

Grape and wine phenolic composition as a result of training system and canopy modification in *Vitis vinifera* L.cv Shiraz.

by

Petrus Johannes de Beer



Thesis presented in partial fulfilment of the requirements for the degree of
Master of Sciences in Agriculture

at

Stellenbosch University

Department of Viticulture and Oenology, Faculty of AgriSciences

Supervisor: Prof Wessel du Toit

Co-supervisor: Dr Albert Strever

December 2015

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 20/11/2015

Abstract

Non-volatile phenols, such as anthocyanins and tannins, are important parameters used in measuring the quality of red wine, as they are the main components influencing red wine colour and astringency. The Smart-Dyson (SD) training system, as developed by Dr Richard Smart and John Dyson, has previously been investigated as an alternative to the vertical shoot positioning (VSP) training system for vigorous vines, as it has the effect of bringing the vine “into balance” and has been shown to increase grape yield. The effects of the SD training system on the non-volatile phenols of the grapes, and how these treatment differences influence the wine, have been investigated in international studies, but limited studies have been done under South African conditions.

The first aim of this study was to assess differences in the non-volatile phenol composition of Shiraz grapes at harvest originating from a Reduced, VSP or SD training system and to assess whether these differences are reflected in the wines between treatments. Between these selected treatments it was found through spectrophotometer and HPLC analysis that the SD system may sometimes lead to a lower concentration of phenols in wine, although the physical structure of the SD system is expected to be more conducive to a better microclimate to enhance the phenolic concentration. The reduced treatment was also added, as it is a method for reducing vegetative growth by physically removing vegetative matter from the plant. This also leads to a better microclimate, but may have a negative effect on the yield.

The second aim of the study was to examine how the differences between the reduced, SD- and VSP treatments in wine were affected by ageing. The reaction rates of the different non-volatile phenols differ and thus their interaction during wine ageing might differ. This will affect the ageing potential, depending on the relative concentrations of the different phenols. However, the relative differences between the treatments remained unchanged during ageing.

The final aim of this study was to look at whether the treatment differences in the wine could be perceived sensorially. As sensory perception is ultimately the main parameter by which wine quality is judged by the consumer, it is important to know if analytical differences are reflected sensorially. When the wines were tasted, the panel could in general not find an association between the treatments.

The results generated from this study show that there were some differences regarding non-volatile phenols between the, Reduced canopy treatment and SD- and the VSP training system treatments. It still has to be investigated how management practices relating to these training systems can affect these differences.

Opsomming

Nie-vlugtige fenole, soos antosianiene en tanniene, is belangrike parameters wat gebruik word om die kwaliteit van rooi wyn te meet, aangesien hulle die vernaamste komponente is wat 'n invloed op die kleur en vrugtheid van rooi wyn het. Die Smart-Dyson (SD) opleistelsel, wat deur dr Richard Smart en John Dyson ontwikkel is, is reeds as 'n alternatief tot die vertikale loot posisionering (VSP) stelsel vir geil wingerdstokke ondersoek, aangesien die effek daarvan is om die wingerdstok in balans te bring en daar is ook getoon dat dit druif opbrengs verhoog. Die invloed van die Smart-Dyson stelsel op die nie-vlugtige fenole van die druive, en hoe hierdie verskille die wyn beïnvloed, is reeds in internasionale studies ondersoek, maar daar is beperkte studies daarvoor onder Suid-Afrikaanse toestande.

Die eerste doelwit van hierdie studie was dus om die verskille in die nie-vlugtige fenol samestelling van Shiraz-druive afkomstig van 'n VSP- of SD-opleistelsel te ondersoek en hoe hierdie verskille in die wyne weerspieël word. Tussen die gekose behandelings van 'n verminderde behandeling, kontrole VSP en Smart-Dyson behandeling is daar gevind dat die SD-stelsel soms kan lei tot 'n laer konsentrasie van fenole deur spektrofotometriese en HPLC analyses, hoewel die struktuur van die SD-stelsel veronderstel is om voordelig te wees vir 'n beter mikroklimaat, wat die fenol konsentrasie sal verhoog. Die verminderde behandeling is ook ingesluit, aangesien dit 'n metode is waarvolgens vegetatiewe groei verminder kan word deur vegetatiewe materiaal fisies van die plant te verwyder. Dit lei ook tot 'n beter mikroklimaat, maar het moontlik 'n negatiewe effek op die opbrengs.

Die tweede doelwit van die studie was om te ondersoek hoe die verskille tussen die SD- en VSP-behandelings deur veroudering beïnvloed word. Die reaksietempo's van die verskillende nie-vlugtige fenole verskil, en dit is dus moontlik dat hulle interaksie tydens wynveroudering ook sal verskil. Dit sal die verouderingspotensiaal beïnvloed op grond van die relatiewe konsentrasies van die verskillende fenole. Daar is wel gevind dat die relatiewe verskille tussen die behandelings dieselfde gebly het met veroudering.

Die finale doelwit van die studie was om ondersoek in te stel na die moontlikheid dat die verskille tussen die behandelings sensories waargeneem kan word. Aangesien sensoriese persepsie die uiteindelijke parameter is waarvolgens wyn deur die verbruiker beoordeel word, is dit belangrik om te weet of analitiese verskille sensories weerspieël word. Toe die wyne geproe is kon die paneel nie tussen die behandelings onderskei nie.

Die resultate wat deur hierdie studie gegenereer is, wys dat daar verskille is met betrekking tot nie-vlugtige fenole tussen die SD- en die VSP-opleistelsels. Daar moet nog ondersoek word hoe bestuurspraktyke wat verband hou met hierdie opleistelsels hierdie verskille kan beïnvloed.

To my family, who supported me throughout this endeavour, my friends Mias, Gys and Albé,
who kept me from working too hard, and my dear wife Marené,
who encouraged me to work when I did not want to.

Biographical sketch

Petri de Beer was born on 20 December 1988 in Potchefstroom, North West, South Africa and matriculated at Potchefstroom Gymnasium in 2007. He obtained his BScAgric degree in Viticulture and Oenology at Stellenbosch University in 2011, and enrolled for his MScAgric in Oenology at the same university in 2012.

Acknowledgements

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

- Prof Wessel du Toit – Supervisor
- Dr Albert Strever – Co-supervisor
- Marisa Nell – for technical assistance
- Edmund and Andy – for help in the experimental cellar
- Riaan Wassung– for the use of his cellar
- All the technical staff at the department
- THRIP and WineTech – for the funding that made all of this possible
- Prof Koos van Rensburg – for technical assistance with the manuscript
- Annelie and Attie de Beer – for their technical assistance

Preface

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

Chapter 1 **Introduction and project aims**

Chapter 2 **Literature review**
Overview of non-volatile phenols in red grapes and wine

Chapter 3 **Results**
Grape and wine phenolic composition as a result of training system and canopy modification in *Vitis vinifera* L.cv Shiraz.

Chapter 4 **General discussion and conclusions**

Table of Contents

Chapter 1: Introduction and project aims

1.1	Introduction	1
1.2	Project aims	2
1.3	Literature cited	3

Chapter 2: Literature review

2.1	Introduction	6
2.2	Grape and wine phenolics	6
2.4	Viticultural factors affecting grape phenolics	8
2.4.1	Microclimate factors.....	8
2.4.2	The effects of yield vs. effectively exposed leaf area on grapes	8
2.4.3	Effect of leaf removal on phenols	9
2.4.4	Overview of training systems investigated in this study	9
2.4.4.1	Vertical shoot positioning.....	9
2.4.4.2	Smart-Dyson.....	10
2.5	Biosynthesis of phenolics throughout the ripening of grapes.....	10
2.6	Extraction of phenolics from grapes to wines	12
2.7	Phenolic reactions in red wine.....	13
2.7.1	Direct condensation reactions.....	13
2.7.2	Acetaldehyde-mediated condensation reactions.....	13
2.7.3	Polymerisation of tannins	14
2.7.4	Phenolic adducts.....	15
2.7.5	Co-pigmentation.....	15
2.8	Effects of SO ₂ bleaching and pH on phenolics in red wine.....	15
2.9	Effect of wine ageing on phenolics in red wine	16
2.9.1	Barrel ageing	16
2.9.2	Non-volatile oak extracts.....	16
2.9.3	Oxidation reduction.....	17
2.9.4	Sorption on wood surface.....	18
2.9.5	Bottle ageing.....	19
2.10	Conclusion.....	20
2.11	Literature cited	21

Chapter 3: Results

3.1	Introduction	27
3.2	Materials and Methods	28
3.2.1	Experimental layout of the vineyard	28

3.2.2	Winemaking	30
3.2.3	Spectrophotometric analyses	31
3.2.3.1	Bovine serum albumin tannin analysis	31
3.2.3.2	Colour density	31
3.2.3.3	Modified colour density	31
3.2.4	HPLC analysis	31
3.2.5	Sensory evaluation	32
3.2.6	Statistics	33
3.3.	Results and discussion	34
3.3.1	Grape berry data	34
3.3.1.1	Tannins, total flavan-3-ols, polymeric phenols, total anthocyanins and polymeric pigments	35
3.3.1.2	Total flavonols	40
3.3.1.3	Hydroxycinnamic acids	40
3.3.2	Wine results	41
3.3.2.1	Colour density and modified colour density	41
3.3.2.2	Tannin analysis, polymeric phenols and flavan-3-ols	46
3.3.2.3	Total anthocyanins and polymeric pigments	49
3.3.2.4	Total flavonols	50
3.3.2.5	Hydroxycinnamic acids	51
3.3.3	Combined phenolic and colour results	52
3.3.4	Sensory evaluation of wine	54
3.3.5	Conclusion	56
3.3.6	Literature cited	57
Chapter 4: Conclusion		
4.1	Concluding remarks and future work	62
4.2	Literature cited	64

Chapter 1

Introduction and project aims

1.1 Introduction

In the South African wine industry there currently is a drive to increase the yield of vines without compromising wine quality. The improvement in grape yield has been investigated for a long time by Stellenbosch University, the Agricultural Research Council and various industry role-players, as smaller yields may lead to lower profit margins. For this reason the Smart-Dyson training system have started to receive more attention as there have been reports of increased yield using this system compared to the vertical shoot positioning system (Bosman, 2011). One of the factors that are considered as an indication of wine quality is the non-volatile phenol concentration of red wine. Grape flavonoids such as anthocyanins are important, as they are responsible for the colour in red wines (Monagas *et al.*, 2005), while tannins influence the mouth-feel and astringency (Vidal *et al.*, 2004). Anthocyanins are present in the skin of red grapes and sometimes in the pulp (Guan *et al.*, 2012). Anthocyanins bind through self-association or with other phenolic compounds in wine to form polymers that are more stable than the monomeric anthocyanins and, in some cases, are more intensely coloured (Boulton, 2001). These changes in the composition of the anthocyanins have a positive effect on the colour profile of the wine (Boulton, 2001; Teissedre & Jourdes, 2013). Tannins in grapes are mostly derived from flavan-3-ols, which associate to form condensed tannins and are responsible for the astringency in wine (Ojeda *et al.*, 2002). The concentration of tannins in a wine can be a positive or negative factor, depending on their mean degree of polymerisation and the concentration present. Increased tannin polymerisation to the point where it cannot bind to the tongue's receptors leads to the wine being perceived as having a softer taste (McRae & Kennedy, 2011). This explains why aged red wines taste less astringent, as these polymerisation reactions occur during ageing over time. Tannins also interact with other components such as anthocyanins to form pigmented polymers that improve the colour stability of the wine. These polymers are a desired form, as they are protected more from oxidation and bleaching and are more resistant to changes in the pH of the wine than the original polymers (Picinelli *et al.*, 1994). Ageing also has an effect on the concentrations of different phenolics in a wine, as different phenols have different reactivity towards other compounds in the wine (Oberholster *et al.*, 2010). Little information exists on phenolics in grapes and how they relate to what occurs in the wine after ageing (Du Toit & Visagie, 2012). For this reason it is important to look at how different concentrations of non-volatile phenols react to ageing.

It is fairly well documented that phenolic compounds are also affected by viticulture practices applied to the vine. The temperature and light to which bunches are exposed to influence the amount of phenols produced (Downey *et al.*, 2006; Nicholas *et al.*, 2011). The total exposed leaf area of a vine influences the synthesis of the phenols, as this area determines the effective leaf area available for photosynthesis (Heyns, 2010). The amount of water and nutrients available to the grapes with regard to the yield and the vigour of the vine will also influence the capability of the vine to synthesise these phenols (Ojeda *et al.*, 2002). All these factors can be influenced by the canopy management strategy,

as well as by the irrigation and fertilisation management that is implemented by the producer (Delgado *et al.*, 2004).

No studies have been done that specifically focus on how the increase in grape yield of the Smart-Dyson training system affects the non-volatile phenols in Shiraz grapes and wines under South African conditions. The results of this study will be valuable to producers who need to make informed decisions about whether it is an economically sound choice to convert to the Smart-Dyson training system when considering the increase in yield against a possible change in non-volatile phenols.

1.2 Project aims

The main aim of this study was to assess the phenolic composition of Shiraz grapes which were obtained from a vineyard which underwent different treatments and especially how this composition evolved in the resulting wines. This work thus focussed more on the oenological aspects of this research as indicated in Chapter 3. This study has been conducted in parallel with another MSc study (Bosman, in preparation) which looked at the viticultural/climate related aspects. Unfortunately data was not available to collaborate the results, and literature was consulted throughout the thesis to attempt to explain observed trends/result.

The specific aims of the study were as follows:

- a) to investigate differences in phenolic concentrations in grapes and wines as a result of viticultural treatments applied in a companion study Smart-Dyson, heavily cut back vines (“Reduced canopy”) and vertical shoot positioning systems, and vertical shoot positioning and double bearer systems on a larger scale;
- b) to assess how these differences in phenolic concentration, if any, are affected by wine ageing (in both bottle and barrel ageing); and
- c) to determine if these treatment differences are perceived sensorially.

1.3 Literature cited

Bosman, D., 2011. Smart-Dyson: A trellis system for improved yield and wine quality. Wynboer August, 5.

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

Delgado, R., Martin, P., Del Alamo, M. & Gonzalez, M., 2004. Changes in the phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilisation rates. *J. Sci. Food Agric.* 84,623-630.

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

Du Toit, W.J. & Visagie, M., 2012. Correlations between South African red grape and wine colour and phenolic composition: Comparing the Glories, Iland and bovine serum albumin tannin precipitation methods. *S. Afr. J. Enol. Vitic.*33, 33- 41.

Guan, L., Li, J., Fan, P., Chen, S., Fang, J., Li, S. & Wu, B., 2012. Anthocyanin accumulation in various organs of a Teinturier grape cultivar (*V. vinifera* L.) during the growing season. *Am. J. Enol. Vitic.*63, 132-138.

Heyns, A.D.M., 2010. The impact of viticulture-trellising systems and lateral removal – Influence on berry composition and wine quality. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

McRae, J.M. & Kennedy, J.A., 2011. Wine and grape tannin interactions with salivary proteins and their impact on astringency: A review of current research. *Molecules* 16, 2348-2364.

Monagas, M., Bartolomé, B. & Gomez-Cordoves, C., 2005. Evolution of polyphenols in red wines from *Vitis vinifera* L. during aging in bottles. *Eur. Food Res. Technol.* 220, 331-340.

Nicholas, K., Matthews, M., Lobell, D., Willits, N. & Field, C., 2011. Effect of vineyard-scale climate variability on Pinot noir phenolic composition. *Agr.Forest.Meteorol.*24, 264-277.

Oberholster, A., Botes, M.P. & Lampbrecht S., 2010. Phenolic composition of Cabernet Sauvignon (*Vitis vinifera*) grapes during ripening in four South African winegrowing regions. *J. Int. Sci. Vine Vin (Macro wine special issue)* June, 33-40.

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

Picinelli, A., Bakker, J. & Bridle, P., 1994. Model wine solutions: Effect of sulphur dioxide on colour and composition during aging. *Vitis* 33, 31-35.

Teissedre, P. & Jourdes, M., 2013. Tannins and anthocyanins of wine: Photochemistry and organoleptic properties. *Nat. Prod.* 67,2255-2274.

Vidal, S., Francis, L., Noble, A., Kwaitkoski, M., Cheynier, V. & Waters, E., 2004. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Anal. Chim. Acta*513, 57-65.

Chapter 2

Literature review

Overview of non-volatile phenols in red grapes and wine

2.1 Introduction

Phenolic compounds are one of the main parameters used for measuring the quality of red wines, as they can give an indication of colour and mouth-feel. A thorough understanding of the factors and reactions that govern phenolic concentrations in grapes and wine are paramount to ensuring red wine quality. Phenolics in grapes and wines are a complex field, but this review provides a brief discussion of different phenolics in red grapes and wine and how they are affected by viticultural and oenological practices.

2.2 Grape and wine phenolics

Significant efforts have been made to characterise and quantify the major phenolics in grapes and where they are found within the berry due to their importance to wine quality (Kennedy, 2008). The main groups of phenolics in white grapes are hydroxycinnamic acids and proanthocyanidins (condensed tannins). These groups of compounds, along with anthocyanins, constitute the main phenolics in red grapes (Kennedy, 2008). **Fig. 2.1** shows the distribution of phenolics throughout the berry. Anthocyanins are located mostly in the skin of the berry, with a few teinturier varieties having anthocyanins in the pulp (Guan *et al.*, 2012). Organic acids such as hydroxycinnamic acid are located in the pulp, and the condensed tannins are distributed in the berry skin and seeds (Kennedy, 2008). The concentration of these phenolics is highly dependent on external factors, such as terroir, and internal factors such as cultivar, diseases, nutrient shortages, etc. The phenol concentrations in grapes may differ greatly between cultivars and winemaking regions. Therefore it is important to have a reference from the specific area and cultivar of interest for future research.

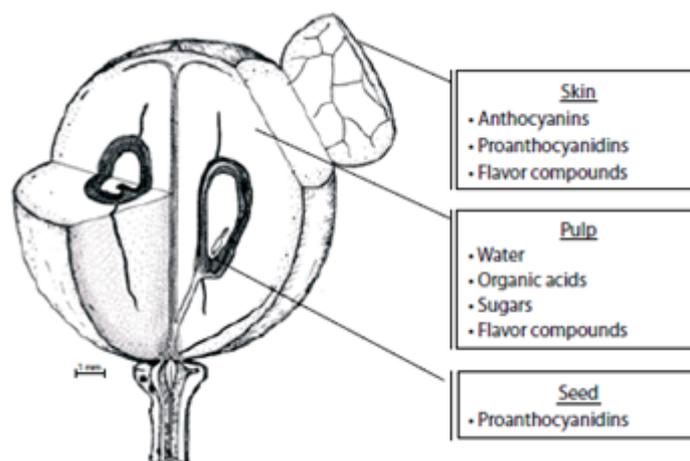


Figure 2.1: The distribution of different organic components throughout the grape structure (Kennedy, 2008).

Wine phenolics can be grouped into two main classes, namely non-flavonoids and flavonoids. Non-flavonoids consist of a single benzene ring with an -OH group with different -R groups attached to the

benzene ring. Non-flavonoids in wine include benzoic acids, cinnamic acids and stilbenes. Of the phenolic acids in wine, the most abundant are the hydroxycinnamic acids, especially caftaric acid. These compounds do not normally have a major impact on the aromatic quality of the wine and only affect the colour and taste (bitterness) of the wine when they are oxidised and react with other phenolic compounds in the wine (Harbertson & Spayd, 2006).

One of the main characteristic of flavonoids is that they consist of two benzene rings connected by a heterocyclic ring of carbon molecules and an oxygen molecule (Packer & Cadenas, 2002) (**Fig. 2.2**). As illustrated in **Fig. 2.2** the flavonoids are grouped into different classes depending on the substitution on the heterocyclic ring of the molecule. Flavonoids can further be divided into anthocyanins, flavan-3-ols and flavonols (Fulcrand *et al.*, 2005). Anthocyanins are responsible for the red colour of grapes and red wine (Timberlake & Bridle, 1976). Flavan-3-ols

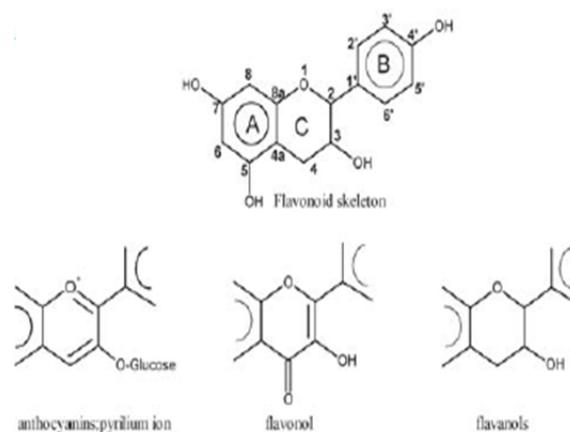


Figure 2.2: Basic form of a flavonoid (Fulcrand *et al.*, 2005)

consist mainly of catechin and epicatechin, which are the monomers of condensed tannins in wine, with the latter constituting almost all of the tannins in unwooded red wines. Flavonols are found in the skins of berries and are synthesised by the vine as protection for the berries against sunburn; they can associate with anthocyanins during ageing (Monagas & Bartolomé, 2005). The most abundant flavonol in grapes is normally kaempferol and its derivatives. Flavonols are not present in large quantities when compared to other phenols, but react with anthocyanins to form co-pigments and may have a bitter taste (Russouw & Marais, 2004). The flavonoids thus are very important components relating to red wine quality aspects, such as colour and astringency (Gawel, 1998).

The concentration of wine phenols may vary drastically and is influenced by many viticultural and vinification factors. Russouw and Marais (2004) have attempted to obtain a better understanding of the concentration and variety of phenols in South African wine.

2.4 Viticultural factors affecting grape phenolics

2.4.1 Microclimate factors

Many factors, such as soil composition, altitude, genetic material, irrigation etc., can have an influence on phenol biosynthesis in grapevines. The two factors that tend to have the largest influence on phenolic concentrations are light and temperature (Spayd *et al.*, 2002). It is hard to separate the effect of light from that of temperature and for a long time the effects of bunch exposure were thought to be due to the light in the bunch zone, but recent studies have shown that the UV irradiation is not as important as the increase in temperature caused by the light exposure (Haselgrove *et al.*, 2000; Spayd *et al.*, 2002; Downey *et al.*, 2006).

Temperature and light

It has been shown that temperature is the main factor influencing the biosynthesis of anthocyanins (Spayd *et al.*, 2002). The optimal temperature for biosynthesis is between 20°C and 30°C (Yamane *et al.*, 2006), with temperatures above 35°C leading to the degradation of phenols (Bergqvist *et al.*, 2001). There also are indications that varying day/night temperature fluctuations have a negative influence on the accumulation of anthocyanins when day/night temperatures vary by more than 10°C (Downey *et al.*, 2006). This is in contrast to studies showing that night-time temperatures below 15°C help with the accumulation of anthocyanins (Mori *et al.*, 2005). It therefore seems the optimum temperature ranges for anthocyanin accumulation to be a day temperature of 20 to 25°C, with a night-time temperature of around 15°C. Tannin and flavonol synthesis is not significantly influenced by higher grape temperatures (Monagas & Bartolomé, 2005), with degradation only taking place above 35°C (Bergqvist *et al.*, 2001; Heyns, 2010). It has also been shown that light has little influence on the tannin concentration of the grapes (Downey *et al.*, 2004) and the concentration of anthocyanins (Downey *et al.*, 2006), but may have a slight positive effect up to 100 mmol/m.

2.4.2 The effects of yield vs. effectively exposed leaf area on grapes

The number of grapes a vine can ripen is limited by the size of exposed leaf area available for photosynthesis (Reay & Lancaster, 2001). Larger fruit yields require more exposed leaf area to ripen the grapes (Kliewer & Dokoozlian, 2005). The minimum leaf area required to still produce good quality grapes for a single cordon training system is between 0.8 and 1.2 m² per kilogram of fruit produced (Kliewer & Dokoozlian, 2005; Petrie *et al.*, 2008). By opening up the canopy, the exposed leaf area is increased while at the same time the density of the canopy is decreased (Gladstone & Dokoozlian, 2003). The increased photosynthesis will lead to the vine being able to synthesize larger quantities of phenols for higher yields from an increase in available energy from metabolic working (Kliewer & Dokoozlian, 2005).

2.4.3 Effect of leaf removal on phenols

Leaf removal before flowering can cause improper set and decrease the berry size of the vines (Tardaguila *et al.*, 2010). The effect on berry size is cultivar specific, with different reactions being reported in different cultivars (Tardaguila *et al.*, 2010). Early growth season leaf removal has the effect of decreasing fruit set and berry size, thereby lowering the crop load and increasing the soluble components in the berry. This increase will lead to higher levels of phenols in the grapes and wine through a better skin-to-pulp ratio (Holt *et al.*, 2008). It has also been shown that leaf removal before véraison will increase the anthocyanin levels in the grapes, as the berries are exposed to more light and higher temperatures (Downey *et al.*, 2006). Holt *et al.*, (2008) found that reduced crop and smaller berry size, with a higher skin-to-juice ratio due to early leaf removal, may lead to slight increases in soluble components in the berries as well as higher levels of phenolics in the wine.

2.4.4 Overview of training systems investigated in this study

2.4.4.1 Vertical shoot positioning

Vertical shoot positioning (VSP) (**Fig. 2.3**) is one of the most widely used training systems around the world for wine grapes due to its ease of vine management and ability to be mechanically harvested (Danehower, 2006). It consists of one or two cordons in a row supported by a cordon wire. The shoots are positioned vertically and held in place by spaced wires. This training system is normally used for low- to medium-vigour vines. The system has some disadvantages, as it is not very well suited for higher-vigour vineyards (Reynolds & Van den Heuvel, 2009)

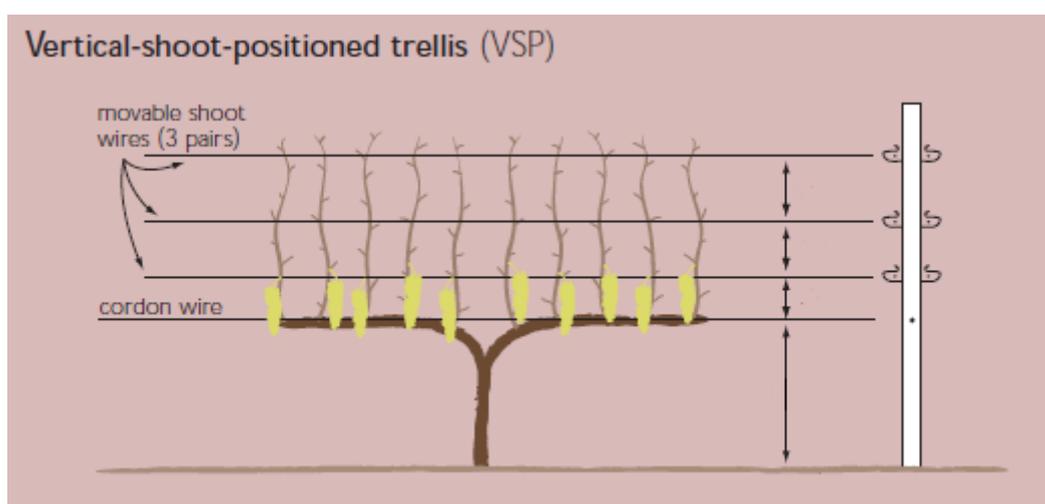


Figure 2.3: Illustration of the training of the VSP system (Dokoozlian & Kliewer, 1995)

2.4.4.2 Smart-Dyson

The Smart-Dyson training system (SD)(**Fig. 2.4**) was developed by Dr Richard Smart and John Dyson. It is similar to the traditional vertical shoot positioning system with the only difference being that there are additional shoots on the canes pointing downwards (Bosman, 2011). This, in effect, doubles the amount of shoots on the vine and has been shown to lead to an increase in fruit production of up to 40 % (Bosman, 2011). There has been a lot of interest in this system lately in South Africa as it is suitable for mechanical harvesting, but also significantly more expensive to set up than the VSP system (Bosman, 2011).

The opened canopy is less dense than the canopy on a similar VSP vine and has increased light on the berries and a better exposed leaf area. This system is only suitable for vineyards with a higher vigour as it provides an increased vegetative growth area that needs to be maintained (Danehower, 2006). However, the advantages of this training system decrease in lower vigour vines. Vines with lower vigour are not able to fill out the larger canopy system required of an SD system optimally. The low vigour will cause vine stress, as the vine has to use reserves from the permanent part of the plant to facilitate the increased growth and higher yield of the SD training system (Howell, 1999). This can lead to a decline in production of the vine and may decrease its life span considerably, as it will experience a nett loss of reserves each year.

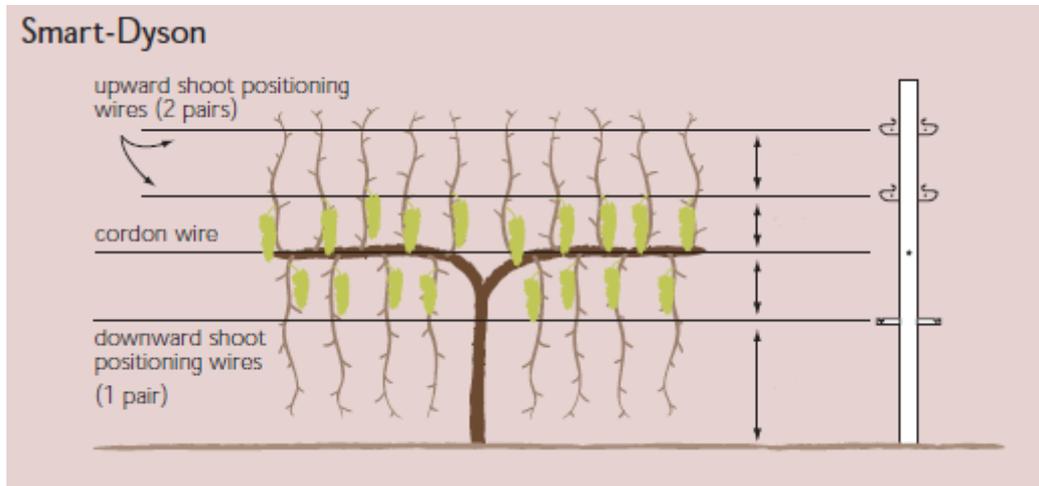


Figure 2.4: Illustration of the training of the SD system (Dokoozlian & Kliewer., 1995)

2.5 Biosynthesis of phenolics throughout the ripening of grapes

The evolution of different compounds during grape ripening can be seen in **Fig. 2.5**. Hydroxycinnamic acids and flavan-3-ols are synthesised early in berry development, from about twenty days after flowering (Kennedy *et al.*, 2001). Although some of the flavan-3-ols increase with the ripening of the berries, the concentration of (+)-catechin, the most abundant of the flavan-3-

ols, reaches a maximum at the green berry stage and then decrease with ripening from véraison, after which it starts to decline through degradation and polymerisation (Kennedy *et al.*, 2001). Since flavan-3-ols decrease with ripening, it will probably not benefit from the more open canopy provided by SD training or comparable systems. This is because their biosynthesis takes place early in the season, when the canopy density problems that sometimes affect vines have not yet become a factor (Kennedy *et al.*, 2001). However, anthocyanin synthesis starts only after véraison and levels normally continue to increase in the berry skin until around commercial harvest (Fig 2.5).

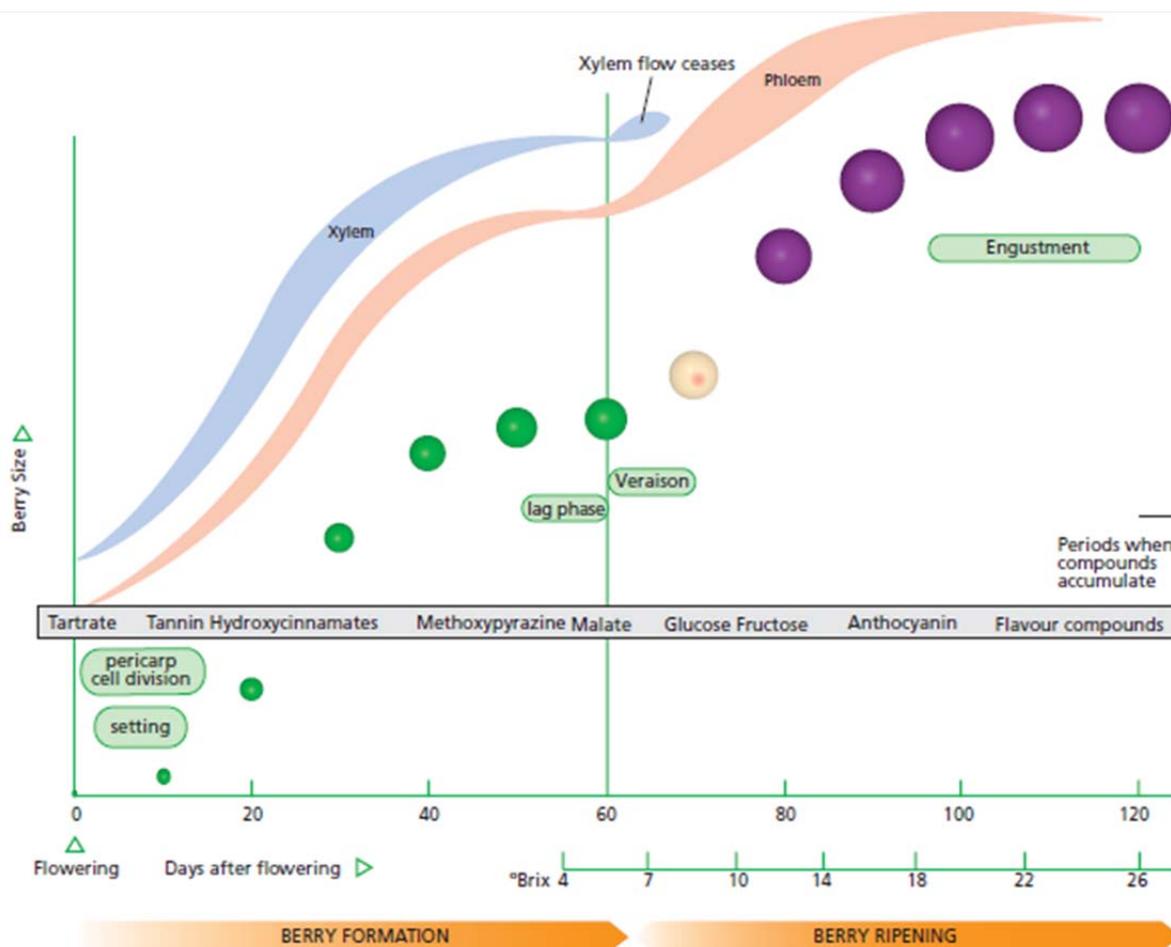


Figure 2.5: Simplified diagram of berry ripening, indicating when different components are synthesised. The main phenols that are present during the ripening of the grape and that have the biggest influence on the wine are also shown (Kennedy *et al.*, 2001).

Flavonols have two peak periods of synthesis during berry development, the first being around flowering and the second right after véraison (Downey *et al.*, 2006). Flavonols can only be found in the skin of the berry with the exemption of teinturier varieties (Guan *et al.*, 2012). This has interesting consequences for different training systems, as less dense canopies will not have a major effect on the quantity of flavonols during the first peak of synthesis, as it is still early in the season and most canopy systems will not have a density problem at this time. During the second period of synthesis,

open canopies could have a major impact, as flavonol synthesis is triggered by UV radiation of the grapes, which means direct sunlight on the berries is necessary for synthesis (Reay & Lancaster, 2001).

2.6 Extraction of phenolics from grapes to wines

The correlation between the grape phenols and the amount of phenols in the wine is believed to depend on the extractability of the phenols during winemaking (Sacchi *et al.*, 2005). It has been reported by Stoyanov *et al.* (2002) that the extractability of grape phenols decreases during the maturation of the grapes. This may be due to the increase in mean degree of polymerisation, which makes phenolics less reactive to other tannins and proteins (McRae & Kennedy, 2011). Furthermore, increases in the concentration of polysaccharides in the berry with which phenolics react during ripening make them more difficult to extract into the wine (Stoyanov *et al.*, 2002). Other authors, such as Liu *et al.* (2010) and Lorrain *et al.* (2013) have found that monomeric phenols, especially flavan-3-ols and flavonols, decrease with grape maturation, while the increase in polymers in the grapes are highly cultivar dependent and may be genetically controlled. With Shiraz, it was found that polymers increase with maturation although not as much as with Cabernet Sauvignon and in contrast to Marselan grapes that showed a decrease (Liu *et al.*, 2010). Liu *et al.* (2010) also showed that an increase in the alcohol percentage increases the extractability of grape phenols into wine. This may be due to the cell wall degradation that is caused by the increased alcohol content, therefore increasing the extractability of the phenols in the skins of riper grapes. Bindon & Kennedy (2011) found that polymeric proanthocyanidins are released into the wine in higher concentrations from riper grapes. This is probably due to proanthocyanins being less reactive with other phenols, such as anthocyanins, in the berry skin. Bindon *et al.* (2013) have also found that phenols in different parts of the grape respond differently to grape ripening. The study noted decreases in seed tannins with ripening and an increase in skin phenolics were observed.

The extraction of phenols from the grapes into the wine is not very effective, with only a small portion normally being extracted. The reactivity of grape phenols is the highest after crushing, after which it decreases during fermentation as the phenols react with other components in the matrix (Sacchi *et al.*, 2005). As can be seen in **Fig. 2.6**, the extraction of tannins from grapes into wine is quite limited during the winemaking process. However, the occurrence of anthocyanins in grapes and wines are often highly correlated (Bindon *et al.*, 2014; Du Toit & Visagie, 2012).

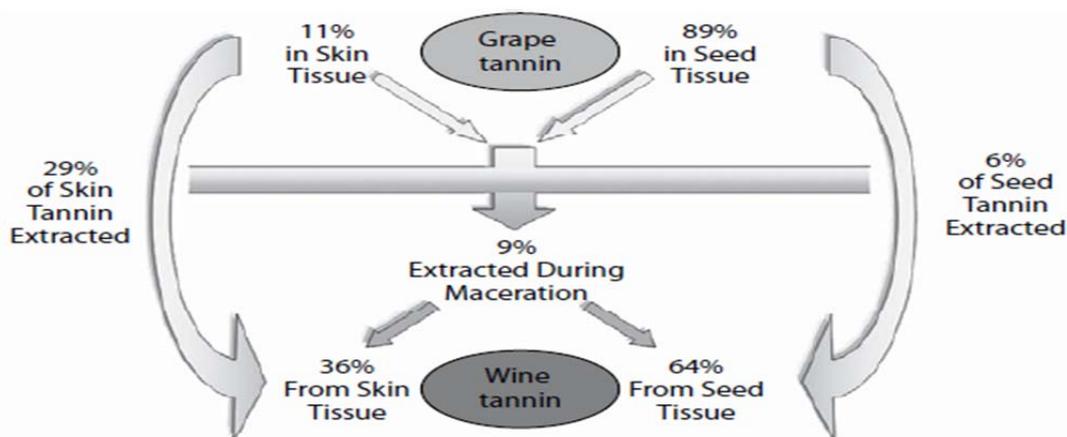


Figure 2.6: Schematic presentation of the percentage of tannins extracted from the grape into the wine (Kennedy, 2008).

2.7 Phenolic reactions in red wine

Phenolics are reactive molecules that are influenced by many other components in the wine matrix. This makes the conformation and concentrations of phenolics in red wines a dynamic system. The interactions between these components and the environment in which they take place determine the components that will be formed (Downey *et al.*, 2006). The components that form can have a major impact on wine quality and it is thus very important to understand these reactions. In sections 2.7.1 to 2.7.5 the focus will fall more on some of the major reactions that involve flavonoids in red wine.

2.7.1 Direct condensation reactions

Direct condensation reactions take place when the coloured, positively charged anthocyanin (flavylium ion) reacts as a cation on the negative nodes (C6 or C8) of the tannin moiety to form a colourless flavene that can change to a red pigmented polymer when oxidised (Ribéreau-Gayon *et al.*, 1983). The polymers that are formed are more stable against decolouration due to oxidation than monomeric anthocyanins.

2.7.2 Acetaldehyde-mediated condensation reactions

When oxygen is absorbed into the wine it oxidises phenols to quinones and H_2O_2 is formed as a by-product (Du Toit *et al.*, 2006b). Acetaldehyde is formed through the reaction between H_2O_2 and ethanol (Fulcrand *et al.*, 2005). The acetaldehyde is highly reactive with flavonoids, ellagitannins and anthocyanins (Oberholster, 2011). The acetaldehyde binds to the C6/C8 position on the A-ring of the flavonoid to form a dimer and expands further in this way to form polymers. **Fig. 2.7** illustrates the binding of an anthocyanin to a catechin molecule in the C8 position via an acetaldehyde-derived orethyl bridge to form a polymerised pigment (Timberlake & Bridle, 1976). The polymers that form between anthocyanins and flavonoids are more stable in a wine-like solution than the free monomeric

anthocyanins (Boulton, 2001). These polymerised molecules are also more resistant to oxidation because they have fewer hydroxyl groups with which oxygen can react in their polymerised form and are also more intensely coloured than free anthocyanins (Gambuti *et al.*, 2010).

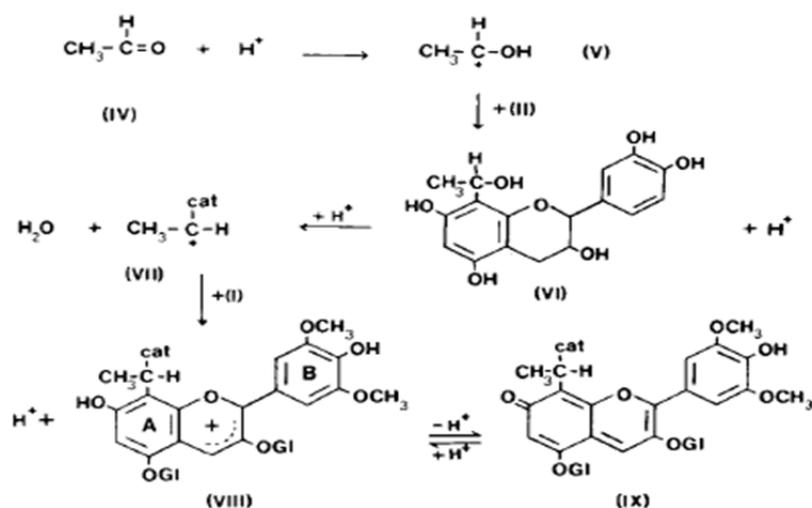


Figure 2.7: Reaction of acetaldehyde (IV) with catechin (II) and malvidin-3,5-diglucoside (I) (Timberlake & Bridle, 1976).

2.7.3 Polymerisation of tannins

The mean degree of polymerisation of tannins determines their astringency and bitterness (Gawel, 1998). When a tannin molecule polymerises beyond a certain degree, it is perceived to be less bitter tasting, because it becomes too large to bind to the taste receptors on the tongue (McRae & Kennedy, 2011). The larger the tannin, the more astringent it is perceived to be until it becomes too large and precipitates out of the solution, causing both the bitterness and astringency of the wine to decrease (Gawel, 1998). Polymerisation can occur through direct or acetaldehyde-mediated condensation reactions between flavan-3-ols and anthocyanins. A study by Monagas & Bartolomé (2005) showed that when an anthocyanin binds at its C8 position to the end of a polymeric chain of flavan-3-ols, the polymerisation reaction ceases at that terminal, as the C6 position of an anthocyanin is much less reactive due to steric hindrance and thus inhibits the binding of any further flavan-3-ols or anthocyanins. This leads to most pigments only having up to two anthocyanins in the polymer (Monagas & Bartolomé, 2005). However, it has been shown by Atanasovan *et al.* (2002) that the C6 position of the anthocyanin is reactive to some extent, although less so than the C8 position, and that polymers formed between anthocyanins in the absence of flavan-3-ols can be due to bonds on the C6 position.

2.7.4 Phenolic adducts

Anthocyanin-vinyl phenol adducts can also form at wine pH. This reaction is initiated by the decarboxylation of *p*-coumaric acid in red wine by the cinnamic decarboxylase of the yeast (Schwarz *et al.*, 2003). These decarboxylated acids react with free anthocyanins on the C4 position during barrel ageing, which leads to the formation of coloured pigments through an oxidation reaction (Monagas & Bartolomé, 2005). Anthocyanin-vinylcatechin products have also been identified, and they possibly form from the reaction between a flavylium ion and a catechin molecule with a vinyl group on its C8 carbon (Du Toit *et al.*, 2006a). These adducts are more stable, are resistant to SO₂ bleaching and are red and orange in colour (Picinelli *et al.*, 1994). This may partially explain the evolution of the colour from red to a browner, tawny colour during red wine ageing in barrels.

2.7.5 Co-pigmentation

Co-pigments consist of coloured anthocyanins such as malvidin-3-glucoside (free anthocyanin) associated with a cofactor consisting of phenolic acids, flavonols, flavan-3-ols or other condensed tannins (Boulton, 2001). Co-pigmentation acts only as a prelude to condensation and polymerisation reactions, as co-pigmentation bonds are not very strong and only serve to render the anthocyanin molecules less reactive through steric hindrance (He *et al.*, 2012). This preserves the anthocyanins from oxidation and other reactions, to later form part of polymerisation and condensation reactions. These reactions have an influence on the colour and taste of the wine, with polymers being browner, thus giving older wines a browner hue than younger wines (De Beer *et al.*, 2005). Co-pigments can comprise up to 50% of the colour observed in young red wines (Boulton, 2001).

2.8 Effects of SO₂ bleaching and pH on phenolics in red wine

Sulphur dioxide (SO₂) has been used to preserve wine since Roman times (Henderson, 2009) and is still considered to be the best all-round antimicrobial/antioxidant additive to use in wine. SO₂ has a substantial influence on the phenols in the wine by what is commonly known as SO₂ bleaching of the anthocyanins. This reversible reaction is present mainly in young red wines. The SO₂ binds with monomeric anthocyanins to form colourless anthocyanin-4-bisulphates (Picinelli *et al.*, 1994). SO₂ also reacts with the acetaldehyde in wine, which helps prevent an oxidative aroma character being perceived, while small amounts of acetaldehyde help to stabilise the colour by forming acetaldehyde-mediated bonds (as discussed in section 2.7.2) between tannins and anthocyanins (Picinelli *et al.*, 1994). The anthocyanin bisulphate bonds break after a while, thus causing the anthocyanins to return to their coloured form. This will have the effect that a wine with recently added SO₂ will seem lighter than before the addition, with the colour returning over time as the bonds break. pH has a large influence on the bleaching effect of SO₂, as the form in which the majority of the free SO₂ can be

found is determined by the pH. At a pH of 3.2, up to 96% of free SO₂ in the wine resides in the bisulphate form, thus greatly increasing the bleaching effect on the wine (He *et al.*, 2012).

The pH of a wine is one of the main factors that has an influence on the colour of the wine that is controllable by the winemaker. The pH influences the colour of the anthocyanins by having an effect on the anthocyanin equilibrium. At lower pH the equilibrium shifts towards the red flavylium ion form, thus increasing the red colour of the wine. At a wine pH of 3.4 to 3.6, only 20 to 25% of the free anthocyanins are in the flavylium ion form. At pH 4, this percentage decreases to 10% due to a shift in the anthocyanin equilibrium towards the colourless carbinol base form at higher pH levels (He *et al.*, 2012).

2.9 Effect of wine ageing on phenolics in red wine

2.9.1 Barrel ageing

Traditionally, most red wine and some white wines are aged in oak barrels because of the positive effect the wood has on the sensory quality of the wine. Many of these changes are due to the modification of the wine's phenolics. Not only is the volatile composition changed by barrel ageing, but also the non-volatile components responsible for the colour, ageing potential, astringency and bitterness of the wine (DelAlamo Sanza *et al.*, 2004). These changes are due to the interactions between the wine and oak phenolics, as well as the oxidation reactions that take place in the barrel because of the physical structure of the barrel and the components extracted from the barrel into the wine (Oberholster, 2011).

2.9.2 Non-volatile oak extracts

Many components that are extracted from the wood have an influence on wine phenolics. These non-volatile components consist mainly of hydrolysable tannins, lignin, triterpenes, coumarins, phenolic acid, gallic acid and polysaccharide-hemicellulose (Oberholster, 2011). The amount and ratio of these different components depend on the wood species, the origin of the wood, the maturation of the wood, the toasting method and the toasting intensity of the barrels (Ribéreau-Gayon *et al.*, 2007; Oberholster, 2011). Of the non-volatiles that are extracted, ellagitannins play an important role in the polymerisation of tannins and the stabilisation of the wine colour. Hydrolysable tannins consist mainly of ellagitannins, which are made up mainly of vescalagin and castalagin (Oberholster, 2011). Grandinin and roburin have also been identified, but are present in smaller quantities. **Fig. 2.8** shows the structure of these compounds and how they polymerise to form ellagitannins (Vivas *et al.*, 1996).

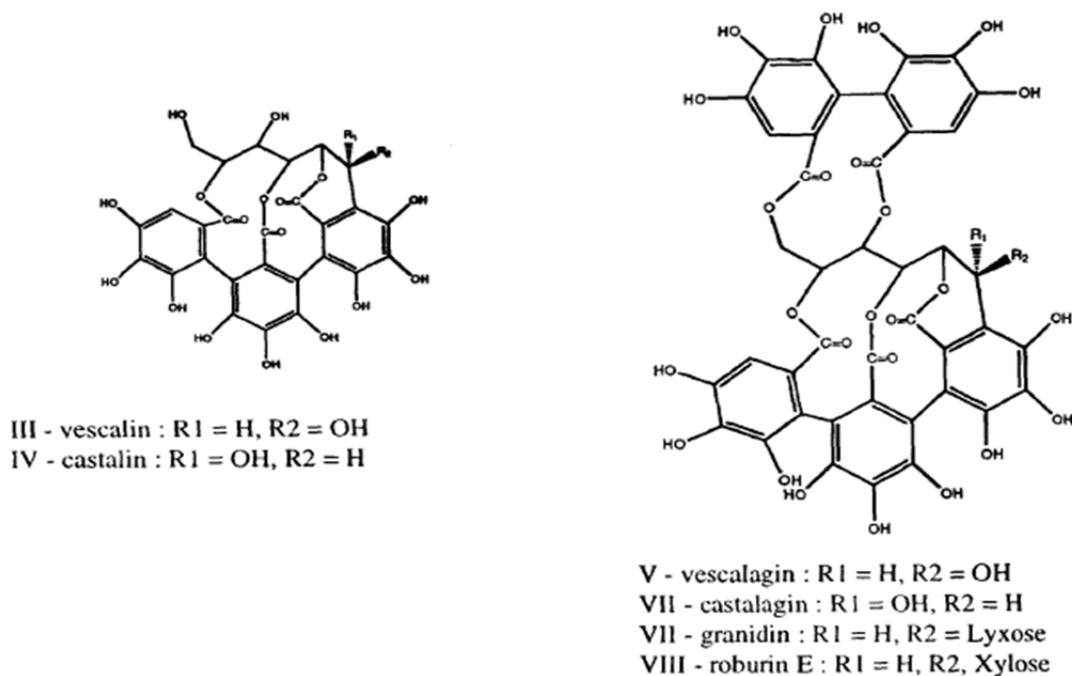


Figure 2.8: Monomeric components of ellagitannins (Vivas *et al.*, 1996).

2.9.3 Oxidation reduction

Wooden barrels provide a porous medium that facilitates the ingress of oxygen (O_2) through to the wine as a result of the structure of the xylem tubes and the spaces between the staves (Vivas *et al.*, 1996). The amount of oxygen to which the wine is exposed due to diffusion through the wood ranges from 1.66 to 2.5 $ml/l^{-1}/month^{-1}$ (Cano-López *et al.*, 2010). The total amount of oxygen diffused through the wood can vary between 20 and 45 $mg/L/year$ for barrel ageing (Du Toit *et al.*, 2006a). This slow exposure of oxygen to wine has a positive effect on the colour and phenol structure of the wine (Cano-López *et al.*, 2010). The ellagitannins extracted from the wood are more oxidisable than the condensed tannins of the wine, thereby outcompeting condensed tannins for the molecular O_2 and thus protecting grape-derived phenolics from oxidation (Vivas *et al.*, 1996). Through the catalisation of Fe^{2+} and Cu^+ , ellagitannins are oxidised and hydrogen peroxide (H_2O_2) is formed. The highly reactive H_2O_2 reacts with the ethanol in the wine to form acetaldehydes, which participate in further phenolic polymerisation reactions in the wine (**Fig. 2.9**), as mentioned previously in section 2.7.2 (Fulcrand *et al.*, 2005). This leaves grape-derived phenols un-oxidised and allow them to take part in other reactions in the wine, such as polymerisation. However, the study by Vivas *et al.* (1996) was done in model wine, and there are many more flavan-3-ols in real wine that can react with O_2 , some of which may be more reactive than ellagitannins. Further studies are therefore needed to be done to determine the effects of these other flavan-3-ols on the oxidation reactions in the wine. (+)-Catechin

moieties are the most common flavan-3-ols found in wine and remain a good indicator of the response the wine might have to oxidation (Monagas & Bartolomé, 2005).

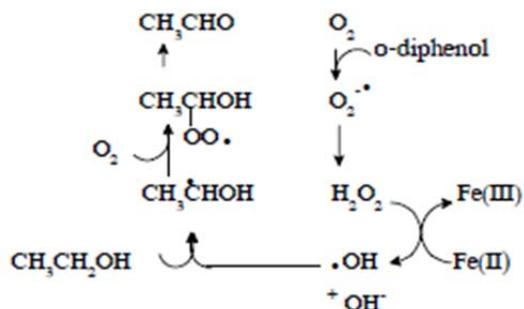


Figure 2.9: Reaction of H₂O₂ with ethanol to form acetaldehyde (Fulcrand *et al.*, 2005).

2.9.4 Sorption on wood surface

Although many wood extracts are dissolved in wine during barrel ageing, many components that can have an effect on the wine through the sorption of wine phenols remain in the wood (Barrera-Garcia *et al.*, 2007). This phenomenon has not been well studied in wine and more detailed experiments are needed to assess its full scope. According to Barrera-Garcia *et al.* (2007), up to 5% of monomeric anthocyanins can be lost due to sorption on the wood. The largest loss reported was for the stilbenes, in particular trans-resveratrol, which showed a decrease of 50% after wood ageing due to sorption on the wood (Barrera-Garcia *et al.*, 2007). The rate of sorption can be seen in **Fig. 2.10**, where the sorption of resveratrol is shown to be much higher than that of the anthocyanins. It was hypothesised that the reaction mechanism of the sorption is driven by the hydrophobicity of the phenols. More hydrophobic compounds are being adsorbed to a higher degree than less hydrophobic compounds like gallic acid, which shows no decrease due to sorption on the wood (Barrera-Garcia *et al.*, 2007).

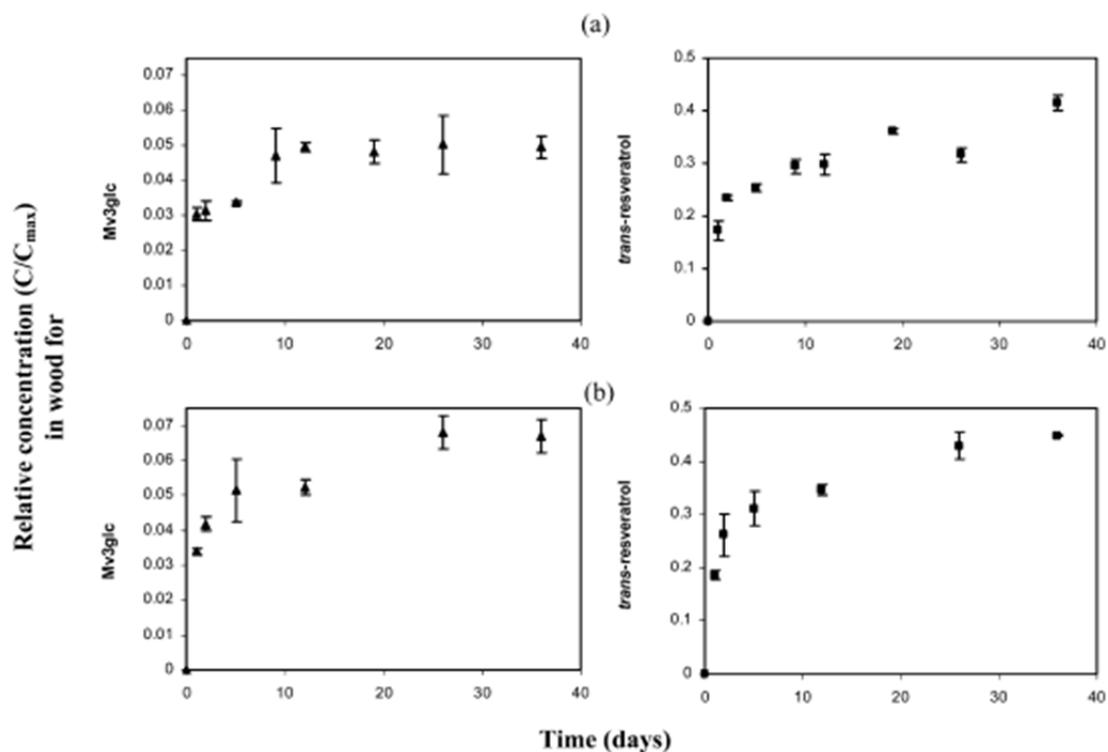


Figure 2.10: Relative concentration of adsorbed malvidin-3-glucoside (Mv3glc) and trans-resveratrol measured after the extraction of wood plates stored for different durations in model wine (a) and real wine (b) (Barrera-Garcia *et al.*, 2007).

2.9.5 Bottle ageing

Placing wine in bottles was first used as a way to transport wine and to store it for later consumption. However, it was later found that prolonged ageing of wine in bottles can have a huge impact on the sensory perception of the wine, thus it has become important to investigate the range of these reactions.

During bottle ageing, the wine is not in direct contact with oxygen, although there is some permeation of oxygen through the closure with time. Therefore most of the reactions that take place are relative anaerobic in nature, involving the co-pigmentation and polymerisation of the anthocyanins (De Beer *et al.*, 2005). It has been shown that most phenolics decrease during bottle ageing during exceeding months, with the exception of hydroxycinnamic acids, which remain constant (De Beer *et al.*, 2005).

Modern wines are made to be drunk within months of being purchased by the consumer, causing the influence of oxygen on bottled wine to be considered minimal. However, the closure type and time that the wine is in the bottle can have a major influence on the oxygen available to the wine. It has been shown that natural cork varies greatly in its permeability, ranging from 0.03 to 0.17 ml/L O₂ per month for the first twelve months, after which it decreases to about 0.002 to 0.007 ml/L O₂ per month (Lopes *et al.*, 2006). This change in permeability can have a great influence on the wine overtime,

which could explain the oxidative nature of old wines. It has been shown that, irrespective of the closure type, the biggest influence on the wine is the storage conditions, in particular the temperature at which the wine is stored (De Beer *et al.*, 2005). This will influence the speed of oxidation, as it influences the reaction kinetics of the oxygen in the wine. Hopfer *et al.*(2013) found that wine stored in bottles at 10°C showed much less of an oxidative character and lower levels of precipitation of the colour in the wine compared to wines stored at 20°C to 40°C.

2.10 Conclusion

A number of studies investigated the effect of environmental and vineyard practices on phenolics in grapes, and how vinification techniques affect their extraction and reactions in wine during ageing. What can be concluded from this is that there are many factors that play a role in determining the phenol composition and concentrations in red grapes and wine and how they correlate to each other. It has also been shown that the responses to these factors are very cultivar specific (Lorrain *et al.*, 2013). Although some factors can be manipulated during grape and wine production, others, such as climate, are difficult to control. This ultimately will have an effect on the final product. Future research should focus on studying the effect of a combination of these factors on the phenolic concentration of the grapes and if or how they correlate with the phenolic and colour composition of the wine at different stages of ageing.

2.11 Literature cited

Atanasova, V., Fulcrand, H., Cheynier, V. & Moutounet, M., 2002. Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Anal. Chim. Acta.* 458, 15-27.

Barrera-Garcia, V.D., Gougeon, R.D., DiMajo, D., De Aaguirre, C., Violley, A. & Chassange, D., 2007. Different sorption behaviours for wine polyphenols in contact with oak wood. *J. Agric. Food Chem.* 55, 7021-7027.

Bergqvist, J., Dokoozlian, N. & Ebisuda, N., 2001. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *Am. J. Enol. Vitic.* 52, 1-7.

Bindon, K. & Kennedy, J.A., 2011. Ripening-induced changes in grape skin proanthocyanidins modify their interactions with cell walls. *J. Agric. Food Chem.* 59, 2696-2707.

Bindon, K., Varela, C., Kennedy, J., Holt, H. & Herderich, M., 2013. Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon: Grape and wine chemistry. *Food Chem.* 138, 1696-1705.

Bindon, K., Kassara, S., Cynkar, W.U., Robinson, E.M.C., Scrimgeour, N. & Smith, P.A., 2014. Comparison of extraction protocols to determine differences in wine-extractable tannin and anthocyanin in *Vitis vinifera* L. cv. Shiraz and Cabernet Sauvignon grapes. *J. Agric. Food Chem.* 62, 4558-4570.

Bosman, D., 2011. Smart-Dyson: A trellis system for improved yield and wine quality. Wynboer August, 5.

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

Cano-López, M., López-Roca, J.M., Pardo-Minguez, F. & Gomez Plaza, E., 2010. Oak barrel maturation vs. micro-oxygenation: Effect on the formation of anthocyanin-derived pigments and wine colour. *Food Chem.* 119, 191-195.

Danehower, C., 2006. Trellising the grape. www.Avalonwine.com

De Beer, D., Joubert, E., Gelderblom, W.C.A. & Manley, M., 2005. Changes in phenolic composition and antioxidant activity of Pinotage, Cabernet Sauvignon, Chardonnay and Chenin blanc wines during bottle aging. *S. Afr. J. Enol. Vitic.* 26, 6-15.

- Del Alamo Sanza, M., Fernandez Escudero, J.A. & De Castro Trio, R., 2004. Changes in phenolic compounds and colour parameters of red wine aged with oak chips and in oak barrels. *Food Sci. Tech. Int.* 10, 233-241.
- Dokoozlian, N.K. & Kliever, W.M., 1995. The light environment within grapevine canopies. I: Description and seasonal changes during fruit environment. *Am. J. Enol. Vitic.*46, 209-218.
- Downey, M.O., Dokoozlian, N.K. & Krstic, M., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. *Am. J. Enol. Vitic.*57, 257-268.
- Downey, M.O., Harvey, J.S. & Robinson, S.P., 2004. Flavonol accumulation and expression of a gene encoding flavonol synthase demonstrates light sensitivity of flavonol biosynthesis in grapevines. In: Hoikkala, A. & Soidinsalo, O. (eds) XXII International Conference on Polyphenols.25-28 August, University of Helsinki, Helsinki, Finland. pp. 59 – 60.
- Du Toit, W.J. & Visagie, M., 2012. Comparing the Glories, Iland and bovine serum albumin tannin precipitation methods. *S. Afr. J. Enol. Vitic.* 33, 33-41.
- Du Toit, W.J., Lisjak, K., Marais, J. & Du Toit, M., 2006a. The effect of micro-oxygenation on the phenolic composition, quality and aerobic microorganisms of different South African red wines. *S. Afr. J. Enol. Vitic.*27, 57-67.
- Du Toit, W., Marais, J., Pretorius, I. & Du Toit, M., 2006b. Oxygen in must: An overview. *African red wines. S. Afr. J. Enol. Vitic.*27, 76-94.
- Fulcrand, H., Dueñas, M., Salas., E. & Cheynier, V., 2005. Phenolic reactions during winemaking and aging. *Am. J. Enol. Vitic.* 57, 289-297.
- Gambutì, A., Capuano, R., Lisanti, M.T., Strollo, D. & Moio, L., 2010. Effect of aging in new oak, one-year-used oak, chestnut barrels and bottle on colour, phenolics and gustative profile of three mono varietal red wines. *Eur. Food Res. Technol.* 231, 455-465.
- Gawel, R., 1998. Red wine astringency: A review. *Aust. J. Grape Wine R.* 4, 74-95.
- Gladstone, E.A. & Dokoozlian, N.K., 2003. Influence of leaf area density and trellis/training system on the light microclimate within grapevine canopies. *Vitis* 42, 123-131.
- Guan, L., Li, J., Fan, P., Chen, S., Fang, J., Li, S. & Wu, B., 2012. Anthocyanin accumulation in various organs of a Teinturier grape cultivar (*V. vinifera* L.) during the growing season. *Am. J. Enol. Vitic.*63, 132-138.

Harbertson, J.F. & Spayd, S., 2006. Measuring phenolics in the winery. *Am. J. Enol. Vitic.* 57, 280-288.

Haselgrove, L., Botting, D., Van Heeswijck, R., Høj, P.B., Dry, P.R., Fort, C. & Land, P.G.I., 2000. Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L cv. Shiraz grape berries. *Aust. J. Grape Wine R.* 6, 141-146.

He, F., Liang, N.N., Mu, L., Pan, Q.H., Wang, J., Reeves, M.J. & Duan, C.Q., 2012. Anthocyanins and their variation in red wine II. Anthocyanin derived pigments and their colour evolution. *Molecules* 17, 1483-1519.

Henderson, P., 2009. Sulfurdioxide: Science behind this anti-microbial, anti-oxidant, wine additive. *Practical Winery and Vineyard Journal.* 1, 1-6.

Heyns, A.D.M., 2010. The impact of viticulture-trellising systems and lateral removal – Influence on berry composition and wine quality. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Holt, H.E., Francis, I.L., Field, J., Herderich, M.J. & Iland, P.G., 2008. Relationships between berry size, berry phenolic composition and wine quality scores for Cabernet Sauvignon (*Vitis vinifera* L.) from different pruning treatments and different vintages. *Aust. J. Grape Wine R.* 14, 191-202.

Hopfer, H., Buffon, P.A., Ebeler, S.E. & Heymann, H., 2013. The combined effect of storage temperature and packaging on the sensory, chemical, and physiological properties of Cabernet Sauvignon wine. *J. Agr. Food Chem.* 61, 3320-3334.

Howell, G.S., 1999. Sustainable grape productivity and the growth-yield relationship: A review. *Am. J. Enol. Vitic.* 52, 165-174.

Kennedy, J.A., 2008. Grape and wine phenolics: Observations and recent findings. *Cienc. Inv. Agr.* 35, 107-120.

Kennedy, J.A., Hayasaka, Y., Vidal, S., Waters, E.J. & Jones, G.P., 2001. Composition of grape skin proanthocyanidins at different stages of berry development. *J. Agr. Food Chem.* 49, 5348-5355.

Kliewer, W. & Dokoozlian, N., 2005. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. *Am. J. Vitic. Enol.* 56, 170-181.

Liu, Y., Pan, Q., Yan, G., He, J. & Duan, C., 2010. Changes of flavan-3-ols with different degrees of polymerization in seeds of ‘Shiraz’, ‘Cabernet Sauvignon’, ‘Marselan’ grapes after veraison. *Molecules* 15, 7763-7774.

- Lopes, P., Saucier, C., Teissedre, P. & Glories, Y., 2006. Impact of storage position on oxygen ingress through different closures into wine bottles. *J. Agr. Food Chem.* 54, 6741-6746.
- Lorrain, B., Ky, I., Pechamat, L. & Teissedre, P., 2013. Evolution of analysis of polyphenols from grapes, wines, and extracts. *Molecules* 18, 1076-1100.
- McRae, J.M. & Kennedy, J.A., 2011. Wine and grape tannin interactions with salivary proteins and their impact on astringency: A review of current research. *Molecules* 16, 2348-2364.
- Monagas, M. & Bartolomé, B., 2005. Updated knowledge about the presence of phenolic compounds in wine. *Crit. Rev. Food Sci.* 45, 85-118.
- Mori, K., Sugaya, S & Gemma, H., 2005. Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. *Sci. Hortic.* 105, 319-330.
- Oberholster, A., 2011. Impact of oak on wine composition and chemistry. *WineBusiness Monthly*, December.
- Packer, L. & Cadenas, E., 2002 (2nd ed). *Handbook of Antioxidants*. Marcel Dekker, New York
- Petrie, P., Trought, M. & Howell, G., 2008. Fruit composition and ripening of Pinot noir (*Vitis vinifera* L.) in relation to leaf area. *Aust. J. Grape Wine R.* 6, 46-51.
- Picinelli, A., Bakker, J. & Bridle, P., 1994. Model wine solutions: Effect of sulphur dioxide on colour and composition during aging. *Vitis* 33, 31-35.
- Reay, P.F. & Lancaster, J.E., 2001. Accumulation of anthocyanins and quercetin glycosides in 'Gala' and 'Royal Gala' apple fruit skin with UV-B-visible irradiation: Modifying effects of fruit maturity, fruit side, and temperature. *Sci. Hortic-Amsterdam* 90, 57-68.
- Reynolds, A. & Van den Heuvel, J.E., 2009. Influence of grapevine training systems on vine growth and fruit composition: A review. *Am. J. Enol. Vitic.* 60, 251-268.
- Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B. & Lonvaud, A., 2007. *Handbook of Enology. The microbiology of wine and vinifications, Volume 2*. John Wiley & Sons Ltd, Chichester.
- Ribéreau-Gayon P., Pontallier P. & Glories Y., 1983. Some interpretations of colour changes in young red wine during their conservation. *J. Sci. Food Agric.* 34, 505-516.
- Russouw, M. & Marais, J., 2004. The phenolic composition of South African Pinotage, Shiraz and Cabernet Sauvignon wines. *S. Afr. J. Enol. Vitic.* 25, 94-104.

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

Schwarz, M., Wabnitz, T.C. & Winterhalter, P., 2003. Pathway leading to the formation of anthocyanin-vinyl phenol adducts and related pigments in red wines. *J. Agric. Food Chem.* 51, 3682-3687.

Spayd, S.E., Tarara, J.M., Mee, D.L. & Ferguson, J.C., 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* 53, 171-181.

Stoyanov, N., Kemilev, S., Spasov, H., Metodieva, R. & Chobanova, D., 2002. Extractability of grape seed and skin phenolic compounds during grape maturity. Department of Winemaking and Brewing, University of Food Technologies, Plovdiv, Bulgaria.

Tardaguila, J., De Toda, F.M., Poni, S. & Diago, M.P., 2010. Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. *Am. J. Enol. Vitic.* 61, 372-381.

Timberlake, C.F. & Bridle, P., 1976. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* 27, 97-105.

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

Vivas, N., Glories, Y., Bourgeois, G. & Vitry, C., 1996. The heartwood ellagitannins of different oak (*Quercus* sp.) and chestnut species (*Castenasativa* Mill.) quantity analysis of red wines aging in barrels. *J. Sci. Tech. Tonnellerie* 2, 51-75.

Yamane, T., Jeong, S.T., Goto-Yamamoto, N., Koshita, Y. & Kobayashi, S., 2006. Effects of temperature on anthocyanin biosynthesis in grape berry skins. *Am. J. Enol. Vitic.* 57, 54-59.

Chapter 3

Results

**Grape and wine phenolic composition
as a result of training system and
canopy modification in *Vitis vinifera*
L.cv Shiraz.**

3.1 Introduction

South African producers are looking for ways to increase grape production without compromising quality, which could possibly be achieved by adapting vineyard training systems. The vertical shoot positioning system (VSP) is currently the most widely used training system in the Stellenbosch area. This system has some disadvantages, however, as it is not well suited to vineyards with higher vigour (Reynolds & Van den Heuvel, 2009). The Smart Dyson (SD) training system seems suitable for South African conditions, as some growers have problems with highly fertile soils that may lead to overly vigorous vine growth that causes dense canopies and excessive vegetative growth. However, these conditions may lead to lower quality grapes. Heavy canopy management is often applied to these over-vigorous vines to increase the quality of the grapes, but this leads to a decrease in yield. The SD training system splits the canopy of the vine, making it less dense while increasing the bud load on the vine, which could lead to increases in yield (Bosman, 2011). This training system is therefore able to accommodate more vigorous vines without the canopy becoming overly dense, which would lead to decreased colour due to less light penetration, lower temperatures and excessive vegetative growth (Dry, 2000). All of these changes may lead to increased fruit yield of a good quality.

Phenolics are critical to the quality of red wine, as they play an important role in the colour and mouth-feel of the wine. Phenolic compounds are formed in secondary metabolic pathways and their biosynthesis is greatly influenced by viticultural management practices and the climatic conditions that these practices can induce in the vine (Downey *et al.*, 2006). It has been shown that the temperature and light exposure of berries have a big influence on the synthesis of phenols (Downey *et al.*, 2006). A study by Monagas *et al.* (2005) showed that increased light exposure led to increases in phenols, especially flavonols, which are directly linked to UV exposure (Monagas *et al.*, 2005). An increase in temperature also has a positive effect on the synthesis of phenolics, although temperatures above 35°C may lead to a breakdown of these compounds (Heyns, 2010). The different concentrations of each of these phenolic compounds also affect the ageing ability of the wine, as they differ in reactivity and thus in their influence on the development of red wine. An increase in yield may lead to a decline in these compounds, as the amount synthesised has to be dispersed to all the grapes on the vine. It has not been reported in the literature whether the less dense canopy of the SD training system, with a more effective and larger leaf area, is able to produce sufficient levels of grape phenolics with the increased fruit yield under South African conditions. Studies are also lacking which investigate the effect of wine ageing on differences in the phenolic compositions of young red wines brought about by different vineyard treatments. The main aim of this study was therefore to investigate the phenolic and sensory composition of red wines produced from Shiraz vines that were exposed to different viticultural management treatments and how these evolved over time. This study forms a small part of a larger one that focuses on a variety of viticultural treatments' effects on phenolics and sensory composition of Shiraz grapes and wines.

3.2 Materials and Methods

3.2.1 Experimental layout of the vineyard

Small-scale experiment

The small-scale experiment was conducted on a block of *Vitis vinifera* L.cv. Shiraz (SH9C clone) on 101-14 Mgt rootstock located on the Welgevallen experimental farm of Stellenbosch University (33°56'S, 18°52'E). The vines were spaced at 2.7 x 1.5m and consisted of a seven-wire training system with movable wires for the VSP training system and the addition of an extra wire 30cm below the cordon for the SD training system. The SD training system was converted from a VSP in the 2011/2012 growing season.

The experimental layout as can be seen in **Figure 3.1** and consisted of a random block design, with 18 vines being randomly assigned to a treatment in the designated block. The SD training system treatments were induced on high-vigour vines that visually exhibited excessive growth and over utilisation of the allocated seven wire VSP training system (Van Noordwyk, 2012; Bosman, 2013). Preliminary findings of the study by Bosman (data not shown) confirm above-average pruning mass values and low canopy light interception values for the original VSP system. Top and bottom shoots were also divided into different treatments [high-vigour SD top shoots (HSDA) and high-vigour SD bottom shoots (HSDB)], as differences between the shoots have been reported (Smart *et al.*, 1990). They were only kept apart to assess the effect on phenols and were calculated together to determine overall yield of the training system. The VSP high-vigour full canopy (HC) used as a control was also selected from among the high-vigour vines to be able to compare them to the SD treatments. The reduced canopy management treatment (R) was not in the original experimental layout, but it was later decided to include this as well. This was done to assess the effect of canopy reduction on vegetative growth to manage vigour. The R treatment was split by a split block design into three different parcels in the vineyard prior to the other treatments from a previous study to assess the extent of vineyard differences. The reduced canopy treatment consisted of removal of the top shoot and its grapes on a two-bud spur around flowering time. Therefore effectively the canopy was halved. The randomised block design was chosen for the HC and HSD treatments to eliminate the possibility of natural heterogeneity of the vineyard. This experiment was repeated for both the 2011/2012 and 2012/2013 growing seasons.

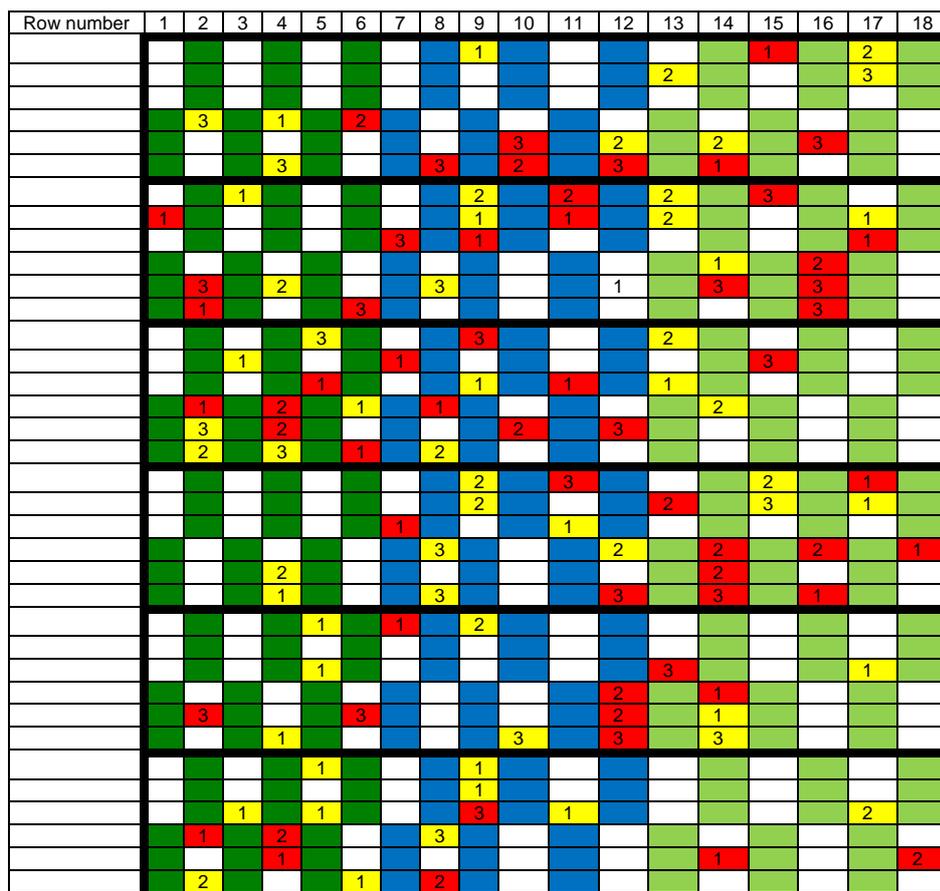


Figure 3.1: The experimental layout of the small scale experiment on the Welgevallen experimental farm where each block represents one vine. The randomized design of the VSP treatment in red and SD treatment in yellow with their repeats indicated by numbers within each block. It also shows the block design of the reduced canopy management treatment with repeat one, two and three being indicated by the dark green, blue and light green blocks respectively.

Commercial-scale experiment

A commercial-scale experiment was also done to assess whether differences in wine phenolics and colour induced by the different training systems were evident during wine ageing in commercial size 225L barrels. It was felt that an experiment in industry sized barrels was needed to best replicate the interactions of the phenols with tannins from the wood and oxygenation speed through the barrels.

This commercial experiment was not conducted the first year (2012) as no suitable vineyards in the industry could be found. It was thus decided to convert a larger part of the Shiraz vineyard to Smart-Dyson on the experimental farm for the second year (2013) of this project. The conversion from VSP to double bearers was done in the 2012/2013 season and was only fully converted to SD in the 2013/2014 season. The grapes were therefore sourced from the same block as those for the small-scale experiment, but from a different section. On a part of the block that was not used for the small-scale experiment, rows were alternated between a VSP training system and the double-bearer pruning system (which would become the Smart-Dyson system, with three repeats randomly drawn from each

training system making up the treatments. Considering that during the first year of the conversion the shoots were not bent down, it could not be referred to as a Smart-Dyson training system and this is why we use the “double bearer training system” naming, as there were two spurs per bearer on the vine.

3.2.2 Winemaking

Harvesting parameters

For both the small and commercial scale experiments the grapes were hand harvested based on the parameters pH, titratable acidity (TA) and total soluble solids, measured in degrees Balling(°B). The grapes were harvested as close as possible to the following values: pH 3.5 to 3.8, TA of 4 to 4.5 g/L, and a sugar concentration of 23 to 25°B (**Table 3.2**). The grapes were harvested on 11 March 2012 for the first season and on 19 March 2013 for the second season. Grape samples were also taken for phenolic analyses at harvest and frozen at -20°C until analysed.

Winemaking procedures

Each treatment repeat was harvested separately and treated as a separate wine from harvest onwards for the entire winemaking procedure. Random samples of grapes were taken from each treatment repeat for analyses, after which they were used to make the experimental wine. This was done to compensate for differences that might have occurred between individual vines. After harvesting, the grapes for the small-scale experiment were crushed, de-stemmed and 20 mg/L SO₂ added before fermentation. The must was then inoculated and fermented with *Saccharomyces cerevisiae* D21 yeast (Lallemand) at 25°C. The co-inoculant, Endoferm Alpha (Lallemand), was used for malolactic fermentation (MLF) and added to the wine 24 hours after yeast inoculation. The skins of the fermenting must were mixed manually (punch down) twice a day, after which the Balling was measured to follow the progression of fermentation. After the wine has fermented dry (residual sugar < 4 g/L), the skins were pressed with a basket press and the press wine mixed with the free-run wine. The wines were then stored in 20 L stainless steel canisters until completion of malolactic fermentation (MLF). MLF was monitored by using a Grapescan FT 120 instrument. After the completion of MLF (<0.3 mg/L malic acid), 30 mg/L of SO₂ were added, while the same amount of SO₂ was added before the wines were bottled in standard 750ml emerald green wine bottles and sealed with screw caps.

For the commercial-scale experiment, 500 kg grapes of each treatment repeat were crushed and the titratable acidity was adjusted to 6g/L for all treatments using tartaric acid. Fermentation took place in plastic bins using *Saccharomyces cerevisiae* D21 yeast (Lallemand). The skins were mixed twice a day (punch downs) and pressed to complete dryness (residual sugar < 4 g/L), after which they were

mixed with the free-run wine in 225L old French oak barrels. The sequential MLF regime (after alcoholic fermentation MLF) was carried out in the barrels using *Oenococcus oeni* PreAc 450 (Laffort), as insufficient co-inoculation bacteria were available. The barrels for the commercial scale experiment were stored in barrel room of Welgevallen Cellar at a constant temperature of 18°C. SO₂ measurements were taken once a month and adjusted to a free SO₂ level of 35 mg/L. Samples for colour and phenolic analyses of the wines were taken at three month intervals after MLF, after six months (for both 2012 and 2013 vintages), and after 12 months (for the 2012 vintage).

3.2.3 Spectrophotometric analyses

3.2.3.1 Bovine serum albumin tannin analysis

The bovine serum albumin analysis (BSA) uses the ability of tannins to form complexes with proteins and to precipitate to determine the tannin concentration in grapes and wines (Harbertson *et al.*, 2002). The specific procedure was as follows: 50 grape berries were randomly selected at harvest for tannin analysis, the grape berries were then homogenised for four minutes in a homogeniser, one gram of the homogenate was mixed with 10ml of a 50% ethanol solution and left to extract for an hour at room temperature. The homogenate was then centrifuged for 5 min at 3500rpm and the supernatant was retained for tannin analysis according to Harbertson *et al.* (2002). For the preparation of the wine sample, one mL of red wine was taken and filtered for analysis according to Harbertson *et al.* (2002).

3.2.3.2 Colour density

Wine samples were measured in a 1mm quartz or glass cuvette at 420 nm, 520 nm and 620 nm to determine the colour density according to Iland *et al.* (2000).

3.2.3.3 Modified colour density

The pH of the wines was adjusted to pH 3.5 using 1M of HCl to acidify the wine or 1M of NaOH to increase the pH of the treatments where necessary. Ten µL of a 10% acetaldehyde solution was then added to one mL of wine sample, mixed and allowed to react for 45 min at room temperature according to Iland *et al.*, (2000). The modified colour density was then measured as described in section 3.2.3.2.

3.2.4 HPLC analysis

Grape sample preparation for the HPLC analysis consisted of homogenising 50 grape berries in a homogeniser for four minutes. One gram of the homogenate was then mixed with 10 ml 50% ethanol solution (at pH 2) and left to extract for an hour. The mixture was then centrifuged for 5 min at 3500 rpm and the supernatant was retained for the HPLC analysis. For wine, sample preparation consisted of filtering one mL of wine sample through a 0.45µm filter prior to the HPLC analysis.

The HPLC analysis was performed on a diode array detector HPLC and adapted from a method developed by Peng *et al.* (2002). The separation was carried out in a polystyrene/divinylbenzene reverse-phase chromatographic column. The mobile phases used were a 1.5% v/v orthophosphoric acid solution in de-ionised water (mobile phase A) and an acetonitrile solution (mobile phase B). The linear gradient used for the two phases was 0min to 73 min; A: 95% and B: 5%, 73 min to 78 min; A: 75.2% and B: 24.8%; and for the remainder of the run it stayed constant at A: 50% and B: 50%. The flow rate was 1mlmin⁻¹ at a constant temperature of 35°C. The standards used were quercetin (Extrasynthèse, France), quercetin-3-glucoside (Fluka), malvidin-3-glucoside (Polyphenols Laboratories AS, Norway), p-coumaric acid (Sigma), caffeic acid (Sigma), gallic acid (Sigma), (-)-epicatechin (Sigma) and (+)-catechin (Fluka). Flavan-3-ols (sum of monomeric and dimeric units) and polymeric phenols were measured as mg/L catechin equivalent units with a quantification limit of 1.5 mg/L. Epicatechin was quantified as epicatechin with a quantification limit of 1.5 mg/L. Gallic acid was quantified as gallic acid with a quantification limit of 0.25 mg/L. Catearic acid and caffeic acid were quantified as mg/L caffeic acid equivalents, while coumaric acid and p-coumaric acid were quantified as mg/L p-coumaric unit equivalents. Flavonol-glycosides and flavonol aglycones were quantified as mg/L quercetin-3-glucoside and mg/L quercetin equivalents respectively, while monomeric anthocyanins and polymeric pigments were quantified as mg/L malvidin-3-glucoside equivalents with a quantification limit of 1.25 mg/L. These compounds were measured at different wave lengths: 280nm (flavan-3-ols), 320nm (hydroxycinnamic acids), 360nm (flavonols) and 520nm (anthocyanins). For simplification and to get a clearer view of the results individual compounds were sometimes summed and are expressed in broader categories, namely the sum of total anthocyanins, total flavan-3-ols, flavonols, polymerized pigments, polymerized phenols and hydroxycinnamic acids.

3.2.5 Sensory evaluation

Because of time and financial constraints it was decided to do sorting testing on the wine rather than descriptive analysis (DA), as it has been shown that the results of these two methods are comparable (Cartier *et al.*, 2006). Sorting is considered a qualitative testing method for identifying differences between treatments, while DA is a quantitative method used to describe the scale of the differences (Chollet *et al.*, 2011). All sensory evaluations were done in standard ISO glasses in a blind manner. All wines were presented to the judges in a randomised order.

In 2012, after six months of ageing, 21 wines from the small-scale experiment were selected for testing. The selection included 12 wines (four treatments x three repeats) that formed part of this Master's study, as well as nine other wines produced in the same manner from the same vineyard, but from low-vigour vines that formed part of another study. This was done due to time and financial constraints. An untrained panel of 30 wine experts, consisting mostly of winemakers, was used, as it

has been shown that there are no significant differences between trained and untrained panels when it comes to using sorting sensory techniques (Cartier *et al.*, 2006). The panel was presented with clear glasses and asked to sort them into groups with similar attributes. No information was given about the experiment, except for the cultivar of the wine.

In 2013, after six months of ageing, only the 12 wines (three repeats of each of HC, HSDA, HSDB and the R treatment) from the small-scale experiment were selected to be used for the sensory evaluation. Again, 30 wine experts were selected for the panel and given no information about the experiment. The wines were sorted in black glasses, as the treatments had significant visual colour differences and it was considered to be a variable that would skew the results. To obtain more descriptive information, the panel was also asked to select descriptors for wines in a group from a list of descriptors provided. The descriptors were selected from a standardised list of Shiraz wine descriptors (Campo *et al.*, 2008). The panellists had to sort the wines in two different sessions. In the first session the wines had to be sorted according to aroma and in the second session according to mouth-feel.

3.2.6 Statistical analysis

Integrations of the separation were done on Chemstation software and the statistics for the ANOVA analyses and biplots were performed on Statistica 10. Multi-dimensional scaling (MDS) graphs were generated through the use of Pearson correlations for the sensory data.

3.3. Results and discussion

Phenolics are influenced by a wide range of factors as broadly defined in Chapter 2. Most of the factors that play a role in the determination of non-volatile phenolics in especially red grapes and to a lesser extent red wine, fall outside the focus of this study. Discussions in terms of grape results are therefore largely based on literature. This is due to treatment differences being induced by viticultural influences that was not the focus of this study. This study therefore assessed the results from an oenological perspective, which forms a small part of a larger study that can ultimately help determine the actual causes of the treatment differences found.

Even though the yield measurements fell outside the scope of this study, they were integral to understanding the results obtained. The yield per vine is presented in **Table 3.1**. The yield of the reduced canopy management (R) treatment over the two years of the study was approximately 50% lower than that of the high vigour vertical shoot positioning (HC) treatment, while the high vigour Smart-Dyson (HSD) top and bottom shoots (sum of A+B) was 40% higher in 2012 and 67% higher in 2013. For the large scale experiment in 2013 the increase in yield of the double bearer (5.4kg per vine) over the VSP (7.5kg per vine) was $\pm 30\%$ (data not shown). The large scale yield results are not of value as it is the first year of conversion and the vine still has to find a new equilibrium when finally converted to SD (Bosman, 2011).

Table 3.1: The yield per vine for the small-scale experiment for 2012 and 2013, with HC taken as control as 0 and the other treatments' yields as a percentage difference from that. Different letters indicate significant differences at $p < 0.05$.

	2012		2013	
	Average yield per vine (kg)	%	Average yield per vine (kg)	%
R	2.75a	-51	3.13a	-52
HC	5.61b		6.55b	
HSD (A+B)	7.84c	40	10.95c	67

3.3.1 Grape berry data

There was attempted to harvest as uniform as possible at a ripeness level that gave the highest phenolic levels in the vineyard in previous studies (Van Noordwyk, 2012) though variability between the repeats were still observed (**Table 3.2**). However, this variability was minimised through the use of the randomised block design, as can be seen in **Table 3.2**. The deviation indicates that although specific harvest parameters were set, slight deviations may influence the results.

Table 3.2: Average pH, TA and °B values of the different treatments at harvest.

	Treatment		2012			2013		
			pH	TA (g/L)	°B	pH	TA (g/L)	°B
Small scale experiment	HC	Avg	3.86	4.28	23.73	3.87	4.62	24.83
		Std dev	0.00	0.15	0.31	0.06	0.09	0.76
	HSDA	Avg	3.75	4.15	24.03	3.65	4.34	22.80
		Std dev	0.06	0.23	0.21	0.02	0.20	1.01
	HSDB	Avg	3.74	4.20	24.03	3.65	4.52	23.57
		Std dev	0.04	0.07	0.15	0.03	0.35	0.23
	R	Avg	3.76	4.24	24.20	3.57	4.53	23.93
		Std dev	0.14	0.20	0.85	0.02	0.03	0.31
Commercial scale experiment	Commercial VSP	Avg	N/A	N/A	N/A	3.61	5.12	23.83
		Std dev	N/A	N/A	N/A	0.05	0.33	0.81
	Commercial double bearer	Avg	N/A	N/A	N/A	3.58	5.51	23.40
		Std dev	N/A	N/A	N/A	0.04	0.61	0.61

3.3.1.1 Tannins, total flavan-3-ols, polymeric phenols, total anthocyanins and polymeric pigments

Tannins, total flavan-3-ols, total anthocyanins, polymeric pigments and polymeric phenols are discussed under one heading as there is interaction between these components and the concentration of one could have an effect on one or more of the others (Monagas *et al.*, 2005).

Small-scale experiment

Tannins, total flavan-3-ols and polymeric phenols

The phenolic grape data of 2012 can be seen in **Table 3.3**. No significant differences were observed in the tannin concentrations of the grapes between the different treatments in 2012 (**Table 3.3**), except for the HSDB treatment, which was lower than the other treatments. This could be ascribed to the reduced vigour of the HSDB treatment's shoots that may have created an environment of shoots having too low an exposed leaf area per kg grape yield (Kliwer & Dokoozlian, 2005; Petrie *et al.*, 2008). This could have the effect of a lower biosynthesis of tannins (Zoecklein *et al.*, 2008). The flavan-3-ol concentration showed that the R treatments were significantly higher in flavan-3-ol phenol concentration than the other treatments (**Table 3.3**). This could be explained by increased sunlight on the R treatment because of the heavy canopy management, which can lead to increases in flavan-3-ol concentrations (Spayd *et al.*, 2002). Polymeric phenols showed significantly higher levels in the HSD (A+B) treatments compared to the other treatments (**Table 3.3**). The reason for this may be that an increased water deficit could lead to an increase in the flavan-3-ol concentration that forms part of the monomers that make up polymeric phenols (Ojeda *et al.*, 2002). The larger leaf area of the HSD

(A+B) treatment could have led to an increase in water stress (Bosman, 2013), increasing the water stress in the HSD (A+B) treatment (Howell, 1999). The high variance in the reduced canopy management was probably due to the experimental layout and indicated how vineyard differences may influence tannin levels, as the one side of the vineyard showed stronger vegetative growth than the other side (Strever, 2012).

During 2013, the second year of the study, differences in the phenolic compounds measured in the grapes were observed between the treatments. The HSDB treatment was significantly higher than the other treatments when considering tannin levels (**Table 3.4**). All the vines were stressed to some degree due to a water deficiency during the growing season of 2013 (Bosman, 2013), and this stress seems to have voided the treatment differences of the high-vigour vines. The higher tannin concentration in the HSDB treatment may be because it had reduced growth due to phototropism. This made the treatment more susceptible to stress as a result of increased vegetative growth, which leads to increased photosynthesis and reduces the vines' capability to handle stress because of excess water loss. This could have a greater influence on the shoots than reduced vigour, which decreases the tannin concentration (Zoecklein *et al.*, 2008). Reduced berry size can also lead to a concentration effect in the treatments (Downey, 2010). However, tannin concentrations in general were significantly lower than those observed in the previous year. The flavan-3-ols showed a big decrease in concentration compared to levels in the previous year between the treatments (**Table 3.3 & 3.4**). In 2013, the treatments showed signs of stress much earlier in the season and much more intensely than in 2012 and, as flavan-3-ols are formed early in berry development, this could explain the lower concentrations, as the degree of stress prohibited the synthesis of these compounds (Kennedy *et al.*, 2001). The stress in 2012 when compared to 2013 was of such a degree that it probably led to a concentration of tannins in the berries as a result of their decreased size and less water content (Ojeda *et al.*, 2002). HSDB was the only treatment that differed significantly from the other treatments in 2013. This is likely due to increased light exposure of the grapes because of the reduced vigour of the HSDB shoots. The increased UV exposure will cause an increase in the flavan-3-ol concentration (Haselgrove *et al.*, 2000; Spayd *et al.*, 2002; Downey *et al.*, 2006). The polymeric phenol concentration showed significantly higher levels in the HC and HSDB when compared to the HSDA and R treatments (**Table 3.4**). The reason for these values could not be established, although it was most likely due to climactic influences. In 2013 the vines were under high water stress and the ripening of the berries probably stopped before phenolic ripeness was achieved. This caused the vines to exhibit very unusual results that could not always be explained. Because of the vintage differences it was difficult to compare the two years' results.

Total anthocyanins and polymeric pigments

In 2012 there were no significant differences in total anthocyanin and grape polymeric pigment concentrations between the treatments (**Table 3.3**). The higher variance in the reduced treatment has to be noted, as this could be due to the large vineyard heterogeneity. Polymeric pigments were very low (**Table 3.3**) compared to the values found in the wine, as the anthocyanins in the grapes are not yet as polymerised through interactions with tannins as those in the wine (**Table 3.3**) (Somers, 1976). Previous studies done by Van Noordwyk (2012) also confirmed these results, with polymeric pigments being very low in the grapes compared to the wine from the same Shiraz vineyard used in this study.

However, the results from the 2013 vintage showed some interesting trends, with only the HSDB treatment being significantly lower in total anthocyanin concentration than the other treatments (**Table 3.4**). This was most likely due to the increased stress the vines experienced in the 2013 season (Bosman, 2013). Because of the increased leaf area and more open canopy configuration of the HSD (A+B) treatments it showed signs of stress earlier in the season compared to the other treatments showed a lower accumulation of anthocyanins (Bergqvist *et al.*, 2001). The HSDB shoots, with their lower vigour, were particularly susceptible to stress, probably even more so than the HSDA shoots. The polymeric pigments were lower in concentration than the 2012 results, with the HSDA treatments being significantly lower than the other treatments. The HC treatment was significantly higher in polymeric pigments than the other treatments. No explanation could be found for these observations regarding the polymeric pigments.

The vintage effect can be seen clearly, as the grapes had greatly reduced levels of tannins during the second year of experimentation. However, smaller differences were observed between polymeric pigment concentrations in the two vintages. This is because the grape polymeric pigments are present at lower levels because tannins and anthocyanins do not react with each other (Somers, 1976) in grapes to the extent that they do in wine. The differences between the two years are most likely due to climatic differences and the serious ripening and water stress problems with the vines in 2013 (Bosman, 2013).

Commercial-scale experiment

Tannins, total flavan-3-ols, polymeric phenols, total anthocyanins and polymeric pigments

The commercial-scale experiment that was only done in 2013 with an increased crop yield for the double-bearer pruning training showed significantly lower levels of anthocyanins, flavan-3-ols, polymeric phenols and polymeric pigments compared to the VSP treatment (**Table 3.5**). The increased yield might have forced the vine to distribute the available nutrients to all the grapes, thus

leading to a dilution effect that also affected the phenol concentration, in this case specifically that of the anthocyanins and flavan-3-ols (Matthews & Nuzzo, 2007). This could also explain the difference in the concentration of polymeric pigments, as they are polymers of anthocyanins and tannins (Cheynier *et al.*, 2006), though these react to a lesser extent in the grapes (Somers, 1976). These results therefore confirm the greater effects that yield has on the phenol concentration compared to the differences in the microclimate, as only the yield and shoots were increased, with no splitting of the canopy and minimal differences in the microclimate. The tannin concentration did not show the same trend, as no differences were observed between treatments. Bravdo *et al.* (1985) found that increased yield had little effect on the concentration of tannins.

Table 3.3: Phenolic grape data of the 2012 small scale experiment. Values are means of triplicate repeats; different letters indicate significant differences at $p < 0.05$.

		Full canopy VSP (HC)	SD bottom shoots (HSDB)	SD top shoots (HSDA)	Reduced (R)	p-value
Tannins (mg/L)	Avg	97.21a	86.93b	95.69a	90.15a	0.05
	Stddev	3.83	1.82	4.93	31.64	
Polymeric pigments (mg/L)	Avg	11.77	13.85	14.15	10.40	0.1
	Stddev	0.98	1.69	1.27	3.22	
Total anthocyanins (mg/L)	Avg	99.27	101.31	110.46	104.06	0.67
	Stddev	6.44	15.72	10.24	35.54	
Polymeric phenols (mg/L)	Avg	154.36b	191.31a	215.17a	156.39b	0.02
	Stddev	14.61	22.54	10.27	59.05	
Total flavonols (mg/L)	Avg	0.25	0.27	0.29	0.39	0.1
	Stddev	0.03	0.01	0.02	0.10	
Total hydroxycinnamic acids (mg/L)	Avg	0.39b	0.42b	0.62b	1.10a	0.02
	Stddev	0.12	0.14	0.17	0.50	
Total flavan-3-ols (mg/L)	Avg	5.04b	4.86b	5.04b	5.94a	0.02
	Stddev	0.66	0.46	0.31	3.07	

Table 3.4: Phenolic grape data of the 2012 small scale experiment. Values are means of triplicate repeats; different letters indicate significant differences at $p < 0.05$.

		Full canopy VSP (HC)	SD bottom shoots (HSDB)	SD top shoots (HSDA)	Reduced (R)	p-value
Tannins (mg/L)	Avg	40.23 b	47.20 a	39.93 b	41.78 b	0.05
	Stddev	0.63	3.85	3.62	2.51	
Polymeric pigments (mg/L)	Avg	11.70 a	10.14 b	8.37 c	9.29 b	<0.01
	Stddev	0.73	1.32	0.38	1.09	
Total anthocyanins (mg/L)	Avg	155.73 a	130.73 b	141.01 a	150.20 a	0.02
	Stddev	14.77	18.51	8.17	8.80	
Polymeric phenols (mg/L)	Avg	253.20 a	243.52 a	202.92 b	209.94 b	<0.01
	Stddev	22.51	17.93	17.87	24.99	
Total flavonols (mg/L)	Avg	0.22 b	0.26 a	0.22 b	0.22 b	<0.01
	Stddev	0.01	0.02	0.00	0.01	
Total hydroxycinnamic acids (mg/L)	Avg	1.24 a	1.19 a	1.14 a	0.99 b	0.01
	Stddev	0.10	0.08	0.22	0.24	
Total flavan-3-ols (mg/L)	Avg	1.56 b	2.45 a	1.58 b	1.67 b	<0.01
	Stddev	0.10	0.14	0.15	0.07	

Table 3.5: Phenolic grape data of the 2013 commercial scale experiment. Values are means of triplicate repeats; different letters indicate significant differences at $p < 0.05$.

		Double bearer	VSP	P-value
Tannins (mg/L)	Avg	35.28	31.07	0.1
	Stddev	2.29	5.15	
Polymeric pigments (mg/L)	Avg	7.14 b	9.63 a	0.07
	Stddev	0.72	1.57	
Total anthocyanins (mg/L)	Avg	112.55 b	130.42 a	0.03
	Stddev	10.53	7.39	
Polymeric phenols (mg/L)	Avg	166.46 b	205.41 a	0.07
	Stddev	7.52	20.06	
Total flavonols (mg/L)	Avg	0.21 b	0.24 a	0.02
	Stddev	0.00	0.02	
Total hydroxycinnamic acids (mg/L)	Avg	1.00	0.97	0.8
	Stddev	0.13	0.11	
Total flavan-3-ols (mg/L)	Avg	1.40 b	2.18 a	<0.01
	Stddev	0.17	0.04	

3.3.1.2 Total flavonols

Small-scale experiment

Flavonols are accumulated through UV radiation exposure of the berries (Monagas *et al.*, 2005). In 2012 there were no significant differences between the treatments in terms of total flavonol concentrations (**Table 3.3**). Therefore it can be hypothesised that the amount of UV that the berries were exposed to was of a similar amount or -nature, or that the degree of exposure was sufficient for maximum flavonol synthesis. The fact that the shoots were bent down in the first year of conversion after the first peak in the synthesis of flavonols during flowering as explained in section 2.4.4 may also explain why there were no differences between the treatments (Downey *et al.*, 2006).

In 2013, total flavonol concentrations were measured in the same range as in 2012 (**Table 3.4**), except for the HSDB treatment that showed higher levels (**Table 3.4**). This could be explained by the fact that flavonols have two accumulation peaks during berry growth, with the second peak occurring during véraison (Downey *et al.*, 2004), when canopy density will have a greater influence on flavonol accumulation. The HSDB treatment with its low canopy density therefore could have been more exposed to UV radiation in 2013 than in 2012.

Commercial-scale experiment

With the increased yield in 2013, the VSP treatment showed a significantly higher concentration of flavonols than the double bearer treatment (**Table 3.5**). This could be explained by a dilution effect of the increased yield on the phenol concentration of the grapes in the double-bearer treatment (Matthews & Nuzzo, 2007). It can also be explained by the increased canopy thickness due to the extra shoots on the double-bearer treatment, which may shield the grapes from the sun, thereby leading to lower flavonol accumulation (Downey *et al.*, 2006).

3.3.1.3 Hydroxycinnamic acids

Small-scale experiment

Although only occurring in small quantities hydroxycinnamic acids could have an influence on the taste (bitterness) of wine (Russouw & Marais, 2004). In 2012, only the R treatment was significantly higher than the other treatments in terms of hydroxycinnamic acid concentrations (**Table 3.3**). Hydroxycinnamic acids are located in the vacuoles of grape cells and it can therefore be postulated that larger berry cells with larger vacuoles may lead to a higher concentration of hydroxycinnamic acids (Monagas *et al.*, 2005). This effect is mitigated by the large positive influence that light and temperature due to a reduced canopy have on the hydroxycinnamic acid concentration (Heyns, 2010). Although the reduced grape berries were smaller, data not shown, (Bosman, 2013), the increased light

and temperature exposure of the grapes increased the concentration of the hydroxycinnamic acid. In contrast to 2012, the only significant difference observed was for the R treatment in 2013 that showed a lower concentration of hydroxycinnamic acid compared to the other treatments (**Table 3.4**). This might be due to the decreased vigour of the reduced treatment shoots (Zoecklein *et al.*, 2008), or differences in water stress.

Commercial-scale experiment

The hydroxycinnamic acid concentrations measured in the grapes of the commercial scale experiment showed no significant differences between the treatments in 2013 (**Table 3.5**). This showed that hydroxycinnamic acid concentration is not affected much by the yield, as it is produced soon after fruit set (± 20 days after set) and thus the increase brought on by increased/decreased berry size will have little effect on the concentration of hydroxycinnamic acids (Kennedy *et al.*, 2001).

3.3.2 Wine results

3.3.2.1 Colour density and modified colour density

Small-scale experiment

Modified colour density basically indicates the colour density of the wines, with the effect of pH and SO₂ on colour negated. The pH influences the colour by determining in which form anthocyanins occur in the wine, with a lower pH resulting in more flavylum ions and a higher pH leading to the more colourless pseudo base (Du Toit *et al.*, 2006; Jurd, 1964).

In 2012, only the R treatment was significantly higher in terms of colour density than the rest of the treatments as expected from the increased temperature and light on the grapes of the R treatment that will lead to higher levels of anthocyanins in the grapes and ultimately the wine (Spayd *et al.*, 2002) (**Table 3.6**). Over the first twelve months after MLF completion there was only a slight, general decrease in the colour density (**Table 3.6**). This phenomenon is supported by literature that shows that large decreases in colour density may still occur after twelve months of ageing as anthocyanins polymerise (Monagas *et al.*, 2005). Because the wine was stored under screw cap, the initial polymerisation that took place was probably acetaldehyde-mediated polymerisation due to the oxygen introduced to the wine during bottling (Kwiatkowski *et al.*, 2007). This reaction will become less important with ageing, as there is little oxygen ingress through screw caps (Duncan & Kleinig., 1999). The main reactions after a few months were therefore probably direct association between anthocyanins and tannins that do not require any oxygen (Ribéreau-Gayon *et al.*, 1983).

The 2013 results showed the same trend as in the previous year, with the R treatment showing significantly higher colour density than the other treatments (**Table 3.7**). This was also found by Van Noordwyk (2012), with the same R treatments leading to significantly higher colour density values compared to the HC treatments. It should be noted, however, that the HSDA and HSDB treatment was significantly lower when colour density was measured than the HC treatment (**Table 3.7**). This difference, although significant, was not large and will probably not influence the visual perception of the wines. After six months of ageing the colour density of all the treatments showed no decline but rather the same range as measured after MLF (**Table 3.7**).

The only difference noted in the 2012 modified colour density measurements for the R treatment was the modified colour density, which was higher than that of the other treatments (**Table 3.6**). When the influence of pH is eliminated, the effect of co-pigmentation and SO₂ bleaching can clearly be seen by the increase in the modified colour density at six months (Picinelli *et al.*, 1994; De *al.*, 2005). At the beginning of ageing, bonds between SO₂ and anthocyanins, as well as between co-pigmented anthocyanins, may break (Boulton, 2001), which may lead to changes in the modified colour density. The colour density then declines with longer ageing due to polymerisation and the possible precipitation of the free monomeric anthocyanins (Monagas *et al.*, 2005).

In 2013 no significant differences were observed after MLF in terms of modified colour density between the treatments. However, after six months of ageing the R- and HC treatment were significantly higher than the HSDA and HSDB treatments (**Table 3.7**). This result differed from that for colour density. This could be due to the higher pH of the HC treatment wines compared to the wines from the HSDA and HSDB treatments (**Table 3.2**), which could also explain the differences observed between R- and HC treatment in terms of colour density and modified colour density. After six months' ageing the modified colour density of the R and HC treatment increased significantly. Again this is probably due to bonds between SO₂ and anthocyanins, as well as between co-pigmented anthocyanins, that break with aging (Boulton, 2001) as also seen in 2012.

Commercial-scale experiment

In 2013, no significant differences were observed between treatments for neither colour density nor modified colour density after MLF (**Table 3.8**). However, colour density decreased significantly regardless of treatment after six months. This could be due to polymerization and precipitation of anthocyanins that leads to the density declining with ageing (Monagas *et al.*, 2005). After six months' ageing, the modified colour density of the double-bearer treatment remained unchanged, while the VSP treatment showed a significant increase in concentration (**Table 3.8**). This increase in the VSP treatment may be due to the polymerisation reactions through the interactions with tannins and acetaldehyde that take place in the wine from the VSP treatment that is aged in barrels, which may happen at a much faster rate than wine aged in bottles (Vivas & Glories, 1996). This is due to the

increased oxygen exposure that helps drive the reaction between tannins and wine phenols (Vivas & Glories, 1996).

Table 3.6: Colour density, modified colour density, HPLC and BSA wine data of the 2012 small scale experiment measured at six month intervals from after MLF to 12 months aging. Values are means of triplicate repeats; different letters indicate significant differences at $p < 0.05$.

		Reduced canopy (R)			Full canopy VSP (HC)			Smart-Dyson top shoots (HSDA)			Smart-Dyson Bottom shoots (HSDB)			p-value
		After MLF	6 months	12 months	After MLF	6 months	12 months	After MLF	6 months	12 months	After MLF	6 months	12 months	
Colour density (AU)	Avg	1.57 a	1.51 a	1.39 b	1.11 c	1.05 c	0.95 d	1.19 c	1.14 c	1.03 c	1.07 c	1.06 c	1.01 c	0.05
	Stddev	0.18	0.07	0.12	0.23	0.14	0.12	0.03	0.06	0.04	0.08	0.12	0.13	
Modified colour density (AU)	Avg	1.69 b	1.87 a	1.67 b	1.12 d	1.26 c	1.13 d	1.14 d	1.30 c	1.11 d	1.19 d	1.28 c	1.14 d	0.06
	Stddev	0.08	0.15	0.15	0.08	0.07	0.08	0.11	0.06	0.08	0.15	0.10	0.09	
Tannins (mg/L)	Avg	101.99 b	163.65 a	115.88 b	35.55 d	45.98 d	34.67 d	75.21 d	58.92 d	44.34 d	58.13 d	77.81 c	31.46 d	0.03
	Stddev	33.86	28.40	14.16	52.43	2.34	47.40	23.55	22.61	32.11	1.81	4.73	9.91	
Polymeric pigments (mg/L)	Avg	64.74 b	64.31 b	75.17 a	53.02 d	50.29 d	57.31 b	49.24 d	50.92 d	56.30 b	50.19 d	51.74 d	55.81 b	0.02
	Stddev	2.22	2.26	3.00	3.01	3.96	5.60	1.75	6.01	5.60	6.13	5.83	9.54	
Total anthocyanins (mg/L)	Avg	782.33 a	652.61 b	416.66 d	605.66 b	499.75 c	315.88 e	614.33 b	511.85 c	309.29 e	603.00 b	496.45 c	308.63 e	0.02
	Stddev	68.00	76.38	32.05	31.35	36.12	28.68	4.57	21.26	11.66	32.08	27.32	11.42	
Polymeric phenols (mg/L)	Avg	554.17 b	563.20 b	653.35 a	458.97 c	438.26 c	516.77 d	460.03 c	425.37 c	499.07 d	433.28 c	443.73 c	486.13 d	0.12
	Stddev	8.92	37.57	14.51	30.08	24.61	40.20	32.17	47.10	58.89	42.55	44.33	60.42	
Total Flavonols (mg/L)	Avg	6.12 c	13.79 b	19.12 a	3.42 c	4.00 c	5.31 c	8.07 c	6.60 c	7.24 c	4.00 c	8.55 c	8.64 c	0.01
	Stddev	1.73	7.89	3.33	0.63	2.00	0.87	5.39	3.14	1.43	0.83	1.20	1.28	
Total Hydroxycinnamic acids (mg/L)	Avg	46.16 b	45.73 b	51.22 a	38.88 c	39.32 c	41.31 b	48.68 b	46.77 b	51.85 a	40.73 a	40.99 b	45.18 b	0.07
	Stddev	5.95	6.47	6.24	4.11	2.54	1.15	2.54	3.23	1.56	0.60	0.44	0.93	
Total Flavan-3-ols (mg/L)	Avg	19.74 b	17.67 c	21.96 a	21.11 a	16.38 c	20.96 a	20.26 b	16.81 c	21.63 a	17.79 b	17.29 c	20.30 a	<0.01
	Stddev	1.74	1.81	2.35	1.12	2.35	1.63	1.51	1.39	1.02	0.23	0.12	0.76	

Table 3.7: Colour density, modified colour density, HPLC and BSA wine data of the 2013 small scale experiment measured at 6 month intervals from after MLF to 12 months aging. Values are means of triplicate repeats; different letters indicate significant differences at $p < 0.05$.

		Reduced canopy VSP(R)		Full canopy VSP(HC)		Smart Dyson top shoots(HSDA)		Smart Dyson bottom shoots(HSDB)		p-value
		After MLF	6 months	After MLF	6 months	After MLF	6 months	After MLF	6 months	
Colour density (AU)	Avg	0.92 a	0.90 a	0.68 b	0.69 b	0.50 c	0.53 c	0.58 b	0.61 b	<0.01
	Stddev	0.15	0.14	0.06	0.07	0.08	0.07	0.07	0.06	
Modified colour density (AU)	Avg	1.30 b	1.40 a	1.15 b	1.31 a	0.90 b	0.96 b	0.89 b	0.97 b	<0.01
	Stddev	0.15	0.15	0.02	0.03	0.06	0.04	0.03	0.07	
Tannins (mg/L)	Avg	271.65 b	371.74 a	119.54 d	224.06 c	83.10 d	170.34 e	88.62 d	152.94 d	<0.01
	Stddev	35.64	53.13	45.56	58.18	39.94	53.38	27.27	30.07	
Polymeric pigments (mg/L)	Avg	28.457 c	44.26 a	29.95 c	46.02 a	21.56 c	32.08 b	22.88 c	32.92 b	0.08
	Stddev	1.77	8.04	4.19	8.20	3.66	3.02	1.20	1.75	
Total anthocyanins (mg/L)	Avg	495.78	503.61	500.69	510.26	460.99	445.25	410.45	410.49	0.92
	Stddev	23.14	30.13	50.45	46.76	102.94	64.26	14.64	7.05	
Polymeric phenols (mg/L)	Avg	324.16 b	521.62 a	369.57 b	521.19 a	233.81 c	336.94 b	252.11 c	338.92 b	0.07
	Stddev	15.04	28.49	46.18	67.86	36.03	19.15	16.48	34.30	
Total Flavonols (mg/L)	Avg	0.02	0.78	0.33	1.44	0.23	1.22	0.15	0.86	0.6
	Stddev	0.00	0.50	0.13	1.45	0.24	0.25	0.13	0.87	
Total Hydroxycinnamic acids (mg/L)	Avg	43.60 c	45.34 b	48.28 a	53.72 a	44.13 b	48.42 a	39.60 c	44.54 b	0.04
	Stddev	1.01	1.86	5.83	4.654	5.82	5.69	1.79	3.49	
Total Flavan-3-ols (mg/L)	Avg	48.59 a	25.50 b	48.55 a	36.13 b	20.38 b	21.36 b	29.41 b	28.13 b	0.04
	Stddev	1.83	1.55	2.18	18.77	9.78	1.30	12.41	13.73	

3.3.2.2 Tannins, polymeric phenols and flavan-3-ols

Small-scale experiment

In 2012 the difference in tannin concentrations between wines made from the R and the other treatments were significant after MLF (**Table 3.6**). Changes in tannin concentrations at the 6 month ageing period of these wines show an increase for the R- and HSDB treatment and no significant changes for the HC can be due to different reasons, which include taking part in initial co-pigmentation reactions and dissociating at a later stage (Boulton, 2001). Other reasons include polymerisation and possible changes in tannin conformation at a later stage, which explain the significant increase in the R and HSDA treatment after six months (Boulton, 2001; Fulcrand *et al.*, 2005). Changes in the molecular mass or shape of tannins might also lead to a different precipitation response with BSA, leading to different tannin levels measured with this precipitation method (Geldenhuys *et al.*, 2012). Otherwise, after 12 months of ageing, tannin levels were still significantly higher in the wines made from the R treatment than the other treatments. R and HSDB treatments showed a significant decrease in tannin concentrations after 12 months aging compared to six months aging, while HC- and HSDA treatments remained near the same levels as after six months aging. The decrease in concentration is most likely due to polymerisation and precipitation of the tannins in the wine (Monagas & Bartolomé, 2005).

In terms of polymeric phenols, again the 2012 R treatment results showed significantly higher levels compared to the other treatments (**Table 3.6**). After six months of ageing there was no significant change in the concentrations between treatments compared to after MLF aging interval. All the treatments showed significant increased levels after twelve months of ageing, following the same trend, with only the R treatment being significantly higher compared to the other treatments. This increase in polymeric phenols is probably due to polymerisation reactions between catechin-derived moieties during ageing (Monagas *et al.*, 2005).

The 2013 tannin results showed the same trends as in the previous year, with the only significant difference between the treatments being the R treatment (**Table 3.7**). The 2013 results followed the same trend as the 2012 results, with an increased tannin concentration after six months of ageing caused by co-pigmentation reactions and dissociating at a later stage (Boulton, 2001) and polymerisation and changes in tannin conformation at a later stage (Boulton, 2001; Fulcrand *et al.*, 2005).

The 2013 polymeric phenols results showed that the R and HC treatments were significantly higher than in the HSDA and HSDB treatments, with significant increases observed after six months' ageing for all treatments with the R- and HC treatment remaining significantly higher than the HSDA and HSDB treatments (**Table 3.7**). These results corroborate those found in a previous study (Van

Noordwyk, 2012) on the same vineyard in which full and reduced canopy treatments were compared, with the latter treatment also leading to a higher level of polymeric phenols in the wine. The same study also showed that differences due to treatments decreased with an increase in vine stress, as was also seen in the 2013 season (Van Noordwyk, 2012). This increase in polymeric phenols is probable due to polymerisation reactions between catechin-derived moieties during ageing (Monagas *et al.*, 2005).

During the 2012 season, the flavan-3-ol concentration of only the HC treatment was significantly higher after MLF compared to the other treatments. After six months of ageing, the flavan-3-ol concentration of all the treatments decreased significantly, but no significant differences were observed between the treatments (**Table 3.6**). This does not correspond with the findings in the literature, which found increases in flavan-3-ol concentrations with ageing (Revilla & Gonzalez-SanJose, 2003; Fang *et al.*, 2007). The possible polymerisation and precipitation of these flavan-3-ols may occur at a faster rate if the concentrations are high enough (Revilla & Gonzalez-SanJose, 2003). This might give a possible explanation for what was observed. All treatments however, showed significant increases in total flavan-3-ols concentrations after 12 months of ageing. This is supported by results obtained by Bido *et al.*, (2006). However, again no significant differences between the treatments were observed. This may be due to the fact that the flavan-3-ol concentrations probably were influenced more by water stress than by canopy management practices (Ojeda *et al.*, 2002).

For the 2013 season the total flavan-3-ols of the R and HC treatments were significantly higher than that measured in the HSDA + HSDB treatments (**Table 3.7**). After six months of ageing, the flavan-3-ol concentration of HSDA and HSDB showed no significant change, while only the R- and HC treatments decreased as observed for 2012. This phenomenon negated any differences between the treatments at this stage during aging. Although other authors have reported an increase in total flavan-3-ols in the early stages of ageing due to their release from co-pigmentation bonds (De *al.*, 2005), the possible polymerisation and precipitation of these flavan-3-ols may occur at a faster rate if the concentrations are high enough (Revilla & Gonzalez-SanJose, 2003). This could explain why the levels in the treatments with high concentrations seemed to decrease after six months, while the treatments with lower concentration showed no difference.

Commercial-scale experiment

Table 3.8: Colour density, modified colour density, HPLC and BSA wine data of the 2013 commercial scale experiment measured at 6 month intervals from after MLF to six months aging. Values are means of triplicate repeats; different letters indicate significant differences at $p < 0.05$.

		Double bearer		VSP		p-value
		After MLF	6 months	After MLF	6 months	
colour density (au)	Avg	0.98a	0.69b	0.95a	0.69b	0.7
	Stddev	0.06	0.06	0.25	0.30	
modified colour density (au)	Avg	0.94b	0.93b	1.03b	1.15a	0.05
	Stddev	0.25	0.21	0.09	0.15	
tannins (mg/l)	Avg	75.55 b	134.00a	100.36 b	141.14a	0.7
	Stddev	29.75	55.30	15.73	53.95	
polymeric pigments (mg/l)	Avg	26.61a	22.23b	26.25a	31.86a	0.05
	Stddev	9.59	1.04	5.77	5.13	
total anthocyanins (mg/l)	Avg	277.33 b	328.48a	245.66b	323.18a	0.8
	Stddev	28.93	9.13	25.00	46.19	
polymeric phenols (mg/l)	Avg	288.39	215.88	305.04	290.48	0.9
	Stddev	100.48	10.15	56.36	53.58	
total flavonols (mg/l)	Avg	0.64	0.80	0.74	1.18	0.62
	Stddev	0.79	0.26	0.24	0.21	
total hydroxycinnamic acids (mg/l)	Avg	39.40	33.64	38.49	34.79	0.7
	Stddev	4.96	2.46	7.25	4.04	
total flavan-3-ols (mg/l)	Avg	43.21a	21.56b	31.12a	39.56a	0.05
	Stddev	0.89	0.49	16.49	2.74	

The 2013 results showed no differences between the training treatments when comparing the tannin concentrations in the wines for both aging intervals (**Table 3.8**). This might be due to it being the first year after the conversion of the vine training system, as differences between systems normally become larger over time as the vine adjusts to the new training system (Pradubsuk & Davenport, 2010). As mentioned before, changes in the molecular mass or shape of tannins might also lead to a different precipitation response with BSA, leading to different tannin levels measured with this precipitation method (Geldenhuys *et al.*, 2012).

In 2013, the flavan-3-ol levels were significantly higher in wines made from the double-bearer treatment after MLF than after six months of ageing (**Table 3.8**). In contrast to the trend of the double bearer treatment the VSP treatments showed no significant difference between the two aging intervals measured. Decreases in total flavan-3-ol concentrations during barrel ageing might be due to acetaldehyde-mediated polymerisation and therefore possible precipitation, as well as the release of the compound from co-pigments (Timberlake & Bridle, 1976; Revilla & Gonzalez-SanJose, 2003).

No differences were observed in terms of polymeric phenols between the training treatments after MLF (**Table 3.8**), and this also was the case after six months of barrel ageing.

3.3.2.3 Total anthocyanins and polymeric pigments

Small-scale experiment

Wines made from the 2012 R treatment had significantly higher concentration of total anthocyanins and polymeric pigments after MLF compared to the other treatments, which did not show any significant difference from each other (**Table 3.6**). Opposite trends were observed between the anthocyanins and the polymeric pigments during ageing (**Table 3.6**). After six months the anthocyanin concentration decreased of all treatments with the R treatment remaining significantly higher than the other treatments. The decrease in concentration persisted at twelve months aging and the R treatment remained significantly higher in concentration than the other treatments. This decrease is most likely due to the polymerization of anthocyanins (Timberlake & Bridle, 1976). As monomeric anthocyanins become polymerised, their concentration decreases, and that of polymeric pigments increases during wine ageing (De Beer *et al.*, 2005).

In 2013 there were no significant differences between the treatments in terms of anthocyanin concentrations neither after MLF nor after six months aging. Although the HC- and R treatments were slightly higher than the HSDA and HSDB treatments (**Table 3.7**). This could be due to the berry size of the HSDA and HSDB treatments being smaller, thus increasing the skin-to-pulp ratio (Holt *et al.*, 2008). The polymeric pigments showed no differences between the treatments after MLF, but after six months of ageing the polymeric pigment concentrations of the wines from all the treatments had increased as the anthocyanins polymerised (**Table 3.7**). At this sampling point, the levels in the HC- and R treatments were significantly higher than in the HSDA and HSDB treatments. This could have been due to greater increased tannin concentrations in these treatments, leading to polymerisation with anthocyanins and therefore an increase in polymeric pigments (De Beer *et al.*, 2005).

Commercial-scale experiment

For the 2013 vintage there was no difference observed between the treatments in terms of anthocyanin concentration after MLF (**Table 3.8**). The berries from the double-bearer treatment were much more shaded than those from the VSP treatment because of the increase in the number of shoots on the vine. These results confirm what has been found by other researchers, namely that temperature and not light are more important in the synthesis of anthocyanins (Spayd *et al.*, 2002; Heyns, 2010). The low anthocyanin concentration of the treatment after MLF may be due to co-pigmentation, as it increased significantly after six months regardless of treatment (Boulton, 2001). This increase was expected as the monomeric anthocyanins broke from their co-pigment bonds leading to an increased concentration

of monomeric anthocyanins (Boulton, 2001) that could be detected by the HPLC, as the current HPLC method cannot detect anthocyanins bound to tannins (Peng *et al.*, 2002).

Total anthocyanins showed the same trend during aging as for the tannins based on this trend of the concentration for both the anthocyanin and tannin, a correlation can be seen in the evolution of their concentration over time, as these components are highly reactive with each other through acetaldehyde-mediated polymerisation in the presence of oxygen. As these components bind to each other, both show a similar trend of a decrease in concentration over time respectively (Fulcrand *et al.*, 2005) (**Table 3.8**).

The polymeric pigments showed no significant differences between the treatments after MLF sampling point (**Table 3.8**). After six months of aging some differences had emerged. The polymeric pigments of the double-bearer treatment showed a significant downward trend after six months ageing, whereas the VSP treatment remained insignificantly changed (**Table 3.8**). It is not known if this downward trend would continue with longer ageing.

3.3.2.4 Total flavonols

Small-scale experiment

In 2012 there were no differences observed between the treatments after MLF in terms of wine flavonol concentrations (**Table 3.6**). As red wine ages, flavonols are released from their co-pigmentation bonds and concentrations could increase (De Beer *et al.*, 2005; Fang *et al.*, 2007a). The flavonol levels of the wine from the R treatment increased significantly during ageing.

Grape sample preparation for the HPLC analyses involved grinding the skin and seeds of the berries. This could have led to a large quantity of flavonols being released from the skins, which would not necessarily happen in wine. This shows that a rethink of the sample preparation method for grape HPLC analysis is needed to better reflect the extractability that happens during fermentation, when the skin are not ground and flavonols are probably not released into the wine in the same manner.

The 2013 results were the same as for 2012, with no significant differences between the treatments over six months aging (**Table 3.7**). Although insignificant, there was a slight upward trend with ageing, as was found in the previous year for the R treatment, and it is speculated that this upward trend will continue with ageing as observed in the 2012 results.

Commercial-scale experiment

The 2013 results showed no difference between the training treatments regarding the flavonol concentration during aging up to six months (**Table 3.8**). The relative stability of the flavonols concentrations might be due to co-pigmentations being released from their bonds and increasing the

concentration (De Beer *et al.*, 2005; Fang *et al.*, 2007a), therefore counteracting the effect of the polymerization of the flavonols that would lead to a decrease in their concentration (Monagas & Bartolomé, 2005).

3.3.2.5 Hydroxycinnamic acids

Small-scale experiment

The only difference observed in terms of hydroxycinnamic acid concentrations between the treatments was for the HC treatment, that showed significantly lower levels compared to the other treatments (**Table 3.6**). All treatments showed a slight increase after twelve months of ageing of which only the HSDB treatment increase was insignificant. This indicates that although hydroxycinnamic acids may slightly increase over time their concentrations remain relatively stable with ageing (Monagas *et al.*, 2005). The hydroxycinnamic acid concentrations in 2013 showed significant differences between all the treatments, with HC having the highest concentration, and HSDB having the lowest concentration. After six months of ageing there was a significant increase in the hydroxycinnamic acid concentration of all the treatments except for the HC treatment (**Table 3.7**). After six months ageing there was no significant difference between the HC and HSDA treatments, with the R and HSDB treatments being significantly lower. These results differ from the previous year which showed no significant differences with ageing (**Table 3.6**).

Commercial-scale experiment

In 2013 there were no significant differences in hydroxycinnamic acid concentrations between the training treatments in the commercial-scale experiment (**Table 3.8**). The concentration remained relatively constant with ageing, with no significant differences between the treatments after six months of ageing. This is in line with literature that found hydroxycinnamic acid to be relatively stable over time (Monagas *et al.*, 2005).

3.3.3 Combined phenolic and colour results

The combined results were represented on biplots so an overall picture of the influences on the treatments could be obtained.

Small-scale experiment

Some differences between treatments were apparent when all the phenolics and colour analyses were represented in a biplot (**Figure 3.2 & 3.3**). The influence of time can be observed with a shift of the treatments away from the monomeric anthocyanins towards the polymeric pigments and polymeric phenols as can be observed by the clumping of the results at MLF that get spread out towards polymeric pigments and phenols with aging. The other major influence that is linked to the polymerisation of the anthocyanins is the polymerisation of the flavan-3-ols to tannins and to monomeric anthocyanins (Gawel, 1998) that can be seen by the movement of the treatment results over time to increased levels of polymeric phenols/pigments. The major changes are only seen after twelve months of ageing (De Beer *et al.*, 2005)(**Figure 3.2**).

When looking at the biplot for the results of the 2013 season, the same development of phenols over time can be observed between treatments (**Figure 3.3**). The treatments showed a shift from the monomeric anthocyanins and flavan-3-ols toward the polymeric pigments and polymeric phenols. These results show that the main interactions that take place during ageing are the interactions between tannins and anthocyanins and the polymerisation of these phenolics (Vivas & Glories, 1996) as happened for all the treatments. These interactions take place more slowly during bottle ageing, which explains the relatively slow progression of these reactions in these small-scale experiments (Monagas *et al.*, 2005).

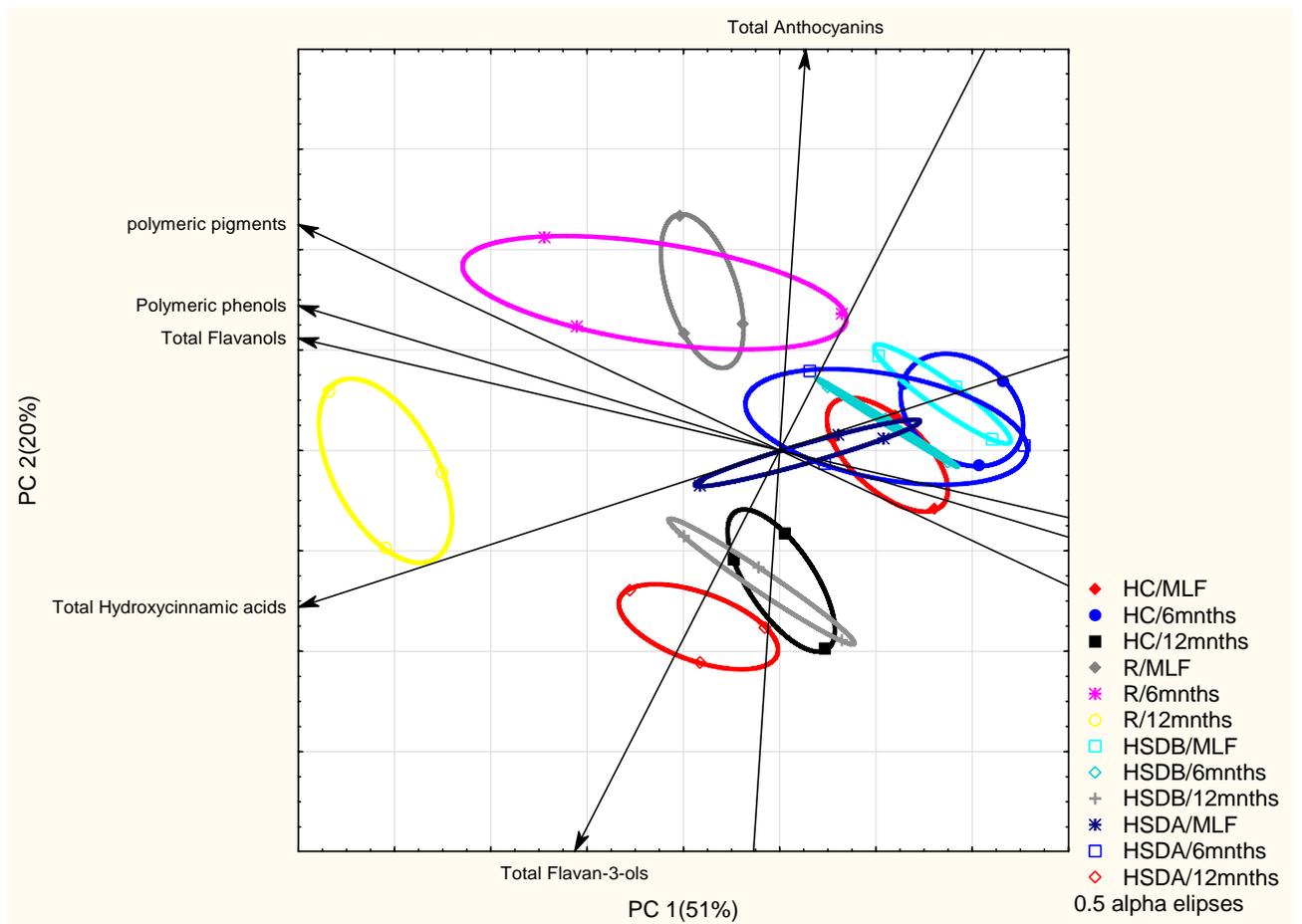


Figure 3.2: Biplot of wine phenolic data from the 2012 small-scale experiment. With PC1 indicating time/aging of the wine. Overlapping circles represent no significant differences between treatments with no overlap representing significant differences. Circles nearer a specific compound indicate higher concentrations of specific compound. The size of the circles indicates standard deviation between repeats of a treatment with larger circles indicating larger standard deviations

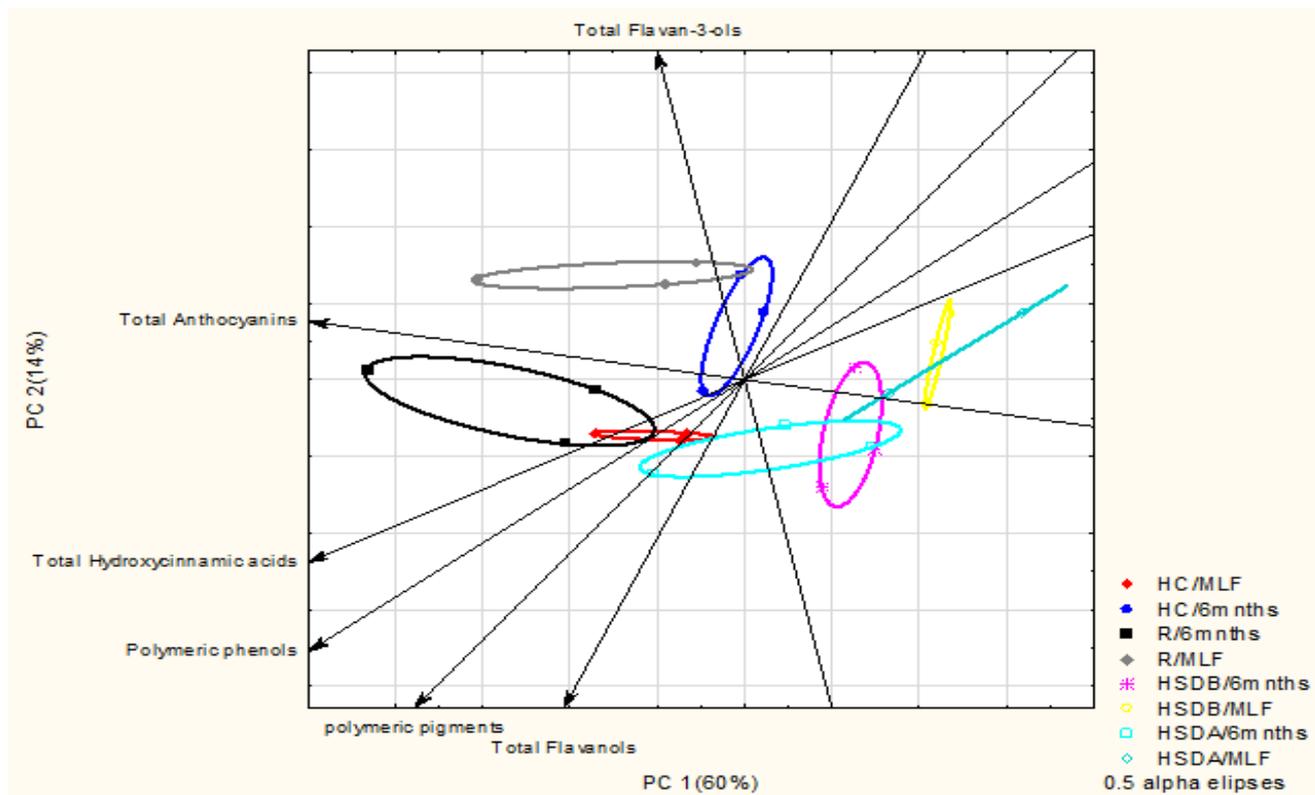


Figure 3.3: Biplot of wine phenolic data from the 2013 small-scale experiment. With PC1 indicating time/aging of the wine. Overlapping circles represent no significant differences between treatments with no overlap representing significant differences. Circles nearer a specific compound indicate higher concentrations of specific compound. The size of the circles indicates standard deviation between repeats of a treatment with larger circles indicating larger standard deviations

3.3.4 Sensory evaluation of wine

Small-scale experiment

Sensory evaluations were done on the wines from both the 2012 and 2013 vintages as shown by **Figures 3.4 & 3.5** respectively. According to the resulting tree diagram and dendrogram, a panel of experts could not find any large differences in aroma or taste between the different treatments (**Figures 3.4 & 3.5**). There were also no relations found between the two vintages tested. This can be observed as there were no specific groupings of treatments, and look almost random indicating there were no differences observed between the treatments. These results show that the phenolic differences induced by some of these treatments probably did not affect the wine to such a degree that it influenced the sensory characteristics to a perceivable extent. It thus seems that converting to an SD training system from a VSP training system under the right conditions can increase the yield of the vine without too great an influence on the sensory aspects of the wine, although the difference in colour was of such an extent that it was observable with the naked eye and may play a role in the perception of the wine although this was not a criteria on which the wine was evaluated.

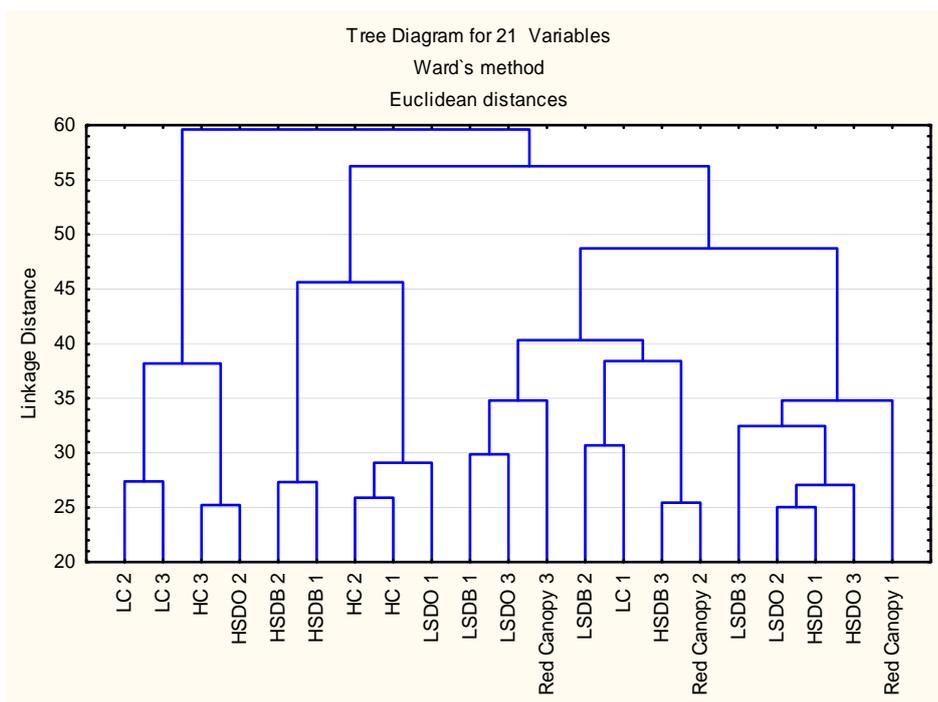


Figure 3.4: Tree diagram of the sensory sorting of the small-scale experiment in 2011/2012. Each treatment had three repeats, indicated by 1, 2 and 3. LC (low-vigour vertical shoot positioning), LSDO (low-vigour Smart-Dyson bottom shoots), LSDB (low-vigour Smart-Dyson top shoots), and LC (low-vigour vertical shoot positioning) were part of a larger study and were included because of financial constraints. HC (high-vigour vertical shoot positioning), HSDO (high-vigour Smart-Dyson bottom shoots), HSDB (high-vigour Smart-Dyson top shoots); Red canopy = reduced treatment.

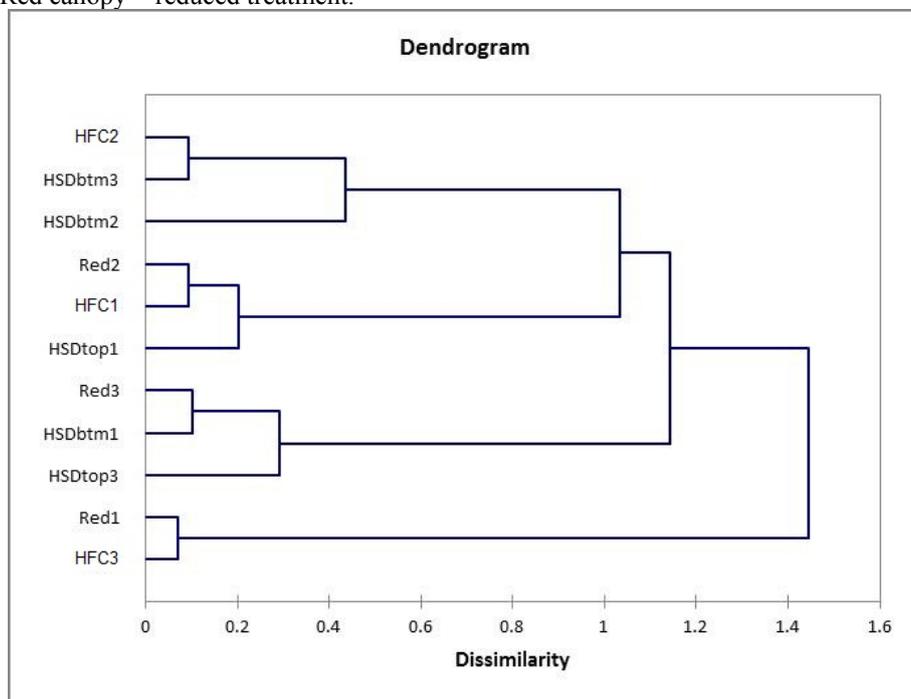


Figure 3.5: Dendrogram of the 2013 sensory data of the small-scale experiment. The three repeats for each treatment are indicated with numbers (1, 2 or 3). HFC (full-vigour vertical shoot positioning (HC)), Red (reduced(R)), HSDbtm (high-vigour Smart-Dyson bottom shoots (HSDB)), and HSDtop (high-vigour Smart-Dyson top shoots (HSDA)).

3.3.5 Conclusion

The R treatment often led to wines with higher levels of some non-volatile phenols than the other treatments. This is not in line with the results found in the grapes, where in most cases the R treatment was not significantly higher than the other treatments. The commercial-scale experiments showed that phenolic compounds in the VSP treatment were higher in almost all cases in both grapes and wine when compared to the double bearer treatment. This strengthens the case that an increase in yield without an increase in effective leaf area could lead to a dilution effect on berry phenolics (Kliewer & Dokoozlian, 2005). The treatment differences observed after MLF remained relatively continuous throughout aging of the wine. The evolution of aging showed no significant differences between the treatments. This shows that the differences in the phenols have a minimal effect on aging with all treatments reacting similarly to the aging process. The commercial experiment showed the same response to ageing as the small-scale experiment, even though they differed in the rate and sequence of phenolic reactions. This work also shows that higher yields can be obtained with an SD training system without having a massive sensory impact when compared to wines made from VSP-trained vines.

3.3.6 Literature cited

Bergqvist, J., Dokoozlian, N. & Ebisuda, N., 2001. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *Am. J. Enol. Vitic.* 52,1-7.

Boido, E., Alcalde-Eon, C., Carrau, F., Dellacassa, E. & Rivas-Gonzola, J. 2006. Aging Effect on the Pigment Composition and Color of *Vitis vinifera* L. Cv. Tannat Wines. Contribution of the Main Pigment Families to Wine Color. *J. Agric. Food Chem.* 54, 6692-6704.

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

Bosman, D., 2011. Smart-Dyson: A trellis system for improved yield and wine quality. Wynboer August, 5.

Bosman, A. 2013. Personal communication

Bravdo, B., Hepner, Y., Loinger, C., Cohan, S. & Tabacman, C., 1985. Effect of irrigation and crop level on growth, yield and wine quality of Cabernet Sauvignon. *Am. J. Enol. Vitic.* 6, 132-139.

Campo, E., Do, B., Ferreira, V. & Valentin, D., 2008. Aroma properties of young Spanish monovarietal white wines: A study using sorting tasks, list of terms and frequency of citation. *Aust. J. Grape Wine R.* 14, 104-115.

Cartier, R., Rytz, A., Lecomte, A., Poblete, F., Krystlik, J., Belin, E. & Martin, N., 2006. Sorting procedure as an alternative to quantitative descriptive analysis to obtain a product sensory map. *Food Qual. Prefer.* 17, 562-571.

Cheyrier, V., Dueñas-Paton, M., Salas, E., Maury, C., Souquet, J., Sarni-Manchado, P. & Fulcrand, H., 2006. Structure and properties of wine pigments and tannins. *Am. J. Enol. Vitic.* 57, 298-305.

Chollet, S., Lelievre, M., Abdi, H. & Valentin, D., 2011. Sort and beer: Everything you wanted to know about the sorting task but did not dare to ask. *Food Qual. Prefer.* 22, 507-520.

De Beer, D., Joubert, E., Gelderblom, W.C.A. & Manley, M., 2005. Changes in phenolic composition and antioxidant activity of Pinotage, Cabernet Sauvignon, Chardonnay and Chenin blanc wines during bottle aging. *S. Afr. J. Enol. Vitic.* 26, 6-15.

Dokoozlian, N.K. & Kliewer, W.M., 1995. The light environment within grapevine canopies. I: Description and seasonal changes during fruit environment. *Am. J. Enol. Vitic.* 46, 209-218.

- Downey, M., 2010. Tannin management in the vineyard. Grape and Wine Research and Development Corporation Fact Sheet. GWRDC Innovators Network, Wayville, South Australia.
- Downey, M.O., Dokoozlian, N.K. & Krstic, M.P., 2006. Cultural practices and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. *Am. J. Enol. Vitic.* 57, 257-268.
- Downey, M.O., Harvey, J.S. & Robinson, S.P., 2004. Flavonol accumulation and expression of a gene encoding flavonol synthase demonstrates light sensitivity of flavonol biosynthesis in grapevines. In: Hoikkala, A. & Soidinsalo, O. (Eds.). XXII International Conference on Polyphenols, August 2004, Helsinki, Finland. pp. 59 – 60.
- Dry, P., 2000. Canopy management fruitfulness. *Aust. J. Grape Wine R.* 6, 109-115.
- Duncan, B., & Kleinig, A., 1999. Oxygen Transmission analysis of wine bottle closures. South Corp Wines internal project, P9703.
- Du Toit, W.J., Marais, J., Pretorius, I.S. & Du Toit, M., 2006. Oxygen in must and wine: A review. *S. Afr. J. Enol. Vitic.* 27, 76-94.
- Fang, F., Li, J., Pan, Q. & Haung, W., 2007a. Determination of red wine flavonoids by HPLC and effect of aging. *Food Chem.* 101, 428-433.
- Fang, F., Li, J., Zhang, P., Tang, K., Wang, W., Pan, Q. & Haung, W., 2007b. Effects of grape variety, harvest date, fermentation vessel and wine ageing on flavonoid concentration in red wines. *Food Res.Int.* 41, 53-60.
- Fulcrand, H., Dueñas, M., Salas, E. & Cheynier, V., 2005. Phenolic reactions during winemaking and aging. *Am. J. Enol. Vitic.* 57, 289-297.
- Gawel, R., 1998. Red wine astringency: A review. *Aust. J. Grape Wine R.* 4, 74-95.
- Geldenhuys, L., Oberholster, A. & Du Toit, W.J., 2012. Monitoring the effect of micro-oxygenation before malolactic fermentation on South African Pinotage red wine with different colour and phenolic analyses. *S. Afr. J. Enol. Vitic.* 33, 150-160.
- Harbertson, J.F., Kennedy, J.A. & Adams, D.O., 2002. Tannin in skins and seeds of Cabernet Sauvignon, Syrah and Pinot noir berries during ripening. *Am. J. Enol. Vitic.* 53, 54-59.
- Haselgrove, L., Botting, D., Van Heeswijck, R., Høj, P.B., Dry, P.R., Fort, C. & Land, P.G.I., 2000. Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L cv. Shiraz grape berries. *Aust. J. Grape Wine R.* 6, 141-146.

- Heyns, A.D.M., 2010. The impact of viticulture-trellising systems and lateral removal – Influence on berry composition and wine quality. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.
- Holt, H.E., Francis, I.L., Field, J., Herderich, M.J. & Iland, P.G., 2008. Relationships between berry size, berry phenolic composition and wine quality scores for Cabernet Sauvignon (*Vitis vinifera* L.) from different pruning treatments and different vintages. *Aust. J. Grape Wine R.* 14, 191-202.
- Howell, G.S., 1999. Sustainable grape productivity and the growth-yield relationship: A review. *Am. J. Enol. Vitic.* 52, 165-174.
- Iland, P., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. Techniques for chemical analysis and quality monitoring during winemaking. Patrick Iland Wine Promotions, Campbelltown, Adelaide.
- Jurd, L., 1964. Reactions involved in sulfite bleaching of anthocyanins. *J. Food Sci.* 29, 16-19.
- Kennedy, J.A., Hayasaka, Y., Vidal, S., Waters, E.J. & Jones, G.P., 2001. Composition of grape skin proanthocyanidins at different stages of berry development. *J. Agr. Food Chem.* 49, 5348-5355.
- Kliewer, W. & Dokoozlian, N., 2005. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. *Am. J. Vitic.Enol.* 56, 170-181.
- Kwiatkowski, M., Skouroumounis, G., Lattey, K. & Waters, A., 2007. The impact of closures, including screw cap with three different headspace volumes, on the composition, colour and sensory properties of a Cabernet Sauvignon wine during two years' storage. *Aust. J. Grape Wine R.* 13, 81-94.
- Matthews, M.A. & Nuzzo, V., 2007. Berry size and yield paradigms on grapes and wines quality. *Proc. Intl. WS on Grapevine.* 31 October. Venosa, Italy.
- Monagas, M., Bartolome, B. & Gomez-Cordoves, C., 2005. Evolution of polyphenols in red wines from *Vitis vinifera* L. during aging in bottles. *Eur Food Res. Technol.* 220, 331-340.
- Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A. & Deloire A., 2002. Influence of pre-and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* 53, 261-267.
- Peng, Z., Iland, P.G., Oberholster, A., Sefton, M.A. & Waters, E.J., 2002. Analysis of pigmented polymers in red wine by reverse phase HPLC. *Aust. J. Grape Wine R.* 8, 70-75.
- Petrie, P., Trought, M. & Howell, G., 2008. Fruit composition and ripening of Pinot noir (*Vitis vinifera* L.) in relation to leaf area. *Aust. J. Grape Wine R.* 6, 46-51.

- Picinelli, A., Bakker, J. & Bridle, P., 1994. Model wine solutions: Effect of sulphur dioxide on colour and composition during aging. *Vitis* 33, 31-35.
- Pradubsuk, S. & Davenport, J.R., 2010. Seasonal uptake and partitioning of micronutrients in mature 'Concord' grapes. *J. Am. Soc. Hortic. Sci.* 135, 474-483.
- Revilla, I. & Gonzalez-SanJose, L., 2003. Compositional changes during storage of red wines treated with pectolytic enzymes: Low molecular-weight phenols and flavan-3-ol derivative levels. *Food Chem.* 80, 205-214.
- Reynolds, A. & Van den Heuvel, J.E., 2009. Influence of grapevine training systems on vine growth and fruit composition: A review. *Am. J. Enol. Vitic.* 60, 251-268.
- Ribéreau-Gayon, P., Pontallier, P. & Glories, Y., 1983. Some interpretations of colour changes in young red wine during their conservation. *J. Sci. Food Agric.* 34, 505-516.
- Russouw, M. & Marais, J., 2004. The phenolic composition of South African Pinotage, Shiraz and Cabernet Sauvignon wines. *S. Afr. J. Enol. Vitic.* 25, 94-104.
- Smart, R.E., Dick, J.K., Gravett, I.M. & Fisher, B.M., 1990. Canopy management to improve grape yield and wine quality – principles and practices. *S. Afr. J. Enol. Vitic.* 11, 3-17.
- Somers, T.C., 1976. Development of pigments during ripening of grapes. *Vitis* 14, 269-277.
- Spayd, S.E., Tarara, J.M., Mee, D.L. & Ferguson, J.C., 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* 53, 171-182.
- Timberlake, C.F. & Bridle, P., 1976. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* 27.3, 97-105.
- Van Noordwyk, M., 2012. Interaction of water deficit, canopy modification and ripening: Effect on phenolic and colour composition of Shiraz grapes and subsequent wine. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.
- Vivas, N. & Glories, Y., 1996. Role of oak wood ellagitannins in the oxidation process of red wines during aging. *Am. J. Enol. Vitic.* 47.1, 103-107.
- Zoecklein, W.B., Wolf, K.T., Pelanne, L., Miller, K.M. & Birkenmaier, S.S., 2008. Effect of vertical shoot-positioned, Smart-Dyson and Geneva double-curtain training systems on Viognier grape and wine composition. *Am. J. Enol. Vitic.* 59, 11-21.

Chapter 4

Conclusion

4.1 Concluding remarks and future work

Phenolic compounds are one of the main parameters used for measuring the quality of red wines, as they can provide an indication of colour, mouth-feel and aroma. A thorough understanding of the factors that affect phenolic concentrations in grapes and wine therefore is of paramount importance to ensuring wine quality.

South African producers are looking for a way to boost grape production without compromising on quality too much, and this possibly could be achieved by adapting vineyard training systems. The vertical shoot positioning system (VSP) currently is the most widely used training system in South Africa. The system has some disadvantages, as it is not very well suited for higher-vigour vineyards (Reynolds & Van den Heuvel, 2009). The conversion of vines from a VSP to a Smart Dyson (SD) training system can have major advantages for the producer. It opens up the canopy, which increases light and temperature in the bunch zones, and this will increase the amount of phenols produced by the vine. More importantly, it could have a large positive effect on the yield of the vines, increasing it up to 40%.

This study aimed more to provide a context for the oenological observations for a related viticultural study on the specific training system modifications and the effect these might have on the sensory composition of the wines. It investigated the effects of the SD training system as well as a Reduced canopy treatment on the non-volatile phenols of wine and was compared to that of the control vertical shoot positioning system; the following conclusions could be drawn.

The trends between the grape and wine analysis (HPLC and Spectrophotometer) were not very clear, with the first year of the study showing little differences between phenols in the grapes, whereas some differences were observed between the wine treatments. The second year's data showed better trends between the grapes and the wine data. This may be due to the cumulative effect of the differences between the treatments over the lifetime of the vines (Pradubsuk & Davenport, 2010).

This study showed that the heavy canopy manipulation of the reduced treatment did have a positive effect on the phenolic concentration of the wines, as the tannins, polymeric pigments, anthocyanins and polymeric phenols generally were higher than those of the other treatments. This improvement came at a cost, however, as the yield of this treatment was much lower than that of the VSP control or the HSD treatment (Bosman, 2013). The R treatment often led to higher levels of certain phenolics, such as tannins and polymeric phenolics, while the HSD treatments sometimes led to lower levels of certain phenolic compounds.

This influence was probably due to the fact that, although the canopy had been opened, the vineyard used for the experiments did not suffer from excessively dense canopies. In the South African context there is usually enough light exposure on the grapes and temperatures are high enough to maximise phenolic production. Thus the effect of the increased light and temperature did not have the drastic effect on the phenolic concentration reported by European scientists (Haselgrove *et al.*, 2000; Heyns, 2010). The HSD treatment did not receive extra fertiliser to help facilitate the bigger canopy and increased yield, and this

could also have had a big influence on the lower phenolic concentrations. The water management of the vineyard was not optimised and, although pulse irrigation was provided to facilitate the larger leaf area and yield of the HSD treatment, it was not enough to keep the vines from severe water stress later in the season. This probably also had a negative effect on the concentration of the phenols produced by the HSD training system.

The wines were aged over a period of 12 months and it was found that trends similar to those observed after MLF were also seen after ageing. The introduction of wood in the commercial size experiment and all the extra factors that were introduced with the barrels showed little effect on the differences between treatments, as the relative differences between the treatments remained the same throughout ageing.

Sensory analysis of the wine showed that, although there were significant differences in terms of phenolics between the treatments in some cases, wine-tasting experts could not differentiate between the treatments. Whether there are possible cumulative effects that will make the differences apparent to wine consumers over the lifetime of the vines is still not known.

The Smart-Dyson training system can thus be beneficial to producers with its increased yields, while it does not seem to decrease phenolics sufficiently for it to be noticeable from a sensory point of view, nor does it have an effect on the aging evolution of the wine. However, this system should only be used under high-vigour conditions and if the necessary adjustments are made to management practices such as water and fertiliser management. The results also are highly cultivar specific(Lorrain *et al.*,2013).

The effect of the larger leaf area and higher yield on the lifespan and productivity of the vines should be investigated further. The effect of varying the irrigation and fertiliser amounts on the wines made from vines converted to Smart-Dyson may have an effect on the volatile composition of the wines, and this also needs further attention. Further investigations should also focus on harvesting grapes from SD and VSP at different ripening levels to assess the effects this might have on the wines' long terms chemical and sensory composition.

4.2 Literature cited

Haselgrove, L., Botting, D., Van Heeswijck, R., Høj, P.B., Dry, P.R., Fort, C. & Land, P.G.I., 2000. Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L cv. Shiraz grape berries. *Aust. J. Grape Wine R.* 6, 141-146.

Heyns, A.D.M., 2010. The impact of viticulture-trellising systems and lateral removal – Influence on berry composition and wine quality. MSc thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Lorrain, B., Ky, I., Pechamat, L. & Teissedre, P., 2013. Evolution of analysis of polyphenols from grapes, wines, and extracts. *Molecules* 18, 1076-1100.

Pradubsuk, S. & Davenport, J.R., 2010. Seasonal uptake and partitioning of micronutrients in mature ‘Concord’ grapes. *J. Am. Soc. Hortic. Sci.* 135, 474-483.

Reynolds, A. & Van den Heuvel, J.E., 2009. Influence of grapevine training systems on vine growth and fruit composition: A review. *Am. J. Enol. Vitic.* 60, 251-268.