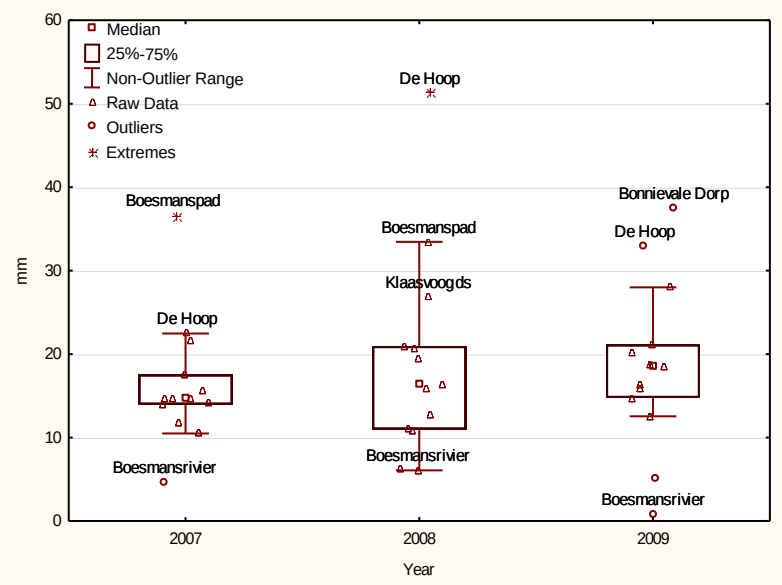


Figure 3.6 Average rainfall and the distribution thereof for the months of January to April during the 2007-2009 harvest seasons.

3.5 Conclusion

In the Robertson region the average monomeric flavanol level of grapes from the Robertson area were found to be much lower than in other studies, despite the wider range. The total grape flavanols were high and could be an indication of the grapes not reaching phenolic maturity, despite the fact that the grapes were otherwise (Sugar, pH, TA) ripe to be harvested. The decision to harvest should be based on the wine style that is desired as well as the sales category the particular wine will fall in. Not only sugar, pH, and TA should be taken into account, but the actual level of ripeness of the flavonoids should be taken into account. In literature various publications exist on the actual measurement techniques available to test for phenolic ripeness. The total colour was also found to be lower than that of other authors. This could be due to higher temperatures and that colour development in the berries was slowed down metabolically. Seasonal impact on the development of colour and phenolic compounds was evident. There were also cultivar and block differences that came into play. Some blocks were outliers due to intrinsic differences in their site or rootstock. The use of colour and phenolic data in the Robertson region for the determination of grape quality at harvest is important due to the fact that such great variability exist. Geographical mapping further demonstrated how vastly the data is distributed through the region. This work can serve as a valuable basis for future research on colour and phenolic characteristics of red grapes in the

Robertson area. Follow on research would entail whether differences observed between vintages, cultivars and blocks are also reflected in the corresponding wines.

3.6 References

Bakker, J., Bridle, P., Timberlake, C.F. & Arnold, G.M., 1986. The colours, pigment and phenol contents of young port wines: Effects of cultivar, season and site. Vitis. 25: 40-52.

Becker, H., 1985. Wine grape varieties for cool climates. In: Proc. Int. Symp. Cool Clim Vitic. Enol. Heatherbell, D.A., Lombard, P.B., Bodyfelt, F.W. & Price, S.F. (Eds.). Oregon State Uni. Exp. Stat. Tech. Pub. No. 7628. pp. 46-62.

Bindon, K., Myburgh, P., Oberholzer, A., Roux, K. & du Toit, C., 2011. Response of grape and wine phenolic composition in *Vitis vinifera* L. cv. Merlot to variation in grapevine water status. S. Afr. J. Enol. Vitic. 32(1); 71-88.

Bramley, R.G.V., 2005. Understanding variability in wine grape production systems (2): Within vineyard variation in quality over several vintages. Aust. J. Grape Wine Res. 11: 33-42.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S. & Tabacaman, H., 1985. Effect of irrigation and crop level on growth, yield and wine quality of Cabernet Sauvignon. Am. J. Enol. Vitic. 36:132-139.

De Freitas, V.A.P. & Glories, Y., 1999. Concentration and compositional changes of procyanidins in grape seeds and skin of white Vitis vinifera varieties. J. Sci. Food Agric. 79: 1601-1606.

Deloire, A., 2011. The concept of berry sugar loading. Wineland Magazine. January. pp. 93-95.

Downey, M.O., Dokoozlian, N.K. & Krstic, M.P., 2005. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. Am. J. Enol. Vitic. 57(3): 257-268.

Du Toit, W.J., Lisjak, K., Marais, J. & Du Toit, M., 2006. The effect of micro-oxygenation on the phenolic composition, quality and microbial composition of South African red wines. S. Afr. J. Enol. Vitic. 27: 57-67.

Freeman, B.M., 1983. Effects of irrigation and pruning of Shiraz grapevines on subsequent red wine pigments. Am. J. Enol. Vitic. 34: 23-6.

Gonzalez-San Jose, M.L., Santa-Maria, G. & Diez, C., 1990. Anthocyanins as parameters for differentiating wines by grape variety, wine-growing region, and wine-making methods. J. Food Comp. Anal. 3: 54-66.

Guidoni, S., Allara, P. & Schubert, A., 2002. Effect of cluster thinning on berry skin anthocyanin composition of Vitis vinifera cv. Nebbiolo. Am. J. Enol. Vitic. 53: 224-226.

Hanlin, R.L. & Downey, M.O., 2009. Condensed tannin accumulation and composition in skin of Shiraz and Cabernet Sauvignon grapes during berry development. Am. J. Enol. Vitic. 60 (1): 13-23.

Harbertson,JF, Picciotto, EA & Adams, DO., 2003. Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching**.** Am. J. Enol. Vitic. 54: 301-306.

Hunter, J.J. & Bonnardot, V., 2011. Suitability of some climatic parameters for grapevine cultivation in South Africa, with focus on key physiological processes. S. Afr. J. Enol. Vitic. 32(1): 137-154.

Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. "Techniques for chemical analysis and quality monitoring during winemaking." (Patric Iland wine Promotions, Campbelltown, Adelaide, Australia).

Jackson, D.I. & Lombard, P.B., 1993. Environmental and management practices affecting grape composition and wine quality-A Review. Am. J. Enol. Vitic. 44(4): 409-430.

Jackson, D., & Schuster, D., 1987. Production of grapes in cool climates. Butterworths, N.Z.

Jensen, J.S, Demiray, S, Egebo, M & Meyer, A.S., 2008. Prediction of wine color attributes from the phenolic profiles of red grapes (Vitis vinifera). J. Agric. Food Chem. 56: 1105-1115.

Kliewer, W.M., 1970. Effect of day temperature and light intensity on coloration of Vitis vinifera L. grapes. J. Am. Soc. Hort Sci. 95: 693-697.

Kliewer, W.M. & Torres, R.E., 1972. Effect of controlled day and night temperatures on coloration of grapes. Am. J. Enol. Vitic. 23: 71-77.

Laget, F., Tondut, J-L, Deloire, A. & Kelly, M.T., 2008. Indications of climate evolution in a Mediterranean area – considerations for the wine and viticulture sectors. J. Int. Sci. Vigne. Vin. 42: 113-123.

Marais, J. & October, F., 2005. Relationship between grape colour and wine quality. Wynboer, 2005. 191: 15-17.

Matthews, M.A., & Anderson, M.W., 1988. Fruit ripening in Vitis viniferaL.: responses to seasonal water deficits. Am. J. Enol. Vitic. 39:313-320.

Mattivi, F., Vrhovsek, U, Masuero, D. & Trainotti, D., 2009. Aust. J. Grape Wine Res. 15: 27-35.

May, P. & Antcliff, A.J., 1963. The effect of shading on fruitfulness and yield of sultana. J. Hort Sci. 38:85-94.

McCarthy, M.G. & Cirami, R.M., 1990. The effect of rootstocks on the performance of Chardonnay from a nematode-infested Barossa Valley vineyard. Am. J. Enol. Vitic. 41:126-130.

McDonald, M.S., Hughes, M., Burns, J., Lean, M.E.J., Matthews, D. & Crozier, A., 1998. Survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. J. Agric. Food Chem. 46: 368-375.

Mercurio, M.D. & Smith, P.A., 2008. Tannin Quantification in Red grapes and wine: comparison of polysaccharide- and protein based tannin precipitation techniques and their ability to model wine astringency. J. Agric. Food Chem. 56: 5528-5537.

Morris, J.R. & Cawthon, D.L., 1982. The effect of irrigation, fruit load, and potassium fertilization on yield, quality, and petiole analysis of Concord (*Vitis labrusca* L.) grapes. Am. J. Enol. Vitic. 33: 145-148.

Myburgh, P.A., 2006. Juice and Wine Quality Responses of *Vitis vinifera* L. *cvs.* Sauvignon blanc and Chenin blanc to timing of irrigation during berry ripening in the coastal region of South Africa. S. Afr. J. Enol. Vitic. 27(1): 1-7.

Ojeda, H., Deloire, A. & Carbonneau, A., 2001. Influence of water deficits on grape berry growth. Vitis. 40(3): 141-145.

Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A. & Deloire, A., 2002. Influence of pre- and postveraison Water Deficit on Synthesis and concentraton of Skin phenolic compounds during berry Growth of Vitis vinifera cv. Shiraz. Am. J. Enol. Vitic. 53: 261-267.

Ollat, N., Diakou-Verdin, P., Carde, J-P., Barrieu, F., Gaudillère, J.P. & Moing, A., 2002. Grape berry development : a review. J. Int. Sci. Vigne. Vin. 36: 109-131.

Ough, C.S., Lider, L.A. & Cook, J.A., 1968. Rootstock-scion interactions concerning wine making. I. Juice composition changes and effects on fermentation rate with St. George and R99 rootstocks at two nitrogen fertilizer levels. Am. J. Enol. Vitic. 19: 213-227.

Peng, Z., Iland, PG., Oberholster, A., Sefton, MA., & Waters, EJ., 2002. Analysis of pigmented polymers in red wine by reverse phase HPLC. Aust. J. Grape Wine Res. 8: 70-75.

Peynaud, E., 1996. The Taste of Wine. The Art and Science of Wine Appreciation. 2nd ed. Wiley & Sons, New York.

Prie, AJG., 1977. Phenolics Accumulation in Red Wine Grapes (*Vitis vinifera* L.). Thesis, University of Sydney.

Revilla, E., Alonso, E. & Kovac, V., 1997. The content of catechins and procyanidins in grapes and wines as affected by agro-ecological factors and technological practices. Am. Chem. Soc., Washington, DC. In Wine: Nutritional and Therapeutic Benefits. T.R. Watkins (ed.). pp. 69-80.

Robichaud, J.L. & Noble, A.C., 1990. Astringency and bitterness of selected phenolics in wine. J. Sci. Food Agric. 53: 343-353.

Romero-Cascales, I., Ortega-Regules, A, López-Roca, J.M., Fernández-Fernández, J.I. & Gómez-Plaza, E., 2005. Differences in Anthocyanin Extractability from grapes to wines according to variety. Am. J. Enol. Vitic. 56(3): 212-219.

Rossi, J.A., Jr. & Singleton,V.L., 1966. Flavor effects and adsorptive properties of purified fractions of grape seed phenols. Am. J. Enol. Vitic. 17:240-246.

Rossouw, M. & Marais, J., 2004. The phenolic composition of South African Pinotage, Shiraz and Cabernet Sauvignon Wines. S. Afr. J. Enol. Vitic. 25(2): 94-104.

Sarneckis, C.J., Dambergs, R.G., Jones, P., Mercuric, M., Herderich, M.J. & Smith, R.A., 2006. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimized tool for grape and wine analysis. Aust. J. Grape Wine Res. 12: 39-49.

Segade, S.R., Rolle, L., Gerbi, V. & Orriols, I., 2008. Phenolic ripeness assessment of grape skin by texture analysis. J. Food Comp. Anal. 21: 644-649.

Singleton, V.L., 1987. Oxygen with phenols and related reactions in musts, wine and model systems: Observations and practical implication. Am. J. Enol. Vitic. 38: 69-77.

Sipiora, M.J. & Gutierriez Grande, M-J., 1998. Effect of Pre-verasion irrigation cutoff and skin contact time on the composition, color and phenolic content of Young Cabernet Sauvignon Wines in Spain. Am. J. Enol. Vitic. 49: 152-162.

Somers, T.C. & M.E. Evans. 1974. Wine Quality: Correlation with colour density and anthocyanin equilibria in a group of young red wines. J. Sci. Food Agric. 25: 1369-1379.

Turner, J. 2009. http://www.howtodothings.com/home-garden/how-to-prevent-grape-growing-mistakes

Van Schalwyk, D., De Villiers, F.E. & De Villiers, F.S., 1999. The influence of production norms on the wine quality of wine grape cultivars in the Robertson area: Ruby Cabernet. Wynboer article. http://wineland.co.za/wynboer_archive/recentarticles/1099influence.html

Wang, Z., Deloire, A., Carbonneau, A., Federspiel, B. & Lopez, F., 2003. Study of Sugar Phloem Uploading in Ripening Grape berry under Water Stress conditions. J. Int. Sci. Vigne Vin. 37(4): 213-222.

Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lieder, L.A., 1974. General viticulture. University of California Press, Berkeley.

Yokotsuka, K. Nagao, A., Nakazawa, K. & Sato, M., 1999. Changes in Anthocyanins in Berry skins of Merlot and Cabernet Sauvignon grapes Grown in two soils modified with limestone or oyster shell versus a native soil over two years. Am. J. Enol. Vitic. 50(1): 2-12.

Chapter 4

Correlations between grapes and wines from the Robertson area of South Africa: Colour, phenolic content and sensorial contribution

Chapter 4: Correlations between grapes and wines from Robertson area of South Africa: Colour, phenolic content and sensorial contribution

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4.1 Abstract

The use of phenolic compounds to increase the number of parameters used for grading of grapes at the point of harvest is accepted as a positive contribution for decision making in wine production. Knowledge of the average, minimum and maximum values of these parameters is the first step to attempt this. Secondly, a positive correlation should exist between the phenolic compounds of grapes and the corresponding wines. In this investigation the grapes and wines of 44 different vineyard blocks of varying sizes was monitored over three seasons (2007-2009). Four main red cultivars were used namely Pinotage, Merlot, Cabernet Sauvignon and Shiraz. Grapes were harvested at approximately 24°B and wines were prepared using standard winemaking techniques. Various spectrophotometric as well as high performance liquid chromatography techniques were employed to determine colour and other phenolic levels of the grapes and wines. The data was analysed from a combined, seasonal and cultivar perspective. It was found that various significant positive correlations exist between the grapes and wines made thereof, while season as well as cultivar can influence this relationship. More positive correlations between grapes and wines were established for colour and anthocyanins than was found for other phenolic compounds like total phenols and tannins. Colour density was found to result in positive correlations in almost all instances (all data together, seasons and cultivars separated) as well as anthocyanins measured with HPLC.

4.2 Introduction

The colour of red wine is the first indicator of its quality when it is evaluated by a potential consumer (Somers & Evans, 1974; Du Toit *et al*., 2006). The mouth feel or structure of a red

wine, which to a large extent is influenced by the tannin and phenolic content, is also an important quality contributor (Gawel, 1998; Rossouw & Marais, 2004; Ristic *et al*., 2007). In grapes, anthocyanins and other phenols are located in the skins and seeds of the berries. In theory, the amount of colour and phenolic compounds occurring in the berry at the point of harvest determines the colour and structure in the corresponding wine produced from these berries. Therefore, the colour/phenolics of the berry should also be indicative of the potential colour and phenolic composition of the resultant wine. Various factors, such as the climate, viticultural practices, vineyard position and cultivar, will influence the development of these compounds during ripening, whilst winemaking techniques could influence the extraction of these important compounds from the berry into the wine.

The use of the anthocyanin and tannin concentration of grapes as a tool to predict wine "quality" has been a topic of interest for some time. In co-operative cellars, the grapes are received from various producers in the surrounding grape-growing areas. These producers are remunerated according to the quality of the vineyard block and the grapes that are delivered to the co-operative cellar. The quality of the vineyard block is determined, in turn, by the viticulturist and the winemaker employed by the co-operative cellar. Therefore, discrepancies concerning remuneration may occur. A quality-grading system should include aspects covering the terroir of a block, the visual appearance of the vineyard at pivotal stages and the chemical constitution of the grapes at harvest. Producers should be able to determine which improvements will lead to increased payment for their grapes on the basis of a standardised, objective and repeatable grading process. In short, quality should be quantified in the vineyard (Gishen *et al*., 2001). The chemical potential of grape juice at harvest is currently not fully determined and therefore cannot be exploited for information on wine potential. The total soluble solid (TSS) indicating the sugar constitution and acid concentration (pH, TA) form the basis of the chemical analyses performed on the grapes. However, there is a need to introduce and apply other factors pertaining to the quality of grapes and wines, of which colour and phenolic compounds could be important examples. In Australia, for instance, colour has been studied and used over the past decade as part of a multivariate system to assess grape maturity and resulting wine quality (Mercurio *et al*., 2010). The time required for the analyses of these compounds was the main reason for not including it as part of routine analyses in big laboratories. An array of wet chemistry steps are necessary to determine colour and phenolic compounds using spectrophotometry (spec) and high performance liquid chromatography (HPLC) (Singleton, 1988; Somers & Vérette, 1988). Lately, Fourier transform infrared spectroscopy has made the prediction based on absorbance spectrum of such compounds extremely easy and rapid. In

South Africa, research over the past few years has shown that anthocyanins and other phenolic compounds in grapes can be determined rapidly without the application of wet chemistry (Dambergs *et al*., 2006; Lochner, 2006).

In South Africa, the use of colour as a quality indicator has been under investigation since 2000, starting with a preliminary study of 16 different Pinotage samples (Marais *et al*., 2001). This work was extended to include Cabernet Sauvignon and Shiraz grapes from the Robertson area, and small-scale wines were produced (no malolactic fermentation) from these grapes for sensory assessment (Marais & October, 2005). It was found in both instances that grape colour theoretically could predict wine quality. Both these studies also stated that future research should include more areas, cultivars and seasons to improve the prediction models that currently exist. This topic has also been under investigation internationally, but consensus on the usability of such predictions has not been reached as yet (Romero-Cascales, 2005; Jensen *et al*., 2008; Mercurio *et al*., 2010).

The aim of this study was to use a large number of samples over three consecutive vintages from the Robertson area to determine the possibility that these compounds in grapes can serve as useful indicators of the colour and phenolic composition of the finished red wines. The correlation between colour and phenolic compounds in the grapes and corresponding wines was thus evaluated. It was also important to determine whether the correlations found were repeatable over seasons and if there were certain cultivars that performed better in this regard. The correlation between the colour and phenolic content of the grapes and wines, as well as both the quality grading of the grape blocks and their wines were assessed.

4.3 Materials and Methods

4.3.1 Selected Vineyards

At the time of the 2007 harvest, Pinotage (C1-6), Merlot (M1-6), Cabernet Sauvignon (C1-11) and Shiraz (S1-11) blocks were selected by Robertson Winery (a large co-operative cellar in the Robertson wine area of South Africa) to be investigated in 2007, 2008 and 2009. The number of selected blocks increased in 2008 and 2009, but included those blocks monitored during 2007. The final number of selected blocks was 9, 12, 12 and 11 for Pinotage, Merlot, Cabernet Sauvignon and Shiraz respectively (Table 4.1). The blocks were selected with the aim of obtaining a sample set with diverse quality grading as allocated to the blocks by the head viticulturist of Robertson Winery. Six rankings in terms of quality are used for red vineyard blocks by Robertson Winery (descending quality order): Vineyard Collection (VC), A blocks, B

blocks, C blocks, Dry Red (DR) blocks and Rosé blocks. The grading values are determined by inspecting the physical aspects of the vineyard throughout the growing season and close to harvest, as well as taking into account the history of each of the blocks with regard to vineyard practices, yield, etc. Each of the selected blocks was divided into three plots containing 40 vines each. The plots were sampled regularly in order to harvest the grapes at approximately 24ºB (refer to Chapter 3 for more detail). In 2007 there were large variations in ripeness level due to unplanned irrigation (raining and irrigation by the farmer) of the grapes at pivotal stages before picking, but for the following two seasons most of these problems did not occur. Grapes from the three plots of each vineyard were combined to form the sample that represents a specific vineyard block. The grapes were combined homogenously and a sample was taken as the grape component, while the rest were vinified to form the wine component of this correlation study.

Table 4.1 The chemical characteristics of the grape juice from Pinotage (P1-9), Merlot (M1-12), Cabernet Sauvignon (C1-12) and Shiraz (S1-11) blocks, chosen for this survey (2007 to 2009) after crushing and the quality grading of the blocks.

	Harvest 2007			Harvest 2008			Harvest 2009			Grading[4]
Sample code:	ºB[1]	pH[2]	TA[3]	ºB	pH	TA	ºB	pH	TA	'07/'08/'09
P1	23.80	3.47	6.21	23.80	3.55	5.69	23.40	3.39	5.88	A
P2	24.20	3.32	6.29	23.40	3.38	6.96	23.30	3.28	6.21	A/A/B
P3	24.30	3.34	6.50	23.60	3.45	6.30	23.70	3.68	5.15	B
P4	26.70	3.66	5.38	22.50	3.63	6.13	21.80	3.59	4.18	VC
P5	21.90	3.30	6.71	22.70	3.37	6.16	25.80	3.55	5.90	B
P6	18.70	3.32	6.03	22.60	3.42	6.26	20.81	3.23	7.27	Rosé
P7	-	-	-	23.30	3.42	5.69	26.00	3.55	5.46	-/B/B
P8	-	-	-	24.00	3.53	5.52	26.30	3.49	5.99	-/B/B
P9	-	-	-	22.10	3.45	6.84	23.80	3.37	5.42	-/B/B
M1	24.80	3.54	5.95	22.90	3.56	4.47	26.10	3.42	7.08	A/A/B
M2	24.60	3.59	5.00	24.30	3.58	8.38	22.40	3.53	4.59	A/A/A
M3	21.90	3.61	5.08	24.80	3.66	4.04	24.40	3.61	4.12	B/B/B
M4	24.40	3.66	6.00	23.20	3.58	4.09	23.8	3.51	5.51	DR/DR/B
M5	24.00	3.53	5.57	22.10	3.58	4.47	24.1	3.46	6.04	C

Table 4.1 (cont.)

	Harvest 2007			Harvest 2008			Harvest 2009			Grading[4]
Sample code:	ºB[1]	pH[2]	TA[3]	ºB	pH	TA	ºB	pH	TA	'07/'08/'09
M6	23.50	3.39	6.15	23.90	3.38	7.61	24.70	3.44	4.38	B
M7	-	-	-	23.30	3.42	4.39	23.40	3.46	5.84	-/B/B
M8	-	-	-	24.00	3.63	4.00	23.10	3.57	5.01	-/B/A
M9	-	-	-	24.70	3.88	4.99	22.90	3.54	4.77	-/DR/C
M10	-	-	-	23.10	3.59	4.54	23.30	3.40	5.14	-/B/B
M11	-	-	-	23.00	3.74	6.28	24.20	3.61	4.06	-/C/DR
M12	-	-	-	25.40	3.76	4.82	24.90	3.64	4.70	-/B/B
C1	23.00	3.54	5.41	24.70	3.65	3.52	24.90	3.59	4.19	A/A/B
C2	21.20	3.66	5.98	24.40	3.60	4.21	24.80	3.68	5.78	B
C3	23.40	4.00	4.84	24.00	3.59	6.40	26.40	3.77	5.40	Rosé/Rosé/DR
C4	25.60	3.83	4.77	23.80	3.48	5.04	24.20	3.46	4.70	A
C5	23.40	3.93	4.87	23.10	3.71	7.00	24.90	3.69	4.35	A/A/A
C6	23.90	4.00	5.08	24.20	3.66	6.79	23.90	3.65	4.36	VC
C7	24.00	4.11	4.44	24.20	3.63	4.93	25.60	3.74	5.08	VC
C8	23.30	3.88	5.59	23.60	3.49	5.14	23.50	3.59	6.45	B/B/C
C9	24.20	3.96	4.73	24.70	4.10	5.51	24.40	3.56	5.05	A/A/A
C10	23.20	3.73	6.13	24.30	3.70	6.24	25.00	3.69	5.79	VC
C11	23.40	3.36	4.03	25.00	3.56	5.91	23.70	3.71	5.27	B
C12	-	-	-	23.90	3.57	4.50	24.50	3.77	5.21	-/B/B
S1	19.70	4.23	4.57	23.80	3.95	5.00	25.20	4.00	3.60	B/B/B
S2	23.80	4.25	4.75	26.30	3.53	7.63	22.20	3.66	4.85	VC
S3	23.10	4.03	4.95	24.10	3.95	6.24	24.90	4.00	3.80	C/C/B
S4	22.80	4.08	4.83	23.90	4.11	5.10	25.70	4.02	4.08	B/B/B
S5	23.50	4.01	3.70	21.80	3.74	6.24	27.00	4.05	3.97	B/B/B
S6	24.00	4.22	3.45	26.00	3.85	4.95	27.00	3.90	4.98	VC
S7	24.50	4.13	3.53	24.00	3.79	5.03	26.00	3.91	3.73	VC
S8	20.90	4.06	3.84	24.80	3.92	4.98	22.80	4.02	3.69	DR

Table 4.1 (cont.)

	Harvest 2007			Harvest 2008			Harvest 2009			Grading[4]
Sample code:	ºB[1]	pH[2]	TA[3]	ºB	pH	TA	ºB	pH	TA	'07/'08/'09
S9	23.20	4.40	4.26	24.60	3.88	5.84	24.40	3.59	4.19	VC
S10	23.60	3.58	4.08	24.80	3.74	5.75	27.00	3.89	4.36	B/B/VC
S11	22.70	4.07	4.78	24.00	3.85	3.48	24.20	3.74	4.00	B/B/B

;
[1] Ripeness of grapes measured in degrees Balling with a hydrometer after crushing of the grapes;
[2] Grape juice pH measured with a Metrohm pH meter;
[3] Titratable acidity of grape juice measured with a Metrohm titrate system and expressed as g tartaric acid /L;
[4] Gradings in order from highest to lowest: Vineyard Collection (VC), A block (A), B block (B), C block (C), Dry Red block (DR), Rosé block (Rosé);
-: blocks not samples during 2007.

4.3.2 Small-scale winemaking

Small-scale wines were prepared at the research winemaking facility of the Department of Viticulture and Oenology, Stellenbosch University by applying standard winemaking practices for all the selected blocks over three seasons. The commercial harvest of the blocks occurred at approximately 24ºB, at which time the representative sample formed by the three plots within each block was used for small-scale winemaking. An average of 22 kg of grapes was used from each block for the winemaking process. Sulphur dioxide (30 ppm, potassium metabisulphite, Everitec, Pramaggiore, Italy) was added at de-stemming and the total acidity of the musts was adjusted to 6.5 g/L using tartaric acid (natural L-(+)-tartaric acid, Bren-O-Kem (PTY) LTD, Wolseley, South Africa). Di-ammonium phosphate (30 ppm) was added as a nutrient two days after the onset of alcoholic fermentation. Alcoholic fermentation (AF) was performed in 20 L food-grade plastic bins, using *Saccharomyces cerevisiae* NT116 (Anchor Yeast, Epping Industrial, Cape Town, South Africa) at 0.3 g/L as described by the supplier. During the alcoholic fermentation, punch-downs were performed on the wines three times a day for two minutes at a time. All the wines thus received the exact same number of punch-downs per day. The skins were pressed to 1.5 bar using a basket press when the sugar concentration reached approximately 5ºB. The wines were then moved to 4.5 L glass bottles to ferment dry. For malolactic fermentation (MLF) the wines were inoculated with Viniflora oenos (CHR-Hansen, Lake International Technologies, South Africa) starter cultures at a rate of 10^6 cfu/mL as was prescribed by the supplier.

4.3.3 Analyses of grape and wine samples

Iland's method of grape homogenisation, extraction, acidification and measurements was used to determine the mg/g berry anthocyanin (Antho/g berry) and mg/g berry total phenolic concentrations (Tphen/g berry) (Iland *et al*., 2000). Homogenised grape samples were kept frozen at -20ºC until analysis.

Spectrophotometric wine colour characteristics [colour density (CD); modified colour density (MCD); total red pigment (TRP)], anthocyanin content (mg/L) and total phenol content (Tphen, 280 nm) were measured using Boulton indexes (Somers & Evans, 1974; Boulton *et al*., 2001) and a method by Ribéreau-Gayon *et al*. (1998) respectively. The grape and wine tannin concentrations were measured using methyl cellulose precipitation (MCP tannins, mg/l) (Sarneckis *et al*., 2006) in 2007 and bovine serum albumin precipitation (BSA tannins, mg/L) (Harbertson *et al*., 2003) in 2008 and 2009 (tannin).

The phenolic content of the 50% v/v ethanol grape (G) extracts obtained from the Iland method and the wines (W) was analysed by High performance liquid chromatography (HPLC). Samples were first centrifuged for five minutes at 13 000 rpm before being analysed with an Agilent 1100 series RP-HPLC system (PLRPS-S 100Å 3µm, 150 x 4.6 mm column) with a diode array detector using the conditions reported by Peng *et al.* (2002). The compounds quantified with the HPLC were divided into monomeric flavanols, polymeric flavanols, total grape flavanols, free anthocyanins, polymeric pigments and total colour pigments, as described in the table below (Table 4.2).

The total acidity and pH of the grapes and wines were determined using a Metrohm analyser (MetrohmSA, Sandton).

Table 4.2 Description of parameters measured with the HPLC for the grape and wine samples.

Parameter	Range
Monomeric flavanols (MonoFlav)	Sum of phenolic compounds at 280 nm, excluding the polymer
Polymeric flavanols (PolyFlav)	Large peak at 280 nm
Total flavanols (TotFlav)	Sum of phenolic compounds at 280 nm
Free anthocyanins (FreeAntho)	Sum of pigments measured at 520 nm, excluding the polymer
Polymeric pigments (Polypigm)	Large peak at 520 nm
Total colour pigments (TotColpigm)	Sum of all colour pigments at 520 nm (FreeAnth and Polypigm)

4.3.4 Chemicals used

To perform Iland's method, hydrochloric acid (1M, 32% HCl, Merck Chemicals PTY, Ltd) and ethanol (50% v/v, absolute ethanol, Merck) were used.

For the MCP method, methyl cellulose (Sigma Aldrich Chemie, Steinheim, Germany) and ammonium sulphate (Merck) were used, with epicatechin (Sigma) as standard.

The BSA tannin precipitation method was performed using glacial acetic acid (Saarchem, Merck), sodium chloride (Saarchem, Merck), potassium tartrate (Fluka, Sigma Aldrich), ethanol (Absolute Ethanol, Merck), triethanolamine (Fluka, Sigma Aldrich), sodium dodecyl sulphate (Fluka, Sigma Aldrich), iron chloride (Radchem), sodium sulphate (Merck) and bovine serum albumin (Sigma) with catechin (Sigma).

For the spectrophotometric colour and phenolic analyses, acetaldehyde (Merck), sodium disulphide (Fluka, Sigma Aldrich), hydrochloric acid and ethanol were used.

All pH adjustments of the buffers for the BSA method were done using either 1 M hydrochloric acid (32% HCl, Merck) or 1 M sodium hydroxide (Saarchem, Merck).

The external standards used to quantify the colour and phenolic compounds with HPLC were: (+)-catechin hydrate (Fluka), (-)-epicatechin (Sigma), and malvidin-3-glucoside (Polyphenols Laboratories AS, Norway). Monomeric, dimeric and polymeric phenols were quantified at 280 nm as mg/L catechin units with a quantification limit of 1.5 mg/L, and epicatechin as epicatechin with a quantification limit of 1.5 mg/L. Anthocyanins, pigments and polymeric pigments were quantified at 520 nm as mg/L malvidin-3-glucoside with a quantification limit of 1.25 mg/L. The detection limit was defined as a signal-to-noise ratio of 3. The quantification limit was determined as the smallest area that could be integrated accurately (< 3% standard deviation).

4.3.5 Wine tasting

After each of the three seasons, approximately four months after the completion of MLF, the wines were evaluated blind by an expert panel. This panel consisted of five members, three of whom were winemakers at Robertson Winery, and all had extensive wine-tasting experience. The same group of tasters was used for all three consecutive years. This was a panel of wine professionals doing profiles of wines from the area as recommended by Perrin *et al.* (2007). The wines were rated on colour intensity, astringency and overall perception of quality, using un-graduated line scales (10 cm) (Lawless & Corrigan, 1993; Gawel *et al*., 2000). The tasters were provided with standards for colour and astringency. The wines with the lowest and highest colour densities (CD) as measured with the spectrophotometer after MLF was used as the reference standards for colour intensity. Alum ($KAl(SO_4)_2.12H_2O$, Merck Chemicals PTY, Ltd) at

a low and high concentration (1 g/L and 10 g/L) was used as a standard for astringency. The wines were tasted per cultivar. All of the wines made from the different blocks of a cultivar were presented at once and evaluated four consecutive times by each panellist. The wines were placed in front of each taster in a randomised manner. This data was then compared to the colour and tannin constitution of the wines after MLF, as measured by the various spectrophotometric and HPLC methods, as well as the actual grading of the blocks given by the co-operative cellar. Only the Pinotage, Cabernet Sauvignon and Merlot wines were tasted in 2007, while all the wines were tasted in 2008 and 2009. In 2007 the data was analysed using normal correlations or scatterplots only. Scatterplots were also used to analyse the data in 2008 and 2009, but the data was also investigated further using PCA bi-plots to get a better overview of the possible correlations.

4.3.6 Statistical analyses

Statistical analyses were done using STATISTICA Software, Version 10 (Statsoft, Inc, Tulsa, Oklahoma, USA).

4.4 Results and Discussion

4.4.1 All cultivars and seasons analysed together

4.4.1.1 Correlations between grape and wine colour composition

When all the data from the three seasons and the four cultivars was used to establish correlations between grape and wine colour, it was found that some measurements showed highly positive correlations ($p < 0.01$; $r^2 > 0.5$), some did not correlate at all ($p > 0.01$), and some correlations were found to be significant ($p < 0.01$) but did not have high positive r^2 values ($r^2 < 0.5$) (Table 4.3).

Strong positive correlations were established especially between grape (G) and wine (W) colour for total colour pigments (G, HPLC) versus CD AF (W, Spec) and MCD AF (W, Spec). The fact that four cultivars and three seasons were included in these correlations shows the robustness of the correlation and therefore broadens the application of the results. Table 4.4 shows the large influence of cultivar and season on the actual values of the colour and phenolic characteristics. When the data was expressed from a seasonal perspective, many measurement parameters showed a significant difference between the three seasons.

The Antho/g berry measurement (G, spec) did not yield very high positive correlations with wine colour characteristics, especially when all the data was correlated together for grapes and their

corresponding wines (Table 4.3). In some of the very first work done on this topic, it was shown that Antho/g berry had a high positive correlation with CD (Iland, 1987). Another group of authors also found an r^2 value of 0.69 between Antho/g berry (Glories method; Glories, 2001) and CD (Romero-Cascales *et al*., 2005). However, these studies mostly investigated a single cultivar or one season. A preliminary study by Marais and October (2005) found not very good correlations between grape and wine colour and phenolic data when all seasons and cultivars were correlated as a unit. They also established that the correlation between grape and wine colour was influenced greatly by the ripeness (in ºB) of the grapes. The r^2 value differed (in their case) by up to 0.2 units between correlating all the data irrespective of degrees Balling, and correlating the data with higher ripeness levels with a smaller range. The same trend was also found during this study. The initial correlation between the grapes and wines was performed using the ripeness levels of the grapes, which varied between 18.7 and 27.0ºB, with an average of 23.8ºB, and included four different cultivars over three seasons. Therefore the data was filtered and correlations between the grape and wine colour of the samples with a sugar level of between 23 and 25ºB were done, with higher correlations being noted in some instances (Table 4.3). The highest correlation remained between total colour pigments (G, HPLC) and CD (W, AF), and MCD (W, AF) and total colour pigments (W, AF), which now yielded r^2 values of 0.87, 0.62 and 0.51, respectively. Most of these improvements have to do with the big impact that ripeness level can have on the colour pigments of grapes. Colour accumulates as the grapes ripen until the full colour potential is reached. The amount of colour measurable at ripeness can still decrease afterwards, mostly as a result of water content deviations and oxidation.

In more recent work that studied the correlation between grape and wine colour/phenols for Barbera and Nebbiolo grapes, positive correlations were also found (Cagnasso *et al*., 2008). However, in this study, the grapes were analysed with the Glories method (Glories, 2001) and the wines were analysed for various colour and phenolic components spectrophotometrically. All the correlations (r^2) were found to be between 0.76 and 0.95. These correlations were somewhat lower when verified against the commercial-scale wines.

It is interesting that, in most cases, the correlations were lower after MLF, although still significant. A correlation between AF and MLF (r^2 of 0.63 and 0.69, respectively and both with $p < 0.01$; Table 1.1 Addendum A) was also established. The same results were obtained by Du Toit and Visagie (2012), who also found a lower correlation between grape and wine phenolic and colour data after MLF. This is probably due to the polymerisation reactions of phenolic compounds taking place during MLF, which could lead to lower levels of anthocyanins after MLF.

Table 4.3 Correlations (r^2 values) between grape and wine colour data of the cultivars Pinotage, Merlot, Shiraz and Cabernet Sauvignon for three seasons (2007 to 2009). Correlations were established for all ripeness levels and also for grapes with a ripeness level of between 23 and 25ºB (only improvements on the first correlation is depicted in the table).

	Grape parameters (G)[1]							
	All ripeness levels[4]				23-25ºB[5]			
	Antho/g berry[6]		Total colour pigments[7]		Antho/g berry[6]		Total colour pigments[7]	
Wine parameters[3] (W)	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val
CD[8] AF	0.21	0.03	0.75	< 0.01	0.37	< 0.01	0.87	< 0.01
CD MLF	0.28	< 0.01	0.41	< 0.01	0.33	< 0.01		
MCD[9] AF	0.40	< 0.01	0.51	< 0.01	0.49	< 0.01	0.62	< 0.01
MCD MLF	0.28	< 0.01	0.24	0.01	0.29	0.01		
TRP[10] AF	0.47	< 0.01	0.12	0.24				
TRP MLF	0.19	0.05	-0.48	< 0.01				
Anthocyanin[11] AF	0.46	< 0.01	0.03	0.75	0.48	< 0.01	0.09	0.71
Anthocyanin MLF	0.23	0.05	-0.06	0.51				
Total colour pigments[12] AF	0.41	< 0.01	0.17	0.10			0.51	< 0.01
Total colour pigments MLF	0.22	0.03	0.14	0.14				

[1] Grape parameters (G): colour measured in the grape samples;
[3] Wine parameters (W): colour measured in the wine after alcoholic fermentation (AF) and after malolactic fermentation (MLF);
[4] Includes all ripeness levels found when the grapes were harvested for winemaking;
[5] Grapes with a ripeness level of between 23 and 25ºB;
[6] Anthocyanin per gram berry measured with the spectrophotometer (Iland *et al*., 2000);
[7] Total colour pigments: all colour pigments measured at 520 nm using HPLC (Peng *et al*., 2002) for grapes and wines (AF and MLF);
[8] CD: Colour density;
[9] MCD: Modified colour density;
[10] TRP: Total red pigments;
[11] Anthocyanin: Anthocyanin in mg/L according to a bleaching method;
[12] Total colour pigments: all colour pigments including the polymeric pigments added together as measured by HPLC (Peng *et al*., 2002);
*r^2 refers to the correlation coefficient between two parameters;
**p value: the probability testing for statistical significance; level of < 0.01 is acceptable.

Table 4.4 Average and standard error of colour and phenolic compounds due to seasonal and cultivar influences for wines from the Robertson area. From a seasonal perspective, the data contains the results of the four cultivars per season, and the data as analysed from a cultivar perspective contains the results of three seasons per cultivar.

	Seasonal perspective[1]			Cultivar perspective[2]			
Compound/ Parameter	2007	2008	2009	Pinotage	Merlot	Cabernet Sauvignon	Shiraz
CD[3] AF	10.2[b] ±0.7	8.29[c] ±0.6	22.3[a] ±0.8	12.7[a] ±0.9	15.2[a] ±0.8	14.2[a] ±0.7	12.4[a] ±0.7
CD MLF	7.95[c] ±0.4	9.74[b] ±0.4	13.9[a] ±0.4	9.49[b] ±0.5	10.5[ab] ±0.5	12.0[a] ±0.4	10.3[b] ±0.4
MCD[4] AF	8.9[c] ±0.8	12.1[b] ±0.6	19.4[a] ±0.9	13.6[ab] ±1.0	13.5[ab] ±0.9	14.4[a] ±0.8	12.3[b] ±0.8
MCD MLF	9.23[c] ±0.5	10.9[b] ±0.4	12.8[a] ±0.5	10.0[b] ±0.6	10.5[b] ±0.5	12.6[a] ±0.4	10.7[b] ±0.5
TRP[5] AF	21.1[b] ±1.0	24.4[ab] ±0.9	26.2[a] ±1.2	26.0[ab] ±1.3	21.0[c] ±1.2	27.0[a] ±1.1	22.3[bc] ±1.1
TRP MLF	14.9[c] ±0.8	24.4[a] ±0.7	19.9[b] ±0.8	20.7[a] ±1.0	17.5[b] ±0.9	22.5[a] ±0.8	18.3[b] ±0.8
Anthocyanin[6] AF	465[b] ±23	605[a] ±20	549[a] ±27	584[a] ±30	471[b] ±28	593[a] ±25	509[b] ±25
Anthocyanin MLF	317[c] ±20	492[a] ±17	432[b] ±19	470[a] ±24	340[b] ±22	449[a] ±19	395[b] ±20
Total colour pigments[7] AF	340[a] ±18	366[a] ±15	382[a] ±20	425[a] ±23	339[b] ±21	385[a] ±19	302[b] ±19
Total colour pigments MLF	241[a] ±10.6	255[a] ±9.0	261[a] ±10	311[a] ±13	206[c] ±12	276[b] ±11	217[c] ±11
Tphen[8] AF	32.7[c] ±1.3	37.7[b] ±1.1	46.5[a] ±1.6	38.9[ab] ±1.7	35.3[b] ±1.6	41.7[a] ±1.5	40.0[a] ±1.5
Tphen MLF	30.7[b] ±1.2	41.3[a] ±1.0	42.2[a] ±1.2	38.0[ab] ±1.5	35.5[b] ±1.4	41.2[a] ±1.2	37.6[b] ±1.3
Tannin[9] AF	154 ±15	290 ±14	192 ±19	200.2[ab] ±25	163[b] ±22	228[a] ±24	191[ab] ±23
Tannin MLF	109 ±12	123 ±10	141 ±12	98.9[a] ±15	128[a] ±14	142[a] ±12	128[a] ±12
TotFlav[10] AF	354[b] ±27	585[a] ±23	612[a] ±32	393[b] ±35	412[b] ±33	664[a] ±30	600[a] ±30
TotFlav MLF	361[a] ±21	506[b] ±18	635[c] ±21	419[b] ±26	458[b] ±23	562[a] ±21	563[a] ±21

[1] Data analysed using season as the influencing parameter. Each season included data from four cultivars: Pinotage, Merlot, Cabernet Sauvignon and Shiraz;
[2] Data analysed using cultivar as the influencing parameter. Each cultivar includes data obtained from three seasons: 2007 to 2009;
[3] Colour density of the wines after alcoholic fermentation (AF) and after malolactic fermentation (MLF) (spec), absorbance units;
[4] Modified colour density measured after AF and MLF (spec), absorbance units;
[5] Total red pigments after AF and MLF (spec, absorbance units);
[6] Anthocyanins after AF and MLF (spec), mg/L;
[7] Total colour pigments after AF and MLF (HPLC), mg/L;
[8] Total phenols after AF (spec), absorbance units;
[9] Tannins after AF and MLF (spec); in 2007, MC precipitation was used and the tannins were expressed as mg/L epicatechin units; in 2008/2009, BSA precipitation was used and the tannins were expressed in mg/L catechin units;
[10] Total flavanols/phenols after AF and MLF (HPLC), mg/L;
[a,b,c] Indicates significant differences between the averages of either the seasonal data or the cultivar data.

4.4.1.2 Correlations between the tannin and total phenolic content of grapes and wines

A variety of correlations could also be seen for the phenolic components (280 nm readings, HPLC and spec) (Table 4.5). Positive correlations were found between Totphen/g berry (G, spec) and Tphen after AF and MLF (W, spec), this was also the case with total grape flavanols AF (W, HPLC), with r^2 values of 0.54, 0.64 and 0.54 respectively. All three of these correlations were also significant ($p < 0.01$). In other studies it has also been found that significant positive correlations exist between the total phenol content of grapes and wines (Romero-Cascales *et al*., 2005; Cagnasso *et al*., 2008; Jensen *et al*., 2008).

In this study, low positive correlations were found for the grape and wine tannins measured using the BSA method (2007 data were excluded). In the literature, BSA has been correlated with other methods. For instance, in the study by Romero-Cascales *et al.* (2005), high positive correlations was found when they compared the Glories method (grape and wine), as well as the Glories method (G) versus the BSA method (W) and BSA (G). They did find that skin tannins had a low negative correlation and seed tannin had a very high positive correlation with grape wine tannins measured by the Glories and BSA methods (Romero-Cascales *et al*., 2005). In our study, an extraction was made for tannin analyses that contained both skin and seed tannin, as would be the case in actual winemaking, which could have decreased the correlation between the grape and wine tannins.

When the total flavanols as measured with the HPLC of grapes were correlated with the different phenolic measurements taken in the corresponding wines, low positive correlations were detected, but some correlations were found to be significant none the less. These were the correlation between total flavanols (G, HPLC) and Tphen AF (W, Spec) and total flavanols (W, HPLC, Table 4.5). The tannins after AF and MLF (excluding 2007 data) showed high negative correlations in almost all instances and were significant when compared to the Tphen/g berry (G, spec) data. All the correlations were significant ($p<0.01$) between the Tphen/g berry (G, Spec) and the wine data post-AF and post-MLF. A few improvements were noted when correlations were made between grape and wine phenols of samples with a sugar level of between 23 and 25ºB. The most important one was between Tphen/g berry (G, spec) and Tphen (W, AF), which had an r^2 of 0.54 and increased to an r^2 of 0.61.

Table 4.5 Correlations between grape and wine phenolic data, including the Pinotage, Merlot, Cabernet Sauvignon and Shiraz cultivars for three seasons (2007 to 2009). Correlations were established for all ripeness levels and also for grapes with a ripeness level of between 23 and 25ºB (only improvements on the first correlation is depicted in the table). (excluding 2007 tannin measurements).

	Grape parameters (G)[1]											
	All ripeness levels[4]						23–25ºB[5]					
Wine parameters[3] (W)	Tphen/g berry[6]		Tannin[7]		TotFlav[8]		Tphen/g berry		Tannin		TotFlav	
	r^{2*}	p-val**	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val
Tphen[9] AF	0.54	< 0.01	-0.74	< 0.01	0.32	< 0.01	0.61	< 0.01	-0.79	< 0.01	0.39	< 0.01
Tphen MLF	0.64	< 0.01	-0.43	< 0.01	0.18	0.06						
Tannin[7] AF	0.32	< 0.01	0.34	< 0.01	0.13	0.29			0.41	< 0.01		
Tannin MLF	0.35	< 0.01	-0.08	0.40	0.23	0.02					0.35	< 0.01
TotFlav[8] AF	0.54	< 0.01	-0.47	< 0.01	0.04	0.68			-0.54	< 0.01		
TotFlav MLF	0.38	< 0.01	-0.52	< 0.01	0.27	< 0.01			-0.58	< 0.01	0.32	< 0.01

[1] Grape parameters (G): flavanols measured in the grapes samples;
[3] Wine parameters (W): phenolic compounds measured in the wine after alcoholic fermentation (AF) and after malolactic fermentation (MLF);
[4] Includes all ripeness levels found when the grapes were harvested for winemaking;
[5] Grapes with a ripeness level of between 23 and 25ºB;
[6] Total phenol per gram berry measured with the spectrophotometer (Iland *et al*., 2000);
[7] Tannins measured with the spectrophotometer in the grapes and wines after AF and MLF (Harbertson *et al*., 2000);
[8] Total grape flavanols: sum of the monomeric and polymeric flavanols measured at 280 nm using HPLC (Peng *et al*., 2002) for grapes and wines (AF and MLF);
[9] Total phenols measured in the wines with the spectrophotometer at 280 nm after AF and MLF;
*r^2 refers to the correlation coefficient between two parameters, with the value 1 being the maximum and perfect linear correlation;
**p value: the probability testing for statistical significance (p < 0 .01).

4.4.2 Seasonal influence on colour and phenolic correlations

4.4.2.1 Distribution of grape and wine data as influenced by season

The distribution of the grape and wine, colour and phenolic data varied between the seasons and also between sample types (Figure 4.1 A, B and C; Table 4.3 and Table 3.4, Chapter 3).

The grape data show that, despite the fact that most of the data was found to be more or less in the same ranges, there seemed to be a wider distribution of some of the parameters investigated in 2007 (Figure 4.1, A). The parameters that drove this wider distribution were polymeric pigments, pH, polymeric flavanols and total phenols. The most important role player here was the level of phenolic ripeness, which had not yet been reached, even though the ripeness level in Balling was already high enough for the grapes to be harvested (refer to Chapter 3, Section 3.3.1 and 3.3.2). In 2007 there were also a couple of vineyard blocks that were sampled below 20°B (refer to Chapter 3, Table 3.4), which could have contributed to the wider distribution. Of the three seasons, 2008 had the most tightly distributed data set. There were a number of parameters that were not significantly different between the 2008 and 2009 seasons, such as polymeric pigments, total phenols and pH. Anthocyanin and total phenols per gram berry (spec), monomeric flavanols and degrees Balling are not shown in the bi-plots. These parameters are not included due to low r^2 values.

The data from the three seasons is clearly separate in the case of the wine samples, with the 2007 data relatively in the middle of the data set and 2008 and 2009 on either side (Figure 4.1, B and C). For the wine samples after alcoholic fermentation certain of the 2008 data had higher values for free anthocyanin (HPLC), polymeric flavanols (HPLC) and total phenols (HPLC), as well as for spectrophotometrically measured data, namely anthocyanins. The 2009 wine post-AF had higher levels in some of the colour data as measured by the spectrophotometer namely CD and MCD.

For the wine samples post-MLF (Figure 4.1, C), the 2008 data showed in some instances to have higher levels of free anthocyanins and total colour pigments (HPLC), as well as for TRP and Antho (anthocyanins via bleaching method), which were analysed with the spectrophotometer. The actual level of total colour pigments (HPLC) between AF and MLF samples in 2008 showed that the prior were slightly higher than the latter (Table 4.4). The 2009 data were higher with regard to polymeric phenols and total flavanols (HPLC), as well as CD (spec) before and after MLF. Therefore the CD

remained highest for the 2009 data between AF and MLF. This is confirmed by an actual correlation between the colour and phenolic data after AF and after MLF (Table 1.2 Addendum A). In 2007, only the total phenols (HPLC) were significantly correlated between AF and MLF, while, in 2008, all the parameters listed in Table 1.2 (Addendum A)were significantly correlated between these stages. In 2009, total phenols (HPLC and Spec) were significantly correlated between AF and MLF (Table 1.2 Addendum A. There are a lot of different opinions on the influence of season on the anthocyanin or phenolic constitution of grapes. One school of thought is that the anthocyanin content of a given cultivar is linked to its genetic inheritance, which is independent of season and production area, while it is also known that even clones of the same cultivar can be different in this regard and that season may well influence anthocyanin content (Fernandez-Lopez *et al*., 1998; Arozarena *et al*., 2002).

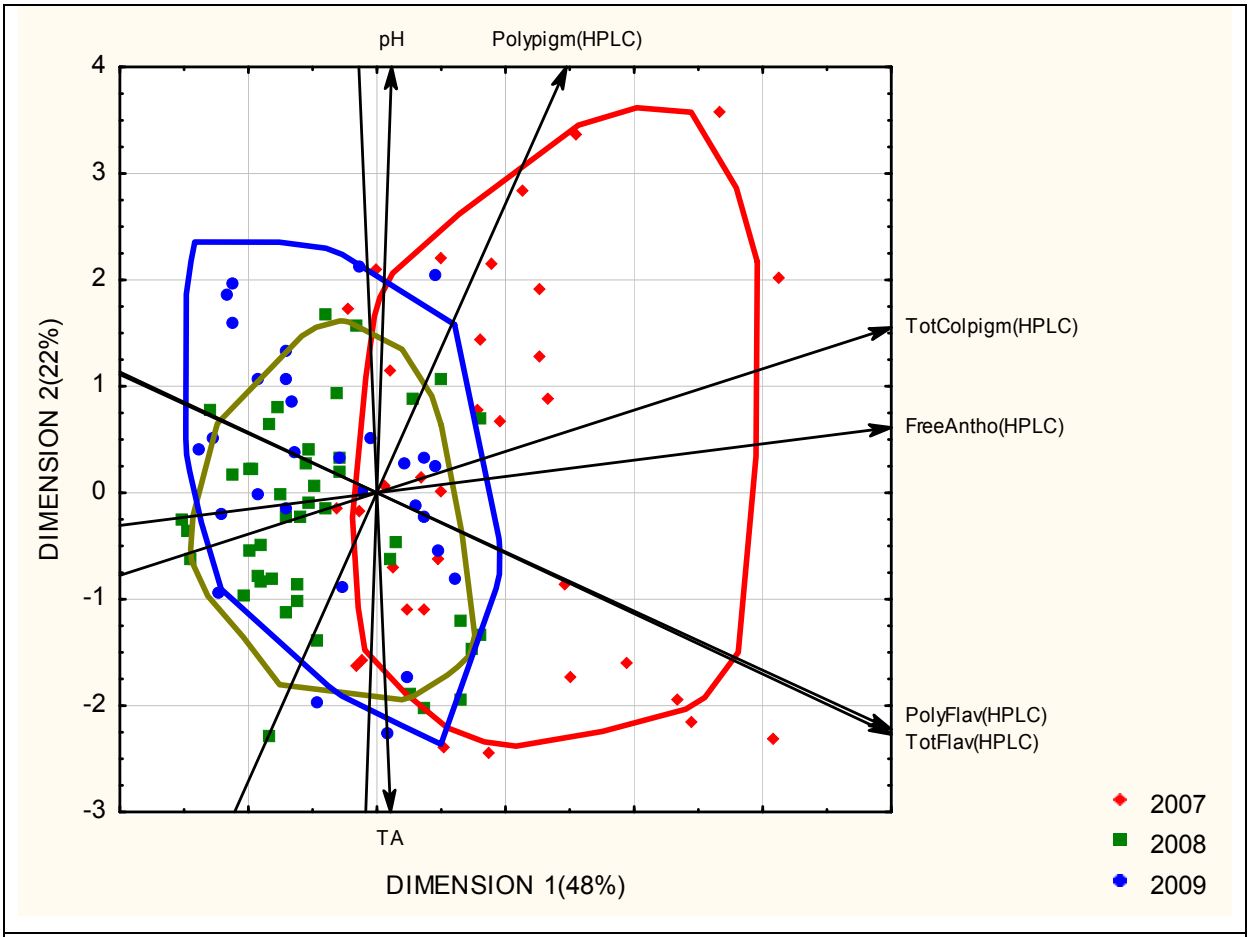


Figure 4.1 A

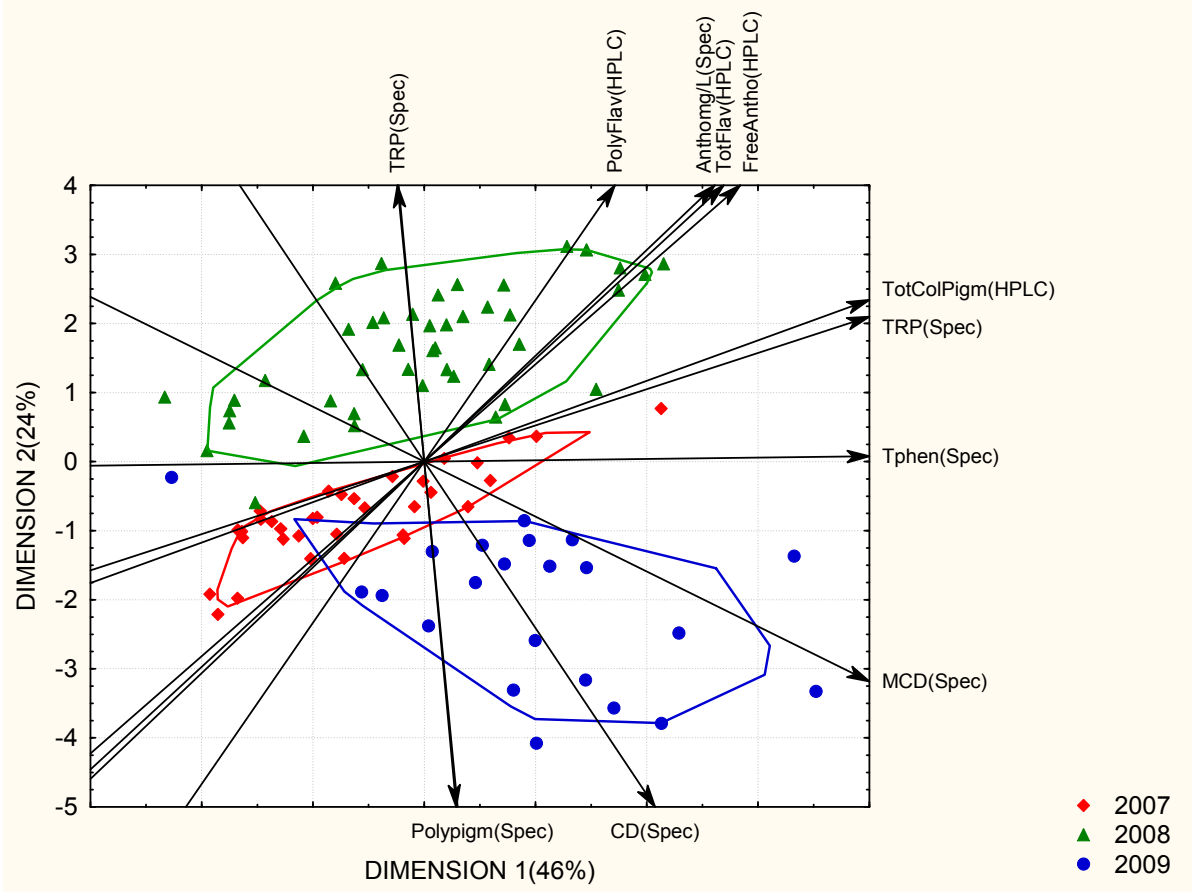


Figure 4.1 B

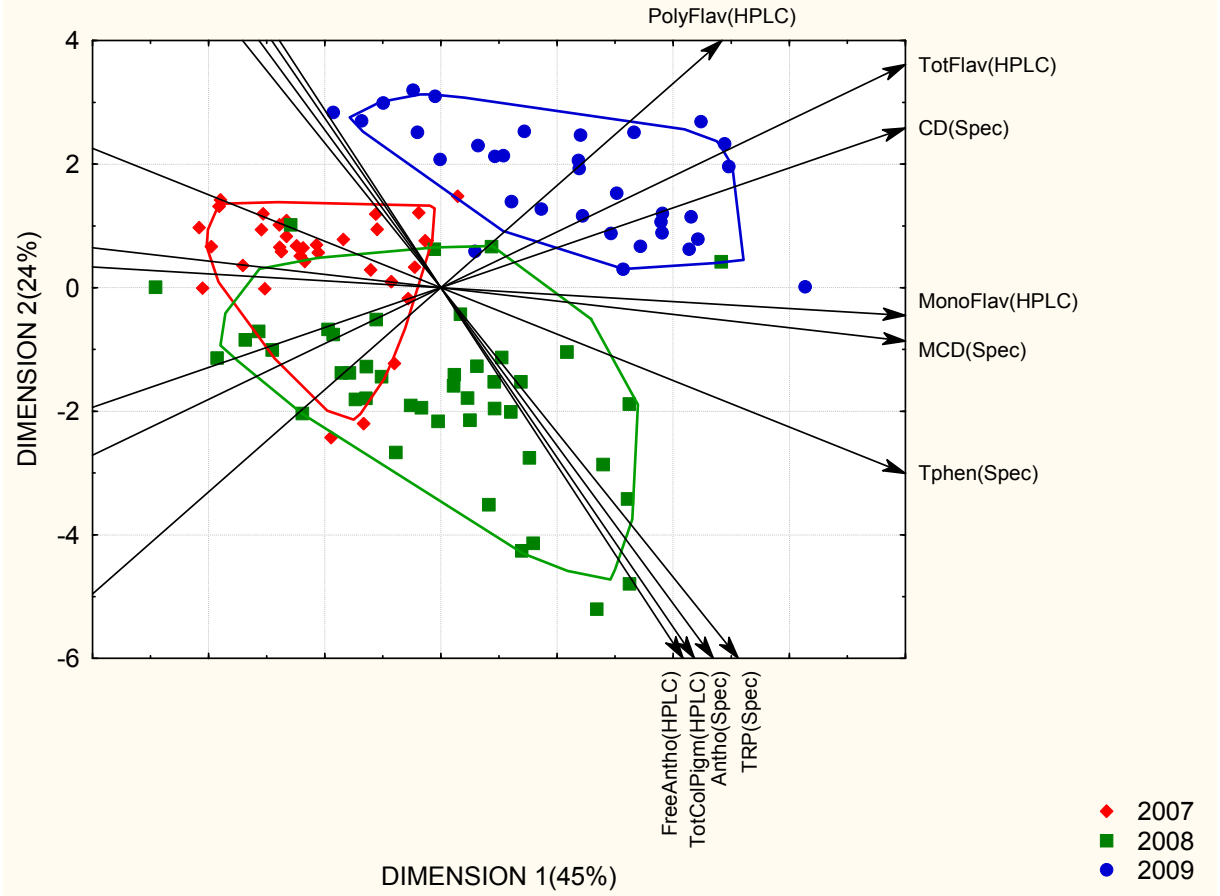


Figure 4.1 C

Figure 4.1 A: PCA bi-plot of grape berry colour and phenolic data for the 2007 to 2009 harvest seasons; B: Distribution of the colour and phenolic data of wines after AF for the 2007 to 2009 harvest seasons; C: Distribution of the colour and phenolic data of the wines after MLF for the 2007 to 2009 harvests. Lines drawn around data points indicate a 95% confidence level ($p < 0.05$); points outside these lines are deemed outliers for the specific season.

4.4.2.2 Correlations between grape and wine colour per season

During 2007, some low positive correlations were found between grape and wine colour, of which only one correlation was significant ($p< 0.01$) (Table 4.6). The grape data distribution in 2007 was completely different from that in the 2008 and 2009 seasons. This was also the season in which the variation in ripeness levels of the grapes (degree Balling, data not shown) was most widely distributed. As mentioned previously in the study by Marais and October (2005), variance in ripeness level influences these correlations quite dramatically.

It appears that there were significant correlations between grape and wine parameters in almost all instances in 2008 (Table 4.6). Anthocyanins as measured with the

spectrophotometer (Antho/g berry) versus CD MLF, MCD AF/MLF, TRP AF/MLF, anthocyanin AF and total colour pigments AF had the highest positive correlations in this group. CD AF, Antho MLF and colour HPLC MLF were also significantly correlated ($p<0.01$), but at lower positive values (0.47, 0.47 and 0.40). Very similar results were found in another study that correlated CD with grape anthocyanins as measured with the spectrophotometer for various cultivars in one season. An r^2 of 0.69 was found, close to the 0.60 that was found in this study (Romero-Cascales *et al*., 2005). Even higher positive correlations were found in other work that correlated all cultivars over one season in this regard (Jensen *et al*., 2008). The specific method (HPLC-phloroglucinolysis) used in the latter study was different than in the study by Romero-Cascales and associates (2005) and for this work where reverse phase chromatography was used.

Total colour pigments (grapes, HPLC) showed the highest positive correlation with TRP AF and total colour pigments HPLC AF. The grape analyses with the spectrophotometer in 2008 were shown to be better correlated with all wine parameters than the HPLC data. It is important to note that the pigments according to the HPLC were looked at collectively; therefore correlations between individual pigments like malvidin-3-glucoside, for instance, could lead to a different result.

In 2009 there were positive and significant correlations between Antho/g berry (G) and MCD AF and Antho/g berry (G) and TRP AF. Positive and significant correlations were also found between total colour pigments (G) and Antho AF and total colour pigments (G) and colour HPLC AF. These correlations were of a similar magnitude to those found in 2008.

The influence of season on correlations between grape and wine colour and phenolic data has also been reported by other authors (Cagnasso *et al*., 2008).

Table 4.6 Correlations between grape and wine parameters for 2007–2009, including all cultivars.

Grape parameters[1]												
	2007[4]				2008[4]				2009[4]			
	Antho/g berry[5]		Total colour pigments[6]		Antho/g berry		Total colour pigments		Antho/g berry		Total colour pigments	
Wine parameters[3]	r^{2*}	p-val**	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val
CD[7] AF	0.41	0.44	0.27	0.12	0.64	< 0.01	0.44	0.01	0.51	0.03	0.61	< 0.01
CD MLF	-0.19	0.37	-0.02	0.92	0.68	< 0.01	0.48	< 0.01	0.08	0.76	-0.38	0.04
MCD[8] AF	0.28	0.20	0.22	0.20	0.59	< 0.01	0.40	< 0.01	0.53	< 0.01	0.42	0.08
MCD MLF	-0.31	0.14	-0.03	0.85	0.70	< 0.01	0.45	< 0.01	-0.10	0.59	-0.44	0.02
TRP[9] AF	0.38	0.07	0.25	0.16	0.68	0.05	0.53	< 0.01	0.48	0.01	0.56	0.01
TRP MLF	-0.38	0.07	-0.01	0.95	0.63	< 0.01	0.40	< 0.01	-0.08	0.67	-0.04	0.85
Antho[10] AF	0.52	< 0.01	0.35	0.05	0.66	< 0.01	0.44	< 0.01	0.29	0.20	0.61	< 0.01
Antho MLF	-0.35	0.09	0.19	0.29	0.47	0.05	0.36	0.02	-0.06	0.76	-0.09	0.65
TotColPigm[6]HPLC AF	0.10	0.63	0.01	0.97	0.63	< 0.01	0.50	< 0.01	0.29	0.17	0.73	< 0.01
TotColPigmHPLC MLF	0.02	0.93	0.15	0.38	0.40	0.05	0.31	0.04	0.01	0.97	0.14	0.50

[1] Grape parameters (G): colour measured in the grape samples;
[3] Wine parameters (W): colour measured in the wine after alcoholic fermentation (AF) and after malolactic fermentation (MLF);
[4] Seasonal data includes all four cultivars and only ripeness levels between 23 and 25°B;
[5] Anthocyanin per gram berry measured with the spectrophotometer (Iland *et al*., 2000);
[6] Total colour pigments: all colour pigments measured at 520 nm using HPLC (Peng *et al*., 2001), for grapes and wines (AF and MLF);
[7] Colour density;
[8] Modified colour density;
[9] Total red pigments;
[10] Anthocyanin in mg/L according to a bleaching method;
*r^2 refers to the correlation coefficient between two parameters;
**p value: the probability testing for statistical significance (significant difference); a level of < 0 .01 is significant.

4.4.2.3 Correlations between grape and wine phenolic compounds per season

In 2007 and 2008 there were low positive, but significant correlations found only sporadically in the case of grape and wine total phenols (Table 4.7). In 2007 the correlations were between Tphen/g berry (G, spec) and tannin MLF (W, spec) as well as between Totphen (G, HPLC) and Tannin AF (W, spec) as well as Totphen MLF (W, spec). In 2008, the correlations were between Tphen/g berry (G, spec) and Tphen MLF (W, spec) as well as Tphen/g berry (G, spec) and Totphen MLF (W, HPLC). No similarities were found in these correlations between 2007, 2008 and 2009.

However, in 2009 there were positive and significant correlations between Tphen/g berry (G, spec) and most of the wine phenolic parameters.

The question thus arises why the 2008 colour data (520 nm spec and HPLC) had more significant positive correlations between grapes and wines, but fewer between the total phenols data (280 nm spec and HPLC), while the opposite is true for 2009? The sites used, as well as all cultivation practices, remained the same over the three seasons. It seems that maximum temperature and rainfall (Refer to Figures 3.5 and 3.6, Chapter 3), as well as ripeness level (sugar and phenolic) and extraction factors, are some of the main role players that should be looked at to try to explain some of the results. With regard to seasonal temperature it was found that, although the temperature averages were above 27°C during ripening in all three seasons, 2008 showed a lower average temperature trend than the other seasons, thus creating conditions that are more suitable for anthocyanin development. According to the rainfall data, 2008 again was the season that showed a trend of being more variable than 2007 and 2009. If only these two weather components are considered, it could be speculated that anthocyanin development and the resulting correlations are influenced more positively by a lower average maximum temperature than is the case in the development of the other phenolic components pertaining to eventual tannin development.

Table 4.7 Correlations between the phenolic data of the grapes and wines for 2007, 2008 and 2009 separately, but for all cultivars collectively.

	Grape parameters[1](G)																	
	2007[4]						2008[4]						2009[4]					
	Tphen/g[5]		Tannin[6]		TotFlav[7]		Tphen/g		Tannin		TotFlav		Tphen/g		Tannin		TotFlav	
Wine parameters[3](W)	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val	r^2	p-val
Tphen[5]AF	-0.16	0.38	-0.31	0.14	-0.33	0.19	0.45	< 0.01	0.16	0.37	0.28	0.06	0.43	0.02	-0.18	0.48	-0.19	0.31
TphenMLF	-0.06	0.76	0.60	< 0.01	0.47	0.02	0.53	< 0.01	0.28	0.11	0.23	0.18	0.63	< 0.01	-0.40	0.1	-0.50	< 0.01
Tannin[6]AF	0.15	0.41	0.52	0.02	0.52	< 0.01	0.38	0.02	0.49	< 0.01	0.31	0.04	0.50	< 0.01	0.55	0.02	0.16	0.42
TanninMLF	0.35	< 0.01	0.26	0.21	0.23	0.02	0.29	0.09	0.32	0.06	0.39	0.02	0.66	< 0.01	0.39	0.11	-0.33	0.08
TotFlav[7]AF	-0.34	0.05	-0.40	0.05	-0.36	0.04	0.53	< 0.01	0.28	0.11	0.28	0.07	0.65	0.02	-0.08	0.79	-0.58	< 0.01
TotFlavMLF	-0.40	0.02	-0.54	< 0.01	-0.48	< 0.01	0.50	< 0.01	0.31	0.08	0.45	< 0.01	0.56	0.03	-0.45	0.09	-0.43	0.03

[1] Grape parameters (G): flavanols measured in the grapes samples;
[3] Wine parameters(W): phenolic compounds measured in the wine after alcoholic fermentation (AF) and after malolactic fermentation (MLF);
[4] Each season includes all cultivars and only samples that had ripeness levels between 23 and 25ºB;
[5] Total phenol per gram berry measured with the spectrophotometer for grapes and wines (280 nm reading) (Iland *et al*., 2000);
[6] Grape and wine (AF and MLF) tannin measured with the spectrophotometer (Harbertson *et al*., 2000);
[7] Total grape flavanols: sum of the monomeric and polymeric flavanols measured at 280 nm using HPLC (Peng *et al*., 2002) for grapes and wines (AF and MLF);
*r^2 refers to the correlation coefficient between two parameters;
**p value: the probability testing for statistical significance (significant difference); a level of < 0.01 is significant.

If these correlations (Table 4.6) between grape and wine colour are compared again to the data that included all the seasons (Table 4.3), the effect of seasonal influence is pertinent. When all the data are considered together, the highest positive correlations are founds between the total colour pigments measured by HPLC and the various wine colour measurements. In 2008, the higher positive correlation was between the spectrophotometric grape data and the wine colour, while there also was a positive correlation between the HPLC grape measurements and the wine colour. In 2009 the results were much more similar to the compiled data.

4.4.3 Correlations between the colour and phenolic compounds of grapes and wines per cultivar

4.4.3.1 Distribution of the grape and wine data (AF and MLF) for Pinotage, Merlot, Cabernet Sauvignon and Shiraz

The distribution of the grape data according to cultivar (Figure 4.2, A) showed that some Pinotage and Merlot grapes samples were higher in total phenols and total acidity, while Cabernet Sauvignon was slightly in between with regard to all components. Shiraz grape, on the other hand, was highest in colour and pH. More or less the same distribution trend was found in the wines after alcoholic fermentation (Figure 4.2, B) – Shiraz and Cabernet Sauvignon were pulled in a similar direction, while Pinotage and Merlot were grouped together to a greater extent, although overlap of these cultivars were also observed. Other authors have also shown that Shiraz grapes are highest in colour (as measured with the HPLC), while Cabernet Sauvignon is average and Merlot has the lowest content, although these differences are not significantly different for all three cultivars (Jensen *et al*., 2008). This trend of Shiraz and Cabernet Sauvignon samples grouping together and similarly Pinotage and Merlot was also followed through to the post-alcoholic fermentation stage for anthocyanin content during this study. Variations were seen in the total phenol content from the berries to the wine. The extraction of phenolic components from the berries into the wine and subsequently winemaking practices, plays a major role in these deviations in the distribution of colour and total phenols. In another study looking at extractions, it was shown that the free anthocyanin and total phenolic extraction did not vary as a result of ripeness content for a specific sample (Fournand *et al*., 2006). It was shown that the more complex the component, the less readily it was extracted. It has also been shown that the extraction index for Cabernet Sauvignon, Merlot and Shiraz is more or less the same (Romero-

Cascales *et al*., 2005). In another study, also on South African grapes (mostly Stellenbosch region), it was shown that the extraction of extractability index of anthocyanins were found to be higher in Cabernet Sauvignon than in Pinotage, Merlot and Shiraz (Du Toit & Visagie, 2012). The reason for the difference in results could be region or method related.

Winemaking techniques that can severely influence extraction are fermentation temperature, SO_2, cold soaking, must or grape freezing, thermovinification, carbonic maceration, pectolytic enzymes, pumping over and punching down, maceration time and yeast selection (Sacchi *et al*., 2005). Although great care was taken during this investigation to apply the same winemaking techniques to each of the fermenting samples, these factors demonstrates just how many variables there are that can influence the extraction of grape components into wine.

In the samples obtained after malolactic fermentation (MLF), the distinction between cultivars with regard to colour and phenolic components was less obvious than after AF (Figure 4.1, C vs. B). Two of the reasons that may explain this are the direct effect from the pH change due to MLF and the conversion of the pigments and phenols into more polymerised components. It was found by other authors as well that MLF changed the correlations that existed between grape and wine with regards to colour (Du Toit & Visagie, 2012). Nevertheless, Pinotage still seemed to have the highest degree of colour of all the cultivars, while Shiraz was higher in total phenol content. This is also evident from the absolute data, where the total colour pigment (of which free anthocyanin forms the largest constituent) is highest in Pinotage, while Shiraz has higher total phenol content than the other cultivars (Table 4.4). When comparing these results it was found that the average malvidin-3-glucoside levels in finished wine were almost double for South African Pinotage than for Shiraz and Cabernet Sauvignon (Rossouw & Marais, 2004).

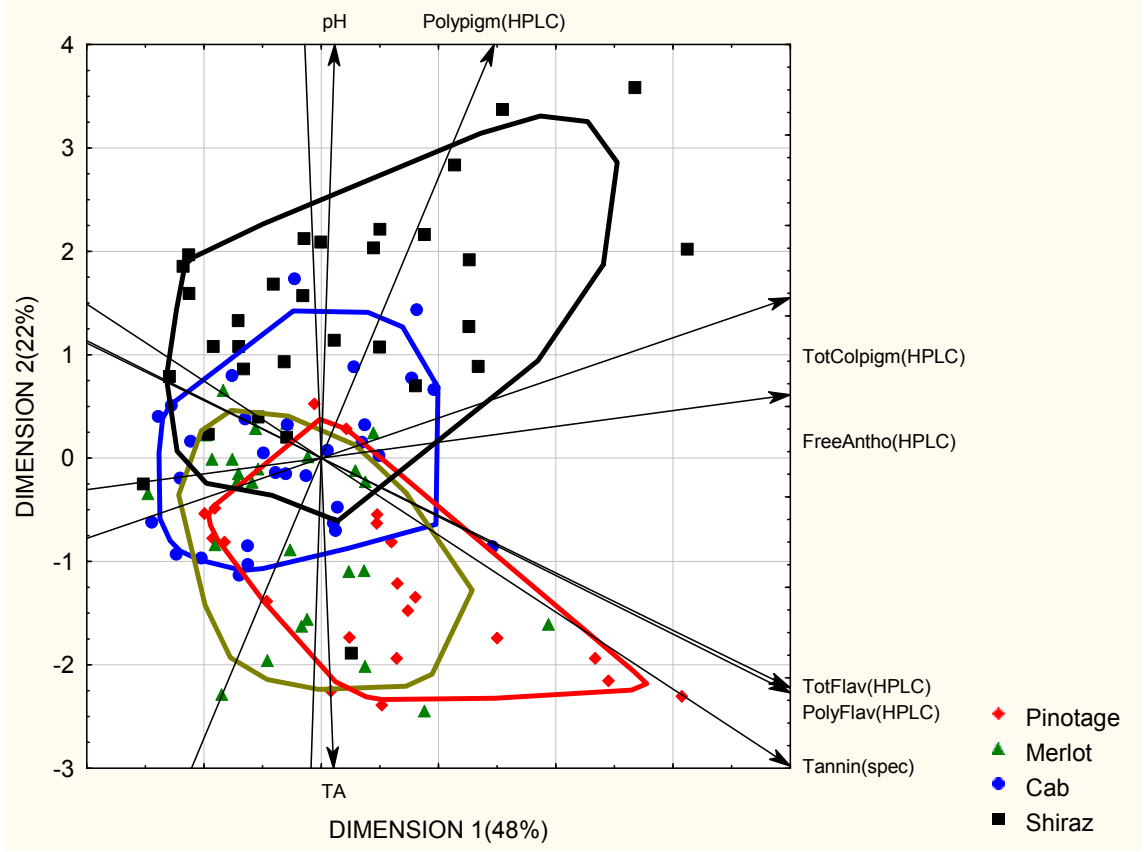


Figure 4.2 A

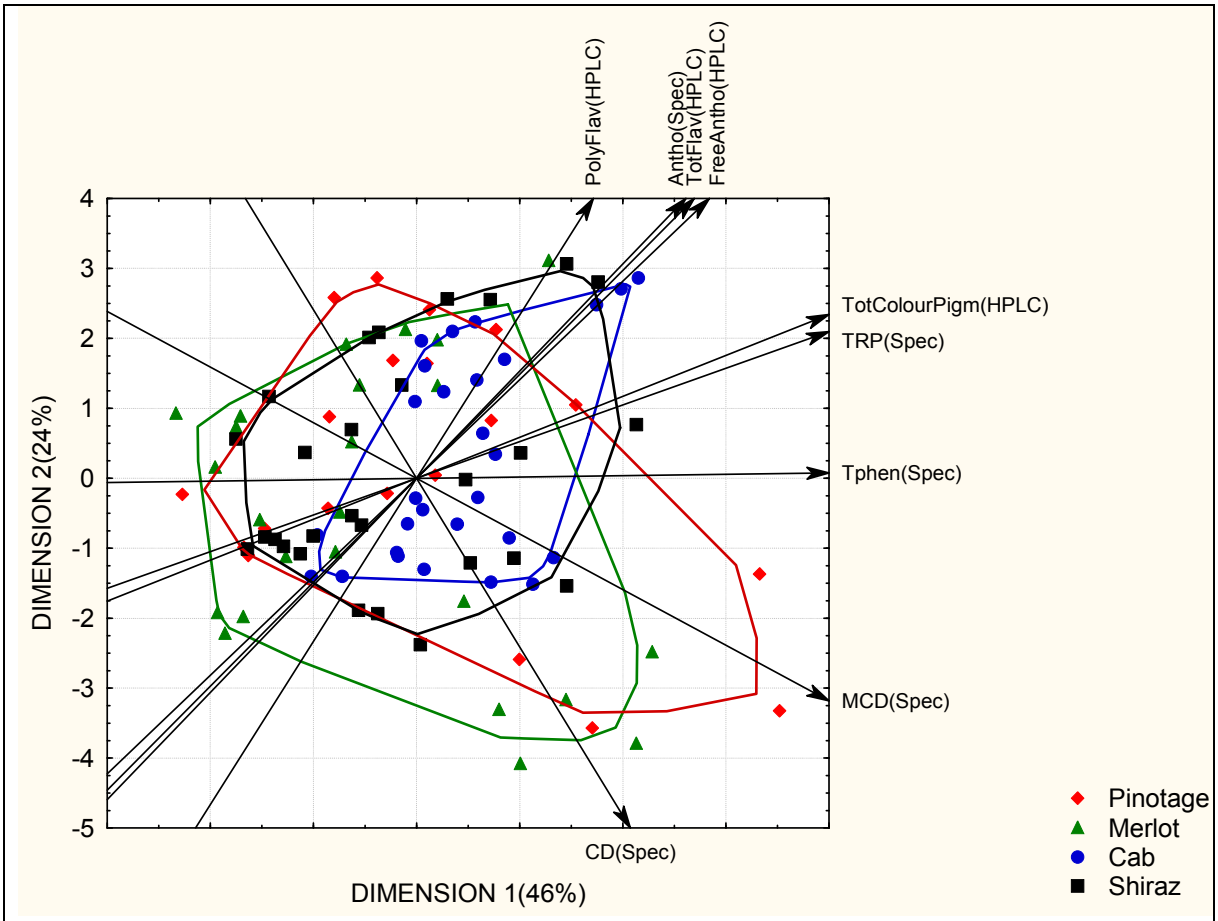


Figure 4.2 B

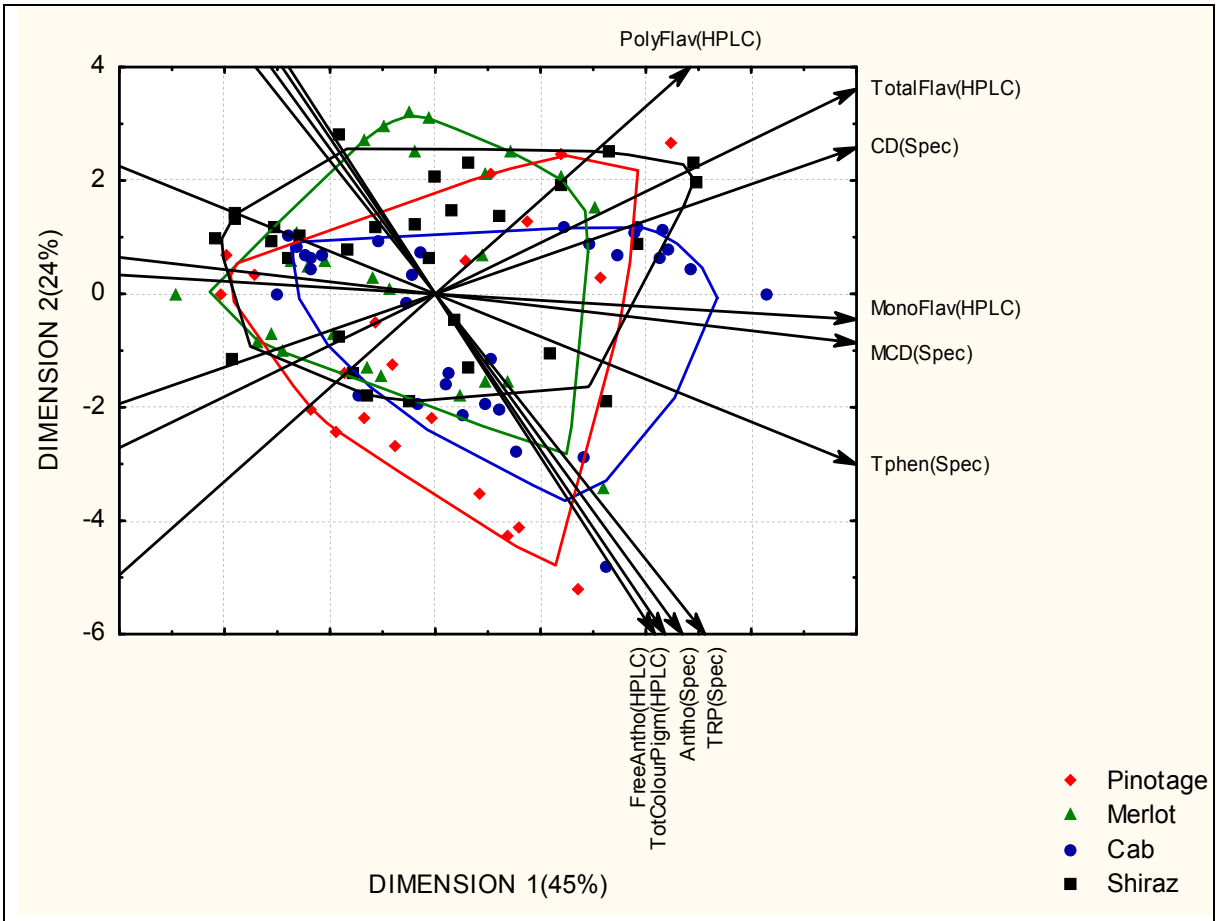


Figure 4.2 C

Figure 4.2 Distribution of grape berry colour and phenolic compounds in Pinotage, Merlot, Cabernet Sauvignon and Shiraz, including the data from the 2007 to 2009 harvest seasons; A: Grape data; B: Wine after alcoholic fermentation (AF); C: Wine after malolactic fermentation (MLF). Lines drawn around data points indicate a 95% confidence level; points outside these lines are deemed outliers for the specific season.

4.4.3.2 Correlations between grape and wine colour and phenolic compounds (AF and MLF) for Pinotage, Merlot, Cabernet Sauvignon and Shiraz grapes

All the cultivars showed high positive correlations between the total colour pigments of the grapes (G, HPLC) and the CD (W, AF and MLF) and MCD (AF) of the wines (Table 4.8). In some instances this seems to give better correlations than when all the cultivars were combined per season. This shows that cultivars should rather be used separately for the prediction of wine colour and phenolic content. In other studies using South African grapes, similar positive correlations between grape colour (Iland *et al*., 2000) and between wine colour intensity and modified colour density (Marais & October, 2005)

were found for these cultivars. Marais & October (2005) found highest and lowest r^2 values (grape colour (spec) versus MCD) of 0.46 and 0.65, 0.63 and 0.77, and 0.68 and 0.69 for Pinotage, Shiraz and Cabernet Sauvignon grapes, respectively. The Shiraz and Cabernet Sauvignon in their study came from the same region as our study. This indicates that there is a strong potential for the use of total grape pigments (HPLC) to predict wine colour in all cultivars. Although HPLC could be somewhat laborious and complex for some commercial cellars to use, under our circumstances the use of spectroscopy to determine the Anth/g berry in Merlot seemed to provide less valuable information.

In the case of phenolic correlations, spectrophotometric analyses can be used for the prediction of at least the phenolic content of Cabernet Sauvignon and Shiraz wines (Table 4.9). Pinotage and Merlot had the lowest positive correlations when phenolic compounds were examined. This could be as a result of the earlier ripening stage of Pinotage and Merlot, which falls at the beginning of the season and is marked by lower pH and higher TAs, high fermentation rates, poor regulation of temperature during fermentation, and therefore too fast extraction or rather poor extraction. The other reason could be that extractability is not optimal due to the ripeness of grapes early in the season.

Table 4.8 Correlations between grape and wine colour parameters for Pinotage, Cabernet Sauvignon and Shiraz (2007 to 2009). Correlation values in bold were also found to be significant at a p value of < 0.01.

	Grape parameters[1]							
	Pinotage[3]		Merlot[3]		Cabernet Sauvignon[3]		Shiraz[3]	
Wine parameters[2]	Antho/g Berry[4]	Total colour Pigments[5]	Anth/g berry	Total colour pigments	Antho/g berry	Total colour pigments	Anth/g berry	Total colour pigments
	r^{2*}							
CD[6] AF	0.31	**0.98**	**0.84**	**0.98**	0.30	**0.81**	0.15	**0.82**
CD MLF	**0.75**	**0.47**	**0.53**	**0.52**	0.36	**0.60**	0.22	**0.82**
MCD[7] AF	0.36	**0.74**	**0.82**	**0.87**	**0.45**	**0.55**	0.27	**0.70**
MCD MLF	0.42	0.10	0.24	0.16	0.32	0.42	0.24	**0.49**
TRP[8] AF	0.43	**0.55**	**0.58**	**0.58**	**0.43**	0.25	0.15	0.31
TRP MLF	0.27	**0.51**	-0.26	-0.57	0.31	-0.44	0.11	-0.48
Antho[9] AF	0.07	0.40	**0.60**	0.28	**0.55**	-0.13	**0.52**	-0.13
Antho MLF	0.33	-0.23	0.08	-0.17	0.31	0.25	0.05	0.14
TotColPigm[5]AF	-0.06	**0.84**	**0.67**	0.39	**0.48**	-0.16	0.19	-0.16
TotColPigmMLF	-0.06	-0.04	0.15	-0.04	0.27	0.36	0.19	0.33

[1] Grape parameters (G): colour measured in the grapes samples;
[2] Wine parameters (W): colour measured in the wine after alcoholic fermentation (AF) and after malolactic fermentation (MLF);
[3] Data separated into individual cultivars, Pinotage, Merlot, Cabernet Sauvignon and Shiraz, with ripeness levels of between 23 and 25°B;
[4] Anthocyanin per gram berry measured with the spectrophotometer (Iland *et al*., 2000);
[5] Total colour pigments: all colour pigments measured at 520 nm using HPLC (Peng *et al*., 2002) for grapes and wines (AF and MLF);
[6] CD: colour density;
[7] MCD: modified colour density;
[8] TRP: total red pigments;
[9] Anthocyanin: anthocyanin in mg/L (bleaching);
*r^2 refers to the correlation coefficient between two parameters, with the value 1 being the maximum and perfect linear correlation..

Table 4.9 Correlations between grape and wine phenolic parameters for Pinotage, Cabernet Sauvignon and Shiraz, including data from all three harvests.

	Grape parameters[1]							
	Pinotage[3]		Merlot[3]		Cabernet[3]		Shiraz[3]	
Wine parameters[2]	Tphen/g[4]	TotFlav[5]	Tphen/g	TotFlav	Tphen/g	TotFlav	Tphen/g	TotFlav
	r^{2*}							
Tphen[6]AF	0.58	0.47	0.38	0.55	0.79	0.43	0.70	0.44
TphenMLF	0.37	0.15	0.52	0.04	0.72	0.26	0.74	0.44
Tannins[7]AF	0.41	0.28	-0.16	0.35	0.52	-0.05	0.52	-0.06
TanninsMLF	0.79	0.45	0.12	0.31	0.48	0.39	0.62	0.15
TotFlav[5]AF	0.49	0.35	0.53	0.16	0.67	0.32	0.53	0.06
TotFlavMLF	0.51	0.37	0.40	0.40	0.34	0.62	0.60	0.33

[1] Grape parameters (G): flavanols measured in the grape samples;
[2] Wine parameters(W): phenolic compounds measured in the wine after alcoholic fermentation (AF) and after malolactic fermentation (MLF);
[3] Each cultivar includes the data from all three seasons and only samples that had ripeness levels of between 23 and 25ºB were used;
[4] Total phenol per gram berry measured with the spectrophotometer for grapes (280 nm reading) (Iland *et al*., 2000);
[5] Total grape flavanols: sum of the monomeric and polymeric flavanols measured at 280 nm using HPLC (Peng *et al*., 2002) for grapes and wines (AF and MLF);
[6] Wine (AF and MLF) tannin measured with the spectrophotometer (Harbertson *et al*., 2000);
*r^2 refers to the correlation coefficient between two parameters, with the value 1 being the maximum and perfect linear correlation; the p value was found to be < 0.01 for all correlations > 0.5;

4.4.4 Correlations between grape and wine data for each cultivar per each season

The colour of the grapes and wines in 2007 showed various high positive correlations, especially for Pinotage and Cabernet Sauvignon (Table 1.4, Addendum). The two grape measurements (Antho/g berry (spec) and total colour pigments (HPLC)) showed high positive correlations with all the wine measurements (spec and HPLC) for these two cultivars. The correlations varied in r^2 values of between 0.56 and 0.92. Another interesting feature observed in the Pinotage samples was that the grape colour analyses by HPLC showed higher correlations with the post-MLF wine samples than with the post-AF samples. The Merlot (2007) had high negative correlations between the grape and wine colour for both spec and HPLC analyses. Most of the correlations between the Shiraz components seemed to be insignificant in 2007.

In 2008 the correlations were different from those in 2007 (Table 1.5, Addendum). Pinotage was the cultivar in which only very low positive and low negative correlations were found, which were insignificant in all instances. On the other hand, the other three cultivars (Merlot, Cabernet Sauvignon, Shiraz) showed high positive correlations in basically all instances between grape and wine colour data, and for all measurement types. These high positive correlations varied in r^2 values of between 0.5 and 0.93.

In 2009, the Pinotage, Merlot and Cabernet Sauvignon samples showed high positive and significant correlations between grape and wine colour in some instances (Table 1.6, Addendum A). Shiraz, on the other hand, showed only low negative and low positive correlations between the colour of the grapes and wines made from them. In 2009 fewer instances of correlations between the colour of the grapes and the wines of Pinotage, Merlot and Cabernet were found (or combinations) than was the case in 2007 (Pinotage and Cabernet) and 2008 (Cabernet and Merlot).

4.4.5 Wine tasting results

4.4.5.1 2007

For the Pinotage, Shiraz and Cabernet Sauvignon of the 2007 harvest, a very high positive correlation was found between the colour and quality grading (r^2 = 1.00, r^2 = 0.99, r^2 = 0.93) by the tasters (Merlot was not tasted in 2007). This phenomenon of the taster linking quality to colour has been found previously (Du Toit *et al*., 2006). A positive correlation ($p < 0.01$ in both instances) was also found between taster's rating of colour and the real colour density for the Shiraz and Cabernet Sauvignon wines (r^2 = 0.86 and r^2 = 0.84) (Figures 4.3and 4.4). In a similar tasting, the correlations between grape colour and wine colour and grape colour and overall wine quality were also found to be highly positive and significant (Marais & Swart, 2001). These correlations are of the highest importance because they could potentially be linked to the colour and quality perceptions of the commercial wine consumer. Positive correlations were also found between taster grading and astringency (data not shown). Astringency has been positively linked to overall wine quality (Boselli *et al*., 2004; Landon *et al*., 2008). It has been shown in the literature that astringency is influenced by various parameters, such as pH, alcohol, sugar content and anthocyanin content (Ishikawa & Noble, 1995; Kallithraka *et al*., 1997; Vidal *et al*., 2004; Boselli *et al*., 2006; Gawel *et al*., 2007; Demiglio & Pickering, 2008; Fontoin *et al*., 2008; Obreque-Slíer *et al*., 2010; Sáenz-Navajas *et al*., 2010), and should rather be tested in coloured wineglasses to avoid the influence of wine colour. Since astringency tasting was not a main focus of this study, dark coloured wineglasses were not used during this tasting.

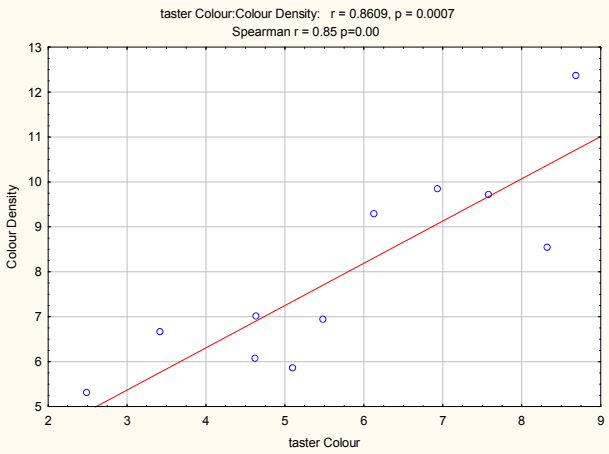


Figure 4.3 Taster colour and colour density for Shiraz samples from 2007 (r^2 value 0.86, n = 12).

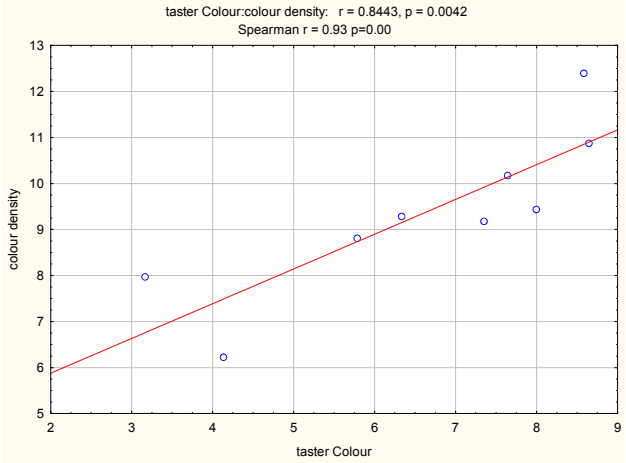


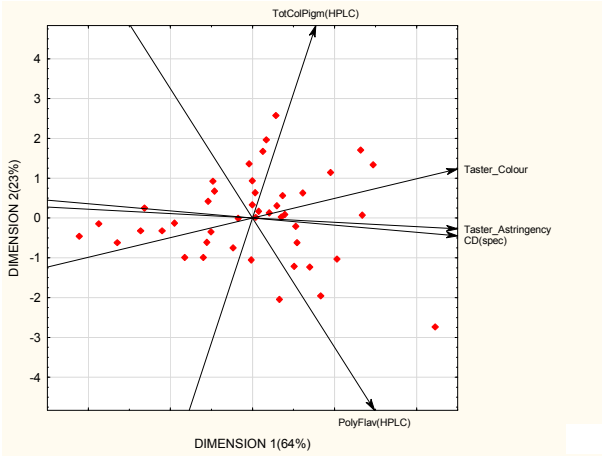
Figure 4.4 Taster colour and colour density for Cabernet Sauvignon samples from 2007 (r^2 value 0.84, n = 12).

4.4.5.2 2008

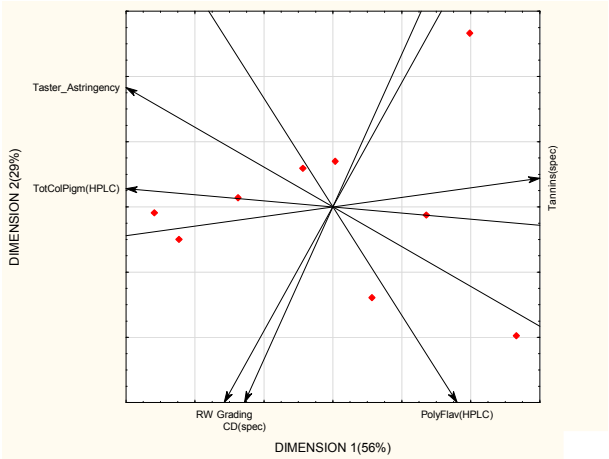
The tasting data of 2008 was analysed using multivariate analyses (Figure 4.5A-E) (Lawless & Heymann, 1998). The following data was considered for these bi-plots: taster_colour/astringency/grading, as well as CD, TRP, Antho, TotColPigm (HPLC), Tphen, Tannins, PolyFlav (HPLC), and the actual grading (RW grading) of each of the vineyards that were selected for this trial. These bi-plots show only the parameters that accurately display the data after a regression was plotted between the actual values of each parameter and the estimated or test value for each parameter.

The bi-plots constructed using all the data, as well as for the Merlot, Cabernet Sauvignon and Shiraz, after the tastings in 2008 (Figure 4.5 A, C-E) showed high associations between taster colour and astringency as well as between taster colour and the real CD of the wines. This was not true of the Pinotage wines. In all the bi-plots (Figure 4.5 A-E) it is seen that the real colour and tannin contents have no positive or very low positive associations with each other, but rather high negative associations.

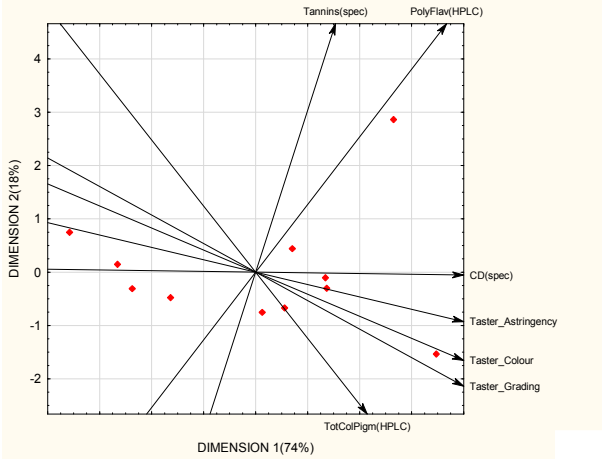
Interesting data from the bi-plot of Pinotage data (Figure 4.5 B) is the very high positive correlation between CD and the grading that was given to the Pinotage vineyard blocks on the basis of the combined visual and historical data of those blocks. The only other situation where a good correlation was found between the actual quality grading of the blocks and a chemical parameter was with tannins in Shiraz. The cultivars that were found to be representative of the combined data were Merlot, Shiraz and to a certain degree Cabernet Sauvignon.



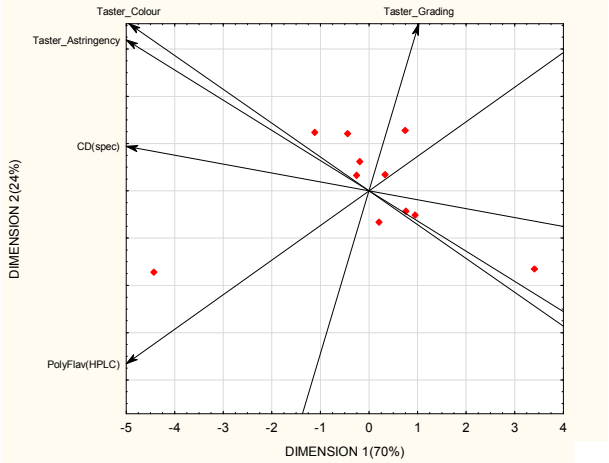
A All cultivars



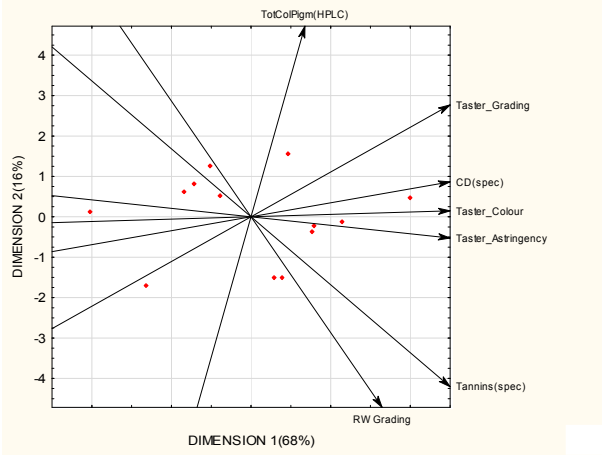
B Pinotage



C Merlot



D Cabernet Sauvignon



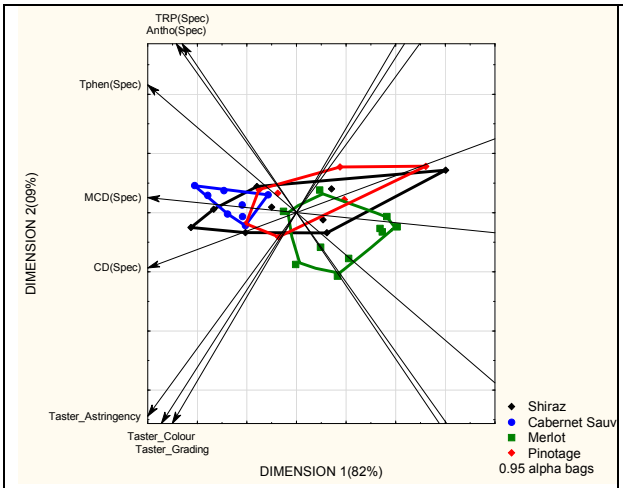
E Shiraz

Figure 4.5 Multivariate analyses of the 2008 data collected by the tasters and the real chemical data from the wines post-MLF

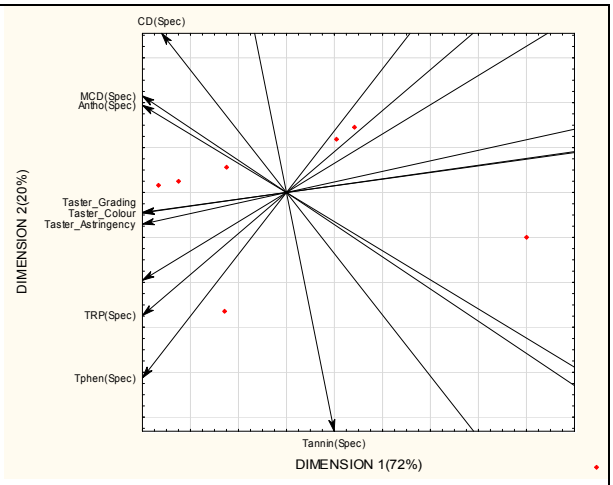
4.4.5.3 2009

In 2009, taster colour, taster astringency and taster grading had high positive associations with each other (Figure 4.5 A-E). These relationships were also statistically significant (results not shown). The collective tasting data was correlated with the actual wine chemical characteristics and it was found that there were no strong positive correlations between the taster data and the chemical data (Figure 4.5 A). Taster colour, taster astringency and taster grading correlated positively, but not statistically significantly, with CD, MCD, TRP, anthocyanin and total phenol (W, spec). It can be deduced from this plot that the Merlot wines had the lowest colour of the cultivars and that the opposite was the case for the Cabernet Sauvignon wines. Shiraz and Pinotage showed a larger range of CD and MCD than the other two cultivars (please refer to Table 4.4 for chemical data). This scenario changed somewhat with the bi-plots of some of the individual cultivars (see also correlations in Tables 1.6 to 1.9, Addendum A). It seems as though the taster grading is strongly related to colour and does not take into account the tannin structure to determine the overall grading.

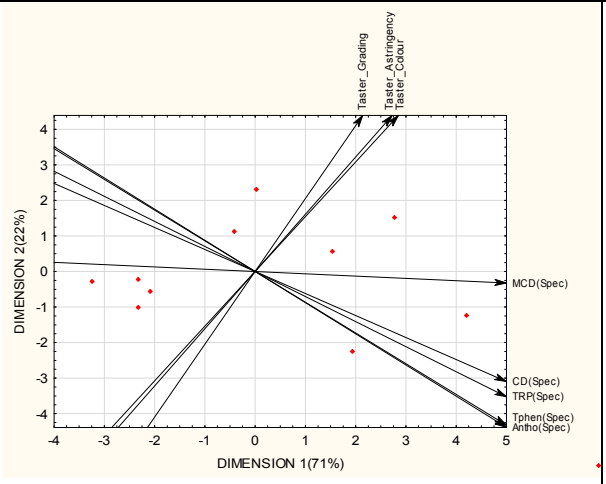
The actual tannin levels were not correlated with taster astringency, and taster grading and real grading also were not correlated (see Tables 1.6-1.9 Addendum A). The only difference between these two sets of tastings was in the case of the Merlot data, which showed a positive correlation between taster grading and the real grading (assignedto the blocks by the winery). The tasters found positive correlations between all taster parameters and the actual quality grading that was given to these blocks by the viticulturalist. Shiraz grapes showed positive correlations between all parameters tasted, and in some cases significant correlations were found (Table 1.9 Addendum A).



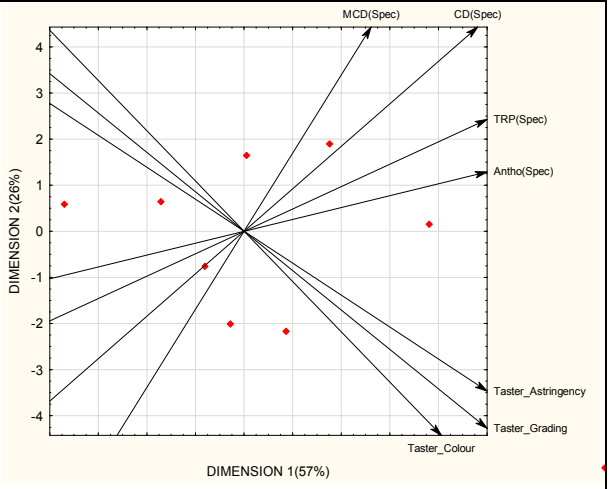
A All Cultivars



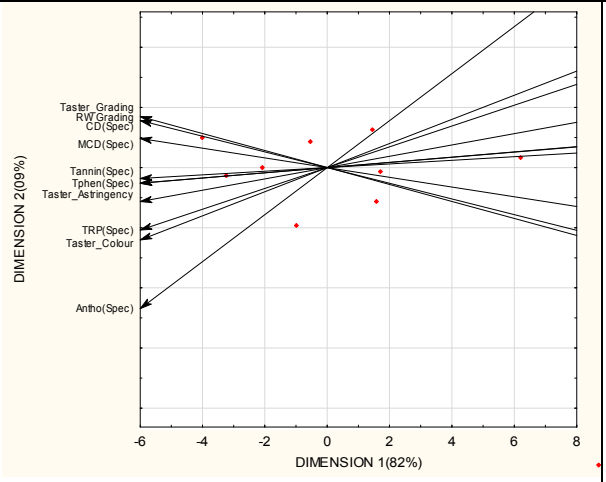
B Pinotage



C Merlot



D Cabernet Sauvignon



E Shiraz

Figure 4.5 (A-E) Bi-plots showing the results from the tasters and their correlation with the actual chemical data of the wines (post-MLF analyses).

4.5 Conclusion

Various positive and statistically significant correlations were established between the grape and wine parameters. These correlations were affected by differences in cultivar, seasonal factors as well as method of analysis of the parameters. Cabernet Sauvignon showed high positive correlations between grape and wine colour parameters over all three seasons, Merlot showed these correlations for two of the seasons in question, and Pinotage and Shiraz showed high positive correlations only for one season. This could be as a result of inherent differences regarding extractability, or it could be linked to the ripening stage. More high positive correlations were found between comparisons of grape anthocyanin determined by HPLC and wine colour density measured with the spectrophotometer. From the perspective of a co-operative cellar, this could make the predictions shown in this study tedious and expensive. Other studies have shown different levels of success using other methods, such as the Glories extraction method for grape colour and phenolic analyses. The Glories method is less costly than the HPLC, but also entails an array of steps to be performed. The ultimate answer to the problem of which analyses are best suited, lies in the use of NIR and MIR technology to measure grapes already in a homogenate phase. Various works has been published in recent years supporting these technologies and their good correlation to HPLC and other methods. The fact that tasters can perceive clear differences between the colour of the wine and also directly link this to quality shows that colour is definitely one of the most important grape characteristics to be used for predicting a wine's quality. Important factors to bear in mind are the seasonal and cultivar influences and, very importantly, the extraction of anthocyanin and phenolic material from the grapes. As is now known, large variations could occur during the vinification step, to such an extent that the grapes with the highest anthocyanin content do not always deliver wine with the best colour. When remunerating a grape producer a cellar should thus keep in mind the level of colour compounds in the grapes as well as their extractability. The findings of this study can contribute to improve the grading and payment systems for grape growers of RW. More consistency in the results will improve the potential for the application of grape colour for remuneration purposes on a commercial scale. More focused research on particular methods and samples from a more even ripening stage could deliver just that.

4.6 References

Arozarena, I., Ayestarán, B., Cantalejo, M., Navarro, M., Vera, M., Abril, I., & Casp, A., 2002. Anthocyanin composition of Tempranillo, Garnacha and CabernetSauvignon grapes from high- and low-quality vineyards over two years. Eur. Food Res.Tech. 214:303-309.

Boselli, E., Boulton, R.B., Thorngate, J.H. & Frega, N.G., 2004. Chemical and sensory characterization of d.o.c. red wines from Marche (Italy) related to vintage and grape cultivars. J. Agric. Food Chem. 52: 3843-3854.

Boselli, E., Minardi, M., Giomo, A. & Frega, N.G., 2006. Phenolic composition and quality of white d.o.c. wines from Marche(Italy). Anal. Chim. Acta. 563: 93-100.

Boulton, R., 2001. Copigmentation of anthocyanins and its role in the colour of red wine: a critical review. Am. J. Enol. Vitic. 52:67-87.

Cagnasso, E., Rolle, L., Caudana, A & Gerbi, V., 2008. Relationship between grape phenolic maturity and red wine phenolic composition. It. J. Food Sci. 20: 365-380.

Dambergs, R.G., Cozzolino, D., Cynkar W.U., Janik L. & Gishen, M., 2006. The determination of red grape quality parametersusing the LOCAL algorithm. J. Near Infr. Spectros. 14: 71-79.

Demiglio, P. & Pickering, G.J., 2008. The influence of ethanol and pH on the taste and mouth feel sensations elicited by redwine. J. Food Agric. Environ. 6: 143-150.

Du Toit, W.J. & Visagie, M., 2012. Correlations between South African Red Grape and Wine Colour and Phenolic Composition: Comparing the Glories, Iland and Bovine Serum Albumin Tannin Precipitation Methods. S. Afr. J. Enol. Vitic. 33: 33-41.

Du Toit, W.J., Lisjak, K., Marais, J. & Du Toit, M., 2006. The effect of micro-oxygenation on the phenolic composition, quality and microbial composition of South African red wines. S. Afr. J. Enol. Vitic. 27: 57-67.

Fernández-López, J.A., Almela, L., Muñoz, J.A., Hidalgo, V. & Carreño, J., 1998. Food Res. **31**: 667-672.

Fontoin, H., Saucier, C., Teissedre, P.-L. & Glories, Y., 2008. Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. Food Qual. Prefer. 19: 286-291.

Fournand, D., Vicens, A., Sidhoum, L., Souquet, J.-M., Moutounet, M. & Cheynier, V., 2006. Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. J. Agric. Food Chem. 54: 7331-7338.

Gawel, R., 1998. Red wine astringency: a review. Aust. J. Grape Wine Res. 4: 74-95.

Gawel, R., Iland, P.G., Leske, P.A. & Dunn, C.G., 2001. Compositional and sensory differences in Syrah wines following juicerun-off prior to fermentation. J. Wine Res. 12: 5-18.

Gawel, R., Oberholster, A. & Francis, I.L., 2000. A 'mouth-feel wheel': terminology for communicating the mouth-feelcharacteristics of red wine. Australian Journal of Grape and Wine Research, 6: 203-207.

Gishen, M., Iland, P.G., Dambergs, R.G., Esler, M.B., Francis, I.L., Kambouris, A., Johnstone, R.S. & Hoj, P.B., 2001. Objective measures of grape and wine quality. Proc. 11th Aust. Wine Ind. Tech. Conf. October 2001, Adelaide, Australia. pp. 188-194.

Glories, Y., 2001. Characterisation of phenolic potential: adaptation during vinification. Prog. Agric. Vitic. pp. 118-347.

Harbertson, J.F., Picciotto, E.A. & Adams, D.O., 2003. Measurement of polymeric pigments in grape berry extracts and winesusing a protein precipitation assay combined with bisulfite bleaching. Am. J. Enol. Vitic. 54: 301-306.

Iland, P., 1987. Predicting red wine colour from grape analyses. The Aust. Grapegrower & Winemaker. 29: 285.

Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. Techniques for chemical analysis and quality monitoring during winemaking. Patric Iland Wine Promotions, Campbelltown.

Ishikawa, I. & Noble, A.C., 1995. Temporal perception of astringency and sweetness in red wine. Food Qual. Prefer. 6: 27-33

Jensen, J.S., Demiray, S., Egebo, M. & Meyer, A.S., 2008. Prediction of wine colour attributes from the phenolic profiles of red grapes (*Vitis vinifera*). J. Agric. Food Chem. 56: 1105-1115.

Kallithraka, S., Bakker, J. & Clifford, M.N., 1997. Effect of pH on astringency in model solutions and wines. J. Agric. Food Chem. 45: 2211-2216.

Landon, J.L., Weller, K., Harbertson, J.F. & Ross, C.F., 2008. Chemical and sensory evaluation of astringency in Washingtonstate red wines. Am. J. Enol. Vitic. 59: 153-158.

Lawless, H.T. & Corrigan, C.J., 1993. Semantics of astringency. In: Kurihara, K., Suzuki, N. & Ogawa, H. (eds). Olfaction andtaste XI. Proc. 11th Int. Symp. Olfaction and Taste and 27th Japanese Symp. on Taste and Smell, MONTH, Tokyo, Japan. pp. 288–292.

Lawless, H.T. & Heymann, H., 1998. Descriptive analysis. In: Hall, C. (ed). Sensory evaluation of food: principles and practices Plenum Publishing, New York. pp. 341–378.

Lochner, I., 2006. The evaluation of Fourier transform infrared spectroscopy (FT-IR) for the determination of total phenolics andtotal anthocyanins concentrations of grapes. Master of Science in Agriculture Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Marais, J. & October, F., 2005. Correlation between grape colour and wine quality. Available: http://wynboer.co.za/recentarticles/200507colour.php3

Marais, J., Iland, P. & Swart, E., 2001. Exploring the correlations between Pinotage grape colour and wine quality – preliminary results. Available: http://wineland.co.za/wynboer_archive/recentartivles/0201 explore.html

Mercurio, M.D., Dambergs, R.G., Cozzolino, D., Herderich, J.J. & Smith, P.A., 2010. Correlation between red wine grades and phenolics. 1. Tannin and total phenolics concentrations. J. Agric. Food Chem. 58: 12313-12319.

Obreque-Slíer, E., Peña-Neira, A. & López-Solís, R., 2010. Enhancement of both salivary proteinenological tannin interactionsand astringency perception by ethanol. J. Agric. Food Chem. 58: 3729-3735.

Peng, Z., Iland, P.G., Oberholster, A., Sefton, M.A. & Waters, E.J., 2002. Analysis of pigmented polymers in red wine byreverse phase HPLC. Aust. J. Grape Wine Res. 8: 70-75.

Perrin, L., Symoneaux, R., Maître, I., Asselin, C., Jourjon, F. & Pagès, J., 2007. Comparison of conventional profiling by atrained tasting panel and free profiling by wine professionals. Am. J. Enol. Vitic. 58(4): 508-517.

Ribéreau-Gayon, P., Glories, Y., Meujean, A. & Dubourdieu, D., 1998. Handbook of enology, Vol. 2. The chemistry of wine stabilization and treatments. John Wiley and Sons, New York.

Ristic, R., Downey, M.O., Iland, P.G., Bindon, K., Francis, I.L., Herderich, M. & Robinson, S.P., 2007. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. Aust. J. Grape Wine Res. 13: 53-65.

Romero-Cascales, I., Ortega-Regules, A.O., Lopez-Roca, J.M., Fernandez-Fernandez, J.I. & Gomez-Plaza, E., 2005.Differences in anthocyanin extractability from grapes to wines according to variety. Am. J. Enol. Vitic. 56: 212-219.

Rossouw, M. & Marais, J., 2004. The phenolic composition of South African Pinotage, Shiraz and Cabernet Sauvignon wines.S. Afr. J. Enol. Vitic. 25: 94-104.

Sacchi, K.L., Bisson, L.F. & Adams, D.O., 2005. A review of the effect of winemaking techniques on phenolic extraction in red wines. Am. J. Enol. Vitic. 56: 197-206.

Sáenz-Navajas, M.P., Tao, Y.S., Dizy, M., Ferreira, V. & Fernández-Zurbano, P., 2010. Relationship between non-volatile composition and sensory properties of premium Spanish red wines and their correlation to quality perception. J. Agric. FoodChem. 58: 12407-12416.

Sarneckis, C.J., Dambergs, R.G., Jones, P., Mercuric, M., Herderich, M.J. & Smith, R.A., 2006. Quantification of condensedtannins by precipitation with methyl cellulose: development and validation of an optimized tool for grape and wine analysis. Aus. J.Grape Wine Res. 12: 39-49.

Singleton, V.L., 1988. Wine phenols. In: Linskens, H.F. & Jackson, J.D. (eds). Wine analysis. 6. Modern methods of plantanalysis. Springer-Verlag, Heidelberg. pp. 173–218.

Somers, T.C. & Evans, M.E., 1974. Wine quality: correlations with colour density and anthocyanin equilibria in a group of youngred wines. J. Sci. Food. Agric. 25: 1369-1379.

Somers, T.C. & Vérette, E., 1988. Phenolic composition of natural wine types. In: Linskens, H.F. & Jackson, J.F. (eds). Wine analysis. 6. Modern methods of plant analysis. Springer-Verlag, Heidelberg. pp. 219–257.

Vidal, S., Courcoux, P., Francis, L., Kwiatkowski, M., Gawel, R., Williams, P., Waters, E. & Cheynier, V., 2004. Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. Food Qual. Prefer. 15: 209217.

Chapter 5

Research results

Rapid measurement of anthocyanin, total phenolic and tannin content in red grape homogenates using near-infrared spectroscopy and chemometric methods

Chapter 5: Rapid measurement of anthocyanin, total phenolic and tannin content in red grape homogenates using near-infrared spectroscopy and chemometric methods

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5.1 Abstract

The current data collected at grape harvest is often insufficient to predict the end-quality of the wines made thereof. Since anthocyanin and tannin of red grapes is commonly accepted as important to wine quality these parameters should be measured during grape intake. More importantly the method of measurement should be fast, efficient and reliable. Fourier transform near infrared spectroscopy (FT-NIR) is such a method and has been applied with success on grape homogenates and especially wines (Ferrer-Gallego *et al*., 2011; Fragoso *et al*., 2011; Laghi *et al.,* 2011; Romera-Fernàndez *et al*., 2012).

Red grape homogenates were analysed using a near-infrared Bruker multipurpose analyser (MPA) spectrophotometer in the 12500-4000 cm^{-1} range. Anthocyanin and total phenol content was spectrophotometrically determined for 241 and 240 red grape homogenate samples respectively. The tannin content of 142 samples was determined using bovine serum albumin precipitation. Partial least squares regression (PLS-R) was used to establish models to predict the anthocyanin, total phenol and tannin content of the grape homogenates. The r^2 and the standard error of cross validation (SECV) for anthocyanin, total phenol and tannin content were 0.81 and 0.10%; 0.61 and 0.19%; 0.59 and 0.26% respectively. The standard error in prediction for anthocyanin, total phenol and tannin was 0.11 mg/g fresh berry weight, 0.18 mg/g fresh berry weight and 0.18 mg/g catechin equivalents, respectively. The use of FT-NIR for the accurate determination of grape anthocyanin content in wineries is therefore possible. More importantly the use of FT-NIR can be applied in high through-put laboratories, often used by co-operative wineries. This type of analyses could possibly be incorporated into an in-line type measurement to even

further improve the practically of such analyses at wineries in South Africa. This work also shows the first calibration for anthocyanins in grape homogenate instead of grape extract.

5.2 Introduction

Phenolic compounds of red grapes and wines are commonly accepted as quality indicating characteristics in a wine (Dambergs *et al*., 2006). These compounds relate to the colour intensity and mouth feel of red wines. Co-operative wineries in particular are increasingly being required to devise objective tests by which wine quality can be predicted when grapes are received at the cellar for vinification (Marais & October, 2005; Cagnasso *et al*., 2008; Celotti & Carcereri de Prati, 2005). The importance of such tests is further driven by the remuneration that is coupled with the quality rating of grapes at intake (van der Merwe *et al*., 2012). Apart from the normal parameters that are considered as important at harvest, such as pH, titratable acidity (TA) and sugar content (Dambergs *et al*., 2003; Gishen *et al*., 2004), additional analyses should be included to strengthen quality rating systems of such cellars. Anthocyanin and tannin concentrations could be a good quality indicator of especially red grapes at harvest (Francis *et al*., 2004; Herderich *et al*., 2004). Publications on the relationship between red grapes and the wines made thereof have shown that it is possible to predict wine colour and phenolic composition already at grape harvest (Dambergs *et al*., 2006; Cozzolino *et al*., 2006; Gishen *et al*., 2005; Herderich & Smith, 2005; Ough & Singleton, 1968).

There are two major obstacles in the way of successfully introducing anthocyanin and tannin analyses for wine quality prediction in a winery system. Firstly, knowledge of the phenolic content of grape samples at ripeness for the particular grape growing area is needed. This will enable the analyst to determine how the data obtained relate to the quality of the grapes and therefore wines. It is, however, difficult to interpret these tests and their potential value for a winery, unless a database with vintage-related trends and correlations between grape and wine parameters is established.

In the second instance, it is of critical importance to establish methods that are able to measure phenolics derived from grapes not only accurately, but also rapidly and cost effectively. Anthocyanin and total phenolic concentrations of red grapes are often spectrophotometrically determined at 520 and 280 nm, respectively, in grape extracts, which requires extensive sample preparation (Iland *et al*., 2000). Preparation time of grape extracts is much longer than that of grape homogenates. The research presented in this chapter, deals with the second aspect, namely establishing a fourier transform infrared spectroscopy (FT-IR) method for fast and accurate measurement of anthocyanin, total phenol and tannin quantities in grape homogenates.

FT-IR has been shown to be a solution for the rapid measurement of routine grape juice and wine analyses. FT-IR can give an enormous amount of parameters at the ease of one scan that takes only a few seconds and the calibrations are very specific for the matrix that's being analysed, such as grape juice, fermenting must, white wine and red wine (Palma & Barroso, 2002; Kupina & Shrikhande, 2003; Moreira & Santos, 2004; Swanepoel *et al.,* 2007). FT-IR can roughly be divided into 2 wavelength regions namely, near infrared (FT-NIR) and mid infrared (FT-MIR). They refer to 12600-4000 cm-1 and the 4000-900 cm^{-1} respectively. The FT-NIR absorptions reflect more overtones and combination bands of fundamental transitions; therefore it can be seen as much less distinct as FT-MIR spectra (Bellon-Maurel & McBratney, 2011). The technology is based on the measurement of the frequencies of chemical bonds in functional groups such as C-C, C-H, O-H, C=O and N-H, upon absorption of radiation in near infrared (NIR) regions. Absorbance is thus directly proportional to the concentration of a particular compound (Skoog *et al*., 1997; Smith, 1999).

The prediction of phenolic compounds in red wine using FT-NIR started over the last decade. In 2004 FT-NIR was used to successfully predict malvidin-3-glucoside, pigmented polymers and tannin in red wines (Cozzolino *et al.,* 2004). A monochromator instrument together with partial least squares (PLS) regression with internal cross validation was used. The r^2_{CAL} > 0.80 and the residual predictive deviation (RPD) ranged from 1.8-5.8. These authors concluded that FT-NIR could be used as a rapid predictive method for phenolic compounds in red wines. FT-NIR was also shown to be suitable for the prediction of wine elements such as Ca, Fe and K with r^2_{CAL} of > 0.86 for all three elements (Cozzolino *et al*., 2007). FT-NIR was applied together with PLS to measure condensed tannin and dry matter in red grape homogenates (r^2_{CAL} > 0.86 for both instances) (Cozzolino *et al*., 2008). FT-NIR has been applied with varying degrees of success for anthocyanin and phenolic measurements directly from the intact grapes in the vineyard using hand held equipment (Cerovic, 2008; Ferrer-Gallego *et al*., 2011; Gonzàlez-Caballero *et al*., 2010; Laghi *et al*., 2011). The most recent work on phenolic compounds of red wine also includes using Raman spectroscopy and preprocessing techniques (Ferrer-Gallego *et al*., 2011). Improvements on prediction error values have also been investigated recently to aid the developments in FT-NIR and FT-MIR with regards to accuracy (Overgaard *et al.,* 2012).

The aim of the present work was to implement FT-NIR spectroscopy for the quantification of anthocyanins, tannins and total phenols in red grape homogenates. The calibration models were specifically designed for red grape cultivars of the Robertson winemaking region, South Africa and included 9 different cultivars and 3 vintages. This calibration provides the wine industry with an analytical tool that gives rapid, reliable results with the absolute minimum sample preparation. The phenolic content of grapes can be determined at intake to aid the winery in classification of grapes into different quality categories.

5.3 Materials and Methods

5.3.1 Grape samples: Origin and preparation

Samples from 52 different vineyards from the Robertson region, South Africa, consisting of 9 different cultivars over 3 vintages (Table 5.1) were used for the calibration. Pinoptage, Merlot, Cabernet Sauvignon and Shiraz are more common varieties in the Robertson region and therefore make up the largest proportion of the samples.

Table 5.1 Cultivars and number of different vineyard blocks used over 3 vintages (2007-2009) to establish the calibration models for anthocyanin, total phenol and tannin content in grapes.

Cultivar	Number of different blocks
Shiraz	13
Merlot	12
Cabernet Sauvignon	12
Pinotage	9
Mouvedre	2
Pinot noir	1
Petit Verdot	1
Grenache	1
Tannat	1

Grape samples at commercial harvest were picked and the berries placed into plastic zip lock bags and transported to a 4°C fridge as soon as possible. The samples remained here for a maximum of 48 hours prior to homogenisation. For homogenisation 50 berries (weighed, Iland *et al*., 2000) from each block sample was used. Homogenisation was done at high speed 24 000 rpm until a smooth paste was formed using an IKA ultra-turrax T18 (IKA – Werke GmBH & Co, Staufen). Post homogenisation samples were stored in a -20°C freezer until analysis. Samples were removed from the -20°C freezer and thawed to reach room temperature of approximately 20°C (±2°C) for scanning and subsequent extraction. Grape extracts (50% ethanol v/v) were prepared separately from each of these homogenates (Iland *et al*., 2000) to use in the reference method measurements.

5.3.2 Reference methods

Anthocyanin and total phenols concentrations (mg/g fresh berry weight) of the grape extract were measured using the Iland method (Iland *et al*., 2000), as described in detail in Chapter 3, Section 3.2.2.

Bovine serum albumin (BSA) is commonly used in spectrophotometric methods for quick tannin analyses in the field of oenology (Harbertson *et al*., 2003). Grape and wine samples were analysed using only the tannin determination leg of the BSA precipitation method (Van der Merwe *et al*., 2012).

Anthocyanin, total phenols and tannin concentrations were determined using a UV-Vis spectrophotometer (Analytic Jena Specord 50 UV/VIS Spectrophotometer; Jena, Germany) in triplicate.

5.3.3 Near Infrared Spectroscopy

5.3.3.1 FT-NIR instrumentation and spectroscopic measurements

A MPA multipurpose analyser FT-NIR instrument (Bruker Optics, GmbH, Germany) was used to collect the spectra. Aliquots (approximately 2 grams) of thawed grape homogenate were placed in the rotating sample cup with a quartz base and diameter of 50 mm, which allowed that a relatively large area of the samples to be scanned. The sample cup was coupled to the rotating integrating sphere of the instrument and spectra in reflectance mode were collected at the PbS detector. The wavenumber range or scanning interval was set from 12500 to 3600 cm^{-1} and 2307 data points per spectrum were recorded. Spectra were taken at a resolution of 8 cm^{-1} and the average of 32 scans per sample was stored. A background spectrum was generated prior to and on an hourly basis during analysis by using exactly the same parameters as for the grape samples, and a gold plate that gave 100% reflectance. This was done to compensate for possible changes in the internal instrumental conditions.

5.3.3.2 PLS-R model construction

PLS was used to establish new calibration models for each of the three chemical parameters, using OPUS 5.5 software (Bruker Optics, GmbH, 2004). PLS-R is a bilinear modelling method whereby the **X** data, referring to the absorbance of grape homogenates at the respective wave numbers are projected onto a small number of underlying variables called partial least squares components. The calculation of PLS components actively uses the reference data values (**Y** data, anthocyanin, total phenol and tannin content in this study) to ensure that the first PLS components are most relevant for predicting these variables (Næs, *et al*., 2002). The relationship between X and Y data is described by this linear equation:

$$y= b_0 + b_1x_1 + b_2x_2 + b_nx_n$$

Where y is the dependent variable, b_1 to b_n is the regression co-efficients, b_0 is the intercept and x_1 to x_n represent the absorbance at the selected wave numbers.

The three y variables were each scaled to unit variance, by multiplication with 1 divided by the standard deviation (1/SD).

Calibration models were developed using randomly selected samples in segmented cross validation, in order to identify outlier samples and to evaluate different spectral pre-processing techniques. The techniques tested were multiplicative scatter correction, first- and second derivatives, vector normalisation, linear offset subtraction, straight line subtraction and combinations of these processes. In addition to the spectral pre-processing, various combinations of the spectral range were also included in the model building stage. More than 350 models per chemical compound were tested and the one with the lowest root mean square error of cross validation (RMSECV), lowest number of PLS components and with high r^2 value was chosen to be used in a subsequent round of independent test set validations.

Final evaluation of the predictive abilities of the respective models was done by repeated rounds of independent test set validation, each time selecting 30% of the full set as validation samples, while ensuring that the minimum and maximum values were in the calibration set. The accuracy of calibration is expressed in standard error of prediction (SEP) of the bias-corrected residuals. The bias refers to the average difference between y and y1 in the prediction set. If the bias becomes 0 the error of validation is equal to the standard deviation (SD). RMSEP was also used to express the accuracy of samples from individual sets and is calculated as the accuracy of the calibration against the reference methods. RPD is determined by the deviation of the SD from the reference data divided by the SEP of the reference data.

5.4 Results and Discussion

5.4.1 Descriptive statistics of phenolic content in grape homogenates

The choice of the reference methods was primarily based on being commonly used by the South African wine industry and easy to apply (Iland *et al*., 2000; Harbertson *et al*., 2003). Descriptive statistics were applied on these compounds in the homogenates to determine if the datasets to be used for the calibration and validation have normal distribution and compares well with levels of these compounds in other literature (Table 5.2). It was previously found that Robertson grapes tend to be lower in anthocyanin and high in total phenol/tannin content at a balling level (24 °B) where grapes is normally considered to be ripe. This is mostly the effect of the extreme heat experienced in Robertson (van der Merwe *et al*., 2012). This was confirmed for the anthocyanins and total phenol ranges that were respectively 0.29-1.40 mg/g and 0.67-2.01 mg/g fresh berry weight. From literature anthocyanins and total phenols can respectively range from 0.1-3.7 mg/g and 0.1-7.1 mg/g

(Bramley, 2005; Romero-Cascales *et al*., 2005; Janik *et al*., 2007; Jensen *et al*., 2008; Segade *et al*., 2008; Hanlin *et al*., 2009; Mattivi *et al*., 2009; Du Toit & Visagie, 2012). The tannin content as measured with the BSA method resulted to a range of 0.72 – 1.96 mg/g catechin equivalents (Table 5.2). According to literature the grape tannin content (BSA precipitation) varies between 0.50-1.10 mg/g (Mercurio & Smith, 2008), but the specific ripeness levels of these samples is not known. The normal distribution of the anthocyanin and tannin concentrations were acceptable (p=0.2 and p=0.4) but the total phenols concentrations were skewed to an extent (p=0.03) (data not shown).

Table 5.2 Descriptive statistics of grape homogenate samples used to develop PLS-R calibration models for the prediction of the anthocyanin, total phenol and tannin content.

Stats*	n	Mean	SD	Min	Max
Anthocyanin (mg/g)	241	0.73	0.25	0.29	1.40
Total phenol (mg/g)	240	1.33	0.30	0.67	2.01
Tannin (mg/g)	142	1.35	0.27	0.72	1.96

*Abbreviations used: n: number of samples; mean: average level of compound determined; SD: Standard deviation; Min: Minimum level for compounds determined; Max: Maximum level for the compounds determined; CV: Coefficient of variation (SD/Aver*100) expressed as %.

FT-NIR spectrum for red grapes homogenate is influenced by water and displays two prominent peaks (Figure 1). This first peak demonstrates the OH stretch and the second one the OH asymmetric stretch and bending combination (Cozzolino *et al*., 2005; Cozzolino *et al*., 2006; Dambergs *et al*., 2006; Osborne *et al.,* 1993).

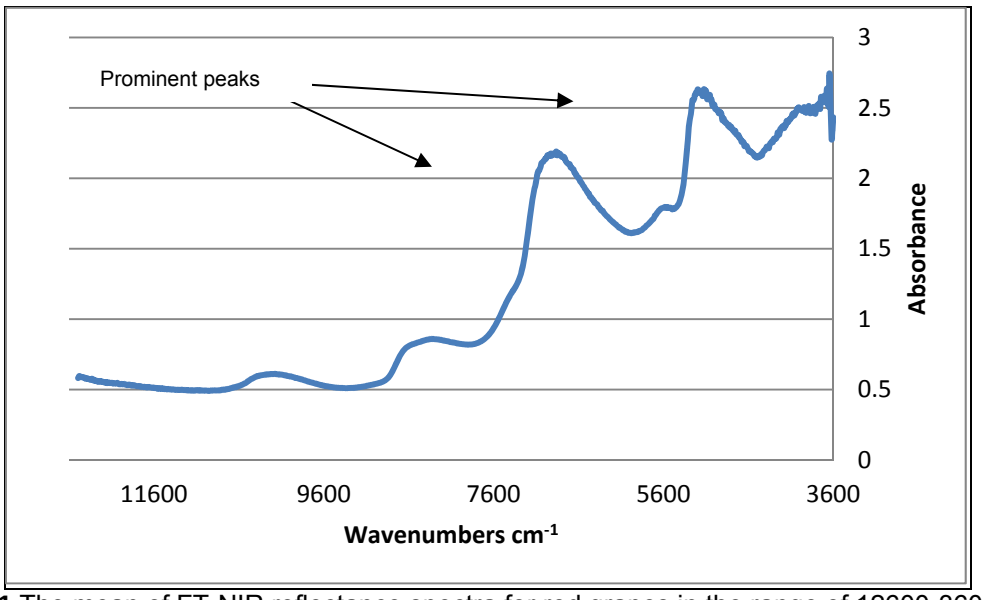

Figure 5.1 The mean of FT-NIR reflectance spectra for red grapes in the range of 12600-3600 cm^{-1}.

5.4.2 Calibration models

Wave number regions were selected for the different phenolic parameters investigated. In the case of anthocyanins and total phenols two regions were selected: 1) 12493.2-7498.3 cm^{-1} and 2) 6102-5774.1 cm^{-1}, while for tannins the wave number region 12493-5446.3 cm^{-1} was selected.

Calibration statistics were developed using cross validation which is considered to be a very useful statistical method to evaluate the predictability of calibration equations. The calibration models developed for anthocyanin, total phenol and tannin contents using UV-VIS and FT-NIR had varying results (Table 5.3).

Table 5.3 Near infrared partial least squares (PLS) calibration and validation statistics for anthocyanin, total phenols and tannin content of grape homogenates from the Robertson grape growing region.

Calibration statistics						
	n^a	r^2_{CAL}	SECV	RPD	PLS Factor	
Anthocyanin (mg/g)[b]	241	0.81	0.10	2.32	8	
Total phenols (mg/g)	240	0.61	0.19	1.60	7	
Tannin (mg/g)	142	0.59	0.26	1.57	9	
Validation statistics						
	n	r^2_{VAL}	SEP	RPD	PLS Factor	Bias
Anthocyanin	80	0.75	0.11	2.04	7	0.006
Total phenols	80	0.61	0.18	1.61	7	-0.006
Tannin	43	0.64	0.18	1.70	6	-0.460

[a]Abbreviations used: n: number of samples; r^2: coefficient of determination in calibration or validation; SECV: standard error of cross validation; SEP: standard error of prediction; RPD: residual predictive deviation: SD/SECV, where SD is the standard deviation of the sample
set; PLS factor: Partial least squares factor.
[b]Wavenumber regions for anthocyanin and total phenol were 12493.2-7498.3 cm^{-1} and 6102-5774.2 cm^{-1} and for tannin 12493.2-5446.3 cm^{-1}.

Of the developed calibrations the highest r^2 values were for anthocyanin content (r^2_{CAL} = 0.81) (Figure 5.2). This is also the component with the lowest standard error of cross validation in combination with the high correlation (SECV = 0.01). The RPD for anthocyanin content is 2.3, underlining the applicability of FT-NIR for determining this parameter (Chang *et al*., 2001; Dunn *et al*., 2002). These authors suggested that RPD values below 1.5 were considered insufficient for most application while FT-NIR calibration models with values greater than 2 were considered excellent. Calibration models with RPD values between 1.5 and 2 were deemed as useful contingents for the determination of the specific parameter in question (Fearn, 2002; Williams, 2001). The determination of anthocyanin content with FT-NIR has been investigated and also applied to a great extent in Australia. Various authors has shown the success of this application and also the practical applicability thereof in the

industry, especially for the segregation of grapes with regards to quality and payment (Dambergs *et al*., 2003; Gishen *et al*., 2005; Cynkar *et al*., 2004). This calibration can be further improved by using other regression methods. For instance the LOCAL algorithm was found to improve the prediction error of especially anthocyanin content and pH, because it lowers the effect of outliers on the highest and lowest concentration of the dataset (Dambergs *et al*., 2006). Artificial neural networks (ANN) have also been applied to improve the prediction of total anthocyanin content in red grape homogenates with success (Janik *et al*., 2006). Other ways of improving a prediction model could be by applying powered partial least squares (PPLS) and backward variable selection of partial least squares (BVSPLS) regression for especially smaller datasets as was more recently shown (Overgaard *et al*., 2012). It was shown that PPLS gave the best predictive performance of all methods and also gave the selections of variables that were most easily assigned to specific chemical bonds.

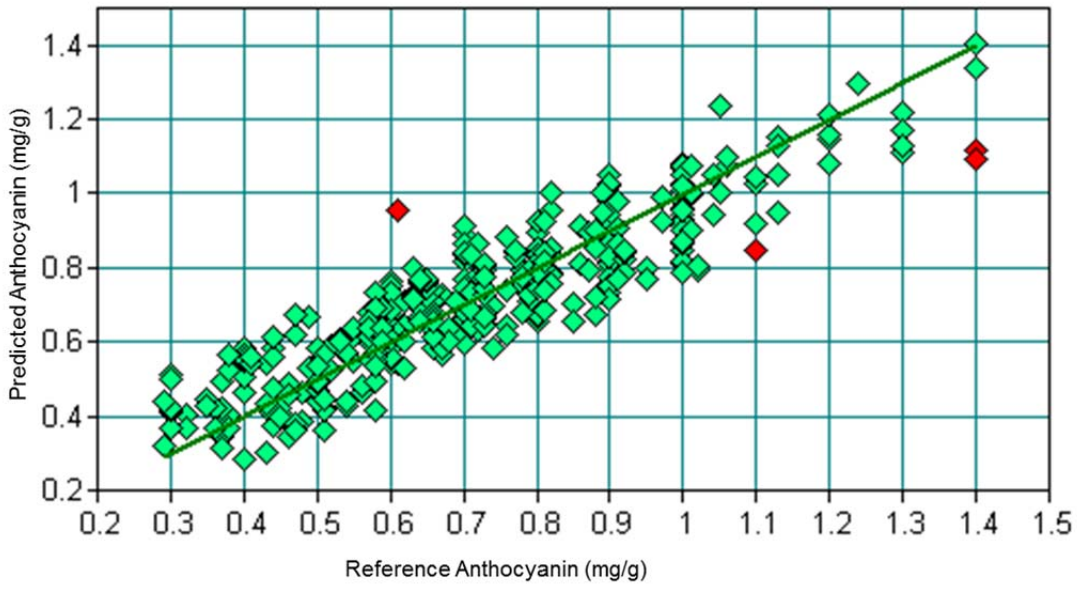

Figure 5.2 Comparison of the anthocyanin concentration (mg/g berry) determined by the reference method, UV-VIS, with those predicted by FT-NIR using partial least squares regression in red grape homogenate samples. The red samples indicate outliers from the regression model.

A r^2_{CAL} value of 0.61 for total phenols, with a SECV 0.19 and RPD of 1.57 (Table 5.3, Figure 5.3). This means that the total phenol prediction is not excellent but still useful for the prediction of the compound, but should be improved (Chang *et al*., 2001; Dunn *et al*., 2002; Overgaard *et al*., 2012). It is highly probable that the lack of specificity of the reference method probably contributed to this lower predictive ability of FT-NIR for use on total phenol content. This method basically consists of measuring an acidified ethanol extracted grape sample at 280 nm with a UV-VIS spectrophotometer. This type of measurement is very

coarse and could include other compounds, such as proteins, that are not related to the phenolic family.

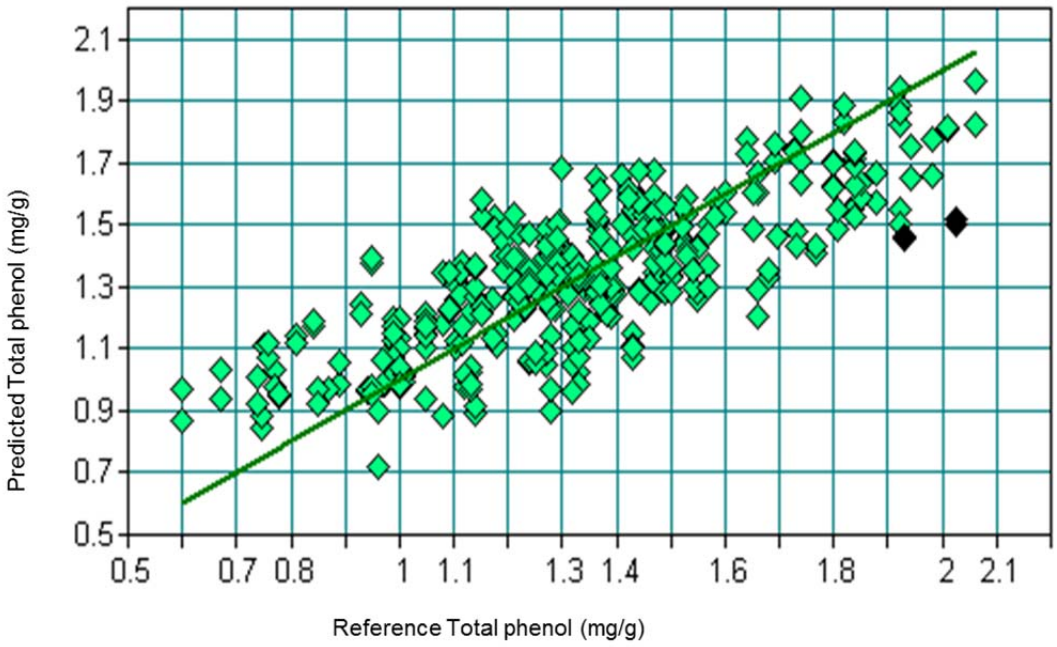

Figure 5.3 Comparison of the concentration of Total phenols (mg/g berry) determined by UV-VIS with those predicted by NIR using partial least squares regression in grape homogenate samples. Samples in black indicate outliers from the regression model.

NIRS on the other hand delivered a slightly better model for tannin content prediction (Table 5.3, Figure 5.4). The RDP of the present calibration was also between 1.5 and 2 deeming it usable for preliminary screening. In other work where condensed tannin (methylcellulose precipitation as reference method) were used to create a calibration model for NIRS the r^2_{CAL}, SECV and RPD was found to be 0.86, 0.46 and 3.3 respectively (Cozzolino *et al*., 2008), showing that it is possible to calibrate for tannin. The sample used in the current study was possibly too small for these more complex structures in grapes to calibrate successfully.

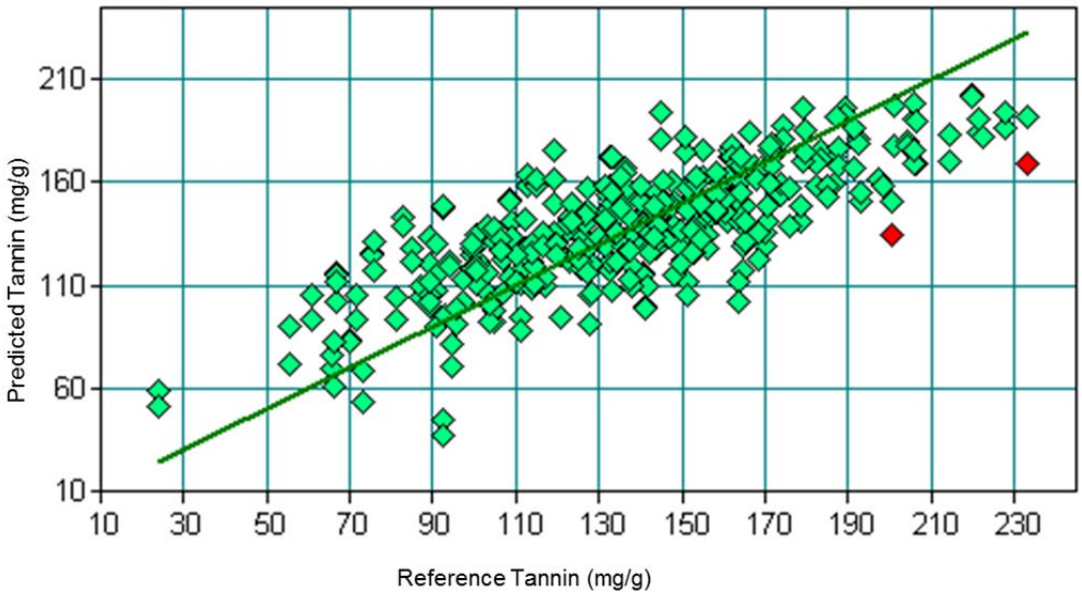

Figure 5.4 Comparison of the concentration of Tannin (mg/L berry) determined by UV-VIS with those predicted by NIR using partial least squares regression in grape homogenate samples. Samples in red indicate outliers from the regression model.

The robustness of the sample set could be an influencing factor on the calibration for total phenols and tannin content. It was previously deduced by other authors that different grape varieties contained in a sample set can influence the calibration to a degree (Gozzolino *et al*., 2004).

Validation of these calibrations was done using repeated rounds of independent test validation by using 30% of randomly selected samples in the validation sets (Figure 5.5). Good predictions were obtained for all three models developed during this investigation. The SEP for anthocyanin, total phenols and tannin content were 0.11 mg/g, 0.18 mg/g and 0.18 mg/g respectively (Table 5.3). This for instance shows that a given grape homogenate can be scanned with NIR technology and give results for anthocyanin content that will be correct within 0.11 mg/g. The best model was developed for anthocyanin prediction and more or less similar levels of accuracy were experienced in the case of total phenols and tannin.

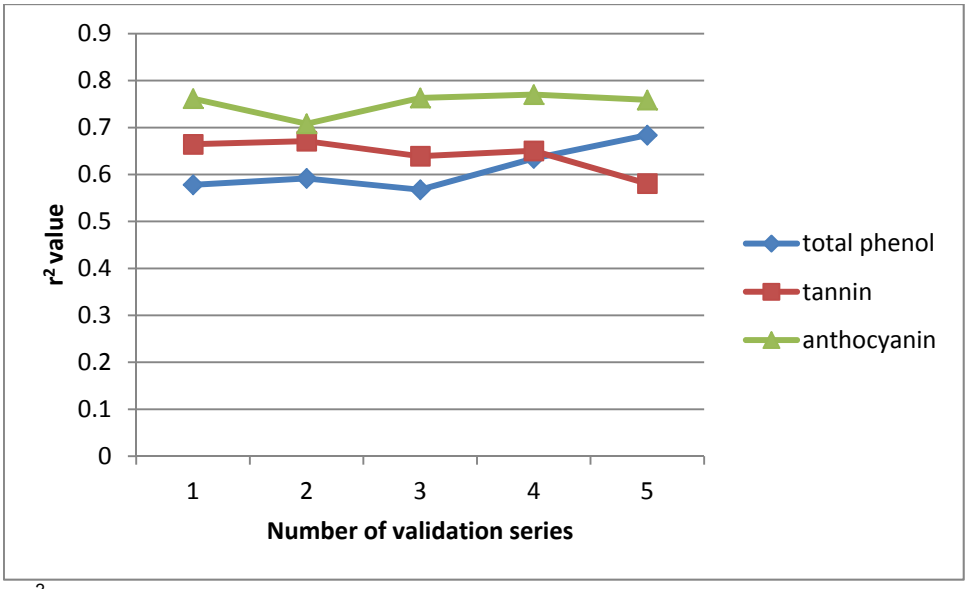

Figure 5.5 r^2 values for validation test series for anthocyanin content, total phenol and tannin content of grape homogenates analysed with FT-NIR using grape homogenates.

In summary, the results obtained in this study demonstrated the potential of the use of NIR spectroscopy to quantify anthocyanin, total phenols and tannin content of red wine grapes. The development of the calibrations for total phenols and tannin could still be improved, probably by either using a greater sample set, samples with a greater distribution in compound concentration or by developing separate calibration models for individual varietals, as well as investigating additional methods for spectral pre-processing.

5.5 Conclusions

The best calibration model was built for anthocyanin content of grape homogenates. However, total phenol and tannin contents of grapes could also be predicted by NIR spectroscopy to a certain extent. Many reasons why total phenol and tannin content showed lower correlations with the calibration models could exist of which molecule complexity and reference method is the most likely ones and important to mention. Calibration models for the rapid determination of anthocyanins, total phenol and tannin content is very important to wine cellars for quality predictions, in particular, the co-operative wineries. In these large winery systems, where grapes are delivered for payment, an increased amount of rapidly obtainable parameters to predict potential grape/wine quality could ensure a more fair remuneration system to grape suppliers.

In South Africa such systems has been lacking, but with the availability of NIR (and other) equipment improving as well as more literature available on the successful application of these calibrations, the grading of grapes may improve. In future a larger sample set could also improve current calibrations. The reasons why setting up a calibration for total phenols

and tannins is more challenging be it due to the current reference methods or the complicated nature of these compounds should also be elucidated.

5.6 References

Bellon-Maurel, V. & McBratney, A., 2011. Near infrared (NIR) and mid-infrared (MIR) spectroscopic techniques for assessing the amount of carbon stock in soils – critical review and research perspectives. J. Soil Biol. Bioch. 43: 1398-1410.

Bramley, R.G.V., 2005. Understanding variability in wine grape production systems (2): Within vineyard variation in quality over several vintages. Aust. J. Grape Wine Res. 11: 33-42.

Cagnasso, E., Rolle, L., Caudana, A. & Gerbi, V., 2008. Relationship between grape phenolic maturity and red wine phenolic composition. Ital. J. Food Sci. 20(3): 365-380.

Celotti, E. & Carcereri de Prati, G., 2005. The phenolic quality of red grapes at delivery: Objective evaluation with colour measurements. S. Afr. J. Enol. Vitic. 26(2): 75-82.

Cerovic, Z.G., Moise, N., Agati, G., Latouche, G., Ghozlen, B. & Meyer, S., 2008. New portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence. J. Food Comp. Anal. 21: 650-654.

Chang, C.W., Laird, D.A., Mausbach, J.J. & Hurburgh, C.R., 2001. Near-Infrared Reflectance Spectroscopy–Principal Components Regression Analyses of Soil Properties Soil Sci. Soc. Am. 65: 480-490.

Cozzolino, D., Cynkar, W.U., Dambergs, R.G., Mercurio, M.D. & Smith, P.A., 2008. Measurement of condensed tannins and dry matter in red grape homogenates using near infrared spectroscopy and partial least squares. J. Agric. Food Chem. 56: 7631-7636.

Cozzolino, D., Dambergs, R.G., Cynkar, W.U., Janik, L. & Gishen, M., 2006. Analysis of grapes and wine by near infrared spectroscopy. J. NIRS. 14: 279-289.

Cozzolino, D., Kwiatkowski, M.J., Dambergs, R.G., Cynkar, W.U., Janik, L.J., Skouroumounis, G. & Gishen, M., 2007. Analysis of elements in wine using near infrared spectroscopy and partial least squares regression. Talanta, doi:10.1016/j.talanta.2007.06.045.

Cozzolino, D., Kwiatkowski, M.J., Parker, M., Cynkar, W.U., Dambergs, R.G., Gishen, M. & Herderich, M.J., 2004. Prediction of phenolic compounds in red wine fermentations by visible and near infrared spectroscopy. Anal. Chem. Act. 513: 73-80.

Cynkar, W.U., Cozzolino, D., Dambergs, R.G., Janik, L. & Gishen, M., 2004. The effects of homogenisation method and freezing on the determination of quality parameters in red grape berries of *Vitis vinifera*. Aust. J. Grape wine Res. 10: 236-242.

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. & New Zeal. Grapegrower Winemaker Ann. Tech. Issue. pp. 69-76.

Dambergs, R.G., Cozzolino, D., Cynkar, W.U., Janik, L., & Gishen, M., 2006. The determination of red grape quality parameters using the LOCAL algorithm. J. NIR spectros. 14: 17-79.

Du Toit, W.J., Visagie, M., 2012. Correlations between South African Red Grape and Wine Colour and Phenolic Composition: Comparing the Glories, Iland and Bovine Serum Albumin Tannin Precipitation Methods. S. Afr. J. Enol. Vitic. 33: 33- 41.

Dunn, B.W., Batten, G.D. & Ciavarella, S., 2002. The potential of near infrared reflectance spectroscopy – a case study from the Riverine plain of South Eastern Australia. Aust. J. Exp. Agric. 42: 607.

Edelmann, A., Diewok, J., Shuster, K.C. & Lendl, B., 2001. Rapid Method for the discrimination of red wine cultivars based on Mid-infrared spectroscopy of phenolic wine extracts. J. Agric Food Chem. 49: 11139-11145.

Fearn, T., 2002. Assessing calibration: SEP, RPD, RER and R2. NIR news. 13: 12-14.

Ferrer-Gallego, R., Hernández-Hierro, J.M., Rivas-Gonzalo, J.C. & Escribano-Bailón, M.T., 2011. Determination of phenlic compounds of grape skins during ripening by NIR spectroscopy. Food Sci. Tech. 44: 847-853.

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

Fragoso, S., Aceña, L., Guasch, J., Busto, O. & Mestres, M., 2011. Application of FT-MIR spectroscopy for fast control of red grape phenolic ripening. J. Agric. Food Chem. 59: 2175-2183.

Francis, I.L, Høj, P.B., Dambergs, R.G., Gishen, M., De Barros Lopes, M.A., Pretorius, I.S., Godden, P.W., Henschke, P.A., Herderich, M.J. & Waters, E.J., 2004. Objective measures of grape quality – are they achievable? In: Blair, R.J., Williams,P. & Pretorius, I.S. (eds). Proceeding of the 12th Australian Wine Industry Technical Conference, July 2004, Melbourne, Australia. pp. 85 – 89.

Gishen, M., Iland, P.G., Dambergs, R.G., Esler, M.B., Francis, I.L., Kambouris, A., Johnstone, R.S. & Høj, P.B., 2004. Objective measures of grape and wine quality. In: Blair, R.J., Williams, P. and Pretorius, I.S. (eds). Proceeding of the 12th Australian Wine Industry Technical Conference, July 2004, Melbourne, Australia. pp. 188 – 194.

Gishen, M., Cozzolino, D. & Dambergs, R.G., 2005. Grape and wine analysis – Enhancing the power of spectroscopy with chemometrics. A review of some application in the Australian wine industry. Austr. J. Grape Wine Res. 11: 296-305.

Givens, D.I. & Deaville, E.R., 1999. The current and future role of near infrared reflectance spectroscopy in animal nutrition: A review. Aust. J. Agric. Res. 50: 1131.

González-Caballero, V., Sánchez, M-T., López, M-I. & Pérez-Marín, D., 2010. First steps towards the development of a non-destructive technique for the quality control of wine grapes during on-vine ripening and on arrival at the winery. J. Food Eng. 101: 158-165.

Hanlin, R.L. & Downey, M.O., 2009. Condensed tannin accumulation and composition in skin of Shiraz and Cabernet Sauvignon Grapes during berry development. Am. J. Enol. Vitic. 60(1): 13-23.

Harbertson, J.F., Picciotto, E.A. & Adams, D.O., 2003. Measurement of polymeric pigments in Grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. Am. J. Enol. Vitic. 54(4): 301-306.

Herderich, M.J. & Smith, P.A., 2005. Analysis of grape and wine tannins: methods, applications and challenges. Aust. J. Grape Wine Res. 11: 205-214.

Herderich, M.J., Bell, S.-J., Holt, H., Ristic, R., Birchmore, W., Thompson, K. & Iland, P.G., 2004. Balanced wine: what might be achieved in the vineyard? In: Blair, R.J., Williams, P. & Pretorius, I.S. (eds). Proceeding of the 12th Australian Wine Industry Technical Conference, July 2004, Melbourne, Australia. pp. 79 – 84.

Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer N., 2000. Techniques for chemical analysis and quality monitoring during winemaking: Determination of red pigments (colour) and total phenolics of grapes berries. P. Iland Wine promotion, Cambelltown.

Janik, L.J., Cozzolino, D., Dambergs, R., Cynkar, W. & Gishen, M., 2007. The prediction of total anthocyanin concentration in red grape homogenates using visible-near-infrared spectroscopy and artificial neural networks. Anal. Chim. Act. 594: 107-118.

Jensen, J.S., Egebo, M. & Meyer, A.S., 2008. Identification of spectral regions for the quantification of red wine tannins with Fourier Transform Mid-Infrared Spectroscopy. J. Agric. Food Chem. 56: 3493-3499.

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

Laghi, L. & Versari, A., 2011. FT-IR spectroscopy and direct orthogonal signal correction preprocessing applied to selected phenolic compounds in red wines. Food Anal. Meth. 4: 619-625.

Marais, J. & October F., 2005. Relationship between grape colour and wine quality. Wynboer 191: 15-17. www.wynboer.co.za/recentarticles/200507colour.php3

Mattivi, F., Vrhovsek, U, Masuero, D. & Trainotti, D., 2009. Aust. J. Grape Wine Res. 15: 27-35.

Mercurio, M.D. & Smith, P.A., 2008. Tannin quantification in red grapes and wine: comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency. J. Agric. Food Chem. 56 (14): 5528-5537.

Moreira, J.L. & Santos, L., 2004. Spectroscopic interferences in fourier transform infrared wine analysis. Anal. Chim. Acta. 513: 81-89.

Næs, T., Isaksson, T., Fearn, T. & Davies, T., 2002. A user-friendly guide to multivariate calibration and classification. NIR publications, Chichester, UK.

Osbourne, B.G., Fearn, T. & Hindle, P.H., 1993. Practical NIR Spectroscopy with application in food and beverage analysis. Longman Publishers, Singapore.

Ough, C.S. & Singleton, V.L., 1968. Wine quality prediction from juice brix/acid ratio and associated compositional changes for “White Riesling” and “Cabernet Sauvignon”. Wine Quality prediction. pp. 129-138.

Overgaard, S.I., Pierna, J.A.F., Baeten, V., Dardenne, P. & Isaksson, T., 2012. Prediction error improvements using variable selection on small calibration sets – a comparison with some recent methods. J. NIRS. 20: 329-337.

Palma, M. & Barossa, C.G., 2002. Application of FT-IR spectroscopy to the characterisation and classification of wines, brandies and other distilled drinks. Talanta. 58: 265-271.

Romera-Fernández, M., Berrueta, L.A., Garmón-Lobato, S., Gallo, B., Vicente, F. and Moreda, J.M., 2012. Feasibility study of FT-MIR spectroscopy and PLS-R for the fast determination of anthocyanins in wine. Talanta. 88: 303-310.

Romero–Cascales, I., Ortega–Regules, A., Roca, J.M., Fernandez, J.I. & Gomez–Plaza, E. 2005. Differences in anthocyanins extractability from grapes to wines according to varieties. Am. J. Enol. Vitic. 56(3): 212-219.

Segade, S.R., Rolle, L., Gerbi, V. & Orriols, I., 2008. Phenolic ripeness assessment of grape skin by texture analysis. J. Food Comp. Anal. 21: 644-649.

Skoog, D.A., Holler, F.J. & Nieman, T.A., 1997. (5th ed). Principles of instrumental analysis. Harcourt Brace College Publishers, USA.

Smith, B., 1999 (1st ed). Infrared spectral interpretation: a systematic approach CRC Press LLC, Florida, USA.

Swanepoel, M., Du Toit, M. & Nieuwoudt, H.H., 2007. Optimisation of the quantification of total soluble solids, pH and titratable acidity in South African grape must using fourier transform mid-infrared spectroscopy. S. Afr. J. Enol. Vitic. 28(2): 140-145.

Van der Merwe, H., Nieuwoudt, H., De Beer, D. & Du Toit, W.J., 2012. Comprehensive survey of the colour and phenolics of different red grape wine vineyard blocks from the Robertson area in South Africa. S. Afr. J. Enol. Vitic. 33(1): 58-71.

Williams, P.C., 2001. Implementation of near0infrared technology, in: Williams, P.C., Norris, K.H. (Ed.), American Association of cereal chemist. St. Paul, Minnesota, USA. pp. 145-169.

Chapter 6

General discussion and conclusions

6. General discussion and conclusions

6.1 Concluding remarks

The Robertson wine region in South Africa experience high temperatures (>27°C) in the critical growth phases post véraison. The developmental influence this has on anthocyanin and overall flavonoid biosynthesis is known to a large extent. At temperatures higher than 27°C anthocyanin biosynthesis slows down and completely stops at 30°C. Site, season, water and temperature is the four ultimate factors that influence phenolic development in grapes (Bakker *et al*., 1986; Gonzalez-San Jose *et al*., 1990; Revilla *et al*., 1997; McDonald *et al*., 1998; de Freitas & Glories, 2000; Guidoni *et al*., 2002; Ojeda *et al*., 2002). The importance of anthocyanin and tannin composition in the quality of wines is widely accepted and therefore using these parameters in quantifying grape quality is of interest (Gishen *et al*., 2001). During grading of grapes a combination of factors such as viticultural practices, historic performance of the blocks and chemical parameters, are used. Some of these factors already influence the variability of anthocyanin and tannin content of grapes. It is important to acknowledge that extracting phenolic compounds from grapes to wine may also introduce variability. The most important factors influencing anthocyanin and tannin extraction is skin contact time, temperature and pressing technique (Bergand Akiyoshi, 1956; Ough & Amerine, 1961; Aubert & Poux, 1969; Scudamore-Smith *et al*., 1990; Mayen *et al*. 1994; Kovac *et al*., 1992; Zimman *et al*., 2002; Aron & Kennedy, 2007). Therefore a large number of variables can influence the colour and phenolic correlations between grapes and wines. Finding situations in which the use of anthocyanin and tannin content of grapes can be successfully employed to predict potential wine quality is thus valid, but does come with a myriad of stumbling blocks to overcome. Internationally, there has been a lot of interest to include more parameters in quality determination of grapes and therefore quality prediction of the wines made there of (Celotti & Carcereri de Prati, 2005; Romero-Cascales, 2005; Cagnasso *et al*., 2008; Jensen *et al*., 2008; Mercurio *et al*., 2010), which has been done with varying degrees of success. Seasonal changes and finding an accurate and fast measuring technique for phenolic compounds in grapes may prove challenging to this model. In South Africa all results on correlations between grape and wine regarding colour has been more positive than for instance correlations regarding total phenol or tannin contents (Marais & October, 2005; Du Toit & Visagie, 2012; van der Merwe *et al*., 2012). Wine colour is the first level of quality that a taster can perceive and therefore it could be seen as an important component to use as red grape quality determination. Grape colour is also more easily measured than grape tannin concentration which is a further reason to focus on this. This research aimed to establish a database of anthocyanin and tannin

contents for Robertson Winery (RW) red grapes from different cultivars over 3 seasons, to establish correlations between these compounds in the grapes and the wines made thereof and to look into new techniques for rapid measurement of these compounds. This work as a unit forms a sound basis for of the prediction of wine anthocyanin and tannin quality already in the grape stage.

During the first part of this work the distribution of anthocyanin and phenolic content of red grapes from the Robertson wine region was investigated. It was found that the average anthocyanin content of these grapes were lower than when compared to grapes from other areas, like for instance Australia (Hanlin *et al*., 2009). Anthocyanin accumulation is hampered by these temperatures (>27°C) and this could probably be managed by manipulating vines through for instance keeping the bunch zone more covered as opposed to the general practice of leaf removal in the bunch zones. Another factor that came to light is the fact that the tannin levels in the grapes were sometimes high which could be indicative of "unripe" phenolic compounds. This, however, was not verified by looking at other parameters or methods to test for the "phenolic ripeness" level. Nevertheless, low, high as well as average levels for all parameters were established for 3 consecutive seasons. A wider range of colour and phenolic data were seen in 2007 versus 2008 and 2009 that showed more similar results. It was also found that the colour and phenolic content of grapes varied due to cultivar. Shiraz was found to be in general highest in colour and Pinotage in total phenolic content. Differences perceived can be further linked to different blocks of the same cultivar and even different rootstocks seemed to influence the overall distribution of the colour and phenolic content. Follow-on work would be to try and define *terroirs* according to colour and phenolic composition for the important cultivars of the Robertson area. Using the aerial mapping as a tool would make the definitions of areas with low and high colour and phenolic content more visual for grape producers.

The second part of this study established correlations between grape and wine colour and phenolic compounds. This resulted in an array of r^2 values that varied in strength considering sample size, cultivar, season and so forth. Situations in which the correlations were considered to be strong were mostly per cultivar per season, except for colour that showed overall good correlation between grapes and wines over different seasons and cultivars. The more variables (cultivars and seasons) that were used to establish correlations between total phenol and tannin content of grapes and wines the lower it became. The type of measurement that was used to determine a specific parameter also seemed to play a role in the success of the correlations. However, it is important to understand that the extraction of anthocyanins and tannins from grapes is influenced by various extrinsic factors such as punch downs, aerations and eventually pressing. If these techniques is not applied correctly or similarly between seasons the resulting wines may still

lack in colour and phenolic compounds even if present at high concentrations in the grapes. Follow on research should further explore the positive correlations that were already established. The occurrence of varying correlations between grape and wine parameters should be further exploited and explained. The construction of a mathematical equation to better describe it should also be considered.

The third part of the research concentrated on establishing FT-NIR as a rapid method for anthocyanin, total phenol and tannin measurement. Calibrations were based on measuring the grape homogenate, thereby skipping the time consuming extraction step. Calibrations were successful in the case of anthocyanin and tannin content, but validation of the total phenol calibration was not as satisfactory at this stage. This is probably as a result of the complex nature of the total phenols as well as the specific reference methods that was used for the analyses. The use of FT-NIR in a high throughput laboratory environment is without question the future. Follow on research should focus on improving calibrations using pre-processing techniques. Accuracy, time efficacy and ease of use are also important considerations to be kept in mind.

This study makes a significant contribution to the improvement of the potential prediction of wine quality already at harvest not only to Robertson Winery, but also for the South African wine industry. It demonstrates the importance of anthocyanin and tannin content of grapes for wines and how these compounds vary across cultivar and season in one region. It also shows how these compounds could be potentially employed in predicting end wine colour, phenolic composition and possibly quality. Lastly it shows that FT-NIR can be used to rapidly obtain grape colour and tannin levels in grapes which could be used at any winery to improve the applicability of grape measurements in red wine production.

6.2 References

Aron, P.M., & Kennedy, J.A., 2007. Compositional investigation of phenolic polymers isolated from *Vitis vinifera* L. cv. Pinot noir during fermentation. J. Agric. Food Chem. 55: 5670-5680.

Aubert, S. & Poux, C., 1969. Extraction descomposés phénoliques du raison II . Taux depassage dans les vins. Ann. Technol. Agric.18: 111-127.

Bakker, J., Preston, N.W. & Timberlake, C.F., 1986. The determination of anthocyanins in aging red wines: Comparison of HPLC and spectral methods. Am. J. Enol. Vitic. 37: 121-126.

Berg, H.W., and M. Akiyoshi. 1956. The effect of contact time of juice with pomace on the color and tannin content of red wines. Am. J. Enol. Vitic. 7: 84-90.

Cagnasso, E., Rolle, L., Caudana, A. & Gerbi, V., 2008. Relationship between grape phenolic maturity and red wine phenolic composition. It. J. Food Sci. 20(3): 365-380.

Celotti, E. & Carcereri de Prati, G., 2005. The phenolic quality of red grapes at delivery: Objective evaluation with colour measurements. Am. J. Enol. Vitic. 26 (2): 75-82.

De Freitas, V.A.P., Glories, Y. & Monique, A., 2000. Developmental changes of procyanidins in grapes of red *Vitis vinifera* varieties and their composition in respective wines. Am. J. Enol. Vitic. 51(4): 397-403.

Du Toit, W.J., Visagie, M., 2012. Correlations between South African red grape and wine colour and phenolic composition: comparing the Glories, Iland and Bovine Serum Albumin tannin precipitation methods. *S. Afr. J. Enol. Vitic.* 33: 33- 41.

Gishen, M., Iland, P.G., Dambergs, R.G., Esler, M.B., Francis, I.L., Kambouris, A., Johnstone, R.S. & Hoj, P.B., 2001. Objective measures of grape and wine quality. Proc. 11th Aust. Wine Ind. Tech. Conf. October 2001, Adelaide, Australia. pp. 188-194.

Gonzalez-San Jose, M.L., Santa-Maria, G. & Diez, C., 1990. Anthocyanins as parameters for differentiating wines by grape variety, wine-growing region, and wine-making methods. J. Food Comp. Anal. 3: 54-66.

Guidoni, S., Allara, P. & Schubert, A., 2002. Effect of cluster thinning on berry Anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. Am. J. Enol. Vitic. 53: 224-226.

Hanlin, R.L. & Downey, M.O., 2009. Condensed tannin accumulation and composition in skin of Shiraz and Cabernet Sauvignon grapes during berry development. Am. J. Enol. Vitic. 60 (1): 13-23.

Jensen, J.S., Demiray, S., Egebo, M. & Meyer, S., 2008. Prediction of wine colour attributes from the phenolic profiles of red grapes (*Vitis vinifera*). J. Agric. Food Chem. 56: 1105-1115.

Kovac, V., Alonso,E., Bourzeix, M. & Revilla, E., 1992. Effect of several enological practices onthe content of catechins and proanthocyanidins of red wines. J. Agric. Food Chem. 40: 1953-1957.

Marais, J. & October, F., 2005. Relationship between grape colour and wine quality. Wynboer, 2005. 191: 15-17.

Mayen, M., Merida, J. & Medina, M., 1994. Free anthocyanins and polymeric pigmentsduring the fermentation and post-fermentationstanding of musts from Cabernet Sauvignon and Tempranillo grapes. Am J. Enol. Vitic.45: 161-166.

McDonald, M.S., Hughes, M., Burns, J., Lean, M.E.J., Matthews, D. & Crozier, A., 1998. Survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. J. Agric. Food Chem. 46: 368-375.

Mercurio, M.D., Dambergs, R.G., Cozzolino, D., Herderich, J.J. & Smith, P.A., 2010. Correlation between red wine grades and phenolics. 1. Tannin and total phenolics concentrations. J. Agric. Food Chem. 58: 12313-12319.

Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A. & Deloire, A., 2002. Influence of pre- and postveraison water deficit on synthesis and concentraton of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. Am. J. Enol. Vitic. 53: 261-267.

Ough, C.S. & Amerine, M.A., 1961. Studies oncontrolled fermentation V. Effects on color, composition and quality of red wine. Am. J. Enol. Vitic. 12: 9-19.

Revilla, E., Alonso, E. & Kovac, V., 1997. The content of catechins and procyanidins in grapes and wines as affected by agro-ecological factors and technological practices. Am. Chem. Soc., Washington, DC. In Wine: Nutritional and Therapeutic Benefits. T.R. Watkins (ed.). pp. 69-80.

Scudamore-Smith, P.D., Hooper, R.L. & McLaran, E.D., 1990. Color and phenolic changes of Cabernet Sauvignon wine made by simultaneous yeast/bacterial fermentation and extended pomace contact. Am. J. Enol. Vitic. 41: 57-67.

Romero-Cascales, I., Ortega-Regules, A., López-Roca, J.M., Fernández-Fernández, J.I. & Gómez- Plaza, E., 2005. Differences in anthocyanin extractability from grapes to wines according to variety. Am. J. Enol. Vitic. 56(3): 212-219.

Van der Merwe, H., Nieuwoudt, H., De Beer, D. & Du Toit, W.J., 2011. Comprehensive survey of the colour and phenolics of different red grape wine vineyard blocks from the Robertson area in South Africa. S. Afr. J. Enol. Vitic. 33(1): 58-71.

Zimman, A., Joslin, W.S., Lyon, M.L., Meier, J. & Waterhouse, A.L., 2002. Maceration variables affecting phenolic composition in commercial scale Cabernet Sauvignon winemaking trials. Am. J. Enol. Vitic. 53: 93-98.

Addendum A

Chapter 4

Additional results

Addendum A

Table 1.1 The correlations between the wine colour and phenolic components after alcoholic and malolactic fermentation for all seasons and cultivars combined.

Wine parameters	r^{2*}	p-val**
CD[1]	0.63	<0.01
MCD[2]	0.69	<0.01
TRP[3]	0.65	<0.01
Antho[4]	0.53	<0.01
TotColPigm[5]	0.52	<0.01
Tphen[6]	0.74	<0.01
Tannins[7]	0.60	<0.01
TotFlav[8]	0.82	<0.01

[1]Colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm without any additions to the sample;
[2]Modified colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm after addition of acetaldehyde;
[3]Total red pigments measure spectrophotometrically at 520 nm after acidification;
[4]Anthocyanin in mg/L according to a bleaching method;
[5]Total colour pigments, including the polymeric pigments added together, as measured by HPLC (Peng *et al*., 2001);
[6]Total phenols as measured with the spectrophotometer at 280 nm (Iland *et al*., 2000));
[7]Tannin in mg/L catechin units as measured with the spectrophotometer using the BSA method (Harbertson *et al*., 2000)(only for 2008/2009);
[8]Total flavanols refers to the large peak at 280 nm as measured with HPLC (Peng *et al*., 2001);
*Correlation expressed as r^2 value between two parameters;
**Significance level of <0.01 was used.

Table 1.2 The correlations between the wine colour and phenolic components after alcoholic and malolactic fermentation (AF and MLF), separated into the seasons (2007 to 2009).

	2007		2008		2009	
Wine parameters	r^{2*}	p-val**	r^2	p-val	r^2	p-val
CD[1]	0.37	0.03	0.68	<0.01	-0.01	0.98
MCD[2]	0.28	0.10	0.63	<0.01	0.16	0.39
TRP[3]	-0.09	0.62	0.70	<0.01	0.43	0.02
Antho[4]	0.01	0.95	0.85	<0.01	0.35	0.06
TotColpigm[5]	0.38	0.03	0.89	<0.01	0.33	0.17
Tphen[6]	0.05	0.80	0.65	<0.01	0.73	<0.01
Tannins[7]	0.15	0.39	0.76	<0.01	0.39	0.04
TotFlav[8]	0.86	<0.01	0.78	<0.01	0.75	<0.01

[1]Colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm without any additions to the sample;
[2]Modified colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm after addition of acetaldehyde;
[3]Total red pigments measure spectrophotometrically at 520 nm after acidification;
[4]Anthocyanin in mg/L according to a bleaching method;
[5]Total colour pigments, including the polymeric pigments added together, as measured by HPLC (Peng *et al*., 2001);
[6]Total phenols as measured with the spectrophotometer at 280 nm (Iland *et al*., 2000));
[7]Tannin in mg/L catechin units as measured with the spectrophotometer using the BSA method (Harbertson *et al*., 2000)(only for 2008/2009);
[8]Total flavanols refers to the large peak at 280 nm as measured with HPLC (Peng *et al*., 2001);
*Correlation expressed as r^2 value between two parameters;
**Significance level of <0.01 was used.

Table 1.3 The correlations between post-alcoholic fermentation and post-malolactic fermentation data for the wines for all seasons together per cultivar.

	Pinotage		Merlot		Cabernet		Shiraz	
Parameters	r^{2*}	p val**	r^2	p val	r^2	p val	r^2	p val
CD[1]	0.63	< 0.01	0.63	< 0.01	0.49	< 0.01	0.76	< 0.01
MCD[2]	0.69	< 0.01	0.41	0.04	0.75	< 0.01	0.95	< 0.01
TRP[3]	0.57	< 0.01	0.12	0.57	0.81	< 0.01	0.81	< 0.01
Antho[4]	0.57	< 0.01	0.44	0.03	0.54	< 0.01	0.31	0.09
TotColPigm[5]	0.46	0.06	0.49	0.01	0.20	0.31	0.44	0.02
Tphen[6]	0.52	0.02	0.46	0.02	0.82	< 0.01	0.91	< 0.01
Tannins[7]	0.74	< 0.01	0.72	< 0.01	0.25	0.31	0.56	< 0.01
TotFlav	0.79	< 0.01	0.94	< 0.01	0.58	< 0.01	0.87	< 0.01

[1]Colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm without any additions to the sample;
[2]Modified colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm after addition of acetaldehyde;
[3]Total red pigments measure spectrophotometrically at 520 nm after acidification;
[4]Anthocyanin in mg/L according to a bleaching method;
[5]Total colour pigments, including the polymeric pigments added together, as measured by HPLC (Peng *et al*., 2001);
[6]Total phenols as measured with the spectrophotometer at 280 nm (Iland *et al*., 2000));
[7]Tannin in mg/L catechin units as measured with the spectrophotometer using the BSA method (Harbertson *et al*., 2000)(only for 2008/2009);
[8]Total flavanols refers to the large peak at 280 nm as measured with HPLC (Peng *et al*., 2001);
*Correlation expressed as r^2 value between two parameters;
**Significance level of <0.01 was used.

Table 1.4 Correlations drawn between colour of the grapes and their corresponding wines after AF and after MLF. Each cell indicates the correlation coefficient (r^2 values) for Pinotage, Cabernet Sauvignon and Merlot from the 2007 harvest. All r^2 values of > than 0.45 had a p-val of < 0.01 and is therefor significant.

	Grape parameters							
	Anthocyanins / g berry[7]				TotColPigm[8]			
Wine parameters	Pinotage	Cabernet	Merlot	Shiraz	Pinotage	Cabernet	Merlot	Shiraz
CD[1]_AF[5]	-0.05	-0.15	-0.11	-0.17	0.26	0.51	0.17	0.27
CD_MLF[6]	-0.11	-0.12	-0.15	-0.12	0.86	0.57	0.39	0.22
TRP[2]_AF	0.69	0.76	-0.44	0.27	0.23	0.77	-0.56	0.07
TRP_MLF	0.87	0.71	-0.15	0.45	0.92	0.68	-0.97	0.54
Antho[3]_AF	0.56	0.78	-0.30	0.34	0.16	0.74	-0.18	0.15
Antho_MLF	0.87	0.71	-0.51	0.25	0.87	0.68	-0.75	-0.1
TotColPigm[4]_AF	0.74	0.71	-0.45	-0.04	0.30	0.66	-0.42	-0.00
TotColPigm_MLF	0.65	0.71	-0.56	0.25	0.84	0.71	-0.62	0.30

[1]Colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm without any additions to the sample;
[2]Total red pigments measure spectrophotometrically at 520 nm after acidification;
[3]Anthocyanin in mg/L according to a bleaching method;
[4]Total colour pigments, including the polymeric pigments added together of the wine as measured by HPLC (Peng *et al*., 2001);
[5]Samples obtained after alcoholic fermentation;
[6]Samples obtained after malolactic fermentation;
[7]Grape colour measured with Iland's method (Iland *et al*., 2000);
[8]Total colour pigments, including the polymeric pigments of the grapes as measured with HPLC (Peng et al., 2001).

Table 1.5 Correlations drawn between colour of the grapes and their corresponding wines after AF and after MLF. Each cell indicates the correlation coefficient (r values) for Pinotage, Cabernet Sauvignon and Merlot from the 2008 harvest. All r^2 values of > than 0.45 had a p-val of < 0.01 and is therefor significant.

	Grape parameters							
	Anthocyanins/g berry[7]				Total colour pigments[8]			
Wines	Pinotag	Caberne	Merlo	Shira	Pinotag	Caberne	Merlo	Shira
CD[1]_AF[5]	0.32	0.91	0.50	0.86	0.09	0.87	0.56	0.85
CD_MLF[6]	0.62	0.93	0.64	0.81	0.20	0.89	0.77	0.82
TRP[2]_AF	0.00	0.65	0.89	0.63	-0.11	0.57	0.90	0.71
TRP_MLF	-0.18	0.76	0.60	0.74	-0.09	0.47	0.75	0.76
Antho[3]_AF	-0.12	0.71	0.81	0.71	-0.05	0.53	0.89	0.73
Antho_MLF	0.05	-0.24	0.78	0.16	-0.04	-0.11	0.80	0.29
TotColPig[4]_AF	-0.15	0.85	0.72	0.77	-0.32	0.75	0.84	0.79
TotColPig_ML	-0.34	-0.22	0.39	0.41	-0.34	-0.15	0.54	0.40

[1]Colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm without any additions to the sample;
[2]Total red pigments measure spectrophotometrically at 520 nm after acidification;
[3]Anthocyanin in mg/L according to a bleaching method;
[4]Total colour pigments, including the polymeric pigments added together of the wine as measured by HPLC (Peng *et al*., 2001);
[5]Samples obtained after alcoholic fermentation;
[6]Samples obtained after malolactic fermentation;
[7]Grape colour measured with Iland's method (Iland *et al*., 2000);
[8]Total colour pigments, including the polymeric pigments of the grapes as measured with HPLC (Peng et al., 2001).

Table 1.6 Correlations between the taster and chemical data post-MLF for the Pinotage wines of 2009.

Wine parameters	Taster colour[1]		Taster astringency[2]		Taster grading[3]	
After MLF	r^{2*}	p-val**	r^2	p-val	r^2	p-val
CD[4]	0.71	0.07	0.64	0.01	0.74	0.06
MCD[5]	0.81	0.03	0.72	0.06	0.83	0.02
TRP[6]	0.79	0.03	0.68	0.09	0.78	0.04
Antho[7]	0.76	0.04	0.65	0.11	0.77	0.04
Tphen[8]	0.79	0.03	0.80	0.03	0.81	0.03
Tannin[9]	-0.04	0.94	-0.18	0.70	-0.04	0.60
Real grading[10]	-0.22	0.63	-0.22	0.63	-0.22	0.63

[1]Colour according to a 1-10 graded scale as perceived by the tasters;
[2]Astringencey according to a 1-10 graded scale as perceived by the tasters;
[3]Quality of the wine as perceived by the tasters;
[4]Colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm without any additions to the sample;
[5]Modified colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm after addition of acetaldehyde;
[6]Total red pigments measure spectrophotometrically at 520 nm after acidification;
[7]Anthocyanin in mg/L according to a bleaching method;
[8]Total phenols as measured with the spectrophotometer at 280 nm (Iland *et al*., 2000));
[9]Tannin in mg/L catechin units as measured with the spectrophotometer using the BSA method (Harbertson *et al*., 2000);
[10]Quality graded of the blocks as given by the viticulturist each year before harvest;
*Correlation expressed as r^2 value between two parameters;
**Significance level of <0.01 was used.

Table 1.7 Correlations between the actual Merlot wine data for 2009 post-MLF and the perceived results from the tasters.

Wine parameters	Taster colour[1]		Taster astringency[2]		Taster grading[3]	
After MLF	r^2	p-val	r^2	p-val	r^2	p-val
CD[4]	0.52	0.13	0.43	0.21	0.32	0.37
MCD[5]	0.70	0.03	0.68	0.03	0.60	0.06
TRP[6]	0.55	0.10	0.43	0.21	0.35	0.16
Antho[7]	0.40	0.25	0.35	0.31	0.25	0.15
Tphen[8]	0.30	0.39	0.36	0.31	0.24	0.35
Tannin[9]	0.10	0.78	0.13	0.73	0.07	0.91
Real grading[10]	0.23	0.52	0.36	0.30	0.42	0.07

[1]Colour according to a 1-10 graded scale as perceived by the tasters;
[2]Astringencey according to a 1-10 graded scale as perceived by the tasters;
[3]Quality of the wine as perceived by the tasters;
[4]Colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm without any additions to the sample;
[5]Modified colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm after addition of acetaldehyde;
[6]Total red pigments measure spectrophotometrically at 520 nm after acidification;
[7]Anthocyanin in mg/L according to a bleaching method;
[8]Total phenols as measured with the spectrophotometer at 280 nm (Iland *et al*., 2000));
[9]Tannin in mg/L catechin units as measured with the spectrophotometer using the BSA method (Harbertson *et al*., 2000);
[10]Quality graded of the blocks as given by the viticulturist each year before harvest;
*Correlation expressed as r^2 value between two parameters;
**Significance level of <0.01 was used.

Table 1.8: Correlations between the taster data and the actual colour and phenolic content of the Cabernet Sauvignon wine after MLF in 2009.

Wine parameters	Taster Colour[1]		Taster Astringency[2]		Taster Grading[3]	
After MLF	r^2	p-val	r^2	p-val	r^2	p-val
CD[4]	0.33	0.43	0.57	0.14	0.25	0.45
MCD[5]	0.16	0.70	0.26	0.53	0.24	0.57
TRP[6]	0.47	0.24	0.64	0.09	0.55	0.16
Antho[7]	0.39	0.34	0.48	0.23	0.54	0.16
Tphen[8]	0.23	0.67	0.35	0.54	-0.18	0.67
Tannin[9]	0.10	0.82	0.24	0.57	-0.14	0.74
RW grading[10]	0.63	0.09	0.52	0.19	0.69	0.11

[1]Colour according to a 1-10 graded scale as perceived by the tasters;
[2]Astringencey according to a 1-10 graded scale as perceived by the tasters;
[3]Quality of the wine as perceived by the tasters;
[4]Colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm without any additions to the sample;
[5]Modified colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm after addition of acetaldehyde;
[6]Total red pigments measure spectrophotometrically at 520 nm after acidification;
[7]Anthocyanin in mg/L according to a bleaching method;
[8]Total phenols as measured with the spectrophotometer at 280 nm (Iland *et al*., 2000));
[9]Tannin in mg/L catechin units as measured with the spectrophotometer using the BSA method (Harbertson *et al*., 2000);
[10]Quality graded of the blocks as given by the viticulturist each year before harvest;
*Correlation expressed as r^2 value between two parameters;
**Significance level of <0.01 was used.

Table 1.9 Correlations between the tasting results and the actual chemical results for phenolic data and Shiraz wines after MLF in 2009.

Wine parameters	Taster colour1		Taster astringency2		Taster grading3	
After AF	r^2	p-val	r^2	p-val	r^2	p-val
CD4	0.87	< 0.01	0.93	< 0.01	0.81	< 0.01
MCD5	0.91	< 0.01	0.95	< 0.01	0.79	0.01
TRP6	0.90	< 0.01	0.90	< 0.01	0.66	0.05
Antho7	0.92	< 0.01	0.90	< 0.01	0.63	0.06
Tphen8	0.82	< 0.01	0.90	< 0.01	0.73	0.06
Tannin9	0.60	0.01	0.87	< 0.01	0.87	< 0.01
RW grading10	0.81	< 0.01	0.89	< 0.01	0.94	< 0.01

[1]Colour according to a 1-10 graded scale as perceived by the tasters;
[2]Astringencey according to a 1-10 graded scale as perceived by the tasters;
[3]Quality of the wine as perceived by the tasters;
[4]Colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm without any additions to the sample;
[5]Modified colour density as the combined spectrophotometric reading at 420 nm, 520 nm and 620 nm after addition of acetaldehyde;
[6]Total red pigments measure spectrophotometrically at 520 nm after acidification;
[7]Anthocyanin in mg/L according to a bleaching method;
[8]Total phenols as measured with the spectrophotometer at 280 nm (Iland *et al*., 2000));
[9]Tannin in mg/L catechin units as measured with the spectrophotometer using the BSA method (Harbertson *et al*., 2000);
[10]Quality graded of the blocks as given by the viticulturist each year before harvest;
*Correlation expressed as r^2 value between two parameters;
**Significance level of <0.01 was used.