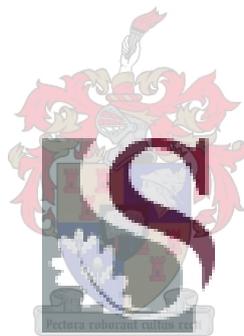


# The influence of commercial tannin additions on wine composition and quality

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by

**Daniël B Keulder**



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

February 2006

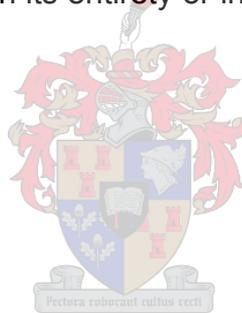
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*Supervisor:*  
WJ du Toit

Co-supervisor:  
A Oberholster

# DECLARATION

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



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**Name of candidate**

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**Date**

## SUMMARY

The quality of wine is influenced by numerous factors of which the grapes, winemaking techniques and effective quality control are to name a few. The use of new techniques should be cost effective and always have a positive influence on the wine quality. The addition of commercial tannins to wine is a fairly new technique and the effects of these additions at the concentrations prescribed have not been investigated in detail. The commercial tannins can be added to wine for different reasons, which may include: stabilisation of colour, increasing the aging potential, to modify aromas, promote precipitation of proteins, limit the effect of laccase activity, substrate for micro-oxygenation, to act as a redox buffer and structural and mouth feel modification. The reason for the addition determines the type of commercial tannin that is used, the timing of the addition and the dosage used.

Limited research has been done on the addition of commercial tannins. Those researchers that have investigated the effect of condensed or hydrolysable tannins on wine, obtained similar results than ours.

Our results indicate that the addition of commercial tannins after destemming of the grapes and before alcoholic fermentation increased the total phenol content of the wine. This effect could still be seen after a year of bottle maturation. The addition of these tannins, however, did not stabilise the colour, when compared to the control. The additions did change the structure and mouth feel of the wine, but depending on the dosage and commercial tannin used this modification were either observed as positive or negative. When the addition of a pectolytic enzyme was compared to the control, one of the cultivars tested showed that this treatment increased the total phenol content and colour density of the wine. When different commercial tannins were added to wines made of the same grapes which received different maceration times, the treatments did not significantly differ from their respective controls, although the differences between the controls decreased. The effect of different commercial tannin additions on wines with different amounts of oxygen was investigated. The tannin additions did influence the phenolic content of the wines to a certain extent, but the effect of the oxygen concentrations was much larger. The composition of the grapes or wine before the addition of the commercial tannins will probably determine whether the addition will have a marked effect. The composition of some of the commercial tannins available on the market was also determined, which indicates that large differences exist.

This study made a valuable contribution to our knowledge regarding the addition of commercial tannins to red wine and raises the question whether the dosages recommended by the suppliers are optimal.

## OPSOMMING

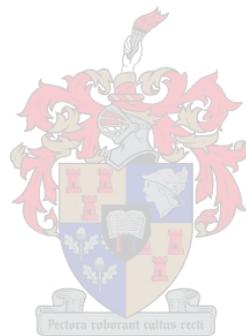
Die gehalte van wyn word beïnvloed deur baie faktore waarvan die duiwe, wynmaak tegnieke en effektiewe gehalte beheer 'n paar is. Die gebruik van nuwe tegnieke moet koste effektief wees en altyd 'n positiewe invloed op die wyn kwaliteit hê. Die byvoeging van kommersiële tanniene tot wyn is 'n relatiewe nuwe tegniek en die nagevolge van die byvoegings, teen die konsentrasies voorgeskryf, is nog nie in diepte ondersoek nie. Die kommersiële tanniene kan tot die wyn toegevoeg word vir verskeie redes, wat onder andere van die volgende insluit: stabilisasie van die kleur, verhoging van die verouderings potensiaal, verandering van die aroma, bevordering van die presipitasie van proteïene, lakkase aktiviteit te verminder, om te dien as substraat vir mikro oksiginase, om te dien as 'n redoks buffer en om struktuur en mondgevoel te verander. Die rede vir die byvoeging van die tannien beïnvloed die tipe kommersiële tannien wat gebruik word, die tyd van byvoeging en die dosis wat gebruik word.

Beperkte navorsing is al gedoen oor die byvoeging van kommersiële tanniene. Die navorsers wat reeds die effek van gekondenseerde of hidroliseerbare tanniene op wyn ondersoek het, het soortgelyke resultate as ons verkry.

Ons resultate dui aan dat die byvoeging van kommersiële tanniene na afmaal van duiwe en voor alkoholiese gisting die totale fenol inhoud van die wyn verhoog. Hierdie uitwerking kon steeds na 'n jaar se bottelveroudering gesien word. Die byvoeging van hierdie tanniene het egter nie die kleur gestabiliseer as dit vergelyk word met die kontrole nie. Die toevoegings van die tanniene het wel die struktuur en mondgevoel van die wyne verander, maar afhangend van die dosis en kommersiële tannien wat gebruik is, was die verandering óf positief óf negatief. As die byvoeging van die pektolitiese ensiem vergelyk is met die kontrole het een van die kultivars wat ondersoek is die totale fenol inhoud en kleurdigtheid van die wyn verhoog. Toe die verskillende kommersiële tanniene toegevoeg is by wyne wat van dieselfde duiwe gemaak is, maar wat verskillende tye van masserasie ontvang het, het die behandelings nie beduidend van hul onderskeie kontroles verskil nie. Die verskil tussen die onderskeie kontroles het wel verminder. Die invloed van kommersiële tannien byvoegings op wyne met verskillende konsentrasies suurstof is ook ondersoek. Die tannien byvoegings het die fenoliese samestelling van die wyne tot 'n sekere mate beïnvloed, maar die invloed van die suurstof konsentrasies was baie groter. Die samestelling van die duiwe of wyn voor die byvoeging van die kommersiële tanniene sal heel moontlik bepaal of die byvoeging 'n merkbare invloed sal hê. Die samestelling van sommige van die kommersiële tanniene wat beskikbaar is in die bedryf was ook bepaal, wat aandui dat daar groot verskille tussen die tanniene bestaan.

Hierdie studie het 'n waardevolle bydra gelewer tot ons kennis rakende die byvoeging van kommersiële tanniene tot rooi wyn. Die vraag word egter geopper of die dosisse wat aanbeveel word deur die verskaffers optimaal is.

This thesis is dedicated to my parents, family and friends for their support



## BIOGRAPHICAL SKETCH

Danie Keulder was born on 9 September 1981 in Malmesbury and matriculated at Swartland High School in 1999. Danie obtained a Bachelors degree in Agricultural Science (Viticulture and Oenology) in 2003 at the University of Stellenbosch. In 2004 he enrolled for a Masters degree in Oenology at the same University.



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

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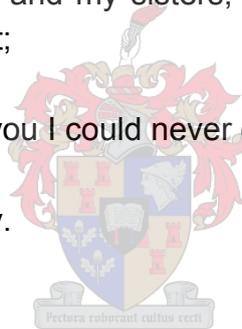
THE STAFF of the Department of Oenology and Viticulture and the Institute of Wine Biotechnology, for their assistance;

MY FRIENDS, especially Hanneli, Lood, Gustav, Andries and John, for their help and support;

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MY PARTNER, Zahn-Mari, without you I could never do this;

THE ALMIGHTY, for this opportunity.



# PREFACE

This thesis is presented as a compilation of six chapters. Each chapter is introduced separately and is written according to the style of the journal [Click *here* and type NAME OF JOURNAL] to which Chapter [Click *here* and type CHAPTER NUMBER] submitted for publication.

**Chapter 1**      **General Introduction and Project Aims**

**Chapter 2**      **LITERATURE REVIEW**

The influence of tannins on the composition of wine

**Chapter 3**      **Research Results**

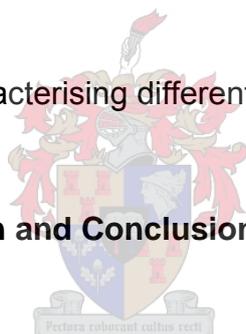
The effect of different commercial tannin and pectolytic enzyme additions to red wine before fermentation

**Chapter 4**      **Research Results**

Evaluating and characterising different commercial tannin additions to wine after fermentation

**Chapter 5**      **General Discussion and Conclusions**

**Chapter 6**      **Appendixes**



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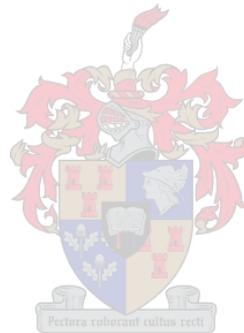
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# Chapter 1

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**INTRODUCTION AND  
PROJECT AIMS**

# CHAPTER 1: GENERAL INTRODUCTION AND PROJECT AIMS

## 1.1 INTRODUCTION

---

The art of winemaking has been known for centuries and evidence of wine production dates as far back as 6000BC, to early Mesopotamian culture. From early times to modern times wine has been produced and enjoyed by anyone from peasants to kings. Winemaking was kept alive through the dark ages due to the fact that Europe lacked reliable drinking water. To this day wine are still consumed for health reasons and can reduce mortality from coronary heart disease due to the presence of ethanol and high concentrations of polyphenols (Gali *et al.*, 1992; Ribéreau-Gayon *et al.*, 1998; Soleas *et al.*, 1997). In the last 90 years the wine industry has seen a revolution in the production of winemaking. This is largely due to the scientific development of wine production. Modern winemakers can now achieve almost total control of every stage of winemaking. Competition in the wine industry, however, has led to the temptation to produce cheaper and larger volumes wine at the expense of quality. Winemakers now face the challenge of producing wine for a larger market without losing character and individual flavour of their wines (Anonymous, 2003). Annually about 26 billion litres of wine are produced from about 8 million hectares of vineyards across the world. There is, however, a decline in consumption, which has led to a worldwide oversupply of 15-20 % (Cape Wine Academy, 2001). Fierce competition for market share has led to increased diversity and innovation within the wine industry (Pretorius, 2000). Commercial tannin additions are one of these innovations used in the industry today.

During red wine production tannins can be extracted from different sources. During fermentation condensed tannins are extracted from the skins and seeds (Riou *et al.*, 2002; Sun *et al.*, 1999; Zimman *et al.*, 2004) or external seeds can be added during the fermentation to increase the condensed tannin concentration (Kovac *et al.* 1992, 1995). Most wines also receive some kind of oak contact during the maturation of the wine whether from barrel maturation, chips or staves. During this stage hydrolysable tannins are extracted (Puech *et al.*, 1999; Quinn and Singleton, 1985; Vivas and Glories, 1996) which, together with oxygen, can induce the indirect polymerisation of proanthocyanidins (Vidal *et al.*, 2004; Vivas and Glories, 1996). An alternative/addition to these tannin extractions are the addition of commercial tannins, which consist of condensed tannins, hydrolysable tannins or mixtures of

both. The influence of these additions has not been scientifically researched thoroughly and the effects of these additions at concentrations prescribed are mostly theoretical speculation and have not been proven.

Different dosages of commercial tannins are added to wine at different stages of the winemaking process, depending on the reason for these additions (Zoecklein, 2005). These reasons may include stabilisation of colour, increasing the aging potential, modifying aromas, promote precipitation of proteins, limit the effect of laccase activity, substrate for micro-oxygenation, acts as a redox buffer and structural and mouth feel modification. The above mentioned properties of the commercial tannin depend on the source of the tannin, purity of the tannin, extraction and process methods used and the degree of oxidation of the tannin.

## **1.2 PROJECT AIMS**

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This study was conducted at the Department of Viticulture and Oenology, Stellenbosch University, to determine the effects of commercial tannin additions and a pectolytic enzyme at the concentrations recommended by the suppliers on South African wine quality. The specific aims of the study were as follows:

- a) to monitor the phenolic composition of different red wines over two vintages after the addition of different commercial tannins and a pectolytic enzyme at different dosages added before the start of fermentation;
- b) to sensorially evaluate a wine where different commercial tannins and a pectolytic enzyme were added at different dosages to must after 20 months of maturation;
- c) to determine the effect of different commercial tannin additions on the phenolic composition of a wine produced from the same grapes which received different maceration times;
- d) to determine the effect of different commercial tannins at different dosages on the phenolic composition of a wine with different amounts of oxygen added;
- e) to investigate the compositions of different commercial tannins.

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mouse skin treated with 12-O-tetradecanoylphorbol-13-acetate in vivo. *Int. J. Cancer.* **51**, 425-432.

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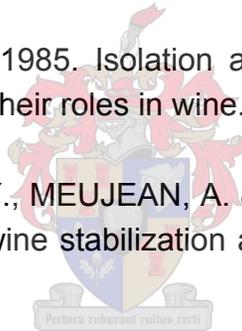
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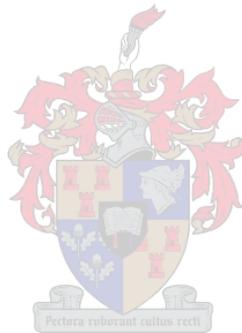
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# Chapter 2

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## LITERATURE REVIEW

### The Influence of Tannins on the Composition of Wine

# CHAPTER 2: THE INFLUENCE OF TANNINS ON THE COMPOSITION OF WINE

## 2.1 INTRODUCTION

---

Phenols and more specific tannins are of great importance in wine. They play an important role in oxidation reactions, the maturation and aging of wine, as well as the organoleptic properties. Tannins can be divided into two groups: 1) condensed tannins or proanthocyanidins that originate from the grapes and can be further divided into procyanidins and prodelphinidins, as well as 2) hydrolysable tannins that are extracted from wood. At this stage ellagitannins are the only hydrolysable tannin that can be extracted from oak (Puech *et al.*, 1999), but gallotannins can be added to wine in the form of commercial tannin extractions from nutgalls.

This report will give a short overview of the major phenols and condensed tannins present in wine and the influence of hydrolysable tannin additions on the wine. There will also be looked at the influence that commercial tannin additions have on wine.

## 2.2 PHENOLS IN GRAPES AND WINE

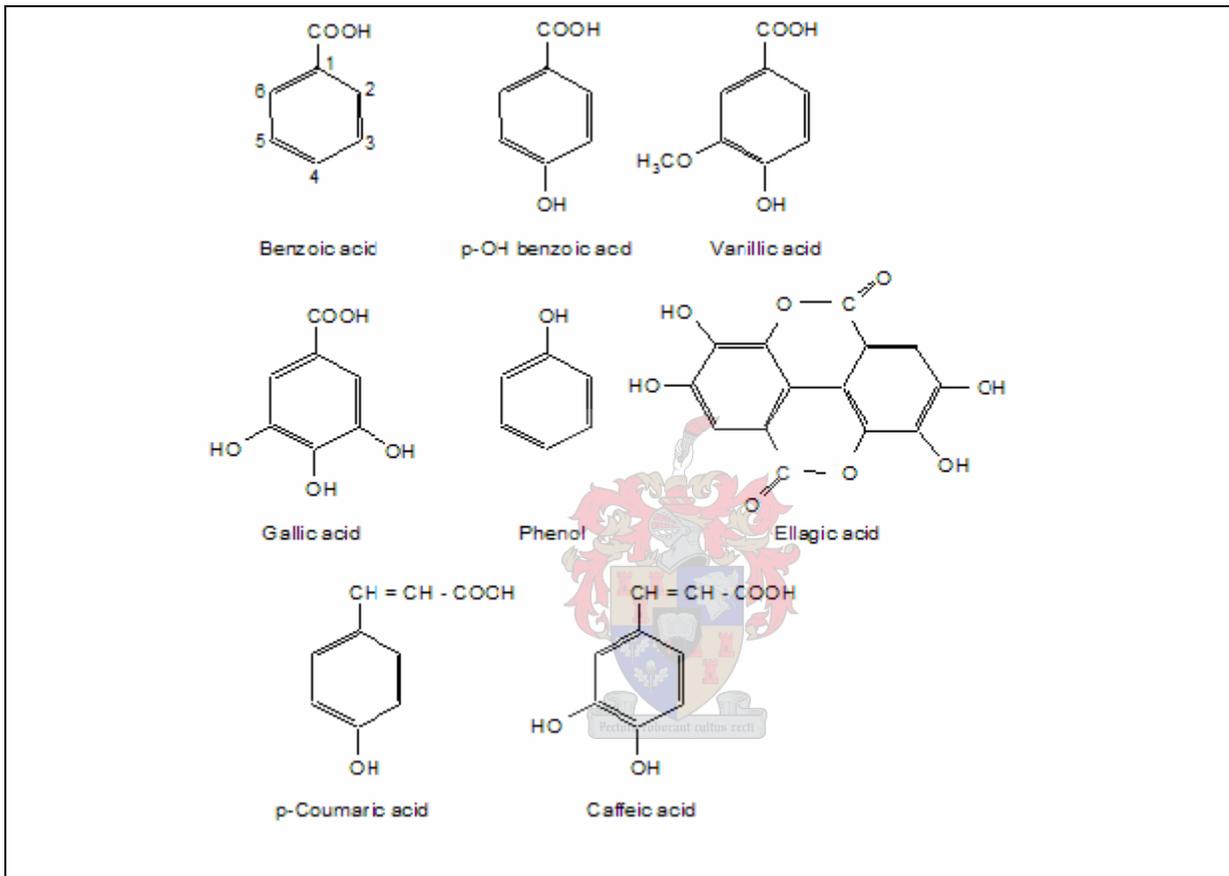
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The phenolic composition of wine depends not only on the phenolic composition of the grapes, but also on the winemaking conditions that influence the extraction of the phenols. Winemaking processes such as cold soaking, maceration temperature and punch downs influence the extraction of phenolics (Oberholster, 2003). The location of the phenols in the grapes is 1% in the pulp, 5% in the juice, 30-50% in the skins with the rest of the phenols in the seeds (Zoecklein *et al.*, 1995). Even during extended skin maceration only 50% of the available phenols in the skins are extracted, while 60% of the available phenols in the seeds are extracted during fermentation (Ribéreau-Gayon *et al.*, 1998; Zoecklein *et al.*, 1995).

Phenols can be divided into two groups namely the non-flavonoids and flavonoids (Ribéreau-Gayon *et al.*, 1998).

## 2.2.1 NON-FLAVONOIDS

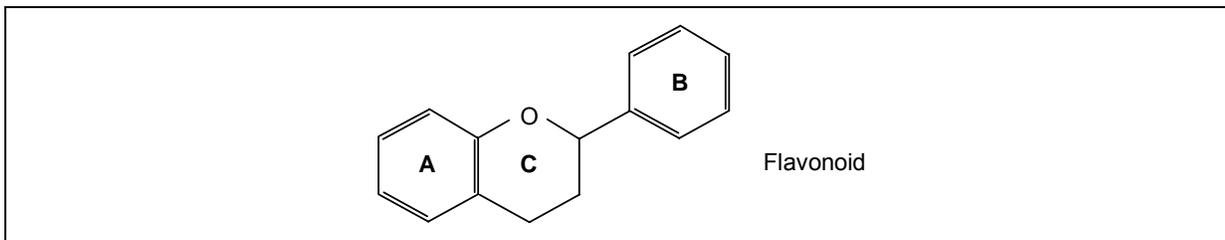
Benzoic acid and cinnamic acid derivatives are the main non-flavonoids present in grapes and wine (Figure 2.1). In grapes these non-flavonoids are usually bound to glucose and esters. Organoleptically they do not have any odour or taste. Non-flavonoid concentrations are in the order of 10-20 mg/L in white wines and 100-200 mg/L in red wines (Ribéreau-Gayon *et al.*, 1998).



**Figure 2.1** Examples of different non-flavonoids in grapes and wines (Ribéreau-Gayon *et al.*, 1998).

## 2.2.2 FLAVONOIDS

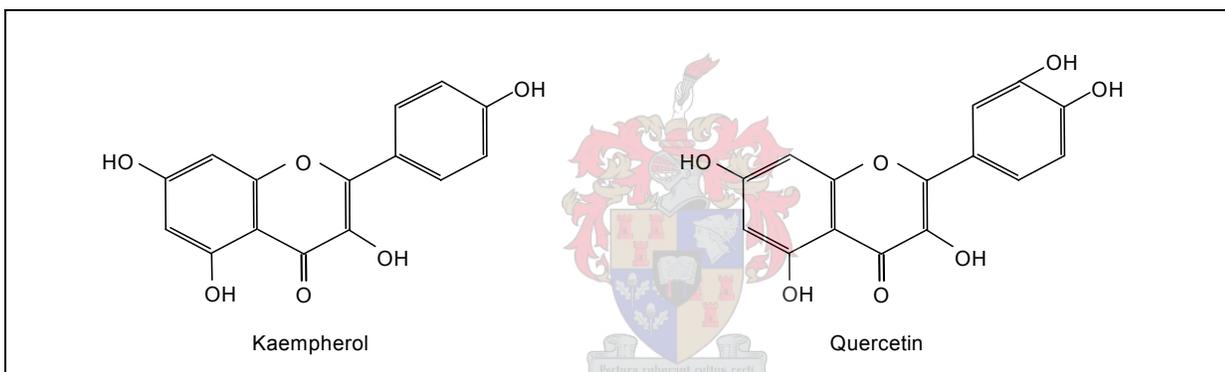
Flavonoids are more complex than non-flavonoids and consist mainly of two benzene cycles bonded by an oxygenated heterocycle (Figure 2.2) (Hagerman, 2002; Monagas *et al.*, 2005; Ribéreau-Gayon *et al.*, 1998).



**Figure 2.2** Flavonoid skeleton with the standard letters system.

### 2.2.2.1 Flavonols

Flavonols (Figure 2.3) are usually esterified to glucose at position 3 of the C ring. They occur mainly in the skins and are yellow in colour. They are efficient UV screens that can protect the bound pigment from photo-oxidative degradation (Sweeny *et al.*, 1981). In white wine (without skin maceration), their concentrations are in the order of 1-3 mg/L and in red wine in the region of 100 mg/L. In red wine, they usually disappear over time. Examples of important flavonols in wine are quercetin and kaempherol (Ribéreau-Gayon *et al.*, 1998).



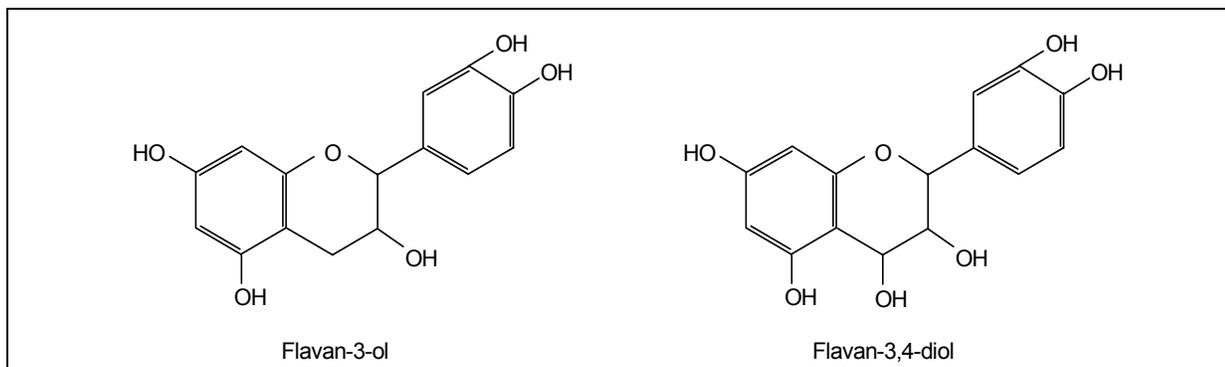
**Figure 2.3** Examples of two flavonols, kaempherol and quercetin, in wine.

### 2.2.2.2 Flavan-3-ols

Flavan-3-ols are characterised by an OH group at position 3 of the C ring (Figure 2.4). Catechin and epicatechin are the natural occurring flavan-3-ols in grapes. When there are three OH groups on the B ring, gallocatechin and epigallocatechin are formed. They can also have a gallic acid acylated at position 3 of the C ring and are then known as catechin-3-O-gallate or epicatechin-3-O-gallate. The polymers of flavan-3-ols are called proanthocyanidins or condensed tannins. Singleton and Esau (1969) found that the catechin and epicatechin concentrations in white wines range from 10-50 mg/L, while it may reach 200 mg/L in red wines.

### 2.2.2.3 Flavan-3,4-diols

Flavan-3,4-diols are characterised by an OH group at position 3 and 4 of the C ring (Figure 2.4). They react in the same manner as flavan-3-ols and can thus also polymerise to form condensed tannins (Ribéreau-Gayon *et al.*, 1998; Zoecklein *et al.*, 1995).



**Figure 2.4** Basic structures of a flavan-3-ol and a flavan-3,4-diol.

### 2.2.2.4 Anthocyanins

When anthocyanidins are esterified to glucose, it is known as anthocyanins (Figure 2.5). This is the stable form that occurs in red grape skins and red wine. The anthocyanins are important for the red colour. Anthocyanin concentrations vary according to the wine age and cultivar. Usually it is present in concentrations between 100 and 1500 mg/L in wine (Monagas *et al.*, 2005; Ribéreau-Gayon *et al.*, 1998; Somers, 1971). Colour of wine is an important quality parameter and is normally associated with the phenolic structure of the wine. Colour extraction normally reaches a maximum before the end of fermentation, but other phenols are still being extracted with extended skin maceration (Gil-Munoz *et al.*, 1999).

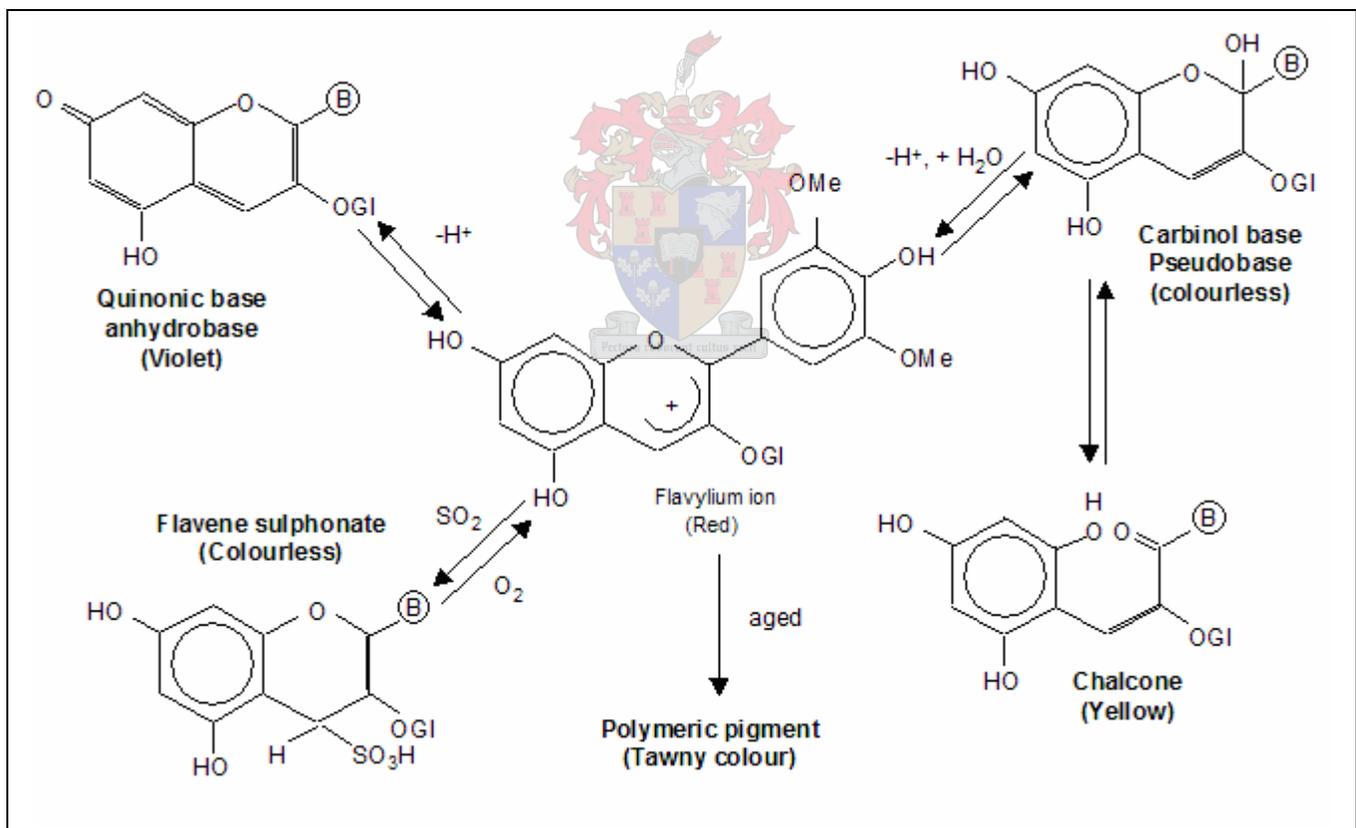
Anthocyanins have a positive (+) charge on the C-ring, which is responsible for the reactivity of the anthocyanin and also for the absorption of green light, with red light being reflected. Wine pH has an influence on the charge and hence the colour of the anthocyanin. Depending on the pH, the anthocyanin can be in four different forms, these are: flavylium ion (red), carbinol base (colourless), chalcone (yellow) and quinoic base (violet) (Figure 2.5). At a pH lower than 2.5 more than 50 % of the anthocyanins are in the red form (flavylium ion) and at a pH higher than 2.5 more than 50 % in the colourless form (carbinol base). At normal wine pH, about 25 % of the anthocyanins are in the red flavylium form. Other components in the wine (copigments) can also influence colour changes by copigmentation which results in a bathochromic shift (change to violet) and hyperchromic shift of the maximum absorbance (increase in intensity) (Ribéreau-Gayon *et al.*, 1998; Zoecklein *et al.*, 1995).

Copigmentation is the association of anthocyanins with copigments to increase and stabilise their colour. These copigments include flavonoids, non-flavonoids, phenols, amino

acids and organic acids. Darias-Martín *et al.* (2001) investigated the copigmentation effect of caffeic acid and catechin on anthocyanins and wine colour. They found that caffeic acid addition enhanced the colour drastically and that catechin addition showed a slight increase over time when these copigments were added before fermentation. Malien-Aubert *et al.* (2002) explained the smaller increase in colour where catechin is added compared to the addition of caffeic acid. They found that when monomers and dimers bind to anthocyanin, yellow coloured xanthylium salts are formed that decreases the red colour. They also showed that the stability of the anthocyanin increases with an increase in polymerisation of the copigment.

### 2.3 ENZYMATIC EXTRACTION OF PHENOLS

Phenols are extracted during fermentation and maceration of the must. Grapes and yeasts both show a limited amount of pectinase activity. Adding pectolytic enzymes during these stages are a common practice to increase the phenolic and aromatic content of the wine.



**Figure 2.5** Equilibria of anthocyanins as affected by pH and polymerisation of the anthocyanin malvidin-3-glucoside, which influences the colour of wine (Brouillard *et al.*, 1978).

Revilla and Gonzalez-SanJose (2003) showed that grape pulp treated with enzymes are richer in catechin and epicatechin and have higher concentrations of flavanol dimers. They also showed that the phenolic aldehydes and acids in the wine increased with the addition of enzymes. During aging of these wines monomer and dimer concentrations decreased, with the highest losses in monomer concentrations occurring in the wines which started with the highest concentration of monomers and dimers. These decreases can be as a result of polymerisation and oxidation of the flavonoids. This indicates that wines with higher phenol content will undergo more polymerisation and thus will end up with an improved tannin structure. Revilla and Gonzalez-SanJose (2003) did, however, not determine the effect of commercial tannin additions on the phenol content of the wine and whether it will initiate precipitation of phenols.

Pardo *et al.* (1999) showed that the addition of enzymes to fermenting wine increased the anthocyanin content and thus the colour density of the wine. Over time the colour density of these wines decreased, but this decline was less in the enzyme treated wines. They also found that the decrease in the anthocyanin concentration over time was higher in the treated wine. This shows that more of the colour in the treated wines was due to the more stable polymerised pigments and thus protected.

## 2.4 TANNINS IN GENERAL

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The term tannin originates from the Celtic word which means oak. It is widely used in commercial leather tanning, protection of fishing nets and protection of metal drainage pipes. Saucier *et al.* (1998) defined tannins as secondary plant metabolites that are water-soluble polyphenols and have the ability to precipitate proteins and complex carbohydrates. This property is only found in polyphenols above a certain molecular weight (500-3000) (Puech *et al.*, 1999).

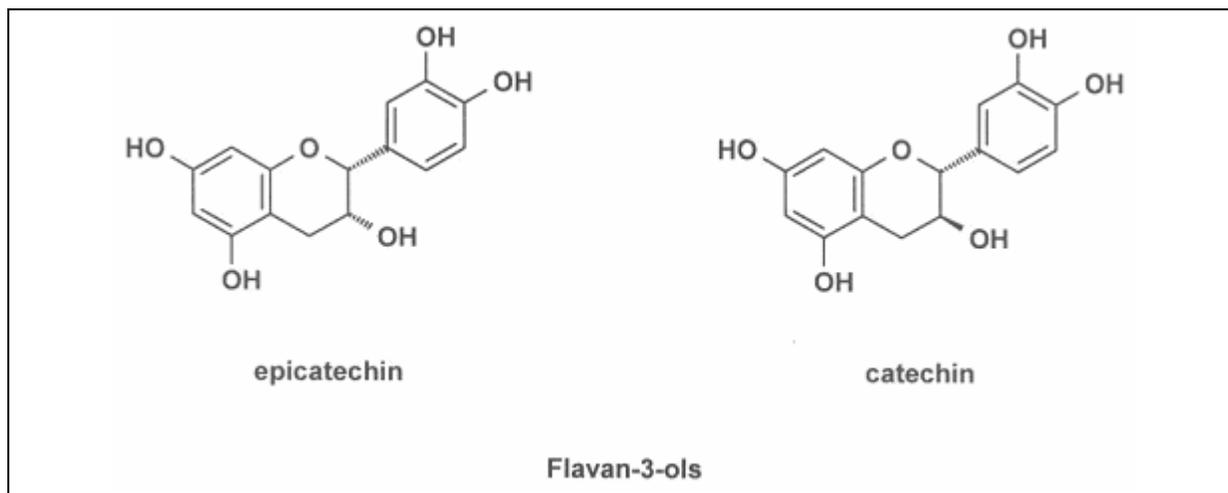
Tannins in wine can be divided into two groups namely hydrolysable and condensed tannins, which originate from oak and grapes respectively.

### 2.4.1 CONDENSED TANNINS

#### 2.4.1.1 Formation, components and structures

Condensed tannins are polymerised flavanol units. These flavanol units consist of catechin, epicatechin, galocatechin, epigallocatechin and epicatechin gallate (Figure 2.6) (Prieur *et al.*, 1994; Souquet *et al.*, 1996). When a third phenolic group is added on the B ring of catechin and epicatechin, galocatechin and epigallocatechin are yielded (Hagerman, 2002). Condensed tannins are also known as proanthocyanidins. Proanthocyanidins are classified either as procyanidins or as prodelfinidins. Procyanidins are catechin- and epicatechin-

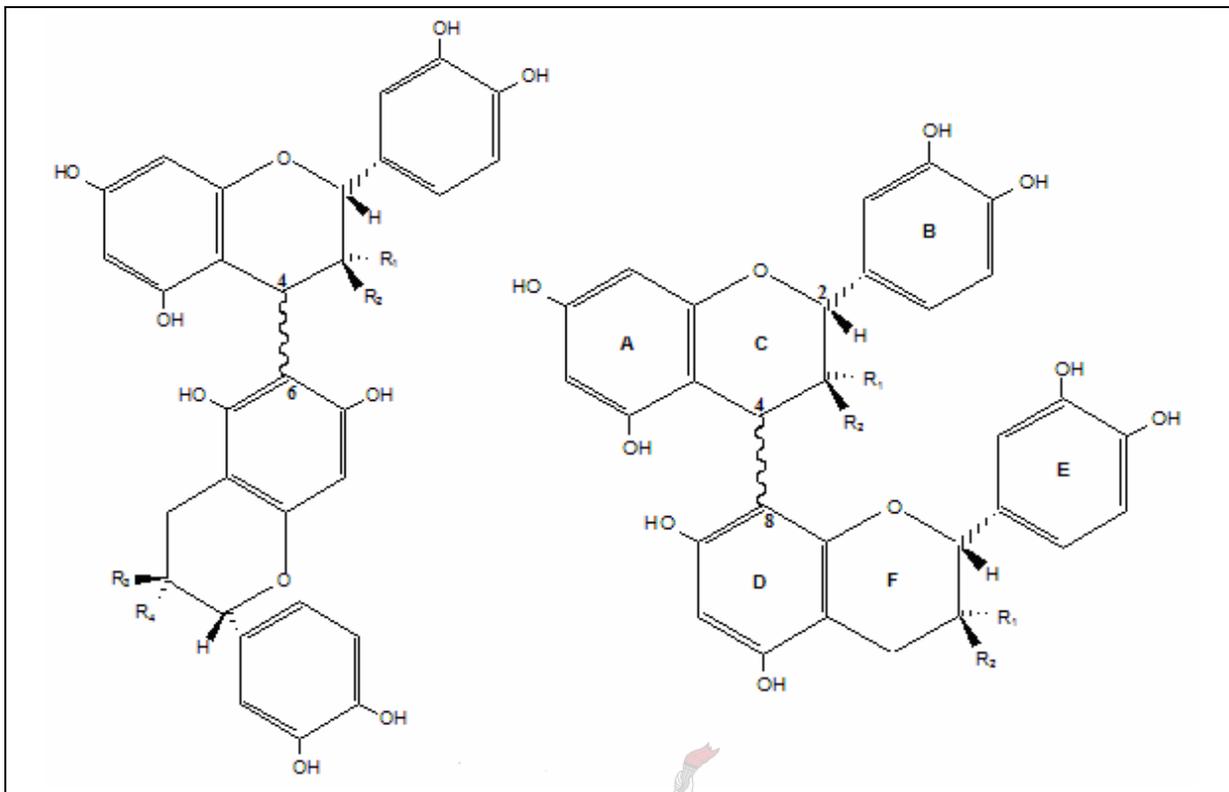
based polymers, while prodelphinidin also contain gallocatechin- and epigallocatechin units in addition to catechin and epicatechin (Hagerman, 2002).



**Figure 2.6** Structures of the monomeric flavan-3-ols, epicatechin and catechin.

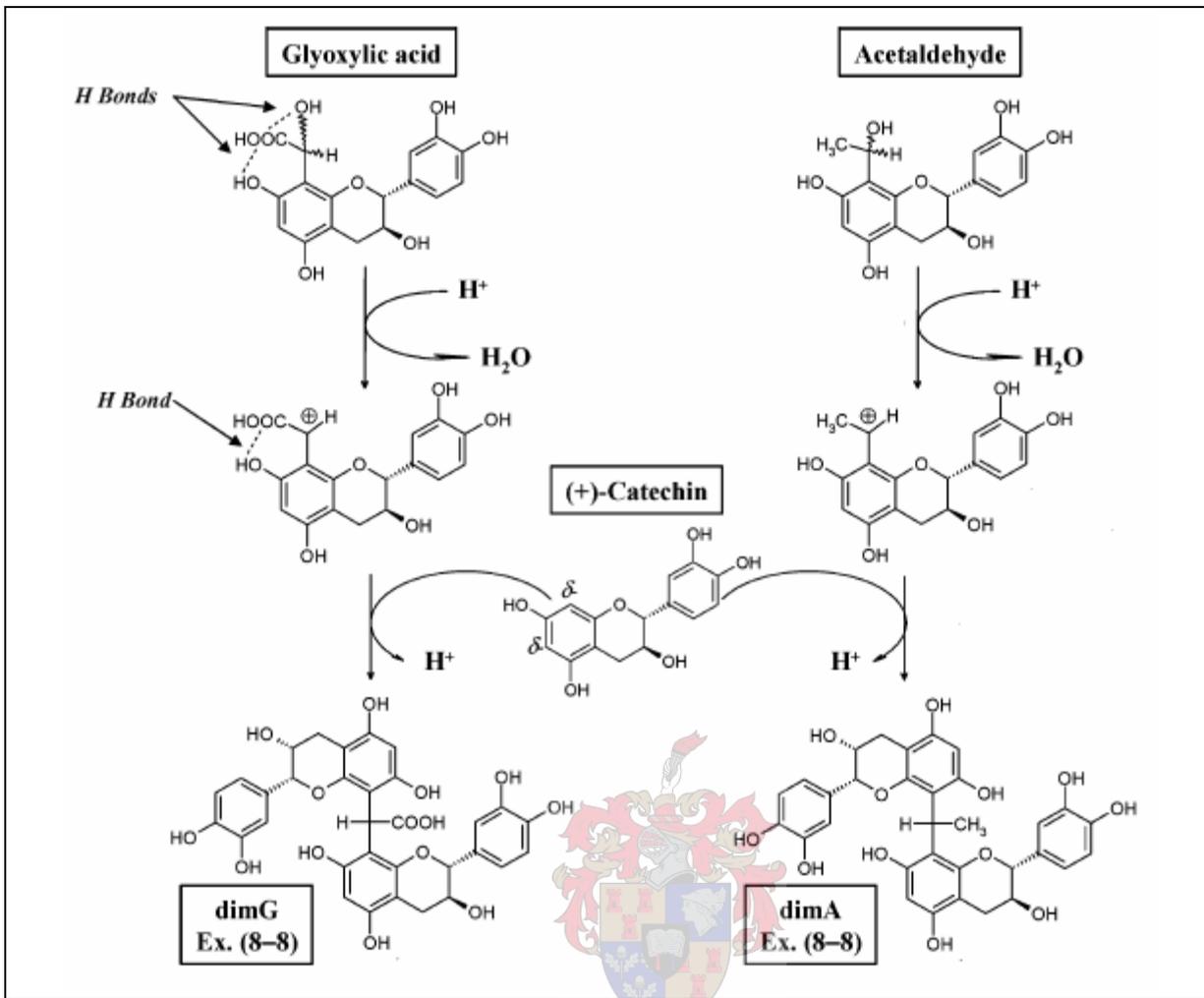
There are a number of different ways that the flavan-3-ols and flavan-3,4-diols can polymerise to form condensed tannins. These are:

Direct polymerisation: The flavonoids are linked by C<sub>4</sub>-C<sub>8</sub> or C<sub>4</sub>-C<sub>6</sub> carbon bonds to form a polymer (Prieur *et al.*, 1994). This reaction is dependent on temperature and the reaction rate increases with an increase in temperature. When dimeric procyanidins are formed with C<sub>4</sub>-C<sub>8</sub> bonds it is identified as B1 to B4 and when C<sub>4</sub>-C<sub>6</sub> bonds are formed it is identified as B5 to B8 depending on the combination of the subunits catechin and epicatechin (Figure 2.7). Type-A dimeric procyanidins can also form when in addition to the C<sub>4</sub>-C<sub>8</sub> or C<sub>4</sub>-C<sub>6</sub> bonds there is an ether bond between either the C<sub>5</sub> or C<sub>7</sub> and the C<sub>2</sub> carbon (Ribéreau-Gayon *et al.*, 1998). Direct polymerisation is a slow process and no oxygen is required. The different linkages will also influence the three dimensional shape of the tannin as well as the way it interacts with other compounds (Allen *et al.*, 1997).



**Figure 2.7** Direct C<sub>4</sub>-C<sub>8</sub> (B1 to B4) and C<sub>4</sub>-C<sub>6</sub> (B5 to B8) bonds.

Indirect polymerisation: Ethanol is oxidised to form acetaldehyde. The acetaldehyde polymerise with the flavonoids to form condensed tannins. This reaction is known as acetaldehyde-mediated condensation and an ethyl-bridge is formed between the flavonoids (Figure 2.8) (Vidal *et al.*, 2004). This form of polymerisation is much faster than direct polymerisation and can also take place between tannins and anthocyanins (Ribéreau-Gayon *et al.*, 1998). Tartaric acid can also be oxidised to yield glyoxylic acid, which then reacts with flavonoids to form polymers. This polymerisation reaction resembles that of acetaldehyde-mediated condensation and can be seen as a form of indirect polymerisation (Drinkine *et al.*, 2005; Fulcrand *et al.*, 1997).



**Figure 2.8** Indirect polymerisation and the formation of dimers (Drinkine *et al.*, 2005).

#### 2.4.1.2 Condensed tannins (proanthocyanidins) in grapes

The concentration, nature and structure of the condensed tannins change with grape maturity and cultivar (Oberholster, 2003). The concentrations in red wine vary between one and four g/L and in white wine between 100 and 300 mg/L (Ribéreau-Gayon *et al.*, 1998). The highest levels occur just before colouration and then decrease up to *veraison* with a further decrease up to harvest (Harbertson *et al.*, 2002; Oberholster, 2003). Optimal ripeness does not occur simultaneously in the seeds and the skins and cooler conditions generally gives higher tannin and anthocyanin concentrations, providing the grapes get ripe (Oberholster, 2003).

Skins and stems contain both procyanidins and prodelphinidins, while the seeds only contain procyanidins (Riou *et al.*, 2002; Souquet *et al.*, 1996). High condensed tannin concentrations in the skins are usually accompanied by a high anthocyanin concentration. After *veraison*, the seed coats harden, which makes the polyphenols less extractable (Oberholster, 2003).

### 2.4.1.3 Extraction of grape phenolics

The main phenolic compounds in red wine are anthocyanins and proanthocyanidins (condensed tannins) (Riou *et al.*, 2002). Between one and four g/L proanthocyanidins are normally extracted from the grapes during fermentation (Ribéreau-Gayon *et al.*, 1998). The concentration, nature and structure of condensed tannins in wine vary with the type of technology used during winemaking. Vigorous crushing, mechanical punch down, pump over treatments, cold soaking and higher maceration temperatures all increase the extraction of phenolic compounds from the grapes to the must or wine (Oberholster, 2003; Sun *et al.*, 1999). Grape skins are the main source of extractable phenols, while only part of the phenols in the seeds are extracted. The seeds contribute only to the monomeric and oligomeric proanthocyanidins, but not to the polymeric proanthocyanidins (Sun *et al.*, 1999). General phenol extraction reaches a maximum at pressing and remains stable during malolactic fermentation and aging. Anthocyanin extraction reaches a maximum after two to three days of fermentation and decreases during storage. During extended skin maceration, the anthocyanin concentration decreases, while the tannin concentration can still increase up to  $\pm 36$  days (Oberholster, 2003; Zimman *et al.*, 2004).

### 2.4.1.4 Tannin-anthocyanin interaction

Red wine colour becomes increasingly associated with polymeric material as the wine ages (Kennedy & Hayasaka, 2004). However, most of the observed colour in red wine still needs to be characterised. Saucier *et al.* (2004) found that as red wine ages the observed colour increases in molecular weight. This is due to the polymerisation between anthocyanins and proanthocyanidins via indirect or direct polymerisation. The polymerisation of proanthocyanidins with anthocyanins also enhances the colour of the wine and protects it from oxidation (Saucier *et al.*, 2004). Polymerisation reactions depends on anthocyanidin composition and the ratio of proanthocyanidin and anthocyanidin (Timberlake and Bridle, 1977). Kennedy and Hayasaka (2004) concluded that ethyl-bridged anthocyanin-proanthocyanin does not play a significant role in colour stabilisation and that ethyl-bridged pigments are unstable and rapidly converted to other pigmented material.

### 2.4.1.5 Acetaldehyde in wine

Dallas *et al.* (1996) and Sims and Morris (1986) reported that practises which enhanced the production of acetaldehyde, in red wine, led to a large increase in colour stability and accelerated polymerisation of tannins and anthocyanins. This will happen in red wine during barrel maturation, provided that the oxidation is not too severe and that the anthocyanin-tannin polymers do not become large enough to precipitate (Sims & Morris, 1986). Dallas *et*

*al.* (1996) has shown that flavanols and anthocyanins can undergo some self-polymerisation in the presence of acetaldehyde.

#### **2.4.1.6 Organoleptic influence**

Condensed tannins are responsible for some of the wine's major organoleptic properties namely astringency, browning and turbidity (Ricardo-da-Silva *et al.*, 1993). The astringency and bitterness of wine is mainly attributed to the presence of phenolics (Vidal *et al.*, 2004) and more specific condensed tannins. Astringency increases with an increase in the degree of polymerisation. Ethyl-bridged flavanols can also increase astringency and bitterness, provided they are present in sufficient amounts. An increase in the degree of trihydroxylation decreases the astringency, while an increase of galloylation increases the coarse perception of the proanthocyanidins (Vidal *et al.*, 2004). Pigmented polyphenolic compounds can also decrease the astringency if they are present at sufficient levels.

### **2.4.2 HYDROLYSABLE TANNINS**

#### **2.4.2.1 Components and structures**

Hydrolysable tannins contain a polyhydric alcohol (typically D-glucose) as a basic structural unit of which the hydroxyl groups have been esterified by gallic acid or hexahydroxydiphenic (HHDP) acid (Hagerman & Butler, 1991; Hagerman, 2002). These tannins are easily hydrolysed either enzymatically or in acid or base conditions to form free gallic acid or HHDP acid. The latter spontaneously hydrolyse to yield ellagic acid. Hydrolysable tannins can be classified as either gallotannins or ellagitannins, according to the type of acid formed (Puech *et al.*, 1999).

#### **2.4.2.2 Tannins in oak wood**

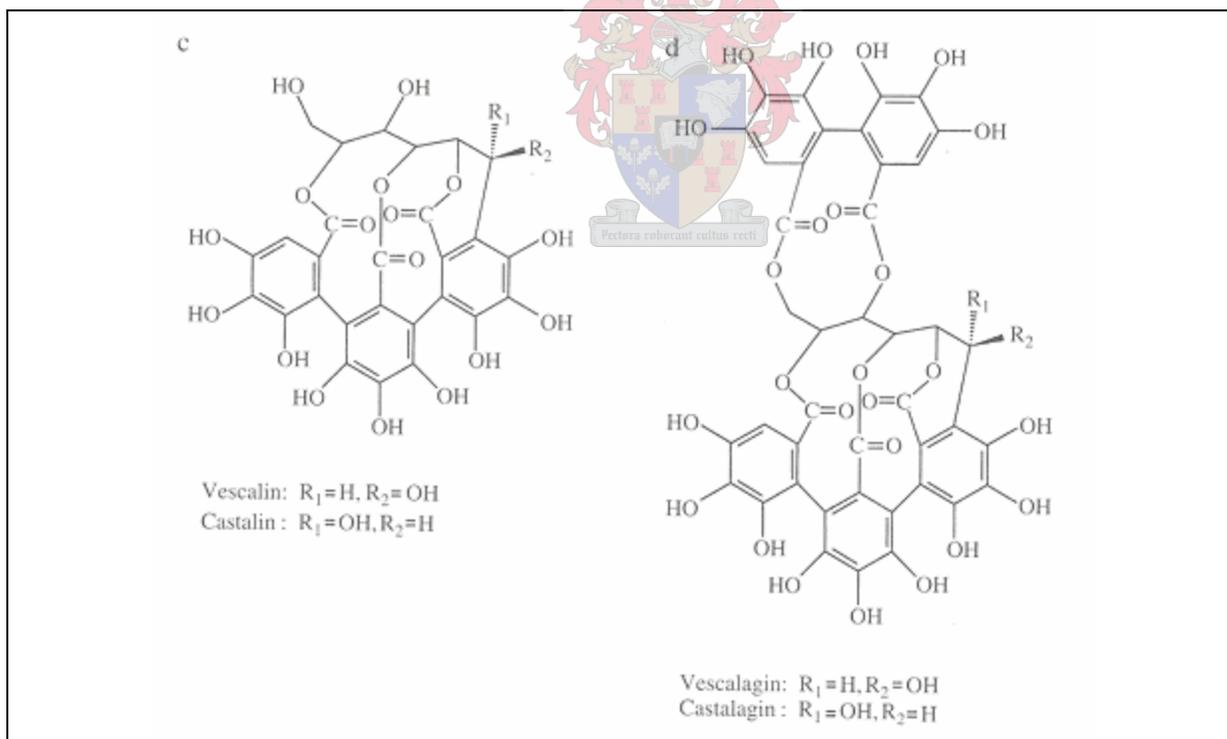
Of the ethanol-extractable phenols in oak wood, 90 percent are non-flavonoids which include lignin, gallic acids, ellagic acids, aromatic acids, aldehydes and hydrolysable tannins (Singleton & Esau, 1969). The non-volatile hydrolysable tannins account for the majority of these non-flavonoids and are 5 to 15 percent of the dry weight of oak wood (Puech, 1984).

Quinn and Singleton (1985) found that the oak extracts of American and European oak species were very similar in the major components, but that American oak has a much lower phenolic content than European oak. They have also shown that ellagitannins are extracted into wine through oak chip or barrel aging of wine.

### 2.4.2.3 Ellagitannins

Ellagitannins constitute up to 10% of the dry weight of oak heartwood. The two most common ellagitannins are vescalagin and castalagin (Figure 2.9). Vescalagin is in general more reactive than castalagin. Six other ellagitannins have also been identified namely roburins A-E and grandinin (Herv'e du Penhoat *et al.*, 1991). Ellagitannins can undergo intermolecular coupling with other hydrolysable tannins to yield dimers (Hagerman, 2002). According to Quinn and Singleton (1985), the tannins of cooperage oak are believed to be ellagitannins, whose products are gallic acid and ellagic acid.

Ellagitannins influence the structure of phenolic compounds and the colour of red wine by speeding up the condensation of procyanidins, while limiting the degradation of procyanidins through oxidation and precipitation (Glories, 1993; Ribéreau-Gayon and Stonestreet, 1965; Vivas, 1993). The ellagitannins also have the ability to combine covalently to grape-derived nucleophilic species such as ethanol, flavanols, anthocyanins and thiols (Quideau *et al.*, 2005). Winemaking practices can influence the reactivity of ellagitannins. Puech *et al.* (1999) stated that the amount of time the wine spends in contact with oak would influence the amount of ellagitannins extracted from the wood. Vivas and Glories (1996) have shown that sulphur dioxide addition to wine limits ellagitannin oxidation by competing with the oxygen.



**Figure 2.9** Structures of ellagitannins in extractions of oak heartwood (Ribéreau-Gayon *et al.*, 1998).

#### 2.4.2.4 Gallotannins

Gallotannins are the simplest hydrolysable tannin and consist of polygalloyl esters of glucose (Hagerman, 2002). Gallotannins have been thought to occur in oak, but no structures of such compounds have yet been identified in oak. The gallic acid found in oak extract may derive from gallotannins, but is more likely to derive from more complex structures containing gallic acid units (Puech *et al.*, 1999). Gallotannins are present in nutgalls and can be added to wine as part of a commercial tannin in certain wine producing countries (Hagerman, 2002; Resolution Oeno, 2002).

#### 2.4.2.5 Reactions of hydrolysable tannins

The presence of several hydroxyl (OH) functions in the *ortho* position led authors to believe that hydrolysable tannins are involved in the oxidation processes in red and white wine (Moutounett *et al.*, 1989). According to Vivas and Glories (1996) when oxygen consumption between ellagitannins and catechin were compared in red wine, the oxygen consumption rate was much faster when the wine were supplemented with ellagitannins than supplemented with catechin. This is probably due to two hydroxyl functions existing in one mole of catechin, opposed to 15 hydroxyl functions existing in one mole of castalgin or vescalagin.

The higher oxidative ability of ellagic tannins generates peroxides faster and in larger quantities, which in turn produce larger quantities of acetaldehyde (the pivoting point of condensation between condensed tannins and anthocyanin) (Vivas & Glories, 1996). Vivas and Glories (1996) found that a solution containing a mixture of catechin and ellagitannins produces a large amount of peroxides, but far less than each component on its own. This is probably due to an inhibition phenomenon, because the two substances are competing for the oxygen. They also found that ellagitannins consume most of the oxygen and hence protect catechin from oxidation.

#### 2.4.2.6 Properties of hydrolysable tannins

The properties of the hydrolysable tannins vary widely due to differences in size, structure and configuration. Many of the properties of the hydrolysable tannins are due to the hydroxyl groups. Both the number and location of these hydroxyl groups will influence the properties of the tannin (Puech *et al.*, 1999).

Hydrolysable tannin varies in size, type and binding reactions. The ester linkages make the tannins highly susceptible to hydrolysis. Puech *et al.* (1999) has shown ellagitannins to be very unstable in hydro-alcoholic solutions. Hydrolysable tannins are hydro soluble and dissolve quickly in wine (Moutounnet *et al.*, 1992). According to Puech *et al.* (1999), the solubility decreases with an increase in molecular weight, thus polymerisation lead to a decrease in solubility.

Hydrolysable tannins are easily oxidised, thereby decreasing the oxygen availability for other reactions. They also chelate metal cations, which are catalysts for oxidation reactions. Hydrolysable tannins are acting as free radical scavengers and the possible combination with quinones also inhibit free radical formation (Puech *et al.*, 1999). Hydrolysable tannins can act as a passive defence against biological decay and have the ability to inhibit the growth of several wood decaying fungi (Scalbert *et al.*, 2005). There is no clear proof of any medicinal beneficial effect of oak tannins, because it is not sure whether the human body absorbs ellagic acid and gallic acid, but they have been shown to inhibit cancer formation (Gali *et al.*, 1992).

#### **2.4.2.7 Influence of hydrolysable tannins on wine quality**

There is great controversy on the organoleptic effects, especially taste properties, of hydrolysable tannins on wine. Quinn and Singleton (1985) postulated that ellagitannins account for much of the astringent taste and mouth feel of wine aged in barrels, but further investigation was needed. Somers (1990) suggested the concentrations of these compounds are too low to contribute to the taste of wine. Herv'e du Penhoat *et al.* (1991) confirmed the astringency of ellagitannins, but came to no conclusive results regarding the sensory impact of the ellagitannin oxidation products. Two authors came to the conclusion that ellagitannins are not responsible for the astringency in wine. Pocock *et al.* (1994) found that oak tannins were present in white wine matured in wood, but that these tannins were near or below their detection limit. Their tasting panel could, however, detect volatile oak constituent in wines that received only low dosages of oak extract. This suggested that oak-derived volatiles provided the primary sensory cue indicating that the wine received oak treatment. Puech *et al.* (1999) suggested that the direct effect of oak tannins on astringency remain uncertain, but only appear likely through a synergistic effect with other wine phenolic compounds. They also found that ellagitannins are astringent, but more bitter than astringent. The hydrolytic products of the hydrolysable tannins are not astringent or bitter (Quinn & Singleton, 1985).

The presence of ellagitannins in oak aged wines is very low due to several reasons. Heating (toasting) of the barrels reduces ellagitannins. The wood structure is changed during toasting, which will influence the extraction of ellagitannins. Ellagitannins also undergo chemical transformation due to oxidation, polymerisation and hydrolysis in wine (Puech *et al.*, 1999). Vivas and Glories (1996) determined that ellagitannins enhanced colour stability in wine and reduced astringency by enhancing tannin-anthocyanin reactions. Barrel fermentation and aging on lees has shown to decrease the polyphenolic content of the wine due to the precipitation of tannins (Dubourdieu, 1992).

## 2.5 TANNIN ADDITION TO WINE

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### 2.5.1 COMMERCIAL TANNIN ADDITION

According to Resolution Oeno (2002), commercial tannins can be extracted from nutgalls, wood rich in tannins or grape seeds. Commercial tannins are recognised as grape derived tannins when the total flavanol content, expressed as (+) catechin, is over 50 mg/g or when its proanthocyanic tannin content is more than 0.5 mg/g. Tannin have its origin from nutgall when the digallic acid content is between 4 and 8 mg/g and its origin from oak when the scopoletine (Solich *et al.*, 1995) content is higher than 4 µg/g (Resolution Oeno, 2002). Commercial tannins are normally extracted by water, ethanol, ether or steam (Zoecklein, 2005; Saucier *et al.*, 1997). It should be noted that the tannin content in the commercial tannin mixture can be very low according to the above mentioned classification and as a result impurities could be added to the wine.

Wine subject to ageing should have enough structure and body to counteract the negative effect that oxygen could have in the case of over oxidation. Any method that increases the total phenols of the wine will support the wine in the aging process.

According to Zoecklein (2005) tannins can be added to wine for the following purposes or problem corrections: redox buffer; sun-damaged fruit; unripe grape tannins; structural/textural, mouth feel modification; increased substrate for micro-oxidation; limit the activity of laccase; assist to precipitate proteins; help to modify aromas, including vegetative aromas; help increase aging potential and help stabilise red wine colour.

Commercial tannins can be added to wine for the above mentioned reasons depending on the different country's legislation. However, it should be noted that the above mentioned properties of commercial tannins have not all been scientifically researched and are mostly claims of the producing companies.

The timing and concentration of the tannin addition is very important, depending on the purpose of the addition. Addition of tannins during fermentation should be higher than during aging due to grape proteins precipitating a portion of the tannins. The degree of precipitation will depend on the grape variety and the season. Investigations into the effect of commercial tannin additions to wine have not yet been scientifically published, but the effects of condensed tannin addition and hydrolysable tannin additions on its own in the wine have been researched (Pocock *et al.*, 1994; Puech *et al.*, 1999; Quinn & Singleton, 1985; Vidal *et al.*, 2004).

### 2.5.2 CONDENSED TANNIN ADDITION

Sims and Morris (1986) found that condensed tannin addition does not significantly affect the chemical age of the wine, but that these additions did increase the total phenolics of the wine.

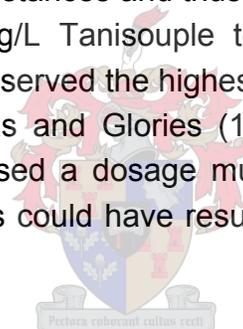
Total phenolics, however, still decreased during storage of the wine. Sims and Morris (1986) also found that condensed tannin additions did not affect the colour intensity of the wines.

Condensed tannins can also be added to the wine by adding grape seeds, which is rich in catechin and other proanthocyanidins. Kovac *et al.* (1992, 1995) showed that the addition of grape seeds to the must led to a small increase in the total phenols of the wine and a dramatic increase in the catechin and proanthocyanidin content. The addition of exogenous seeds also resulted in an increase in colour density when added at a dosage of 60 g seeds per kg of grapes. A higher dosage, however, produced a slight decrease in colour density. Kovac *et al.* (1995) in addition found that exogenous seed addition resulted in wines with a more pronounced variety character and a more intense flavour and aroma.

### 2.5.3 HYDROLYSABLE TANNIN ADDITION

Vivas and Glories (1996) added 1 g/L ellagitannin to Merlot noir wines. They found that the total phenol content of the wines decreased slightly. The wine also became less astringent and more tannin-anthocyanin combinations were present. The ellagitannins also slowed down the degradation process of colour substances and thus stabilised the colour.

Díaz-Plaza (2002) added 20 mg/L Tanisouple to Monastrell wines. This oenological tannin is rich in ellagitannins. They observed the highest total phenol content in the wines with added tannin. The reason why Vivas and Glories (1996) noted a slight decrease in total phenols is probably because they used a dosage much higher than what the suppliers of commercial tannins recommend. This could have resulted in the precipitation of the phenols due to excessive polymerisation.



## 2.6 OXYGEN AND WINE

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Oxygen can come into contact with certain wines during storage. This can happen during pump over of the wine, ageing in oak barrels, or due to micro oxygenation of the wine. According to Singleton (1987) the solubility of oxygen in wine at room temperature and atmospheric pressure is roughly 6 ml/L or 8 mg/L. Castellari *et al.* (2000) reported that oxygen addition to wine reduces the total phenolics of the wine with a simultaneous increase in the red polymeric pigments. Thus with an addition of oxygen an increase in the colour density and colour stability is expected.

Oxygen is important for the formation of acetaldehyde which plays a pivotal role in the indirect polymerisation (acetaldehyde mediated polymerisation) of condensed tannins. This polymerisation of the flavonoids are responsible for the decrease in the gallic acid, caffeic acid, ferulic acid, (+)-catechin, (-)-epicatehin and *trans*-resveratrol concentrations in wines where oxygen was added (Castellari *et al.*, 2000).

Atanasova *et al.* (2002) investigated the effect of oxygen on wine colour. They found that when wine is exposed to oxygen, anthocyanin degradation is enhanced and that reactions involving acetaldehyde leading to indirect polymerisation is favoured. Malien-Aubert *et al.* (2002) found that in the presence of oxygen more xanthylium pigments form which will also contribute to the loss of red colour. The presence of hydrolysable tannins in the wine can also increase the acetaldehyde concentration. This happens because hydrolysable tannins are more easily oxidised than condensed tannins. This oxidation of the hydrolysable tannins generates peroxides, which in turn generates large quantities of acetaldehyde (Gomez-Plaza *et al.*, 2004).

## 2.7 PHENOL ANALYSIS

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### 2.7.1 COLOUR ANALYSIS

The spectral absorbance of red wines has a maximum at 520 nm (red colours) and a minimum in the region of 420 nm (brown colours). For younger wines 620 nm (blue colours) should also be taken into account (Ribéreau-Gayon *et al.*, 1998). Colour density represents the amount of colour, thus the sum of 420 nm, 520 nm and 620 nm measurements. The hue indicates the development of the colour towards orange, therefore the 420 nm value divided by the 520 nm value (Somers & Evans, 1977). Young wines normally have hue values in the order of 0.5 to 0.7, while older wines have values in the order of 1.2 to 1.3. White wines do not have a defined maximum in the visible range and absorption ranges from 280 nm to 500 nm, but have a maximum in the UV range. Oxidised white wines can be measured using the 420 nm values (Ribéreau-Gayon *et al.*, 1998).

Boulton (2001) described a method to determine the different fractions in which the colour can be classified namely: copigmented, free anthocyanins and polymeric pigments. When sulphur dioxide is added to wine, it binds quickly to the C<sub>4</sub> site of the anthocyanin. This reaction produces a stable colourless bisulphite addition product (Timberlake & Bridle, 1967). When acetaldehyde is added to wine, it preferentially binds to sulphur dioxide and thus removes the bleaching effect that sulphur dioxide have on the colour. Using this method the modified colour density, in other words colour density without sulphur dioxide, and sulphur dioxide resistant pigments can be determined by measuring the red colour after the addition of excess sulphur dioxide. With these values the different fractions that contribute to the colour of red wine can be calculated (Boulton, 2001).

The total red pigments of a wine can be determined when a spectrophotometric measurement is taken of the wine at a pH < 1.0 at 520 nm. At this low pH all the anthocyanins are in the coloured flavylium form (Figure 2.5). Thus the anthocyanins that are in the red form

and other anthocyanins that are copigmented are measured (Boulton, 2001; Somers & Evans, 1977).

### 2.7.2 TOTAL PHENOL CONTENT

There are various ways to determine the phenol content of wines, of which the Folin-Ciocalteu value (Folin & Ciocalteu, 1927; Singleton *et al.*, 1999) and 280 nm absorbance (Somers & Ziemelis, 1985; Ribéreau-Gayon *et al.*, 1998) are the most reproducible. Other assays include permanganate titration, reaction with iron salts and the Prussian blue assay. The Folin-Ciocalteu method uses oxidation-reduction reactions, where the phenolate ion is oxidised while phosphotungstic-phosphomolybdic compounds are reduced to a blue molybdenum-tungsten complex that is then measured at 760 nm. The phenol content is determined by using the standard gallic acid as reference (Singleton & Rossi, 1965; Singleton *et al.*, 1999). This method can result in an over estimation of phenols because all hydroxyl groups are oxidised. The second method is based on the absorption of benzene A cycles of the phenols at 280 nm. This method is much easier and faster to perform, but certain phenols, such as cinnamic acids and chalcones are not measured (Ribéreau-Gayon *et al.*, 1998).

### 2.7.3 ANTHOCYANINS

Anthocyanins are present in different forms in wine namely: free pH-dependant anthocyanin forms, quinine (blue), flavylum (red) and carbinol base, anthocyanins associated with copigments and anthocyanins bleached by sulphur dioxide (Figure 2.5). Anthocyanins have a maximum absorption in the range of 520 nm. An estimation of the total anthocyanin concentration can be obtained by bleaching all the anthocyanins with sulphur dioxide. The difference between the bleached and not bleached measurements will give an estimation of the total anthocyanins (Ribéreau-Gayon & Stonestreet 1965; Somers & Evans, 1977).

### 2.7.4 TANNINS

Condensed tannins: Porter *et al.* (1986) described an acid butanol assay where the total condensed tannin concentration of a wine can be determined. This assay work on the principle that the bonds of procyanidins are broken when they are heated in an acid medium. The resulting carbocations are then partially converted into cyanidin when they are oxidised. This method only gives an approximate value and normally over-estimates the tannin concentration of the wine. There are also several modifications to this method to overcome the interference by pigments. Other methods include vanillin method (Price *et al.*, 1978) and phloroglucinol method (Foo & Karchesy, 1991).

Hydrolysable tannins: The hydrolysable tannin content of a wine can be determined by converting the hydrolysable tannin to methyl gallate via methanolysis. The methyl gallate then

reacts with  $\text{KIO}_3$  to yield a chromophore. This method gives a good estimate of the gallotannin concentration, but normally underestimates the ellagitannin concentration (Hartzfeld *et al.*, 2002). Gallotannins can also be determined with the rhodanine assay and ellagitannins can be determined with the nitrous acid method. HPLC analysis is the most often used for the determination of hydrolysable tannins.

### 2.7.5 INDEXES

Gelatine index: The sensory sensation known as astringency is as a result of tannins in the wine binding and precipitating the mucus proteins in the saliva. In this index gelatine is added to the wine to bind and precipitate the tannins in the wine. By taking measurements with and without gelatine additions, the protein-binding strength of the tannins can be determined. Values of 25 to 80 are obtained, where values above 60 is seen as high and values below 35-40 is seen as low. The gelatine index might give an indication of astringency, but have unfortunately not been effectively correlated with the degree of astringency in a wine (Ribéreau-Gayon *et al.*, 1998).

HCl index: The degree of polymerisation of the procyanidins in wine will determine the rate at which the procyanidins will precipitate in an HCl medium. Normally values of 5 to 40 are obtained. Lower values indicate light wines, medium values indicate balanced wines and high values indicate highly polymerised phenols. This index indicates the state of polymerisation of the tannins of the wine (Ribéreau-Gayon *et al.*, 1998). We had, however, found in our laboratory that the HCl index does not always work efficiently in a young red wine.

Ethanol index: This index precipitates condensed anthocyanin polysaccharides by the addition of an excess ethanol. This index depends on the age of the wine and is related to the organoleptic characteristics such as softness and fullness of the wine. The value of this index rises with the age of the wine and is comparable to the HCl index (Glories, 1978).

PVPP index: This index measures the percentage of anthocyanins bounded to tannins. The bounded anthocyanins are absorbed by the PVPP and the free anthocyanins are washed out. This index increases with the age of a wine (Glories, 1978).

### 2.7.6 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Wine phenolic compounds can be separated and measured by means of high performance liquid chromatography (HPLC). Normally this separation is done by reverse phase columns packed with spherical particles of silica bonded with octadecyl (C18) chains (Lamuela-Raventós & Waterhouse, 1994). Castellari *et al.* (2002) described a method using a monolithic column. The monolithic column reduced the time of analysis, due to a faster separation time, shorter wash time, quicker re-equilibration and higher flow rate. By using a

slow gradient the monomeric pigments and non-pigmented phenols, as well as up to the trimeric procyanidins can be separated as individual peaks. A sharp increase in gradient after their elution, elutes the remaining phenolic material as a large peak that are defined as the non-pigmented (280 nm) and pigmented (520 nm) polymeric peak (Price *et al.*, 1995; Peng, 2002).

## 2.8 CONCLUSION

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Condensed tannins are responsible for the organoleptic properties of wine. They are able to bind with anthocyanins, which protect the colour of the wine against oxidation and precipitation.

Hydrolysable tannins are present in too low concentrations in wine to seem to have a direct influence on the taste properties, but may have a synergistic effect with other phenols in the wine to indicate the wine underwent wood maturation. Hydrolysable tannins may, however, protect the wine against oxidation and promote condensed tannin formation.

Commercial tannin additions still need to be investigated to determine the exact influence of these additions on the wine quality. The amount of tannin added by producers in the wine industry also needs to be explored to determine whether these amounts are optimal.

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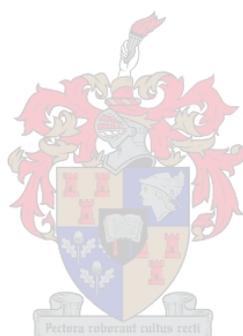
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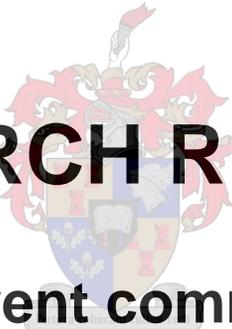
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# Chapter 3

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## RESEARCH RESULTS

**The effect of different commercial tannin and pectolytic enzyme additions to red wine before fermentation**

This manuscript will be submitted for publication in  
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**The effect of different commercial tannin and  
pectolytic enzyme additions to red wine before  
fermentation**

**DANIËL B. KEULDER, ANITA OBERHOLSTER, WESSEL J. DU TOIT**

Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, 7602,  
Matieland, South Africa, facsimile +27 21 808 4781, email [wdutoit@sun.ac.za](mailto:wdutoit@sun.ac.za)

### **Abstract**

The effects of five commercial tannins (hydrolysable, or a mixture of condensed and hydrolysable tannins) and a pectolytic enzyme were monitored on Shiraz (2004 and 2005), Merlot (2004 and 2005) and Cabernet Sauvignon (2004) wines when added according to the suppliers recommendation at the beginning of fermentation. The colour density, hue, sulphur dioxide resistant pigments, modified colour density, total red pigments, colour composition, total anthocyanins, total phenols, total tannins and gelatine index were monitored after fermentation and after malolactic fermentation (MLF). The Shiraz was also monitored during the following year of bottle maturation. HPLC analysis was done on all the cultivar wines after fermentation and additionally the Shiraz wines after a year of bottle maturation. The Shiraz was also sensorially evaluated after 18 months of bottle maturation. The addition of the enzyme enhanced the colour of the Shiraz in the 2004 season and this difference could still be observed after a year of maturation. The different tannin additions showed small differences regarding the above mentioned analysis at the middle and end of fermentation, but these differences diminished over time in all the wines. The addition of some of the commercial tannins changed the benzoic acid fraction of all the wines. Differences between the treatments could also be observed at the sensorial evaluation of the 2004 Shiraz, but did not contribute significantly to the phenolic composition of the different wines.

### **Abbreviations**

**MLF** malolactic fermentation; **LGC** Lafase Grant Cru; **TR** Tanenol Rouge; **Oeno** Oenotan; **VR S** VR Supra; **VR NF** VR Supra NF, **Cntrl** Control

**Keywords:** Commercial tannins, fermentation, maturation, colour composition, total anthocyanins, total phenols, total tannins, gelatine index, HPLC

### 3.1 INTRODUCTION

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Phenols and more specific tannins are of great importance in wine. They contribute to the organoleptic properties and play an important role during the maturation and aging of wine. Tannins can be divided into two groups: 1) Condensed tannins or proanthocyanidins that originate from the grapes, 2) Hydrolysable tannins present in oak. Ellagitannins are the only hydrolysable tannin that can be extracted from oak (Puech *et al.*, 1999), while gallotannins are extracted from nutgalls and can be added to wine in the form of commercial tannin additions.

Wine subjected to aging should have enough structure and body to counteract the negative effect of over-oxidation. Any method that increases the total phenols of the wine will increase the aging capability of the wine (Díaz-Plaza *et al.*, 2002). Sims and Morris (1986) showed that the addition of condensed tannins to wine increased the total phenol concentration of the wine. The addition of exogenous tannins can thus help the wine in the aging process. Exogenous condensed tannins can be added to wine in the form of grape seeds or commercial tannin additions. The addition of hydrolysable tannins also increases the total phenolics of a wine when added in moderate dosages (Díaz-Plaza *et al.*, 2002), but when they are added at very high dosages total phenolic concentrations may decrease (Vivas and Glories, 1996).

Commercial tannins can be extracted from nutgalls, wood rich in tannins and grape seeds. Tannins can be added to wine before fermentation to counteract unripe grape tannins, limit laccase activity, help stabilise colour compounds and modify structural/textural mouth feel properties of the wine (Zoecklein, 2005). Commercial tannins can be added to wine for the above-mentioned reasons depending on the different country's legislation. However, it should be noted that the aforementioned properties of commercial tannins have not all been scientifically researched and are mostly claims of the producing companies.

The objective of this work was to determine the effects that commercial tannin addition before fermentation have on red wine phenolic composition and quality.

## 3.2 MATERIALS AND METHODS

### 3.2.1 PREPARATION OF MUST

Shiraz grapes from the Stellenbosch area, Merlot grapes from the Vredendal area and Cabernet Sauvignon grapes from the Robertson area were used in 2004. Grapes from the same Shiraz and Merlot blocks were again used in 2005. All these wine production areas are in the Western Cape, South Africa.

One thousand kg of each cultivar was destemmed and crushed; the skins and pulp were separated from the juice and equally divided into 46 x 25 L plastic buckets. The juice was then equally divided into these 46 buckets. This resulted in  $\pm$  20 kg skins, pulp and juice per treatment. The juice and skins were separated to ensure every treatment received the same amount of skins and juice.

The pH and titratable acid (TA) were determined using a Motrohm titration unit (Metrohm Ltd., Switzerland) and the TA were adjusted, if necessary, to between 5 and 7 g/L. SO<sub>2</sub> were added at 50 mg/L and the different tannin additions were made as indicated in Table 3.1. The dosages used were those recommended by the suppliers, except for VR Supra 1000 and VR Supra NF 1000 that were much higher than recommended. The tannins were prepared and added to the grape must according to the supplier's recommendations. Every treatment was done in triplicate and the control was done in quadruplicate.

**Table 3.1** Commercial tannin addition dosages

Treatment and source	Type of tannin/enzyme according to the supplier	Dosage (mg/L)		
Lafase HE Grand Cru (LGC) (Laffort Oenologie)	Pectolytic enzyme	30	50	
Tanenol Rouge (TR) (C.J. Petrow chem.)	Condensed & hydrolysable	100	300	
Oenotan (Oeno) (Columbit)	Hydrolysable	100	300	
QCTN (Warren chem.)	Hydrolysable	100	300	
Tanin VR Supra (VR S) (Laffort Oenologie)	Condensed & hydrolysable	300	500	1000
Tanin VR Supra NF (VR NF)	Condensed & hydrolysable	300	500	1000

(Laffort Oenologie)

Control (Cntrl)

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### **3.2.2 WINE FERMENATIONS**

The musts were inoculated with the yeast strain Actiflore C (Laffort Oenologie) at 20 g/hL and were re-hydrated with the fermentation activator Superstart (Laffort Oenologie) (2004) and Dynastart (Laffort Oenologie) (2005) at 30 g/hL. The fermentations were conducted at 20°C. The skin cap of the wines was punched down and the sugar content determined twice a day with a Balling meter. The wines were fermented dry on the skins, and were pressed within five days after the completion of fermentation. A small scale basket press was used to press all the skins at 1.5 Bar. The wine of each treatment was collected after pressing and kept in separate 4.5 L glass bottles.

### **3.2.3 MALOLACTIC FERMENTATION**

All the wines underwent malolactic fermentation, except for the 2004 Merlot and 2004 Cabernet Sauvignon. A malolactic nutrient Malostart (Laffort Oenologie) was added at 20 g/hL 24 hours before inoculation with lactic acid bacteria. The wines were inoculated with MBR B1 (Laffort Oenologie) (*Oenococcus oeni*) at 1 g/hL. Malolactic fermentation (MLF) was conducted at 20°C. The malic and lactic acid concentrations were monitored on a grapescan FT 120 instrument (Foss Electric, Denmark) (Nieuwoudt *et al.*, 2004). MLF was considered to be completed when the malic acid concentrations were lower than 0.3 g/L. The free SO<sub>2</sub> levels were then adjusted to 50 mg/L. SO<sub>2</sub> concentration was determined with a Metrohm titration unit (Metrohm Ltd., Switzerland).

### **3.2.4 BOTTLING AND MATURATION**

The 2004 Shiraz wines were bottled in 750 mL screw cap bottles (five per treatment) a month after the completion of MLF and stored at 15°C until analysis.

### **3.2.5 SPECTROPHOTOMETRIC ANALYSIS**

The musts from 2004 were monitored for colour density and hue, total red pigments, sulphur dioxide resistant pigments, modified colour density, total phenols, total anthocyanin concentration, total tannins and gelatine index. The colour density, hue, sulphur dioxide resistant pigments and modified colour density were measured at pH of 3.6 that were adjusted with sodium hydroxide or hydrochloric acid. These analyses were done in the middle and end of alcoholic fermentation as well as after MLF. The 2004 Shiraz was also monitored

after six months and one year of maturation. Colour density and total phenols were monitored every day of fermentation. The musts from the 2005 season were monitored for colour density and hue, total red pigments, SO<sub>2</sub> resistant pigments, modified colour density, total phenols, total anthocyanin concentration, total tannins and gelatine index. These analyses were completed at the end of alcoholic fermentation and after MLF.

All spectrophotometric analysis was performed using a Helios spectrophotometer (Thermo Electron Corporation Ltd., United Kingdom). Depending on the density of the wine or wavelength of the analysis, 10 mm quartz cuvettes, 1 mm glass cuvettes or 10 mm plastic cuvettes were used. The total tannins, gelatine index and total anthocyanins were determined using the method described by Ribéreau-Gayon *et al.* (1998). The colour density and hue, total red pigments, SO<sub>2</sub> resistant pigments, modified colour density and total phenols were determined using the methods described by Boulton (2001) and Iland *et al.* (2000).

### 3.2.6 HPLC ANALYSIS

Reverse Phase High Performance Liquid chromatography were performed on a Agilent 1100 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). Data processing was done with Chemstation software (Hewlett-Packard, Waldbronn, Germany). A 100 mm x 4.6 mm Chromolith Performance RP-18e column and pre-column was used (Merck).

The mobile phases used were solvent A, containing water adjusted to a pH of 2.04 with *ortho*-phosphoric acid (Reidel-de Haën), and solvent B, that consisted of acetonitrile (Chromasolve, Reidel-de Haën) with 20% of Solvent A. A flow rate of 2 mL/min was used and column temperature was maintained at 35°C. The gradient profile used is shown in Table 3.2.

**Table 3.2** Gradient profile

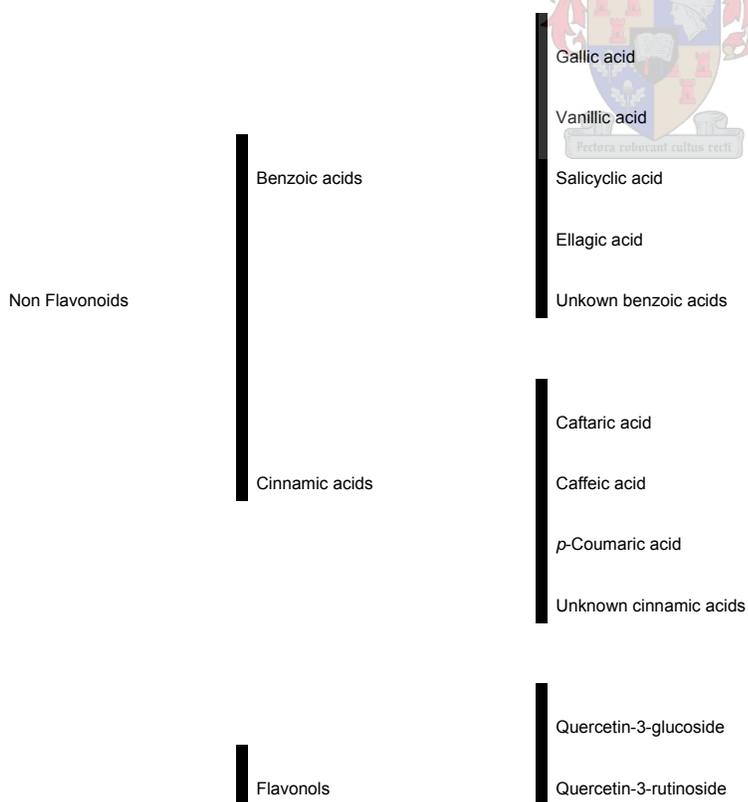
Time (min)	% Solvent A	% Solvent B
0	99	1
2	99	1
17	96	4
31	90	10
55	84	16
75	75	25
80	20	80

84	20	80
85	99	1

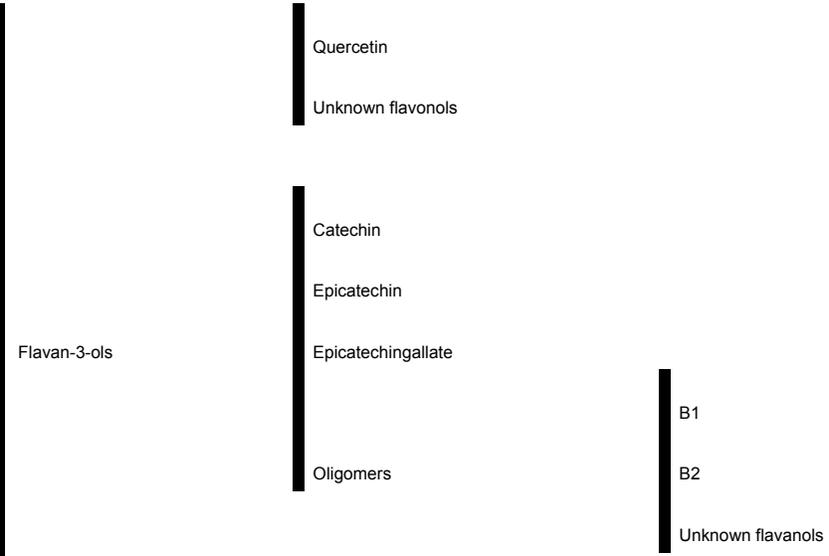
Phenols were quantified using external standards: (+)-catechin hydrate (Fluka), gallic acid (Fluka), vanillic acid (Fluka), *p*-coumaric acid (Sigma), malvidin-3-glucoside (Fluka), ellagic acid (Fluka), quercetin-3-glucoside (Fluka) and quercitin (Extrasynthèse).

Flavan-3-ols and polymeric phenols were quantified at 280 nm as mg/L catechin units with a quantification limit of 10 mg/L, benzoic acids at 280 nm as mg/L vanillic acid units with a quantification limit of 4 mg/L, cinnamic acids at 320 nm as mg/L *p*-coumaric acid units with a quantification limit of 0.8 mg/L, anthocyanins, pigments and polymeric pigments at 520 nm as mg/L malvidin-3-glucoside with a quantification limit of 5 mg/L, flavonol-glycosides at 360 nm as mg/L quercetin-3-glucosides with a quantification limit of 4 mg/L and flavonol aglycones at 360 nm as mg/L quercetin units with a quantification limit of 3 mg/L. Gallic acid and ellagic acid had quantification limits of 1 mg/L and 4 mg/L respectively. The compounds measured were divided into different groups as indicated in Table 3.3. When the compound concentration was absent or below the quantification limit, its value were not used.

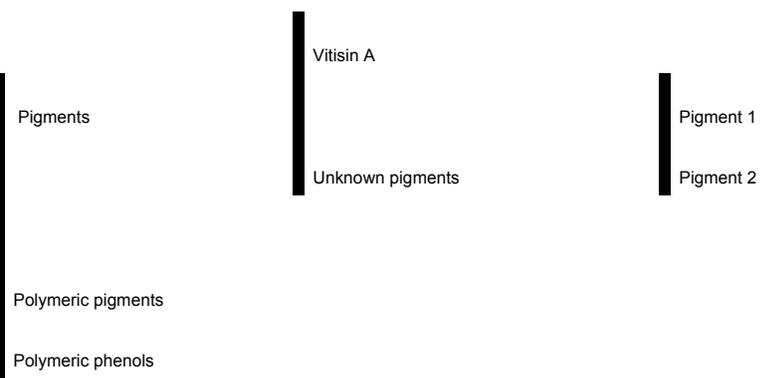
**Table 3.3** Division of compounds measured by the HPLC. The gallic acid, vanillic acid, salicylic acid, ellagic acid and unknown benzoic acids were added to calculate the benzoic acids and the benzoic and cinnamic acids were combined to calculate the non-flavonoids.



Flavonoids



Polymers



### 3.2.7 SENSORY ANALYSIS

A preference test was done on the 2004 Shiraz wines after 20 months of maturation. The panellists had to identify and rank the three most preferred and the three least preferred wines out of the 15 different treatments that were presented to them. The other nine wines were not ranked. The panel consisted out of 15 panellists of the Viticulture and Oenology Department and the Institute for Wine Biotechnology of Stellenbosch University. The wines were presented to the judges in random order and to each sample a random number between 20 and 99 were assigned. The tasting was conducted at 20°C in separate booths in standard ISO glasses.

### 3.2.8 STATISTICAL ANALYSIS

The statistical significance of the difference between the treatments versus the control was obtained using Statistica 7 (StatSoft Inc.) software. One-way analysis of variance (ANOVA) and the Bonferroni test was used to determine differences between the different treatments. A two dimensional box plot graph were used to evaluate the sensorial data.

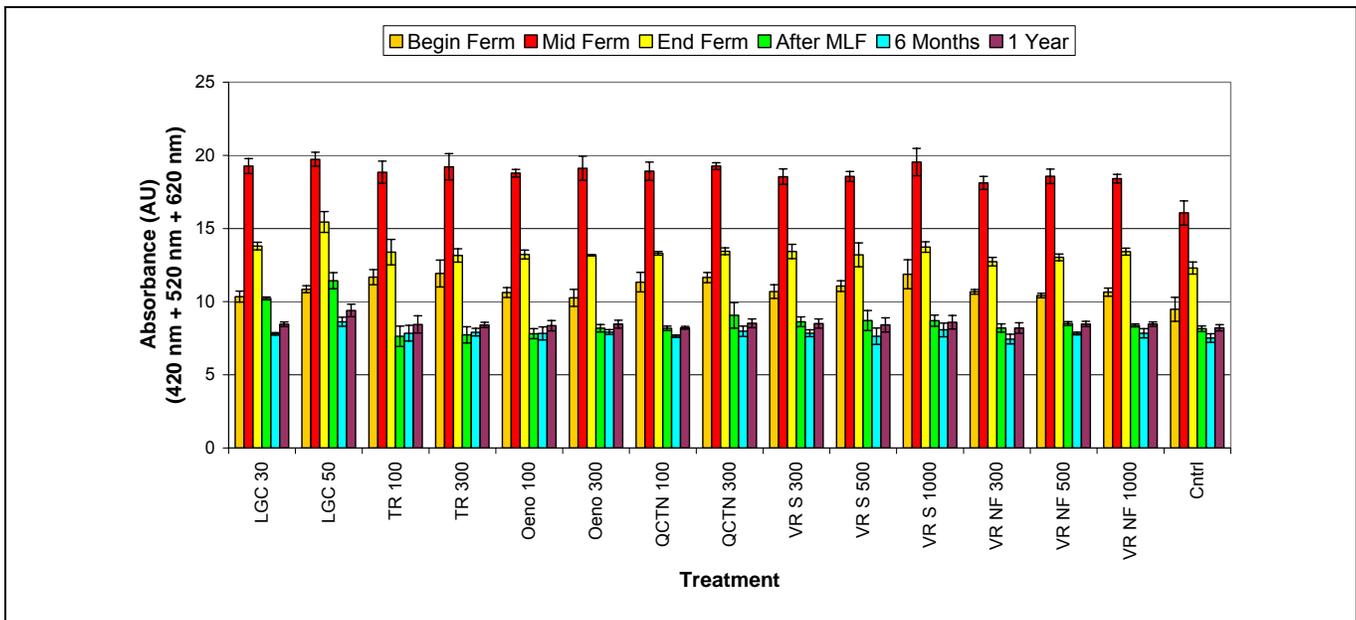
## 3.3 RESULTS AND DISCUSSION

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The numbers after each treatment denote the concentration of commercial tannin that was added in mg/L.

### 3.3.1 COLOUR DENSITIES AND HUES

All the treatments showed an increase in colour density from the beginning of fermentation to the middle of fermentation. (Appendix A, Tables 6.1 and 6.2) This is due to the extraction of anthocyanins from the skins into the must. At the end of fermentation colour densities decreased to almost the same value as at the beginning of fermentation. This is in agreement with what other authors have found (De Freitas *et al.*, 2000; Gómez-Cordovés and González-SanJosé, 1995; Mazza *et al.*, 1999; Sims and Bates, 1994). In the 2004 Shiraz, 2005 Shiraz and 2005 Merlot a further decrease in colour density can be seen until completion of MLF (Appendix A, Tables 6.1 and 6.2). This is possibly due to the oxidation of the anthocyanins and polymerisation and copigmentation of the anthocyanins with tannins (Atanasova *et al.*, 2002; Gómez-Plaza *et al.*, 2004; Somers, 1971; Somers and Evans, 1979). In the 2004 Shiraz the colour density remained constant after MLF up to a year of bottle maturation (Figure 3.1). The modified colour densities of all the wines showed the exact same tendencies as the colour densities of the wines, with only higher absorption values. Significant differences observed in the colour densities of the wines were also observed for the modified colour densities of the wines (Appendix A, Tables 6.2 and 6.3).



**Figure 3.1** Evolution of colour density of the 2004 Shiraz from the beginning of fermentation up to a year of maturation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.

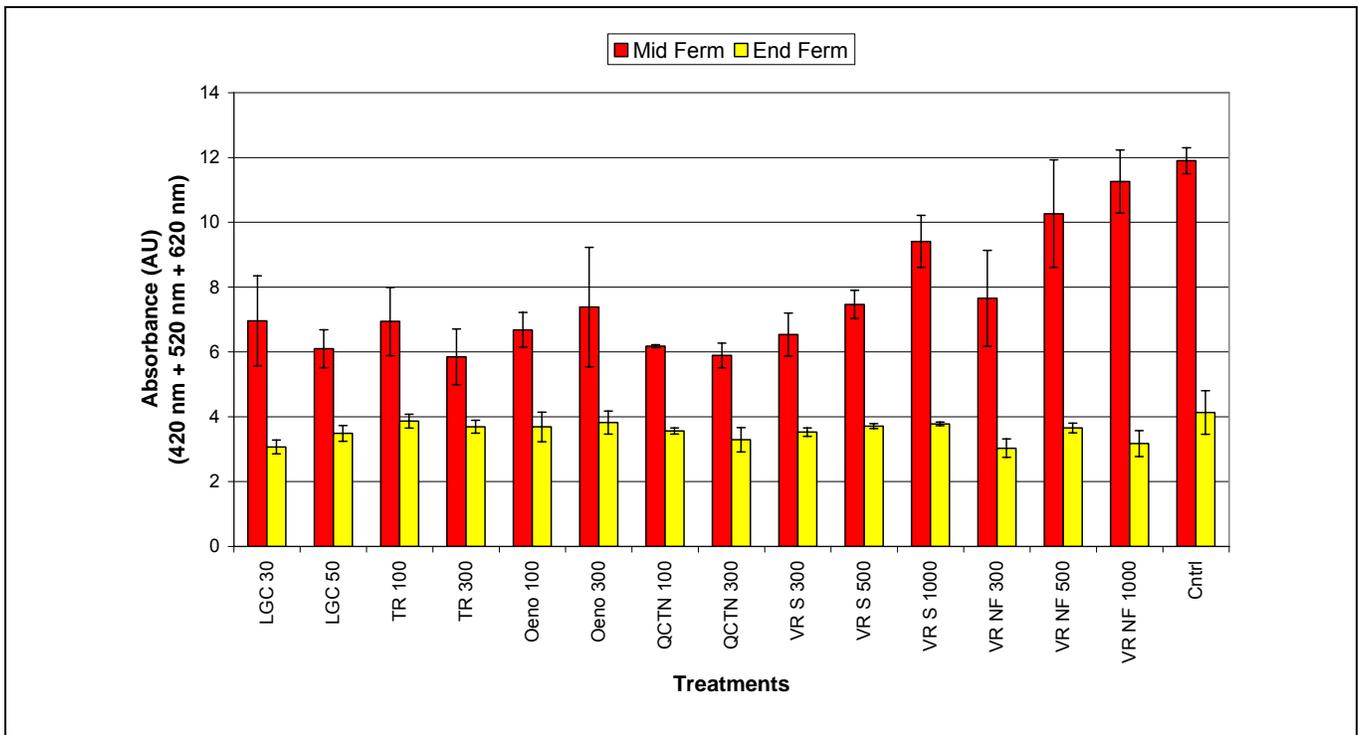
The hue of all the wines increased over time (Appendix A, Tables 6.4 and 6.5) mostly due to an increase in the brown colour resulting from the oxidation of phenols (Castellari *et al.*, 2000; Perez-Prieto *et al.*, 2003), the formation of xanthylium salts (orange) (Dallas *et al.*, 1996; Malien-Aubert *et al.*, 2002) and polymerisation of anthocyanins with tannins (Castillo-Sánchez *et al.*, 2005).

In the 2004 Shiraz the only difference in colour density between the treatments and the control was observed at the middle of fermentation, where all the treatments were higher than that of the control (Appendix A, Table 6.1). No further differences between the colour densities of the treatments and the control were observed, except for the Lafase HE Grant Cru 50 treatment that was significantly higher than the control for up to a year of maturation. The difference between the Lafase HE Grant Cru 50 and the control decreased over time in the 2004 Shiraz (Figure 3.1). The 2005 Shiraz also showed no difference in colour density between the treatments and the control after fermentation or MLF, except for VR Supra NF 300 that was significantly lower than the control (Appendix A, Table 6.1). The Hue of the 2004 Shiraz increased from 0.35 in the middle of fermentation to 0.75 after a year of maturation (Appendix A, Table 6.4), due to the increase in brown colour as a result of oxidation and polymerisation reactions. As with the colour density the only differences between the control and the treatments could be seen at the middle of fermentation. After fermentation and up to a year of maturation there were no differences between the treatments and the control. Lafase HE Grant Cru 30 and Lafase HE Grant Cru 50 also had a lower hue than the rest of the wines, but this difference in hue decreased with aging. In the 2005 Shiraz the control had

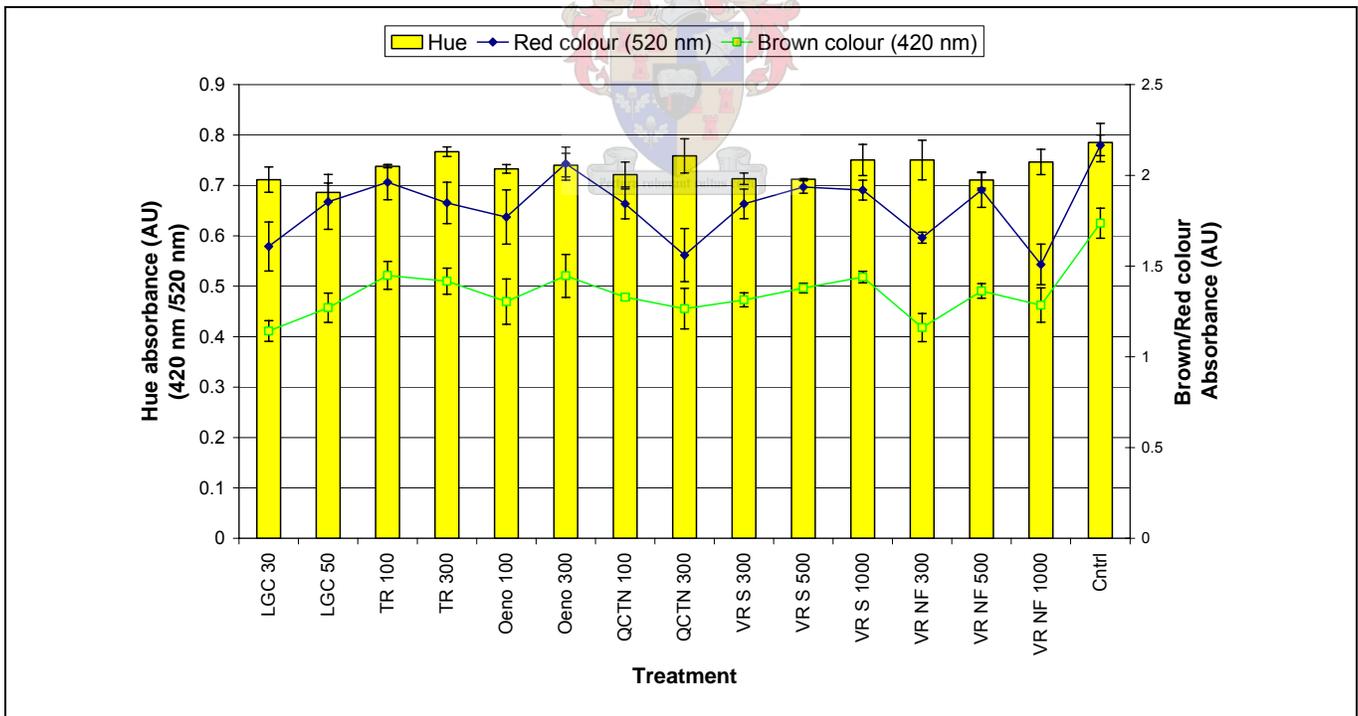
a significantly lower hue than the treatments with Oenotan 300, QCTN 100, QCTN 3000 and VR Supra 300 at the end of MLF (Appendix A, Table 6.4).

In both the 2004 and 2005 Merlot there was no significant differences in colour density between the treatments and the control after fermentation or after MLF (Appendix A, Tables 6.1 and 6.2). The hue of the 2004 Merlot showed treatments Lafase HE Grant Cru 30, Lafase HE Grant Cru 50 and Tanenol Rouge 100 had a lower value than the control at the end of fermentation (Appendix A, Table 6.4). The rest of the treatments showed no differences between the treatment and the control. In the 2005 Merlot there was no difference between any of the treatments and the control in hue values after fermentation or after MLF (Appendix A, Table 6.5).

With the 2004 Cabernet Sauvignon larger differences in colour densities could be seen between the treatments at the middle of fermentation. A clear increase in colour density was observed with an increase in tannin dosage in the Oenotan, VR Supra and VR Supra NF treatments, although all values were less than those of the control (Figure 3.2; Appendix A, Table 6.2). The fact that the treatments were lower than the control could be due to low concentration of tannins in the wine. The higher value of the control could also be partly due to the higher 420 nm (brown colour) value (Figure 3.3). The Cabernet Sauvignon exhibited a large percentage of rot and the grapes were probably highly infected with *Botrytis cinerea*. This could have resulted in higher oxidation in the wine, especially in the middle of fermentation where the laccase activity was more active than at the end of fermentation. At higher dosages the added tannins probably reacted with other tannins through acetaldehyde mediated polymerisation (indirect polymerisation) (Saucier *et al.*, 1997; Saucier *et al.*, 2004; Vivas de Gaulejac *et al.*, 2001). This will also explain why VR Supra 1000 and VR Supra NF 1000 gave better results. At the end of fermentation these differences decreased and only Lafase HE Grant Cru 30 and VR Supra NF 300 were significantly lower than the control. These results can change significantly during maturation and further trails should investigate the effect of tannin additions to low phenol wines and wines made from grapes with high laccase activity. The 2004 Cabernet Sauvignon already had a very high hue value (0.7 to 0.8) at the end of fermentation (Figure 3.3; Appendix A, Table 6.5). This value possibly would have continued increasing during aging (Castillo-Sánchez *et al.*, 2005; Ribéreau-Gayon *et al.*, 1998).



**Figure 3.2** Evolution of colour density of the 2004 Cabernet Sauvignon from the middle of fermentation to end of fermentation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.



**Figure 3.3** Hue, red and brown colour of the 2004 Cabernet Sauvignon at the end of fermentation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.

The results show that the addition of commercial tannins to these musts did not protect or stabilise the colour density more effectively compared to the wine where no commercial tannin were added under our conditions. The addition of colour extracting enzymes, however, resulted in lower hue values (more red colour) that could still be seen after a year of maturation. Pardo *et al.* (1999) reported the same results with the addition of pectinase enzymes to Monastrell musts. It is speculated that in a wine with high laccase activity a higher tannin dosage may be more effective as tannins inhibit oxidation enzymes. In the specific Cabernet Sauvignon tested the control and treatments with higher concentrations of tannin added, exhibited the highest colour densities.

### **3.3.2 SULPHUR DIOXIDE RESISTANT PIGMENTS**

The SO<sub>2</sub> resistant pigments of the wines increased from the middle to the end of fermentation (Appendix A, Tables 6.5 and 6.6). This increase is due to the polymerisation of the anthocyanins with tannins and other compounds in the wine (Adams *et al.*, 2004; Lee *et al.*, 2004; Saucier *et al.*, 2004; Somers and Evans, 1977). After fermentation the SO<sub>2</sub> resistant pigments of the wines decreased up to MLF and then stabilised. This decrease could be due to the rapid degradation of ethyl-linked pigments after fermentation (Lee *et al.*, 2004), or the precipitation of the polymeric pigments formed during fermentation (Adams *et al.*, 2004).

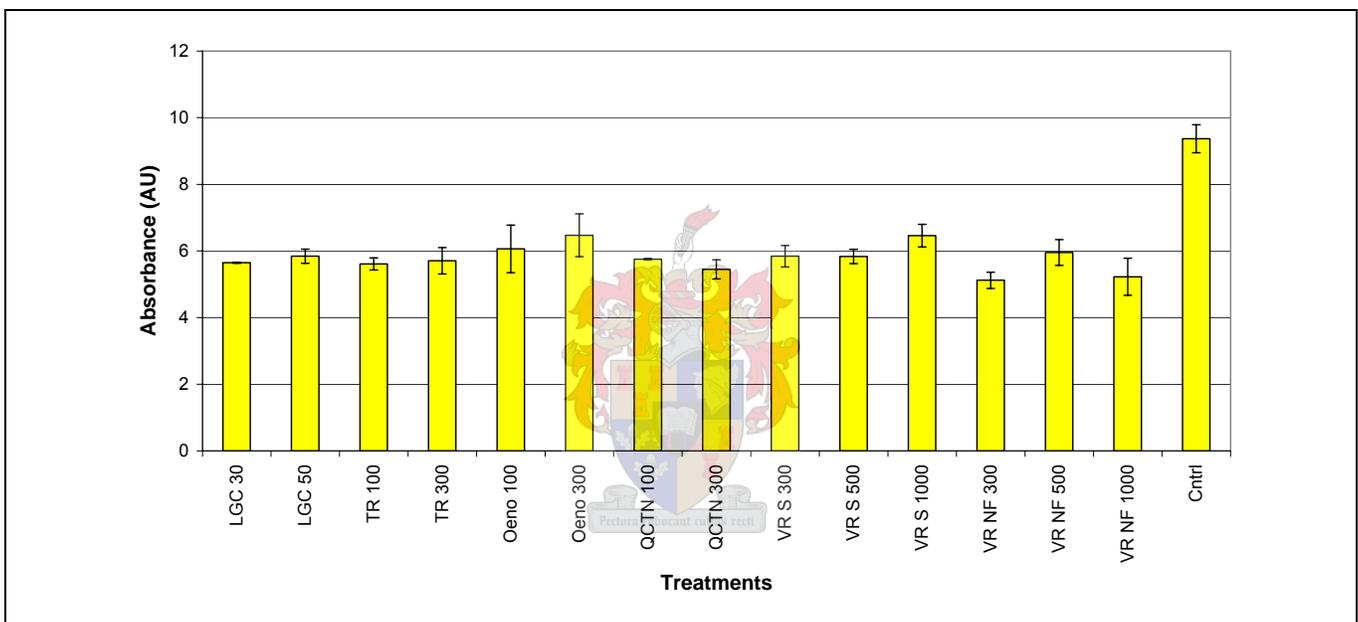
In the 2004 Shiraz at the end of fermentation the Lafase HE Grant Cru 50, Tanenol Rouge 100 and VR Supra 300, 500 and 1000 treatments were significantly higher than the control, with the enzymatic treatment giving the best results. After fermentation the sulphur dioxide resistant pigments decreased significantly up to MLF and stabilised up to a year after MLF. Lafase HE Grant Cru 50 was the only treatment that stayed significantly higher than the control after a year of aging. VR Supra NF 300, however, decreased to the same level as the control (Appendix A, Table 6.5). In the 2005 Shiraz no differences could be seen after MLF between the control and the treatments, except for VR Supra NF 300 that was significantly lower than the control (Appendix A, Table 6.6).

In the 2004 Merlot the sulphur dioxide resistant pigments also increased from the middle of fermentation to the end (Appendix A, Table 6.6). The 2005 Merlot also showed an increase in sulphur dioxide resistant pigments from the end of fermentation until the completion of MLF (Appendix A, Table 6.6). This consistent increase can be explained by the fact that this must had a lower phenol content than the Shiraz must and thus less potential complexes that could precipitate. This value could also have decreased over time, but was not monitored during maturation. In the 2004 Merlot at the middle of fermentation treatments Lafase HE Grant Cru 30, Lafase HE Grant Cru 50 and Tanenol Rouge 100 and at the end of fermentation treatments Lafase HE Grant Cru 30 and Lafase HE Grant Cru 50 were significantly lower than

the control. The rest of the treatments showed no differences between the control and the treatments in both the 2004 and 2005 Merlot.

In the 2004 Cabernet Sauvignon all the treatments had much lower sulphur dioxide resistant pigment values than the control (Figure 3.4). This could be due to competition of the added tannins with anthocyanins during polymerisation with grape tannins, or due to higher oxidation of the control by the laccase.

Over time the addition of commercial tannins showed no differences in the sulphur dioxide resistant pigments, except for the 2004 Cabernet Sauvignon, where the addition of commercial tannins lowered the sulphur dioxide resistant pigments. It also seems as if the VR Supra NF 300 treatment were not ideal for the Shiraz in terms of SO<sub>2</sub> resistant pigments, as it resulted in lower values than the rest of the treatments in 2004 and 2005.



**Figure 3.4** Sulphur dioxide resistant pigments of the 2004 Cabernet Sauvignon at the end of fermentation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.

### 3.3.3 TOTAL RED PIGMENTS

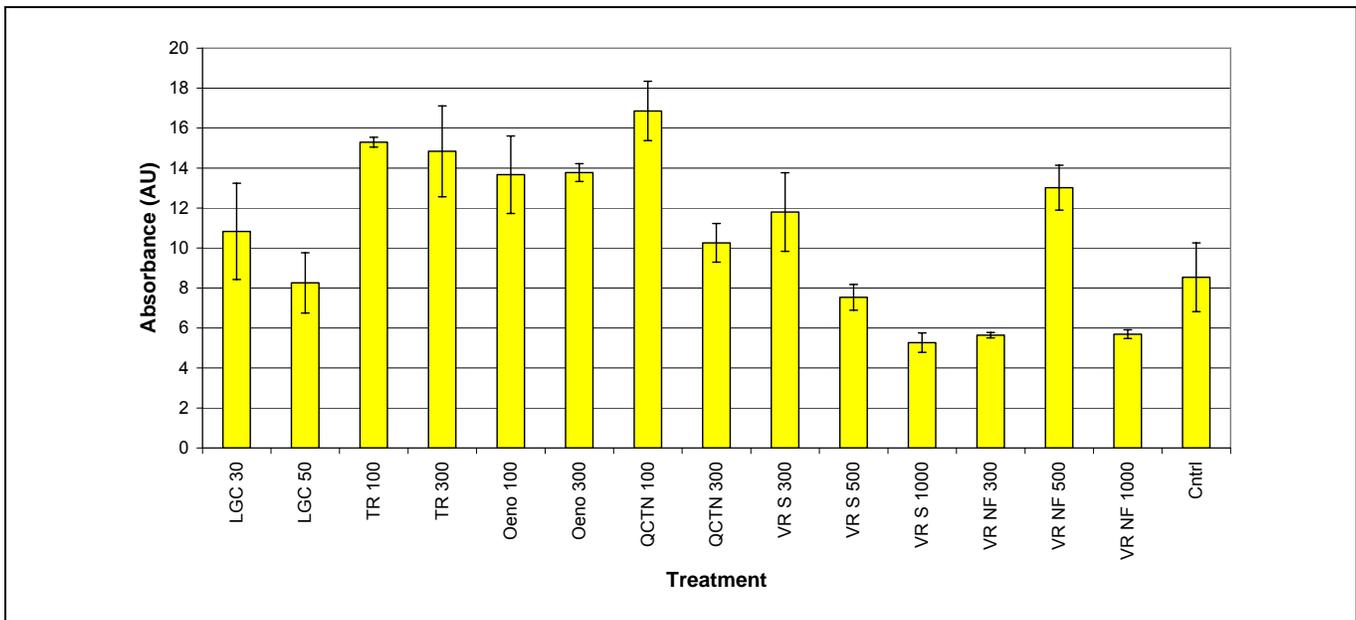
This measurement lowers the pH below 1.0, where all the anthocyanins are in its coloured flavylium form. Thus all the anthocyanins, copigmented anthocyanins and pigments are measured. The total red pigments of all the cultivars showed an increase from the beginning of fermentation and reached a maximum around the middle of fermentation (Appendix A, Tables 6.7 and 6.8). This is due to the extraction of anthocyanins from the skins (González-Neves *et al.*, 2004; Sims and Bates, 1994). The total red pigments decreased to the end of MLF and then stabilised as illustrated by the 2004 Shiraz data. A loss in red colour exhibited

by the pigments will be expected as a result of their polymerisation (Saucier *et al.*, 2004) and differences in the extinction coefficients of the newly formed pigments compared to the monomeric anthocyanins (Boulton, 2001). The loss of red pigments over time can also be explained by the oxidation (Gómez-Plaza *et al.*, 2004) and precipitation of the red pigments (Gil-Munoz *et al.*, 1997; Perez-Prieto *et al.*, 2003).

In the 2004 Shiraz all the treatments were higher than the control at the middle of fermentation, except for the VR Supra 300, VR Supra 500 and all the VR Supra NF treatments. By the end of fermentation (or after 6 months of aging) there were no significant differences between the treatments and the control. (Appendix A, Table 6.7). Similarly, there was no difference between the treatments and the control for the 2005 Shiraz as well as the 2004 and 2005 Merlot (Appendix A, Tables 6.7 and 6.8).

In the 2004 Cabernet Sauvignon there was no significant differences between the treatments and the control at the middle of fermentation. By the end of fermentation, however, there were increased differences between the treatments and control with treatments Tanenol Rouge 100, Tanenol Rouge 300 and QCTN 100 being significantly higher than the control (Appendix A, Table 6.8). It thus seems as if the treatments Tanenol Rouge, Oenotan and QCTN, of which two are hydrolysable tannins, protected the red colour better than the rest of the treatments in this wine made from rotten grapes. This could be due to the hydrolysable tannins being oxidised itself by the laccase rather than the natural grape phenols (Vivas and Glories, 1996).

Commercial tannin addition in general did not influence the total red pigments of any of the wines, except in the Cabernet Sauvignon that had a high rot percentage where some of the tannins had a positive and other a negative influence on the total red pigments content of the wines. The total red pigments concentrations were in general higher at the middle of fermentation where the tannin was added. This could be due to the latter protecting the pigments initially or serving as copigments. At the end of alcoholic fermentation, however, other phenolic compounds extracted from the grapes might have taken over this role, thus negating this effect.

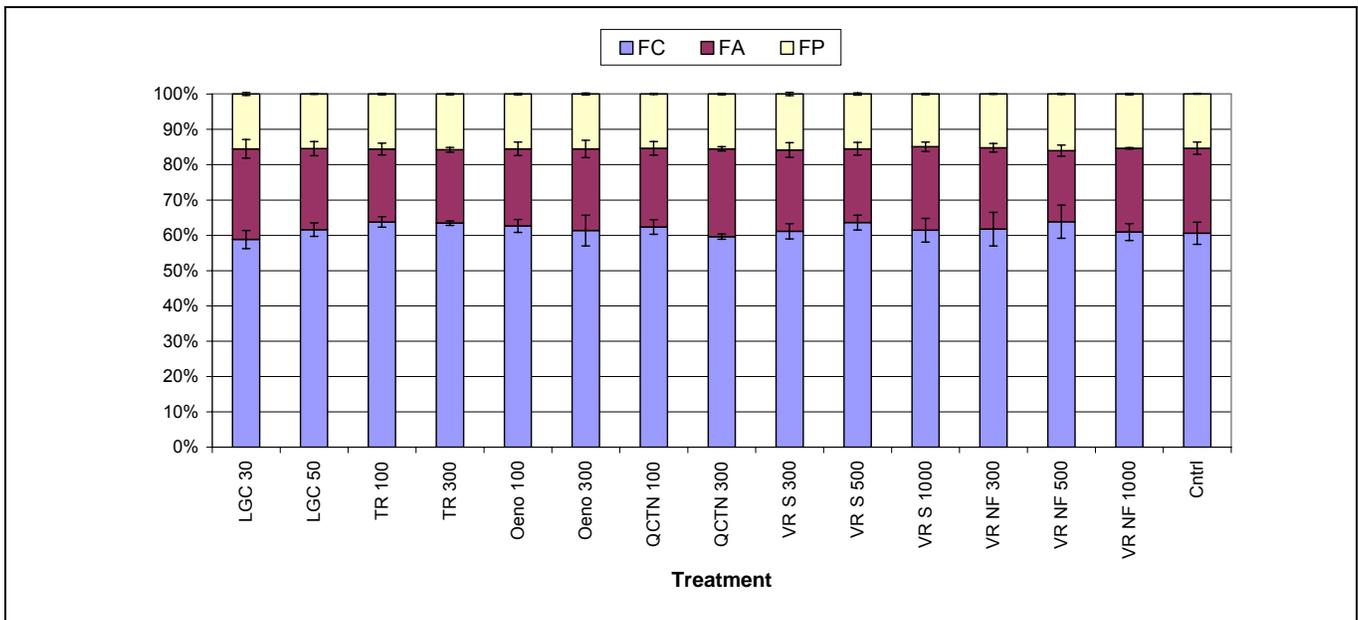


**Figure 3.5** Total red pigments of the 2004 Cabernet Sauvignon at the end of fermentation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.

### 3.3.4 COLOUR COMPOSITION

The colour due to polymeric pigment of all the wines increased over time, except for the 2004 Shiraz that decreased after the end of fermentation and then remained constant for up to a year (Figure 3.6; Appendix A, Tables 6.8 and 6.9). This increase over time is due to the direct (Kennedy and Hayasaka, 2004) and indirect (Bishop and Nagel, 1984; Somers, 1971; Somers and Evans, 1977; Saucier *et al.*, 1997) polymerisation of anthocyanins with other flavonoids. The decrease in polymeric pigments of the 2004 Shiraz from the end of fermentation to the end of MLF (Figure 3.6; Appendix A, Table 6.8) is due to the precipitation of some of the pigmented polymers (Gil-Munoz *et al.*, 1997; Perez-Prieto *et al.*, 2003). The colour due to copigmentation mostly decreased over time as this association was replaced by covalent bonds during polymerisation, except in the Merlot where this fraction of the colour stayed the same (Figure 3.6; Appendix A, Tables 6.10 and 6.11). The colour due to free anthocyanins remained constant in the 2005 Shiraz and the 2004 Cabernet Sauvignon; decreased in the 2004 Merlot; increased in the 2004 Shiraz and the 2005 Merlot (Figure 3.6; Appendix A, Tables 6.11 and 6.12).

In all the cultivars only small differences, if any, could be seen between the different treatments and the control, which diminished over time. Most of the differences seen in the fractions of the colour are possibly due to differences in seasons and compositions of the musts.



**Figure 3.6** Different fractions of the colour of 2005 Shiraz after MLF with different treatments as referred to in Table 3.1. FC = fraction of colour due to copigmentation, FA = fraction of colour due to polymeric pigments, FP = fraction of colour due to polymeric pigments. The error bars denote the standard deviation from the mean of the triplicate treatments.

### 3.3.5 TOTAL ANTHOCYANINS

The total anthocyanin concentration decreased with time in all the cultivars, except for the 2004 Merlot where there was a large increase in anthocyanin concentration from the middle of fermentation to the end of fermentation. This increase in anthocyanin concentration is probably because anthocyanins were still being extracted toward the end of fermentation (Appendix A, Tables 6.13 and 6.14). The decrease observed in the rest of the wines could be due to polymerisation and precipitation of the anthocyanins (Gonzalez-Neves *et al.*, 2004; Monagas *et al.*, 2006) and also association of the anthocyanins with yeast cells (Medina *et al.*, 2005). This decrease in total anthocyanins were stabilised after six months of maturation in the 2004 Shiraz. The 2004 and 2005 Shiraz had similar concentrations of anthocyanins at the end of fermentation, namely 700 – 850 mg/L (Appendix A, Table 6.13). In the 2004 Shiraz there were no differences between the control and the treatments, except in the middle of fermentation where Lafase HE Grant Cru 30, Lafase HE Grant Cru 50, QCTN 300 and Tanin VR Supra 1000 that were significantly higher than the control. In the 2005 Shiraz, however, certain treatments were lower than the control, but only the VR Supra 300 treatment exhibited a significant lower total anthocyanin concentration at the end of fermentation (Appendix A, Table 6.13). In the 2005 Shiraz after MLF it was only VR Supra NF 300 that was lower than the control, but not significantly. After a year the total anthocyanin concentration decreased to 350 mg/L in the 2004 Shiraz.

The treatments Tanenol Rouge 100, QCTN 300 and Tanin VR Supra NF 300 were significantly lower than the control in the 2004 Merlot at the middle of fermentation. These differences diminished toward the end of fermentation, where none of the treatments differed significantly from the control. The 2005 Merlot had a higher total anthocyanin concentration than the 2004 Merlot after fermentation but this difference disappeared after MLF (Appendix A, Table 6.14). At the end of fermentation treatments Lafase HE Grant Cru 50, Tanenol Rouge 100, Tanenol Rouge 300, Oenotan 100, Oenotan 300, QCTN 100, QCTN 300, VR Supra NF 300 and VR Supra 500 were significantly lower than the control for the 2005 Merlot. These differences diminished toward the end of MLF.

There were no significant differences between total anthocyanins of the different treatments and the control for the 2004 Cabernet Sauvignon at the end of fermentation (Appendix A, Table 6.14).

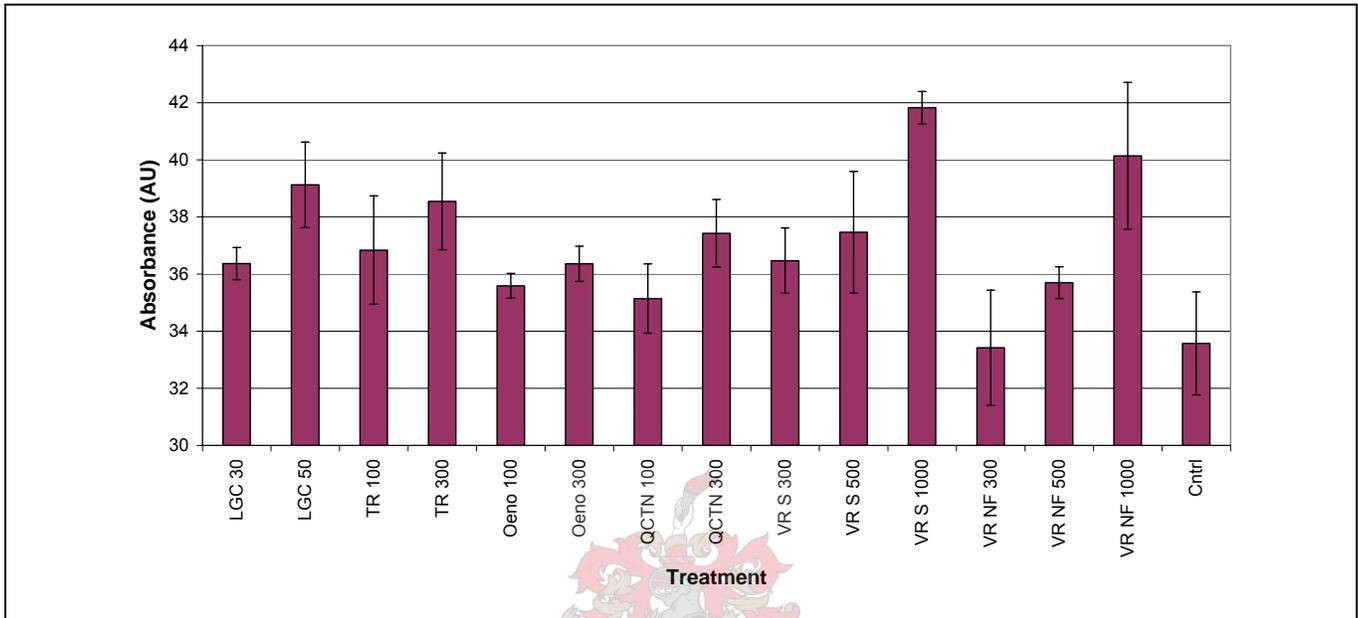
The fraction of coloured anthocyanins increases after being bounded to tannin molecules with subsequent oxidation (Monagas *et al.*, 2005). Commercial tannin additions are also supposed to “protect” anthocyanins, but our results did not support this. This could be due to some dosages being too low or the natural grape phenolics constituting this role.

### 3.3.6 TOTAL PHENOLS

In all the cultivars the total phenol concentrations decreased after the middle of fermentation (Appendix A, Tables 6.14 and 6.15). This is due to polymerisation and the resultant change in extinction coefficients and possible precipitation (Mazza *et al.*, 1999; Perez-Prieto *et al.*, 2003).

In the 2004 Shiraz all the treatments, except for Tanenol Rouge 100, were higher than the control in the middle of fermentation (Appendix A, Table 6.14). At the end of fermentation only the total phenols of Lafase HE Grant Cru 50, Tanenol Rouge 300, VR Supra 1000 and VR Supra NF 1000 were significantly higher than the control (Appendix A, Table 6.14). A clear increase in total phenols could be seen with an increase in commercial tannin dosage. The total phenols of the treatments VR Supra 1000 and VR Supra NF 1000 were also significantly higher than the rest of the treatments. Although the differences between the treatments and the control decreased during aging the effect of the increase in tannin dosage could still be seen after a year of aging. After a year of maturation the treatments were similar to the control, except for the Lafase HE Grant Cru 50, Tanenol Rouge 300, VR Supra 1000 and VR Supra NF 1000 which were significantly higher. VR Supra 1000 and VR Supra NF 1000 were also significantly higher than most of the other treatments (Figure 3.7). There were no significant differences between the treatments and the control for the 2005 Shiraz at the end of fermentation or MLF, although treatments VR Supra 1000 and VR Supra NF 1000 had the highest values (not significant) (Appendix A, Table 6.15).

In the 2004 and 2005 Merlot increases in the total phenol concentrations could be seen with increasing tannin dosages (Appendix A, Table 6.15). VR Supra 1000 and VR Supra NF 1000 were also significantly higher than the rest of the treatments, where VR Supra 1000 was also significantly higher than the control in both seasons after fermentation. In the 2005 Merlot these differences diminished after MLF.



**Figure 3.7** The total phenol content of the 2004 Shiraz after a year of maturation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.

In the 2004 Cabernet Sauvignon an increase in total phenols can be seen with an increase in commercial tannin dosage added (as in the Shiraz and Merlot) (Appendix A, Table 6.13). At the middle of fermentation Tanenol Rouge 300, VR Supra 1000, VR Supra NF 500 and VR Supra NF 1000 were significantly higher than the control. At the end of fermentation these differences could still be seen, although they were not significant.

The addition of pectolytic enzymes will increase the phenolic content of a wine (Revilla and González-Sanjosé, 2003). This effect will be most prominent during the fermentation. This increase in phenol content could be seen in the 2004 Shiraz during the middle of fermentation and after a year of bottle fermentation. In the rest of the cultivars the phenol contents of the enzyme treatments were the same as the controls and the tannin treatments.

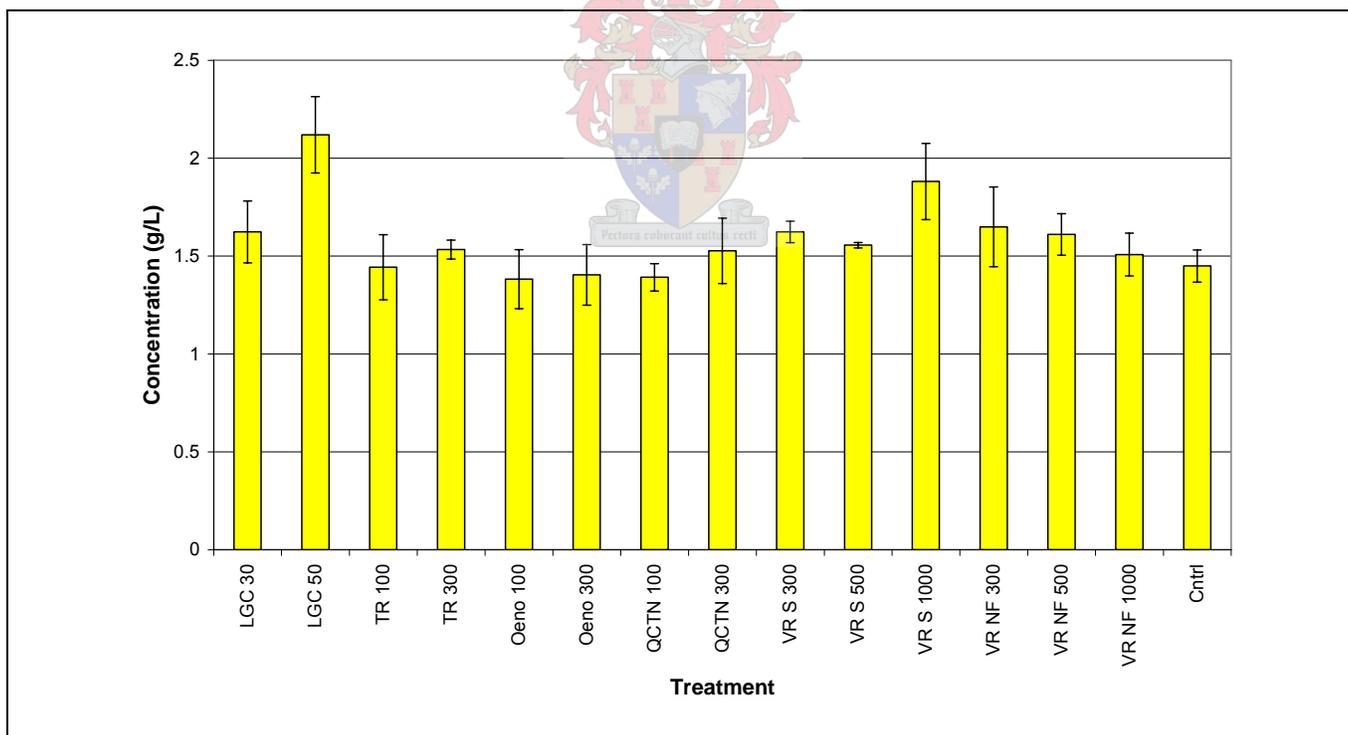
In all the cultivars it was mainly the higher dosages that led to higher total phenol concentrations compared to the control. This raises the question whether the supplier's recommendations is not too low to significantly increase the total phenol concentrations of a wine. VR Supra 1000 resulted in the highest total phenol concentrations in most of the wines investigated.

### 3.3.7 TOTAL TANNINS

In all the cultivars the total condensed tannins decreased with time (Appendix A, Tables 6.16 and 6.17). This is possibly due to polymerisation and subsequent precipitation of the tannins.

In the 2004 Shiraz (Appendix A, Table 6.16) at the middle and end of fermentation there were slight differences between the treatments, but after MLF these disappeared. After a year all the treatments had lower concentrations of tannins than the control, but none of these differences were significant. Treatments VR Supra 1000 and VR Supra NF 1000, however, were significantly higher than the control. The 2004 Shiraz had, after a year of maturation, a total tannin concentration between 0.65 and 1.2 g/L. In the 2005 Shiraz (Appendix A, Table 6.16) the treatments Lafase HE Grant Cru, Tanenol Rouge, QCTN and Oenotan all were about 0.5 g/L lower in tannin concentration than the control at the end of fermentation. After MLF no differences could be seen between the treatments and the control.

In the 2004 Merlot (Figure 3.8) only Lafase HE Grant Cru 50 and VR Supra 1000 had significantly higher tannin concentrations at the end of fermentation compared to the control. In the 2005 Merlot only VR Supra NF 1000 were significantly higher than the control at the end of fermentation (Appendix A, Table 6.17), but after MLF no significant differences could be observed between any of the treatments.



**Figure 3.8** The total tannin content of the 2004 Merlot at the end of fermentation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.

In the 2004 Cabernet Sauvignon (Appendix A, Table 6.17) at the middle of fermentation the Oenotan 100, QCTN 100, VR Supra 1000 and VR Supra NF 1000 treatments gave significantly higher tannin concentrations than the control, After fermentation all the treatments had values lower than the control, where treatments Lafase HE Grant Cru 50, VR Supra 1000 and VR Supra NF 1000 were the only treatments that were not significantly lower than the control. The 2004 Cabernet Sauvignon already had the same tannin concentration after fermentation as that of the 2004 Shiraz after a year's maturation.

The only larger differences could be seen at the middle and end of fermentation. This could be due to different amounts of condensed tannins extracted from the grapes. This also shows that the amounts of tannins added are small compared to the amounts of tannins that are extracted from the grapes.

### **3.3.8 GELATINE INDEXES**

The gelatine index gave inconsistent results and thus only the tendencies of this index were evaluated. The gelatine index decreased for some treatments from the middle of fermentation to the end of MLF and for other treatments increased in the same period (Appendix A, Tables 6.17 and 6.18). This indicates differences in the composition of the grapes and differences in the composition of the commercial tannins added. In the 2004 Shiraz could be seen that over a year of maturation all the treatments' gelatine index decreased from the end of MLF. It can be expected that this would have happened in all the cultivars.

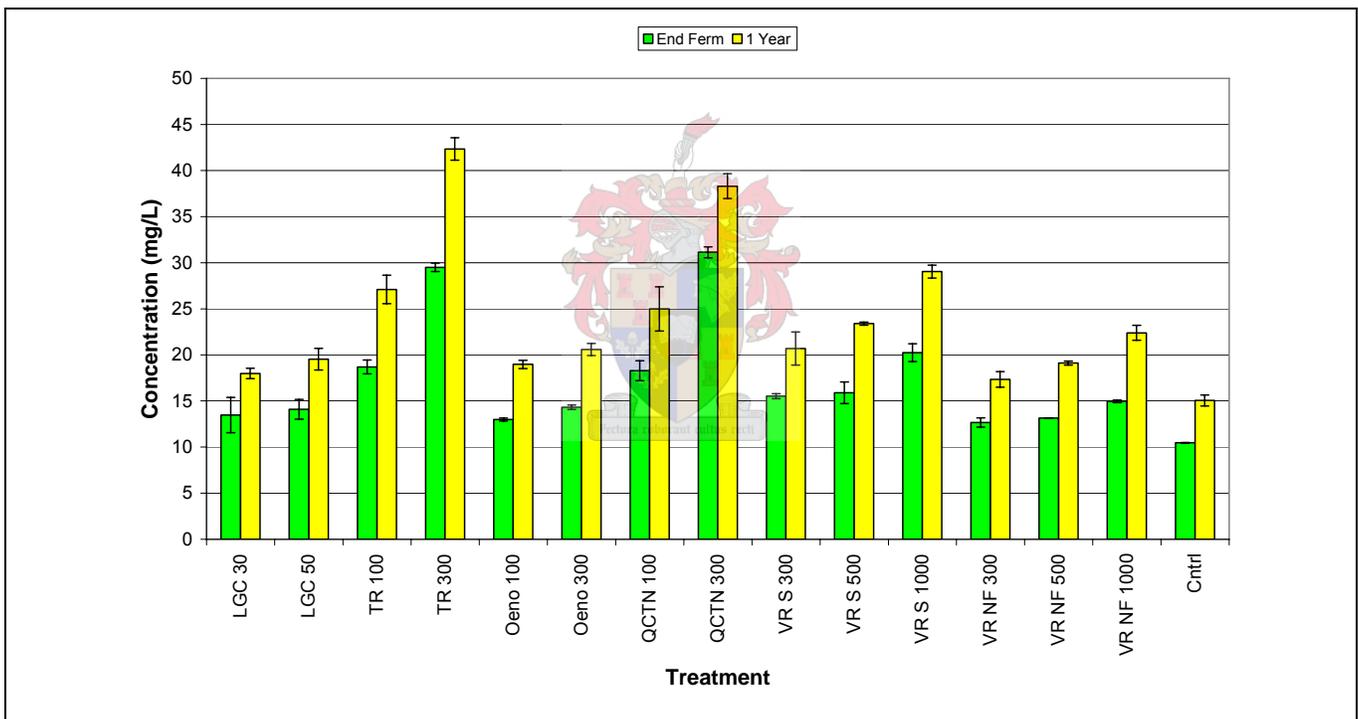
In the 2004 Shiraz (Appendix A, Table 6.17) the different treatments' gelatine indexes peaked either after MLF or after 6 months of maturation. There was a tendency that the lower dosages peaked later than the higher dosages. This could be because most of the reactive tannins already precipitated after MLF in the higher addition wines. In the 2005 Shiraz (Appendix A, Table 6.18) all the treatments were the same as the control after fermentation and after MLF.

In the 2004 Merlot (Appendix A, Table 6.18) there were no significant differences between any of the treatments at the middle of fermentation or at the end of fermentation. In the 2004 Cabernet Sauvignon all the treatments, except VR Supra NF 1000 were lower than the control. At the end of fermentation the results were not repeatable due to large errors between the repeats.

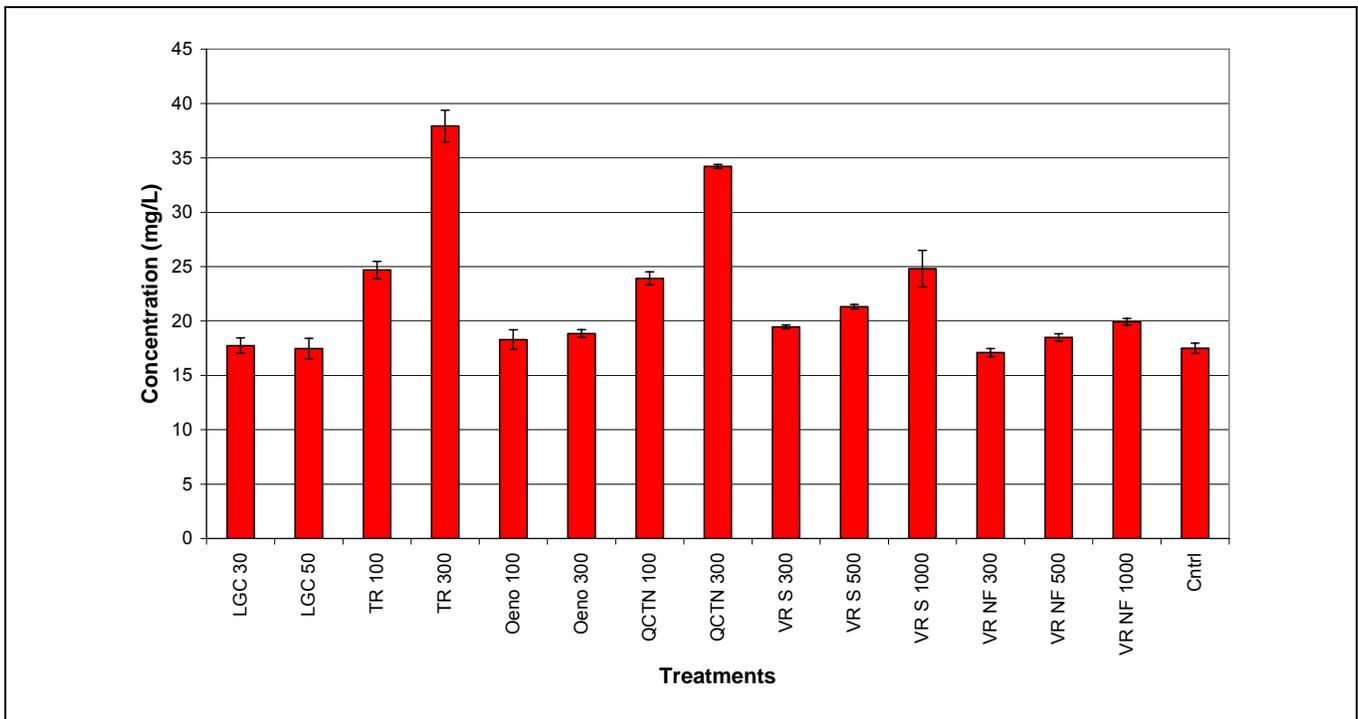
### **3.3.9 HPLC ANALYSIS**

The non-flavonoid concentrations (Appendix A, Tables 6.19 – 6.23) of the treatments Tanenol Rouge 300 and QCTN 300 were significantly higher than the control in most of the different cultivars and seasons tested. This indicates that the addition of these tannins at these dosages increased the non-flavonoid content, and more specific the benzoic acid, especially

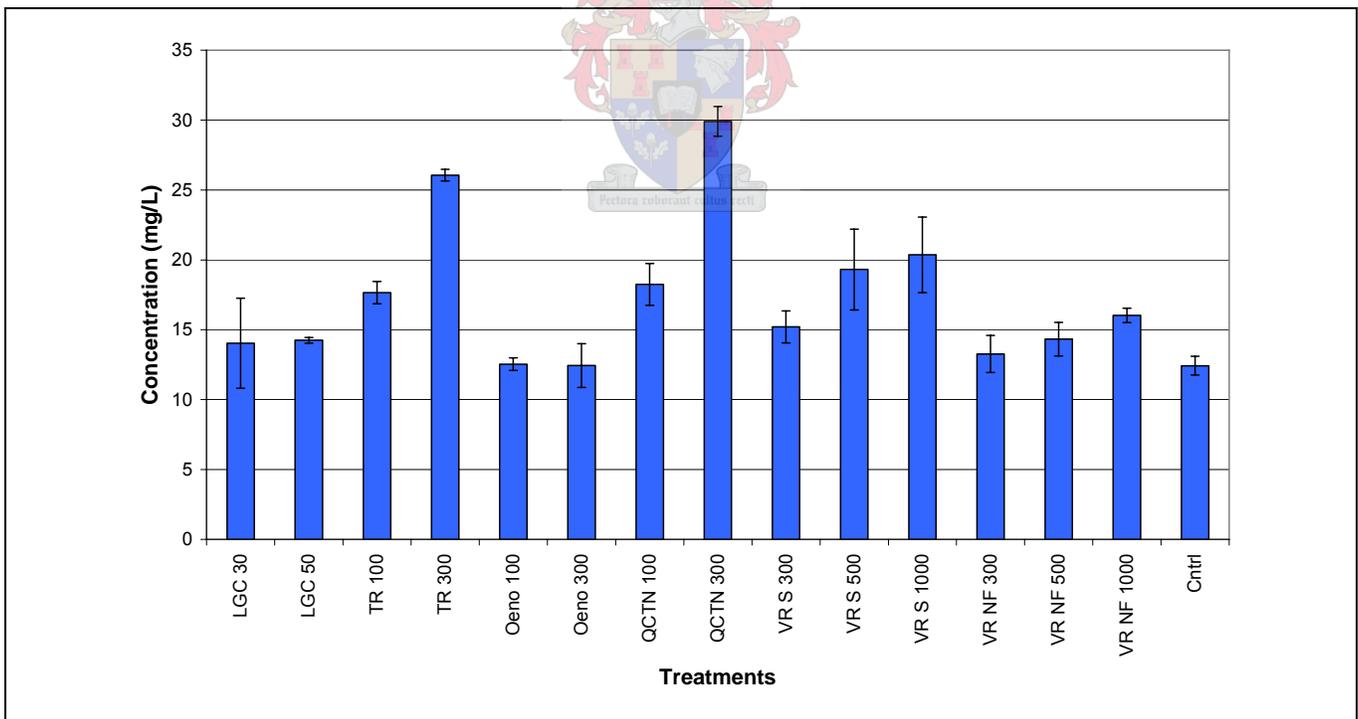
the gallic acid contents (Figure 3.9 – 3.11), of the wines tested. The increase of the gallic acid concentration could be due to hydrolysis of the hydrolysable tannin of which these tannins have high concentrations of (Chapter 4). In the 2004 Shiraz (Appendix A, Table 6.20), after a year of maturation, most of the treatments exhibited a significantly higher concentration of gallic acid in the 2004 Shiraz. The Lafase HE Grant Cru 50 treatment also contained significantly higher concentrations of the non-flavonoids compared to the control, specifically gallic and p-coumaric acid, even after a year of maturation. The 2004 Cabernet Sauvignon and 2005 Merlot (Appendix A, Tables 6.22 and 6.23) also showed that the VR Supra 300 and VR Supra 500 treatments were higher in gallic acid (not significant) than the control. In most of the wines increases in the gallic acid concentration could be seen with an increase in tannin addition (Appendix A, Tables 6.19, 6.20 and 6.23). The cinnamic acid concentrations did not exhibited any significant differences in any of the treatments (Appendix A, Tables 6.19 - 6.23).



**Figure 3.9** Gallic acid content of the 2004 Shiraz at the end of fermentation and after a year of maturation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.



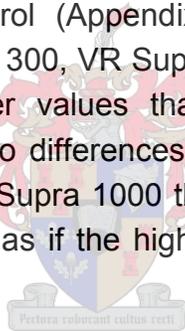
**Figure 3.10** Gallic acid content on the 2005 Shiraz at the end of fermentation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.



**Figure 3.11** Gallic acid content on the 2005 Merlot at the end of fermentation with different treatments as referred to in Table 3.1. The error bars denote the standard deviation from the mean of the triplicate treatments.

The only difference in the total flavonoid concentrations (Appendix A, Tables 6.24 – 6.32) could be seen in the 2004 Shiraz at the end of alcoholic fermentation. In the 2004 Shiraz (Appendix A, Tables 6.24 and 6.25) all the treatments contained more flavonoids than the control after alcoholic fermentation with only the treatments treated with the Lafase Grand Cru enzyme containing significantly higher concentrations. After a year of aging no differences could be seen between the treatments and the control (Appendix A, Tables 6.26 and 6.27). The flavonol concentrations of the Lafase treatments for Shiraz 2004 were higher than the rest of the treatments after alcoholic fermentation and year of aging although these differences were not significant (Appendix A, Tables 6.24 and 6.26). The flavan-3-ol and anthocyanin concentrations of the Lafase treatments were significantly higher than the control after fermentation (Appendix A, Tables 6.24 - 6.27), but these differences disappeared after a year (Appendix A, Table 6.26).

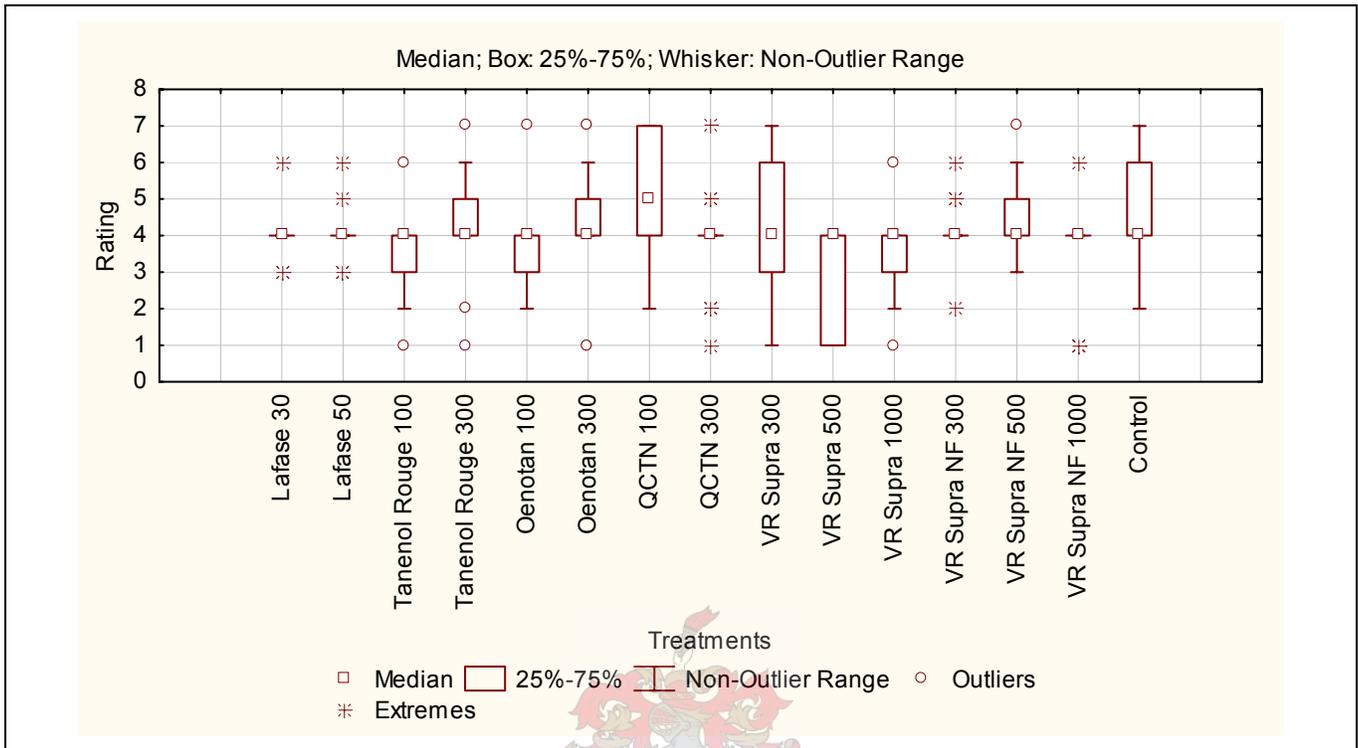
In the 2004 Shiraz no significant differences could be seen between the different treatments in polymeric content (pigmented or non-pigmented polymers) after fermentation or a year of maturation (Appendix A, Tables 6.33 and 6.34). The 2005 Shiraz showed that the Tanenol Rouge and Oenotan treatments both had significantly higher polymeric pigment concentrations compared to the control (Appendix A, Table 6.34). In the 2005 Merlot (Appendix A, Table 6.34) the VR Supra 300, VR Supra 500, VR Supra NF 500 and VR Supra 1000 had significantly higher polymer values than the control. In the 2004 Cabernet Sauvignon (Appendix A, Table 6.35) no differences in the polymeric pigments or polymeric phenols could be seen, except for VR Supra 1000 that was significantly lower in both cases than the control. In this wine it seems as if the high dosage accelerated the polymerisation and precipitation of the polymers.



### **3.3.10 SENSORY RESULTS OF 2004 SHIRAZ**

The addition of commercial tannins helps to stabilise the colour and to enhance the structure and thus the taste of the wine according to the suppliers. The wine treated with QCTN 100 was the most preferred wine by the tasting panel, where most of the panel scored this wine between most preferred wine and not giving the wine a score. Only three panellists scored the wine as second least preferred. Treatments VR Supra 300 and the control were rated as the second most preferred wines. The treatment VR Supra 500 was the least favoured wine with all the panellists either not scoring the wine or scoring the wine as least preferred (Figure 3.12). The main difference between the treatments VR Supra 300 and VR Supra 500 is the gelatine index and amount of polyphenols present according to HPLC determination. Treatments QCTN 100 and VR Supra 300 both have high polymeric phenol concentrations but low gelatine indexes. These tannins and dosages thus motivated the formation of less reactive, soluble complexes which seemed to exhibit positive sensory characteristics. One

should also keep in mind that this is preliminary results and should be investigated in more detail in the future.



**Figure 3.12** Box plot of the 2004 Shiraz preference tasting where 1 is least preferred and 7 is most preferred (different treatments as referred to in Table 3.1).

### 3.4 CONCLUSION

According to the suppliers of the commercial tannins the addition of these tannins will stabilise the colour, modify taste, balance flavour, enhance structure and aging potential, react with proteins, facilitates clearing and inhibit laccase activity.

The commercial tannins tested in this experiment did not significantly change most of the colour and other phenolic characteristics tested by us. Differences seen were mostly during or after fermentation. These differences diminished over time. The Lafase HE Grant Cru treatments performed better than the tannin additions in the 2004 Shiraz due to higher extraction of phenolic material. The enzyme additions did not, however, improve the light bodied wines. The 2004 Cabernet Sauvignon showed the most significant differences regarding total red pigments, but the change in composition of this wine was not tested during maturation. It thus seems that commercial tannin additions may be justified when rotten grapes are being used. The total phenol measurements exhibited the most differences between treatments. Here it was mostly the higher dosages, especially VR Supra 1000 and

VR Supra NF 1000, with significant higher values than the control. The tasting data showed that there are sensorial differences between the different treatments, but did not show that tannin additions improved the quality of the 2004 Shiraz. Varying results in each cultivar and season demonstrate the influence of the wine's unique composition. The influence of commercial tannin additions on laccase activity should still be investigated, as well as the influence of these additions on the maturation time. The influence of higher commercial tannin dosages should also be investigated in the future and whether the recommended dosages are optimal.

### 3.5 ACKNOWLEDGEMENTS

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# Chapter 4

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## RESEARCH RESULTS

**Evaluating and characterising different  
commercial tannin additions to wine after  
fermentation**

This manuscript will be submitted for publication in  
**Australian Journal of Grape and Wine Research**

# Evaluating and characterising different commercial tannin additions to wine after fermentation.

**DANIEL B. KEULDER, ANITA OBERHOLSTER, WESSEL J. DU TOIT**

Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, 7602, Matieland, South Africa, facsimile +27 21 808 4781, email [wdutoit@sun.ac.za](mailto:wdutoit@sun.ac.za)

## Abstract

The effect of three commercial tannins (hydrolysable, condensed and a mixture of hydrolysable and condensed tannins) were monitored on a wine that received no post fermentation maceration and a wine that received extended post fermentation maceration made from the same grapes, when added according to the suppliers' recommendations. The effect of three other commercial tannins (hydrolysable and mixtures of condensed and hydrolysable tannins) were also monitored on a wine where different additions of oxygen were made. The colour density, hue, sulphur dioxide resistant pigments, modified colour density, total red pigments, colour composition, total anthocyanins, total phenols, total tannins and gelatine index were monitored in both experiments. HPLC analyses on certain non-flavonoid, flavonoids and polymers were also done for both these experiments. In addition, the composition of different commercial tannins used in the wine industry were analysed for brown colour, total phenol content, Folin-Ciocalteu values, condensed tannin and hydrolysable tannin contents. The addition of the tannins to the different maceration time wines did not exhibit significant differences when compared to their respective controls, but when compared to each other the additions of the tannins decreased the differences in colour density, hue, sulphur dioxide resistant pigments, modified colour density, total anthocyanin concentrations and gelatine index between the two control wines. Tannin additions to a wine with different oxygen additions showed small differences, but the effect of the oxygen was much greater than those of the tannin additions in terms of the above mentioned analyses. The commercial tannins also differed significantly regarding brown colour, total phenolic content, condensed and hydrolysable tannin concentrations.

## Abbreviations

**MLF** malolactic fermentation; **HPLC** High performance liquid chromatography; **NM** No post fermentation maceration; **EM** extended post fermentation maceration; **Oeno** Oenotan; **Bio** Biotan; **VR S** VR Supra; **Cntrl** Control; **Tan Rich** Tanenol Rich; **AU** Absorbance units

**Keywords:** Commercial tannins, phenols, oxidation, colour composition, total anthocyanins, total phenols, total tannins, gelatine index, HPLC

## 4.1 INTRODUCTION

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Tannins in wine contribute to the organoleptic properties and oxidation reactions and play an important role during the maturation and aging of wine. Both condensed tannins and hydrolysable tannins can be present in wine. Condensed tannins or proanthocyanidins originate from the grapes and hydrolysable tannins, more specifically ellagitannins are extracted from oak (Puech *et al.*, 1999), while the hydrolysable gallotannins can be added to wine in the form of commercial tannin additions (prepared from nutgalls).

Commercial tannins are added to wine at different stages and different dosages depending on the purpose of the addition. Tannin additions during aging should be lower than those during fermentation, as less tannin will be precipitated by grape proteins during aging. Commercial tannin additions during aging are normally made during the racking of a wine to help mix the tannin thoroughly with the wine. Commercial tannin additions during aging can modify the structural/textural mouth feel properties, act as substrate for micro-oxidation, precipitate proteins, increase the aging potential and stabilise red wine colour (Zoecklein, 2005). It should be noted that the above mentioned properties of commercial tannins have not all been scientifically researched and are mostly claims of the producing companies.

Scientific publications of commercial tannin additions have been limited. However, the effects of condensed and hydrolysable tannin additions on its own in the wine have been investigated (Pocock *et al.*, 1994; Puech *et al.*, 1999; Quinn and Singleton, 1985; Vidal *et al.*, 2004). Sims and Morris (1986) found that the addition of condensed tannin did not affect the chemical age or the colour intensity of wines. The additions did, however, increase the total phenolic content of the wine. Vivas and Glories (1996) added 1 g/L ellagitannin to Merlot noir wines. They found that the total phenol content of the wines decreased slightly and that the wine became less astringent. Tannin-anthocyanin combinations increased and the ellagitannins also slowed down the degradation process of colour substances, thus stabilising the colour. Díaz-Plaza (2002) added 20 mg/L Tanisouple (rich in ellagitannins) to Monastrell wines. They observed the highest total phenol content in the wines with added tannin. The reason why Vivas and Glories (1996) noted a slight decrease in total phenols is probably because they used a dosage much higher than what the suppliers of commercial tannins recommend, or that will normally occur in wine that came into contact with oak. This could have resulted in the precipitation of the phenols due to excessive polymerisation.

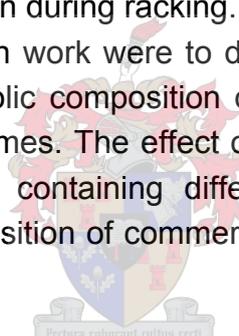
Alcoholic fermentation entails maceration of the solid parts of the grape. Phenols are extracted as a result of the production of alcohol, skin contact and other winemaking practices. Phenols are extracted throughout the alcoholic fermentation and up to 32 days

after the completion of alcoholic fermentation, while anthocyanin extraction reaches a maximum before the end of fermentation (Gil-Munoz *et al.*, 1999).

Oxygen normally comes into contact with wine during storage. This can happen during pump over of the wine, ageing in oak barrels, or due to micro-oxygenation of the wine. According to Singleton (1987) the solubility of oxygen in wine at room temperature and atmospheric pressure is 6 ml/L or 8 mg/L. Oxygen is important for the formation of acetaldehyde. The acetaldehyde is used in indirect polymerisation (acetaldehyde mediated polymerisation) of condensed tannins and other phenols. The polymerisation of the flavonoids are responsible for decreases in the gallic acid, caffeic acid, ferulic acid, (+) catechin, epicatechin and *trans*-resveratrol concentrations in wines where oxygen was added (Castellari *et al.*, 2000). The presence of hydrolysable tannins in the wine can also increase the acetaldehyde concentration. This is due to the fact that hydrolysable tannins are more easily oxidised than condensed tannins. Oxidation of the hydrolysable tannins generates peroxides, which in turn generates large quantities of acetaldehyde (Gomez-Plaza *et al.*, 2004).

Some producers of commercial tannins recommend different dosages for wines with different phenolic compositions, as well as adding these tannins when the wine comes into contact with oxygen as would happen during racking.

The objectives of the undertaken work were to determine the effects that commercial tannin additions have on the phenolic composition of wine made from the same grapes that received different maceration times. The effect of commercial tannin additions on the phenolic composition of red wine containing different amounts of oxygen was also investigated. Additionally the composition of commercial tannins used in the wine industry was evaluated.



## **4.2 MATERIALS AND METHODS**

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### **4.2.1 WINES WITH DIFFERENT MACERATION TIMES**

#### **4.2.1.1 Preparation of must**

Merlot grapes from the Constantia area and Cabernet Sauvignon grapes from the Franschhoek area of the 2005 season were used. Both these wine production areas are in the Western Cape, South Africa. Six hundred kg of each cultivar were destemmed, mixed thoroughly and were equally divided into four 200 L tanks. The pH and titratable acid (TA) were determined using a Motrohm titration unit (Metrohm Ltd., Switzerland) and the TA were adjusted to 6 g/L. Sulphur dioxide were added at 50 mg/L.

#### **4.2.1.2 Wine fermentation**

The four tanks were inoculated with the yeast strain Actiflore C (Laffort Oenologie) at 20 g/hL and were re-hydrated with a fermentation activator Dynastart (Laffort Oenologie) at 30

g/hL. The fermentations were conducted at 20°C. The skin cap of the wines was punched down and the sugar content determined twice a day with a Balling meter. Two of the tanks were pressed at 6 degrees Balling, which resulted in a wine that received no post fermentation maceration (NM). The other two tanks underwent extended skin maceration for 10 days after the completion of fermentation, resulting in a wine that received extended post fermentation maceration (EM). A small scale basket press was used to press the skins to 1.5 Bar. The wine from each treatment was collected after pressing and the two wines that received no post fermentation maceration were thoroughly mixed as well as the two wines that received extended post fermentation maceration in two separate 100 L tanks.

#### 4.2.1.3 Malolactic fermentation

A malolactic nutrient Malostart (Laffort Oenologie) was added at 20 g/hL 24 hours before inoculation with lactic acid bacteria. The wines were inoculated with MBR B1 (Laffort Oenologie) (*Oenococcus oeni*) at 1 g/hL. Malolactic fermentation (MLF) was conducted at 20°C. The malic and lactic acid concentrations were monitored on a grapescan FT 120 instrument (Foss Electric, Denmark) (Nieuwoudt *et al.*, 2004). MLF was considered to be completed when the malic acid concentrations were lower than 0.3 g/L. The free SO<sub>2</sub> levels were then adjusted to 50 mg/L. SO<sub>2</sub> concentrations was determined with a Metrohm titration unit (Metrohm Ltd., Switzerland).

#### 4.2.1.4 Addition of commercial tannins

The no extended maceration (NM) and extended skin maceration (EM) wines were each racked from the MLF lees and then divided into 13 x 4.5 L glass bottles. The different commercial tannin additions were made as indicated in Table 4.1. The preparation, addition and dosage of the tannins were done according to the suppliers' recommendations. Every treatment was done in triplicate and the controls were done in quadruplicate. The wines were kept at 20°C in a dark room for a month and then analysed.

**Table 4.1** Commercial tannin additions and dosages used.

Treatment and commercial source	Type of tannin according to the supplier	Dosage (mg/L)	
		Extended post fermentation maceration (EM)	No post fermentation maceration (NM)
Oenotan	Hydrolysable	100	100

(Colombit)			
Biotan	Condensed	100	100
(Laffort Œnologie)			
Tanin VR Supra	Condensed &	100	100
(Laffort Œnologie)	hydrolysable		
Control		0	0

## 4.2.2 TANNIN ADDITIONS IN COMBINATION WITH DIFFERENT OXYGEN LEVELS

### 4.2.2.1 Wine preparation and the addition of the commercial tannins

Three hundred L 2004 Merlot, from Asara wine estate in the Stellenbosch area (Western Cape, South Africa), that completed MLF were used. One hundred L of the wine were 80% saturated with oxygen by letting it run from one tank to another. To another 100 L of the wine 40% oxygen was added in the same manner. The third 100 L of wine were kept without the addition of oxygen and were designated the 0% oxygen addition and served as the control. The oxygen in the wine was measured using an Oxi 330i/set, WTW (Merck) according to the supplier's recommendation. The tannin dosages, preparation and additions made were those recommended by the suppliers (Table 4.2).

Each of the three 100 L tanks was allocated into 22 x 4.5 L glass bottles. The different commercial tannin additions were made as indicated in Table 4.2 to each oxygen level. Every treatment was done in triplicate and the controls were done in quadruplicate.

The wines were kept in the bottles at 20°C in a dark room for a month and then analysed. After two months the same amounts of oxygen and tannins were added again, left for three months and analysed. The HPLC analysis was done on the wines five months after the first addition of oxygen and commercial tannins. Only the 0% and 80% oxygen addition treatment wines were analysed.

**Table 4.2** Commercial tannin additions and dosages used in combination with 0%, 40% and 80% oxygen addition.

Treatment and commercial source	Type of tannin according to the supplier	Dosage (mg/L)	
Oenotan (Colombit)	Hydrolysable	25	50
Tanenol Rich (C.J. Petrow chem.)	Condensed & hydrolysable	50	100
Tanin VR Supra (Laffort Œnologie)	Condensed & hydrolysable	25	50
Control			

### 4.2.3 DETERMINATION OF THE COMPOSITION OF THE COMMERCIAL TANNINS

Eight commercial tannins of different compositions (Table 4.3) and companies were compared to each other using different spectrophotometric analyses. An artificial wine medium were prepared that consisted of distilled water, 12% v/v ethanol (Merck), 5.5 g/L

tartaric acid (Protea chemicals) and were saturated with potassium bitartrate (Sigma). The pH of the medium was adjusted to 3.6 using hydrochloric acid (Merck) or sodium hydroxide (Saarchem) and was filtered (0.45µm). Each tannin (1 g/L) were dissolved in the artificial wine medium and analysed immediately afterwards.

**Table 4.3** Composition of different commercial tannins tested.

Commercial tannin and source	Type of tannin according to the supplier		
	Condensed tannin	Ellagitannin	Gallotannin
Tanenol Rouge (C.J. Petrow chem.)	x	x	
Oenotan (Colombit)		x	
QCTN (Warren Chem.)			x
Tanin VR Supra (Laffort Œnologie)	x	x	
Tanin VR Supra NF (Laffort Œnologie)	x	x	
Tanenol Rich (C.J. Petrow chem.)	x	x	
Biotan (Laffort Œnologie)	x		
Tanin Galacool (Laffort Œnologie)			x

#### 4.2.4 SPECTROPHOTOMETRIC ANALYSIS

The wine were monitored for colour density and hue, total red pigments, sulphur dioxide resistant pigments, modified colour density, total phenols, total anthocyanin concentration, total tannin concentrations and gelatine index. For the commercial tannins composition the brown colour (420 nm), total phenolics, Folin Ciocalteu value, total tannins (condensed tannins) and hydrolysable tannin concentrations were determined.

All spectrophotometric analyses were performed using a Helios spectrophotometer (Thermo Electron Corporation Ltd., United Kingdom). Depending on the density of the wine or wavelength of analysis, 10 mm quartz cuvettes, 1 mm glass cuvettes or 10 mm plastic cuvettes were used.

The total tannin concentrations, gelatine index and total anthocyanin concentrations were determined using the method described by Ribéreau-Gayon *et al.* (1998). The methods described by Boulton (2001) and Iland *et al.* (2000) were used to determine the colour density and hue, total red pigments, SO<sub>2</sub> resistant pigments, modified colour density and total phenols. The Folin-Ciocalteu value was done according to the method described by Folin and Ciocalteu (1927) and modified by Singleton (1999), while the hydrolysable tannin concentrations were measured according to the method outlined by Hartzfeld *et al.* (2002).

#### 4.2.5 HPLC ANALYSIS

Reverse Phase High Performance Liquid chromatography were performed on a Agilent 1100 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). Data processing was done with Chemstation software (Hewlett-Packard, Waldbronn, Germany). A 100 mm x 4.6 mm Chromolith Performance RP-18e column and pre-column was used (Merck).

The mobile phases used were solvent A, containing water adjusted to a pH of 2.04 with *ortho*-phosphoric acid (Reidel-de Haën), and solvent B, that consisted of acetonitrile (Chromasolve, Reidel-de Haën) with 20% of Solvent A. A flow rate of 2 mL/min was used and column temperature was maintained at 35°C. The gradient profile employed is shown in Table 4.4.

**Table 4.4** Gradient profile.

Time (min)	% Solvent A	% Solvent B
0	99	1
2	99	1
17	96	4
31	90	10
55	84	16
75	75	25
80	20	80
84	20	80
85	99	1



Phenols were quantified using external standards: (+)-catechin hydrate (Fluka), gallic acid (Fluka), vanillic acid (Fluka), *p*-coumaric acid (Sigma), malvidin-3-glucoside (Fluka), ellagic acid (Fluka), quercetin-3-glucoside (Fluka) and quercetin (Extrasynthèse).

Flavan-3-ols and polymeric phenols were quantified at 280 nm as mg/L catechin units with a quantification limit of 10 mg/L, benzoic acids at 280 nm as mg/L vanillic acid units with a quantification limit of 4 mg/L, cinnamic acids at 320 nm as mg/L *p*-coumaric acid units with a quantification limit of 0.8 mg/L, anthocyanins, pigments and polymeric pigments at 520 nm as mg/L malvidin-3-glucoside with a quantification limit of 5 mg/L, flavonol-glycosides at 360 nm as mg/L quercetin-3-glucosides with a quantification limit of 4 mg/L and flavonol aglycones at 360 nm as mg/L quercetin units with a quantification limit of 3 mg/L. Gallic acid and ellagic acid had quantification limits of 1 mg/L and 4 mg/L respectively. If any of the phenols measured were below the quantification limit, their concentrations were not used.

## 4.2.6 STATISTICAL ANALYSIS

The statistical significance of the difference between the treatments and the control was obtained using Statistica 7 (StatSoft Inc.) software. One-way analysis of variance (ANOVA) and the Bonferroni test was used to determine differences between the different treatments.

## 4.3 RESULTS

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### 4.3.1 WINES MADE WITH DIFFERENT MACERATION TIMES

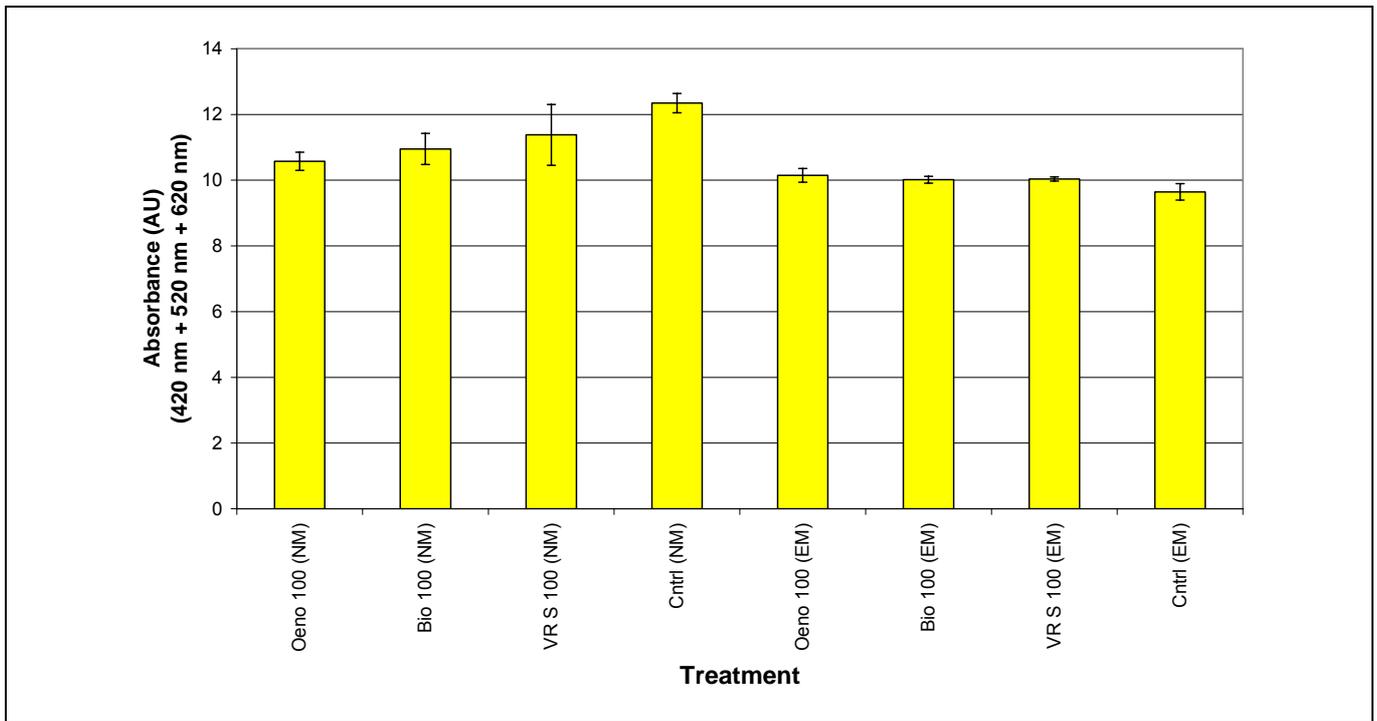
#### 4.3.1.1 Spectrophotometric analysis

The total anthocyanin and total tannin concentrations of the wines were determined before addition of the tannins. The total anthocyanin content of the wines was 650 mg/L (data not shown) and no differences could be observed in total anthocyanin concentrations between the two wines.

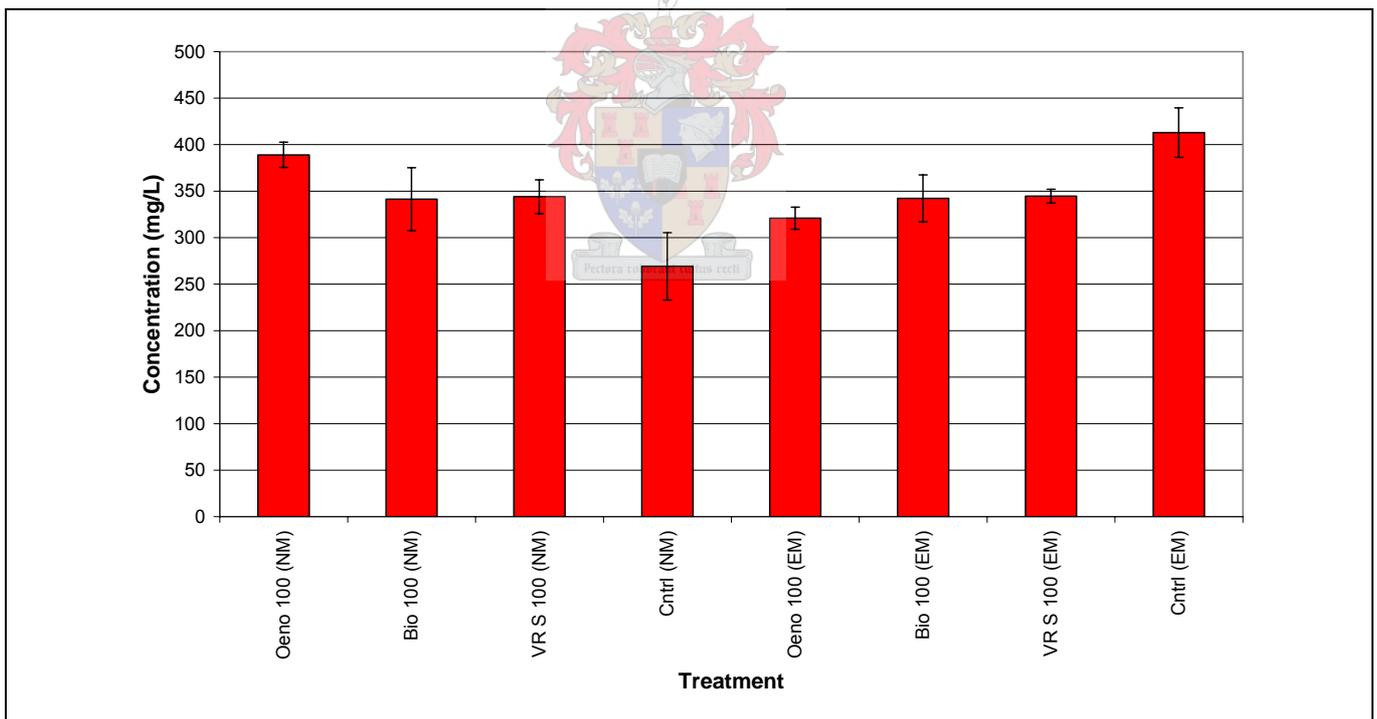
The NM and the EM wines showed no differences between any of the treatments and their respective control wines in terms of colour density, hue, sulphur dioxide resistant pigments, modified colour density, total red pigments, total anthocyanins, colour composition, total phenols, total tannins or gelatine index a month after the tannin additions were made (Appendix B, Tables 7.1 and 7.2).

When the NM and EM wine are compared to each other significant differences could be seen in the colour density, hue, sulphur dioxide resistant pigments, modified colour density, total anthocyanins, colour composition and gelatine index. Most of these differences were between the NM and EM control wines.

The colour density of the NM wines was in general higher than the EM wines (Figure 4.1). The sulphur dioxide resistant pigments showed no differences between the treatments, except for the control (NM) that was significantly higher than the rest of the treatments (Appendix B, Table 7.1). The total anthocyanin concentration of the control from the NM treatment was lower than that of the control from the EM treatment (Figure 4.2) (Appendix B, Table 7.1). The colour composition of the wines showed no differences except for the fraction of colour due to polymeric pigments where the control (NM) had significantly more colour due to polymeric pigments than the control (EM) (Appendix B, Table 7.2). The control (NM) also had a higher gelatine index than the control (EM) (Appendix B, Table 7.2).



**Figure 4.1** The colour density of the NM and EM wines with different treatments as referred to in Table 4.1. The error bars denote the standard deviation of the mean of the triplicate treatments. The number after each treatment denotes the concentration of the tannin added (mg/L).



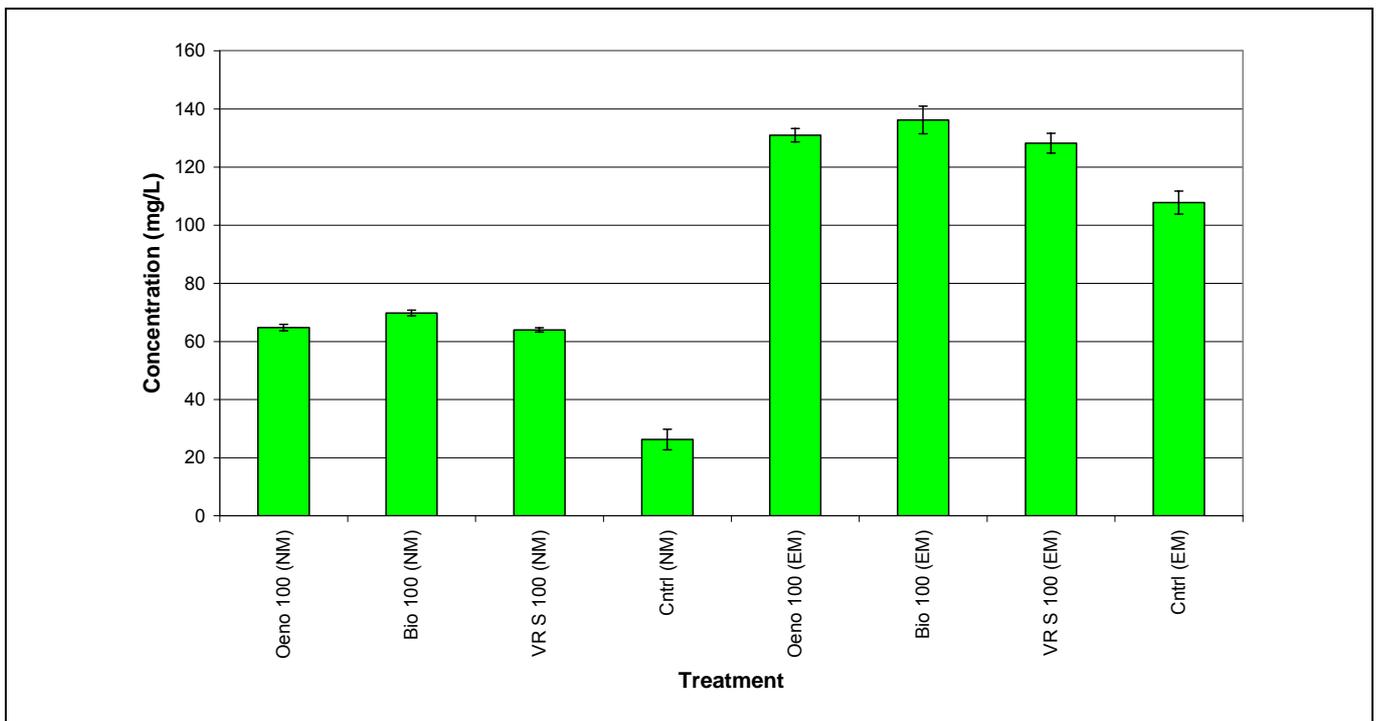
**Figure 4.2** The total anthocyanin concentrations of the NM and EM wines with different treatments as referred to in Table 4.1. The error bars denote the standard deviation of the mean of the triplicate treatments. The number after each treatment denotes the concentration of the tannin added (mg/L).

#### 4.3.1.2 HPLC analysis

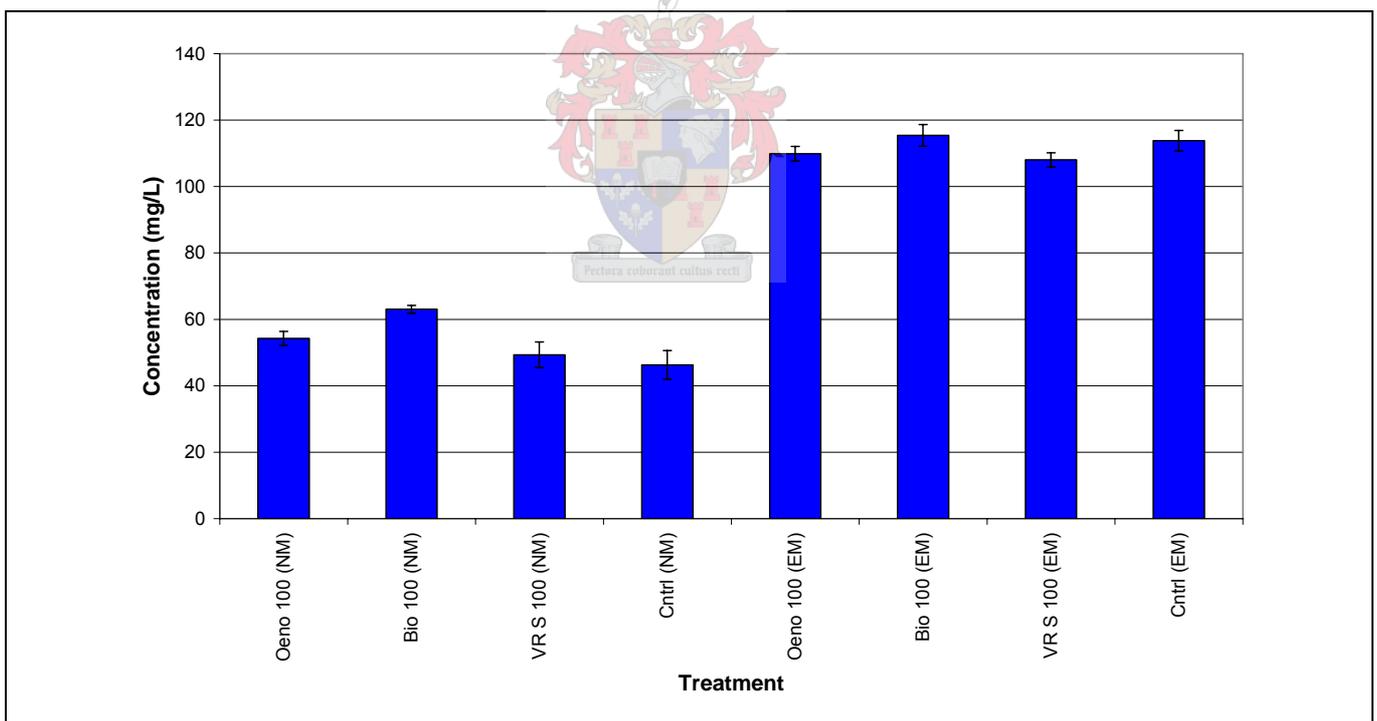
The wines that received different maceration times mostly showed significant differences in the NM wines. In the NM wine the benzoic acids (gallic acid and unknown benzoic acids) concentration was responsible for the difference in non-flavonoid concentrations (Appendix B, Table 7.3). The gallic acid concentrations of all the treatments were significantly higher than the control, but only Biotan's benzoic acid concentration were significantly higher than the control. In the EM wine no significant differences could be observed in the non-flavonoid concentrations, but the gallic acid concentrations of the Biotan and VR Supra treatments were significantly higher than the control. Although no significant differences could be observed in the cinnamic acid concentrations, the treatments of the NM wines were significantly lower in caffeic acid concentrations. Oenotan and Biotan were also significantly higher in unknown cinnamic acid concentrations.

In the EM wine the Oenotan treatment had a significant higher concentration of flavonols (quercetin-3-glucoside and myrecetin). In the NM wine the Oenotan treatment also had a higher concentration of quercetin. Of the flavan-3-ols it was only catechin, epicatechin and the dimers ( $B_1$  and  $B_2$ ) that showed significant differences (Figures 4.3 and 4.4) (Appendix B, Tables 7.4 and 7.5). In both the NM and EM wines only the Biotan treatment yielded a higher catechin concentration (significant in the NM wine) than their respective controls, but the epicatechin concentrations of all the treatments (Oenotan, Biotan and VR Supra) were significantly higher than their respective controls. In the NM wines the Biotan treatment also had a significantly higher dimer ( $B_1$  and  $B_2$ ) concentration than the control (NM) (Figure 4.4). The total anthocyanin concentrations of the treatments were significantly higher and the polymer concentrations significantly lower than their control in the NM treatment. This is due to lower concentrations of polymeric pigments and polymeric phenols.

The non-flavonoid and flavonoid concentrations of the EM wines were higher than the NM wines (significant between the NM and EM controls) (Appendix B, Tables 7.3, 7.4 and 7.5). In the non-flavonoid fraction the benzoic acid (gallic acid) concentration of the EM wines was higher than the NM wines, while the cinnamic acid (caftaric acid) concentrations of the NM wines were higher than the EM wines. Of the flavonoid fraction, catechin, epicatechin and the dimers  $B_1$  and  $B_2$  were responsible for the higher concentration of flavonoids in the EM wines (Appendix B, Table 7.6).



**Figure 4.3** The epicatechin concentrations of the NM and EM wines with different treatments as referred to in Table 4.1. The error bars denote the standard deviation of the mean of the triplicate treatments. The number after each treatment denotes the concentration of the tannin added (mg/L).



**Figure 4.4** The dimer (B<sub>1</sub> and B<sub>2</sub>) concentrations of the NM and EM wines with different treatments as referred to in Table 4.1. The error bars denote the standard deviation of the mean of the triplicate treatments. The number after each treatment denotes the concentration of the tannin added (mg/L).

## 4.3.2 TANNIN ADDITIONS IN COMBINATION WITH DIFFERENT OXYGEN LEVELS

### 4.3.2.1 Spectrophotometric analysis

The colour density, hue, sulphur dioxide resistant pigments, modified colour density, colour composition, total phenols, total tannins and gelatine index were measured before the different tannin additions were made (Table 4.5).

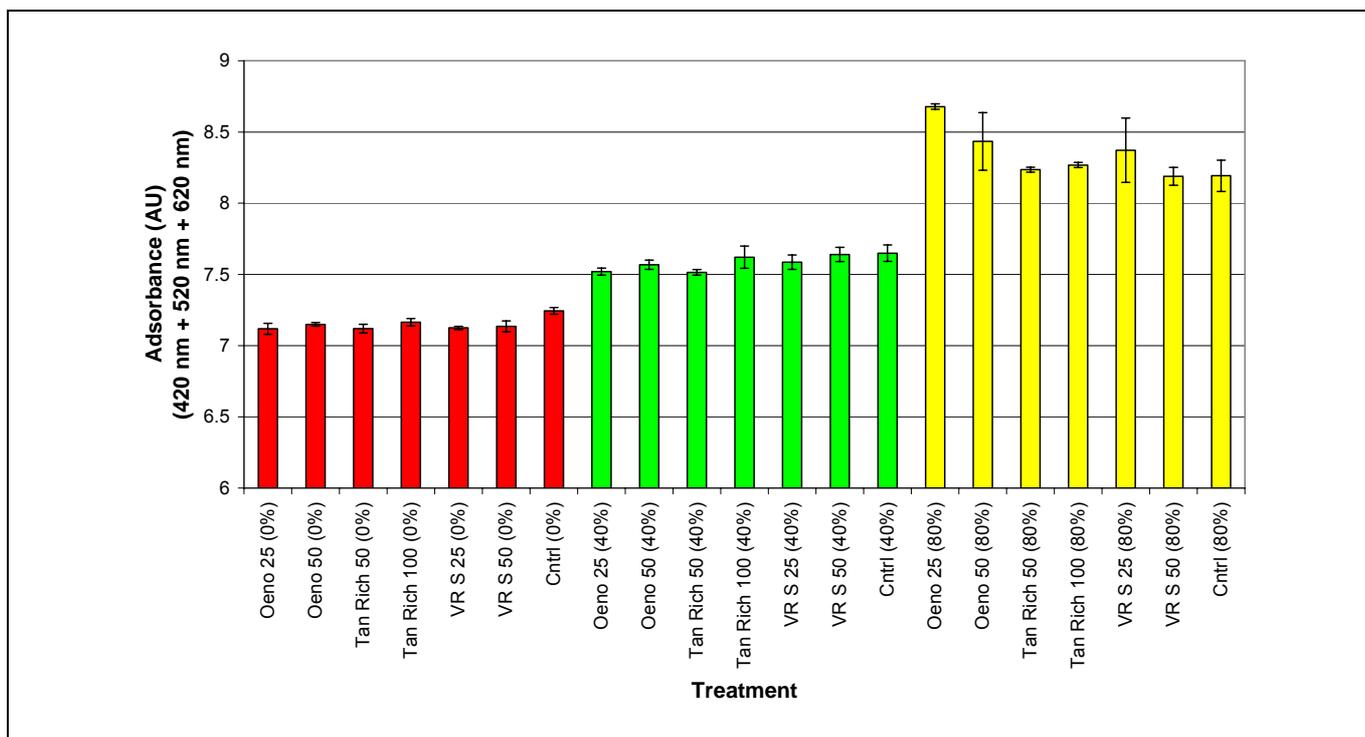
**Table 4.5** Analysis done before the addition of the different tannins and oxygen.

Colour density (AU)	7.73 ± 0.15
Hue (AU)	0.68 ± 0.00
SO <sub>2</sub> resistant pigments (AU)	1.81 ± 0.02
Modified colour density (AU)	10.42 ± 0.18
Total anthocyanins (mg/L)	471.53 ± 32.71
Fraction of colour due to copigmentation (AU)	0.68 ± 0.03
Fraction of colour due to free anthocyanins (AU)	0.16 ± 0.01
Fraction of colour due to polymeric pigments (AU)	0.17 ± 0.00
Total phenols (AU)	46.45 ± 2.01
Total tannins (g/L)	2.15 ± 0.20
Gelatine index (AU)	51.32 ± 3.81

(AU Absorbance units)

The additions of the commercial tannin only resulted in changes in the colour density, hue, modified colour density and the colour composition of the wines. A month after the addition of the tannins and oxygen, the treatments to which 0% oxygen were added were significantly lower in colour density than their control (Figure 4.5) (Appendix B, Table 7.7). In the 80% oxygen addition the Oenotan 25 mg/L treatment had significantly higher colour density than the rest of the 80% oxygen treatments. Five months after the first additions no differences could be seen between the different treatments for each oxygen addition, except for the Tanenol Rich 100 mg/L treatments at 40% oxygen addition (Appendix B, Table 7.7).

In the 0% oxygen treatments the hue of all the treatments were significantly higher than their control after a month, with the Oenotan (25 and 50 mg/L) treatments being the highest. At the 40% oxygen level the Oenotan (25 and 50 mg/L) treatments as well as the Tanenol Rich (50 and 100 mg/L) treatments were significantly higher than the control. At the 80% oxygen level the 25 mg/L Oenotan treatment was significantly lower than the control. After five months the 0%, 40% and 80% oxygen levels showed no significant differences, except at the 40 % oxygen level where Tanenol Rich were added at 100 mg/L that were significantly higher than the rest of the treatments (Appendix B, Table 7.7).



**Figure 4.5** The colour density after one month of the different oxygen level wines with different treatments as referred to in Table 4.2. The error bars denote the standard deviation of the mean of the triplicate treatments. The number after each treatment denotes the concentration of the tannin added (mg/L).

The modified colour density showed differences after a month. At the 0% oxygen addition all the treatments were significantly higher than the control, except for the VR Supra treatment that was added at 50 mg/L. At the 40% oxygen level no differences could be observed. At the 80% oxygen level the Oenotan (25 and 50 mg/L), Tanenol Rich 50 mg/L and VR Supra 25 mg/L treatments were significantly higher than the control (Appendix B, Table 7.7).

Total red pigments decreased slightly with an increase in oxygen dosage, especially where tannins were added to. The SO<sub>2</sub> resistant pigments, however, increased with oxygen, but the tannin addition did not change this significantly.

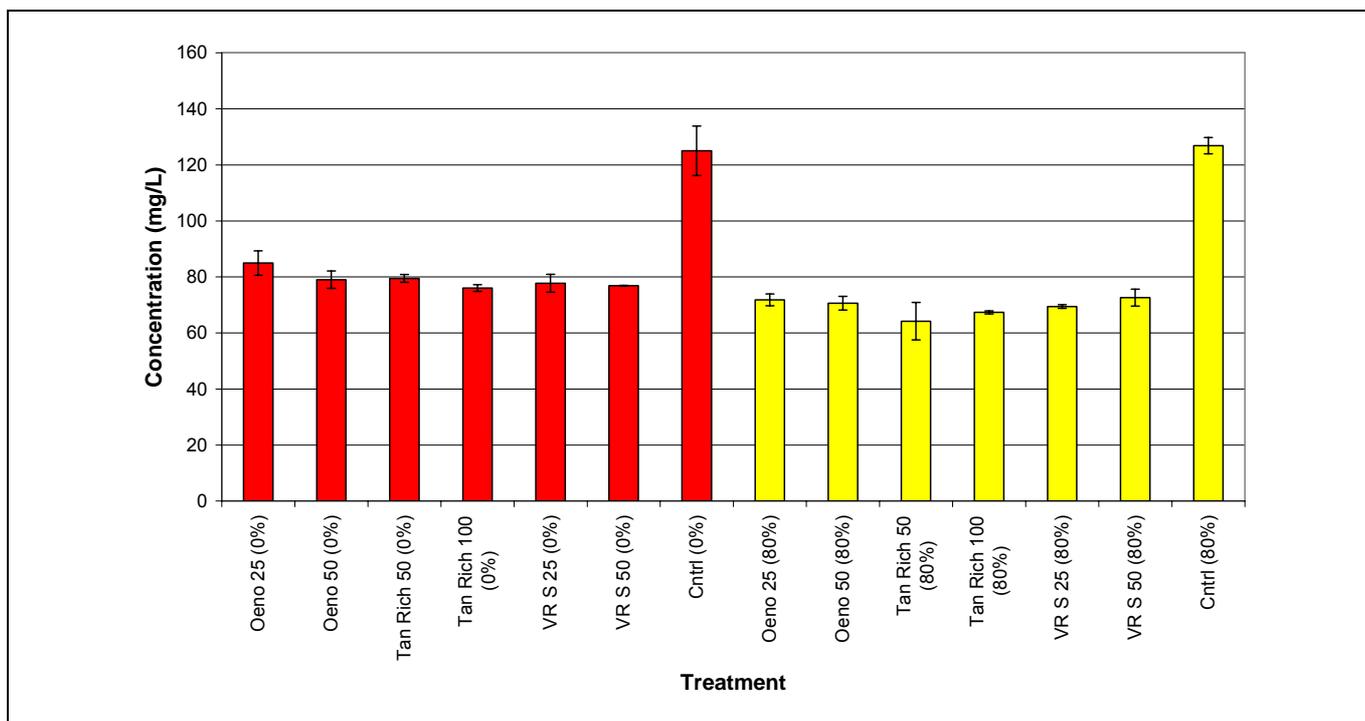
In the colour composition differences could only be observed in the 0% and 80% oxygen levels. At the 0% oxygen level after a month Tanenol Rich 100 mg/L had a significant higher fraction of colour due to copigments and a significant lower fraction of colour due to free anthocyanins compared to the control. At the 0% oxygen level the control had a higher fraction of colour due to polymeric pigments after a month. After five months there were no significant differences at the 0% oxygen level. At the 80% oxygen level the Oenotan 50 mg/L and Tanenol Rich 100 mg/L treatments contained significantly higher fraction of colour due to copigmentation and lower fraction of colour due to free anthocyanins than the control after five months. After a month the Oenotan (25 and 50 mg/L) treatments had a significant lower fraction of colour due to polymeric pigments than the control and after five months the Oenotan 50 mg/L and Tanenol Rich 50 mg/L treatments exhibited significantly lower fractions of colour due to polymeric pigments

(Appendix B, Table 7.8). The total phenol contents of the wines did not change significantly with the tannin additions. The total tannin concentrations also decreased, especially after five months, with an increase in oxygen addition. After five months the addition of commercial tannins led to slightly higher tannin concentrations, but these were not significant. No clear tendency could be established for the gelatine index (Appendix B, Table 7.9)

#### 4.3.2.2 HPLC analysis

The non-flavonoid concentrations of all the treatments were significantly higher than the control in the 0% oxygen addition (Appendix B, Table 7.10). In the 80% oxygen level the non-flavonoids showed no differences, except the Oenotan 25 mg/L treatment that was significantly lower than the control. The benzoic acid concentrations of all the treatments (0% oxygen and 80% oxygen addition) were also significantly higher than their respective controls, except for the Oenotan (25 and 50 mg/L) treatments. The unknown benzoic acids were mostly responsible for these higher benzoic acid concentrations. The Oenotan (25 and 50 mg/L) treatments were significantly lower than their controls in the 0% and 80% oxygen additions in gallic acid concentration. All the tannin treatments had a significantly lower vanillic acid concentration compared to their controls (0% and 80% oxygen additions). The cinnamic acid concentrations showed no differences between the treatments in the 0% and 80% oxygen levels, but the caffeic acid concentration of Oenotan (25 and 50 mg/L) and Tanenol Rich (50 and 100 mg/L) were significantly higher than the control at the 0% oxygen addition. The *p*-coumaric acid concentrations of all the treatments were also significantly higher than their controls in both the 0% and 80% oxygen additions. The Oenotan (25 and 50 mg/L) treatments and VR Supra (25 and 50 mg/L) were also significantly lower in unknown cinnamic acid concentrations.

The flavonoid concentrations of all the treatments were lower than the control in both the 0% and 80% oxygen treatments (Appendix B, Tables 7.11 and 7.12). The flavonol concentrations of the treatments of the 80% oxygen addition were significantly lower than that of the control, except for the Tanenol Rich 100 mg/L treatment. The significantly lower quercetin concentrations were responsible for this. The significantly lower flavan-3-ol concentrations of the treatments were also responsible for the lower flavonoid concentrations of the treatments. With and without the addition of oxygen the tannin treatments led to significant lower concentrations of epicatechin (Figure 4.6) and oligomers. The polymers of the Oenotan treatments showed significant higher concentrations for the 0% and 80% oxygen addition treatments compared to their controls (Appendix B, Table 7.13). The rest of the treatments were also higher than the control, although not significant, at the 0% oxygen addition. The polymeric phenols of all the treatments were higher than the control for the wines in the 0% oxygen addition, but for the 80% oxygen addition there were no differences, except for the Oenotan (25 and 50 mg/L) treatments that were significantly higher than the control.



**Figure 4.6** The epicatechin concentrations of the 0% and 80% oxygen level wines with different treatments as referred to in Table 4.2. The error bars denote the standard deviation of the mean of the triplicate treatments. The number after each treatment denotes the concentration of the tannin added (mg/L).

### 4.3.3 COMPOSITION OF COMMERCIAL TANNINS

The brown colour of Tanenol Rouge, Tanenol Rich, VR Supra and VR Supra NF were the same, while the brown colour of Oenotan and QCTN were significantly higher than the before mentioned. Tanin Galacool had very little brown colour, while Biotan had the second lowest brown colour value (Table 4.6).

The total phenols according to the method described by Boulton (2001) and the Folin-Ciocalteu value were much higher for Tanin Galacool than the rest of the tannins tested. QCTN were also the second highest regarding both characteristics. According to Boulton’s (2001) method Biotan and VR Supra NF had the lowest phenol content, in contrast the Folin-Ciocalteu value of VR Supra NF and VR Supra was the lowest (Table 4.6).

**Table 4.6** Brown colour, total phenols, Folin-Ciocalteu value, condensed tannins and hydrolysable tannins of the commercial tannins referred to in Table 4.4.

Commercial tannin and source	Brown colour	Total phenols	Folin Ciocalteu	Condensed tannins	Hydrolysable tannins
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	(AU)	(AU)	(mg GAE/L)	(mg/g)	(mg/g)
Tanenol Rouge (C.J. Petrow chem.)	0.38	0.20 ± 0.01	628.19 ± 9.48	183.75 ± 64.97	2.69 ± 0.10
Oenotan (Colombit)	0.87	0.19 ± 0.00	657.64 ± 5.29	113.04 ± 78.30	1.01 ± 0.06
QCTN (Warren Chem.)	0.83	0.21 ± 0.01	695.42 ± 1.18	217.60 ± 29.31	2.26 ± 0.05
Tanin VR Supra (Laffort CEnologie)	0.42	0.15 ± 0.00	600.69 ± 10.42	327.61 ± 155.58	0.59 ± 0.02
Tanin VR Supra NF (Laffort CEnologie)	0.41	0.13 ± 0.01	573.47 ± 20.76	492.27 ± 74.27	0.51 ± 0.00
Tanenol Rich (C.J. Petrow chem.)	0.42	0.16 ± 0.00	669.03 ± 3.76	450.92 ± 70.10	0.69 ± 0.06
Biotan (Laffort CEnologie)	0.16	0.13 ± 0.01	644.86 ± 2.10	568.99 ± 87.92	0.77 ± 0.03
Tannin Galacool (Laffort CEnologie)	0.01	0.43 ± 0.02	790.14 ± 3.47	128.74 ± 35.53	9.56 ± 0.14

(AU Absorbance units; GAE gallic acid equivalents)

The condensed tannin content of Biotan was the highest, while that of Oenotan and Tanin Galacool was the lowest. The hydrolysable tannin concentration of Tanin Galacool was at 9.5 mg/g, much higher than the rest of the tannins. The two tannins that contained the second highest amount of hydrolysable tannins were Tanenol Rouge and QCTN at respectively 2.7 and 2.2 mg/g. VR Supra and VR Supra NF had the lowest hydrolysable tannin concentration of all the tannins tested (Table 4.6).

## 4.4 DISCUSSION

### 4.4.1 WINES MADE WITH DIFFERENT MACERATION TIMES

The analysis done before the different tannins were added confirmed that both the NM and EM wines had initially the same amount of anthocyanins. This was expected because normally all the anthocyanins are extracted within the first three to seven days of fermentation (Gil-Munoz *et al.*, 1999). A difference in the total amount of phenolics would also be expected due to the extended skin maceration that the EM wine underwent. Phenols and tannins are extracted throughout fermentation and post fermentation skin maceration (Ribéreau-Gayon *et al.*, 1998).

The difference in colour density and modified colour density a month after the commercial tannins were added could be due to polymerisation and precipitation of the colour in the EM wine. The lower colour density could also be the result of a higher concentration of xanthylum salts. The higher concentrations of dimers (B<sub>1</sub> and B<sub>2</sub>),

catechin and epicatechin (as can be seen in the HPLC results; Appendix B, Tables 7.4 and 7.5) are responsible for the formation of xanthylium salts (Dallas *et al.*, 1996; Malien-Aubert *et al.*, 2002). This increase in xanthylium salts, which have an orange/brown colour, in the EM wines, will also explain their higher hue values.

The lower SO<sub>2</sub> resistant pigment values in the NM treatments, compared to the control (not significant), could result from polymerisation due to acetaldehyde mediated association and precipitation, which also coincides with the lower colour densities of this wines.

Differences in the total anthocyanin content of the wines were probably due to different levels of polymerisation of the anthocyanins (Gonzalez-Neves *et al.*, 2004; Monagas *et al.*, 2006; Saucier *et al.*, 2004) as indicated by the higher fraction of colour due to polymeric pigments and polymeric pigment according to HPLC analysis. This will also explain the higher fraction of colour due to polymeric pigments of the NM control wine.

The higher gelatine index of the NM wines was due to more reactive tannins towards proteins that were present in these wines. The tannins were probably less polymerised in the NM wines than in the EM wines leading to this difference (Ribéreau-Gayon *et al.*, 1998). This corresponded with the higher dimer (B<sub>1</sub> and B<sub>2</sub>) and lower polymeric phenol concentrations in the EM wines. Extended skin maceration also leads to the extraction of polysaccharides from the skins, which can lower the astringency of red wine due to association with polyphenols (Ribéreau-Gayon *et al.*, 1998).

The commercial tannins that were added to the wines increased the non-flavonoid concentrations. This increase is probably due to benzoic and gallic acids present in the commercial tannins. The higher flavan-3-ol concentrations of the Biotan treatments in both the NM and EM wines are as a result of the fact that Biotan is a condensed tannin, which is extracted from grape seeds. The higher anthocyanin concentration of the treatments in the NM wines compared to the control is possibly due to enhanced protection of the anthocyanins by the added tannins. This also explains the lower polymeric pigment concentration of the treatments. Promotion of tannin-tannin polymerisation and subsequent precipitation could lower account for the lower polymeric phenol concentrations compared to that of the control for the NM wines.

#### **4.4.2 TANNIN ADDITIONS IN COMBINATION WITH DIFFERENT OXYGEN LEVELS**

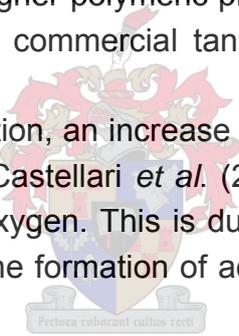
None of the tannin additions enhanced the colour density of the treatments where no oxygen was added when compared to the control, with all the hue values of the treatments higher than the control. This could possibly be due to polymerisation and precipitation of the anthocyanins with the tannins that were added (Atanasova *et al.*, 2002; Gómez-Plaza *et al.*, 2004; Somers 1971; Somers and Evans, 1979). The modified colour density of the treatments was higher than their controls in the 0% and 80% oxygen level, which indicates that more of the anthocyanins of the treatments were bleached by sulphur dioxide present in the wine. At the 80% oxygen level the Oenotan 25 mg/L addition seems to be the best

treatment for this specific wine regarding colour density. Oenotan was probably able to protect the colour by being oxidised itself, rather than oxidising other grape phenols present in the wine due to more hydroxyl functions present in hydrolysable tannins (Vivas and Glories, 1996). Over time these differences diminished and no differences could be seen after five months.

In both the 0% and 80% oxygen additions the Tanenol Rich 100 mg/L treatment exhibited a higher fraction of colour due to copigmentation and a lower fraction of colour due to free anthocyanins when compared to their respective controls. This could be due to more effective polymerisation in these treatments as a result of more acetaldehyde produced by this hydrolysable tannin (Castellari *et al.*, 2000; Vivas and Glories, 1996). Over time these differences also diminished.

This increase in non-flavonoid concentrations could also be seen in the wines that received different maceration times. There were no differences between the cinnamic acid concentrations of the treatments. These results are in agreement with results obtained from the extended post fermentation maceration and no post fermentation maceration experiment. The lower flavonoid concentrations of the treatments are possibly due to improved polymerisation of the monomers where the tannins were added. This increase in polymerisation can be seen in the higher polymeric phenol concentrations for both the 0% and 80% oxygen treatments where commercial tannins were added compared to their respective controls.

With an increase in oxygen addition, an increase in colour density and sulphur dioxide resistant pigments were observed. Castellari *et al.* (2000) reported the same increase in colour density with the addition of oxygen. This is due to enhanced polymerisation of the colour fraction of the wines due to the formation of acetaldehyde (Atanasova *et al.*, 2002; Castellari *et al.*, 2000).



#### 4.4.3 COMPOSITION OF COMMERCIAL TANNINS

Commercial tannins can be extracted from grapes (condensed tannins), nutgalls (gallotannins and oak (ellagitannins)). The tannins are recognised as grape derived when the total flavonoid concentration is higher than 50 mg/g, nutgall derived when the digallic acid content is between 4 to 8 mg/g and oak derived when the scopoletine content is higher than 4 µg/g (Resolution Oeno, 2002). The hydrolysable tannins had a browner colour than the mixtures of condensed tannins and hydrolysable tannins, except for tannin gallacool. The condensed tannin, Biotan also has very little brown colour. The two gallotannins, QCTN and Tannin Galacool had the highest total phenol content and Folin-Ciocalteu value. The condensed tannin value of the condensed tannin (Biotan) is the highest, while the two hydrolysable tannins have the lowest value. This confirms the suppliers' description of the tannins, except for QCTN that contained fairly high concentrations of both condensed tannin and hydrolysable tannin. The concentrations of the condensed and hydrolysable tannins indicates that there are other compounds present

in the tannin powder and that the actual amount of condensed or hydrolysable tannins added to the wine could be very low. The properties of the commercial tannins also depend on the composition of the tannin, the extraction method used and the origin of the tannin (Zoecklein, 2005).

#### 4.5 CONCLUSION

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The commercial tannin additions did not affect most of the colour characteristics of the NM and EM wines. The differences between the NM and EM wines decreased with the addition of commercial tannins. The effect of these additions should, however, still be monitored on different cultivars and during maturation.

The addition of commercial tannins to wines that underwent oxidation is speculated to protect and stabilise the phenolic compounds present in the wine. In our experiment it was clear that the effect on the phenolic composition of the oxidation itself was much larger than that of the added tannin. Thus the question arises whether the protection and stabilisation that the added tannins gave to the wine were enough to not lower the quality of the wine.

The commercial tannins that are used in the wine industry differ in composition due to different origins and extraction methods used. These differences will result in differences in the wine composition. The different commercial tannins used in the wine industry should be analysed thoroughly to determine their exact composition and the effect that the unknown components added can have on the wine quality.

#### 4.6 ACKNOWLEDGEMENTS

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# Chapter 5

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## **GENERAL DISCUSSION AND CONCLUSION**

## CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

### 5.1 CONCLUDING REMARKS AND OTHER PERSPECTIVES

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Wine making are not only an art form, but also a complicated science. The end product is influenced by different biochemical, chemical and physical processes working together to form one of the most complex beverages known to humankind. Wine consists of a wide spectrum of flavours, aromas and other organoleptic experiences. Phenolic compounds are mostly responsible for the reactions that take place in wine and the resulting complexity of the wine (Pretorius, 2000; Zoecklein *et al.*, 1995).

Producing larger quantities of less expensive wine, without lowering the quality, is one of the largest challenges facing the modern winemaker today. New technologies in the wine industry can help the winemaker with this challenge, if proper research is done to evaluate it. One of the new technologies used today, solely to reduce the production costs of wine, is the use of alternative wood maturation products. These alternative wood maturation products include: chips, staves, wood extract, wood powder and finally the addition of commercial tannins. The addition of commercial tannins are not only used as an alternative to wood maturation, but can also be added to help control the winemaking process and modify the wine's composition (Zoecklein, 2005).

Phenols and more specific condensed tannins are mostly responsible for the organoleptic properties of red wine (Glories, 1988; Ricardo-da-Silva *et al.*, 1993; Vidal *et al.*, 2004). They take part in numerous reactions that change the composition and structure of the resulting wine. Hydrolysable tannins can only be added to wine by the extraction of hydrolysable tannins during oak maturation, or by the addition of commercial tannins. Ellagitannins are the only hydrolysable tannin that can be extracted from oak, while gallotannins can be added in the form of commercial tannin additions (Quinn & Singleton, 1985). Hydrolysable tannins probably do not have any direct sensory effect on a wine, but contribute to some reactions present in the wine (Pocock *et al.*, 1994; Puech *et al.*, 1999). Examples of these reactions are: protecting the wine against oxidation, formation of acetaldehyde and promoting indirect polymerisation of condensed tannins (Vivas & Glories, 1996).

The effect of the addition of different commercial tannins and a pectolytic enzyme at different dosages on the phenolic composition of red wine was monitored during the alcoholic fermentation for the 2004 season. This was conducted on three different cultivars (Merlot, Shiraz and Cabernet Sauvignon) of different areas. The 2004 Shiraz was also monitored for up to a year of maturation. During the 2005 season the same commercial tannins and pectolytic enzyme and dosages were used on the same Merlot and Shiraz grapes. These wines were monitored after alcoholic fermentation up to the end of malolactic fermentation. Differences in the phenolic composition between the control and

treatments were observed at the middle and end of alcoholic fermentation, but after malolactic fermentation most of these differences diminished. One of the only differences that could still be seen after a year of maturation was in the total phenol concentrations, where it was mostly the higher dosages that had higher values than the control. The 2004 Cabernet Sauvignon was highly infected with *Botrytis cinerea*. This cultivar showed the most significant differences after alcoholic fermentation, but was not monitored further. The enzyme tested showed enhanced total phenols, sulphur dioxide resistant pigments, modified colour density, colour density and total anthocyanin concentrations for the 2004 Shiraz. This indicates that depending on the composition of the grapes, the addition of enzymes can give similar or even better results than that of the tannin additions in terms of certain phenolic compositions. Differences between the treatments were also sensorially detected after 20 months. The commercial tannin additions were seen as positive in some cases and negative in other.

The effect of different commercial tannin additions were also monitored on two red wines made from the same grapes, which received different maceration times. The addition of the tannins did not significantly change the composition of the colour of the two wines, but when the two wines were compared the differences between the controls were decreased by the addition of certain commercial tannins.

The effect of commercial tannin additions at different dosages were also monitored on a wine where different amounts of oxygen were added. The addition of tannins did influence the phenolic compounds to a certain extent, but the effect of the oxygen was much more prominent.

When the compositions of some of the different commercial tannins that are available on the market were investigated, large differences were observed in colour and phenolic composition. These differences are probably due to different sources of the tannins, different extraction methods used, differences in purity of the tannin and differences in the degree of oxidation of the tannins.

When one consider the low concentrations of condensed and/or hydrolysable tannins that are present in the commercial tannin powders, the question arises whether these amounts, their recommended dosages, could make any colour and other phenolic differences in the resulting wine. In further research higher dosages should be investigated and whether these higher dosages precipitate some of the existing phenols in the wine.

It is clear that further work needs to be done on the influence that commercial tannin additions have on wine quality. Future work should also include the determination of the effect of different commercial tannin additions on laccase activity. The experiment where the commercial tannins were added before alcoholic fermentation gave an indication that in the case of laccase activity the commercial tannins have larger influences (either positive or negative). The effect of commercial tannin additions on laccase activity and on wine in general should also be monitored in the presence of micro-oxygenation and over a more extended period of time. The stability of commercial hydrolysable tannins in a water alcohol medium should be determined. Another interesting aspect will be to investigate

whether commercial tannins are able to associate and stabilise anthocyanins in the wine. A detailed study should also be conducted on the composition of the commercial tannins that are available on the market

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