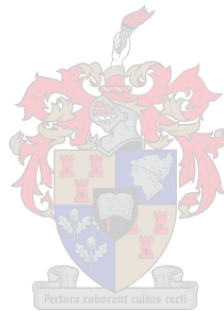


Evaluation of parameters to determine optimum ripeness in Cabernet Sauvignon grapes in relation to wine quality

by

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*Thesis presented in partial fulfilment of the requirements for the degree of
Master of AgriSciences at Stellenbosch University.*

March 2009

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DECLARATION

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SUMMARY

South Africa is the eighth largest wine producing country in the world and face stiff competition on the world market. Cabernet Sauvignon is the most planted red cultivar in the world as well as in South Africa and can be seen as the wine by which countries are judged.

The aim of this study was to investigate suitable, practical maturity parameters or combinations thereof to determine the optimal time to harvest Cabernet Sauvignon grapes under South African conditions. The following parameters were investigated during this study: seed lignification, maturity indexes, anthocyanin concentration per berry, sensory criteria (grape skins tasting and wine) and phenolic content.

Berry development in four Cabernet Sauvignon vineyards in different South African winegrowing areas were investigated over the 2003, 2004 and 2005 seasons. The first parameter to be investigated was seed lignification percentages. Seasonal differences at commercial harvest were observed with values of 2004 varying between 73% and 91% compared to 59% and 80% for the 2003 and 2005 seasons but commercial harvest was two weeks later during the 2004 season. During this study it was found that seeds never reached 100% lignification for Cabernet Sauvignon as was found in previous work to indicate grape maturity. The development of anthocyanins also peaked well before the maximum seed lignification was reached. It therefore appears that seed lignification is not suitable for the determination of grape maturity for Cabernet Sauvignon grapes under South African conditions.

The second parameter to be investigated was maturity indexes (Balling / Titratable Acidity (TA), Balling \times pH, Balling \times pH²). The best wine values were used to determine the optimal maturity index values. Morgenster was the only vineyard to consistently give values that corresponded to previously reported data (index values). Anhöhe and Plaisir de Merle reported higher maturity values than that reported in literature and seasonal variation was observed. Maturity index values for the best wines varied between 88 and 101 (Balling \times pH) for Anhöhe during 2003 and 2005 seasons, but increased too between 97 and 107 (Balling \times pH) for 2004. The maturity index values were found to be vineyard and season dependant, with warmer areas reaching higher values. From this study it appears that maturity index values as a singular maturity parameter does not give a good indication of berry maturity in all seasons or vineyards.

Thirdly, the berry anthocyanin concentration (mg / berry and mg / g berry) were investigated and comparable trends were found between the four vineyards. However vineyards in warmer, drier regions (Anhöhe) tended to have higher anthocyanin concentrations per gram berry. The more vigorous vineyard of Morgenster consistently exhibited a higher anthocyanin concentration per berry. This can be explained by the ratio of skin to pulp between small berries (Anhöhe, 0.95 g - 2004) and larger berries

(Morgenster, 1.82 g – 2004). Wine colour density (A420+A520) followed the same trend as the anthocyanin concentrations of the homogenate.

Grape skins (G) were used to make an artificial wine that was evaluated by an expert panel to determine the development of the grapes. Wines (W) made from sampled batches were also evaluated by an expert panel for: colour intensity, vegetative, red berry, black berry with spice, acidity, astringency and general quality. Vegetative aromas and acidity decreased and red and black berry with spice increased during ripening for both berries and wine. Colour intensity also increased, corresponding to an increase in perceived general quality score. Correlations between general quality of both the grape skins tasting and wines were investigated. Balling showed a strong correlation with general quality of the grape skins tasting ($r = 0.76$; $p = 0.00$) but not as strongly with subsequent wines ($r = 0.57$; $p = 0.00$). Anthocyanin concentration (mg / g berry) of the berries ($r = 0.36$; $p = 0.00$), perceived colour intensity of grapes ($r = 0.69$; $p = 0.00$) and wine ($r = 0.84$; $p = 0.00$) correlated with general wine quality. The tasting panel identified wines that were statically better than the rest for each season and vineyard. Maximum berry anthocyanin concentration coincided with wines rated as the best by the tasting panel. More than one wine was identified during the maximum anthocyanin peak that did not differ statistically from the best wine. It appears from this study that a window period exists at the maximum anthocyanin peak, where wines of comparable quality, but different style, can be produced.

Principal component analysis (PCA) was used to determine the least number of suitable parameters that could distinguish between unripe and ripe grapes in order to establish a grape maturity model. These differences were successfully described by Balling, TA, pH, potassium (K^+), tartaric and malic acid. Anthocyanin concentration could further distinguish between ripe and overripe grapes in the model. From these parameters the minimum and maximum values were used to construct a universal ripeness model containing data from all four vineyards. Variation between the four vineyards caused too much overlapping in the universal model data as the vineyards were situated in different climatic regions according to the Winkler temperature model. On a per vineyard basis this did not occur to the same extent. The best rated Cabernet Sauvignon wines correlated strongly with soluble solid content; colour and quality perceptions of grapes, but large seasonal differences resulted in larger grape compositional variances than that of the individual vineyards in the different climatic zones. This illustrated the difficulty of pinpointing a specific parameter to indicate optimal ripeness. From this study it is clear that a universal maturity model for Cabernet Sauvignon berries is not attainable at present, but individual vineyard models shows the most potential.

A preliminary study into the differences of the phenolic composition was done using reverse phase high performance liquid chromatography (RP-HPLC) on the homogenate and wine. Malvidin-3-glucoside and total anthocyanins followed comparable trends to that found for the Hland method. Strong correlations ($r > 0.9$) were found between the malvidin-3-glucoside and malvidin-3-glucoside-acetate and p-coumarate; this was also true for the

total anthocyanins in both homogenate and wine. Wines identified by a tasting panel to be the best quality, corresponded with the maximum anthocyanin concentration (mg / L) peak in the homogenate. Dense canopies at the Morgenster vineyard over the three seasons lead to lower total anthocyanin and quercetin-3-glucuronide concentrations compared to the Anhöhe and Plaisir de Merle vineyards. The shading of bunches by the dense canopy most likely contributed to this.

Catechin, epicatechin, proanthocyanidin and polymeric phenol concentrations decreased significantly from veraison until harvest. Seasonal differences were noted in the four vineyards. No correlations could be found between the general wine quality and the phenolic compounds, but a weak trend was observed for total anthocyanins in the homogenate. A trend was found with the total flavan-3-ol to anthocyanin ratio determined by RP-HPLC analysis of the grape homogenates ($r = 0.40$, $p = 0.00$). This ratio varied between 1 and 3 for the wines rated as being the best quality. Phenols by themselves do not give a clear indication of optimal harvest time.

From this study it appears that no single parameter could consistently indicate optimal ripeness over the seasons or per vineyard, but the maximum berry colour (anthocyanin concentration) did give an indication of optimal harvesting time. It is clear that a combination of parameters could predict the optimal time more precisely as with the above mentioned model but more research is needed to this end.

OPSOMMING

Suid-Afrika is die agste grootste wynproduserende land in die wêreld en is blootgestel aan strawwe kompetisie van die res van die internasionale wynmark. Cabernet Sauvignon is die mees aangeplante rooi kultivar in die wêreld asook in Suid-Afrika. Cabernet Sauvignon word gesien as die kultivar waardeur wynlande geëvalueer word.

Die doel van hierdie studie was om gepaste, praktiese rypheids parameters of kombinasies daarvan te evalueer, om die optimale oestyd van Cabernet Sauvignon druive onder Suid-Afrikaanse toestande te bepaal. Die volgende parameters is tydens hierdie studie geëvalueer: saadlignifikasie, rypheidsindekse, antosianien konsentrasie per korrel, sensoriese evaluasie (druifdop proe en wyn) en fenoliese konsentrasie.

Korrelontwikkeling is in vier Cabernet Sauvignon wingerde in verskillende Suid-Afrikaanse wynproduserende gebiede geëvalueer gedurende die 2003, 2004 en 2005 seisoene. Saad lignifikasie is die eerste parameter wat ondersoek is. Seisoenale verskille tydens kommersiële oes is waargeneem. Tydens 2004 wissel die waardes tussen 73% en 91% wanneer dit vergelyk word met die 59% en 80% in 2003 en 2005. Kommersiële oestyd was twee weke later gedurende die 2004 seisoen. Hierdie studie het gevind dat sade van Cabernet Sauvignon nooit 100% lignifikasie, soos in vorige studies gerapporteer is as rypheids indikator, bereik nie. Die ontwikkeling van antosianiene het 'n maksimum bereik voor die maksimum saad lignifikasie. Dit bewys dat saad lignifikasie nie geskik is vir die bepaling van druifrypheid vir Cabernet Sauvignon onder Suid-Afrikaanse toestande nie.

Rypheidsindekse ($\text{Balling} \setminus \text{Titreerbare suur (TS)}$; $\text{Balling} \times \text{pH}$; $\text{Balling} \times \text{pH}^2$) is die tweede parameter wat ondersoek is. Die beste wyn waardes is gebruik, om die optimale rypheidsindeks waardes te bepaal. Morgenster was die enigste wingerd wat konstant waardes opgelewer het wat ooreenstem met vorige gerapporteerde data (indekswaardes). Anhöhe en Plaisir de Merle het hoër rypheidswaardes gelewer as wat in vorige literatuur gerapporteer is. Seisoenale variasie is gevind. Tydens 2003 en 2005 seisoene het die rypheidsindeks waardes vir die beste wyne vir Anhöhe gewissel tussen 88 en 101 ($\text{Balling} \times \text{pH}$), maar het toegeneem na tussen 97 en 107 ($\text{Balling} \times \text{pH}$) in 2004. Die rypheidsindeks waardes is wingerd en seisoen afhanklik, met die warmer areas wat hoër waardes bereik het. Uit hierdie studie blyk dit dat rypheidsindekse as 'n enkele rypheids parameter nie 'n goeie enkele indikasie vir druif rypheid in alle seisoene en wingerde gee nie.

Derdens is die korrel antosianien konsentrasie (mg / korrel en mg / g korrel) ondersoek. Ooreenstemmende tendense is gevind tussen die vier wingerde. Wingerde in die warmer, droër gebied (Anhöhe) het hoër antosianien konsentrasies per gram korrel gehad. Die geiler wingerde van Morgenster het weer konstant 'n hoër antosianien konsentrasie per korrel gelewer. Dit kan verduidelik word aan die hand van die dop tot pulp verhouding tussen die klein (Anhöhe, 0.95g – 2004) en groot (Morgenster, 1.82g –

2004) korrels. Wynkleur digtheid (A420+A520) het dieselfde tendens gevolg as die antosianien konsentrasie van die homogenaat.

Kunsmatige wyne is berei van druifdoppe (G). Dit is deur 'n ekspert paneel geëvalueer, om die ontwikkeling van die druife te bepaal. Wyne (W) is ook geëvalueer deur 'n ekspert paneel vir: kleurintensiteit, vegetatiewe, rooi bessie, swart bessie met spesserye aromas, suurheid, vrangkheid en algehele kwaliteit. Vegetatiewe aromas en suurheid het afgeneem en rooi en swart bessie met spesserye aromas het toegeneem tydens rypwording vir beide die korrels en wyn. Kleurintensiteit het ook toegeneem, wat ooreenstem met 'n toename in algehele kwaliteit. Korrelasies tussen algehele kwaliteit vir beide die proe van druifdoppe en wyn is ondersoek. Daar is 'n sterk korrelasie gevind tussen Balling en algehele kwaliteit vir die druifdop proe ($r = 0.76$, $p = 0.00$), maar nie so 'n sterk korrelasie met die wyn ($r = 0.57$, $p = 0.00$) nie. Korrel antosianien konsentrasie (mg / g korrel) ($r = 0.36$, $p = 0.00$), waargeneemde kleurintensiteit van die druifkorrels ($r = 0.69$, $p = 0.00$) en wyn ($r = 0.84$, $p = 0.00$) het gekorreleer met algehele wynkwaliteit. Die proepaneel het wyne vir elke wingerd en seisoen geïdentifiseer wat statisties beter as die res was. Maksimum antosianien konsentrasie van die korrels stem ooreen met die beste wyne soos bepaal deur die proepaneel. Meer as een wyn is geïdentifiseer tydens die maksimum antosianien piek wat nie statisties verskillend was van die beste wyn nie. Hierdie studie wys dat daar 'n venster periode is by die maksimum antosianien piek, waar wyne van soortgelyke kwaliteit maar verskillende style gemaak kan word.

"Principle component analysis" (PCA) is gebruik, om die minste geskikte parameters te bepaal, wat kan onderskei tussen onryp en ryp druife, om sodoende 'n rypheidsmodel daar te stel. Die verskille is suksesvol beskryf deur Balling, TS, pH, kalium (K^+), wynsteen- en appelsuur. Antosianien konsentrasie kon verder tussen ryp en oorryp druife onderskei in die model. Minimum en maksimum waardes is vanaf die parameters gebruik om 'n uniersiele rypheidsmodel saam te stel wat al die data van die wingerde bevat. Die variasie tussen die vier wingende het tot te veel oorvleueling gelei in die uniersiele model data. Die rede vir die variasie lê in die verskillende klimaatsgebiede, soos volgens die Winkler temperatuur model, van die wingerde. Oorvleueling is nie tot dieselfde mate waargeneem per wingerd nie. Die beste Cabernet Sauvignon wyne het sterk gekorreleer met die oplosbare vaste stof inhoud, kleur en kwaliteits persepsie van die druife, maar seisoenale verskille het groter druif samestelling variasies tot gevolg gehad as die individuele wingerde in die verskillende klimaatgebiede. Dit beklemtoon hoe moeilik dit is om 'n spesifieke parameter te kies as 'n indikator van optimale rypheid. Hierdie studie wys dat 'n uniersiele rypheidsmodel vir Cabernet Sauvignon druife nie op die oomblik haalbaar is nie, maar dat individuele wingerd modelle wel potensiaal het.

'n Voorlopige studie oor die verskille in fenoliese samestelling in die homogenaat en wyn is gedoen deur hoëdoeltreffendheidsvloeistofchromatografie (HPLC). Malvidien-3-glukosied en die totale antosianiene het vergelykbare tendense gevolg soos gevind is vir die lland metode. Sterk korrelasies ($r > 0.9$) is gevind tussen malvidien-3-glukosied en malvidien-3-glukosied-asetaat en p-kumaraat; dit is ook vir totale antosianiene in beide

homogenaat en wyn gevind. Die beste kwaliteit wyn soos geïdentifiseer deur die proefpaneel het ooreengestem met die maksimum antosianien konsentrasie (mg/L) piek in die homogenaat. Digter lower by Morgenster oor die drie seisoene het gelei tot laer antosianiene en kwersetien-3-glukuronide konsentrasies wanneer vergelyk word met Anhöhe en Plaisir de Merle wingerde. Die beskaduwing van die trosse a.g.v. die digte lower het moontlik daartoe bygedra.

Katesjien, epikatesjien, proantosianidien en polimeriese fenol konsentrasie het betekenisvol afgeneem van deurslaan tot oes. Seisoenale verskille is waargeneem in al vier wingerde. Geen korrelasies is gevind tussen algemene wynkwaliteit en fenoliese komponente nie, maar 'n swak tendens is gesien vir totale antosianiene in die homogenaat. 'n Tendens is gevind vir die totale flavan-3-ol tot antosianien verhouding soos bepaal deur RP-HPLC vir die druifhomogenaat. ($r = 0.4$, $p = 0.00$). Die verhouding het gewissel tussen 1 tot 3 vir die beste kwaliteits wyne. Fenole op hul eie gee nie 'n goeie indikatie van optimale oestyd nie.

Die studie wys dat geen enkele parameter konstant optimale rypheid kon aandui oor die seisoene of per wingerd nie, maar die maksimum korrelkleur (antosianien konsentrasie) het wel 'n aanduiding van optimale oestyd gegee. Dit is duidelik dat 'n kombinasie van parameters die optimale tyd vir oes meer akkuraat kan voorstel soos met die bogenoemde model, maar verdere navorsing is nodig.

This thesis is dedicated to my family for all their support and prayers.

Hierdie tesis is opgedra aan my familie vir al hulle ondersteuning en gebede.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

Prof Marius Lambrechts of the Department of Viticulture and Oenology, for acting as my supervisor and his guidance, motivation and encouragement;

Dr Anita Oberholster of the Department of Viticulture and Oenology, for acting as my co-supervisor and for her guidance, encouragement, motivation and enthusiasm;

Mr Riel Tredoux of the Department Quality, Management and Research at Distell for acting as my co-supervisor and his encouragement and support;

My wife Anél Botes for her support, understanding and encouragement;

My parents, sister and friends for their support, motivation and reassurance throughout my studies;

Freddie le Roux of Plaisir de Merle, Kosie de Villiers of Morgenster, Retief Joubert en NW Hanekom of Anhöhe and Guillame Kotzé of LNR Infruitec- Nietvoorbij for their cooperation in the vineyards;

My colleagues at the Department Quality, Management and Research at Distell for their assistance, encouragement and guidance;

The students who assisted in the sample preparation and sampling;

Winetech for the financial assistance during this study;

God, for giving me opportunities in life and the ability to complete this goal.

PREFACE

This thesis is presented as a compilation of five chapters. Each chapter is introduced separately and is written according to the style of the journal *South African Journal of Enology and Viticulture* to which Chapters three and four will be submitted for publication.

Chapter 1 **General Introduction and Project Aims**

Chapter 2 **Literature Review**

Methods used in the determination of grape maturity

Chapter 3 **Research Results**

Evaluation of grape parameters to determine grape maturity for Cabernet Sauvignon in four South African wine growing regions

Chapter 4 **Research Results**

A preliminary study of the phenolic composition of Cabernet Sauvignon (*Vitis vinifera*) grapes during ripening in four South African wine growing regions

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Chapter 1

GENERAL INTRODUCTION AND PROJECT AIMS

1. GENERAL INTRODUCTION AND PROJECT AIMS

1.1 INTRODUCTION

South Africa has a rich winemaking heritage that started with the first wine in the Cape on 2 February 1659, seven years after the founding of a Dutch settlement by Jan van Riebeeck (Du Plessis and Boom, 2005). From these meagre beginnings some 350 years ago, South Africa has grown to become the eighth largest wine producing country in the world (Robinson 1994). After the 1994 elections that brought about the re-emergence of South Africa onto the world stage; wine exports of South African wines into the world markets have increased to over 240 million litres (Du Plessis and Boom, 2005). The increase of market share for South Africa lies not only in the price of each litre of wine sold abroad but also the quality of that wine. Ever fiercer competition from other wine exporting countries drives the expectation of consumers to higher quality wines. Cabernet Sauvignon is the most planted red cultivar in the world (Robinson 1994), as well as in South Africa (Du Plessis and Boom, 2005) and as such has tremendous impact on the perception of South African wines. The diversity of *terroir* in South Africa has given the wine industry the opportunity to produce numerous Cabernet Sauvignon wine styles.

The style and quality of wine that can be produced are influenced by the degree of maturity of the grapes (Du Plessis and Van Rooyen, 1982). It then stands to reason that for each style there should be an optimal ripeness level at which point the grapes are to be harvested for maximum quality. What does optimal ripeness mean? Bisson (2001) defined optimal maturity as the time when the synthesis of desirable enological characteristics ceased, and the subsequent deterioration begins in the berry. Archer (1981) stated that optimal ripeness is the level at which the maximum grape quality also coincides with the maximum wine quality. For this study the following definition regarding optimal ripeness was used: It is the stage of maturity in the berry where all components are in balance and the resulting wine gives maximum quality for the specific wine style. The wine quality at different stages of grape maturity has been investigated extensively over the years (Amerine and Winkler, 1941; Berg, 1958; Ough and Singleton, 1968; Ough and Alley, 1970; Du Plessis and Van Rooyen, 1982; Van Rooyen *et al.*, 1984 and Marais *et al.*, 1999). However to some extent only one indicator at a time has been correlated to the quality of the wine by the above-mentioned authors.

Berry maturity is influenced by the following: temperature (Buttrose *et al.*, 1971; Pirie, 1979); light exposure (Rojas-Lara and Morisson, 1989; Spayd *et al.*, 2002); water availability (Van Zyl, 1981; Ginestar *et al.*, 1998; Ribéreau-Gayon *et al.*, 2001a; Roby *et al.*, 2004); and viticultural practices (Archer, 1981). The changes brought about by these above mentioned factors needs to be measured objectively and accurately. To this end measuring tools have been developed: soluble solids (Ribéreau-Gayon *et al.*, 2001a and b), titratable acidity (Boulton *et al.*, 1996), pH (Boulton *et al.*, 1996; Iland *et al.*, 2000), combinations of the aforementioned (maturity index) (Amerine and Winkler, 1941; Du Plessis and Van Rooyen, 1982; Van Rooyen *et al.*, 1984), seed lignification percentage (Ristic and Iland, 2005), Ilands method (Iland *et al.*, 2000), glycosyl-glucose (G-G) method (Francis *et al.*, 1998, 1999),

extractability potential (Glories, 2001) and the pH shifting and SO₂ bleaching first used by Ribereau–Gayon and Stonestreet (Ough and Amerine, 1988). Archer (1981) noted that regional differences have an impact on the criteria used for measuring ripeness. For example, in cooler wine regions with lower temperatures and less sunlight sugar accumulation is important for quality and measuring sugar concentrations is a good indicator of maturity; as opposed to a warm wine region where ample sunlight and higher temperatures favour sugar accumulation and measuring sugar concentration would not give a good indication of maturity by itself (Archer, 1981). What this means for the South African wine industry, is that measuring tools used overseas under those climatic and viticultural practices does not necessarily work under South African conditions. Methods thus need to be validated under South African conditions for Cabernet Sauvignon to give the wine industry a means to compare the accuracy of a given method with data from the originating wine regions. Judging optimal berry maturity under South African conditions, and adjustments to optimize Cabernet Sauvignon quality for a given wine style, can then be made more easily and thus adapting quicker to world trends.

1.2 PROJECT AIMS

This project forms part of a larger industry driven vision of Winetech to optimize the strategic approach in the South African wine industry towards world competitiveness. Cabernet Sauvignon is seen as the cornerstone of the world wine industry and as the right of passage for upcoming wine countries (Robinson, 1994). Judging the right time to harvest grapes for optimal quality is the aim of all winemakers and viticulturists. The aim of this project was to determine suitable parameters, or combinations thereof, for the measurement of optimal ripeness in Cabernet Sauvignon under South African conditions.

In order to achieve the abovementioned goal, the objectives of this study included the following:

- i) the identification of suitable Cabernet Sauvignon vineyards in four wine regions of the Western Cape;
- ii) the evaluation of seed colouration as a possible indicator of grape maturity;
- iii) the evaluation of grape skin tasting as grape maturity indicator;
- iv) the evaluation of total soluble solids (TSS), total titratable acidity (TTA), pH, potassium (K⁺), tartaric and malic acid as grape maturity indicators;
- v) the evaluation of maturity index values as grape maturity index values;
- vi) the evaluation of the effect of fruit maturity on anthocyanin concentration in the grape and wine;
- vii) the evaluation of grape development on sensory and quality evaluation of wines made from grapes at different ripeness levels;
- viii) to determine the influence of ripening on the phenolic profile of Cabernet Sauvignon

grapes from different climatic zones;

ix) to determine the correlations between grape colour development and optimal harvest time;

x) the development of a optimal ripeness model for Cabernet Sauvignon grapes.

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Chapter 2

LITERATURE REVIEW

Methods used in the determination of grape maturity

2. LITERATURE REVIEW

2.1 INTRODUCTION

Wine has been part of our civilization for the past 5000 years (Johnson, 2002). South Africa has been part of wine history for the last 350 years. From the time of those ancient cultures to our modern consumers, there has been a demand for ever higher quality wines. These demands lead winemakers and growers to find more precise criteria to judge the optimal time to harvest, for different styles of wine. With our increasing scientific knowledge, new ways have been identified to measure different berry components, with ever increasing accuracy.

This literature review will briefly examine the components of the berry as well as their development. The focus will however be on some methods developed to measure and quantify the components deemed good indicators of ripeness.

2.2 PHYSIOLOGY OF THE GRAPE DURING DEVELOPMENT

2.2.1 BERRY DEVELOPMENTAL CYCLES

Berry development consists of two successive sigmoidal growth periods that are separated by a lag phase (Coombe and McCarthy, 2000). This first period lasts from bloom till approximately 60 days afterwards and ends at véraison (Kennedy, 2002). The number of cells in the berry is established during the first few weeks by rapid cell division and formation of the seed embryo. This also has bearing on the eventual size of the berry (Harris *et al.*, 1968). The accumulation of solutes gives rise to the expansion of the berries during this period of development. Of these solutes, tartaric and malic acid are the most prevalent (Possner and Kliewer, 1985; Kennedy, 2002). During the first growth period there is also an accumulation of hydroxycinnamic acids in the flesh and skins (Romeyer *et al.*, 1983) and monomeric flavan-3-ols in the seeds and skins (Kennedy *et al.*, 2000a; 2001). Other solutes that accumulate are minerals, amino acids, micronutrients and aroma compounds (Kennedy, 2002).

The softening and colouring of the berries mark the beginning of the second growth phase. Between véraison and harvest the berry volume doubles. Solute accumulated in the first development period remains in the berry, but are reduced in concentration by the berry enlargement. Tannins, malic acid and aroma components decrease during the second period (Kennedy, 2002). The reduction in seed tannins could be due to the oxidation of the tannins as they are fixed to the seed coat (Kennedy *et al.*, 2000b). According to Kennedy *et al.* (2001) skin tannins are modified with pectins and anthocyanins. The two most notable changes during the ripening phase after véraison is the influx of sugars, glucose, fructose and sucrose, as well as the anthocyanin production in the skin cells of red grapes (Kennedy, 2002). Sucrose is hydrolyzed into glucose and fructose in the berry (Robinson and Davies, 2000).

Growth regulators are important in the development of the grape, (cytokinin, abscisic acid), are supplied to the berry through the xylem from the roots (Greenspan *et al.*, 1994).

Kataoka *et al.* (1982) found that the growth regulator abscisic acid contributed to the accumulation of anthocyanins in the skins.

2.2.2 CARBOHYDRATES

Winkler *et al.* (1974) found that during the first two stages of berry development there was no significant accumulation of carbohydrates in the berries. The small amount present was offset by respiration and berry growth. Carbohydrates in the skin tissue, varies from the pulp and is not related to sugar levels in the pulp (Pirie, 1979). Shading of leaves has a negative effect on photosynthesis and carbohydrate transport in the vine (Morrison and Noble, 1990). Guidoni *et al.* (2002) hypothesized that the sugar content influenced the anthocyanin composition of the berry. They based their hypothesis on the proposition of Pirie and Mullins (1977), that flavonoid accumulation in grape berries could be regulated by the sugar content. Rojas-Lara and Morrison (1989) worked on the differential effects of shading fruit or foliage and found that shaded treatments were on average two weeks later than treatments with exposed leaves.

2.2.3 ORGANIC ACIDS

2.2.3.1 Tartaric acid

This acid is specific to grapes (Zoecklein *et al.*, 1995; Boulton *et al.*, 1996; Ribéreau-Gayon *et al.*, 2001a) and synthesized in an early stage of berry development, with very little synthesis or catabolism after véraison. During experiments conducted by Morrison and Noble (1990) they noted that tartrate accumulation was fastest during the first four weeks of berry development. It tends to accumulate in the outer part of the developing berry (Kennedy, 2002) as L-(+)-tartaric acid (Zoecklein *et al.*, 1995; Boulton *et al.*, 1996; Ribéreau-Gayon *et al.*, 2001a). The acid is found in concentrations from 5 to 10 g/L according to Boulton *et al.* (1996), while Ribéreau-Gayon *et al.* (2001) estimated it between 4 to 16 g/L in colder wine growing areas of the world at harvest.

Ruffner (1982a) and Ribéreau-Gayon *et al.* (2001) stated that tartaric acid is formed as a secondary product of sugar metabolism with ascorbic acid playing a pivotal role. In trials the transformation rate was 70% for ascorbic acid to tartaric acid in grape berries (Saito and Kasai, 1969; 1982; 1984; Malipiero *et al.*, 1987). Saito and Kasai (1978) concluded that tartaric acid is formed from glucose via galacturonic, glucuronic and ascorbic acid. In a further study in 1984 they concluded that the pathway seemed to follow the reactions: L-ascorbic acid → 2-keto-L-idonic acid → idonic acid → L- (+)-tartaric acid.

Tartaric acid occurs in berries in three forms as an undissociated acid (H₂T) and two salt forms, potassium bi-tartrate (KHT) and di-potassium tartrate (K₂T). The salt forms are in dispute though. Some said that tartaric acid salts occurred as calcium salts (Hale, 1977; Ruffner, 1982a), but others believe that potassium are more likely, because of the abundance of potassium in grape berries (90% of total cations) (Boulton, 1980; Iland, 1987a and b; Ribéreau-Gayon *et al.*, 2001). The ascorbic-tartaric acid conversion is well

understood, but the origin of ascorbic acid still eludes researchers for the past 30 years (Ribéreau-Gayon *et al.*, 2000).

Saito and Kasai (1969) demonstrated that tartrate synthesis required light exposure of the berries. In 1989, Rojas-Lara and Morrison reported that the tartaric acid was significantly lower in heavily shaded treatments, which supported the Saito and Kasai (1968) theory. The shading of clusters or leaves, however does not seem to have a significant influence on the accumulation of tartaric acid (Morrison and Noble, 1990).

There is no proof of catabolism of tartaric acid during maturation of the berries (Ribéreau-Gayon *et al.*, 2001). Tartaric acid can however be degraded at a pH above four by a few bacteria strains, or converted to glucuronic acid by *Botrytis cinerea* (Boulton *et al.*, 1996).

2.2.3.2 Malic acid

Malic acid is the most widespread fruit acid especially in green apples (Ribéreau-Gayon *et al.*, 2001). L-(+)-malic acid are found in grapes (Boulton *et al.*, 1996; Ribéreau-Gayon *et al.*, 2000) and tends to accumulate in the flesh just before véraison (Kennedy, 2002). Accumulation of the acid peaks at véraison in the berry after which it starts to decline. The acid is synthesized via pyruvic acid from glucose (Boulton *et al.*, 1996). Ribéreau-Gayon *et al.* (2000) explained the synthesis of malic acid as follows: CO₂ is assimilated from the air by C₃-mechanism. During the dark phase of photosynthesis, the green grapes fixate CO₂ on ribulose-1,5-diphosphate to produce phosphoglyceric acid, which leads to phosphoenolpyruvic acid after dehydration. In the last reaction oxaloacetic acid is formed by the catalyzing of PEP carboxylase. Malic acid is then formed by the reduction of oxaloacetic acid. The shading of leaves influenced the accumulation and decline of malic acid. Morrison and Noble (1990) reported a slower increase, pre-véraison and a decrease, post-véraison. The shift in respiratory substrate, from sugars to organic acids after véraison has been proposed by Harris *et al.* (1971). Figure 2.1 shows the Krebs cycle and the importance of malic acid in it. The degradation of malic acid is temperature dependent and is well documented (Radler, 1965; Kliewer, 1971). Lakso and Kliewer (1975, 1978) contributed the degradation of malic acid to an increase in the activity of the malic enzyme post-véraison. Ruffner (1982b) cited that the gluconeogenic catabolism of malate by phosphoenolpyruvate carboxykinase (PEPCK) appeared not to be temperature sensitive. Kliewer and Lider (1970), Reynolds *et al.* (1986) and Rojas-Lara and Morrison (1989), all reported a faster decrease in malate in exposed canopies. Concentrations between 2 to 4 g/L are generally formed in grape berries in cool growing areas. Boulton *et al.* (1996) commented that malic acid levels as high as 6 g/L in the cool areas or well below 1 g/L in warm areas, was possible.

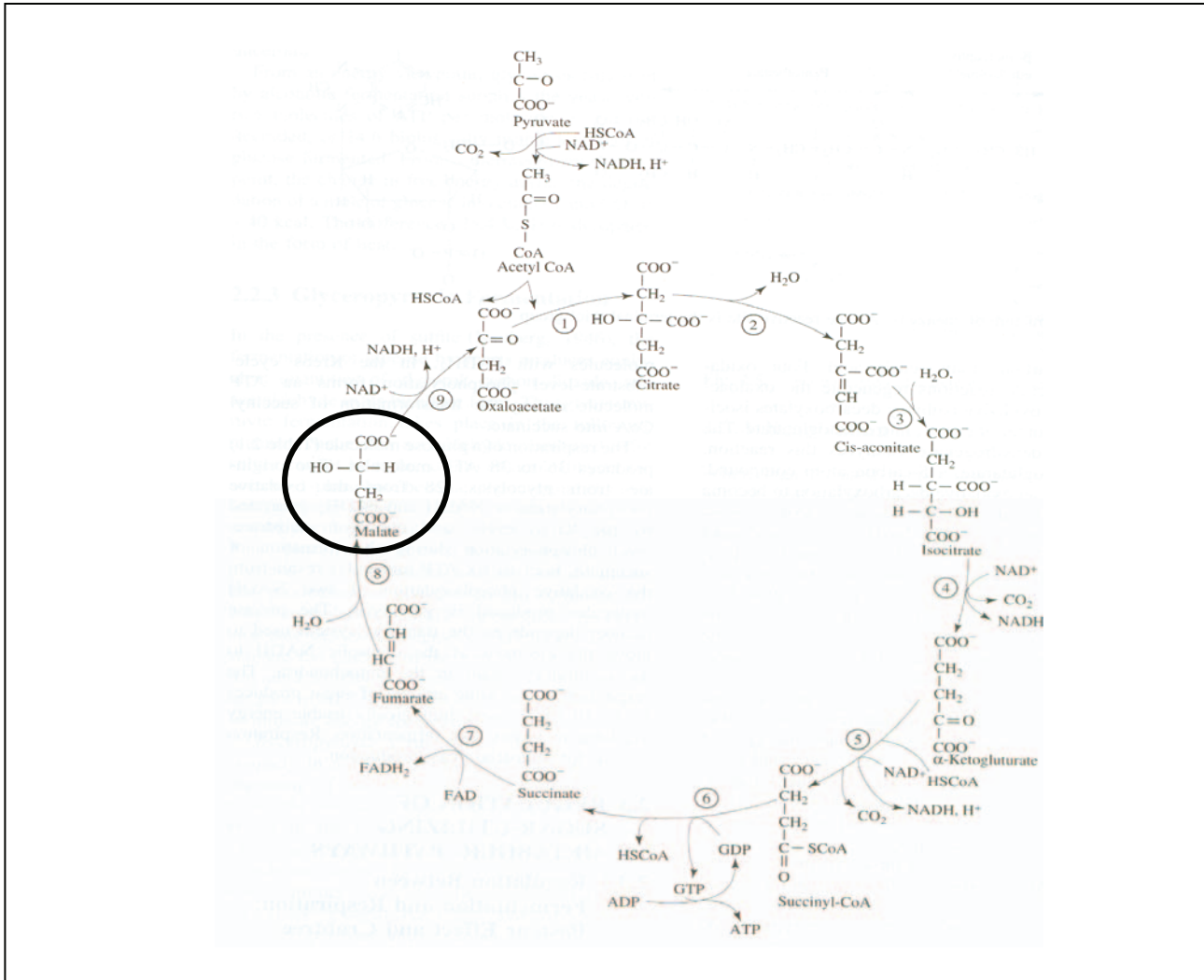


Figure 2.1 Schematic of the Krebs cycle to illustrate the importance of malic acid in the grape (Ribéreau-Gayon *et al.*, 2000).

2.2.4 PHENOLIC COMPOUNDS

Phenolic compounds are an integral part of grapes and wine, as they contribute to the colour, taste (mouthfeel) and stability of wines. As antioxidants, tannins and anthocyanins are beneficial to human health. Grape tannins are predominantly condensed tannins also called proanthocyanidins and made up of subunits joined together. The composition of the polymers differs between the skin and seeds. Grape skins contain polymers with subunits that average about 20 to 30 subunits and seeds 4 to 6 subunits. These differences play a role in the extractability of the components and their impact on mouthfeel (Ribéreau-Gayon *et al.*, 2000; Robinson and Walker, 2006). See Figure 2.2 for the biosynthetic pathway of phenolic compounds as described by Ribéreau-Gayon *et al.* (2000).

Phenolic subunits are formed via the flavonoid pathway by sequential enzymatic transformations from one intermediate to the next (Figure 2.3). These enzymes are as follows: chalcone synthase (CHS); chalcone isomerase (CHI); flavanone-3-hydroxylase (F3H); dihydroflavonol reductase (DFR); leucoanthocyanidin dioxygenase (LDOX); UDP-glucosyl:flavonoid glycosyltransferase (UFGT); leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR). UFGT gene is only expressed in red grapes after véraison (Robinson and Walker, 2006).

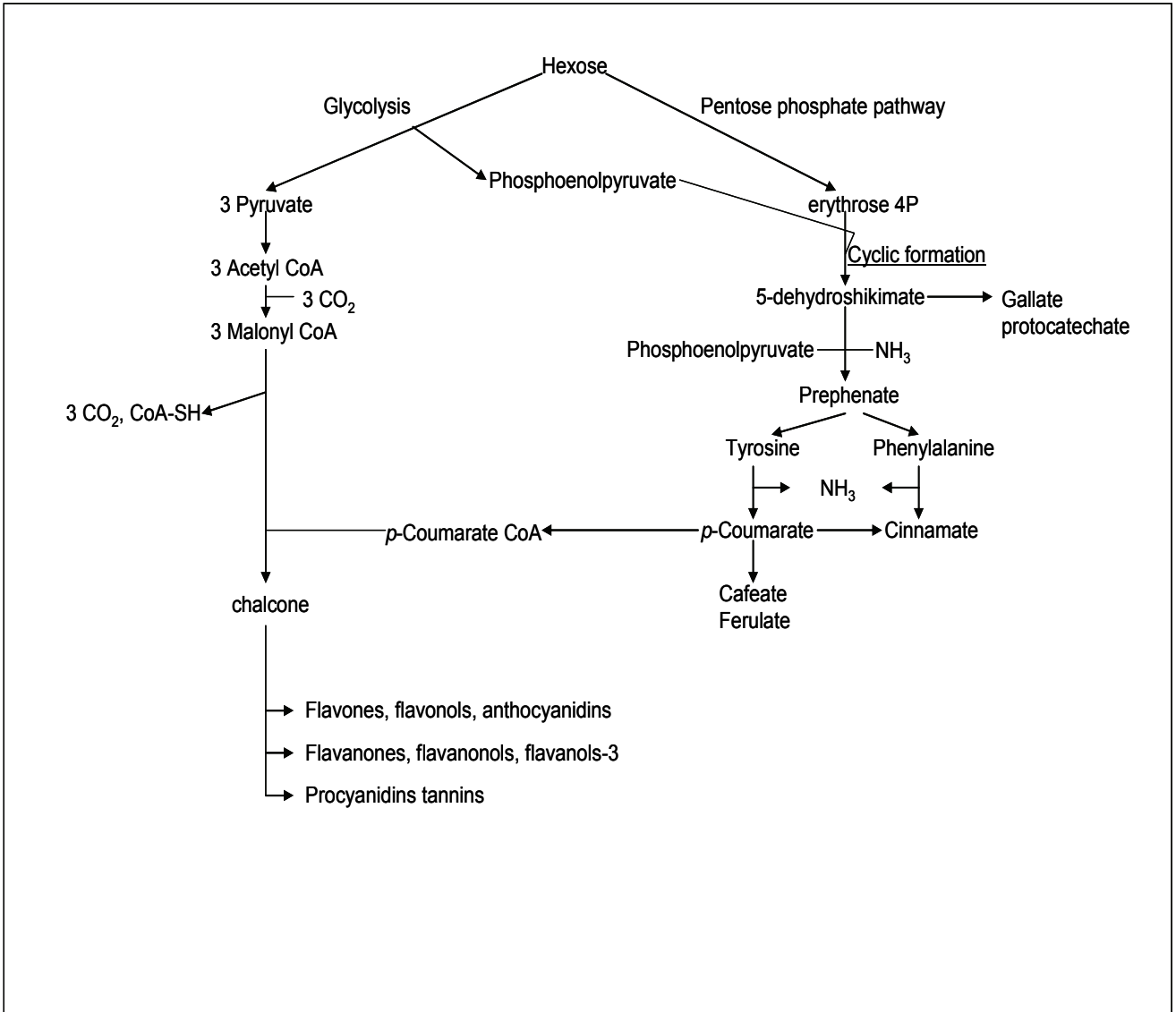


Figure 2.2 Schematic of the biosynthetic pathway of phenolic compounds (Ribéreau-Gayon *et al.*, 2000).

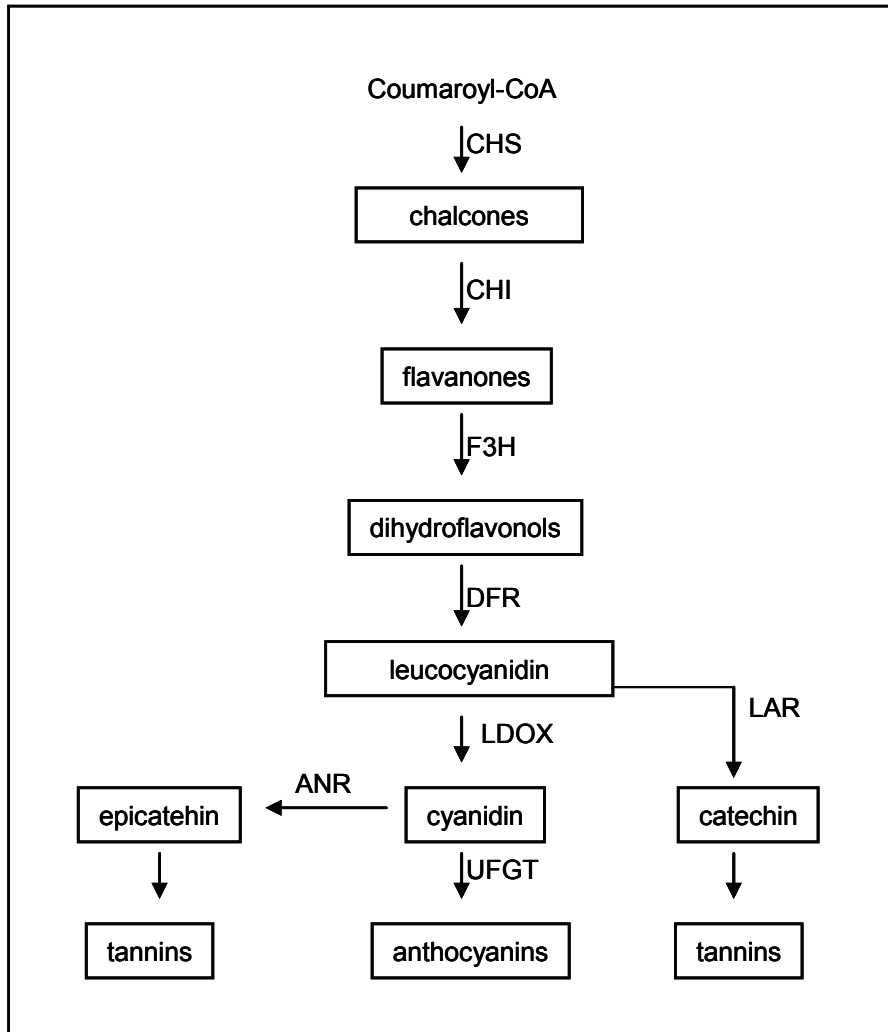


Figure 2.3 Schematic of the flavonoid pathway for the production of anthocyanins and tannins in grapes (Robinson and Walker, 2006).

2.2.4.1 Phenolic acids

Two forms, benzoic acid (C_6-C_1) and cinnamic acid (C_6-C_3) are present (Figure 2.4; Table 2.1). Benzoic acids is found in grapes in combination with glucose and esters (gallic and ellagic acid). Cinnamic acids (*p*-coumaric acid, caffeic acid, ferulic acid) are found mainly esterified with tartaric acid (Ribéreau-Gayon *et al.*, 2000). These are the main phenolic acids found in grapes and wine.

It is found in wine to the order of 100-200 mg/L in red and 10-20 mg/L in white wine (Ribéreau-Gayon *et al.*, 2000). Phenolic acids are colourless, but may become yellow due to oxidation. They have no particular flavour or odour, but are precursors of volatile phenols produced by certain microorganisms such as *Brettanomyces* and bacteria. They play a significant role in flavour properties according to Pocock *et al.* (1994).

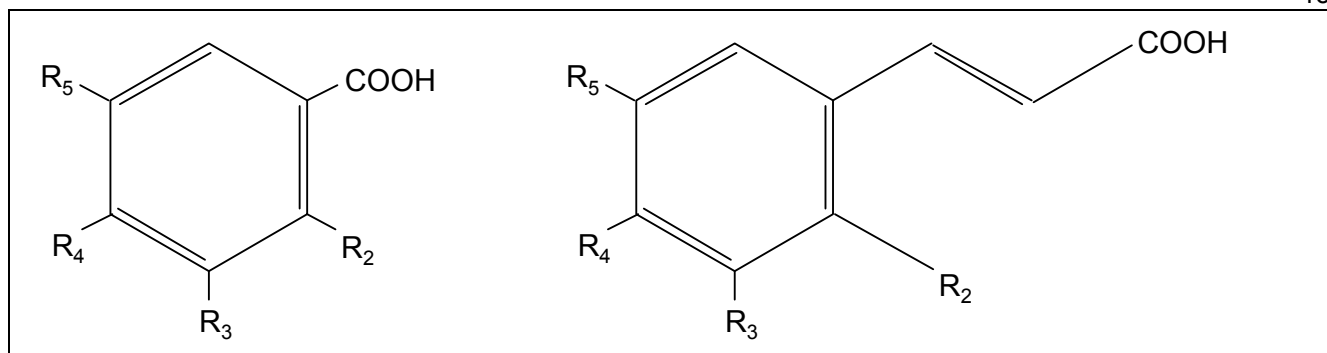


Figure 2.4 Phenolic acids found in grapes: Benzoic acid (left) and Cinnamic acid (right) (Ribéreau-Gayon *et al.*, 2000).

Table 2.1 Structures of phenolic acids (Ribéreau-Gayon *et al.*, 2000).

Benzoic acid	R ₂	R ₃	R ₄	R ₅	Cinnamic acid
<i>p</i> -Hydroxybenzoic acid	H	H	OH	H	<i>p</i> -Coumaric acid
Protocatechuic acid	H	OH	OH	H	Caffeic acid
Vanillic acid	H	OCH ₃	OH	H	Ferulic acid
Gallic acid	H	OH	OH	OH	
Syringic acid	H	OCH ₃	OH	OCH ₃	Sinapic acid
Salicylic acid	OH	H	H	H	
Gentisic acid	OH	H	H	OH	

2.2.4.2 Flavonols

These are intensely yellow pigments, as well as the most widespread compounds found in the skins of both red and white grapes. Figure 2.5 shows three pigments, kaempferol, quercetin and myricetin. All three of the above are found in red grapes, but only kaempferol and quercetin are found in white grapes (Ribéreau-Gayon *et al.*, 2000). Concentrations vary from 100 mg/L in red to 3 mg/L in white wine, according to cultivar.

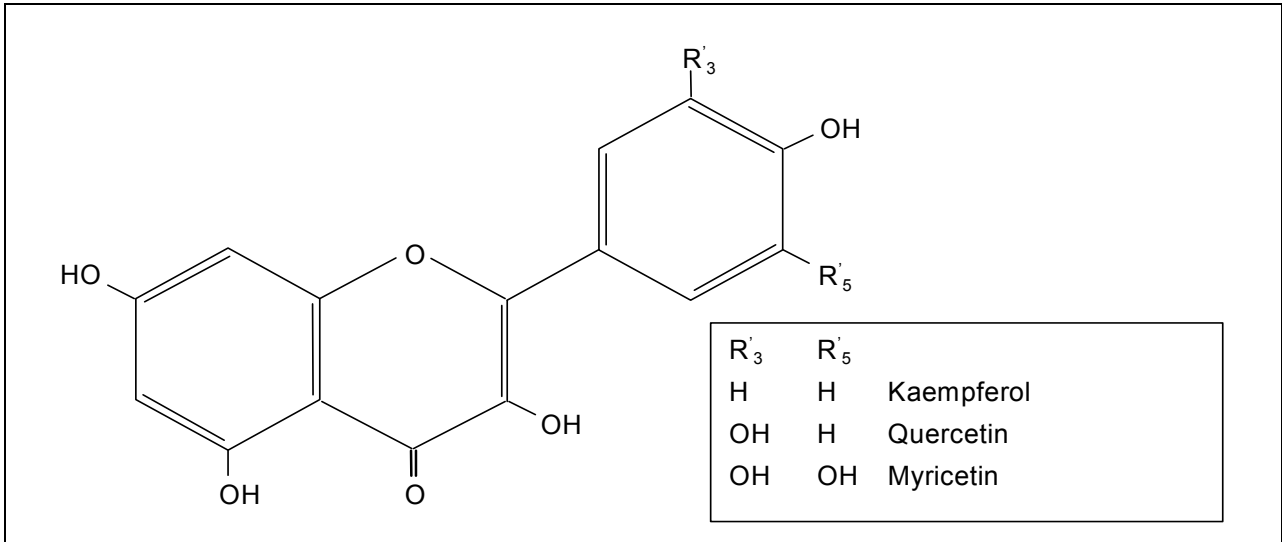


Figure 2.5 Structures of flavan-3-ols found in grapes.

2.2.4.3 Tannins

By definition, tannins are substances capable of producing stable combinations with proteins and other plant polymers such as polysaccharides. Chemically they are bulky phenol molecules, produced by the polymerization of elementary molecules with phenolic functions. Their molecular weights range from 600 to 3500 (Ribéreau-Gayon *et al.*, 2000).

Tannins are divided into two groups, the hydrolysable and condensed tannins. These tannins differ from each other by their elementary molecules (Figure 2.6). See Figure 2.7 for comparison of structure. Hydrolysable tannins consist out of gallotannins and ellagitannins that release gallic acid and ellagic acid respectively. Hydrolysable tannins are not present in grapes and are only present in wine due to extraction from oak or other additives and would not be discussed further. Gallic acid is found naturally in skins and seeds (Ribéreau-Gayon *et al.*, 2000). They are mostly linked by C₄-C₈ and C₄-C₆ interflavan bonds (Prieur *et al.*, 1994; Souquet *et al.*, 1996).

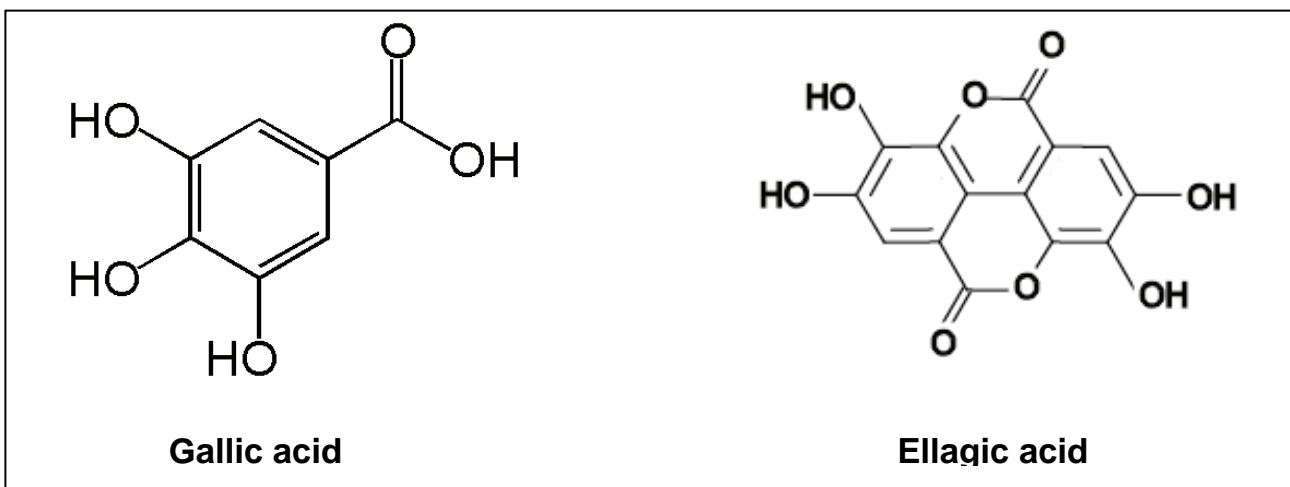


Figure 2.6: Structures of gallic and ellagic acids (Ribéreau-Gayon *et al.*, 2000).

Condensed tannins in grapes are polymers of flavan-3-ols, mainly catechin, epicatechin and epicatechin-3-O-gallate (Figure 2.7) and are responsible for the bitter and astringent

properties of red wines (Robichaud and Noble, 1990; Gawel, 1998). The source of catechin is thought to be leucocyanidin that is transformed by the enzyme leucoanthocyanidin reductase (LAR) to catechin, while epicatechin is transformed from cyanidin by anthocyanidin reductase (ANR) (Robinson and Walker, 2006). Monomeric catechin units may not be considered as tannin as their molecular weight is too low and have restricted properties in relation to proteins. These molecules and their polymers are also referred to as proanthocyanidins, because they have the ability to convert to red cyanidins and delphinidins when heated in an acid medium (Zoecklein 1995; Boulton *et al.*, 1996; Ribéreau-Gayon *et al.*, 2000). Catechin is the major constituent of flavan-3-ols in the seed coat and skin (Thorngate and Singleton, 1994; De Freitas and Glories, 1999). Proanthocyanidins are found in their highest concentrations during the early development stages of the berry up to véraison, with a decrease in the extractability during post véraison (De Freitas and Glories, 1999; Kennedy *et al.*, 2000a). Bogs *et al.* (2005) found that the concentration of condensed tannins were the highest 1 - 2 weeks before flowering and concluded that synthesis was already ongoing before the berry was even formed. After berry set and subsequent berry development the levels of tannin was maintained during this phase. In skins catechin can be four times as much as epicatechin, but in seeds the concentration stays similar (De Freitas and Glories, 1999). Downey *et al.* (2003) found that tannin levels reached a maximum 1 - 2 weeks before véraison in skins, but in seeds the maximum was reached 2 weeks after véraison. After véraison the polymers become chemically conjugated to other compounds during the maturation phase and less extractable.

Kennedy *et al.* (2000a) reported that after véraison the colour change in seeds was consistent with polyphenol oxidation, which lead to the decline in the extractability. Kennedy *et al.* (2000a) divided polyphenol development in seeds into four distinct stages: stage 1) procyanidin biosynthesis; stage 2) flavan-3-ol monomer biosynthesis; stage 3) programmed oxidation; stage 4) non-programmed oxidation. The biosynthesis of procyanidin coincides with the initial rapid growth period of the berry as per stage 1 until véraison. Flavan-3-ol biosynthesis increases pre-véraison and coincided with a decrease in procyanidin biosynthesis rate. Changing seed colour (green to brown) and increase in phenoxyl radical generation introduce stage 3. During this stage Kennedy *et al.* (2000a) stated that flavan-3-ol monomers decreased to a greater extend than procyanidins. The cessation of the stage is closely related to the maximum berry weight and completion of seed desiccation. Stage 4 is characterised by maximal berry weight, non-anthocyanin glucoside accumulation, complete desiccation of the seed and the levelling in phenolic extraction and composition (Kennedy *et al.*, 2000a).

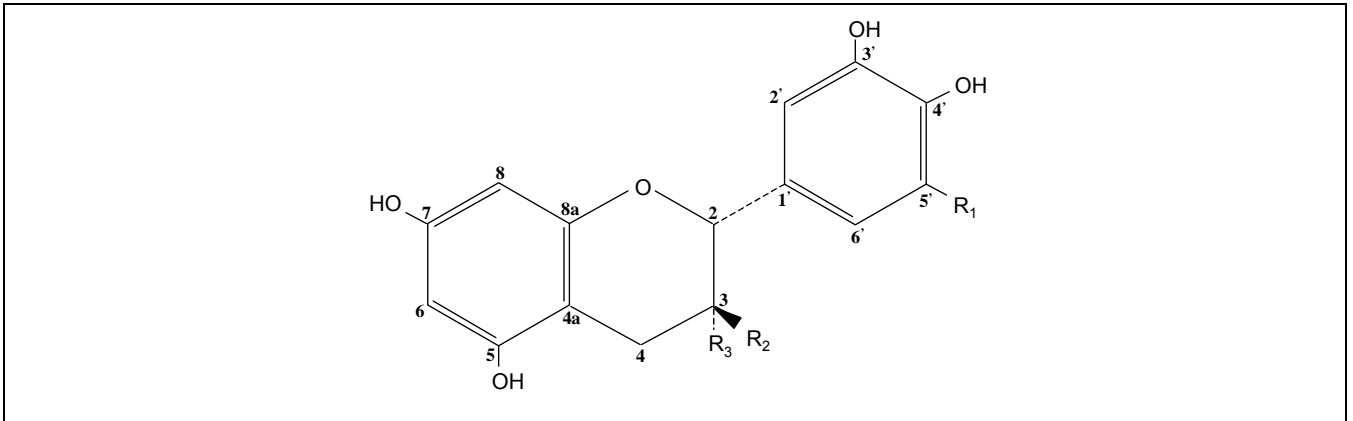


Figure 2.7 Basic structure of flavan-3-ols (Ribéreau-Gayon *et al.*, 2000).

Table 2.2 Structures of flavan-3-ols (Moutounet *et al.*, 1996)

R' ₁	R' ₂	R' ₃	
H	OH	H	(+) – catechin
OH	OH	H	(+) – galocatechin
H	H	OH	(-) – epicatechin
OH	H	OH	(-) – epigallocatechin

2.2.4.4 Anthocyanins

Anthocyanins are the red pigments in the skins of grapes and in some cultivars in the flesh as well, for example the cultivar Pontac (Zoecklein, 1995; Boulton *et al.*, 1996; Ribéreau-Gayon *et al.*, 2000). The concentration of anthocyanins is an important fruit – quality parameter, by affecting both colour quality and intensity in the wine (Guidoni *et al.*, 2002). According to Winkler *et al.* (1974) anthocyanins accumulate in the dermal cell layers and Amrani-Joutei (1993) found that the molecules located in the skin cells had a concentration gradient from inside towards the outside of the grape. Anthocyanins are also found in great quantities in the leaves at the end of the growing season (Boulton *et al.*, 1996; Ribéreau-Gayon *et al.*, 2000). See Figure 2.8 for the structure of the five types of anthocyanins present in grapes. The molecules are found in the stable glycoside (anthocyanin) form, while the aglycone (anthocyanidin) is unstable. *Vitis vinifera* only have significant concentrations of the anthocyanin monoglucosides, of which malvidin-3-O-glucoside and its derivatives may be acylated with *p*-coumaric, caffeic and acetic acid (Wulf and Nagel, 1978; Roggero *et al.*, 1986; Boss *et al.*, 1996a). Pinot noir is a *Vitis vinifera* cultivar that does not contain all the anthocyanin derivatives. Diglucosides have been detected below quantification limits with new analytical methods. Acids form acylated anthocyanins by the esterification of acetic, *p*-coumaric and caffeic acid with the glucose of the glycoside (Ribéreau-Gayon *et al.*, 2000). Boss *et al.* (1996a) studied the genetic control of anthocyanin production in grapes during development (Boss *et al.*, 1996b), in other grape tissues (Boss *et al.*, 1996c) and in

grapevine mutations (Boss *et al.*, 1996a). All three studies concluded that UDP glucose-flavonoid 3-O-glucosyl transferase (UGFT) was the controlling point for anthocyanins synthesis (Boss *et al.*, 1996a).

The colour depends on the environment and Pirie (1979) determined that warm, not hot days influenced the metabolism of anthocyanins. Rojas-Lara and Morrison (1989) reported that treatments with shaded foliage started to accumulate anthocyanins two weeks later than exposed treatments. Morisson and Noble (1990) also found that the shading of clusters had a greater effect on anthocyanin and total phenol content than the shading of the leaves.

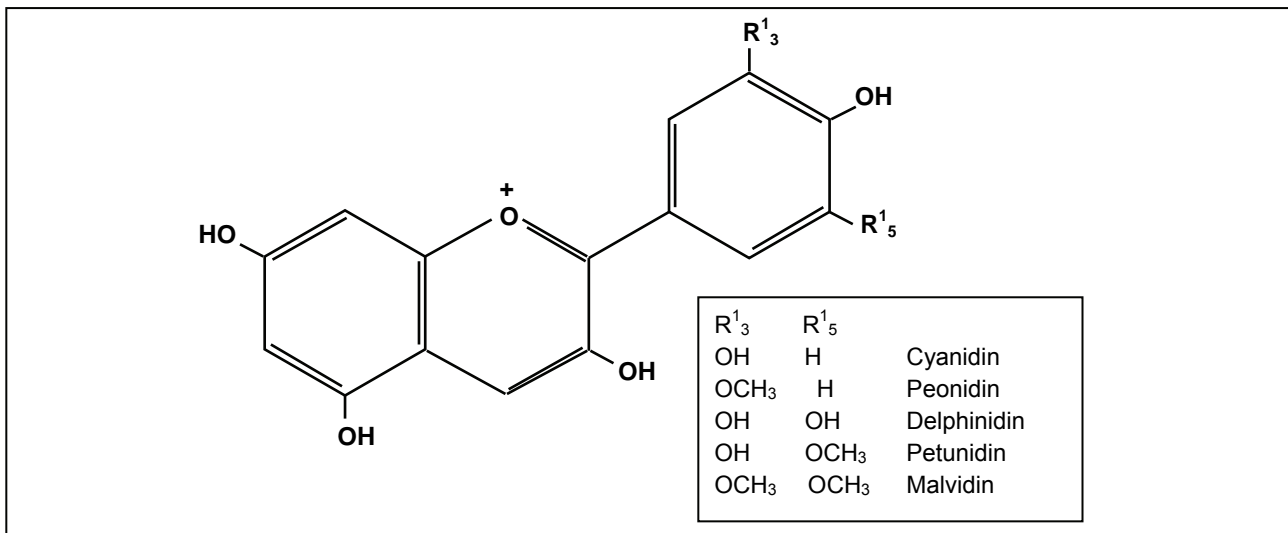


Figure 2.8 Structures of anthocyanidins found in grapes (Ribéreau-Gayon *et al.*, 2000).

2.2.5 POTASSIUM (⁺K)

Potassium (⁺K) ranks with nitrogen and phosphorus in importance as mineral nutrient (Iland, 1988). According to Butzke and Boulton (1997) the potassium levels in Californian grapes range from 560 to 2785 mg/L, with levels of 9000 mg/L in the skins. The skin makes up only 10% of the berry weight but contributes 30% to 40% of the potassium in the berry (Butzke and Boulton, 1997). It has four physiological-biochemical roles: 1) enzyme activation; 2) membrane transport process; 3) anion neutralization and 4) osmotic potential regulation, (Clarkson and Hanson, 1980). Boulton *et al.* (1996) proposed that the enzyme potassium/hydrogen adenosine triphosphatase imported the potassium into the cell (Iland, 1988; Boulton *et al.*, 1996). He suggested that uptake of monovalent metal cations from soil is achieved by adenosine triphosphatase (ATPase) activity in the roots of grapevines. The presence of ATPase in berries also enables cation transport across the plasmalemma in exchange for protons derived from the organic acids. This exchange of protons for potassium (and other cations) in grape berries is partly the reason for the increase in juice pH and titratable acidity during ripening (Iland, 1987a; 1987b). The cause of high juice pH is often due to excessive potassium uptake by the berry (Clarkson and Hanson, 1980; Iland, 1988). Rojas-Lara and Morrison (1989) reported that vines with heavily shaded foliage tended to have the highest potassium concentration at harvest. Potassium also plays an important part in the tartrate stability of wine, with the formation of potassium bitartrate. This

leads to the lowering of acidity and increase in pH (Iland, 1988; Zoecklein, 1995; Boulton *et al.*, 1996; Ribéreau-Gayon *et al.*, 2001).

2.2.6 AROMATIC COMPOUNDS

Aromatic compounds are important because they give cultivars their distinctive varietal aromas. Four categories of grape derived aromatic compounds are found, terpenes, C₁₃ norisoprenoids, methoxypyrazines and sulphur compounds (thiol) (Ribéreau-Gayon *et al.*, 2001).

Terpenes comprises of some 4000 compounds of which 40 have been identified in grapes. These compounds have low perception thresholds and have a synergistic influence on each other. The most odoriferous monoterpene alcohols are linalool, nerol, geraniol, citranellol and α -terpinol. Monoterpenes plays a significant role in wines made from grapes of the Muscat family. Terpenes are also found in Cabernet Sauvignon, Syrah etc. but are below the perception threshold (Ribéreau-Gayon *et al.*, 2001).

Norisoprenoids are degradation compounds of carotenoids, produced by either enzymatic or chemical means. They are divided into two main forms, oxygenated megastigmane (β -damascenone and β -ionone) and none-megastigmane (Vitispirane, Actinidol and 1,1,6-trimethyl-1,2-dihydronaphtalene (TDN)). β -damascenone was first identified in Riesling. It has a recognition threshold of 5000 ng/L in red wines. β -ionone has a recognition threshold of 1.5 μ g/L and is like β -damascenone present in all cultivars. TDN plays a major role in the kerosene odor of old Riesling wines with a threshold 20 μ g/L. Norisoperinoids increases after colour change in grapes as the carotenoid concentration decreases (Ribéreau-Gayon *et al.*, 2001).

Methoxypyrazines contribute the green pepper, asparagus and earthy aromas of wines and are produced by the metabolism of amino acids. In red wines these aromas are considered to be of under ripe grapes. These compounds have very low thresholds and are found in many plants. Bayonove, Cordonnier and Dubois first identified 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon in 1975. They conclude that 2-methoxy-3-isobutylpyrazine was located in the skin of the grape as press wines contained more than the free run juice. Concentrations have been reported to vary in juice and wine between 0.5 to 50 ng/L for Sauvignon blanc and Cabernet Sauvignon. Light exposure in the bunch area decreases the concentration of methoxypyrazines (Ribéreau-Gayon *et al.*, 2001).

Sulphur compounds (mercaptans) are held responsible for defects in wines, but have been found to contribute to Sauvignon blanc aroma. The mecaptopentone gives an aroma of broom or boxtree to the wine. Five odouriferious thiols have been identified in Sauvignon blanc: 4-mercapto-4-methyl-pentan-2-one, 4-mercapto-4-methyl-pentan-1-ol, 3-mercapto-3-methyl-butan-1-ol, 3-mercaptohexan-1-ol and 3-mercaptohexanol-acetate (Ribéreau-Gayon *et al.*, 2001).

2.3 ENVIRONMENTAL FACTORS

2.3.1 TEMPERATURE

Pirie (1979) hypothesized after experimenting in the field and controlled environments that temperature, together with, high carbohydrate status in vines and berries, growth regulators applied before and during ripening, genetic effects and berry size, influenced the pigment content of wine grapes. Buttrose *et al.* (1971) reported that Cabernet Sauvignon colour development was greater at day temperatures of 20°C than 30°C even with a constant night temperature of 15°C in both cases, but berries at higher day temperatures had higher concentrations of proline and malate. The optimum temperature for Shiraz and Cabernet Sauvignon was between 20 - 26°C day temperature or an average, minimum/maximum, temperature between 17.5 and 23.5°C. This agreed with Pirie (1979) that found that regions with an average temperature summation of 1600 - 1900 day-degrees C were more likely to produce highly pigmented grapes. Spayd *et al.* (2002) concluded on the other hand that excessive absolute bunch temperatures reduced anthocyanin concentrations, rather than the difference of ambient fruit temperatures.

2.3.2 LIGHT EXPOSURE

Spayd *et al.* (2002) concluded that in hot regions full bunch exposure should be avoided, but not totally as sunlight is needed for maximum anthocyanin synthesis and balance of other berry components. Rojas-Lara and Morisson (1989) reported that the period of rapid berry growth at véraison was delayed by two weeks in treatments with shaded leaves. The growth curves of shaded treatments were more gradual, with berry growth and cell enlargement still occurring at commercial harvest. Berry size was also influenced by shading of leaves and tended to be smaller than the exposed treatments (Rojas-Lara and Morisson, 1989).

2.4 METHODS USED FOR DETERMINATION OF MATURITY IN GRAPES

2.4.1 pH

It is called an abstract concept by Ribéreau-Gayon *et al.* (2000), but stands central to the microbiological and physicochemical stability of juice and wine (Boulton *et al.*, 1996). Ribéreau-Gayon *et al.* (2000) refers to pH as the true acidity. pH is the molar concentration of the hydrogen ion (H^+), given as the negative log of H^+ . The pH scale ranges between 0 and 14 (Iland *et al.*, 2000).

A low pH has the following advantages: increase the effectiveness of sulphur dioxide; inhibit reactions associated with oxidation and microbial spoilage; increase colour intensity and hue; increase effectiveness of the action of enzymes and bentonite and enhance aging potential (Iland *et al.*, 2000).

Acids can dissociate and produce free hydrogen ions and anions eg. tartaric acid:



Only a very small percentage; 1 to 3% (Zoecklein, 1995; Iland *et al.*, 2000) of organic acids dissociate, the rest stay in their parental form (Plane *et al.*, 1980).

2.4.2 SOLUBLE SOLIDS

At maturity levels above 18°Brix the levels of soluble solids are within 1% of the actual sugar content, below 18°Brix, soluble solids can vary between 4 to 5% of actual sugar content (Crippen and Morrisson, 1986; Zoecklein *et al.*, 1995). Soluble solids provide an indication of the level of maturity, the potential alcohol content of the resulting wine and there are legal standards for certain wine types (Zoecklein *et al.*, 1995). The scales mainly used for the measurement of soluble solids are the Baume, Balling, Brix and Oechsle scales. Two other scales also mentioned in the literature are the Plato scale (Brewing) and the Klosterneuberg scale (Boulton *et al.*, 1996). In this section we will only concentrate on the four prominent scales used in winemaking (Brix, Balling, Baume and Oechsle).

According to Boulton *et al.* (1996) these scales are generally amplifications of the changes in the specific gravity (s.g.) of solutions to that of water. Specific gravity (s.g.) is defined as the ratio of density of a solution to that of the density of water (Zoecklein *et al.*, 1995; Boulton *et al.*, 1996). The scales are measured by two methods, refractometry and hydrometry, which is both correlated to the density of a solution. Antoine Baume first developed the practice of calibrating hydrometers on the basis of weight percent in the late 1700's (Boulton *et al.*, 1996).

The Baume scale is related to the approximate potential ethanol in percent by volume if the non-sugar extract is ignored (Zoecklein, 1995; Boulton *et al.*, 1996). It corresponds fairly well to the percentage alcohol, at least between 10% and 12% (v/v) (Ribéreau-Gayon *et al.*, 2000). The early scale was based on the concentration of salt solutions, where each degree of the scale corresponded to 1% by weight of salt at 12,5°C. It ranged from 0 (water) to 15 with each degree being equal length on the hydrometer stem (Boulton *et al.*, 1996). The scale was recalibrated in recent times to a new reference temperature of 20°C. Where 1 degree Baume is approximately 1,8 degrees Brix (Balling) (Zoecklein, 1995).

The Balling scale is calibrated against the concentration of sucrose at 17,5°C. The scale was superseded by the Brix scale with a reference temperature of 20°C (Boulton *et al.*, 1996).

The Brix scale was developed by recalculating Balling scale to a reference temperature of 15,5°C. In modern times the scale was recalculated again to the reference temperature of 20°C (Boulton *et al.*, 1996). Ribéreau-Gayon *et al.* (2000), defines Brix as the weight of must sugars, in grams per 100 g of must. It is thus a percentage of the dry matter in the must. The measurement of the scale is only valid after 15°B, because polyphenols, organic acids, amino acids interfere with the reflectance (Ribéreau-Gayon *et al.*, 2000).

The Oechsle scale simply amplifies the density contribution of the solute over that of water by a factor of 1000, at a reference temperature of 20°C (Boulton *et al.*, 1996). According to Zoecklein *et al.* (1995) the scale is based on the difference in weight of 1 L of

must be compared to 1 L of water. The first three figures of the decimal fraction of a specific gravity equal the Oechsle equilibrium.

Ribéreau-Gayon *et al.* (2000) defines Oechsle as corresponding to the third decimal of the relative density (D). With the calculation below the sugar concentration (g/L) could be evaluated as follows:

$$\text{Sugar (g/L)} = (D-1) \times 2000 + 16$$

Sugar content is measured by two densimetric methods, hydrometry and refractometry.

Hydrometry is based on the principle that an object will displace an equivalent weight in any liquid in which it is placed. The volume displaced by an object is inversely proportional to its density. Hence a solution of high density will show less displacement than one of lower density (Zoecklein *et al.*, 1995).

Refractometry is based on the principle that the passing of a ray of light from one medium to another with a different optical density causes the incident ray to change its direction. The index of refraction is defined by Zoecklein *et al.* (1995) as the ratio of the sine of the angle of incidence to the sine of the angle of refraction. The reference wavelength for the refractive index was set with monochromatic sodium light at 589 nm and a temperature of 20°C (Zoecklein *et al.*, 1995).

2.4.3 MATURITY INDICES

Extensive research has been done on the field of maturity indices locally and abroad. Some of these indices are still in use after more than 60 years (Du Plessis, 1984). Zoecklein *et al.* (1995) however commented that soluble solids, titratable acidity and pH were not specific physiological indicators or potential wine quality characters and that considerable variation in these parameters can be found depending on the season, soil moisture, crop loading etc.

Amerine and Winkler (1941) determined Balling/Acid ratios as indicator of maturity in wine grapes. They classified grapes into three groups depending on their varying Balling. Thus taking into account the area and above mentioned grouping of the cultivar, the grapes would either be suitable for table or desert wine. This was a very helpful tool in the preliminary classification of grapes (Amerine and Roesler, 1958; Du Plessis, 1984). The Balling/Acid ratio was used with great success in Switzerland and Romania to determine maturity by Reuthniger (1972) and Tudosie *et al.* (1972). Berg (1958) advocated the use of the Balling/Acid ratio as a credible means of judging maturity, as Balling, by itself, was deemed practically useless as a measurement of the potential quality in California. Du Plessis (1984) found that the Balling/Acid values of the best wines were between 2.4 and 2.6 for Chenin blanc and 4.0 for Pinotage during trials conducted in South Africa. Ough and Alley (1970) suggested a Brix / Acid ratio of 35:1 when the acid is expressed in gram tartaric acid (H₂ta)/100mL. If we consider the acid to be expressed as H₂ta/L then the value would be 3.5:1. This value of Ough and Alley (1970) is mid way between the values found by Du Plessis (1984).

The Acid / Sugar (Balling) ratio was already in use with grapes by 1905 by Tietz according to Copeman (1928) (Jordan *et al.*, 2001). Biolethin (1925) discussed the sugar

(Balling) / Acid ratio as a means of extending quality standards that had been entirely based on Balling alone (Jordan *et al.*, 2001).

Balling / Acid ratio has one fundamental fault on which Boulton (1996) and Jordan *et al.* (2001) agree. Boulton *et al.* (1996) used the following example to explain the danger of only a single value based on the above mentioned ratio; an over cropped late harvest may be so deficient in sugar and acid that it has a proper ratio but cannot make acceptable wine. Jordan *et al.* (2001) used the example of two solutions analysed, one with 10°B and 1% acid, and one with 20°B and 2% acid have the same ratio of 10:1 but differ considerably in palatability. Both authors advocate the inclusion of the two components of the ratio to make an informed choice. Archer (1981) commented that skin contact in white grapes has an influence on the Balling/Acid index, because potassium in the skin and seeds can initiate cation exchange and acid neutralization. These reactions lower the acid concentration of the must and increase the pH. Thus the optimal Balling/Acid index is reached earlier than for free run juice.

Other ratios were also used namely, Brix x pH², Brix x acid, Brix x pH. Brix x pH² was judged to be a better indicator of quality at harvest than Brix/Acid, Brix x Acid or Brix x pH, in South Australian winemaking (Coombe *et al.*, 1980). Coombe *et al.* (1980) reported the best wines for Brix x pH² had values of 200 – 270. The occurrence of high potassium, high pH and higher acid are considered by the pH value. According to Boulton *et al.* (1996), pH has a greater effect on fermentation and metabolic pathways than titratable acidity. The bigger value for pH in the Balling x pH² index can be motivated by the significant role it plays during fermentation and ultimately wine stability (Coombe *et al.*, 1980).

Sinton *et al.* (1978) found Brix x pH to be the most practical indicator of aroma intensity in Zinfandel wines, even though no significant correlation could be found between this ratio and the overall sensory scores. Van Rooyen, Ellis and du Plessis (1984) concluded that Balling x pH gave a better indication of optimum maturity in Pinotage and Cabernet Sauvignon than Balling or Balling/Total Titratable Acidity. Balling x pH values of 85 – 95 corresponded with the best quality wines for the two cultivars.

Du Plessis and van Rooyen (1982) found that Balling/Acid ratios indicated a rapid attainment of optimum quality followed by a rapid decrease. Differences between cultivars were noted. Studies also showed that in some seasons a clear maximum wine quality could be found (Du Plessis, 1984)

pH, sugar (Balling) and titratable acidity values are not consistent during ripening from one season to the next, and this makes it difficult to determine optimum maturity especially in warmer areas where irrigation plays a role. Irrigation leads to fluctuations in the relatively steady increase in pH during ripening which leads to inaccuracy in determining optimum maturity (Du Plessis, 1984).

Out of the above it is clear that no single value could indicate the optimum maturity in all growing areas around the world, but only as a supporting means in making a decision. Maturity must be seen in relative terms, dependant on the style and type of wine, as it is a multidimensional phenomenon with no perfect synchronization of desirable components (Zoecklein *et al.*, 1995).

2.4.4 GLYCOSYL – GLUCOSE METHOD

The glycosyl-glucose (G-G) method was developed to measure the composition of grapes, juice and wines (Francis *et al.*, 1998; 1999). The G-G method measures the pool of glycosides in the grapes, by hydrolyzing the glucose unit and determining the glucose by spectrophotometry (Iland *et al.*, 2004). The G-G values are presented as micromoles of glucosides per gram fresh weight ($\mu\text{mol/g fw}$) or micromoles per berry ($\mu\text{mol/berry}$) (Francis *et al.*, 1998).

G-G studies have shown that berry colour and berry G-G have a positive correlation (Ilands *et al.*, 2004). The total glycosides of red grapes consist of between 70% to 80% anthocyanins (Iland *et al.*, 2004). Berry colour is easier, cheaper and more practically to measure as a routine parameter than G-G in red grapes (Francis *et al.*, 1999; Iland *et al.*, 2004). For white grapes there is no comparable method to G-G to quantify the flavour potential (Francis *et al.*, 1999; Iland *et al.*, 2004). Iland *et al.* (1996) worked on optimizing the G-G method for use on black grapes by removing phenolic interferences of the seeds with a C18 RP cartridge prior to enzymatic analyses. The red-free G-G method gives an estimate of the glucoside concentration other than anthocyanins and is applicable only to fruit where monomeric anthocyanin monoglucoside pigments predominate (Iland *et al.*, 1996). Zoecklein *et al.* (2000) modified the G-G method so that the phenolic glycosides were separated from the aroma and flavour glycosides, giving a “phenolic –free” G-G.

From a study done by Francis *et al.* (1998, 1999), they reported that grapes with higher G-G per gram fresh weight values resulted in wines with high G-G concentration values. Small berries may also give high G-G per gram values even though they may have low G-G per berry values. G-G values for white grapes were in the region of $0.81 \mu\text{mol/g fw}$ and for red grapes $5.2 \mu\text{mol/g fw}$ (Francis *et al.*, 1998). Ilands *et al.* (1996) found that values for Pinot noir and Shiraz varied widely during preliminary studies, from 1 to $1.56 \mu\text{mol/g}$ for Pinot noir and 2.38 to $3.87 \mu\text{mol/g}$ for Shiraz. The method is not suitable for black non-vinifera cultivars with diglucosides anthocyanins as the fruit gives erroneously high G-G values (Iland *et al.*, 1996).

2.4.5 TITRATABLE ACIDITY

Titrateable acidity (TA) measures all the available hydrogen ions present, those free as H^+ or bound to undissociated acids (tartaric acid (H_2T) and malic acid (H_2M)) and anions (HT^- and HM^-) by titrating with an alkaline solution (NaOH) (Zoecklein *et al.*, 1995; Boulton *et al.*, 1996; Iland *et al.*, 2000; Ribéreau-Gayon *et al.*, 2000). Titrations with a strong base give a true end point greater than pH 7 usually between 7.8 and 8.3 (Iland, 2004).

When titrating with an alkali solution (NaOH) a point would be reached where all the available hydrogen ions in the sample reacted with the alkali, this particular pH point is termed the equivalence point or end point (Iland *et al.*, 2000). The weak acid solution is titrated with a strong base, thus the equivalence point is reached at a pH greater than 7.0. Iland *et al.* (2000) gives the range as between pH 7.5-8.4, but taken at pH 8.2. The Methods of analysis for wine lab (2002) gives the range between pH 7.8-8.3. In South Africa samples are titrated to pH 7.0 but in Australia and the United States a pH of 8.2 is used. In France

titratable acidity is expressed as gram per liter sulfuric acid and in the United States Australia, South Africa and New Zealand grams per liter tartaric are used (Zoecklein *et al.*, 1995; Boulton *et al.*, 1996; Iland *et al.*, 2000; Ribéreau-Gayon *et al.*, 2000). Titratable acidity is always less than expected from organic acid concentrations, because the acids could be in less active forms like potassium bitartrate (Boulton *et al.*, 1996).

2.4.6 PHENOLIC ANALYSES

2.4.6.1 Total phenols (Folin-Ciocalteu)

The Folin-Ciocalteu method measures the total phenol content and lacks specificity as it measures the number of hydroxyl groups (–OH) (potential oxidizable phenolic groups) present. A problem common to UV and visible spectrophotometric measurements is that different phenolic components can have significantly different molar absorptivities (Zoecklein *et al.*, 1995).

Folin-Ciocalteu reagent replaced the Folin-Denis reagent. The differences between the two methods are the presences of hydrochloric acid, the higher percentage of molybdate in the complex, is more easily reduced and the use of lithium sulfate, which prevents precipitation problems that plagued the Folin-Denis method (Ough and Amerine, 1974; 1988). Gallic acid is used as standard reference compound and results are expressed in Gallic Acid Equivalent (GAE). Folin-Ciocalteu reagent reduces phenolic compounds with a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). The phenolate anion is required to reduce the Mo(VI) and W(VI) ions and the heteropoly Molybdenum and Tungsten molecules gives a blue colour as apposed to the yellow unreduced molecules. The colour change of the mixture is read at an absorbance of 789 nm (Zoecklein *et al.*, 1995).

2.4.6.2 Protein precipitation assays

Condensed tannins are the most abundant class of phenolics in the berry and are primarily found in the skin and testa of the seed (Adams and Habertson, 1999; Habertson *et al.*, 2002). Grapes and wines present a formidable challenge to analyse, due to the large number of unique chemical structures formed by the monomeric subunits (Adams and Habertson, 1999). Several assay procedures have been described to measure tannins. These assays relied on the ability of plant polyphenols to crosslink or precipitate animal proteins (Makkar *et al.*, 1988; Adams and Harbertson, 1999).

Bate-Smith (1973) used an innovative procedure to determine the astringency of wine by utilizing the ability of hemoglobin to precipitate tannins. The use of blood to fine wine can be traced back through history, but this practice is unacceptable by modern wine preparation standards. Bate-Smith (1977) reported in a later publication that saponins and other plant metabolites interfered with the assay. Herderich and Smith (2005) stated that haemanalysis was not widely adopted.

Hagerman and Butler (1978) measured the tannin precipitated by bovin serum albumin (BSA) followed by the colourimetric shift of ferrichloride ($FeCl_3$) at 510 nm after the pellet was resolubilized. The value was expressed in terms of absorbance units per gram of

extracted grain. Harbertson *et al.* (2003) expanded the BSA assay to incorporate the quantification of small polymeric (non bleachable and non precipitable) and large polymeric pigments (non bleachable and precipitable) by incorporating the bleaching effect of bisulfite.

Asquith and Butler (1985) used dye-labeled protein to measure the amount of protein precipitated by a given amount of tannin, while Earp *et al.* (1981) used the cleavage of a starch bound dye by amylase. Makkar *et al.* (1988) measured the tannin to protein ratio. Ittah (1991) as well as Adams and Harbertson (1999) determined tannin concentration indirectly by measuring alkaline phosphatase and Dick and Bearn (1988) the inhibition of β -galactosidase. McNabb *et al.* (1998) used the digestion of protein by trypsin and chymotrypsin to indirectly measure tannins.

Herderich and Smith (2005) stated that reproducibility and comparison between studies are hindered by the inability to measure the removed tannin directly and the variability of proteins. The isoelectric point, ionic strength, pH, temperature and protein conformation are all potential sources of analytical variation.

2.4.7 GRAPE COLOUR MEASUREMENT

The most problematic part of measuring anthocyanin content by spectrophotometry is the separation of the polymeric and monomeric fractions (Ough and Amerine, 1988). According to Ough and Amerine (1988), changes occur in both fractions that invalidate conversions of direct colour measurements into quantitative values, during the separation methods. Ribéreau-Gayon *et al.* (2000) and Stonestreet standardized the pH shifting and bisulfite bleaching methods (Ough and Amerine, 1988). The principle of the two methods lay in the changes to the absorbance of the unpolymerised pigments relative to the polymerized pigments by the change in pH and SO₂ addition (Ough and Amerine, 1988).

The colour of anthocyanins is directly linked to their pH, being red at low pH and losing colour with an increase in pH (Ribéreau-Gayon *et al.*, 2000). According to Ribéreau-Gayon *et al.* (2000) this is attributed to the flavylium form, which has a stable oxonium cation that is stabilized by resonance across the entire cycle (Figure 2.9). Anthocyanins are strongly bleached by SO₂, especially at pH below 3.2, as the sulphur consists mainly of the HSO₃⁻ anions that react with flavylium cations (Ribéreau-Gayon *et al.*, 2000). Bisulphite attacks exclusively the 4-position of the anthocyanin and forms a stable colourless adduct (Jones and Asenstorfer, 1998).

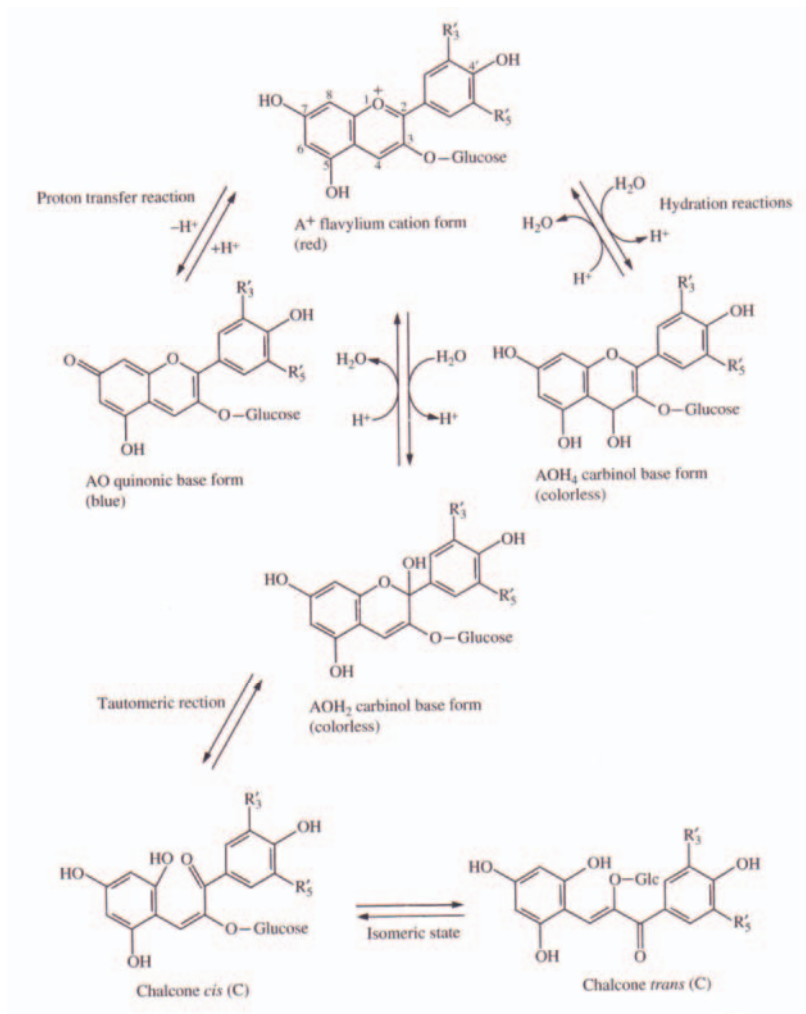


Figure 2.9 Various forms of anthocyanins (R₃' and R₅' see figure 2.8) (Ribéreau-Gayon *et al.*, 2000).

2.4.7.1 Ilands method

The method to determine anthocyanins and total phenols in grape berries as described by Iland *et al.* (2000) is derived from work done by Somers and Evans (1974). In future we will refer to this method as the Ilands method.

From studies done previously berry colour had shown good correlation with, wine total anthocyanins concentration, wine colour density, wine score or grade and intensity of specific aroma descriptors. Iland *et al.* (2000) gives a guide to berry colour as 0.3 to 2.5 mg per g berry weight for Cabernet Sauvignon and Shiraz and 0.2 to 1.4 mg per g berry weight for Pinot noir. When berry colour is above 1.6 mg per g berry weight then colour appears to be less discriminating in the prediction of wine style.

The method entails the homogenation of a specified number of grapes (50 berries). It is then extracted with 50% v/v aqueous ethanol, pH 2.0 and centrifuged after one hour. The extract is read at 280 nm, 520 nm and 700 nm and the mg anthocyanins per berry, mg anthocyanins per gram berry weight are calculated.

The calculation of the berry colour uses the extinction coefficient of malvidin-3-glucoside (500) and is expressed as equivalents of malvidin-3-glucoside. Somers and Evans (1974)

expressed the extinction coefficient of malvidin-3-glucoside in units of g/100 mL, but milligrams are more common to grape berries and the value of 1000 is included in the calculation. The absorbance value at 700 nm gives an indication of turbidity and a typically a value should be under 0.01. The absorbance at 280 nm gives an estimate of the phenolic concentration per 100 mL. The values are arbitrary and can only be used for comparison between samples (Iland *et al.*, 2000).

2.4.7.2 Extractability method (Glories method)

Grapes with high concentrations of anthocyanins, theoretically gives wines that are rich in colour, but this is not always true (Ribéreau-Gayon *et al.*, 2000; Glories, 2001). Glories (2001) commented that grapes have a “colour extraction potential” or extractability that varies according to cultivar and maturity level. The extractability of anthocyanins is a function of the degree of maturity and is determined by the degradation of the skin cells (Glories, 2001). If all conditions during winemaking are equal, then optimal or slightly overripe grapes will give wines with higher anthocyanin content than grapes prior to that maturity levels (Ribéreau-Gayon *et al.*, 2000).

The principle of the method lies in the difference of the extraction results at pH 1 and pH 3.2 that can be related to the fragility of the cell membranes and their potential for extraction (Ribéreau-Gayon *et al.*, 2000). To facilitate the extraction of anthocyanins, acidity is used as the vector, to rupture the proteophospholipid membrane of the vacuoles. Extraction at pH 1 gives an indication of the total potential anthocyanins present in the cells, as all anthocyanins are extracted and solubilized in the pH 1 solution (Glories, 2001). The extraction at pH 3.2 is comparable to the extraction during vinification. During ripening, grape enzymes degrade the cell walls and facilitate release of anthocyanins from the vacuoles to the same degree as at pH 1 (Ribéreau-Gayon *et al.*, 2000; Glories, 2001).

The anthocyanin extraction percentage (AE%) values vary between 70 and 20, depending on the cultivar, maturity level and vineyard practices (Ribéreau-Gayon *et al.*, 2000). The AE% decreases during ripening (Ribéreau-Gayon *et al.*, 2000; Glories, 2001). Cabernet Sauvignon has a tougher skin than Merlot and accordingly Cabernet Sauvignon have higher values than Merlot (Glories, 2001).

Tannins are also extracted under the same conditions as anthocyanins from the skins (Glories, 2001). According to Ribéreau-Gayon *et al.* (2000) anthocyanins can be used as markers for the tannins in the skins. Ratios from the pH 3.2 extract of the absorbance at 280 nm to the absorbance of the anthocyanins at 520 nm are between 35 and 45 for ripe grapes of all cultivars. The contribution of the seeds (MP%) gives an indication of the risk of the negative “green tannin “ flavour on the wine. The MP% decreases during ripening with values between 60 and 0, according to cultivar, maturity and number of seeds (Ribéreau-Gayon *et al.*, 2000; Glories, 2001).

Potential anthocyanin (mg/L) varies between 500 and 2000 mg/L depending on cultivar. Glories studied the phenolic maturity of Cabernet Sauvignon from different vineyards in France over time and found that the potential anthocyanin (pH1) increased from 1318 to 1982 mg/L in Saint-Emilion; 1185 to 1758 mg/L Médoc and 1472 to 1745 mg/L in Graves.

He also found that the contribution of seed tannins (MP%) decreased during the same time from 34 to 17 in Saint-Emilion; 39 to 13 in Médoc and 34 to 14 in Graves.

2.4.8 EVALUTION OF SEED COAT COLOUR

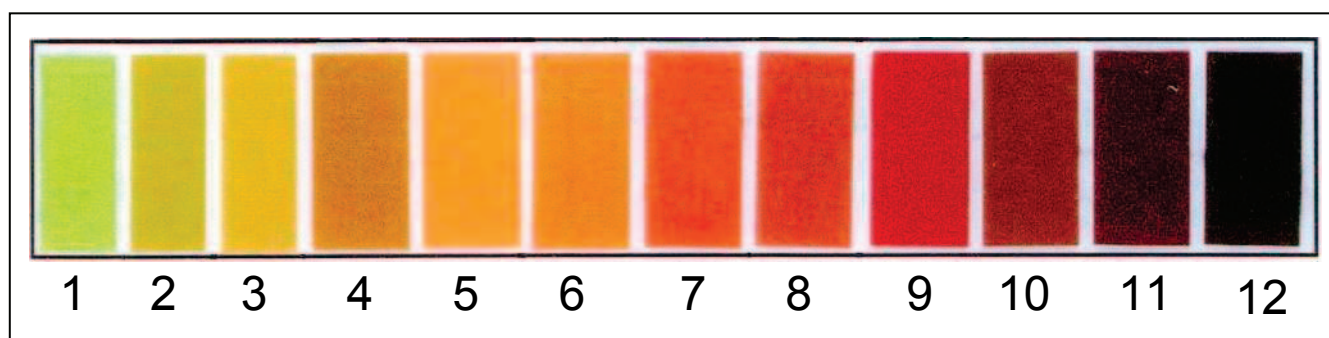


Figure 2.10 Colour chart proposed by Ristic and Iland (2005) indicating changes in grape seed colour during development and maturation.

During the 1999/2000 and 2000/2001 seasons Ristic and Iland (2005) embarked on a study of the developmental changes in seed morphology and phenolic composition of Shiraz. On the basis of strong correlations of seed coat colour changes and extractable seed tannins during seed development, they proposed using the colour value as a indicator of seed maturity. They developed a colour chart (Figure 2.10) with 12 colours representing the colour changes seen in seed development.

The relationships between the seed coat value and seed tannin level (mg catechin eq /berry and mg catechin eq/seed), total anthocyanins (mg/berry) and total skin phenolics (AU/berry) were assessed. The level of seed tannins per seed gave a higher coefficient of determination (r^2) 0.81, than the seed tannins per berry (0.77). Total anthocyanins had a significant correlation with seed maturity (Ristic and Iland, 2005). These researchers stressed that the index should be used as a general indication and that phenolic compounds should be assessed using analytical methods. The index is new and not widely used as of yet, but gives a general quantifiable alternative to merely guessing in the vineyard.

2.4.9 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSES

HPLC analyses are considered an effective and accurate technique for monomeric and some oligomeric phenol analyses from seed, grape as well as wine extracts. Peng *et al.* (2001) found a large degree of variability in the content of seed procyanidins even at near identical sugar levels by using HPLC analyses.

Kennedy *et al.* (2000a) used normal phase HPLC to study the polymerisation of procyanidins in seeds during ripening. They observed a reduction in the mean degree of polymerization (mDP) during thiolysis but an increase in concentration during HPLC measurement as the products increased in molecular size.

HPLC measurements work well with monomeric polyphenols such as catechin and malvidin-3-glucoside (Peng *et al.*, 2002). Herderich and Smith, 2005, commented that the condensed tannins might reflect the method used, as well as revealing the composition of the sample. De Beer *et al.*, 2004, commented on the difficulty of comparing tannin data

derived from different analytical methods. The differences in the extraction of tannins could be affected by any variation in the homogenization technique and this is found particularly in grape seeds, but is less of an issue in the readily extraction of anthocyanins from grape skins (Cynkar *et al.*, 2004; Herderich and Smith, 2005). From literature it is clear that a robust and efficient method for the analysis too quantify grape and wine tannins is still a priority (Herderich and Smith, 2005).

2.5 SUMMARY

Measurement has reached new heights during the last century for grape components and has become indispensable for the winemaker. Traditional, subjective method of tasting berries or seeds has been surpassed by measuring of sugar (Brix, Balling and Oeshle), titratable acidity and pH. These three measurements are the corner stone of determining the level of ripeness of grapes and the subsequent quality of a wine. But during recent years grape colour (Iland and Glories methods) has come to play an ever increasing role in the determination of optimal ripeness of grapes, as their positive correlations with wine quality has been shown (Iland *et al.*, 2000). The focus on more in-depth analyses by HPLC, GC-MS and the Winescan has broadened our understanding of the composition of grapes and their development during ripening.

Unfortunately these methods require specialized skills and expensive apparatuses. They are thus not practical for use in the cellar. The viticulturist and winemaker need inexpensive, quick and reliable methods that can be applied easily. The combination of traditional analyses (maturity indexes) and the determination of berry colour will play an important role in the coming years. In an ever more competing wine market, precise determination of harvest times for specific wine styles will become the norm in the not to distant future.

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Chapter 3

RESEARCH RESULTS

Evaluation of grape parameters to determine grape maturity for Cabernet Sauvignon in four wine growing regions.

This manuscript was submitted for publication in
South African Journal of Enology and Viticulture

3. EVALUATION OF GRAPE PARAMETERS TO DETERMINE GRAPE MATURITY OF CABERNET SAUVIGNON IN FOUR SOUTH AFRICAN WINEGROWING REGIONS

3.1 ABSTRACT

Chemical and phenolic compositional changes of Cabernet Sauvignon grapes were investigated during ripening, to identify the optimal harvest time for the cultivar. Four commercial blocks of Cabernet Sauvignon were investigated in different South African climatic zones over a period of 3 years. Grapes were sampled weekly from just after véraison until 2 weeks after commercial harvest. Seed lignification was followed over the 3 seasons. The lignification percentages were found to vary between 59% and 80% over seasons 2003 and 2005 at commercial harvest. But due to the longer ripening season of 2004, these values increased to between 73% and 91% at commercial harvest. Red and white must were obtained and analysed for Balling (°B), pH, titrable acidity (g/L), potassium (mg/L), malic and tartaric acid (g/L). Comparable trends were found to that of previous literature, but red must had higher levels of potassium and pH, as well as lower titrable acidity, compared to the white must, due to an increase in salification. Anthocyanin levels were investigated and comparable trends were found between the four vineyards, but those in warmer, dryer regions tended to have higher values. Wine colour density (A420+A520) followed the same trend as the anthocyanin levels. Grape skins (G) were used to make an artificial wine that was evaluated by an expert panel. Wines (W) made from sampled batches were also evaluated by an expert panel for: colour intensity, vegetative, red berry, black berry with spice, acidity, astringency and general quality. Vegetative aromas and acidity decreased and red and black berry with spice increased during ripening. Colour intensity also increased corresponding to an increase in perceived general quality score. Correlations between general quality of both the grape skins tasting and wines were investigated. Balling showed a strong correlation with general quality of the grape skins tasting ($r=0.76$; $p=0.00$) and subsequent wines ($r=0.57$; $p=0.00$). Anthocyanin concentration (mg/g berry) ($r=0.36$; $p=0.00005$), perceived colour intensity of grapes ($r=0.69$; $p=0.0000$) and wine ($r=0.84$; $p=0.0000$) correlated with general wine quality. The best rated Cabernet Sauvignon wines correlated strongly with soluble solid content; colour and quality perceptions of grapes, but large seasonal differences resulted in larger grape compositional variances than that of the vineyards in the different climatic zones. This illustrated the difficulty of pinpointing a specific parameter to indicate optimal ripeness.

3.2 INTRODUCTION

Wine quality today is paramount to the export success of wine producing countries throughout the world, including South Africa. The quality of wines have increased over the past 4000 years and with it the demands to judge the right (optimal) time to harvest.

Sugar has been widely used as a maturity criteria defined in Brix, Balling, Oechsle or Baume, depending on the country (Boulton *et al.*, 1995; Ribereau–Gayon *et al.*, 2001a). The use of sugar as the only criteria does not give a true indication of ripeness, due to the climatic impact on the accumulation of sugar in different regions of the world (Archer, 1981). Titratable acidity and pH have also been in use around the world as maturity indicators. Changes in the titratable acidity are caused by changes in the organic acid (tartaric, malic and other acids) concentration due to climatic conditions, transformation of organic acids to sugars, dilution in the berries and salification of the acids by potassium (Archer, 1981). pH increases are similarly influenced by the acid concentrations. Several studies have investigated the use of combinations of sugar, acidity and pH to determine optimal ripeness (maturity) (Amerine and Winkler, 1941; Berg, 1958; Coombe *et al.*, 1980; Du Plessis and Van Rooyen, 1982).

In red wine, colour is seen as a quality indicator (Somers and Evans, 1974; Jackson *et al.*, 1978). Several chromophores contribute to the red wine colour, which include cation forms of anthocyanins, co-pigmentation complexes, anthocyanin-adducts and larger polymeric pigments (Boulton, 2006). Malvidin monoglucoside is the dominant pigment in grapes and is considered to form the basis of grape and ultimately wine colour (Ribereau–Gayon *et al.*, 2001). Methods for the evaluation of grape quality have been developed over the years. The Ilands method (Iland *et al.*, 2000), glycosyl-glucose (G-G) method (Francis *et al.*, 1998, 1999), extractability potential (Glories, 2001) and the pH shifting and SO₂ bleaching first used by Ribereau–Gayon and Stonestreet (Ough and Amerine, 1988) have all contributed to our understanding of grape composition and harvesting time. Iland *et al.* (2004) showed a positive correlation between berry colour and the berry glycosyl-glucose (G-G) levels. It is cheaper and quicker to do the berry colour measurement than the G-G method on a routine basis (Francis *et al.*, 1999; Iland *et al.*, 2004). Glories (2001) commented that the extraction of anthocyanins is a function of the degree of maturity and is determined by the degradation of the skin cells. Thus if all conditions during winemaking are equal, then optimal or slightly overripe grapes will give wines with higher anthocyanin content than grapes prior to that maturity levels (Ribereau-Gayon *et al.*, 2000a).

The term optimal ripeness has been used in the South African wine industry over the years with varying meaning and interpretation. For the context of this work we will define optimal ripeness as follows: the maturity level of a specific grape cultivar, where all metabolic and physiological components are in balance, to produce a wine of superior quality for a specific wine style. The aim of this study was to evaluate the maturity level of Cabernet Sauvignon grapes at different times, using a range of methods to identify the optimal harvest time for the cultivar under South African climatic conditions (terroir).

3.3 MATERIALS AND METHODS

3.3.1 ORIGIN OF GRAPES

Four commercial vineyards of *Vitis vinifera* L. cv. Cabernet Sauvignon grafted on Richter 99 rootstock were selected in four different winegrowing wards, Simonsberg-Stellenbosch (Nietvoorbij), Simonsberg-Paarl (Plaisir de Merle), Wellington (Anhöhe) and Durbanville (Morgenster) (Table 3.1) of the Coastal Wine Region, Western Cape.

Five rows by eight inter-pole-spaces per vineyard were identified and marked with danger tape. Each row of these blocks was three inter-pole-spaces from the nearest access road. The anchor post at each end of the row was also marked and numbered.

Table 3.1 Experimental vineyard block data from four farms

	Anhöhe	Morgenster	Nietvoorbij	Plaisir de Merle
Winkler	V	III	IV	IV
Average °C	15.8*	15*	15.6*	16.3*
Average °C_{max}	23*	20*	21.2*	20.9*
Average rainfall (mm)	55*	153*	193*	205*
Row direction	East-West	North-South	North-South	North-South
Row width (m)	3.4	3.1	2.7	2.9
Inter-pole-space length (m)	7.4	8.6	7.4	7.4
Clone	46	169	37B	336B
Slope direction	East	West	West	East
Trellis	5 wire lengthened Perold	5 wire lengthened Perold	5 wire lengthened Perold	3 wire hedge
Number of vines per block	240	245	239	232

* Climatic data as obtained from the closest weather station.

3.3.2 SAMPLING AND PREPARATION OF GRAPES

Vineyards were sampled during the 2003, 2004 and 2005 harvest seasons from just after véraison until two weeks after commercial harvest. One bunch was randomly sampled from each vine in the designated experimental block from each vineyard. A berry sample of 2.5 kg was then randomly selected from these pooled bunches for each vineyard. A hundred berries were selected from this 2.5 kg sample and used to determine the berry weight and volume. The 100 berries were then added back to the remainder of the 2.5 kg sample. From this 2.5 kg sample, 150 berries were used for the grape skins tasting and 50 berries for seed lignification and seed extract experiments. A second sample of 700 g was used for the

phenolic and colour measurements and a third sample, consisting of the rest of the original sample, was used to prepare white must for routine analyses.

A 700 g grape sample was homogenised for 20 seconds using a colloid mill (Fryma AG, Rheinfelden, Switzerland). The homogenate was stirred for 20 seconds before a sample was taken. This was done to re-suspend any sediment that might have formed at the bottom of the beaker while standing before sampling.

3.3.3 MUST PREPARATION AND ANALYSES

The white must was obtained, by crushing the grapes by hand and separating the pulp and juice with a strainer. The red must was obtained from the homogenate. Both musts were centrifuged (Beckman, Model J2-21, Beckman Instruments Inc., Palo Alto, CA, USA) at 10000 rpm for 15 minutes and the supernatant used for analyses. The white and red must were analysed for Balling, pH, titrable acidity, organic acids (tartaric and malic), total phenols and anthocyanin content as described by Iland *et al* (2000).

3.3.4 SEED LIGNIFICATION

Seeds were obtained from the pulp of the grape skins tasting. Fifty seeds were randomly selected and the pulp washed off with distilled water and dried using tissue paper. Seed lignification was followed by using the Munsell colour charts for plant tissues (Munsell, 1952) and expressed as percentage of the 50 seeds with the corresponding colour code. Photographs were taken of each batch of seeds using a digital camera (Nikon Coolpix 4500, Nikon Corporation Inc., Japan) for future reference. A Munsell colour chart was required to interpret the results each time, but an easier method was sought to interpret the lignification status of the seeds over the harvest seasons.

The Ristic and Iland (2005) method of following seed lignification was used to determine the degree of seed lignification over the subsequent ripening seasons. It was found to be more meaningful than the Munsell method as a value was given to the degree of lignification. The lignification was followed using the colour chart as shown in figure 3.1.

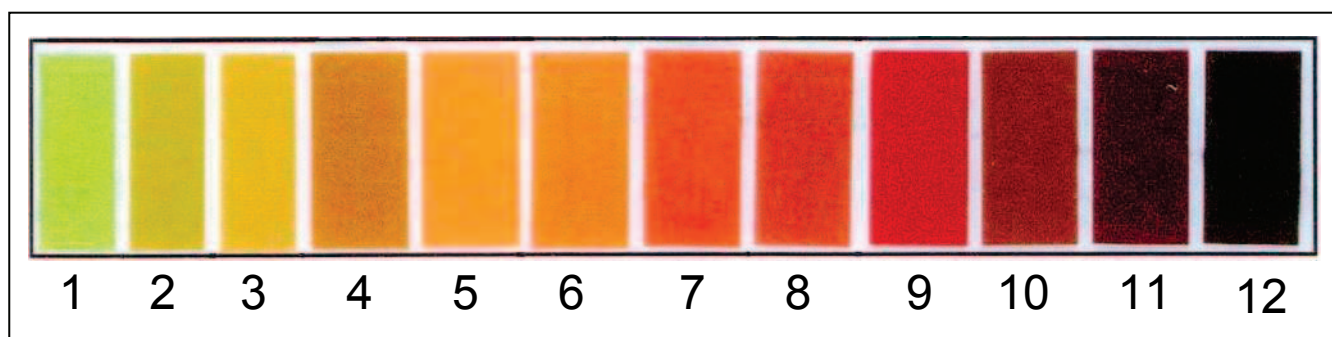


Figure 3.1 Generic colour classification chart developed by Ristic and Iland (2005) for the classification of seed development and maturity determination.

The following steps were followed as per Iland and Ristic (2005):

- Step 1:** Counted and recorded 20 random seeds
- Step 2:** All seeds were turned ventral surface up.
- Step 3:** The colour of each seed was matched to the colour chart (Table 1) and recorded.
- Step 4:** For each group of seeds with the same colour, the number of seeds were multiplied with the corresponding colour number on the seed colour chart.
- Step 5:** The separate values obtained in step 4 were added, and divided by the total number of seeds to give the ventral seed colour.
- Step 6:** The seeds were all turned over and the dorsal colour was determined as in step 3 to 5.
- Step 7:** The average of the values of ventral and dorsal surface colour was obtained to give the overall seed colour.
- Step 8:** The overall seed colour percentage was determined by dividing the overall seed colour by the reference colour 11.

3.3.5 ANTHOCYANIN DETERMINATION

3.3.5.1 Weight and volume per berry

One hundred berries were randomly taken from the 2.5 kg sample and weighed using a digital scale (Mettler, model PB 3000, Mettler Instruments AG, Zurich, Switzerland). The value was divided by 100 to give the weight per berry. A two liter measuring cylinder was used to determine the volume of 100 berries. The cylinder was filled with water to the one liter level (Volume 1), the berries were added and the volume noted (Volume 2). The volume per berry was determined by subtracting volume one from volume two and dividing the answer by 100. This gave the berry volume per berry.

3.3.5.2 Iland Method

Anthocyanins were determined as described by Iland *et al.* (2000) with small modifications. The berry weight as previously determined was used for the calculations by dividing the weight of 100 berries by two. The homogenate, prepared as described in section 3.3.3, was used for the extraction. The procedure was done in triplicate and 2 g of homogenate was used for each extraction. Twenty mL of 50% ethanol adjusted to pH 2 with hydrochloric acid (HCl) (MERCK Chemicals (PTY) Ltd, Wadeville, Gauteng, RSA) was added to each 50 mL centrifuge tube containing the homogenate. The solution was centrifuged (Beckman, Model J2-21, Beckman Instruments Inc., Palo Alto, CA, USA) at 10000 rpm for 10 minutes. The extract as described by Iland *et al.* (2000) was pipeted (0.5 mL) into test tubes and 5 mL of 1 M HCl (MERCK Chemicals (PTY) Ltd, Wadeville, Gauteng, RSA) were added. The mixture was allowed to extract for three hours at 20°C. A ThermoSpectronic spectrophotometer (ThermoSpectronic, Helios Gamma) was used to measure the absorbance at 280 nm, 520 nm and 700 nm. The values obtained from this method were given as milligram anthocyanin

per gram berry (mg anthocyanin / g berry) or milligram anthocyanin per berry (mg anthocyanin / berry).

3.3.6 GRAPE SKIN TASTING

A sample of 150 berries was randomly selected from the 2.5 kg berry sample and weighed using a three decimal scale (Precisa, Type. 280-9826, PAG Oerlikon AG, Zurich, Switzerland). The pulp and seeds were removed by squeezing the berries between the thumb and forefinger into a beaker of distilled water. Seeds were used for colour classification as described in section 3.3.4. The weight of the skins was noted and 75 mL citric acid solution (9.25 g/L) was added to it. The skins and citric acid solution were blended using a handheld stick blender (KRUPS, Pro Mix, Silver Plus, Germany) for four minutes. The slurry was poured into a pre-tared beaker. Any residue left on the blade was washed with distilled water and added to the skin slurry. The skin slurry was fortified to 8% v/v with 96% v/v neutral wine spirits, calculated on the original mass of the 150 berries. Distilled water was used to adjust the blend to the original weight of the 150 berries and mixed. The turbid solution was sealed with Parafilm (Pechiney Plastic Packaging, Menasha, WI, USA) and left for 24 hrs at 20°C, after which it was centrifuged (Beckman, Model J2-21, Beckman Instruments Inc., Palo Alto, CA, USA) at 10000 rpm for 20 minutes. The supernatant was decanted into a sample bottle and stored at 0°C until tasted.

An industry panel of winemakers was used to assess the supernatant using line scales. The grape skins were assessed by the following criteria: colour intensity, aroma (vegetative, red berry, black berry and black berry with spice) and tannin concentration.

3.3.7 SMALL SCALE WINEMAKING.

Small scale wines were made during the harvest seasons of 2003, 2004 and 2005. Grapes were weighed at the cellar and the crate mass subtracted. After the 2.5 kg sample for chemical analysis was taken, each vineyard batch was divided into duplicates of equal weight. Each batch was put through a destemmer-crusher. Thirty five parts per million (ppm) of sulfur dioxide (SO₂) was added to the crushed grapes at the crusher. Twenty five grams per hecto liter (g/hL) hydrated yeast (WE372, Anchor Yeast, Cape Town, RSA) was added. The must was fermented at 20°C and the cap was punched down three times daily. The fermenting grape must was pressed at four degrees balling (°B) using a 20 liter hydro press (Fratelli Marchisio S.p.A., Pieve di Teco, Italia) to press the grapes to three bar. The wine was transferred to a canister to finish primary fermentation. Wines were deemed dry when the residual sugar level was lower than 5 g/L. Sixty ppm SO₂ was added after the wine was fermented dry. Samples were taken after the SO₂ addition and analysed for alcohol level, residual sugar, volatile acidity, pH, titratable acidity, free and total sulfur. The wines were left for one week to settle at 4°C after which the wines were racked off the lees. Final adjustments were made before filtration. The total sulfur level was raised to 100 ppm and the acid level adjusted to 6 g/L. The wines were filtered through a sheet-filter using Seitz K200 (PALL Corp., East hills, New York, USA) sheets into screw cap wine bottles. Duplicates were

blended together before bottling and evaluated as a single wine. The wines were labeled and stored at 15°C until tasted by the panel.

3.3.8 WINE TASTING

An industry panel of winemakers evaluated the wines at the end of each year. Forty millilitres of wine was poured into each glass. Wines of the four vineyards were tasted in a random order by the panel in flights of six wines at a time with a break of five minutes in between each flight. The panel was asked to use a line scale to record their impression of each criterion. The scale was ranged from non detectable at its minimum to intense for the aroma components: vegetative, red berry and black berry with spice as well as for acidity. Colour intensity was scaled from light coloured to intense colour. The overall quality of the wine was determined by each taster in the panel after taking all the preceding criteria into account. Each line scale was measured and the distance used as the degree of liking by the taster.

3.3.9 STATISTICAL ANALYSIS

Data was analysed using Statistica Version 7.1 (Tulsa, OK., U.S.A). Spearman and Pearson correlations were done with the datasets and ANOVAS investigated for trends between different grape and wine variables.

Principle component analysis (PCA) was done on the data by using The Unscrambler Version 9.2 (CAMO Process AS, Oslo, Norway). The data was centred and scaled during PCA analysis.

3.4 RESULTS AND DISCUSSION

3.4.1 SEED LIGNIFICATION

In all four vineyards the percentage of lignification of the berry seeds increased after véraison and corresponds to that found by Ristic and Iland (2005) (Figure 3.2). Kennedy *et al* (2000) also reported a change in the seed colour after véraison from green pliable seeds to dark brown hardened seeds, which coincided with a decrease in flavan-3-ol monomer biosynthesis and the desiccation of the seeds. The higher the number of seeds corresponding to the dark brown colour classification method (Figure 3.1) of Ristic and Iland (2005), the higher the maturity level of the seeds and thus the berry it self. Ristic and Iland (2005) followed berry development and maturity by the corresponding Eichhorn and Lorenz growth stages (4mm pepper corn stage through harvest ripe growth stage), as mentioned in Coombe (1995). They reported seed weight and phenolic composition, as well as total skin phenolic and anthocyanins (mg/berry) at the different colour chart classifications (Figure 3.1).

Table 3.2 shows that grape seeds from the 2004 season had a higher percentage of seed lignification when compared to the 2003 and 2005 seasons, at commercial harvest. Balling values over the three years were comparable. It should also be noted that the

commercial harvest dates were almost three weeks later than the 2003 and 2005 seasons. Plaisir de Merle however did not follow the same trend with the seed lignification percentage higher in 2005. This could not be explained. The 2004 season followed a rapid increase in seed lignification early in the season after veraison, but then levelled off during the remainder of season (Figure 3.2). Ristic and Illand (2005) stated in their protocol that dark brown (Figure 3.1 values 10, 11 and 12) seeds corresponded with Brix values of between 23 and 24 degrees and that the colour value of 11 was chosen as reference point for the lignification percentage equation. Overall seed colour values of the four vineyards followed the same trend as the lignification percentage, but never reached 100% the colour classification value of 11 as stated above (data not shown). The longer growing season and slower accumulation of anthocyanin during the season could be a contributing factor to the similarity of the 2004 trend between the vineyards and commercial harvest. The variation of these values with the data from Ristic and Illand (2005) might be attributed to the difference in the varieties (Shiraz vs. Cabernet Sauvignon) used and the difference in irrigation regime (irrigated Shiraz vs. non-irrigated Cabernet Sauvignon). The berry may reach a maturity level similar in terms of seed colour (dark colour values 10 to 12) or TSS, but have low anthocyanin concentrations. Although Ristic and Illand (2005) concluded that seed and berry maturity could be evaluated, our results are not so conclusive and further research is needed to determine the value of using this technique.

The maximum anthocyanin concentration per berry (mg anthocyanin / berry) were reached, well before the overall seed colour reached a maximum during 2003 and 2005 as illustrated in figure 3.3. The 2004 season almost had a comparable maximum between anthocyanin concentration and overall seed colour.

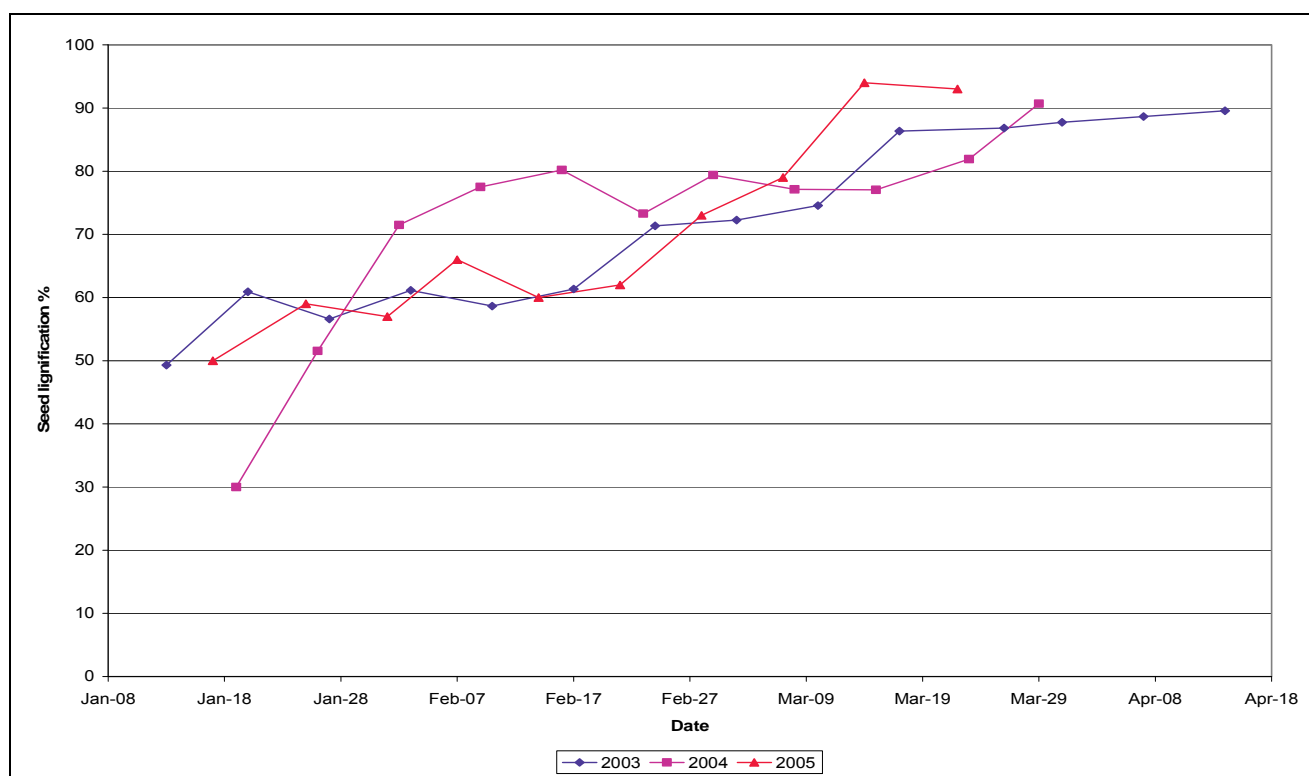


Figure 3.2 Seed lignification percentage (%) of Plaisir de Merle as calculated over the three harvest seasons using the method of Ristic and Illand (2005).

Table 3.2 Seed lignification percentage (%) values and Balling (°B) at commercial harvest.

Vineyard	2003			2004			2005		
	Harvest	°B	%	Harvest	°B	%	Harvest	°B	%
Anhöhe	14/02	26	63	03/03	26	85	22/02	26	67
Morgenster	02/04	25	71	07/04	25	90	17/03	25	71
Nietvoorbij	19/03	26	62	02/04	26	91	03/03	25	80
Plaisir de Merle	10/02	26	59	23/02	24	73	07/03	25	79

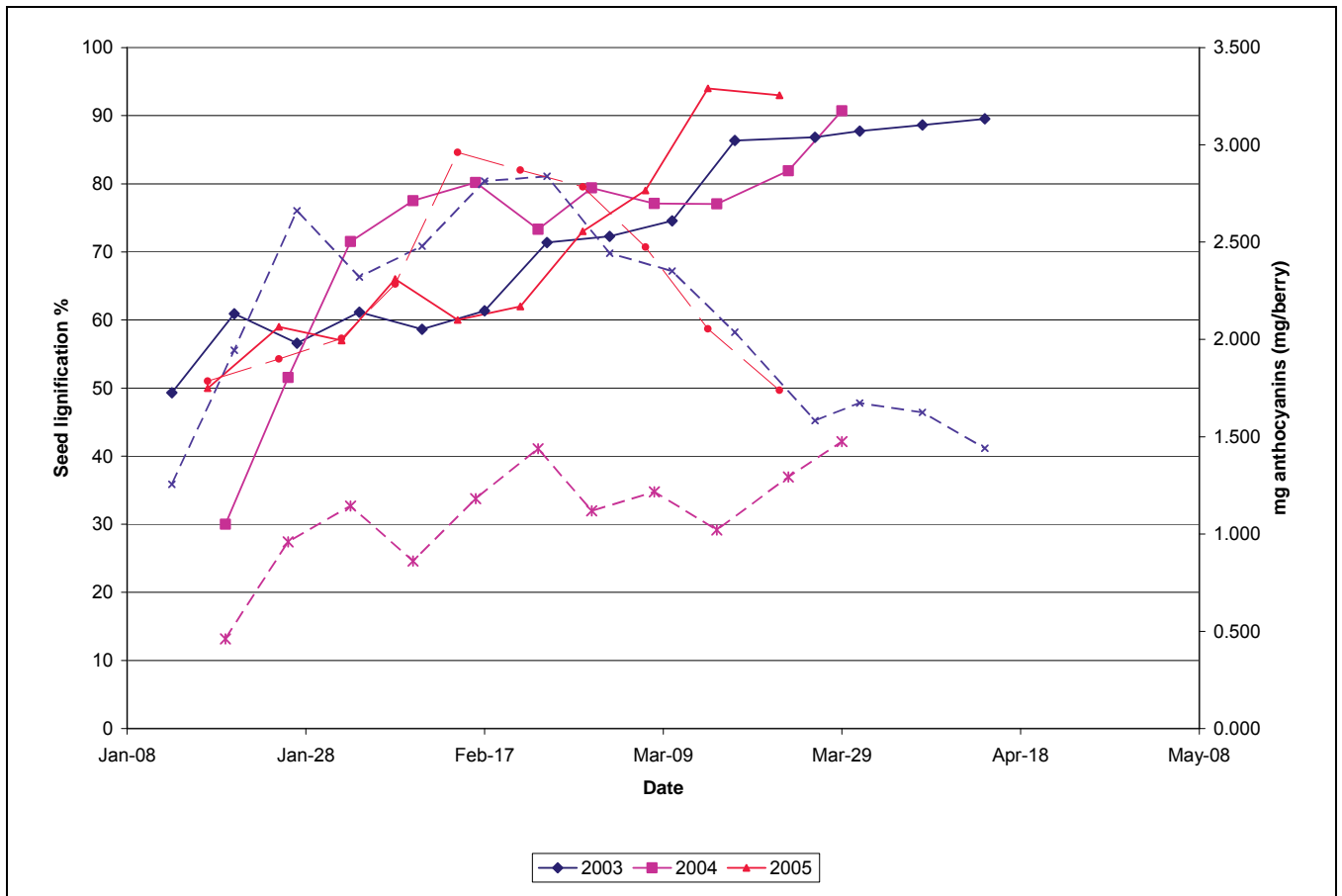


Figure 3.3 Comparison of the maximum anthocyanin (mg anthocyanin / berry) concentration (---) reached and the maximum overall seed colour (—) as derived from the colour chart for Plaisir de Merle over the three seasons.

3.4.2 GENERAL MATURITY PARAMETERS

During the three years the soluble solids (Balling) increased over the season in all four vineyards, following the same trend (Figure 3.4). There was no difference in the pattern of the vineyards when the white must and red must were compared over the three years. This agrees with the literature on the accumulation of soluble solids (Ribéreau-Gayon *et al.*, 2001; Boulton *et al.*, 1996).

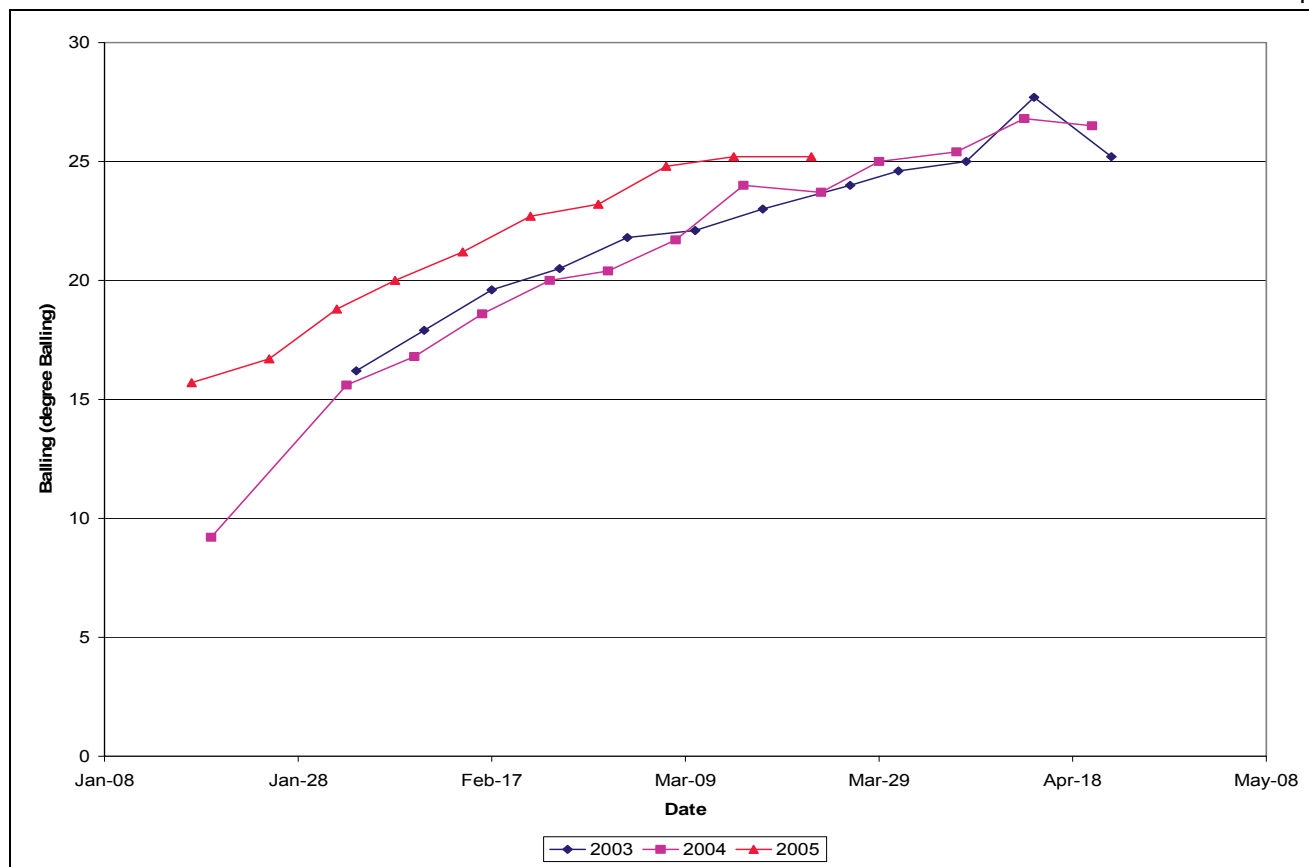


Figure 3.4 Accumulation of soluble solids (Balling) from véraison in the grapes harvested at Morgenster.

Titrateable acidity decreased after véraison and stabilised after 4 to 5 weeks in all vineyards following the same pattern. Red must have a lower titrateable acidity value than the white must for all vineyards over the three years. This can be contributed to the higher concentration of potassium that was released during the homogenation process from the skin cells. The extra potassium combines with the tartaric acid to form potassium bitartrate that does not influence the titrateable acidity as much as the free acid (Ribéreau-Gayon *et al.*, 2001).

Potassium (K^+) concentration was monitored over the harvest seasons in both the white and red must. We found in both musts an increase in potassium concentration over the seasons. The white must started at a level of between 1000 and 1500 mg/L K^+ and peaked at between 2000 and 2500 mg/L. Anhöhe reached levels during the end of the 2005 season of 3500 mg/L. Concentration of the red must were found to be higher at the beginning of the sampling period as well as throughout. Concentrations at the beginning of the sampling period averaged between 1500 and 2500 mg/L and peaked at between 2500 and 3000 mg/L. Plaisir de Merle had lower levels of potassium at the end of the 2003 and 2004 seasons of respectively 1840 and 2500 mg/L compared to the other four vineyards.

The pH increased over the harvest seasons in all vineyards (Table 3.3). The increase in pH can be contributed to the increase of potassium after véraison (Saayman, 1981). This is also supported, by the fact that the white must mostly had a lower pH value than the red must (Table 3.3). The pH values also markedly increase from the beginning to the end of harvest. The potassium values discussed previously indicate that excess potassium is liberated from the destroyed skin cells and contribute to salification of the organic acids in

red must. This happens to a lesser extent in the white must where the extraction increases due to the degradation of cell walls during berry maturation and not manual destruction.

Table 3.3 pH values of the white and red must at the beginning and end of the harvest seasons.

Harvest season	Anhöhe		Morgenster		Nietvoorbij		Plaisir de Merle	
	WMH ^{***}	RMH [□]	WMH ^{***}	RMH [□]	WMH ^{***}	RMH [□]	WMH ^{***}	RMH [□]
2003 Beginning*	2.82	3.20	2.86	3.41	2.78	3.01	2.84	2.95
2003 End**	4.18	4.03	3.41	3.83	3.82	4.16	3.78	3.86
2004 Beginning*	2.95	3.17	2.74	2.85	2.88	3.03	2.89	2.91
2004 End**	3.82	4.42	3.52	3.65	3.76	3.97	3.80	3.78
2005 Beginning*	3.11	3.14	2.86	3.03	2.98	2.98	3.01	3.10
2005 End**	4.01	4.41	3.84	3.90	3.53	3.80	3.88	3.80

* Beginning: The first sample taken from vineyard

** End: The last sample taken from vineyard

*** WMH: White must

□ RMH: Red must

3.4.3 ORGANIC ACIDS

3.4.3.1 Tartaric acid

According to Ribéreau-Gayon *et al.* (2001) tartaric acid reaches a maximum concentration before véraison with no significant accumulation afterwards. During the three years of observation the concentration of tartaric acid decreased after véraison (Figure 3.5). This was true for all vineyards (data not shown). The decrease in concentration can be due to the enlargement of the berries after véraison or the salification of the acid by potassium (Archer, 1981).

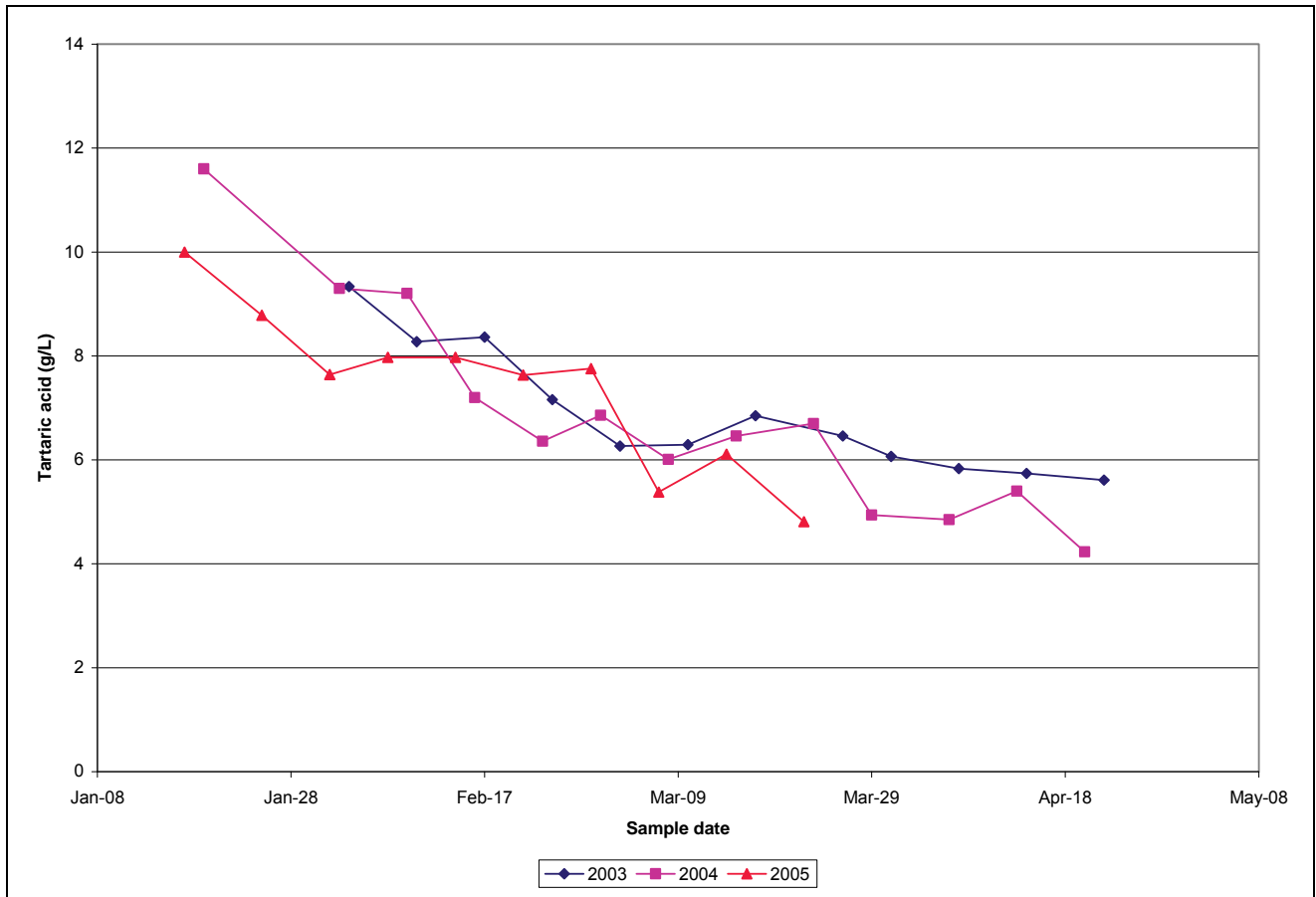


Figure 3.5 Tartaric acid (g/L) concentration of grapes from Morgenster during ripening over three years.

3.4.3.2 Malic Acid

Malic acid decreased after véraison in all four vineyards (data not shown). Malic acid decreased to below 1 g/L at Anhöhe and Plaisir de Merle, but at Morgenster and Nietvoorbij vineyards the acid concentration never decreased below 2 g/L. A higher respiration level at the warmer vineyards together with a less dense canopy were the most likely contributory factors to the lower malic acid values at these two vineyards (Archer, 1981).

3.4.4 MATURITY INDEX

Sugar, acid and pH are well known and extensively studied criteria for grape maturity and can be assessed with ease and accuracy. Combination of sugar, acid and pH (Balling/TA, Balling \times pH, Balling \times pH²) has been studied over the years (Coombe *et al.*, 1980; Du Plessis and Van Rooyen, 1982; Rooyen *et al.*, 1984). The best wines according to the sensory evaluation of the 2003, 2004 and 2005 harvest season are indicated in red in tables 3.4 to 3.7.

Sugar acid ratios range from 3.2 to 8.8. Morgenster was the only vineyard that had values comparable to that found by Du Plessis and Van Rooyen (1982). The lower maturity index values at Morgenster could be influenced by vigorous growth. The accumulation of soluble solids and decrease of acids, especially malic acid is influenced by vegetative growth and dense canopies (van Zyl, 1981). As discussed previously the malic acid concentration

never fell under 2 g/L and could contribute to a higher total acid level in the must. Balling values during the ripening period for Morgenster rarely exceeded 25°B (data not shown). The sugar acid ratio has been evaluated in the past for the determination of optimal grape maturity. Trials conducted in the Stellenbosch area by Du Plessis and Van Rooyen (1982) found sugar acid ratios between 2.4 and 2.6 for Chenin blanc and ± 4.0 in the case of Pinotage at its optimal maturity. From literature it is clear that differences between cultivars, as well as seasons do influence ratios. This was confirmed during this trial over the different seasons (Table 3.4 to 3.7).

During the evaluation of sugar \times pH index, Morgenster (Table 3.5) was found to be the only vineyard to consistently fall in the ranges of van Rooyen *et al.* (1984) during all three seasons. Both the best wines from Plaisir de Merle fell in the range of van Rooyen *et al.* (1984) in the 2004 season, while only one wine did so during the 2005 season (Table 3.7). During the 2004 season, Anhöhe reached a value of greater than 85 (16th February), well before the date of the best wine (8th March) at an index value of 97 (Table 3.4). Van Rooyen *et al.* (1984) reported that sugar \times pH was a better measurement for optimum ripeness in Cabernet Sauvignon and Pinotage from areas of Stellenbosch, Durbanville, Robertson and Elephants River, than sugar by itself or the sugar : acid ratio. He advocated values of between 85 and 95 for the two above mentioned cultivars. With recent trends towards picking grapes at higher sugar levels, these values derived from sugar \times pH were thought too to be too low. It was found not to be the case in all years or vineyards; however vineyards in warmer areas were more likely to have higher values (Table 3.4 to 3.7).

Coombe *et al.* (1980) reported that sugar \times pH² was an even better indicator of optimal ripeness, with the best wines ranging in values between 200 and 270. In comparison to this study, the values of the best wines were in all cases well above the maximum value (270) as can be seen in Tables 3.4 to 3.7.

Table 3.4 Maturity indexes as derived from white must analyses of Anhöhe over three harvest seasons.

Anhöhe 2003				Anhöhe 2004				Anhöhe 2005			
Date	Balling/TA	Balling × pH	Balling × pH ²	Date	Balling/TA	Balling × pH	Balling × pH ²	Date	Balling/TA	Balling × pH	Balling × pH ²
2001	1.3	49	138	1901	0.7	41	122	1701	1.4	58	180
2701	2.7	67	209	2601	1.0	54	170	2401	1.8	63	205
0302	3.7	78	256	0202	3.2	67	220	3101	2.5	66	221
1002	4.8	87	294	0902	4.8	83	297	0702	3.7	85	310
1702	5.4	88	294	1602	5.5	88	328	1402	4.2	85	310
2402	5.6	94	343	2302	6.6	88	322	2102	5.0	101	389
0303	6.7	101	386	0103	7.4	99	379	2802	5.6	103	396
1003	7.6	116	486	0803	7.1	97	370	0703	6.5	115	455
				1503	8.8	109	430	1403	5.4	119	476
				2303	8.1	113	461				
				2903	7.7	104	395				

Table 3.5 Maturity indexes as derived from white must analyses Morgenster over three harvest seasons.

Morgenster 2003				Morgenster 2004				Morgenster 2005			
Date	Balling/TA	Balling × pH	Balling × pH ²	Date	Balling/TA	Balling × pH	Balling × pH ²	Date	Balling/TA	Balling × pH	Balling × pH ²
0302	0.8	46	133	1901	0.3	25	69	1701	0.7	45	128
1002	1.2	54	160	0202	0.8	47	139	2401	1.3	52	162
1702	1.7	58	173	0902	1.0	51	155	0202	1.7	61	200
2402	1.9	66	210	1602	1.7	60	193	0702	1.9	66	215
0303	2.6	71	233	2302	2.2	65	209	1402	2.3	73	249
1003	3.9	82	307	0103	2.3	66	214	2102	3.1	80	283
1703	3.2	77	258	0803	2.7	73	244	2802	3.2	83	296
2603	3.3	82	282	1503	3.5	84	297	0703	3.9	92	338
3103	4.0	84	284	2303	3.4	84	297	1403	4.0	95	360
0704	3.9	83	272	2903	4.6	91	328	2203	4.3	97	372
1404	4.9	94	322	0604	3.7	92	337				
2204	4.0	81	258	1304	4.5	94	326				
				2004	5.4	93	328				

Table 3.6 Maturity indexes as derived from white must analyses of Nietvoorbij over three harvest seasons.

Nietvoorbij 2003				Nietvoorbij 2004				Nietvoorbij 2005			
Date	Balling/TA	Balling × pH	Balling × pH ²	Date	Balling/TA	Balling × pH	Balling × pH ²	Date	Balling/TA	Balling × pH	Balling × pH ²
2701	0.7	44	121	1901	0.4	33	96	1701	0.8	42	124
0302	1.3	50	140	2601	0.6	36	106	2401	1.1	51	154
1002	1.6	58	173	0202	0.9	45	132	0202	1.8	59	192
1702	2.6	66	197	0902	2.2	61	192	0702	2.4	68	224
2402	3.1	73	237	1602	1.8	59	192	1402	3.1	71	239
0303	4.0	83	282	2302	3.0	69	226	2102	3.4	79	286
1003	6.0	103	396	0103	4.0	74	249	2802	4.0	83	289
1703	5.9	103	406	0803	5.4	90	339	0303	5.0	86	304
2603	4.8	95	340	1503	5.7	94	344				
3103	5.3	91	331	2303	5.6	96	355				
0704	5.9	99	366	2903	4.3	88	317				
1404	6.1	100	354	0604	5.0	101	391				
2204	6.2	115	439	1304	6.4	98	371				
				1904	6.5	103	386				

Table 3.7 Maturity indexes as derived from white must analyses of Plaisir de Merle over three harvest seasons.

Plaisir de Merle 2003				Plaisir de Merle 2004				Plaisir de Merle 2005			
Date	Balling/TA	Balling × pH	Balling × pH ²	Date	Balling/TA	Balling × pH	Balling × pH ²	Date	Balling/TA	Balling × pH	Balling × pH ²
2001	1.4	51	144	1901	0.7	41	119	1701	1.5	59	177
2701	2.6	59	171	2601	1.0	46	135	2401	2.4	67	214
0302	3.0	73	234	0202	2.1	63	198	3101	2.4	66	217
1002	4.5	84	279	0902	3.3	71	228	0702	2.9	77	255
1702	4.7	86	278	1602	3.9	71	232	1402	3.6	77	253
2402	5.9	102	359	2302	5.1	79	261	2102	4.2	88	323
0303	6.6	106	383	0103	5.1	80	259	2802	4.9	87	303
1003	7.1	118	465	0803	5.4	88	306	0703	5.1	92	339
1703	6.6	108	399	1503	6.2	94	336	1403	5.4	101	384
2603	6.4	111	418	2303	6.0	97	349	2203	6.5	107	416
3103	7.3	122	467	2903	7.6	106	404				
0704	7.5	135	525								
1404	7.4	118	444								

3.4.5 GRAPE COLOUR

The vineyard of Anhöhe illustrates the trend of anthocyanin (antho) accumulation over the three seasons as observed in terms of berry weight (mg anthocyanin / g berry) and the whole berry (mg anthocyanin / berry)(Figure 3.6 and 3.7). These accumulation trends agree with that found by Ginestar *et al.* (1998) and Kennedy *et al.* (2002). In figure 3.7 the abnormal high peak at 27 January 2003 and the lower anthocyanin values that follow could not be explained by any means except that a sampling error was made.

All four vineyards followed comparable grape colour (mg anthocyanin / g berry and mg anthocyanin / berry) trends. Only Anhöhe had a significantly higher grape colour during 2003 compared to the other seasons investigated, while the other vineyards had comparable 2003 and 2005 values (data not shown). The 2004 season had the lowest grape colour at all four vineyards and followed a very slow accumulation over the season. Anhöhe was the only vineyard to exceed 1.5 mg anthocyanin / g berry during 2004 (Table 3.8).

Comparison of the maximum anthocyanin concentration (mg anthocyanin / g berry) attained during the three seasons, revealed that Anhöhe consistently produced the most anthocyanin per gram berry weight (Table 3.8). However, when the anthocyanin content of the berry (mg anthocyanin / berry) as a whole is taken into consideration, then Morgenster reached the highest concentration of anthocyanin (Table 3.9). This can be attributed to the average berry size differences between Anhöhe and Morgenster (Table 3.10). The ratio between skin and pulp is higher in smaller berries as mentioned by Kennedy, 2002, and gives wines with higher proportion skin and seed compounds. Ojeba *et al.*, (2002) illustrated that water deficits reduce berry size and consequently increase the concentration of phenolic components by increasing the skin-to-pulp ratio.

During the 2003 season leave water potential readings were taken in the vineyards before harvest as an indication of the level of water stress (data not shown). Anhöhe and Plaisir de Merle had leaf water potentials of -1.9 MPa and -1,5 MPa compared to that of Morgenster and Nietvoorbij of -0.95 MPa and -1.1 MPa respectively. This corresponds with findings of Roby *et al.* (2004) and Ginestar *et al.* (1998), where anthocyanin concentrations were higher in vines that experienced a water deficit. Roby *et al.* (2004) reported that midday leave water potential of -1.2 MPa was not sufficient to inhibit berry growth, but under -1.5 MPa water stress did inhibit berry growth by about 15% compared to vines at -1.0 MPa. The leave water potential of Plaisir de Merle was close to this reported value and could explain why the berry weight did not differ as much as those of Anhöhe from Morgenster and Nietvoorbij. Ribéreau-Gayon *et al.* (2001a) and Van Zyl (1981) reported that water stress before véraison can seriously affect normal grape development, even to the point that after véraison, a substantial water supply could not reverse it. Ginestar *et al.* (1998) reported that treatments where water deficits were more severe later in the season had higher concentrations of anthocyanin at harvest. Taiz and Zeiger (2002) also report, that the rate of photosynthesis is inhibited by dehydration of

mesophyll metabolism, which under severe water stress, affects the translocation of assimilates. This can account for differences in berry size between Morgenster and Anhöhe and effect the accumulation of anthocyanins and might explain the similar trend as illustrated by table 3.8 for Anhöhe and Plaisir de Merle. The winter of the 2003 season was uncharacteristically dry, followed by a cool spring that delayed budding by 10 to 14 days (Du Plessis and Boom, 2005). Uneven berry set and grape ripening of bunches was reported (Du Plessis and Boom, 2005), and could explain lower colour in the 2004 season that followed as seen in figure 3.5 and 3.7.

Archer (1981) reported that the optimal temperature for photosynthesis was between 25°C and 28°C if all other environmental factors remained the same. Buttrose *et al.* (1971) reported temperature values for maximum colour accumulation of between 17.5°C and 23.5°C for Cabernet Sauvignon, while Kliewer and Torres (1972) reported that Cabernet Sauvignon were the most tolerant of high temperatures and anthocyanin production increased up until temperatures of 35°C. Jackson and Lombard (1993) reported that night time temperatures of above 15°C and mean temperatures above 20°C during ripening stage of berry development, contribute to low anthocyanin levels. Our temperature values, as determined by the nearest weather station, were higher than 15°C during most nights of ripening and the mean temperatures well above 20°C in all years (data not shown). Day temperatures also fell outside the optimal range of Archer (1981) and Buttrose *et al.* (1971) over extended periods in the vineyards. Pre-season water deficits could in conjunction with temperature influence photosynthesis negatively and could explain the lower accumulation of anthocyanin during the 2004 season.

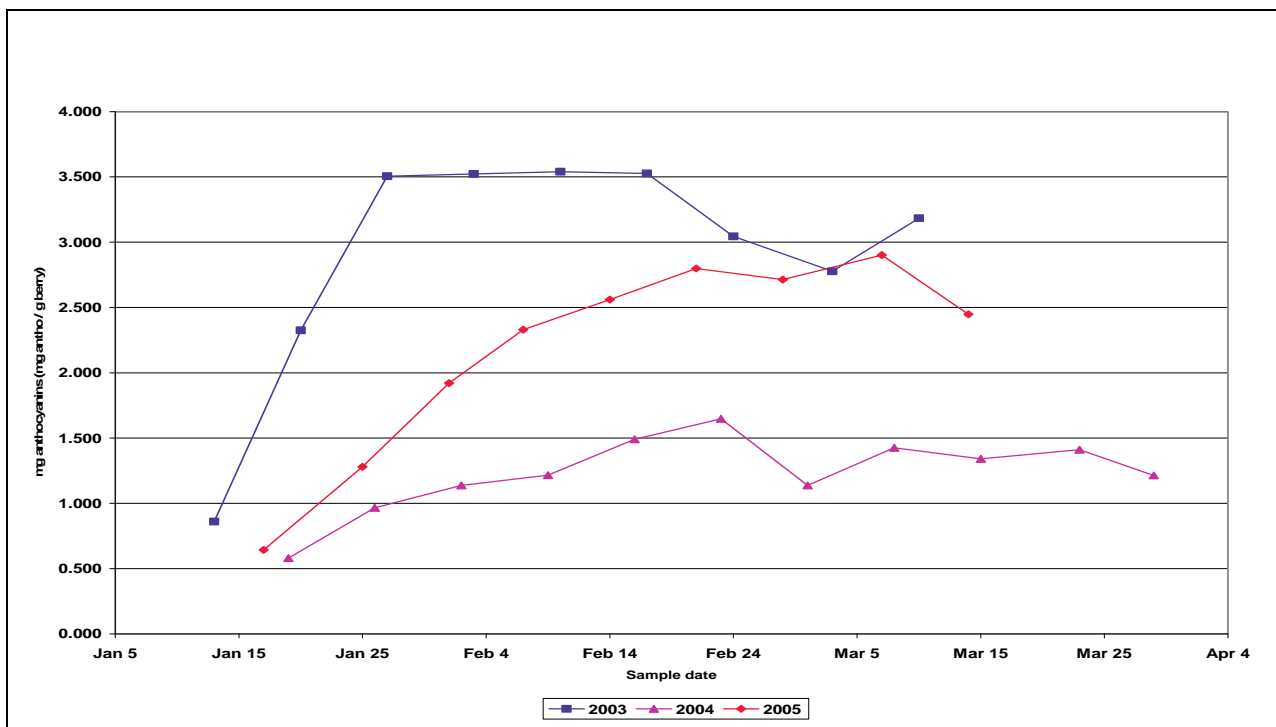


Figure 3.6 Grape colour measured as anthocyanin concentration (mg anthocyanin / g berry) per berry weight for Anhöhe over a period of 3 years.

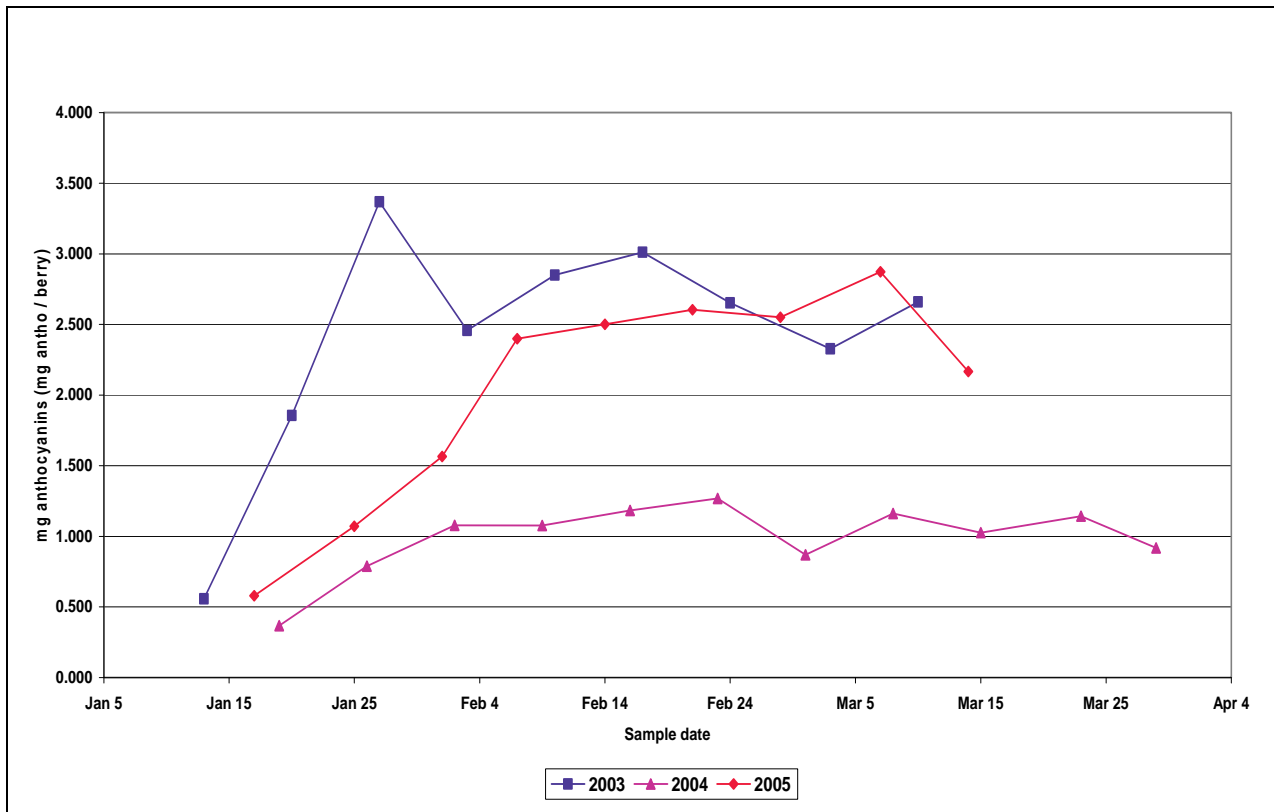


Figure 3.7 Grape colour measured as anthocyanin concentration (mg anthocyanin / berry) per berry for Anhöhe over a period of 3 years.

Table 3.8 Maximum anthocyanin concentration (mg anthocyanin / g berry) obtained in the four vineyards.

Vineyard	2003	2004	2005
Anhöhe	3.54	1.65	2.90
Morgenster	2.69	1.33	2.31
Nietvoorbij	2.35	1.49	2.75
Plaisir de Merle	2.84	1.48	2.96

Table 3.9 Maximum anthocyanin concentration (mg anthocyanin / berry) obtained in the four vineyards.

Vineyard	2003	2004	2005
Anhöhe	3.37	1.27	2.87
Morgenster	4.47	2.41	4.02
Nietvoorbij	3.27	2.29	3.75
Plaisir de Merle	3.61	2.22	4.12

Table 3.10 Maximum berry weight (g) of the vineyards during the three seasons.

Vineyard	2003	2004	2005
Anhöhe	0.96	0.95	1.03
Morgenster	1.70	1.82	1.80
Nietvoorbij	1.58	1.72	1.60
Plaisir de Merle	1.43	1.67	1.59

The possibility of a significant correlation between the anthocyanin measurements (mg anthocyanin / g berry and mg anthocyanin / berry) and the berry weight (g) and volume (mL) were investigated. Firstly all the vineyards, irrespective of year or region were investigated. The grape colour, expressed as mg anthocyanin / berry did not have strong correlations with either berry weight ($r = 0.29$, $p < 0.001$) or berry volume ($r = 0.26$, $p < 0.001$). This was also true for mg anthocyanin / g berry where the berry weight and volume gave a negative trend ($r = -0.17$, $p = 0.0167$; $r = -0.19$, $p = 0.0056$). Possible correlations within each vineyard, irrespective of year were also investigated (data not shown). Anhöhe was the only vineyard that had a strong correlation between mg anthocyanin / berry and berry weight ($r = 0.65$, $p = 0.000003$) or berry volume ($r = 0.52$, $p = 0.0005$). There were no correlations with mg anthocyanin / g berry for any of the other vineyards (data not shown).

From a quality aspect high alcohol levels in the final wine is undesirable, while maximum colour is. Thus a balance between maximum grape colour and sugar level need to be found. Table 3.11 show the sugar level (°B) at the maximum grape colour (mg anthocyanin / berry) peak. Plaisir de Merle reached a grape colour (mg anthocyanin / berry) maximum at 28.4°B and 27.2°B during 2003 and 2004 respectively. Shrivelled berries were observed at this time in bunches at Plaisir de Merle and could be the most likely cause for the high Balling values. Table 3.9 illustrates that sugar levels (°B) used in isolation does not give a reliable indication of maturity.

Table 3.11 Balling (°B) values as measured in the red must at the maximum grape colour (mg anthocyanin / berry) for the three years.

Vineyard	2003	2004	2005
Anhöhe	25.7	24.2	26.8
Morgenster	23.1	24.1	23.9
Nietvoorbij	21.7	26.8	25.6
Plaisir de Merle	28.4	27.2	24.4

3.4.6 WINE COLOUR DENSITY

Wine colour density (A420 + A520) followed a comparable trend with that of grape colour (mg anthocyanin / g berry) (Figure 3.6 and 3.8). In three of the four vineyards, 2004 had the lowest grape colour (mg anthocyanin / g berry) and wine colour density (A420+A520).

Nietvoorbij was the only vineyard where the grapes from the 2003 and 2004 seasons produced comparable wine colour. This can be attributed to the leaf-roll virus infection in the vineyard (Figure 3.9).

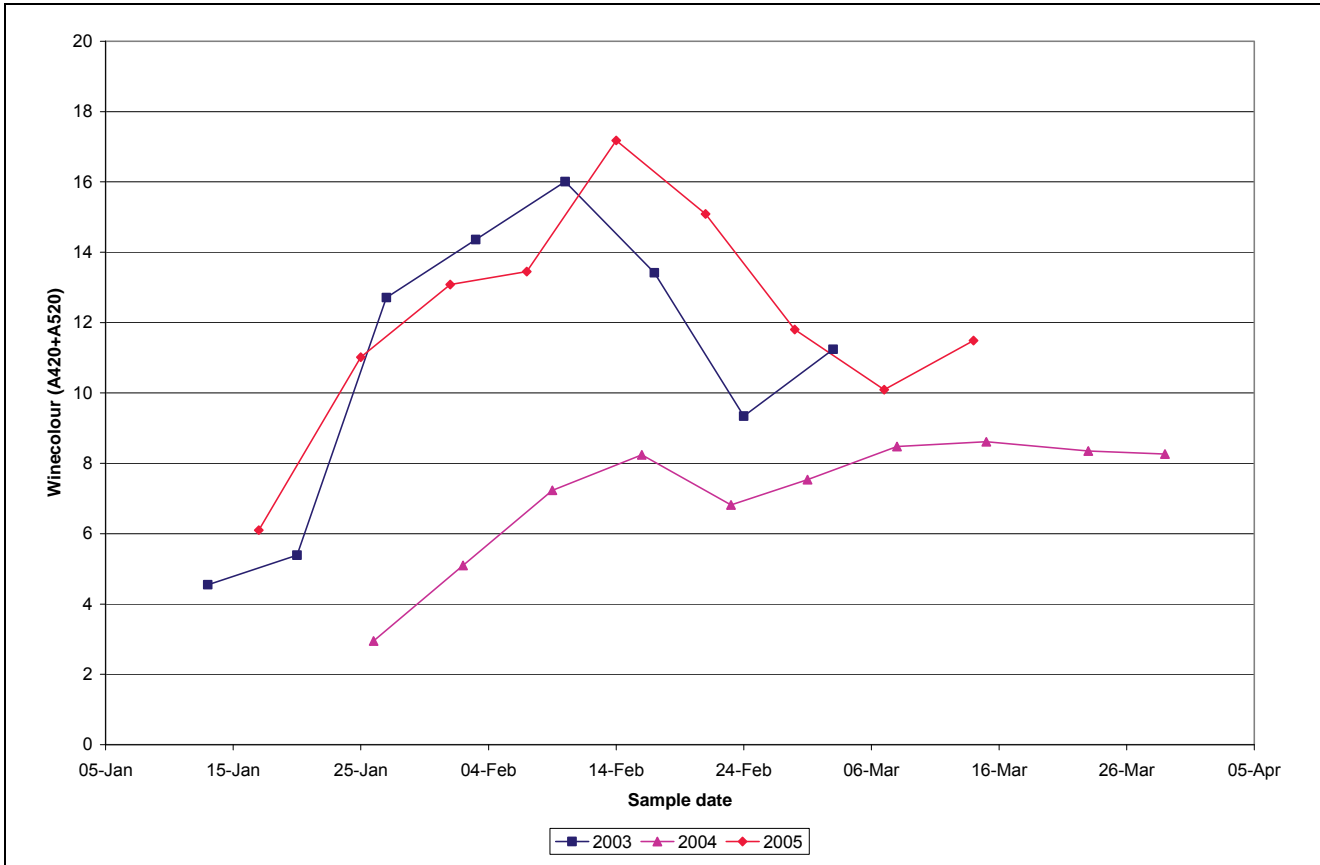


Figure 3.8 Wine colour density (A420+A520) from Anhöhe over the three seasons.

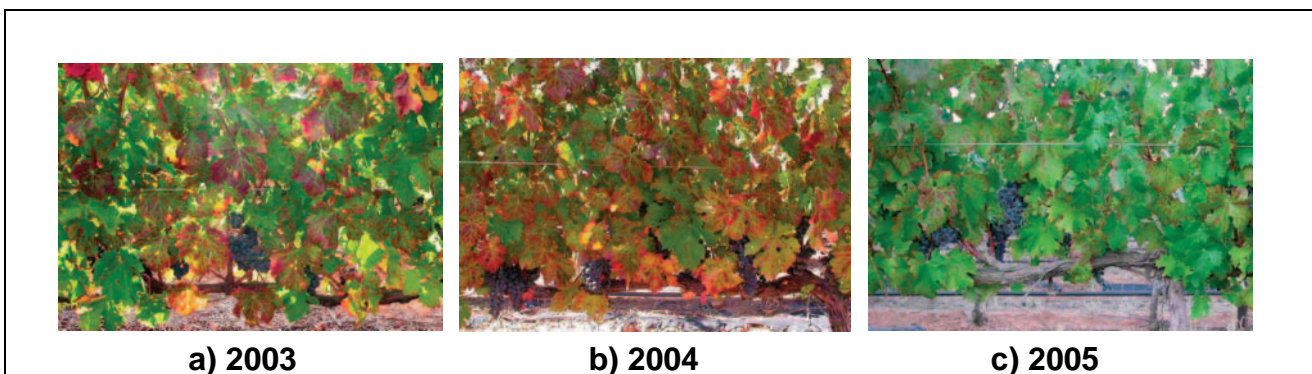


Figure 3.9 Leaf-roll virus infection expressions in Nietvoorbij vineyard during 2003, 2004 and 2005.

In 2005, the wine colour density for Nietvoorbij was higher than previous years (Figure 3.10). This can be attributed to an improved vineyard management strategy that was implemented during the season (personal communication). Unfortunately no post

commercial harvest trends could be followed in the Nietvoorbij experimental block as it was accidentally harvested in 2005 on the 3rd of March.

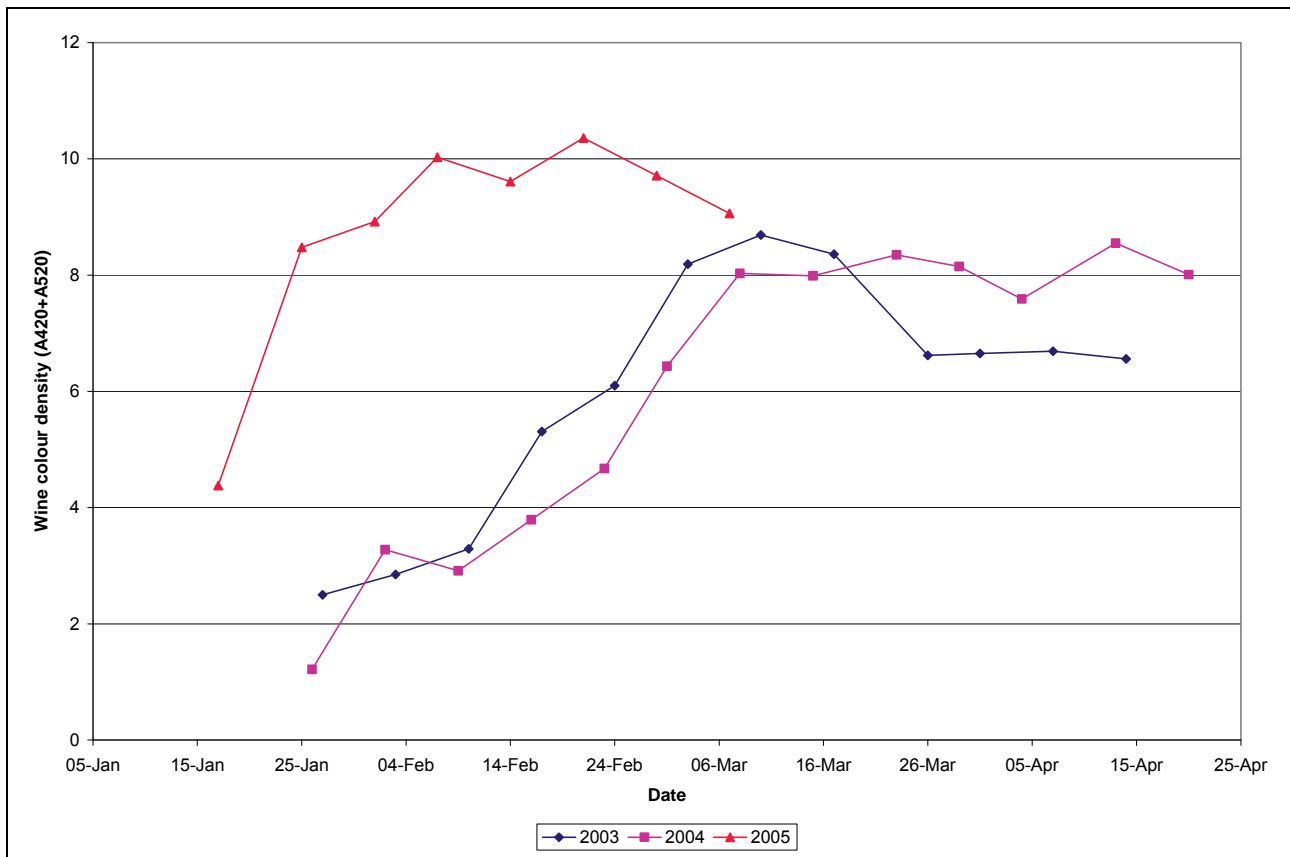


Figure 3.10 Wine colour density of Nietvoorbij during the three seasons.

Wine colour density of the grapes from Morgenster decreased from the middle of the season during 2003 (Figure 3.11). Dense canopies in the Morgenster vineyard compared to Anhöhe and Plaisir de Merle, during the 2003 season, resulted in entirely shaded grape bunches. This could be an important contributing factor to the lower wine colour obtained in Morgenster compared to Anhöhe and Plaisir de Merle during the 2003 season (Figure 3.12). DeGaris (2003), comments on the importance of light exposure in cooler areas for better photosynthesis. The shading of bunches was reported by Ginestar *et al.* (1998) and Morrison and Noble (1990) to have a greater influence on grape colour than only shading of the foliage as reported in 1989 by Rojas-Lara and Morrison, but the shading of bunches could have contributed to the higher humidity at Morgenster when compared to the other vineyards. High humidity within the vineyard due to the dense canopy also contributed to the downy mildew infection of bunches and leaves that was observed during the 2003 season. A decrease in effective leave area due to downy mildew infection would lead to a decrease in the formation of grape colour components and this coupled to the shading effect could explain the low colour in the wines observed at Morgenster in 2003.

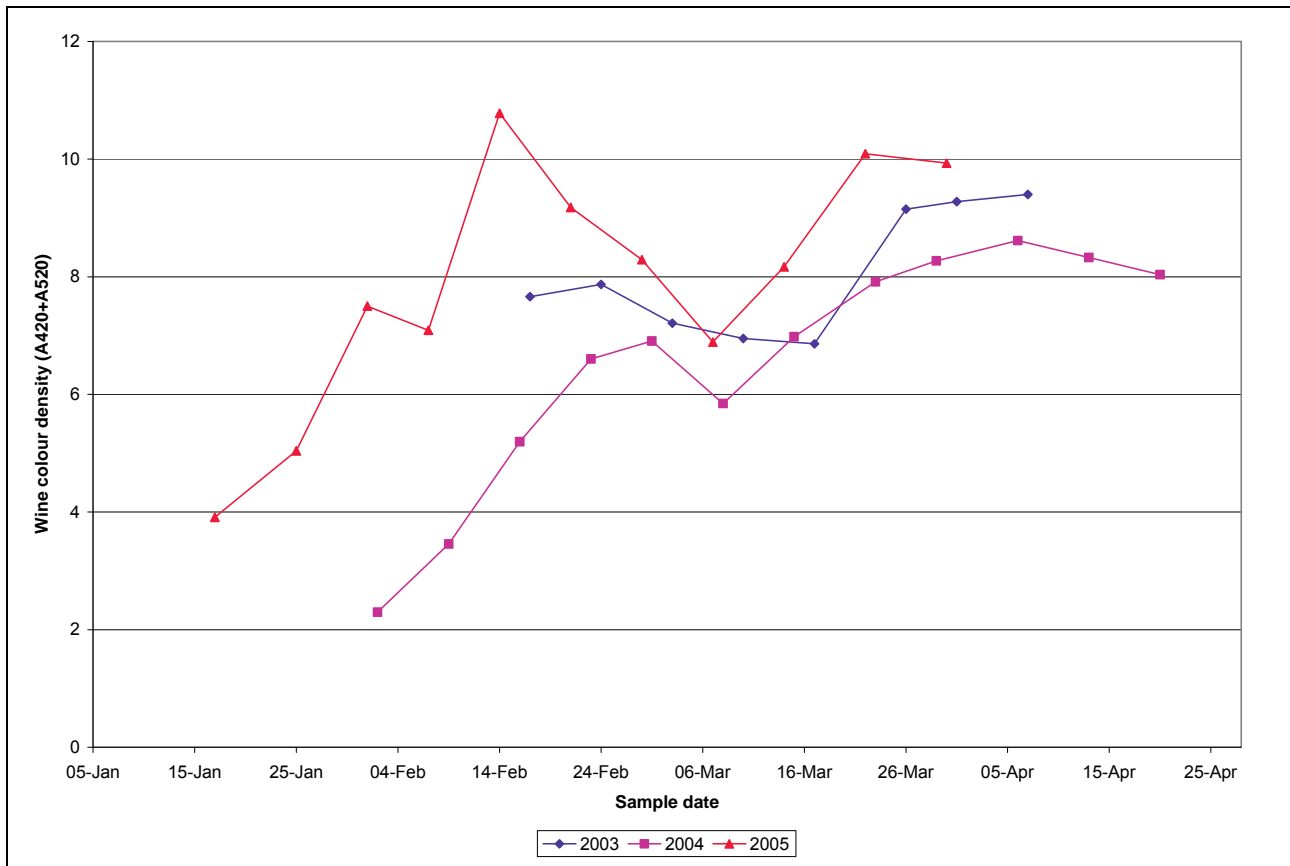


Figure 3.11 Wine colour density of Morgenster during the three growing seasons.

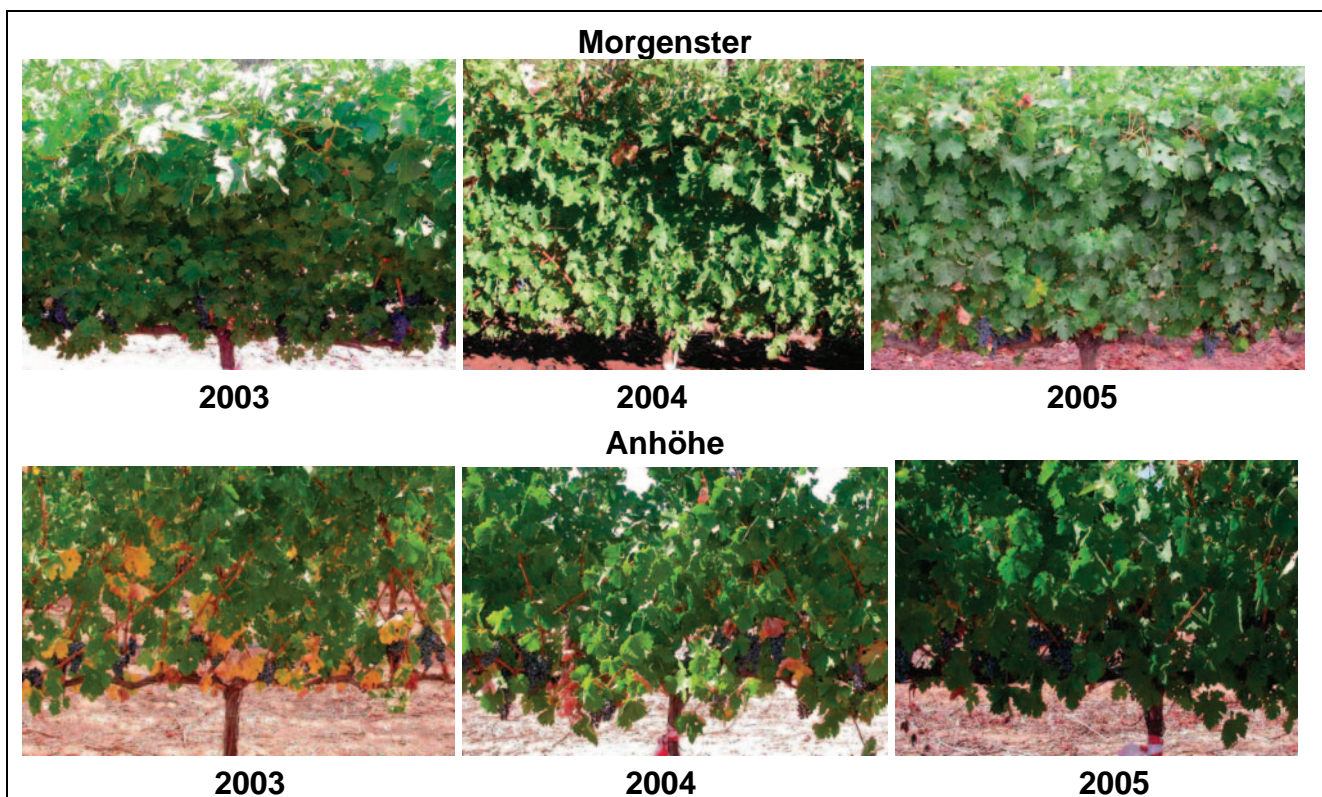


Figure 3.12 Comparison of dense canopies at Morgenster during 2003, 2004 and 2005 seasons, showing the shaded bunches, with the less dense canopies at Anhöhe over the same period.

3.4.7 GRAPE AND WINE SENSORY DATA

Small scale wines were evaluated by an expert panel of tasters. The statistically best wines were used to determine the dates at which the grape composition were optimal. It was important to determine whether the changes in the grape composition during ripening translated into corresponding changes in the wine. Correlations between the rated wine attributes (colour intensity, acidity, astringency, vegetative, red berry, black berry and spicy aroma, general wine intensity, fullness and overall quality) and that of the maturity indicators were investigated.

Significant trends between degree Balling ($^{\circ}$ B) and the following wine attributes were found: vegetative aroma ($r = -0.32$, $p < 0.001$); berry aroma ($r = 0.42$, $p < 0.0001$), spicy aroma ($r = 0.50$, $p = 0.0001$), general aroma intensity ($r = 0.61$, $p < 0.0001$) and general wine quality ($r = 0.56$, $p = 0.0$).

Correlations between maturity index values and general wine quality were also investigated for white must (Table 3.10). Morgenster showed a better correlation between the maturity index values and general wine quality (Table 3.10). Variations between the vineyards are illustrated by table 3.10. The variation accounts for the average correlation found when all the vineyard data are pooled for each season.

Table 3.10 Correlation between maturity index values and general wine quality of the four vineyards separately and together for white and red must.

Maturity index	Anhöhe		Morgenster		Nietvoorbij		Plaisir de Merle		Together	
	r	p	r	P	r	p	r	P	r	P
Balling($^{\circ}$B)/TA(g/L) WMH^a	0.533	0.007	0.702	0.000	0.598	0.000	0.575	0.001	0.558	0.000
Balling($^{\circ}$B)\timespH WMH^a	0.685	0.000	0.746	0.000	0.575	0.001	0.464	0.013	0.609	0.000
Balling($^{\circ}$B)\timespH² WMH^a	0.698	0.000	0.748	0.000	0.595	0.000	0.451	0.016	0.615	0.000

a - white must

Anthocyanin concentration of grape berries showed trends with the general wine quality (mg anthocyanin / berry; $r = 0.348$, $p = 0.0001$ and mg anthocyanin / g berry; $r = 0.3626$, $p < 0.0001$). General wine quality was compared with both the grape skins tasting (G) and the wine tasting (W) data. The four vineyards were evaluated together irrespective of the harvest season as well as separately (Table 3.11 and 3.12).

Table 3.11 Correlations between grape skins tasting characteristics and general wine quality of the four vineyards individually and together over all seasons

	Anhöhe		Morgenster		Nietvoorbij		Plaisir de Merle		Together	
	r	p	R	p	r	p	r	P	r	p
Colour Intensity	0.641	0.001	0.704	0.000	0.755	0.000	0.761	0.000	0.694	0.000
Vegetative aroma	-0.257	0.225	-0.076	0.675	-0.258	0.148	-0.051	0.798	-0.159	0.085
Red berry aroma	-0.066	0.761	0.017	0.927	0.007	0.971	0.266	0.172	-0.010	0.911
Black berry aroma	0.624	0.011	0.605	0.000	0.116	0.522	0.582	0.001	0.383	0.000
Black berry with spice	0.559	0.005	0.473	0.006	0.694	0.000	0.645	0.000	0.549	0.000
Tannin concentration	-0.221	0.299	0.047	0.796	0.080	0.657	0.213	0.278	0.000	0.994

Table 3.12 Correlations between wine tasting characteristics and general wine quality of the four vineyards individually and together over all seasons

	Anhöhe		Morgenster		Nietvoorbij		Plaisir de Merle		Together	
	r	p	r	p	r	p	r	P	r	p
Colour Intensity	0.858	0.000	0.940	0.000	0.792	0.000	0.809	0.000	0.841	0.000
General aroma intensity	0.739	0.001	0.850	0.000	0.818	0.000	0.827	0.000	0.818	0.000
Vegetative aroma	-0.009	0.968	0.079	0.661	0.550	0.001	0.551	0.002	0.344	0.000
Berry aroma	0.799	0.000	0.834	0.000	0.779	0.000	0.823	0.000	0.811	0.000
Spicy aroma	0.557	0.005	0.796	0.000	0.848	0.000	0.792	0.000	0.768	0.000
Acidity	-0.886	0.000	-0.783	0.000	-0.840	0.000	-0.825	0.000	-0.819	0.000
Fullness	0.911	0.000	0.962	0.000	0.929	0.000	0.882	0.000	0.924	0.000
Astringency	0.258	0.224	0.664	0.000	0.813	0.000	0.513	0.005	0.620	0.000

Colour intensity of grape skins tasting ($r = 0.69$; $p < 0.001$) correlated with general wine quality (Table 3.11). The black berry aroma of Anhöhe and Morgenster correlated better with general wine quality than the black berry aroma with spice (Table 3.11). Nietvoorbij and Plaisir de Merle correlated better with the black berry with spice aroma than with only the black berry aroma (Table 3.11). Vegetative and red berry aroma as well as tannin concentration did not show any significant correlations with general wine quality (Table 3.11). Variability between the vineyards influenced the significance of the criteria together.

The colour intensity of the wine ($r = 0.84$; $p < 0.001$) showed a better correlation than the colour intensity of the grape skins tasting ($r = 0.69$; $p < 0.001$) to general wine quality (W) (Table 3.11 and 3.12). Vegetative aroma did not show significant correlations in any vineyard for the wines (Table 3.12). General aroma intensity, berry, spicy aroma and fullness correlated with general wine quality (Table 3.12). Astringency for Anhöhe was not significant, but no explanation could be found (Table 3.12). Similarity between the colour intensity, berry and spicy aroma of the grape skin tasting and wine does give an indication of potential quality when used, but the labour and time intensive protocol of the grape skin tasting does not make it viable as a sole indicator of grape maturity or in the operation of large cellars. Wine colour differences between the samples were not concealed during tasting. This could have influenced the perception of quality of the panel. Pangborn *et al.* (1963) found that experienced wine tasters were more influenced by colour than

inexperienced wine drinkers. But not all researchers found consistent results (Lawless and Heymann, 1998). Parr *et al.* (2003) found that although the expert tasters' judgements were more accurate when the colour of the wine was masked, colour differences only induced olfactory bias to a limited degree.

Wines were divided into classes according to the significant differences in the general wine quality scores as rated by the tasting panel and plotted on radar charts (Figure 3.13). The classes were: class 1, unripe; class 2, ripe (no significant difference from best wine); class 3, overripe and class 4, did not correspond with any of the preceding classes. In figure 3.13 it is clear that the panellist rated the acidity of unripe (green) wines similar in 2004 and 2005. The vegetative aroma and acidity decreased in all seasons and the perceived colour intensity and berry and spicy aromas and fullness increased with ripening. Palomo *et al.* (2007) is in agreement that vegetative aromas decrease with an increase in maturity. Astringency ratings increased with ripening of the grapes which were possibly the result of higher acidity of wines made from unripe grapes which masked the perception of it from the panel earlier. However it should be note that the attributes were increased between the 2003 and 2004 season.

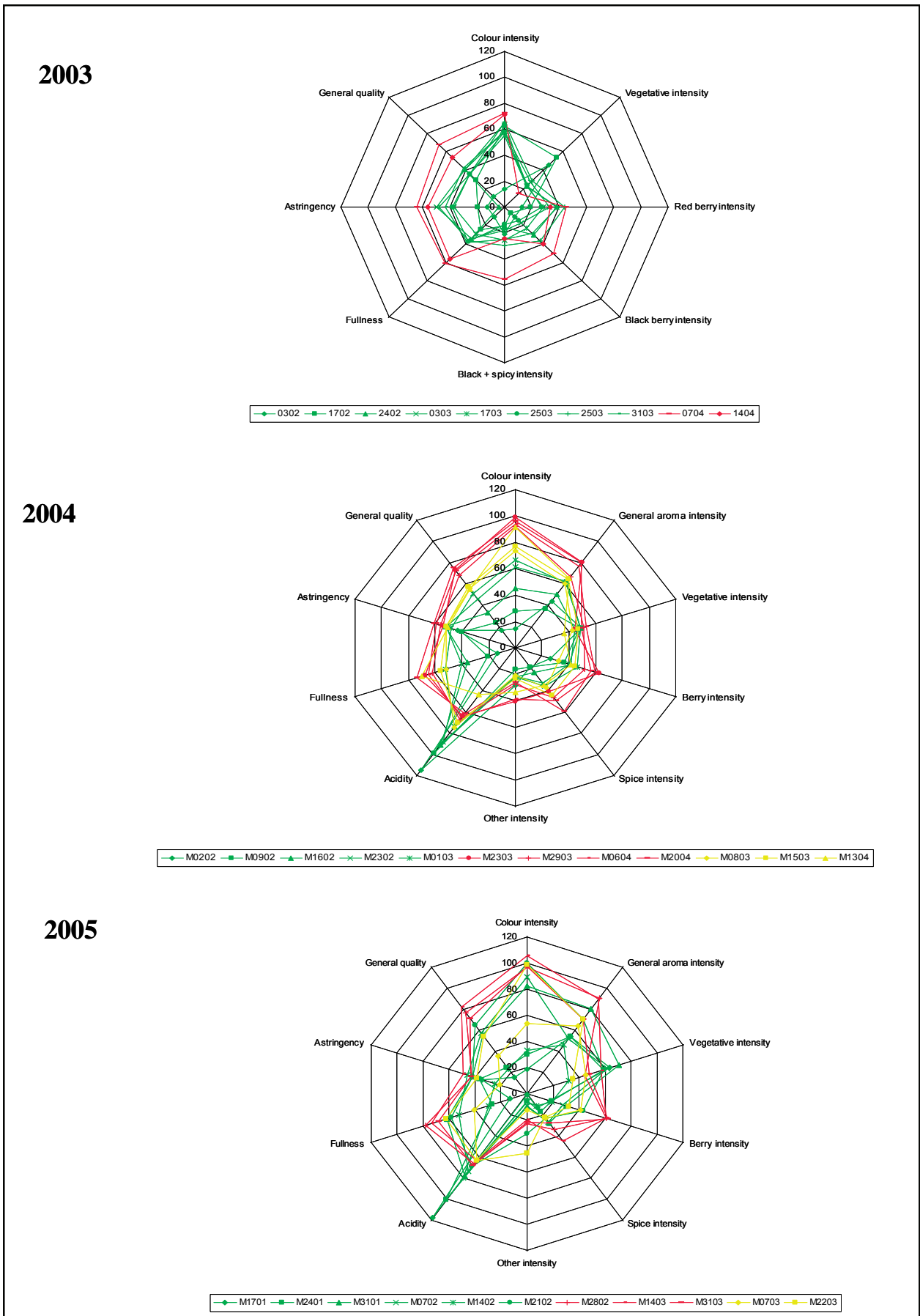


Figure 3.13 Radar chart of tasting results from Morgenster divided into ripening classes: 1(unripe - green), 2(ripe - red), 3(overripe - yellow) and 4(odd - blue).

The coloured lines in figure 3.14 indicate the wines that did not differ significantly from the best wine during the specific season, as described in terms of general quality. Same results were found in the other three vineyards as well (data not shown). Thus wines made during the highest grape colour peak did not differ significantly from the wine of the previous maturity date. However this does not mean that other sensory criteria did not change significantly.

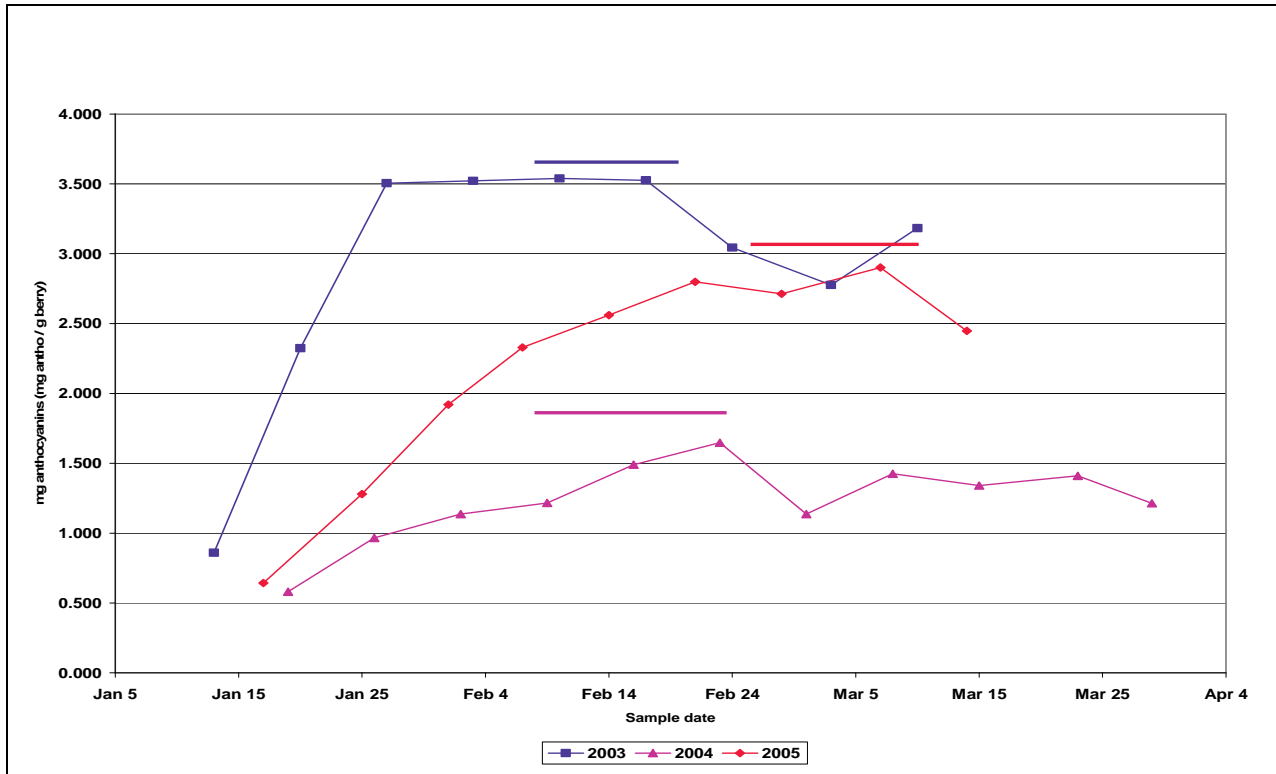


Figure 3.14 Grape colour measured as anthocyanin concentration (mg anthocyanin / g berry) per berry weight for Anhöhe over a period of 3 years and the preferred wines are illustrated by the 3 coloured bars for each season.

3.4.8 OPTIMAL RIPENESS MODEL

In order to establish what grape parameters are important to distinguished unripe from ripe grapes, a PCA of the grape data was done to investigate ripeness classes and seasonal variations (Figure 3.15 and 3.16). All investigated parameters were initially used in the model, but was taken out one by one to determine the least number of parameters to distinguish between unripe and ripe grapes. The wines classified as unripe separated from the ripe and overripe wines, PC1 (Figure 3.15). Limited separation was found between ripe and overripe classes on the PC2 axis. The ripeness levels were separated by the following parameters: Titratable acidity (g/L), malic acid (g/L) and tartaric acid (g/L), A420, A520, A420+A520, Balling, pH, K (mg/L) and anthocyanin concentration (mg anthocyanin/ g berry and mg anthocyanin/ berry) Titratable acidity (g/L), malic acid (g/L) and tartaric acid (g/L) were associated with unripeness and the Balling, A420, A520, A420+A520, pH, K

(mg/L) and anthocyanin concentration (mg anthocyanin / g berry and mg anthocyanin/ berry) with ripeness and overripeness (Figure 3.17). A420, A520, A420+A520 and K (mg/L) mostly distinguished between the ripe and overripe classes, although there was significant overlapping (Figure 3.17).

Seasonal differences were not as pronounced between 2003 and 2004, but some separation between the 2005 season was observed on PC2 axis (Figure 3.16). Balling, pH and K from the red must have little influence on the separation between the ripe and overripe classes or the seasonal classes but show potential as important parameters to use for the indication of ripe fruit compared to non-ripe (Figure 3.17).



Figure 3.15 Score plot of the ripeness classes of the grape components where PC1 explains 44% of the variance in the data and PC2 17%.

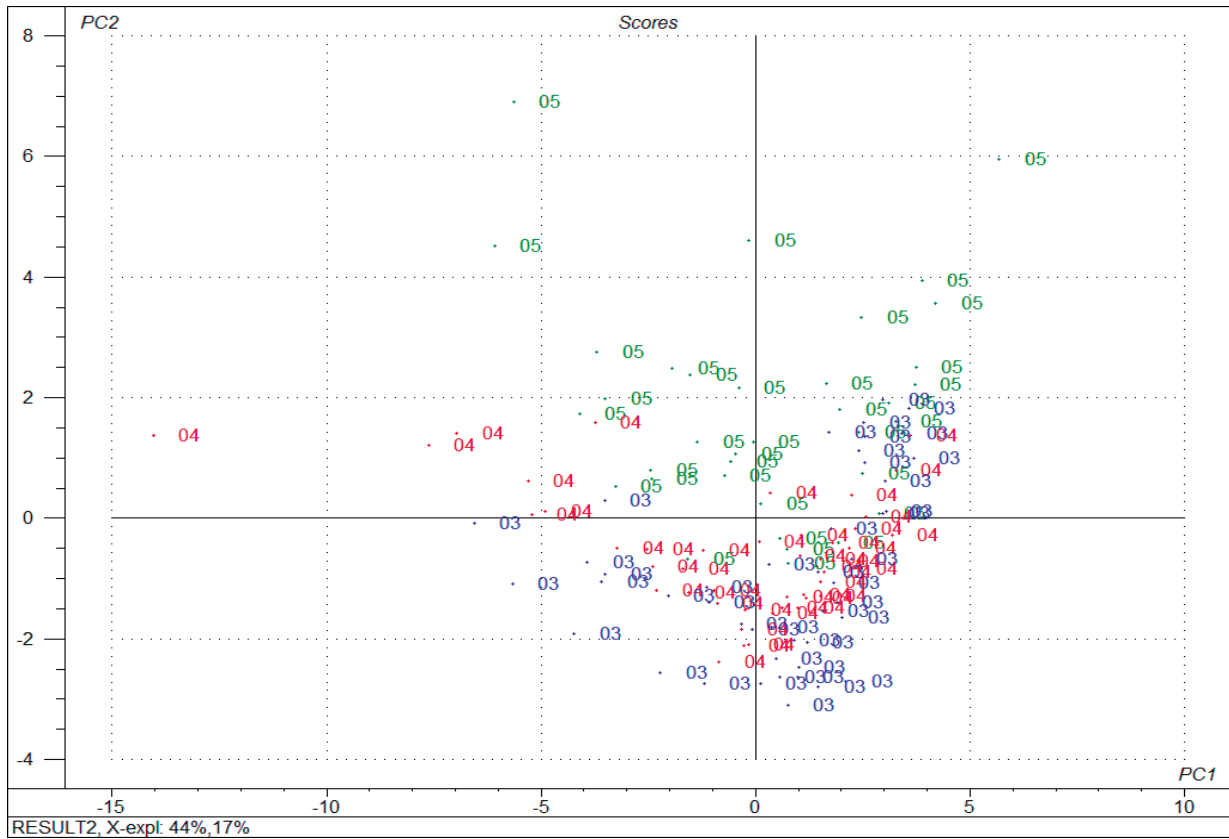


Figure 3.16 Score plot of different seasons of the grape data where PC1 and PC2 explain 44% and 17% of the variance in the data respectively.

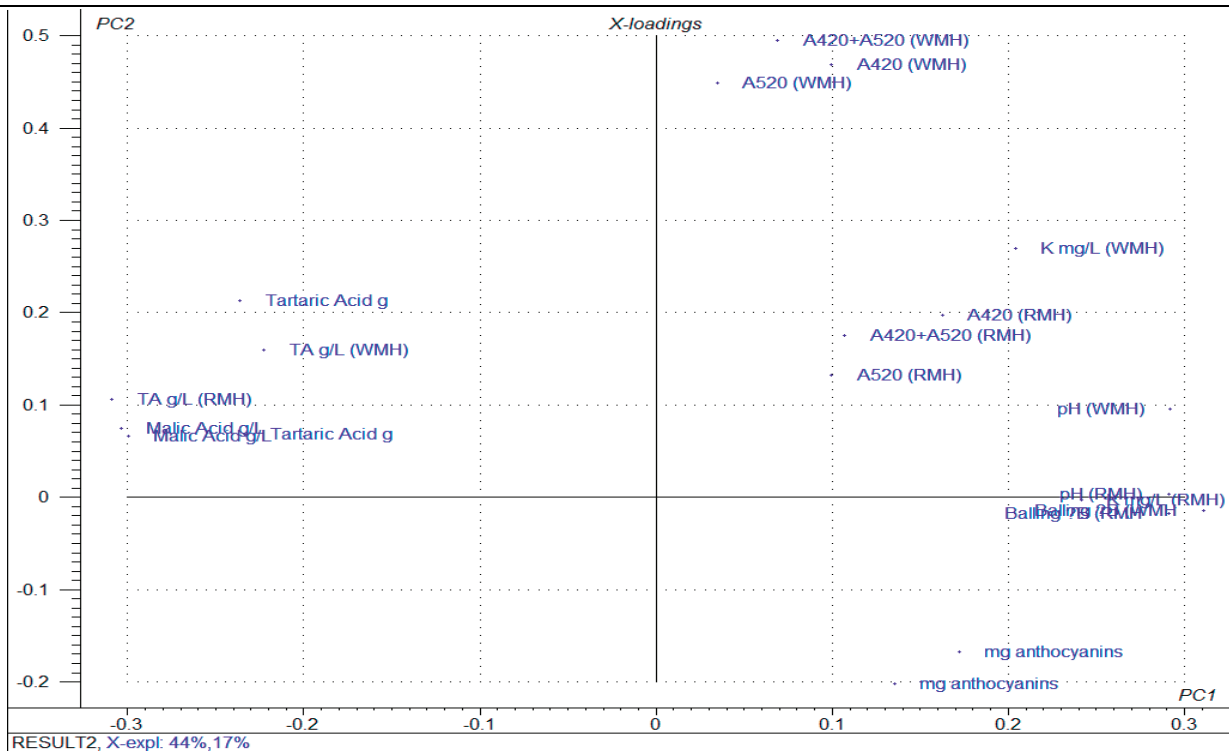


Figure 3.17 Loading plot of red and white grape must components where PC1 and PC2 explain 44% and 17% of the variance in the data respectively.

The important maturity parameters determined with the PCA were then used to establish a ripeness model. The minimum and maximum values of the ranges of unripe and ripe grapes were determined by taking values of each of the four classes and arranging the values in ascending order (Table 3.13).

Table 3.13 Grape component minimum and maximum ranges of the ripeness classes as used in the component analyses.

	Unripe		Ripe		Overripe	
	Min	Max	Min	Max	Min	Max
Balling (°B) WMH	9	24	20	29	25	35
Balling (°B) RMH	8	24	21	28	23	42
TA (g/L) WMH	6.30	34.86	3.64	8.46	3.15	6.40
TA (g/L) RMH	5.11	31.85	3.24	7.67	3.08	5.46
pH WMH	2.74	3.63	2.91	3.95	3.20	4.18
pH RMH	2.85	3.71	3.32	4.14	3.63	4.42
K (mg/L) WMH	580	2320	850	2740	1320	3500
K (mg/L) RMH	800	3120	1620	3360	1760	3575
Tartaric acid (g/L) WMH	4.90	12.30	3.40	8.79	4.10	7.20
Tartaric acid (g/L) RMH	4.00	13.20	4.48	8.85	1.40	6.97
Malic acid (g/L) WMH	2.39	23.50	0.92	4.77	0.87	3.31
Malic acid (g/L) RMH	1.90	24.70	1.21	4.19	0.84	3.17
mg Anthocyanin per berry	0.11	3.64	0.75	4.07	0.78	3.57
mg Anthocyanin per g berry	0.09	2.34	0.97	3.53	0.57	3.18

Table 3.13 shows that there is significant overlap in the minimum and maximum value ranges of the grape components used in the PCA. In figure 3.15 the unripe class overlapped slightly with the ripe class but not the overripe class. By using data of the vineyards separately, the overlapping of the values was minimized (Table 3.14).

Table 3.14 Grape component minimum and maximum ranges of the ripeness classes as used in the component analyses for Plaisir de Merle.

Plaisir de Merle	Unripe		Ripe		Overripe	
	Min	Max	Min	Max	Min	Max
Balling (°B) WMH	14.20	23.40	23.70	26.90	27.60	34.50
TA (g/L) WMH	5.60	21.46	4.28	5.73	3.68	4.95
pH WMH	2.84	3.31	3.23	3.80	3.52	3.93
K (mg/L) WMH	850	2000	1120	2440	1380	2280
Tartaric acid (g/L) WMH	3.78	11.10	4.50	7.76	4.86	6.20
Malic acid (g/L) WMH	2.46	13.60	1.46	1.66	0.87	1.79
mg Anthocyanin per berry	0.42	3.42	0.75	3.79	1.07	3.61
mg Anthocyanin per g berry	0.33	2.77	0.97	3.04	1.41	2.95

The grape components correspond with literature on the basis of berry development (Coombe, 1995 and Ribéreau-Gayon *et al.*, 2001).

Ripeness classes were defined in this study by Balling and not on the basis of grape development stages. The anthocyanin concentration could give a good indication of the potential quality of the grapes depending on the wine style. To be able to define precise ranges for optimal harvest of Cabernet Sauvignon we need to expand our number of vineyards and regions.

3.5 CONCLUSION

Through the decades our understanding of the grape vine has evolved, and with it our interpretation of quality. Berry maturity is influenced by seasonal variation as seen during this study and counting on a single parameter for all vineyards and seasons did not yield a complete picture of berry maturity.

Seed lignification percentage did increase over the seasons, but did not reach their prescribed maximum till after the maximum anthocyanin concentration and best wines. Seasonal differences were illustrated during the 2004 season in which the seed lignification percentages for all four vineyards were higher due to the longer ripening season. In order to employ this method as a potential maturity indicator for Cabernet Sauvignon in South Africa, more research is needed comprising more vineyards in different regions and the effective use outside of pure scientific research still needs to be seen.

Soluble solids (TSS), titratable acidity (TA) and pH are the corner stones of berry maturity. We can not disregard these parameters as shown during this trial as Balling correlated with general wine quality. These measures are quick, simple and cheap to implement in small wineries. Potassium (K^+) levels did influence pH and TA during the study as homogenized must pH values were higher than non-homogenized must samples. Stress conditions and the length of ripening lead to concentrations up to 3500 mg/L in grapes. Malic acid levels were found to be higher in Morgenster, than the other vineyards where canopies were less dense.

Maturity index values derived from different combinations of TSS, TA and pH were found to correlate with wine quality and support that found in previous literature. Seasonal differences between the vineyards were found and values of the best wines were higher than previous reported values, but differences between the cultivars needs to be investigated further in more vineyards and regions.

Anthocyanin concentrations were followed over the three seasons. Seasonal differences between the vineyards were observed, with the 2004 season values the lowest. The wine colour density confirmed this seasonal variation. Morgenster reported the highest levels of anthocyanins per berry, but not per gram berry. Trends were found between general wine quality. The best wines were found to correspond with the maximum anthocyanin concentrations in the grapes for all vineyards. More than one wine of superior quality was found during the maximum anthocyanin peak. It was found that wines made just before the colour peak and with lower sugar levels made wines with no significant

difference from the best wine when evaluated by an expert panel. This however does not mean that other wine components, such as aroma or flavour, did not significantly change over that period.

This study showed that colour intensity played an important role in the interpretation of quality during this study. This parameter followed the same trend from the grape colour through to the wine. Good correlations between colour intensity and general wine quality for both the grape skin tasting and wine was found. Berry and spicy aromas in both grape skin tasting and wine correlated with general wine quality. The grape skin tasting method could be used to give an indication of the potential of grapes at certain maturity levels, but the time consuming grape skin tasting method makes it unsuitable for implementation in large wineries.

PCA analysis of all the data was done and the minimum suitable parameters that could distinguish between unripe and ripe grapes were identified. Balling, TA, pH, K⁺, tartaric and malic acid successfully described the differences. Anthocyanin concentration was found to not only distinguish between unripe and ripe grapes, but could further be used to distinguish ripe from overripe grapes. With the minimum and maximum values of the identified parameters, a ripeness model was constructed. It was found that variation between seasons and vineyards led to, too much overlapping of the minimum and maximum values of all the vineyard data together. Better results were found when the ripeness model was constructed per vineyard. To optimize such a general model for Cabernet Sauvignon would require more research within more regions and vineyards.

Temperature, irrigation, vineyard practices, disease status and crop loading influence berry development. The parameters that we have followed are easy to analyse and can be used to find optimal maturity levels. It is important to use these analyses in conjunction with the history of the vineyards, as was illustrated by the low colour levels found in 2004. Expanding the number of vineyards, clones and regions could give an improved indication on the optimal time to harvest Cabernet Sauvignon in South African conditions.

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Chapter 4

RESEARCH RESULTS

A preliminary study of the phenolic composition of Cabernet Sauvignon (*Vitis vinifera*) grapes during ripening in four South African wine growing regions

This manuscript was submitted for publication in
South African Journal of Enology and Viticulture

4. A PRELIMINARY STUDY OF THE PHENOLIC COMPOSITION OF CABERNET SAUVIGNON (*VITIS VINIFERA*) GRAPES DURING RIPENING IN FOUR SOUTH AFRICAN WINE GROWING REGIONS

4.1 ABSTRACT

The changes in the main phenolic components in grapes were followed during ripening in four different wine growing regions of South Africa by reverse phase high performance liquid chromatography (RP-HPLC). Concentrations of malvidin-3-glucoside and total anthocyanins increased from after veraison until a maximum is reached and started to decline with over ripeness. Strong trends were found between malvidin-3-glucoside and other monoglucoside anthocyanins except for cyanidin. Wines identified by a tasting panel to be the best quality, corresponded with the maximum anthocyanin concentration in the homogenate. Dense canopies at the Morgenster vineyard over the three seasons lead to lower total anthocyanin and quercetin-3-glucuronide concentrations compared to the Anhöhe and Plaisir de Merle vineyards. Catechin, epicatechin, proanthocyanidin and polymeric phenol concentrations decreased significantly from veraison until harvest. Seasonal differences were noted in the four vineyards. No correlations could be found between the general wine quality and the phenolic compounds, but a weak trend was observed for total anthocyanins in the homogenate. A trend was found with the total flavan-3-ol to anthocyanin ratio determined by RP-HPLC analysis of the grape homogenates ($r = 0.40$, $p = 0.00$). This ratio varied between 1 and 3 for the wines rated as being the best quality. No single parameter could consistently indicate optimal ripeness over the seasons and per vineyard. A combination of parameters could predict the optimal time more precisely but more research is needed to this end.

4.2 INTRODUCTION

Cabernet Sauvignon is one of the most planted cultivars in the world as well as in South Africa (Robinson, 1994; Du Plessis and Boom, 2005). The South African wine industry has grown to the eighth largest producer of wine in the world (Robinson, 1994). Cabernet Sauvignon thus plays an important part in the perception of quality of South African wines abroad. The diversity of terroir in South Africa has given the wine industry the opportunity to produce numerous Cabernet Sauvignon wine styles. Du Plessis and Van Rooyen, (1982) stated that the degree of maturity of grapes influences the style and quality of the wine that could be produced from those grapes. Phenolic compounds have a major impact on the colour, mouth feel and taste of red wines (Gawel, 1998; Rossouw and Marais, 2003).

The most abundant class of phenolics in grapes is the flavan-3-ols. This class consists of monomers and oligomeric to polymeric flavan-3-ols containing mostly the monomers catechin, epicatechin, epicatechin-gallate and epigallocatechin, although galocatechin and epigallocatechin gallate has also been detected, that is linked by C₄-C₆ or C₄-C₈ interflavan bonds (Prieur *et al.*, 1994; Souquet *et al.*, 1996). The monomeric and polymeric flavan-3-ols (also known as proanthocyanidin) can induce both an astringent and bitter sensation that has been reported extensively (Thorngate and Noble, 1995; Kallithraka *et al.*, 1997; Gawel, 1998; Peleg *et al.*, 1999). The biosynthesis of tannins start with the onset of fruit set and the accumulation occurs separately in seeds and skins and reaches maximum levels prior to veraison (Downey *et al.*, 2003). Kennedy *et al.* (2000b) reported that from the onset of veraison all polyphenols decreased with ripening and that oxidation played a role. However, other research reported that the total phenolics of the skin increased after the onset of veraison, while the phenol concentration in the seeds decreased (Pirie and Mullins, 1977 and 1980; Ribéreau-Gayon *et al.*, 2001b). Boulton *et al.* (1996) reported that the phenolic concentration in the pulp is very low.

The concentration of individual phenolic components also changes over the course of ripening. Monomeric and polymeric flavan-3-ols follow a declining trend from the start of the ripening stage until maturity (Lee and Jaworski, 1989; Ribéreau-Gayon *et al.*, 2001b; Downey *et al.*, 2003). A steady increase in the degree of polymerisation of skin proanthocyanidin was reported during ripening and the rapid decrease of the catechin concentration from veraison (Kennedy *et al.*, 2001; Kennedy *et al.*, 2002; Downey *et al.*, 2003). Downey *et al.*, (2003) also reported an increase in epicatechin over the period. The hydroxycinnamic-tartaric acid concentration of the must decreased during ripening while the benzoic and other cinnamic acids and their aldehydes showed an increase in the skin (Lee and Jaworski, 1989; Fernández de Simón *et al.*, 1992 and 1993).

Ribéreau-Gayon *et al.* (2001a) stated that phenolic composition is influenced by cultivar, terroir, maturity level and disease status of the vineyard. The skin flavonol concentration also increases from veraison until reaching a maximum around maturity, followed by a subsequent decrease (Fernández de Simón *et al.*, 1993; Kennedy *et al.*,

2002;). Copigmentation and polymerisation occurs in the cells of seeds and skins after veraison (Kennedy *et al.*, 2000b).

Anthocyanins are the pigments that are responsible for the red colour of grapes and wines (Roggero *et al.*, 1986; Fernández-López *et al.*, 1998). These pigments are located in the vacuoles of the first four cell layers of the hypodermis (Núñez *et al.*, 2004). The pigment content in these cells increase from after veraison until maturity and in some years cells adjacent to the pulp might also be coloured (Ribéreau-Gayon *et al.*, 2001a). Anthocyanins in *Vitis vinifera* are monoglucosides consisting of an anthocyanidin linked at position 3 with a glucose molecule (Ribéreau-Gayon *et al.*, 2001a and b; Núñez *et al.*, 2004). The glucoside can be acylated at position 6 of the sugar molecule with acetic, *p*-coumaric or caffeic acid (Ribéreau-Gayon *et al.*, 2001a, Núñez *et al.*, 2004). Malvidin-3-glucoside and its derivatives contribute 57% to 69% to the content of the anthocyanins in the grapes and wines (Núñez *et al.*, 2004). The composition and profile of anthocyanins in red grapes are influenced by seasonal conditions, maturity level, soil conditions, cultivar, canopy management, crop load, irrigation and genetic disposition (Mazza, 1995; Keller and Hrazdina, 1998; Esteban *et al.*, 2001; Ribéreau-Gayon *et al.*, 2001a; Ryan and Revilla, 2003).

The aim of this trial was to investigate the changes in phenolic composition with maturity in the grapes and how this influences the subsequent wines. Furthermore possible correlations between phenolic composition and the other maturity parameters were investigated.

4.3 MATERIALS AND METHODS

4.3.1 ORIGIN OF GRAPES

Four commercial vineyards of *Vitis vinifera* L. cv. Cabernet Sauvignon grafted on Richter 99 rootstock were selected in four different wine growing regions, Simonsberg-Stellenbosch (Nietvoorbij), Simonsberg-Paarl (Plaisir de Merle), Wellington (Anhöhe) and Durbanville (Morgenster) (Table 4.1) of the Coastal Wine Region, Western Cape.

Five rows by eight inter-pole-spaces per vineyard were identified and marked with danger tape. Each row of these blocks was three inter-pole-spaces from the nearest access road. The anchor post at each end of the row was also marked and numbered.

Table 4.1 Experimental vineyard block data from the four farms

	Anhöhe	Morgenster	Nietvoorbij	Plaisir de Merle
Winkler	V	III	IV	IV
Average °C	15.8*	15*	15.6*	16.3*
Average °C_{max}	23*	20*	21.2*	20.9*
Average rainfall (mm)	55*	153*	193*	205*
Row direction	East-West	North-South	North-South	North-South
Row width (m)	3.4	3.1	2.7	2.9
Inter-pole-space length (m)	7.4	8.6	7.4	7.4
Clone	46	169	37B	336B
Slope direction	East	West	West	East
Trellis	5 wire lengthened Perold	5 wire lengthened Perold	5 wire lengthened Perold	3 wire hedge
Number of vines per block	240	245	239	232

* Climatic data as obtained from the closest weather station.

4.3.2 SAMPLING AND PREPARATION OF GRAPES

Vineyards were sampled during the 2003, 2004 and 2005 harvest seasons from just after véraison until two weeks after commercial harvest. One bunch was randomly sampled from each vine in the designated experimental block from each vineyard. A berry sample of 2.5 kg was then randomly selected from these pooled bunches for each vineyard. A hundred berries were selected from this 2.5 kg sample and used to determine the berry weight and volume. The 100 berries were then added back to the remainder of the 2.5 kg sample. A second sample of 700 g was used for the phenolic and colour measurements. The 700 g grape sample was homogenised for 20 seconds using a colloid mill (Fryma AG, Rheinfelden, Switzerland). The homogenate was stirred for 20 seconds before a sample was taken. This was done to re-suspend any sediment that might have formed at the bottom of the beaker while standing before sampling.

4.3.3 ANTHOCYANIN DETERMINATION

4.3.3.1 Iland Method

Anthocyanins were determined as described by Iland *et al.* (2000) with small modifications. The berry weight as previously determined (section 3.3.5) was used for the calculations by dividing the weight of 100 berries by two. The homogenate, prepared as described in

section 4.3.2, was used for the extraction. The procedure was done in triplicate and 2 g of homogenate was used for each extraction. Twenty mL of 50% ethanol adjusted to pH 2 with hydrochloric acid (HCl) (MERCK Chemicals (PTY) Ltd, Wadeville, Gauteng, RSA) was added to each 50 mL centrifuge tube containing the homogenate. The solution was centrifuged (Beckman, Model J2-21, Beckman Instruments Inc., Palo Alto, CA, USA) at 10000 rpm for 10 minutes. The extract as described by Iland *et al.* (2000) was pipeted (0.5 mL) into test tubes and 5 mL of 1 M HCl (MERCK Chemicals (PTY) Ltd, Wadeville, Gauteng, RSA) were added. The mixture was allowed to extract for three hours at 20°C. A ThermoSpectronic spectrophotometer (ThermoSpectronic, Helios Gamma) was used to measure the absorbance at 280 nm, 520 nm and 700 nm. The values obtained from this method were given as milligram anthocyanin per gram berry (mg anthocyanin / g berry) or milligram anthocyanin per berry (mg anthocyanin / berry).

4.3.4 HPLC ANALYSES OF GRAPES AND WINES

Reverse phase high performance liquid chromatography (RP-HPLC) was performed using an Agilent 1100 series HPLC system with a diode array detector (Agilent Technologies Inc., Palo Alto, CA, USA). A 250 mm × 4.6 mm Lichrosorb RP18 column and pre-column (Phenomenex, Torrance, CA, USA) were used. Data processing was done with ChemStation[®] software (Agilent Technologies Deutschland, Waldbronn, Germany).

The mobile phase consisted of 80% HPLC grade acetonitrile (Sigma-Aldrich, Steinheim, Germany) with 20% of solvent B (Solvent A) and deionised water adjusted to pH 1.7 using 70% perchloric acid (Solvent B)(Saarchem, Merck (PTY) Ltd., Midrand, RSA). Solvent B was filtered through a 0.45 µm cellulose filter (Millipore Corp., Bedford, MA, USA) before use. The following gradient was established: 0 min, A 0%, B 100%; 12 min, A 12%, B 88%; 28 min, A 14%, B 86%; 74 min, A 35%, B 65%; 80 to 84 min, A 80%, B 20% and 90 min, A 0%, B 100%. The column was flushed with 100% solvent B between samples for 10 minutes to equilibrate the system and ensure a stable baseline. The column temperature was maintained at 35°C and the flow rate was one mL/min throughout. The spectra were recorded at 280 nm, 320 nm, 365 nm and 520 nm.

Compounds were identified by their spectra and retention times compared to the following authentic standards: gallic acid, (+)-catechin, (-)-epicatechin, epicatechin gallate, epigallocatechin, proanthocyanidin B1, proanthocyanidin B2, caffeic acid, *p*-coumaric acid, quercetin-3-glucoside (Sigma-Aldrich (PTY) Ltd., Johannesburg, RSA), ferulic acid, quercetin, quercetin-3-O-galactoside, quercitrin, myricetin, kaempferol and malvidin-3-O-glucoside chloride (Extrasynthèse, Genay, France) and literature (Price *et al.*, 1995; Peng *et al.*, 2002).

Flavan-3-ols were quantified at 280 nm as mg/L catechin units, cinnamic acids at 320 nm as mg/L caffeic acid, flavonols at 365 nm as quercetin-3-O-galactoside and anthocyanins at 520 nm as mg/L malvidin-3-O-glucoside.

The homogenate extracts were prepared in triplicate as described previously in 50% EtOH adjusted to pH 2. The solution was centrifuged and the supernatant was filtered into HPLC vials through a 0.45 μm AsetatePlus[®] filter (Osmonics Inc., Minnetonka, MN, USA) and stored at 0°C. The wines were similarly filtered before HPLC analysis. All analyses were completed within a week of sample preparation.

Caftaric and coutaric acid as well as proanthocyanidin dimers and trimers were identified according to their spectra and molecular mass by liquid chromatography – mass spectrometry (LC-MS). LC-MS was performed using a Waters API Quattro Micro system with a Waters 2695 alliance and 996PDA detector. Chromatographic separation was carried out on a Lichrosorb RP18 column (100Å, 250 × 4.6mm, 5 μm , Phenomenex, Torrance, CA, USA) using 80% HPLC grade acetonitrile (Sigma-Aldrich) with 20% of solvent B (solvent A) and water with 2% formic acid (Merck) (solvent A) as mobile phase. Elution was performed with the same gradient conditions as described before. The phenols were detected with an electrospray ionization (ESI) system in the positive mode. A cone voltage of 15 V and capillary voltage of 3.5 kV was used with a desolvation temperature of 400°C.

4.3.5 REPEATABILITY AND LIMIT OF QUANTIFICATION

The repeatability was determined by injecting standards six times at test concentrations. The relative standard deviation (RSD) was calculated with the acceptable level of 3% as criteria. The limit of quantification was taken as the lowest amount giving a signal-to-noise ratio of 7:1.

4.3.6 SMALL SCALE WINEMAKING.

Small scale wines were made during the harvest seasons of 2003, 2004 and 2005. Grapes were weighed at the cellar and the crate mass subtracted. After the 2.5 kg sample for chemical analysis was taken, each vineyard batch was divided into duplicates of equal weight. Each batch was put through a destemmer-crusher. Thirty five parts per million (ppm) of sulfur dioxide (SO₂) was added to the crushed grapes at the crusher. Twenty five grams per hecto liter (g/hL) hydrated yeast (WE372, Anchor Yeast, Cape Town, RSA) was added to the must. The must was fermented at 20°C and the cap was punched down three times daily. The fermenting grape must was pressed at four degrees balling (°B) using a 20 liter hydro press (Fratelli Marchisio S.p.A., Pieve di Teco, Italia) to press the grapes to three bar. The wine was transferred to an 18 L canister to finish primary fermentation. Wines were deemed dry when the residual sugar level was lower than 5 g/L. Sixty ppm SO₂ was added after the wine was fermented dry. Samples were taken after the SO₂ addition and analysed for alcohol level, residual sugar, volatile acidity, pH, titratable acidity, free and total sulfur. The wines were left for one week to settle at 4°C after which the wines were racked off the lees. Final adjustments were made before filtration. The total

sulfur level was raised to 100 ppm and the acid level adjusted to 6 g/L TA. The wines were filtered through a sheet-filter using Seitz K200 (PALL Corp., East hills, New York, USA) sheets into screw cap wine bottles. Duplicates were blended together before bottling and evaluated as a single wine. The wines were labeled and stored at 15°C until tasted by the sensory panel.

4.3.7 WINE TASTING

An industry panel of winemakers evaluated the wines at the end of each year. Forty millilitres of wine was poured into each glass. Wines of the four vineyards were tasted in a random order by the panel in flights of six wines at a time with a break of five minutes in between each flight. The panel was asked to use a line scale to evaluate each criterion. The scale ranged from non detectable at its minimum to intense at its maximum for the aroma components: vegetative, red berry and black berry with spice as well as for acidity. Colour intensity was scaled from light coloured to intense colour. The overall quality of the wine was determined by each taster in the panel after taking all the preceding criteria into account. Each line scale was measured and the distance used as the degree of liking by the taster.

4.3.8 STATISTICAL ANALYSIS

Data was analysed using Statistica Version 7.1 (Tulsa, OK., U.S.A). Spearman and Pearson correlations were done with the datasets and ANOVAS investigated for trends between different grape and wine variables.

4.4 RESULTS AND DISCUSSION

4.4.1 MALVIDIN-3-GLUCOSIDE AND TOTAL ANTHOCYANINS IN HOMOGENATE

Malvidin-3-glucoside is the most abundant anthocyanin in *Vitis vinifera* grapes (Núñez *et al.*, 2004) and as such was deemed to be the best indicator of colour development during the ripening period. Grapes from all four vineyards followed a comparative pattern after veraison until harvest, with some seasonal differences. Correlations between malvidin-3-glucoside and the glucosides of delphinidin ($r = 0.70$, $p = 0.00$), petunidin ($r = 0.82$, $p = 0.00$) and peonidin ($r = 0.41$, $p = 0.00$) were found, but the concentration of cyanidin ($r = 0.08$, $p = 0.42$) was too low to correlate. Strong correlations were also found between malvidin-3-glucoside and malvidin-3-glucoside-acetate ($r = 0.85$, $p = 0.00$) and malvidin-3-glucoside-*p*-coumarate ($r = 0.93$, $p = 0.00$). The correlations also persisted for the total anthocyanins (data not shown). Total anthocyanins (mg/L) are the sum of all the

monomeric anthocyanins quantified by RP-HPLC. Thus only malvidin-3-glucoside and total anthocyanins will be discussed for the homogenate below.

During the 2003 season, Anhöhe was the only vineyard to show an ongoing increase in the malvidin-3-glucoside and total anthocyanin (mg/L) concentration until the end of sampling (Figure 4.1 and 4.2). This trend was not observed in the other three vineyards during the 2003 season. This could not be explained by berry shrivelling, as the berry weight (mg) and volume (mL) did not decrease significantly over the corresponding period (data not shown), nor did the anthocyanin concentration increase correspond to data obtained with the Iland method (section 3.3.5). A possible explanation for differences between the methods is that the Iland method measures the total grape colour and the HPLC measures the individual anthocyanin concentrations. The only plausible explanation for the increase in anthocyanin concentration is that anthocyanins were still being synthesized at the Anhöhe vineyard. Anthocyanin accumulation from veraison until ripeness and the subsequent decrease afterwards as seen for 2004 and 2005, corresponded with that found in literature (Ribéreau-Gayon *et al.*, 2001a and 2001b; Fournand *et al.*, 2006). The degradation of anthocyanin could be attributed to enzymatic activity that was reported to increase at ripeness by Cash *et al.* (1976).

During 2003 season, grapes from Plaisir de Merle showed a very slow increase in anthocyanin concentration over the first three weeks after veraison. Seasonal differences were observed at Plaisir de Merle during the 2005 season, where the maximum concentration of anthocyanins was reached about 2 weeks earlier than in previous seasons. This was also observed in the other vineyards (data not shown). The 2005 season was earlier than the previous seasons due to a warm spring resulting in earlier budding (Du Plessis and Boom, 2006). The average temperature over the growing period, from November until February, was also higher in 2004/2005 (21.87°C) than in the 2002/2003 (20.87°C) season.

Morgenster showed a gradual increase in malvidin-3-glucoside concentration in the early part of the season and then started to decline briefly before increasing again until a maximum concentration was reached (data not shown). This pattern was repeated in both 2004 and 2005 season. Rainfall was suspected to play a role in the decrease during mid-season, but no recorded rainfall corresponded to it nor just prior to the dates (data not shown). An increase in berry weight (g) was however observed over the corresponding periods. Mid season irrigation by the producer is left as the only plausible reason. The irrigation could have lead to the excessive vigour as observed and could be counter productive to the production of anthocyanins and quality wines. Boulton *et al.*, (1996) stated that dense canopies can shade bunches, leading to a lowering in anthocyanin concentration and promote disease and pests.

Total anthocyanins (mg/L) are the sum of all the monomeric anthocyanins quantified by RP-HPLC. Graphs of total anthocyanins (mg/L) followed comparable patterns during the three seasons in all four vineyards (data not shown), similar to the accumulation

and decrease seen for malvidin-3-glucoside. Figure 4.3 illustrates the differences between the four vineyards over the three seasons.

There was significant second order interaction between the seasons and the farms investigated (Figure 4.3). Grapes from the 2003 season had the lowest total anthocyanin concentration per berry, except for Morgenster (Figure 4.3). Cooler temperatures at Morgenster appeared to have buffered the vineyard better against prevailing drier conditions as the denser canopies illustrated (Figure 3.12). Plaisir de Merle progressively produced more anthocyanin per berry per year but the difference between 2004 and 2005 seasons were not significant. During the 2003 season, Plaisir de Merle had the lowest berry weight and volume (Figure 4.4) compared to the 2004 and 2005 seasons. Water stress caused by the drier than usual conditions (Du Plessis and Bloom, 2005) that prevailed during the 2003 season could have had an effect on the berry size found during the season at Plaisir de Merle compared to the 2004 and 2005 seasons. Anthocyanin concentration at Anhöhe was comparable during all three seasons and the berry volume and weight (data not shown) were also comparable over all three seasons. Water stress in the vineyards during the growing period is the most likely cause for the smaller berries as the influence of water stress on the berry development is well known (Van Zyl, 1981; Ribéreau-Gayon *et al.*, 2001a).

In order to investigate the influence of the ripeness level on the total anthocyanin concentration in the grapes, arbitrary ripeness levels we defined based on sugar level. The grapes were divided into three ripeness classes according to sugar levels (unripe, < 23.5 °B; ripe, 23.6 °B to 26.5 °B; overripe, > 26.6 °B) where 25 °B was used as the reference ripeness level, as it included all four vineyards over the three seasons. Figure 4.5 shows the differences in total anthocyanin content during the three ripening stages for all vineyards and seasons together. The ease of extraction of anthocyanins out of the cells at ripe or slight overripe conditions are due to the decay of the cell walls and supports the increase in their concentration during ripening in the four vineyards (Ribéreau-Gayon *et al.*, 2001b).

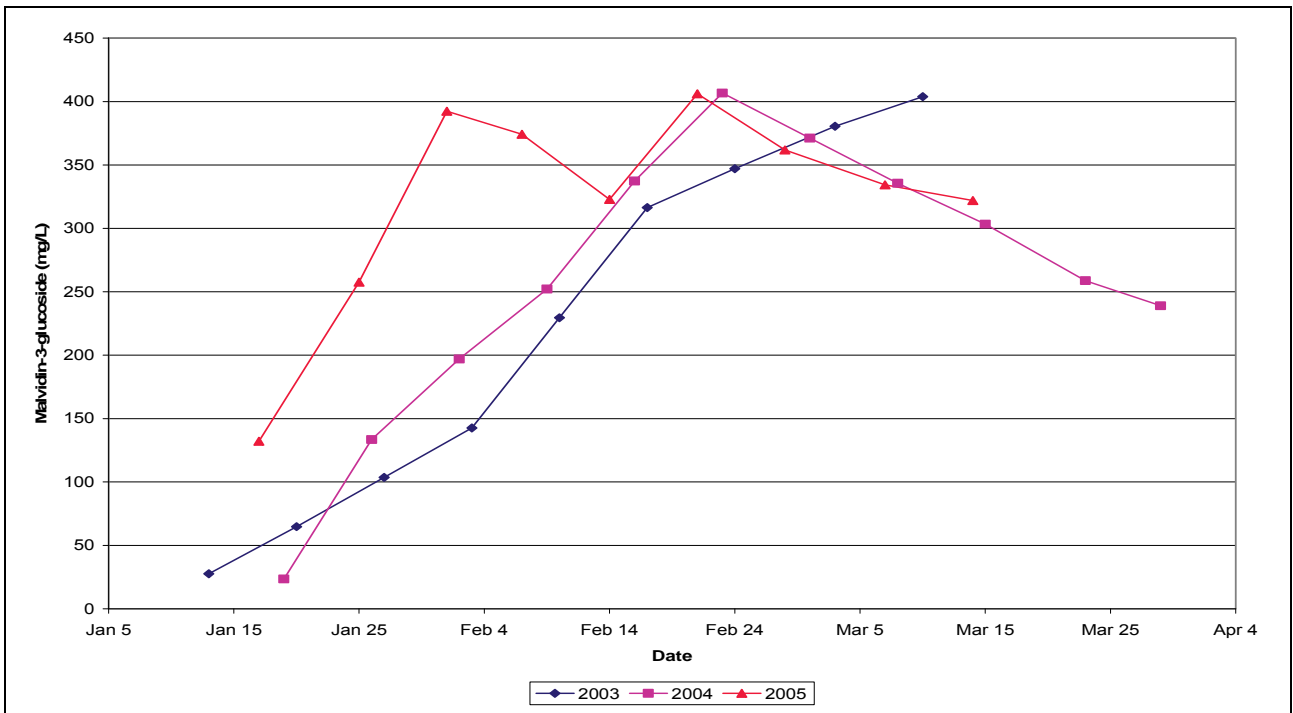


Figure 4.1 Malvidin-3-glucoside (mg/L) concentration of Anhöhe grapes over three seasons as measured by RP-HPLC in the homogenate during ripening.

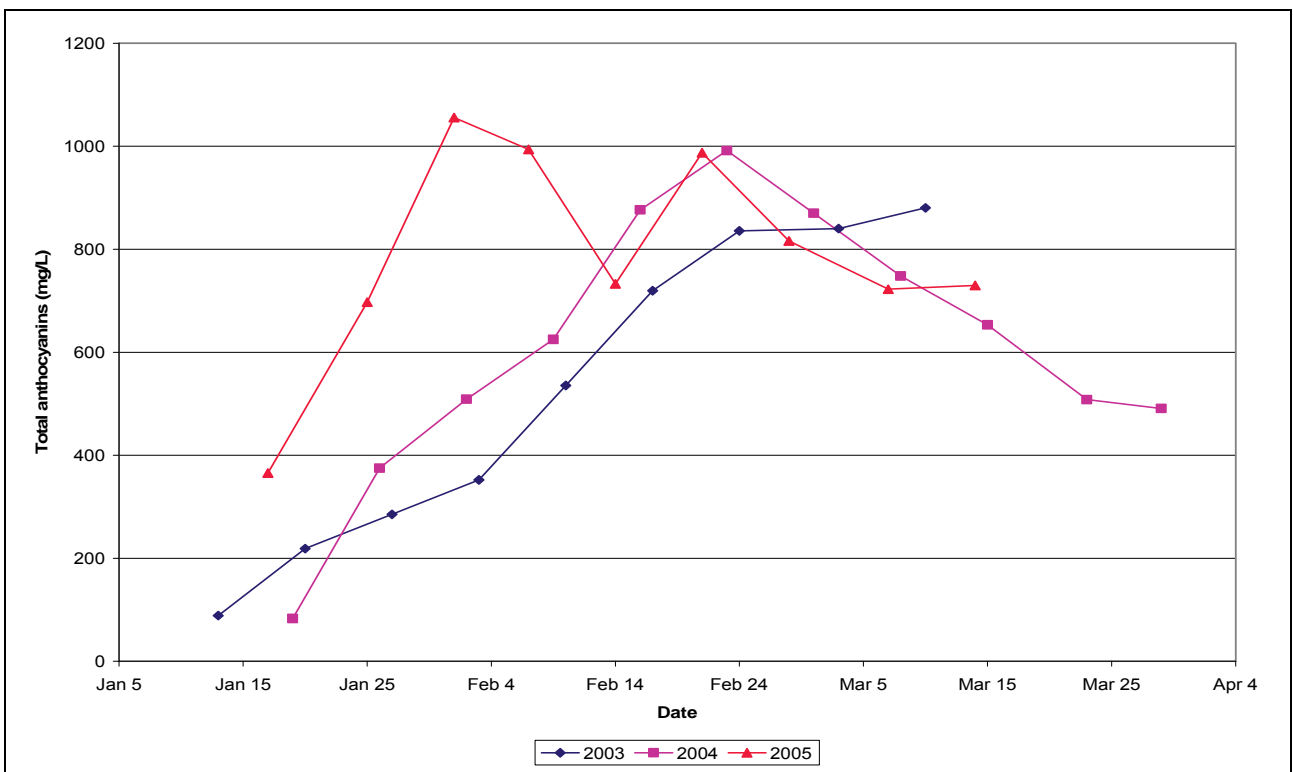


Figure 4.2 Total anthocyanins (mg/L) of Anhöhe grapes over three seasons as followed by RP-HPLC in the homogenate during ripening.

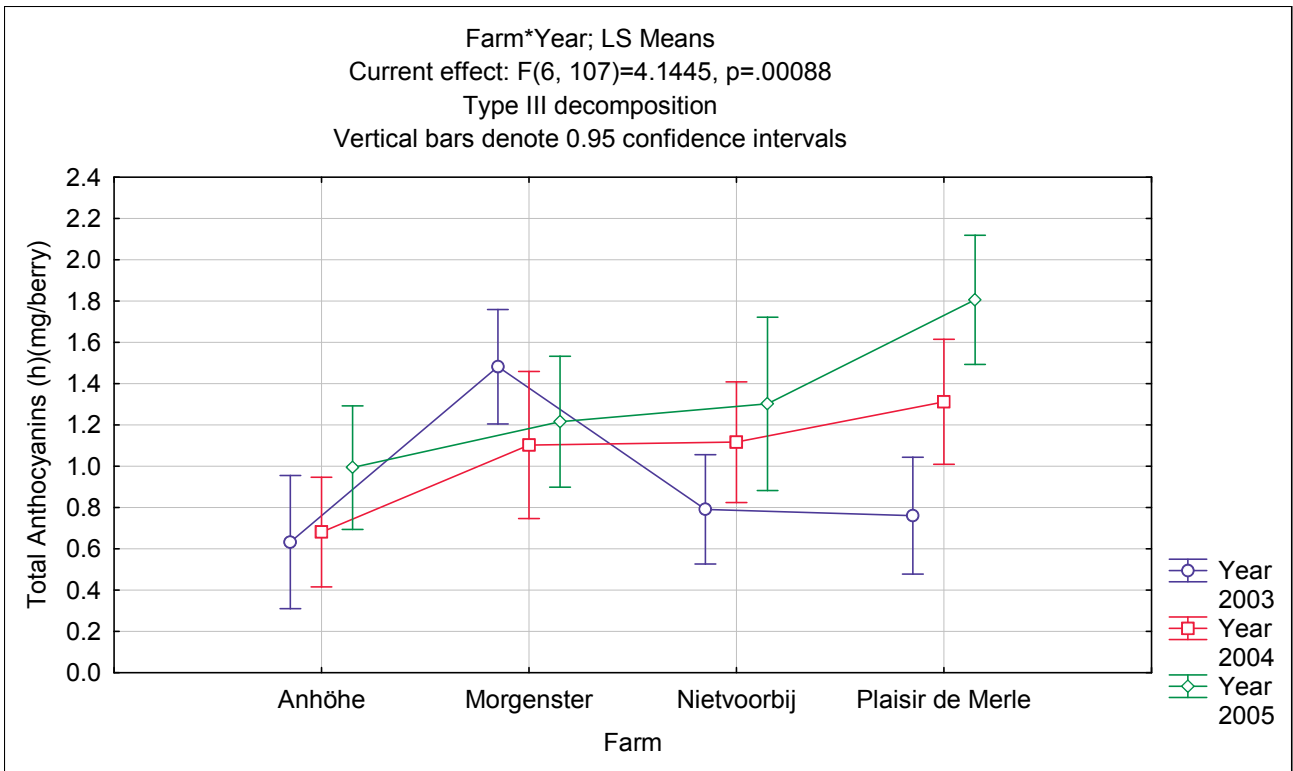


Figure 4.3 Seasonal differences in total anthocyanin (mg/berry) content from the homogenate of the four vineyards over three seasons as determined by RP-HPLC.

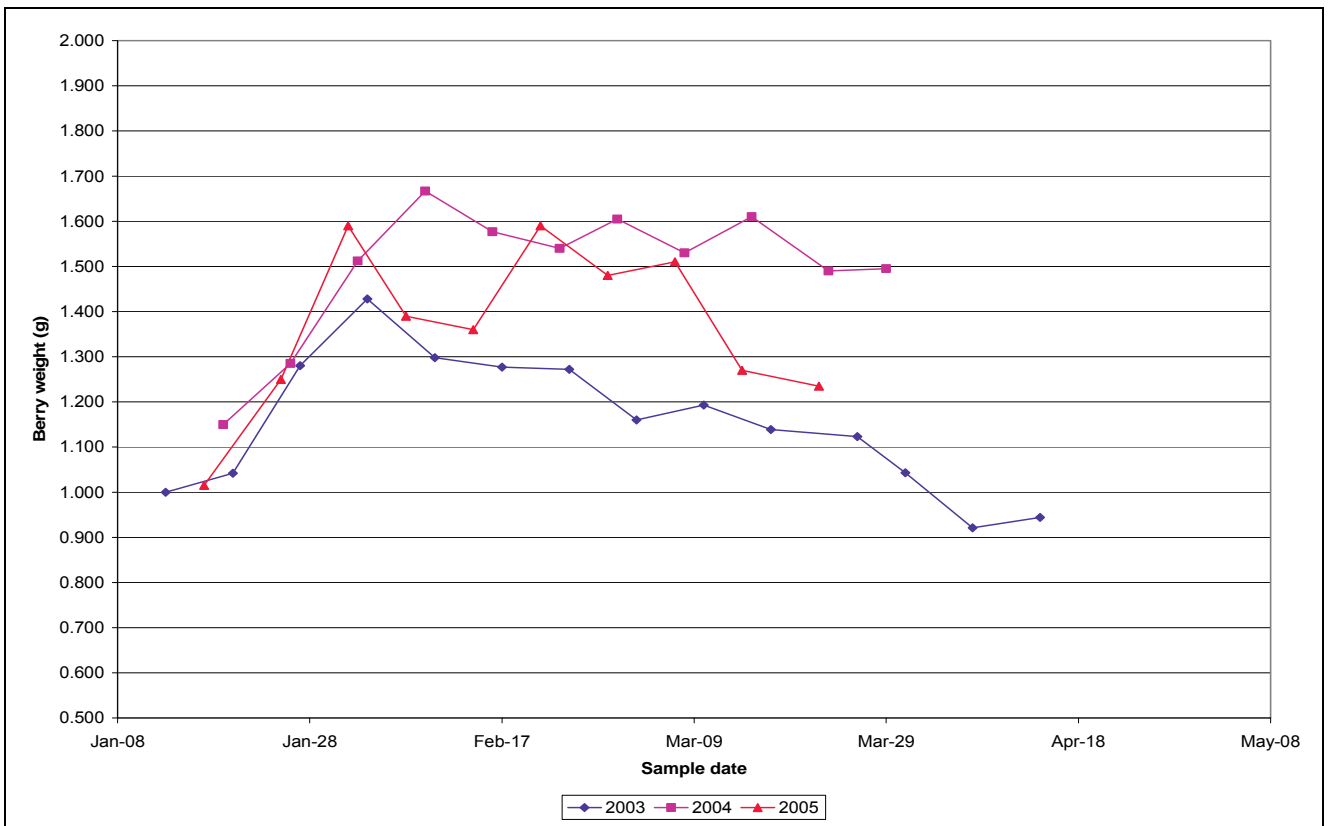


Figure 4.4 Berry weight (g) of Plaisir de Merle over the three seasons.

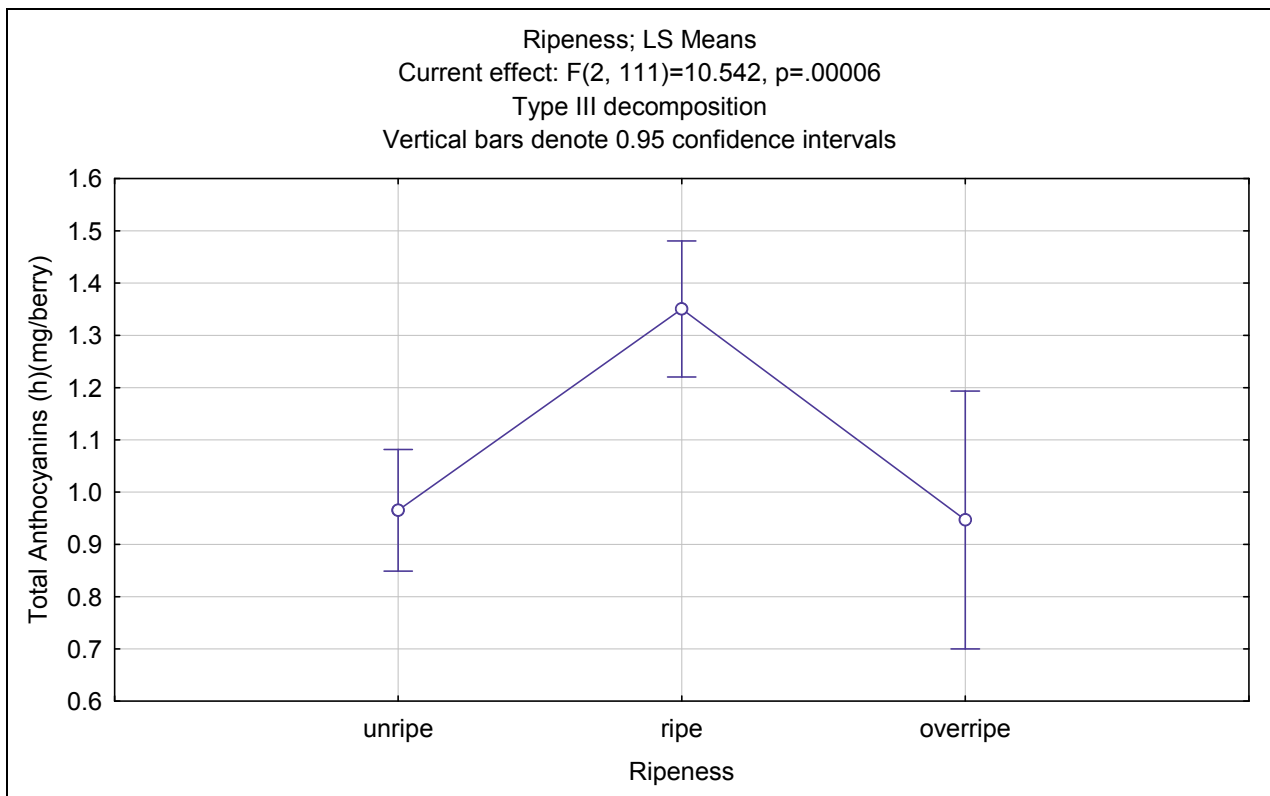


Figure 4.5 Graph illustrating the total anthocyanin content during three ripening stages (unripe, < 23.5 °B; ripe, 23.6 °B to 26.5 °B; overripe, > 26.6 °B) for seasons 2003 till 2005 and for all four vineyards..

4.4.2 COMPARISON OF TOTAL ANTHOCYANINS DETERMINED BY HPLC AND ILAND METHOD

Total anthocyanins as determined by HPLC were converted to mg per berry to compare with the values obtained by the Iland method (Iland *et al.*, 2000). Trends were comparable for all vineyards, but the total anthocyanins determined by RP-HPLC were much lower than the values found with the Iland method (Figure 4.6 and 4.7). The method of Iland (Iland *et al.*, 2000) calculates the anthocyanin concentration from the total amount of colour at 520 nm in the grape berry. It has been shown that small polymeric pigments (SPP) and large polymeric pigments (LPP) are also present in grapes (Vidal *et al.*, 2004) in addition to monomeric pigments (MP). Harbertson *et al.* (2003) determined that the SPP and LPP made a significant contribution (>30%) to wine colour at pH 4.9 in Cabernet Sauvignon. The contribution of non-monomeric pigments to fruit colour could explain the discrepancies between the anthocyanin concentrations determined by Iland's method and RP-HPLC. The anthocyanin concentration increases during ripening as illustrated in Figure 4.8, and corresponds with literature on the basis of metabolism of anthocyanin (Ribéreau-Gayon *et al.*, 2001a).

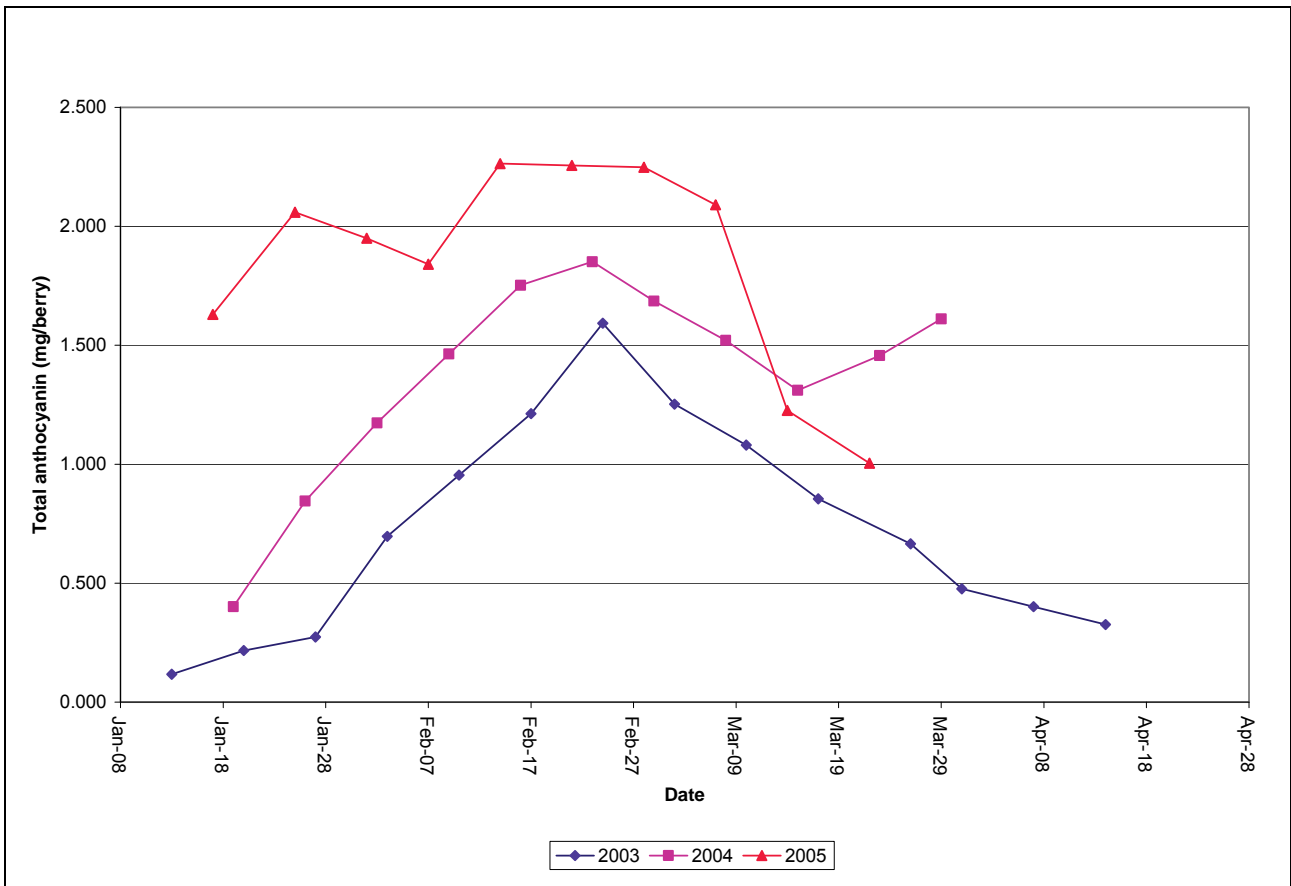


Figure 4.6 Total anthocyanins (mg/berry) graph of HPLC from Plaisir de Merle over the 3 seasons.

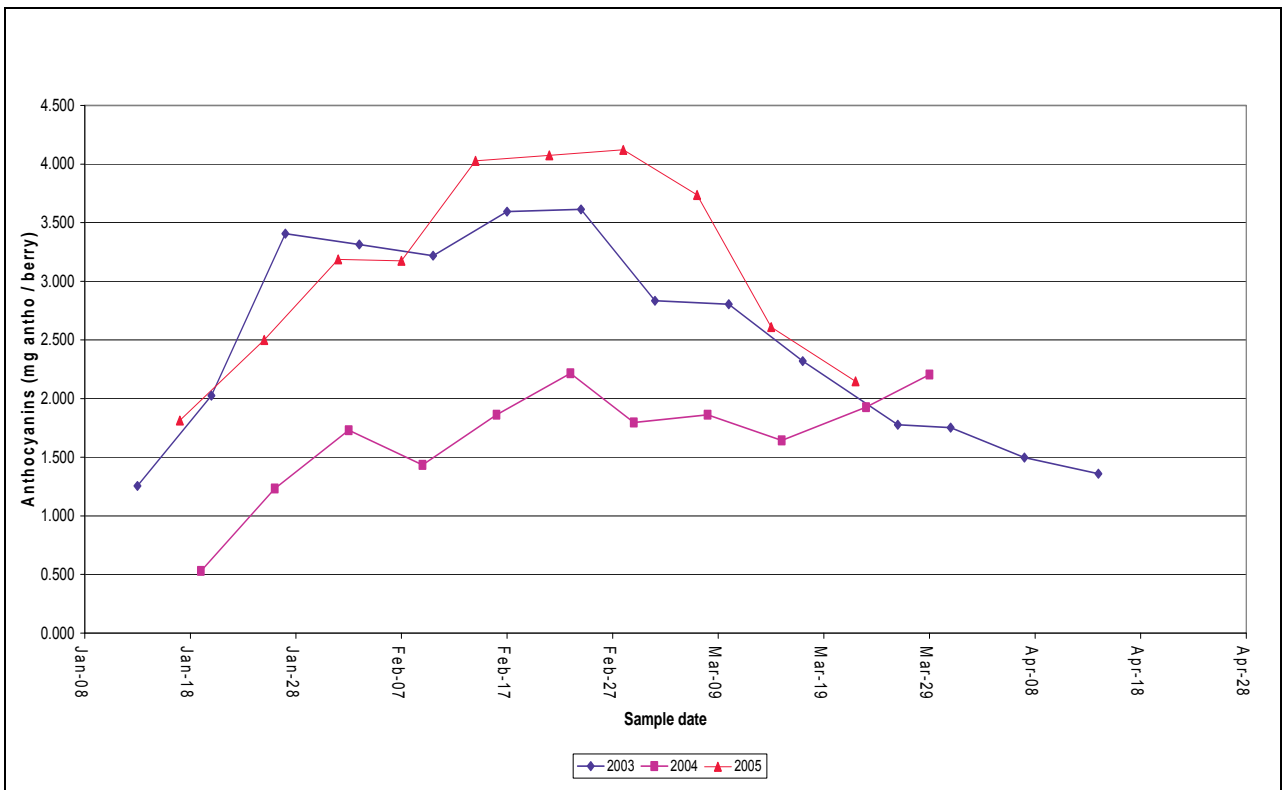


Figure 4.7 Total anthocyanin concentration (mg/berry) of Plaisir de Merle as determined using the method of Iland *et al.* (2000) over three seasons.

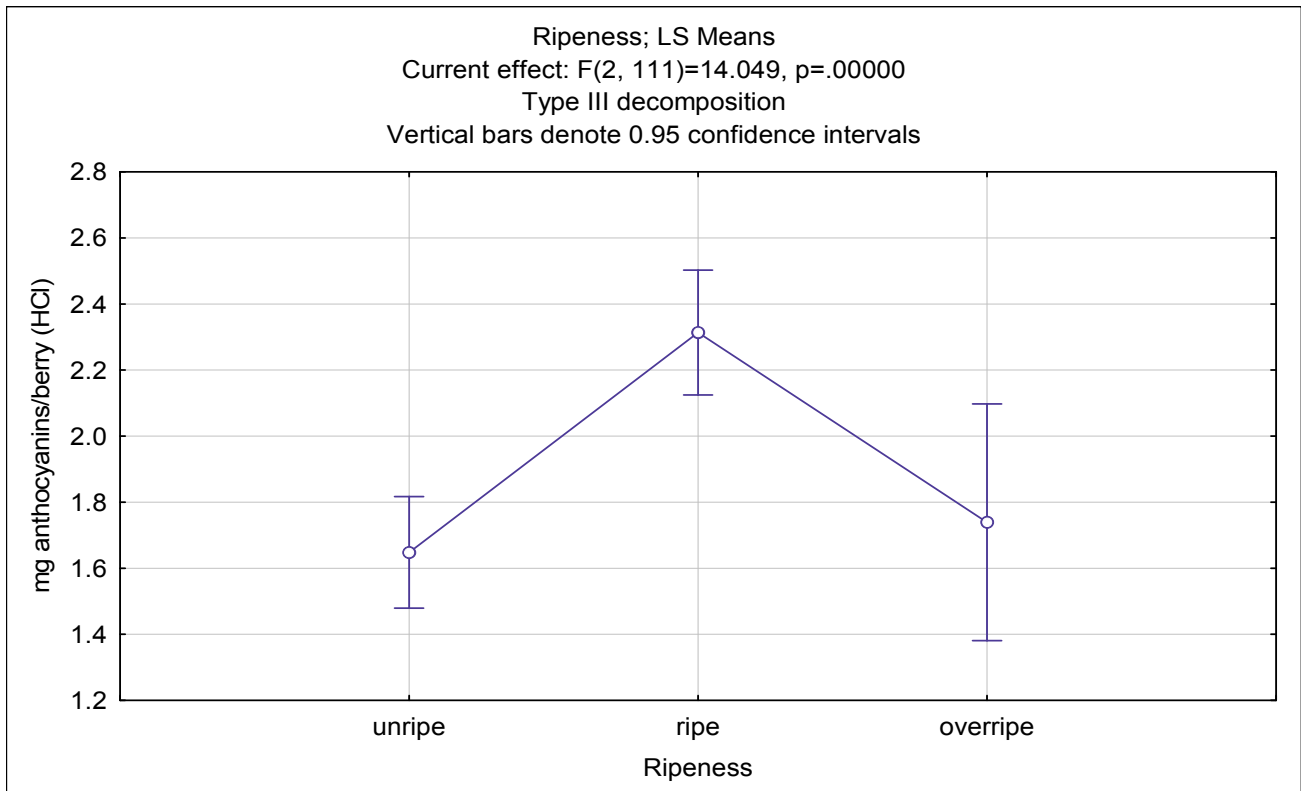


Figure 4.8 Graph illustrating the total anthocyanin content during three ripening stages as done with Iland method for all vineyards and seasons.

4.4.3 MALVIDIN-3-GLUCOSIDE AND TOTAL ANTHOCYANINS IN WINE

The malvidin-3-glucoside concentrations in the homogenates were much higher than those of the wines. This could be due to the absorption of the anthocyanins onto solids during fermentation; the formation of tannin-anthocyanin complexes or anthocyanin breakdown reactions (Ribéreau-Gayon *et al.*, 2001b). HPLC analysis of malvidin-3-glucoside and total anthocyanins correlated strongly with malvidin-3-glucoside-acetate ($r = 0.98, p = 0.00$; $r = 0.97, p = 0.00$) and malvidin-3-glucoside-p coumarate ($r = 0.91, p = 0.00$; $r = 0.90, p = 0.00$) in the wines. Only delphinidin-3-glucoside ($r = 0.35, p = 0.00$; $r = 0.55, p = 0.00$) and petunidin-3-glucoside ($r = 0.64, p = 0.00$; $r = 0.79, p = 0.00$) followed a trend with malvidin-3-glucoside and total anthocyanin. Cyanidin-3-glucoside and peonidin-3-glucoside did not correlate with either malvidin-3-glucoside or total anthocyanin. The reason for this might be due to conditions in the ferment as discussed previously. As with the preceding section 4.4.1, only malvidin-3-glucoside and total anthocyanin will be discussed further below.

During the 2003 season the malvidin-3-glucoside values of the wines from Anhöhe (Figure 4.9) and Plaisir de Merle (Figure 4.10) reached a maximum concentration earlier in the season when compared to Nietvoorbij (Figure 4.11) and Morgenster (Figure 4.12). Both Anhöhe and Plaisir de Merle have earlier seasons compared with Morgenster and

Nietvoorbij as seen in their harvest dates (Table 4.2). Nietvoorbij was harvested earlier by accident by the producer during the 2005 season (Table 4.2).

Nietvoorbij reached a maximum turning point on the 3rd of March 2005 compared with the continued increase of Morgenster (Figure 4.11 and 4.12). The 2004 season showed differences between the vineyards of Anhöhe (Figure 4.12) and Plaisir de Merle (Figure 4.10) to that of Morgenster (Figure 4.12) and Nietvoorbij (Figure 4.11). In the latter two vineyards, the malvidin-3-glucoside kept on increasing over the season, while for the first two vineyards it increased rapidly at the beginning of the season. After which the anthocyanin concentration increased slower than the other two seasons (Figure 4.9). Graphs of total anthocyanin concentration over the seasons and vineyards mirrored that of malvidin-3-glucoside in the wine.

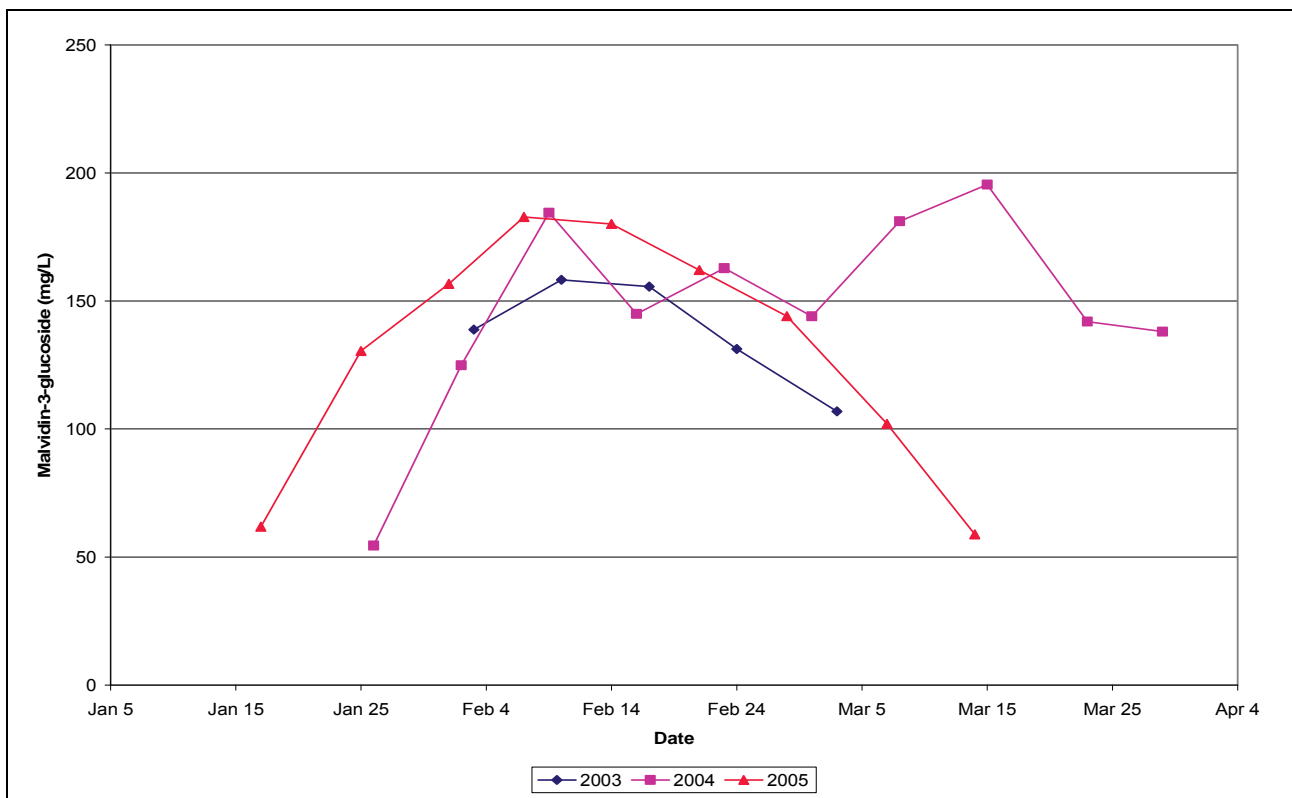


Figure 4.9 Malvidin-3-glucoside (mg/L) concentration of Anhöhe as determined by HPLC over three seasons in the wines.

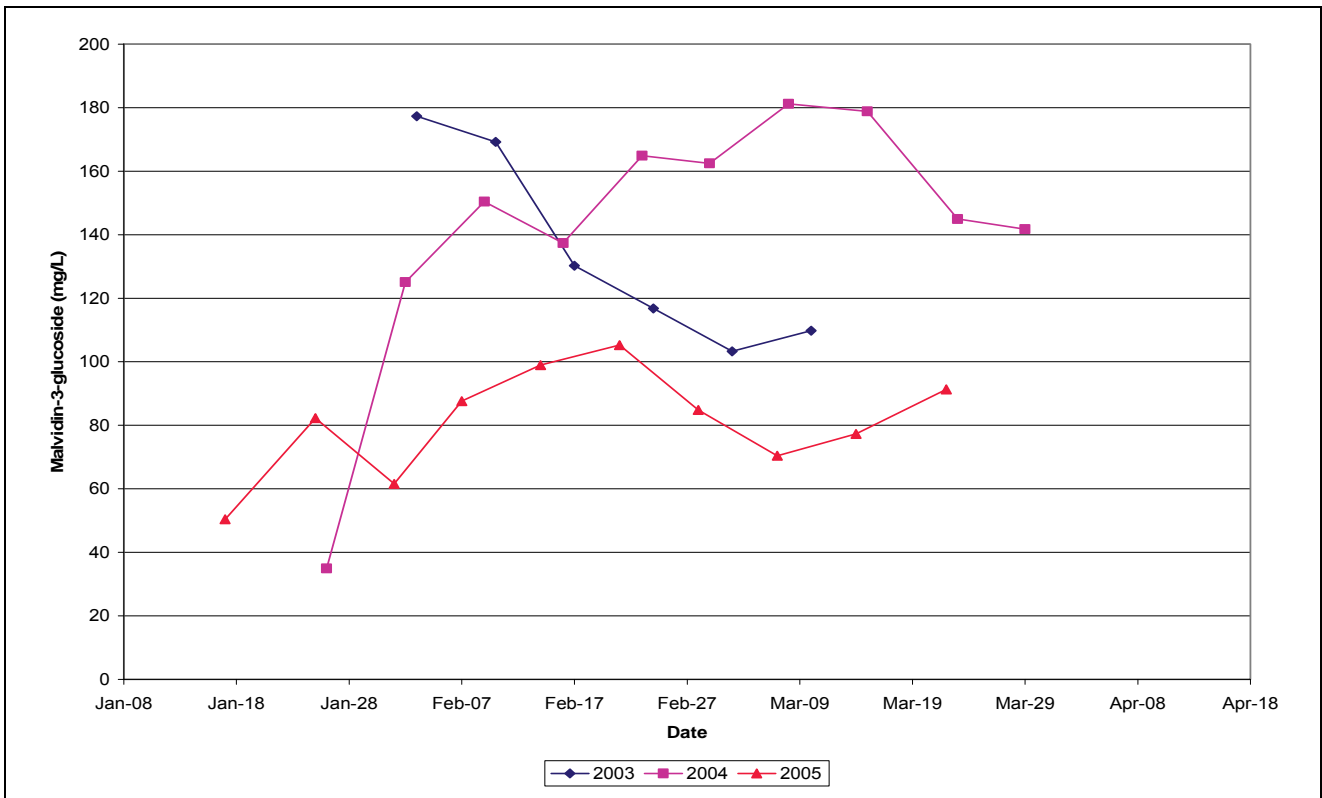


Figure 4.10 Malvidin-3-glucoside (mg/L) concentration of Plaisir de Merle as determined by HPLC over three seasons in the wines.

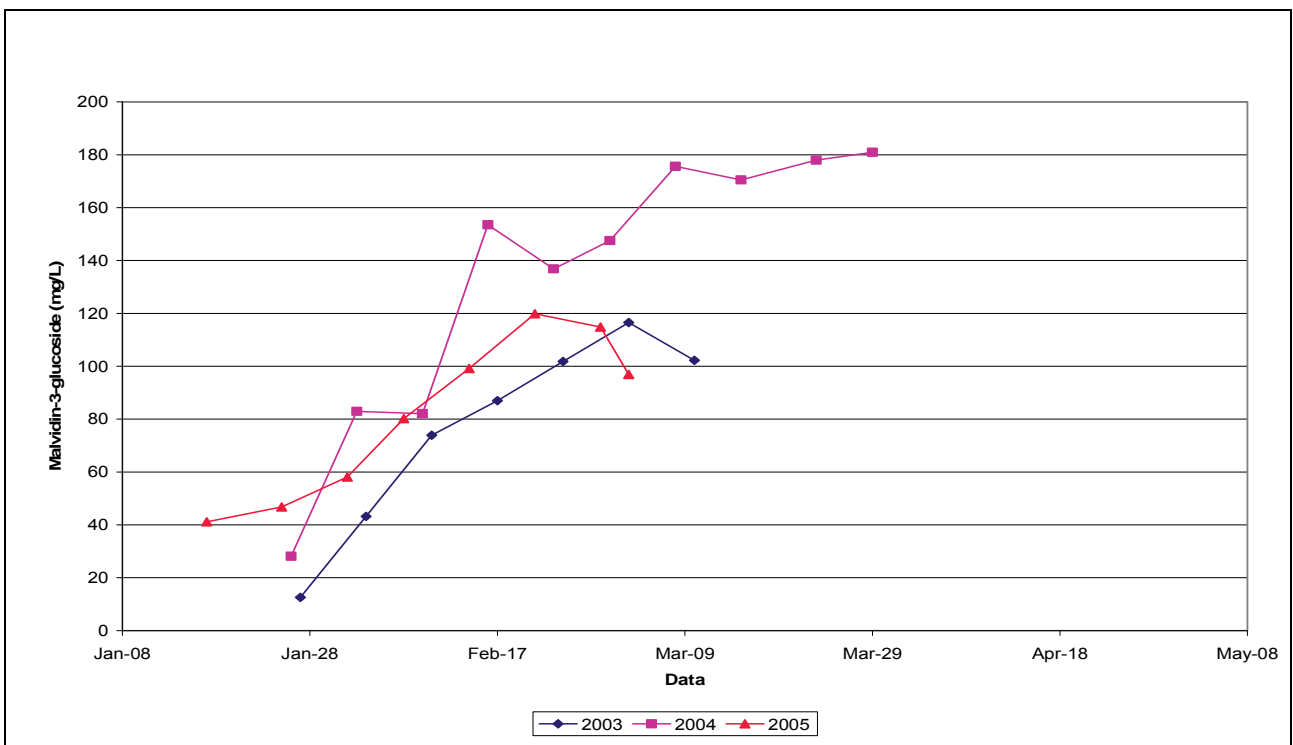


Figure 4.11 Malvidin-3-glucoside (mg/L) concentration of Nietvoorbij as determined by HPLC over three seasons in the wines.

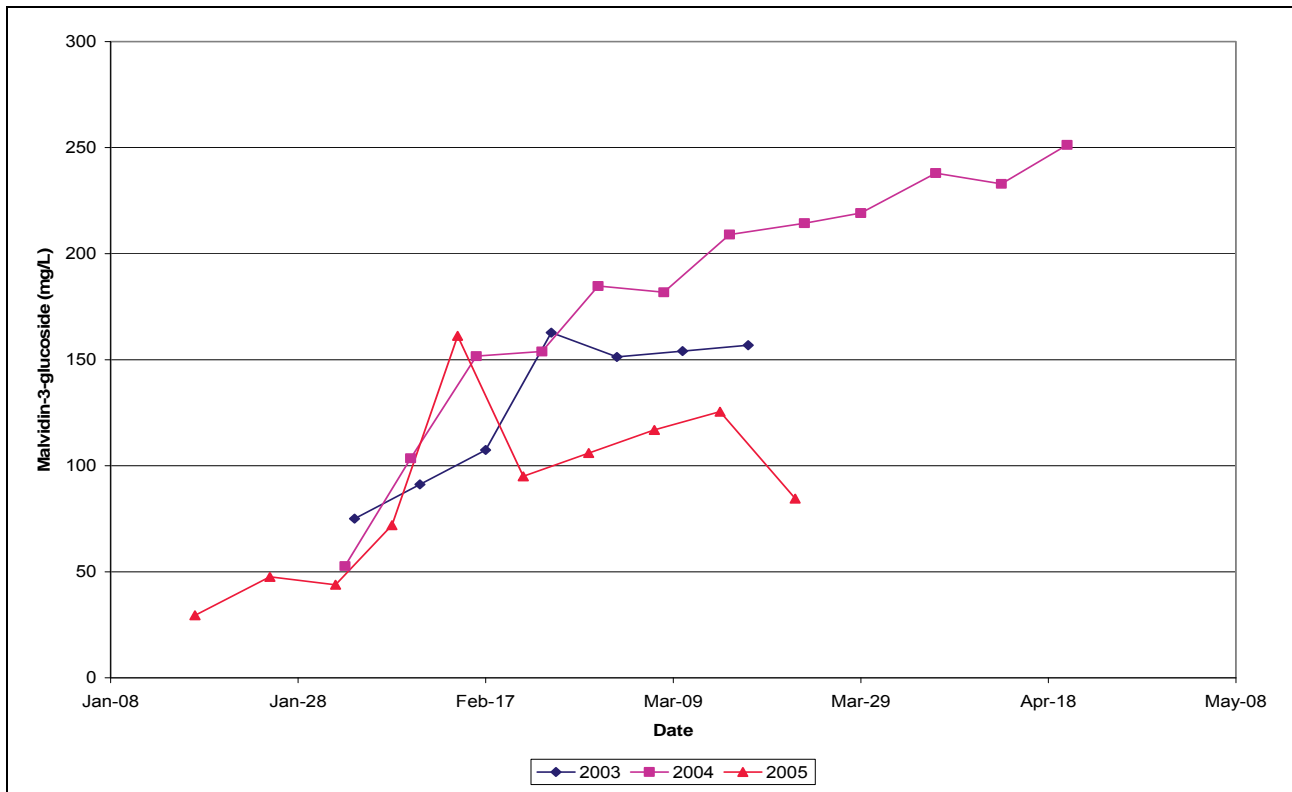


Figure 4.12 Malvidin-3-glucoside (mg/L) concentration of Morgenster as determined by HPLC over three seasons in the wines.

Table 4.2. Commercial harvest (CH) dates of the four vineyards

Vineyard	Area	CH 2003	CH 2004	CH 2005
Anhohe	Paarl	14/02	03/03	22/02
Morgenster	Durbanville	02/04	07/04	17/03
Nietvoorbij	Stellenbosch	19/03	02/04	03/03
Plaisir de Merle	Simondium	10/02	23/02	07/03

Total anthocyanins (mg/L) in the wines increased from onset of ripening until ripeness, but the increase in the wine was not found to be significant (Figure 4.13). However, the total anthocyanin (mg/L) of the wine during the 2005 season was significantly lower than the previous seasons (data not shown). This did not correspond to that found in the homogenate for the 2005 season where it was the highest (data not shown). Higher level of copigmentation or polymerization of the free anthocyanins during the 2005 season could be a possible reason or the variability of the pooled data between the vineyards influenced the significance of the increase.

Nietvoorbij had significantly lower levels of anthocyanins compared to the other three vineyards (Figure 4.14). A possible reason is that the leafroll-associated closterovirus complex reduced photosynthetic capacity through the reduction in carbon dioxide (CO₂) diffusion capacity, through the mesophyll and impacting on the carboxylation capacity of the vine (Sampol *et al.*, 2003). No leafroll virus symptoms were observed at the other vineyards during the three seasons.

No correlations between the total anthocyanin concentrations of the homogenate and the wine ($r = 0.01$, $p = 0.93$) was found.

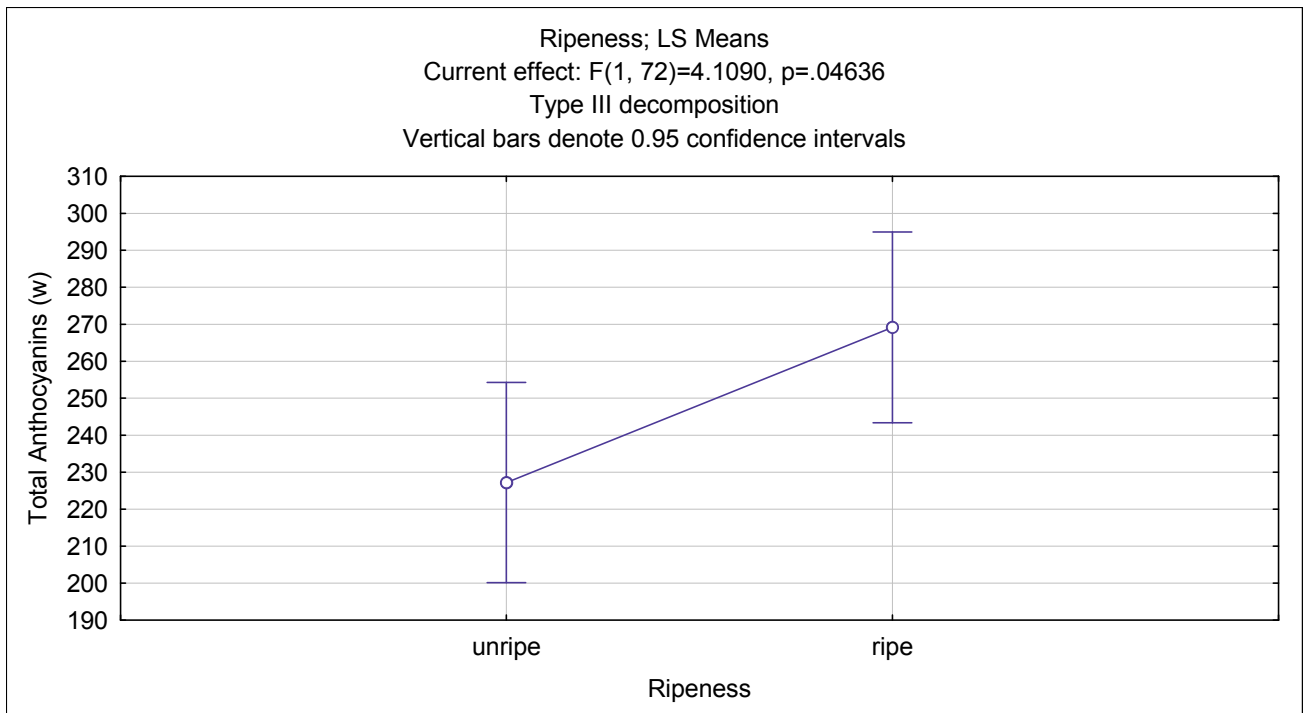


Figure 4.13 Total anthocyanin (mg/berry) increase found in wine by RP-HPLC analysis during the ripeness stages.

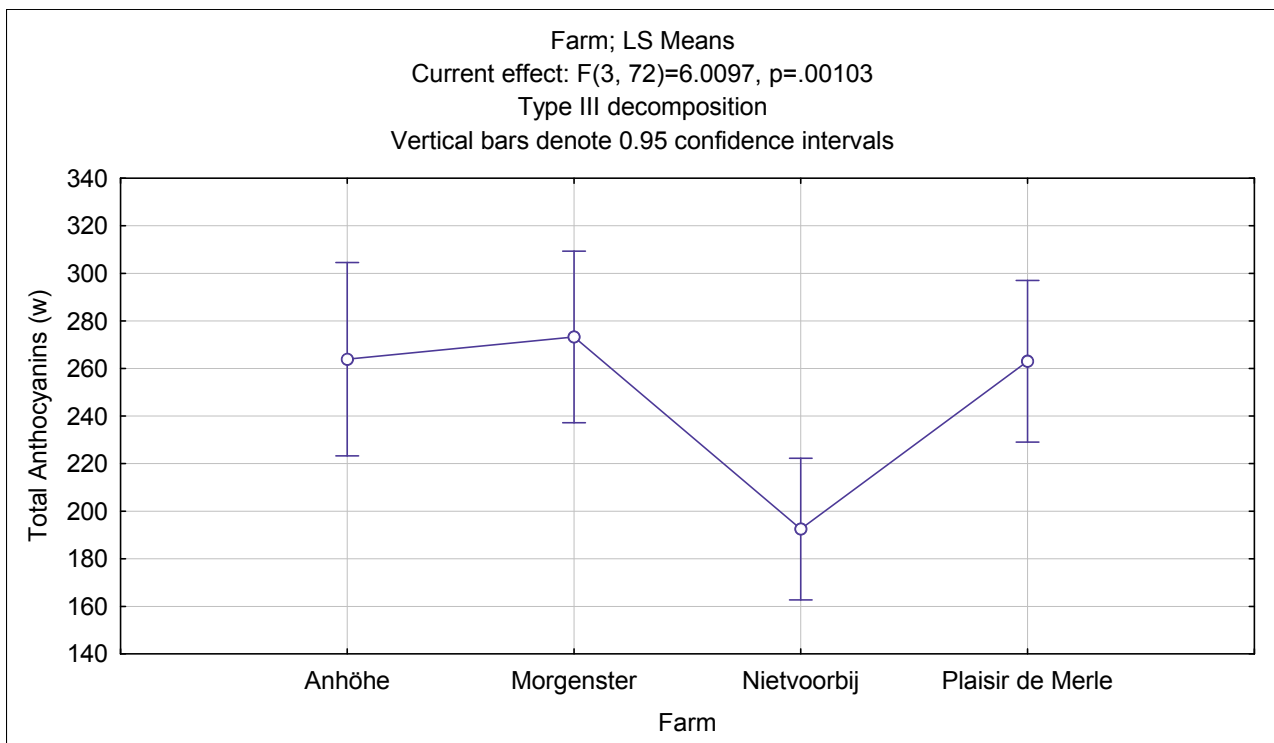


Figure 4.14 The average total anthocyanin (mg/berry) concentration of the different vineyards found in the wine by RP-HPLC analysis over all seasons.

4.4.4 PHENOLIC CONTENT

4.4.4.1 Flavan-3-ols and polyphenols

Catechin, epicatechin, proanthocyanins and polyphenols all decreased significantly with an increase in ripeness for all three seasons in all the vineyards (Table 4.3 to 4.6). This decrease of grape tannins during ripening corresponds with work done by other researchers on Cabernet Sauvignon and Shiraz (Kennedy *et al.*, 2000a; Kennedy *et al.*, 2000b; Kennedy *et al.*, 2001 and Downey *et al.*, 2003). Figure 4.15 illustrates the decrease of the catechin concentration per berry during the different ripening stages in the homogenate. The same trend was found in the corresponding wines (Figure 4.16). No significant difference was found between the seasons or the different vineyards for catechin concentration in the homogenate and wine at the different farms (data not shown).

Table 4.3 Phenolic components per berry as quantified by RP-HPLC in the homogenate for Anhöhe.

Date	Catechin	Epicatechin	Polyphenols	Sum proanthocyanidin	Quercetin-glucuronide	Sum of flavonols	Caftaric acid	Caffeic acid	Sum of hydroxycinnamic acid
	mg/berry								
Anhöhe 2003									
1301	0.064	0.018	0.429	0.075	0.011	0.011	0.019	0.007	0.029
2001	0.047	0.017	0.523	0.060	n/d	0.009	0.010	0.005	0.016
2701	0.043	0.013	0.617	0.047	n/d	0.046	0.003	n/d	0.005
0302	0.082	0.046	0.541	0.134	n/d	0.074	0.004	n/d	0.010
1002	0.069	0.033	1.565	0.108	n/d	0.056	n/d	n/d	0.002
1702	0.082	0.055	1.619	0.162	n/d	0.069	0.003	n/d	0.003
2402	0.042	0.063	0.433	0.163	0.019	0.080	n/d	n/d	n/d
0303	0.034	0.049	0.397	0.129	0.015	0.068	n/d	n/d	n/d
1003	0.034	0.049	0.443	0.130	0.018	0.078	n/d	n/d	n/d
Anhöhe 2004									
1901	0.419	0.087	0.035	0.673	0.000	0.026	0.050	0.016	0.079
2601	0.114	0.037	0.563	0.344	0.000	0.022	0.007	0.006	0.013
0202	0.129	0.064	0.616	0.324	0.026	0.076	0.016	0.008	0.027
0902	0.069	0.027	0.415	0.192	0.030	0.084	0.010	0.005	0.020
1602	0.091	0.046	0.105	0.191	0.023	0.064	0.011	0.006	0.020
2302	0.042	0.020	0.072	0.149	0.026	0.053	n/d	n/d	n/d
0103	0.030	n/d	0.082	0.038	0.015	0.037	n/d	n/d	n/d
0803	0.052	n/d	0.092	0.127	0.014	0.034	n/d	n/d	n/d
1503	0.043	n/d	0.061	0.124	0.012	0.027	n/d	n/d	n/d
2303	0.084	0.059	0.125	0.249	0.013	0.029	0.004	n/d	0.004
2903	0.029	0.060	0.121	0.212	n/d	0.023	n/d	n/d	n/d
Anhöhe 2005									
1701	0.095	0.083	0.545	0.256	0.000	0.064	0.004	n/d	0.004
2501	0.075	0.076	1.526	0.183	0.018	0.084	n/d	n/d	n/d
0102	n/d	0.057	0.945	0.137	0.011	0.069	n/d	n/d	n/d
0702	n/d	n/d	1.498	0.058	0.014	0.066	n/d	n/d	0.005
1402	0.031	n/d	0.750	0.066	0.018	0.035	n/d	n/d	n/d
2102	0.064	n/d	1.082	0.201	0.032	0.090	n/d	n/d	n/d
2802	0.056	0.076	1.080	0.348	0.064	0.127	n/d	n/d	n/d
0703	0.038	0.083	1.129	0.372	0.020	0.080	n/d	n/d	n/d
1403	0.100	0.076	1.167	0.494	0.055	0.110	n/d	0.000	n/d

* Values denote the average data for specific samples where the std was smaller then 10 % for triplicate measurement.

Table 4.4 Phenolic components per berry as quantified by RP-HPLC in the homogenate for Morgenster.

Date	Catechin	Epicatechin	Polyphenols	Sum proanthocyanidin	Quercetin-gluconoride	Sum of flavonols	Caftaric acid	Caffeic acid	Sum of hydroxycinnamic acid
	mg/berry								
	Morgenster 2003								
0302	0.229	0.051	2.874	0.152	n/d	0.047	0.056	0.029	0.100
1002	0.181	0.035	3.249	0.070	n/d	0.045	0.042	0.028	0.074
1702	0.117	0.083	1.339	0.209	n/d	0.050	n/d	n/d	0.016
2402	0.082	0.070	0.424	0.139	n/d	0.071	n/d	n/d	0.006
0303	0.070	0.022	0.434	0.045	n/d	0.081	n/d	n/d	0.004
1003	0.055	0.054	0.372	0.107	n/d	0.057	n/d	n/d	0.004
1703	0.072	n/d	0.381	n/d	n/d	0.048	n/d	n/d	0.006
2603	0.069	n/d	0.399	n/d	n/d	0.085	n/d	n/d	0.006
3103	0.068	n/d	0.461	n/d	0.006	0.078	n/d	n/d	0.005
0704	0.072	n/d	0.434	n/d	n/d	0.071	n/d	n/d	0.003
1404	0.063	0.058	0.477	0.116	n/d	0.058	n/d	n/d	0.005
2204	0.076	0.076	0.497	0.187	n/d	0.064	n/d	n/d	0.003
	Morgenster 2004								
1901	0.668	0.194	0.068	1.331	n/d	0.025	0.091	0.031	0.147
0202	0.581	0.260	0.844	0.652	n/d	0.029	0.044	0.019	0.074
0902	0.296	0.117	0.424	0.363	0.018	0.078	0.043	0.015	0.071
1602	0.161	0.045	0.458	0.131	0.000	0.043	0.040	0.016	0.068
2302	0.102	0.033	0.192	0.141	0.027	0.117	0.008	0.009	0.020
0103	0.080	n/d	0.188	0.004	0.021	0.074	0.005	0.009	0.015
0803	0.096	n/d	0.176	0.000	n/d	0.045	0.019	n/d	0.019
1503	0.106	n/d	0.145	0.000	0.015	0.049	0.019	n/d	0.019
2303	0.120	n/d	0.378	0.129	0.018	0.049	0.015	n/d	0.015
2903	0.080	0.128	n/d	0.296	n/d	0.036	n/d	n/d	n/d
0604	0.107	0.163	n/d	0.365	n/d	0.021	0.016	n/d	0.016
1304	0.071	0.162	n/d	0.323	0.018	0.052	n/d	n/d	n/d
2004	0.081	0.144	n/d	0.287	0.012	0.056	n/d	n/d	n/d
	Morgenster 2005								
1701	0.345	0.150	0.931	0.651	n/d	0.130	n/d	n/d	0.008
2501	0.129	0.113	2.219	0.350	n/d	0.152	n/d	n/d	0.000
0102	0.096	n/d	1.996	n/d	0.027	0.275	n/d	n/d	0.008
0702	n/d	n/d	2.121	n/d	n/d	0.135	n/d	n/d	0.007
1402	n/d	n/d	1.997	n/d	n/d	0.083	n/d	n/d	0.000
2102	0.056	n/d	1.773	0.134	n/d	0.077	n/d	n/d	0.008
2802	n/d	n/d	1.712	0.185	0.054	0.160	n/d	n/d	n/d
0703	n/d	n/d	2.118	0.231	0.077	0.197	n/d	n/d	0.009
1403	n/d	n/d	1.519	0.217	0.070	0.230	n/d	n/d	0.008
2203	n/d	n/d	1.657	0.235	0.051	0.105	n/d	n/d	n/d
3003	n/d	n/d	1.241	0.164	n/d	0.036	n/d	n/d	n/d

* Values denote the average data for specific samples where the std was smaller then 10 % for triplicate measurement.

Table 4.5 Phenolic components per berry as quantified by RP-HPLC in the homogenate for Nietvoorbij.

Date	Catechin	Epicatechin	Polyphenols	Sum proanthocyanidin	Quercetin-gluconoride	Sum of flavonols	Caftaric acid	Caffeic acid	Sum of hydroxycinnamic acid
	mg/berry								
	Nietvoorbij 2003								
2701	0.255	0.043	2.277	0.164	n/d	0.047	0.066	0.025	0.106
0302	0.217	0.015	3.095	0.091	n/d	0.047	0.058	0.028	0.096
1002	0.129	0.000	3.093	n/d	n/d	0.061	0.007	n/d	0.008
1702	0.130	0.016	2.743	0.087	n/d	0.062	0.007	n/d	0.007
2402	0.054	0.029	0.384	0.088	0.017	0.086	n/d	n/d	0.003
0303	0.062	n/d	0.513	0.010	n/d	0.062	n/d	n/d	0.005
1003	0.065	0.058	0.544	0.155	0.013	0.079	n/d	n/d	n/d
1703	0.059	n/d	0.369	0.033	0.016	0.071	n/d	n/d	n/d
2603	0.045	n/d	0.372	0.025	n/d	0.043	n/d	n/d	n/d
3103	0.059	n/d	0.323	0.034	0.011	0.058	n/d	n/d	n/d
0704	0.055	n/d	0.477	0.036	0.000	0.053	n/d	n/d	n/d
1404	0.055	0.052	0.555	0.169	0.000	0.046	n/d	n/d	n/d
2204	0.076	0.060	0.574	0.187	0.000	0.035	n/d	n/d	n/d
	Nietvoorbij 2004								
1901	0.697	0.176	0.503	1.465	n/d	0.038	0.092	0.030	0.146
2601	0.654	0.110	0.962	1.128	n/d	0.058	0.052	0.020	0.086
0202	0.536	0.169	0.900	0.477	0.028	0.138	0.047	0.018	0.079
0902	0.146	0.041	0.447	0.119	n/d	0.087	0.019	0.012	0.038
1602	0.072	0.028	0.293	0.088	n/d	0.056	0.041	0.019	0.075
2302	0.164	0.040	0.167	0.101	0.044	0.124	0.025	0.013	0.044
0103	0.071	n/d	0.190	n/d	0.046	0.134	0.023	n/d	0.023
0803	0.061	n/d	0.175	n/d	0.020	0.075	0.008	n/d	0.008
1503	0.055	n/d	0.130	0.032	0.024	0.074	0.009	n/d	0.009
2303	0.052	0.087	n/d	0.219	0.021	0.064	n/d	n/d	n/d
2903	0.119	0.107	n/d	0.269	0.024	0.082	n/d	n/d	n/d
0604	0.058	0.123	n/d	0.281	0.037	0.120	n/d	n/d	n/d
1304	0.064	0.115	n/d	0.259	n/d	0.026	n/d	n/d	n/d
1904	n/d	0.114	n/d	0.228	n/d	n/d	n/d	n/d	n/d
	Nietvoorbij 2005								
1701	0.207	0.156	1.154	0.517	0.000	0.116	0.023	0.026	0.049
2501	0.077	n/d	1.557	0.031	0.013	0.132	n/d	n/d	n/d
0102	0.063	n/d	1.672	0.000	0.035	0.236	n/d	n/d	n/d
0702	0.054	n/d	1.890	0.040	0.035	0.246	n/d	n/d	0.005
1402	0.064	n/d	1.918	0.000	0.052	0.186	n/d	n/d	n/d
2102	0.056	n/d	1.437	0.097	0.052	0.164	n/d	n/d	n/d
2802	n/d	n/d	1.616	0.123	0.087	0.337	n/d	n/d	n/d
0303	n/d	n/d	1.716	0.133	0.084	0.275	n/d	n/d	n/d

* Values denote the average data for specific samples where the std was smaller then 10 % for triplicate measurement.

Table 4.6 Phenolic components per berry as quantified by RP-HPLC in the homogenate for Plaisir de Merle.

Date	Catechin	Epicatechin	Polyphenols	Sum proanthocyanidin	Quercetin-gluconoride	Sum of flavonols	Caftaric acid	Caffeic acid	Sum of hydroxycinnamic acid
	mg/berry								
	Plaisir de Merle 2003								
1301	0.060	0.013	0.680	0.045	n/d	0.018	0.015	0.008	0.027
2001	0.052	0.013	0.649	0.046	n/d	0.035	0.008	0.006	0.016
2701	0.044	0.015	0.792	0.029	n/d	0.045	0.016	0.008	0.028
0302	0.145	0.055	2.798	0.157	n/d	0.111	0.020	n/d	0.027
1002	0.118	0.013	2.595	0.043	n/d	0.091	0.010	n/d	0.012
1702	0.102	0.030	2.339	0.068	n/d	0.106	0.003	n/d	0.003
2402	0.068	0.059	0.430	0.152	0.028	0.132	n/d	n/d	n/d
0303	0.045	0.046	0.357	0.109	0.021	0.107	n/d	n/d	n/d
1003	0.052	0.028	0.570	0.087	0.018	0.125	n/d	n/d	n/d
1703	0.053	0.052	0.580	0.141	n/d	0.050	n/d	n/d	n/d
2603	0.060	0.043	0.291	0.116	n/d	0.019	n/d	n/d	n/d
3103	0.055	0.035	0.527	0.100	n/d	0.038	n/d	n/d	n/d
0704	0.093	0.058	0.599	0.213	n/d	0.038	n/d	n/d	n/d
1404	0.105	0.052	0.480	0.191	n/d	0.027	n/d	n/d	n/d
	Plaisir de Merle 2004								
1901	0.997	0.342	0.930	2.054	0.005	0.055	0.086	0.025	0.132
2601	0.285	0.078	0.698	0.819	0.007	0.082	0.074	0.022	0.114
0202	0.198	0.090	0.983	0.215	0.041	0.123	0.054	0.018	0.091
0902	0.183	0.080	0.795	0.192	0.036	0.110	0.072	0.022	0.113
1602	0.126	0.039	0.180	0.111	0.032	0.123	0.040	0.013	0.060
2302	0.052	0.030	0.133	0.123	0.034	0.111	0.008	n/d	0.008
0103	0.112	n/d	0.169	n/d	0.015	0.061	0.032	n/d	0.032
0803	0.118	n/d	0.106	0.025	0.024	0.071	0.032	n/d	0.032
1503	0.094	n/d	0.163	n/d	0.029	0.099	0.027	n/d	0.027
2303	0.079	n/d	n/d	0.043	0.020	0.069	0.012	n/d	0.012
2903	0.097	n/d	n/d	0.232	0.025	0.068	0.017	n/d	0.017
	Plaisir de Merle 2005								
1701	0.113	0.068	1.627	0.224	0.015	0.190	n/d	n/d	n/d
2501	0.044	n/d	0.950	n/d	0.020	0.148	n/d	n/d	n/d
0102	n/d	n/d	2.044	n/d	0.039	0.257	n/d	n/d	n/d
0702	0.051	n/d	1.668	0.078	0.019	0.114	0.003	n/d	0.009
1402	n/d	n/d	1.566	0.089	n/d	0.099	n/d	n/d	n/d
2102	0.106	n/d	1.939	0.187	0.046	0.153	0.007	n/d	0.007
2802	0.060	n/d	1.768	0.148	0.064	0.214	n/d	n/d	n/d
0703	0.063	n/d	1.619	0.197	0.049	0.185	n/d	n/d	n/d
1403	0.044	n/d	1.206	0.038	0.047	0.132	n/d	n/d	n/d
2203	n/d	n/d	1.309	0.135	0.038	0.089	n/d	n/d	n/d

* Values denote the average data for specific samples where the std was smaller then 10 % for triplicate measurement.

Epicatechin (mg/berry) significantly decreased from the unripe to the ripe stage but showed an increase in the overripe stage; however this increase is not significant (Figure 4.17 and Tables 4.3 to 4.6). Singleton and Esau, (1969) reported that epicatechin are also found in the skin of grapes in varietally variable concentrations. Berry shrivelling could be responsible, as the concentrating effect could lead to an increase in concentration.

However, this was not observed in the other components except for the sum of proanthocyanidins. In homogenate and wine, Anhöhe had the highest epicatechin concentration, although it was not significant (data not shown).

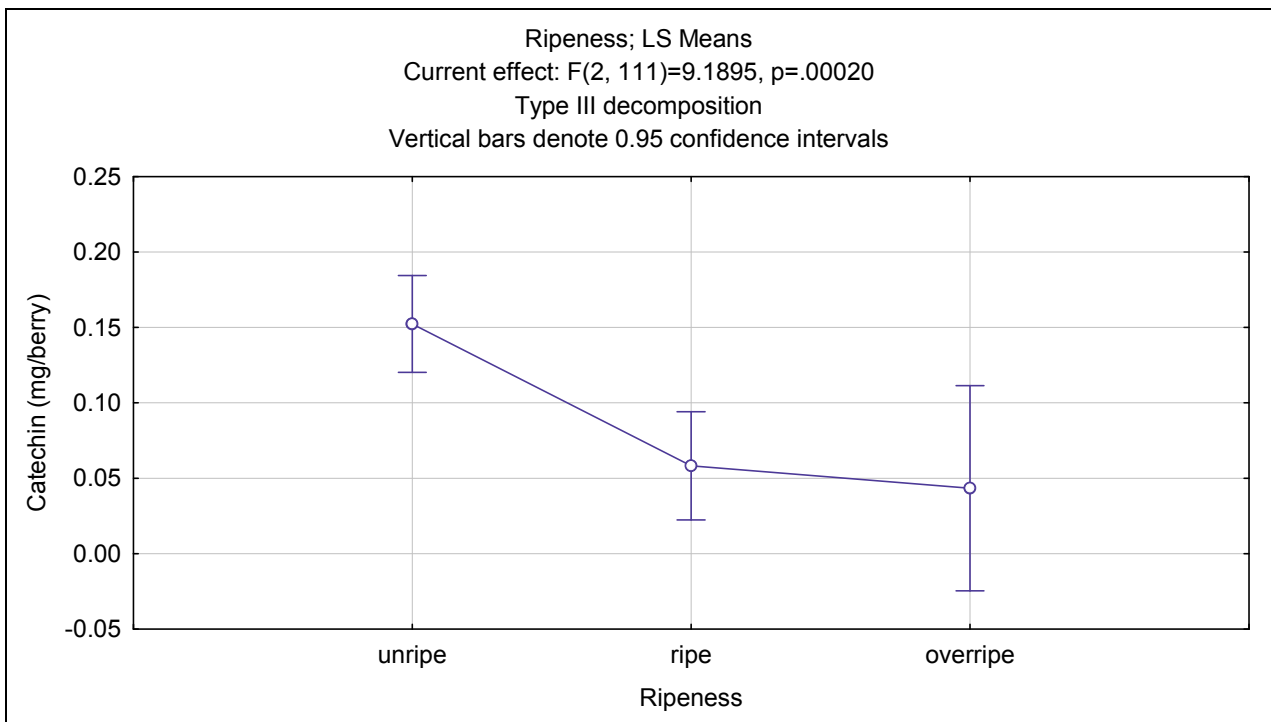


Figure 4.15 Graph showing the decrease of catechin during ripening in the homogenate.

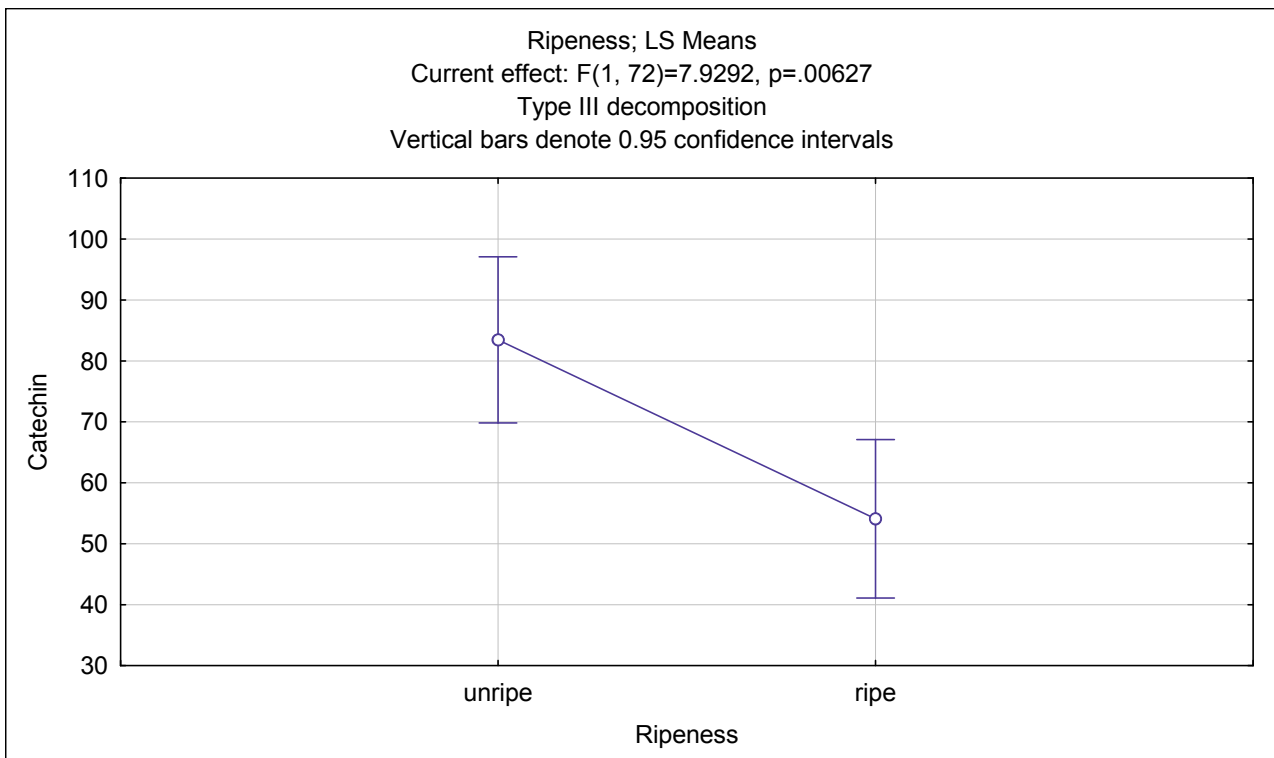


Figure 4.16 Graph showing the decrease of catechin (mg/L) during ripening in the corresponding wine.

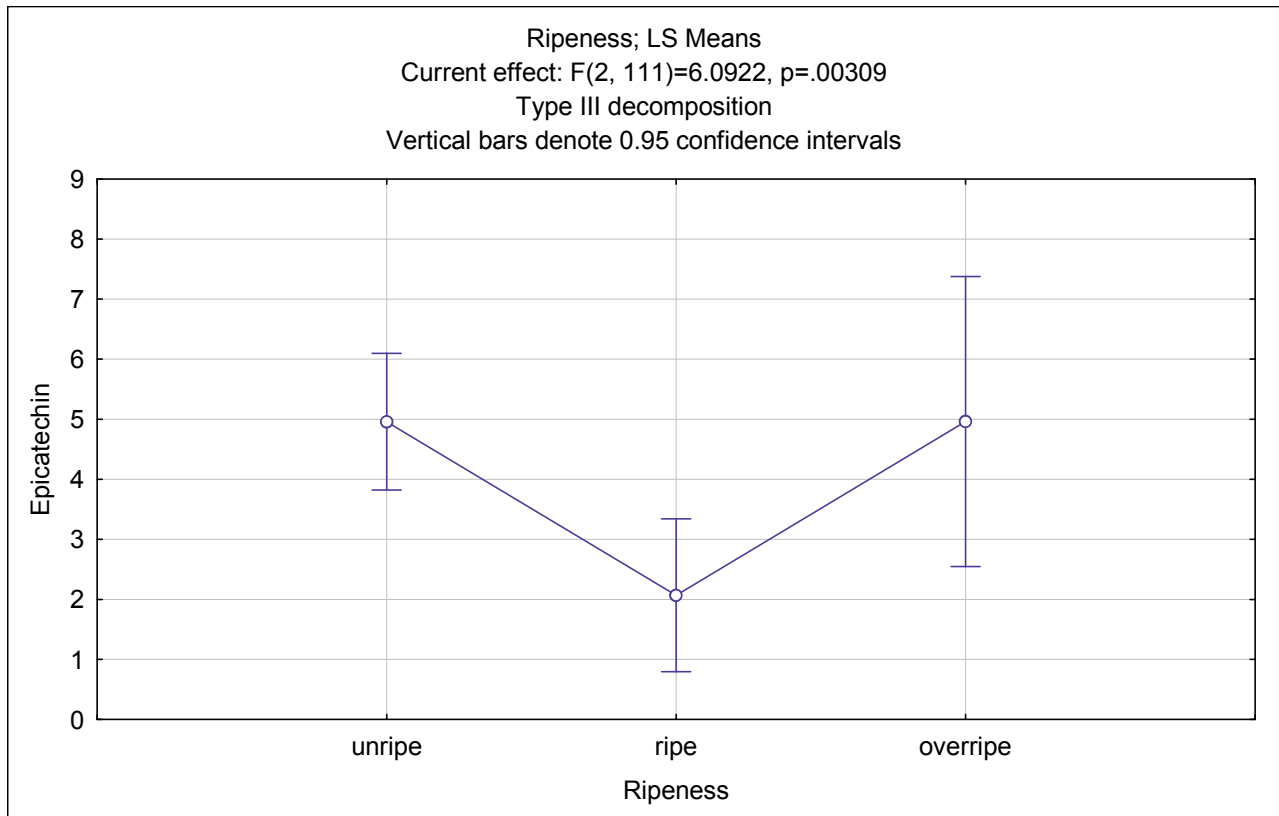


Figure 4.17 Epicatechin concentration (mg/berry) over the ripeness stages in the homogenate.

The sum of proanthocyanidins of the homogenate includes all non-monomeric flavan-3-ols that separated in individual peaks by using RP-HPLC separation (Kennedy *et al.*, 2001). A significant decrease in mg/berry was observed between unripe and ripe berries (data not shown). The 2004 season also differed significantly from the 2003 and 2005 seasons (Figure 4.18). During 2003 Anhöhe had a significantly higher proanthocyanidin concentration than Morgenster but not Nietvoorbij and Plaisir de Merle during 2003. Morgenster showed no significant difference between the 2003 and 2004 season (Figure 4.18). This decrease of grape tannins during ripening corresponds with work done by other researchers on Cabernet Sauvignon and Shiraz (Kennedy *et al.*, 2000a; Kennedy *et al.*, 2000b; Kennedy *et al.*, 2001 and Downey *et al.*, 2003). Figure 4.18 illustrates that the seasonal differences had a more significant impact than difference between vineyards.

Polyphenols followed the same trend during the ripening stages as seen for catechin and epicatechin (data not shown). The 2004 season contained significantly less polyphenols than the other seasons.

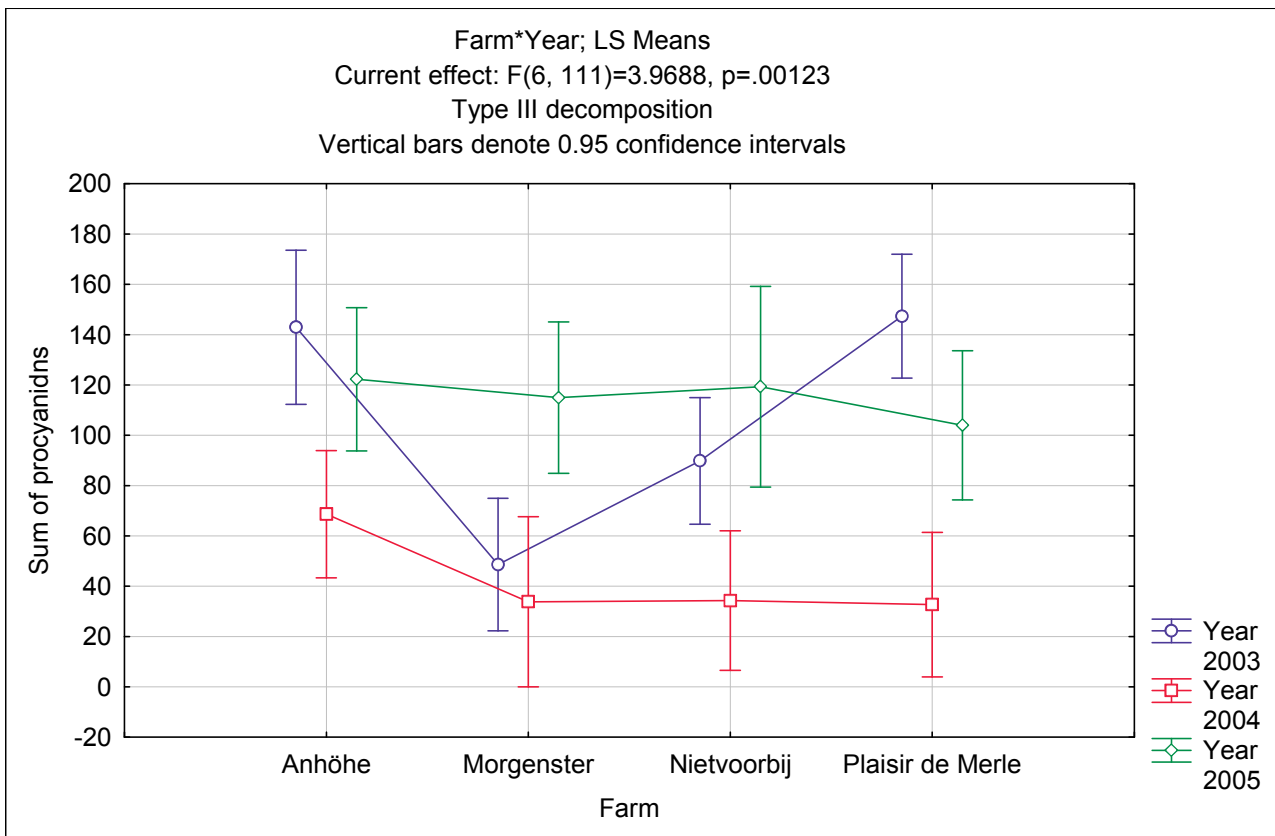


Figure 4.18 Sum of proanthocyanidins (mg/L) from the four vineyards illustrating the difference between seasons in the homogenate.

4.4.4.2 Flavonols

The sum of flavonols was measured using RP-HPLC in the homogenate and wine. The sum of flavonols is the sum of all flavonols, identified and unidentified. Quercetin-3-glucoside (Q3G) decreased significantly during ripening in the homogenate and wine (data not shown). Quercetin-3-glucuronide however increased during the ripening period with some seasonal variation (Table 4.3 to 4.6). During the 2005 season the sum of flavonols were significantly higher in the homogenate, but no significant difference was found in the wine (data not shown). Plaisir de Merle had a significantly higher flavonol concentration than Morgenster when all three seasons are pooled together (Figure 4.19). Morgenster had a denser canopy, during all three seasons, which shaded bunches to a greater degree compared to Plaisir de Merle and Anhöhe. Even though Anhöhe had a higher concentration of flavonols compared to Morgenster it was not significant (Figure 4.19). This shading effect could explain the lower levels observed at Morgenster and corresponds with data reported by Downey *et al.*, (2004), on decreased flavonol synthesis. Haselgrove *et al.*, (2000), reported that moderate exposure of bunches enhance the formation of quercetin post-veraison which could explain the higher concentrations at Anhöhe and Plaisir de Merle with the less dense canopies. Temperature was reported by Spayd *et al.*, (2002), to have little or no effect on the accumulation of flavonols. Flavonols

play an important part at the beginning of fermentation where they are copigmented to anthocyanins according to Ribéreau-Gayon *et al.*, (2001b).

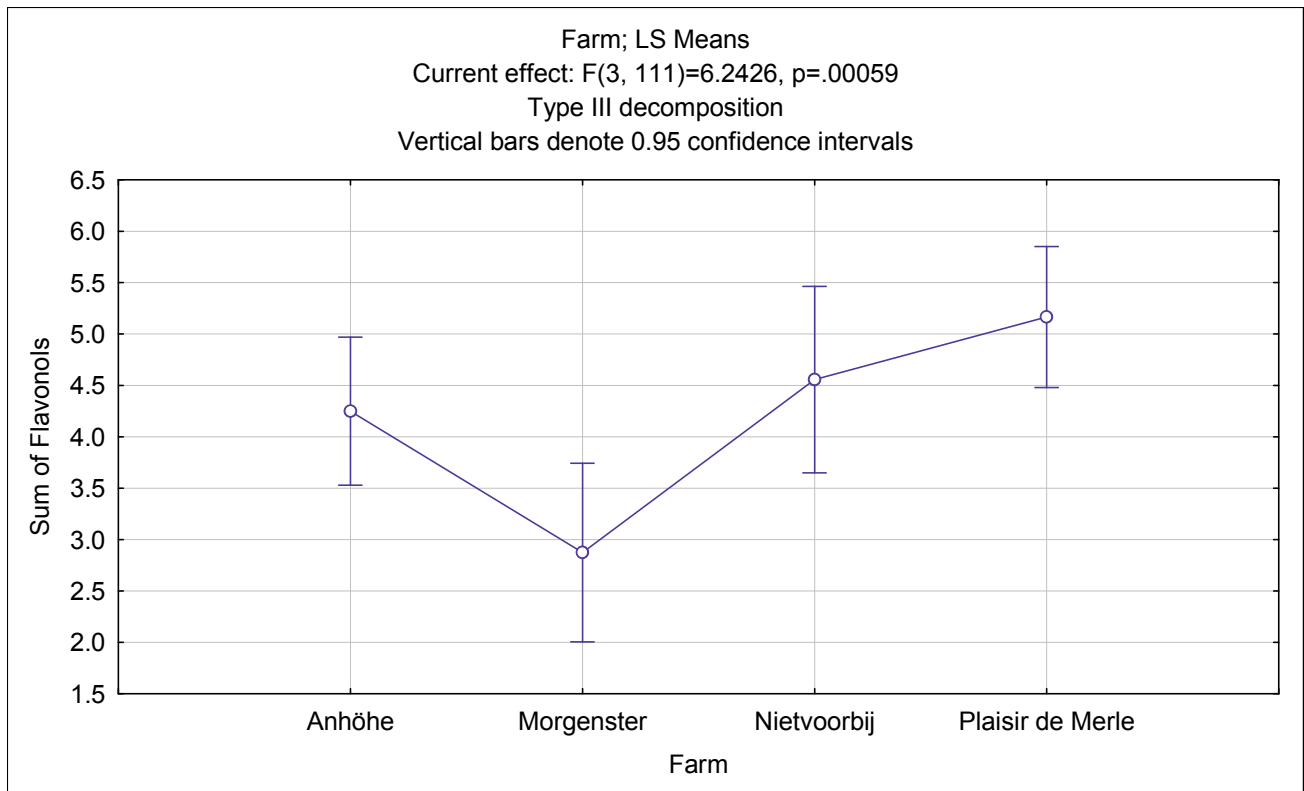


Figure 4.19 Differences in concentration of the sum of flavonols (mg/berry) of the homogenate between the vineyards.

4.4.4.3 Hydroxycinnamic acids

Caftaric acid decreased significantly from the beginning of the ripening period until ripeness in both homogenates and wines (data not shown). Caffeic acid followed the same trend as caftaric acid in the homogenate but no significant differences were found in the wine between unripe and ripe grapes (data not shown). No significant differences were found for both caftaric and caffeic acid concentration between the vineyard or seasons in either the homogenate or wine (data not shown). Boulton *et al.*, 1996, stated that caftaric acid losses are dependant on polyphenoloxidase (PPO) and exposure to air. The potential of exposure of the samples to oxygen during preparation could have an influence on the final concentration.

The sum of the hydroxycinnamic acids in the homogenate followed the same trends as both caftaric and caffeic acids (data not shown).

4.4.5 SENSORY EVALUATION

The wines were evaluated by a tasting panel. The best wines as determined by the panel corresponded to the maximum peaks in anthocyanin concentration as determined by the HPLC method (Figure 3.14). Spearman correlations between general wine quality and different phenolic components, incorporating all vineyards and seasons, were calculated as seen in table 4.7 for the homogenate and the wine. No strong correlations were found for the homogenate or wine with general wine quality (Table 4.7). Individual phenolic compounds did not correlate to any of the wine attributes evaluated (data not shown). A possible explanation for this could be the variability between the different vineyards and seasons used to determine the correlations.

The ratios between the total flavan-3-ol concentrations at 280 nm and anthocyanin concentrations at 520 nm were determined by using data obtained by the HPLC method and RP-HPLC in the homogenates and wines (Table 4.7). Wine quality correlated the strongest with the total flavan-3-ol to anthocyanin ratio determined by RP-HPLC analysis of the grape homogenates ($r = 0.40$, $p = 0.00$). This ratio varied between 1 and 3 for the wines rated as being the best quality.

Table 4.7 Spearman correlations between RP-HPLC analyzed components and general wine quality of all vineyards and seasons.

Homogenate		r	P
Catechin	mg/kg	-0.37	0.000
Epicatechin	mg/kg	-0.11	0.220
Polyphenols	mg/kg	-0.38	0.000
Sum of proanthocyanindins	mg/kg	-0.05	0.600
Total phenols	mg/kg	-0.39	0.000
Total anthocyanins	mg/kg	0.26	0.000
Wine		r	P
Catechin	mg/L	-0.33	0.000
Epicatechin	mg/L	-0.11	0.300
Polyphenols	mg/L	0.41	0.000
Sum of proanthocyanindins	mg/L	0.33	0.000
Total phenols	mg/L	0.27	0.000
Total anthocyanins	mg/L	0.14	0.200
Ratio		r	P
280:520 (HPLC)		-0.24	0.010
280:520 (h)		0.40	0.000
280:520 (w)		-0.31	0.000

In order to establish which phenolic grape parameters are important to distinguish unripe from ripe grapes, a principle component analysis (PCA) of the HPLC and tasting data was

done to investigate ripeness classes and variations (Figure 4.20 and 4.21). The homogenate classified as unripe separated from the ripe and overripe homogenate by PC1, although further separation was also found on PC2 (Figure 4.20). Limited separation was found between ripe and overripe classes on the PC2 axis. The ripeness stages of the grapes were separated by the following: catechin, epicatechin, procyanidins, caffeic and caftaric acids, sum of hydroxycinnamic acids, malvidin-3-glucoside and the individual anthocyanins (Figure 4.21).

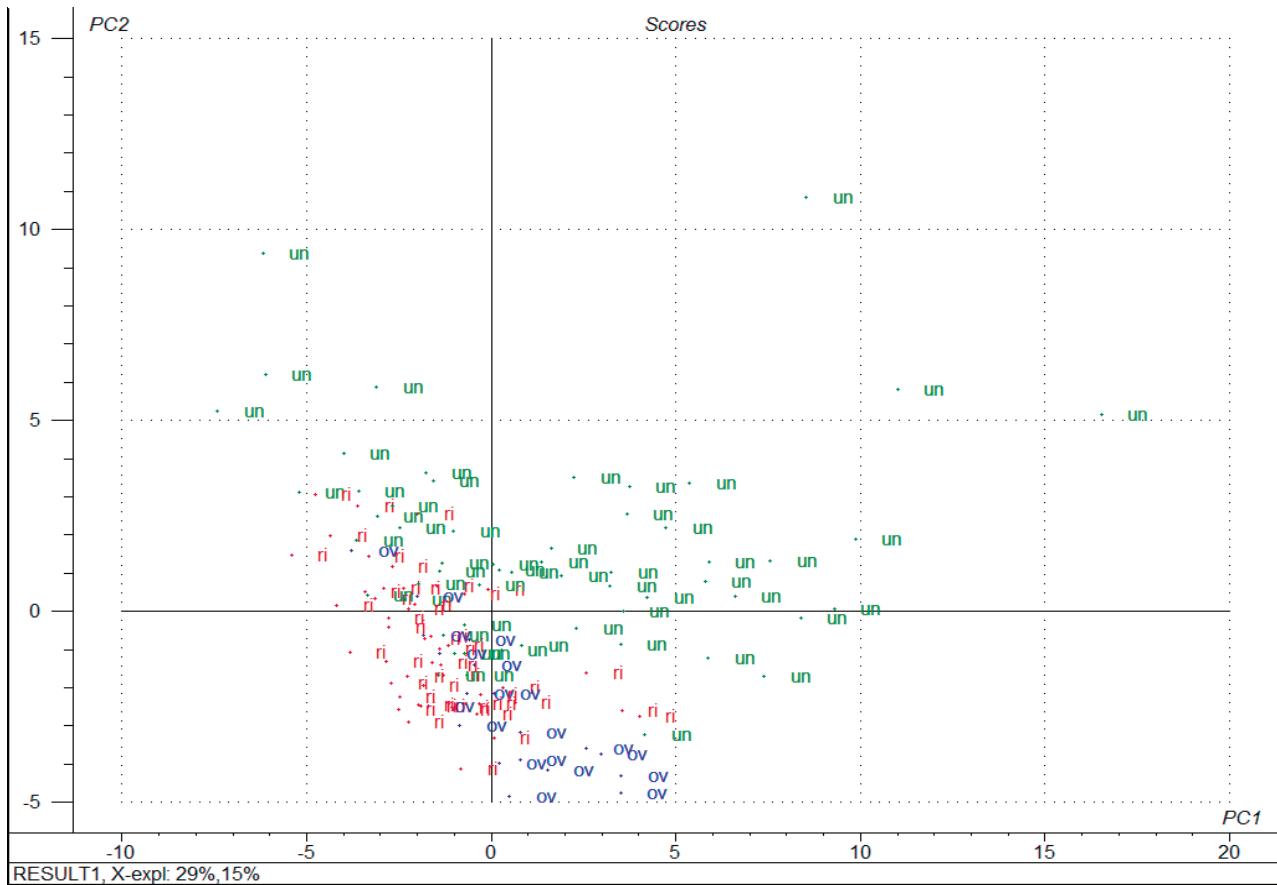


Figure 4.20 Score plot of the ripeness classes of the grape components where PC1 explains 29% of the variance in the data and PC2 15%.

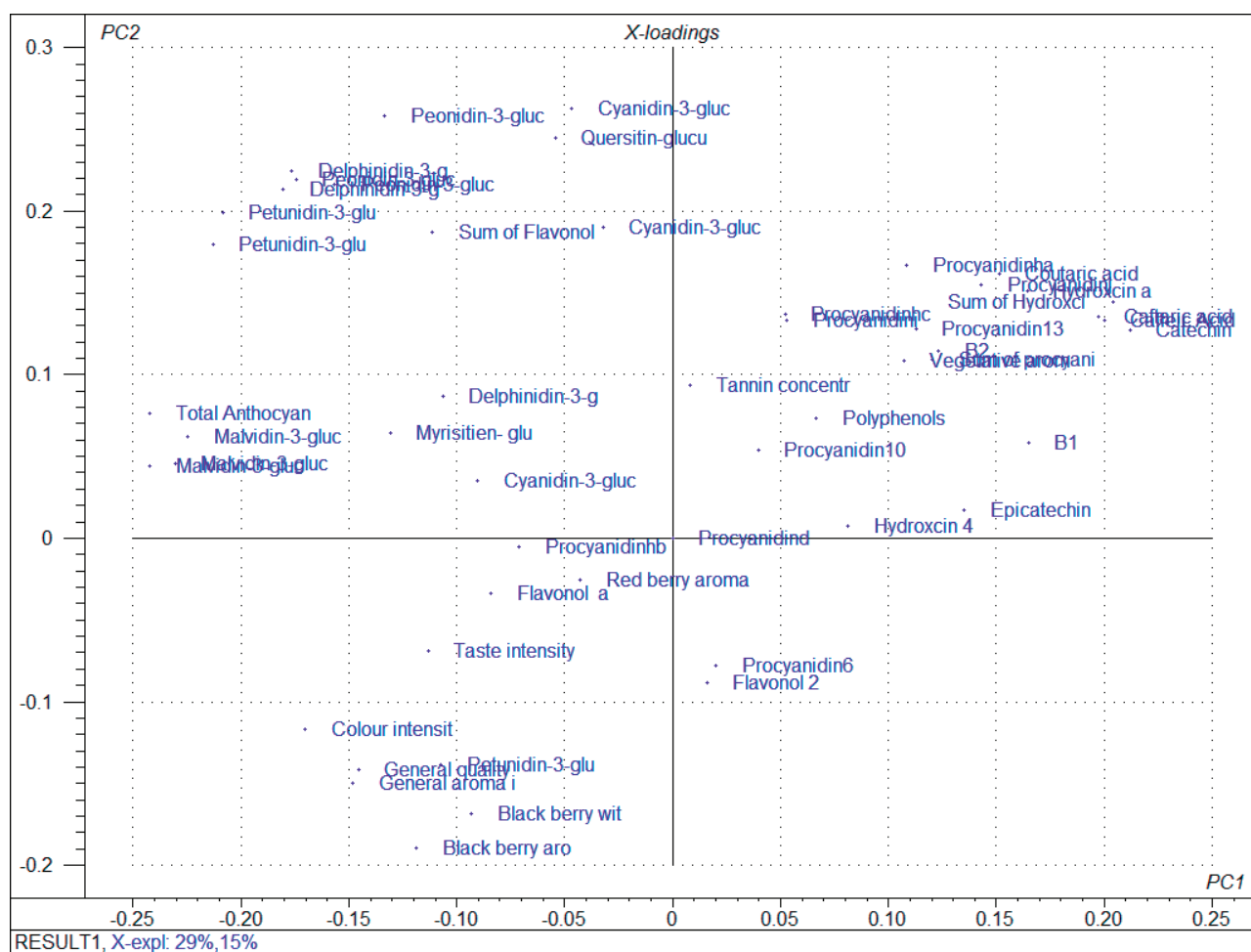


Figure 4.21 Loading plot of red and white grape must components where PC1 and PC2 explains 29% and 15% of the variance in the data respectively.

4.5 CONCLUSION

HPLC analyses of malvidin-3-glucoside and total anthocyanin content were followed in grapes and wines over three seasons in the four different wine growing areas. Strong trends were found for malvidin-3-glucoside and total anthocyanin when compared with delphinidin, petunidin and peonidin in the homogenate. This trend was not observed for peonidin in the wine as well as the trends were not as strong. Cyanidin did not have a correlation for either the homogenate or wine. Variability was found between the different ripeness levels as unripe grape anthocyanin levels significantly differed from the ripe level. Seasonal differences were also observed between the different vineyards but no significant difference could be identified. A larger data pool over at least 10 years might give a better indication of differences between the vineyards.

HPLC and HPLC determination of anthocyanin concentration followed comparable trends. Anthocyanin levels only correlated significantly in the homogenate with the general wine quality as determined by the sensory panel.

Flavan-3-ols (catechin and epicatechin) decreased from their maximum at veraison until harvest. Seasonal variations were observed. Sensory analyses did not show strong correlation between the flavan-3-ol compounds determined by RP-HPLC and the general wine quality. This does not mean that individual components did not influence the perception of the quality, but is most probably an indication that the phenolic compounds have a synergistic effect. Principal component analysis was able to group the different ripeness levels. The loadings plot illustrated the complexity of wine and that it is impossible to use only a single component to determine ripeness in grapes.

It is clear that further in-depth statistical analysis is needed to unravel the optimum combination of measurable components.

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Chapter 5

GENERAL DISCUSSION AND CONCLUSIONS

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5.1 DISCUSSION AND CONCLUSION

South Africa has a rich winemaking heritage that spans almost 350 years. The South African wine industry has grown into the eighth largest wine producer in the world and finds itself in an increasingly competitive world market where consumers expect wines of higher quality. Cabernet Sauvignon is the most planted red cultivar in the world as well as in South Africa and can be seen as the wine by which countries are judged. Finding the right (optimal) time to harvest grapes for wine of highest quality has been the quest of winemakers, viticulturists and agricultural scientists over centuries. In this quest to measure berry maturity objectively and accurately, tools have been developed to determine: total soluble solids (TSS) (Ribéreau-Gayon *et al.*, 2001a and b), titratable acidity (TA) (Boulton *et al.*, 1996), pH (Boulton *et al.*, 1996; Iland *et al.*, 2000), combinations of the afore mentioned (maturity index) (Amerine and Winkler, 1941; Du Plessis and Van Rooyen, 1982; Van Rooyen *et al.*, 1984), seed lignification percentage (Ristic and Iland, 2005), total berry anthocyanin concentration (Iland *et al.*, 2000), glycosyl-glucose (G-G) method (Francis *et al.*, 1998, 1999), extractability potential (Glories, 2001) and the pH shifting and SO₂ bleaching, first used by Ribéreau-Gayon and Stonestreet (Ough and Amerine, 1988). However regional differences play an important role in the accuracy of a particular maturity indicator under a specific set of climatic conditions or for a wine style. Thus, criteria found suitable for one region or wine style in a specific region can not be imposed on another region to the same effect.

The aim of this study was to evaluate suitable, practical maturity parameters or combinations thereof, for Cabernet Sauvignon under South African conditions. This study focused on effective measurements (seed lignification, Balling, pH, titratable acidity, maturity index, phenolic and anthocyanin concentration) of changing berry maturity and prediction of general wine quality.

Investigation of seed lignification as a maturity indicator has been carried out in Australia by Ristic and Iland (2005), but no literature was found to the effect of implementation in South Africa. Seed lignification percentages were found to vary between 59% and 80% over seasons 2003 and 2005 at commercial harvest. But due to the longer ripening season of 2004, these values increased to between 73% and 91% at commercial harvest. Balling values at the commercial harvest dates of all three season were however similar, ranging between 24°B and 26°B. Seasonal variability influenced lignification of the seeds significantly but not the TSS. Investigation over a longer period and with different viticultural practices could determine the optimal window for berry maturity of Cabernet Sauvignon under South African conditions using seed lignification. From this study however it was clear that seed lignification was not a good indicator for Cabernet Sauvignon berry maturity.

TSS and pH increased and TA decreased during all seasons and vineyards and conformed to that found in previous literature (Boulton *et al.*, 1996; Ribéreau-Gayon *et al.*, 2001a). Organic acids (tartaric and malic acid) decreased during ripening over all seasons. Malic acid concentrations in the grapes from Anhöhe and Plaisir de Merle had malic acid levels that decreased to less than 1 g/L over the season, but in the grapes from Morgenster never decreased to below 2 g/L. The dense canopies observed at Morgenster when compared to Anhöhe or Plaisir de Merle most likely contributed to this. Potassium (K^+) levels followed over the seasons varied between 1500 mg/L at the beginning of ripening and 3500 mg/L in overripe berries at Anhöhe over all three seasons. These high levels were also observed at the other vineyards. The increase in concentration coincided with an increase in pH and the salification of the organic acids most likely contributed to this.

Three maturity indexes (Balling / TA; Balling \times pH; Balling \times pH²) were investigated, that had been evaluated previously in South Africa. The best wine values were used to determine the optimal maturity index values for this study. Maturity values for the best wines varied between 88 and 101 (Balling \times pH) for Anhöhe during 2003 and 2005 seasons, but increased too between 97 and 107 (Balling \times pH) for 2004. Morgenster was found to be the only vineyard to consistently give values that corresponded with previously reported data. The higher malic acid values and TSS levels that never exceeded 25°B in this vineyard could be responsible for this. With a trend towards picking grapes at higher TSS levels for phenolic ripeness, the expectation would be to find consistently higher values compared to those from the past. This was not the case in all vineyards or seasons. Anhöhe and Plaisir de Merle had maturity levels higher than 4.0 (Balling / TA) range set in previous research. Pinotage was used during these trails and the difference between that reported cultivar and Cabernet Sauvignon used in this study could account for the difference, as it was previously shown that cultivars differ.

Anthocyanin concentrations were determined in the vineyards over the three seasons using the method described by Iland *et al.*, (2000). Seasonal differences were found in all four vineyards. The anthocyanin concentration per gram berry was higher at Plaisir de Merle and Anhöhe across all seasons. However anthocyanin concentration per berry at Morgenster was found to be the highest. The difference in berry size between Morgenster (1.82 g – 2004) and Anhöhe (0.95 g - 2004) influenced the concentration. The ratio between, skin-to-pulp is higher in the smaller berries from Anhöhe and leads to an increase in phenolic concentration compared to the bigger berries from Morgenster. It corresponds to previous research, that water deficits during early stages of berry development influences the final berry size. The leaf water potential of Anhöhe (-1.9MPa) compared to that of Morgenster (-0.95MPa) during the 2003 season illustrates this.

Grape skins (G) were used to make an artificial wine that was evaluated by an expert panel. Small scale wine were also made from the grapes and evaluated by the same tasting panel and rated for colour intensity, acidity, astringency, vegetative, red berry, black berry and spicy aroma, general wine intensity, fullness and overall quality. TSS

(Balling) followed trends with spicy aroma ($r = 0.50$, $p = 0.0001$), general aroma intensity ($r = 0.61$, $p < 0.0001$) and general wine quality ($r = 0.56$, $p = 0.0$), confirming that TSS (Balling) alone cannot be used as a good quality indicator. For Balling to be seen as a singular quality indicator, r – values would have to be above 0.95. The maturity indexes also followed trends well with general wine quality for all vineyards together (Balling (°B) X pH^2 , $r = 0.62$; $p = 0.00$) or separately. Colour intensity as derived by the tasting panel for grape skin tasting correlated well with the general wine quality ($r = 0.69$, $p = 0.00$). Differences between vineyards were also observed.

Colour intensity, berry aroma and spicy aroma attributes showed a correlation with general wine quality in the grape skins tasting as well as the small scale wines. Seasonal and regional differences influenced the perception of the criteria evaluated by grape skins tasting. Grape skins tasting could be used to give an indication of potential quality at the specific sampling date. But the time consuming preparation of samples and variability between tasters make this method not suitable for large commercial cellars.

Tasting values from the small scale wines showed good correlations with general wine quality for colour intensity ($r = 0.84$; $p < 0.001$), general aroma intensity ($r = 0.82$; $p < 0.0001$), berry aroma intensity ($r = 0.81$; $p = 0.0001$), spicy aromas ($r = 0.76$; $p < 0.0001$) and fullness ($r = 0.92$; $p < 0.0001$). The wines that were rated significantly better than others from the same farm corresponded to those made from grapes at maximum anthocyanin and colour levels. Thus in this study grape and wine colour influenced the perception of general wine quality.

In order to establish what parameters were important to distinguish between unripe and ripe grapes, principle component analysis (PCA) were performed on all analytical and sensory data. Parameters were removed from the data set, until the unripe and ripe grapes could still be separated with the fewest possible parameters. A combination of the following parameters, TSS, TA, pH, K^+ , tartaric and malic acid separated the unripe and ripe grapes, although the addition of grape colour improved the separation even better. The complexity of berry maturity can not be measured by only one single parameter. Even if a specific parameter works for a specific site or vineyard, it may not guarantee that it would work for another vineyard somewhere else.

RP-HPLC analysis was also done on the homogenates and wines to determine individual phenolic and anthocyanin components during ripening in the four vineyards. Malvidin-3-glucoside and total anthocyanin followed comparable trends to that found for the Iland method. The increase in total anthocyanin per berry in the homogenate for all vineyards was significant from the unripe to ripe stage. This was not found in wine as the increase proved not to be significant. This is most likely because the wine from Nietvoorbij had total anthocyanin levels that were significantly lower than the other vineyards. Leafroll virus infection was observed in the vineyard during the three seasons. No correlation could be found between the total anthocyanin concentration of the homogenates and the wines ($r = 0.06$, $p = 0.56$). The best wines were plotted on the anthocyanin curves of the homogenate and wines and corresponded to that found with the Iland method. Best wines

were found at the maximum anthocyanin concentrations for the vineyards. Flavan-3-ols (catechin, epicatechin) and polymeric phenols were found to significantly decrease from after veraison until ripeness in all vineyards. This trend was also observed for flavonols (including quercetin-3-glucuronide) and hydroxycinnamic acids (including caffeic and ferulic acid). The known phenolic compounds analysed by RP-HPLC were used to investigate if a correlation existed between the individual components and general wine quality. No correlations were found for any of the components and the general wine quality. However a trend ($r = 0.40$, $p=0.00$) was observed between the ratio of total flavan-3-ols (RP-HPLC) and total anthocyanins (RP-HPLC) and wine quality.

There were strong correlations between sugar content, colour and quality perception in grapes and the resulting wines. The best Cabernet Sauvignon wines were made from grapes rated highly for colour intensity, red berry and black berry with spice aromas. Seasonal differences resulted in larger variance in grape composition than grapes originating from vineyards in different climatic zones. This highlights the difficulties in pinpointing a specific parameter to indicate optimal maturity. It is much more likely that a group of parameters will be found which can effectively indicate the optimal time to harvest for a specific wine style. The range of identified parameters gave promising results and could differentiate to some extent between ripeness levels of grapes and wine. The parameters that could consistently predict ripeness over seasonal and climatic variance will be investigated further in the coming seasons.

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