The influence of different barrels and oak derived products on the colour evolution and quality of red wines

by

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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.

B.A. Fourie

Date
Winemakers or producers are under constant pressure to reduce the operational costs for the production of wine. The reason for this being threefold: Firstly, the number of cellars and estates locally, and internationally, has grown immensely. Thus, the competition on both the national and international front has put producers under pressure to reduce their production cost in order to survive the explosion of brand possibilities on the consumer’s horizons. Secondly, the exchange rate has been one of the most unpredictable variables which highlight the importance of scenario planning and sufficient financial planning in order to overcome significant exchange rate fluctuations which may cause financial strain for the business. Thirdly, the benefits of using expensive oak barrels for the maturation of premium quality wines are undisputable. These benefits include the clarification and decarbonation of wines, decrease in the astringency of tannins ("softening of tannins"), the evolution of colour, providing complementary oak aromas which increase the complexity and finesse of these wines and stabilising the colloid structure of the maturing wine. The greatest disadvantages remain nevertheless that it is a costly and labour intensive operation. Oak is an essential part of the flavour profile of many premium quality wines, and there is still a growing market demand for these wines. The use of oak barrels for the maturation of premium red wines, incorporates a specific flavour dimension to the wine (and adding value), has been the traditional way of storing wines. Well known cellars and Estates all over the world have build remarkable profiles and identities for their brands. To alter any principles regarding the use of oak will alter the style of the wines and will beyond any doubt influence their brand image negatively.

Chapter 1 gives an introduction and the main aims of the study. The phenolic composition of grapes and oak are a complex mixture of different molecules which are discussed in Chapter 2. These include those responsible for the colour (anthocyanins), taste (mainly the tannins) and aroma of the wine. The composition of the oak barrel can be influenced by its origin, seasoning of the oak, toasting of the staves, age of the barrel etc. These factors all affect the chemical composition of the oak (lactones, volatile phenols, carbohydrate volatiles, hydrolysable tannins etc.) and ultimately, the wine. The chemical reactions taking place in wine during ageing are also complex, which makes maturation and research of wine in oak a complex business.

The challenge, thus, developed to sustain the flavour profiles (oak attributes) and to reduce the operational costs, without influencing the identity or profile of the brand negatively. The use of oak derived products like chips,
dust, blocks, staves, balls and even extracts was proposed, although it is considered by-products of the cooperage profession. Some of the major disadvantages of the use of oak derived products still remain the limited control over the production process. This may lead to inconsistency in the quality of these products. Nevertheless, the use of oak derived products and the availability of these products have grown significantly and with it a need for research on this field.

Chapter 3 address the results obtained from the maturation of Pinotage, which is uniquely South African, and Shiraz wines, in different oak type barrels (French vs. American vs. Russian), as well as treatments with various oak derived products, and the influence of these oak products on the evolution of colour, phenolic development and quality of the wines during ageing.

The results obtained in this project correlated with findings in the literature. The colour density, percentage of red pigment colour and SO₂ resistant pigments increased, especially in the first three to six months during ageing in barrels as a result of ellagic tannin extraction from the barrels and polymerisation reactions, but decreased later as maturation progresses as a result of precipitation. In some cases the use of oak increased the colour density significantly with almost 40%. The colour density, total red pigment colour and total phenolic composition decreased during bottle maturation as a result of polymerisation. No significant colour differences between the barrel types could, however, be detected. The use of oak derived products showed the same tendencies for all colour dimensions, although no significant differences could be detected between the treatments. The most significant difference was that new oak barrels contributed more to the development of colour compared to the oak treatments.

The Gelatine index gives an indication of the degree of polymerisation of tannins; higher values will indicate tannins which are highly reactive and will cause an astringent mouth feel. The values should decrease as maturation progresses and tannins polymerise. Little differences between barrel types (French vs. American vs. Russian) were found in the one Shiraz, but in the two Pintage wines the index was the lowest in the wines matured in French oak after 36 months bottle ageing.

Sensorial tests indicated little quality differences after 36 months between the different oak types. The American oak barrels had, in general, a more "oaky bouquet", even after 36 months in the bottle. Pinotage matured with different alternative oak products showed that chips added to barrels tended to give a higher oak aroma, with a lower fruit compared to the other treatments. The same wine matured in new barrels, as expected, also had a very high oak aroma compared to the same wine matured in older barrels. This led to a higher fruit perception in the latter wines.
Lees contact is a practice more commonly used in the production of white wines, but may be a dangerous practice in the production of red wines. Chapter 4 reports on the effect of lees contact during ageing of red wine on the micro flora and colour after 12 month maturation. No significant differences in microbial analysis were detected during of barrel maturation between the wines matured in the lees and the filtered wines for acetic acid, yeast and lactic acid bacteria. The initial pH differences during cold maceration and fermentation, however, did influence these numbers, with higher numbers being observed at higher pH values. However, colour differences could be detected. The concentration of yellow brown pigments (measured at 420 nm) was lower in the lees matured wines than in the filtered wines which underwent malolactic fermentation in the barrel. Other colour differences that could be detected were higher colour density and total red pigment colour in wines as a result of longer skin contact (due to slower alcoholic fermentation rate).

Thus, manipulation of the skins during alcoholic fermentation, the use of barrels and/or oak derived products in various stages of vinification and maturation of wines and additional practices as maturation on lees may be thus be used to set a certain style of wine which meet a specific market demand, trend or style. Chapter 5 summarises the results obtained and future research to be done on wood and wine. This study, however, clearly showed that the origin and oak type does not influence the colour as much as the aroma and taste of red wine under South African conditions.
OPSOMMING

Wynmakers of produsente word onder gedurige druk geplaas om die produksiekoste van wyn te verlaag. Die redes hiervoor is drieledig: Eerstens het die getal kelders en landgoedere op nasionale en internasionale vlak geweldig toegeneem. Dus, die kompetisie op beide nasionale en internasionale gebied plaas produsente onder druk om die produksie koste van die wyn te verminder om sodoende finansiële die ontploffing van nuwe moontlike wyne op die horizon van die verbruiker te oorleef. Tweedens, die wisselkoers was altyd een van die meer onvoorspelbare veranderlikes wat die belangrikheid van scenario-beplanning en weldeurdagte finansiële beplanning beklemttoon om sodoende groot veranderinge in die wisselkoers, wat moontlike finansiële druk in die besigheid mag veroorsaak, te oorkom. Derdens, een van die grootste uitgawes in die produksie proses van is die verouderingskomponent van kwaliteitswyn.

Die voordele van die gebruik van eikehout vate in die verouderingsproses van rooiwyne is welbekend. Hierdie voordele sluit in die suiwering van die wyn in terme van soliede partikels, dekarbonisering (CO₂-ontgasting), afname in die vrank smaak (“sagtheid van die tanniene”), die ontwikkeling van die kleur van die wyn, toevoeging van complementêre hout boeketstowwe wat bydrae tot die kompleksiteit van die wyn, asook die stabilisering van die kolloïdale struktuur van die wyn.

Die grootste nadeel bly, onteenstaande, die geweldige koste verbonde aan vate, asook die hantering en onderhoud daarvan. Eike hout verwante boeketstowwe is ’n onmisbare komponent van die aroma profiel van baie kwaliteitswyne. Daar is ’n groeiende mark vir hierdie tipe wyne. Die gebruik van eikehout vate vir die veroudering van kwaliteitswyne, behalwe die bydrae van belangrike geurstowwe (een waarde), was nog altyd ’n tradisionele stap in die proses. Beroemde kelders reg oor die wêreld het uitmuntende mark identiteite vir hulle self geskep, asook ’n “spesifieke identiteit” vir hulle wyn. Om enige beginsels aangaande die gebruik van eikehout te verander, sal sonder twyfel die wynstil verander en moontlik ’n negatiewe effek op die kelder se identiteit hê.

Hoofstuk 1 gee ’n inleiding en die hoof doelstellings van die studie. Die fenoliese samestelling van druiwe en eikehout bestaan uit ’n komplekse mengsel van verskillende molekules wat in Hoofstuk 2 beskryf word. Hierdie sluit in daardie verantwoordelik vir die kleur (antosianiene), die smaak (hoofsaaklik tanniene) en die aroma van die wyn. Die samestelling van die eikevat kan beinvloed deur die oorsprong, veroudering van die hout, roostering, ouderdom van die vat ens. Hierdie faktore beinvloed almal die samestelling van die houtvat (laktone, vlugtige fenole, hidroliseerbare...
tanniene ens.) en uiteindelik, die wyn. Die chemiese reaksies wat plaasvind tydens die veroudering van wyn is ook kompleks, wat die veroudering en navorsing van wyn in vate 'n komplekse proses kan maak. Die uitdaging, dus, het ontstaan om die organoleptiese profiel van die wyn (eikehout boeketstowwe) te behou en terselfdertyd die produksiekoste van die wyn te verlaag. Die gebruik van eikehout verwante produkte soos skaafsels, blokkies, stawe en balke en selfs eikehout ekstrakte is aanbeveel, alhoewel dit gesien word as afvalprodukte van die kuiperye. Een van die grootste nadele van eikehout alternatiewe produkte is egter die beperkte kwaliteitskontrole oor die produksieproses. Dit mag lei tot wisselvalligheid in die kwaliteit van hierdie produkte. Nieteenstaande het die gebruik van eikehout verwante produktes en die beskikbaarheid van hierdie produktes geweldig toegeneem en tesame daarmee die behoefte aan navorsing hieroor.

Hoofstuk 3 bespreek resultate van die gebruik van verskillende eikehout tipe vate (Frans vs Russies vs Amerikaans), asook die gebruik van eikehout verwante produktes, op die kleur ontwikkeling, fenoliiese samestelling en kwaliteit van Pinotage (eie aan Suid-Afrika) en Shiraz wyne gedurende vat en bottelveroudering.

Die ontwikkeling van kleur het verskeie unieke dimensies en resultate wat verkry is in hierdie projek stem ooreen met literatuur. Die kleurdigtheid, persentasie rooi pigment en SO₂ weerstandbiedende pigmentte het toegeneem met veroudering in houtvate (veral in die eerste drie tot ses maande) as gevolg van ellagitanniene wat geëxtraaer word en gepolimerisasie, maar het later gedurende veroudering afgegeneem as gevolg van presipitasie reaksies. In sekere gevalle het die kontak met hout die kleurdigtheid met tot 40% vermeerder. Die kleurdigheid, totale rooi pigmentte en totale fenole het tydens bottelveroudering afgegeneem a.g.v. polimerisasie. Geen merkwaardige verskille kon egter tussen die verskillende vat tipes gevind word nie. Verskille tussen die houtverwante produktes was ook klein en het diezelfde tendense getoon as die vate. Nuwe vate se impak op die kleurprofiel en ontwikkeling van verouderde rooiwyne was egter meer beduidenswaardig as die gebruik van gebruikte vate en behandelings met eikehout verwante produktes.

Die gelatien indeks verwys na die toestand van polimerisasie van tanniene. Hoër waardes kan verwag word in jong wyne en dui op hoog reaktiewe tanniene. Die waarde sal afneem soos tanniene polimere vorm met veroudering en sal dus 'n veel minder vrank mondgevoel tot gevolg hê. Klein verskille tussen die vat tipes (Frans vs Amerikaans vs Russies) is opgemerk in die een Shiraz, maar in die twee Pinotage wyne was die indeks die laagste in die wyne verouder in Franse eikehout na 36 maande se bottelveroudering.
Sensoriese toetses het aangedui dat daar klein verskille gevind is tussen die verskillende eiketipes. Die wyn verouder in Amerikaanse eik het, in die algemeen, 'n sterker eikehoutkarakter gehad na 36 maande bottelveroudering. Wyn verouder met verskillende alternatiewe houtprodukte het aangetoon dat eikehouskaafels in vate 'n sterker eikehoutkarakter aan die wyn gegee het met 'n laer vrugtige aroma in vergelyking met die ander behandelings. Dieselfde wyn verouder in nuwe vate het ook, soos verwag, 'n sterk hout boeket gehad in vergelyking met dié verouder in ouer vate. Dit het dan ook geleë dat laasgenoemde wyne 'n sterker vrug aroma gehad het.

Veroudering van wit wyne op moer is 'n algemene praktyk, alhoewel dit minder toegespits word op rooiwyne as gevolg van verwante gevare. Hoofstuk 4 bespreek die effek van moerkontak op die mikrobiese en kleursamestelling van rooiwyn tydens 12 maande se houtveroudering. Geen noemenswaardige verskille is egter verkry tussen die wyne verouder op die moer en die filtreerde wyne vir asynsuur-, melksuurbaterieë en gisgetalle nie. Aanvanklike pH verskille tydens koue masserasie en alkoholiee fermentasie het egter hierdie getalle beinvloed, met hoër getalle verkry in die hoër pH wyn. Die wyne wat appelmelksuurgisting in die vate ondergaan het en verouder is op die moer het egter 'n laer konsentrasie geelbruin pigmente (gemeet by 420 nm) as die gefiltereerde wyne. Ander kleurverskille sluit in hoër kleurdigtheid en totale rooi pigmente as gevolg van langer dopkontak (stadiger alkoholiee fermentasie).

Manipulering van die doppe voor en tydens alkoholiee gisting, die gebruik van eikehoutvate of eikehoutverwante produkte, of kombinasies daarvan tydens verskillende produkcie fases en ander praktyke soos moer kontak, kan dus gebruik word om 'n sekere styl wyn op markversoek daar te stel. Hoofstuk 5 som die resultate op, asook toekomstige navorsingsmoontlikhede wat op die gebied van hout en wyn gedoen kan word. Hierdie studie het egter duidelik aangedui dat die eikehout oorsprong en tipe die kleur nie so baie beinvloed soos die aroma en smaak van die wyn onder Suid-Afrikaanse kondisies nie.
THIS THESIS IS DEDICATED TO MY FAMILY, ESPECIALLY MY PARENTS SAKKIE AND SANTA, MY WIFE CORLEA AND DAUGHTER NELLEKE, FOR THEIR SUPPORT.
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THE WORLD’S GREATEST WINEMAKER, for bunches of blessings, opportunities and for letting me be a winemaker...
This thesis is presented as a compilation of four chapters. Each chapter is introduced separately and Chapter 3 will be submitted for publication.

**Chapter 1**  General Introduction and Project Aims

**Chapter 2**  LITERATURE REVIEW
Grape and Wine Phenolics: Complex pigments and reactions that influence the quality and complexity of wines.

**Chapter 3**  Research Results
The influence of different barrel origins (French oak, American oak and Russian oak) and oak derived products on the colour and phenolic evolution and on the organoleptic profile and quality of Pinotage and Shiraz wines in South-Africa.

**Chapter 4**  Research Results
The influence of lees contact and pH on the development of micro flora and colour of wine during conservation in barrels.

**Chapter 5**  General Discussion and Conclusions
Chapter 1

General Introduction and Project Aims
1. GENERAL INTRODUCTION AND PROJECT AIMS

1.1 INTRODUCTION

The technique of maturing wine in barrels made from wood was introduced at the beginning of the 18th century. The cooperage profession has used different species of wood, such as chestnut, beech wood, acacia, ash and oak for vinification, transportation, ageing and storage of wine (Vivas, 1995).

The main principles in the cooperage of barrels used for the conservation of wine have hardly changed, but knowledge obtained through trial and error has undoubtedly enabled us to use it to better advantage and to avoid serious setbacks. Today the quality of oak wood is accepted and only the species susceptible to improving the quality of wine are being used.

Although the demand for oak barrels is widely recognized, it remains nevertheless true that making wines in this fashion is an expensive operation and only a proportion of the wine, rarely 100%, can benefit from maturation in new oak. The challenge facing producers of fine wines is thus to decrease production costs, while maintaining organoleptic quality and preserving consistency in the product. Although already used especially in New World countries, the effects of addition of different oak elaborated products have not yet been studied intensively. Although some researchers, especially in the New World countries, proofed beneficial effects on wine, some winemakers were unable to retrieve the fineness of flavor given by a new oak barrel.

Nevertheless incorporating oak in the maturation of fine wines is not an end in itself. It is merely a process through which the wine acquires the ability to age in the bottle or to set a specific style. The aim of using oak barrels and oak elaborated products is to flavor and to provoke a number of chemical and physical changes which are indispensable for the final product. Firstly the wine acquires clarity and stability during the maturation phase. Any solids, derived from the physical processes of alcoholic fermentation, malolactic fermentation and finings, chemical processes of hydrolysis and polymerization and tartaric precipitations (of the colouring matter) and microbiological matter for example dead yeast cells and bacteria, descends to the bottom under the force of gravity (Pontallier, 1992). Secondly the bouquet of the wine develops and becomes more refined and complex as the wood releases into the wine specific substances or toasting derived flavours (Sefton et al., 1990; Sefton 1991; Sefton et al., 1993). Thirdly oak barrels (and to a lesser extent the addition of staves, chips and blocks to wine in used barrels or stainless steel tanks), ensures the effect of micro-oxygenation estimated at 30-40mg O₂ per year in new oak barrels (Pontallier, 1992). The oxygenation process stimulates oxidation of anthocyanin and tannin molecules, which make up the colour matter in red wine and can lead to precipitation under the effect of continued oxidation.

Maturation of red wines in barrels results in a spectrum of phenolic transformations of which some can be organoleptically detected. An increase in the colouring intensity occurs, the hue evolves (the colour of red wine takes on ruby
nuances), stabilization of colour takes place (i.e. tannin/anthocyanin pigments formed during polymerization reactions are more stable than the anthocyanin molecules which alone are responsible for the colour in young red wine), precipitation of large tannin molecules and a decrease in astringency and hardness takes place (Somers, 1971; Somers and Evans, 1974; Somers 1978; Somers and Evans, 1986; Somers, 1990).

Many cooperage related processes and vinification techniques remain non-specific, such as phenolic evolution during the transversion of grapes to wine and during the conservation phase of wine in barrels. Although many studies have been conducted on the cooperage profession, the chemistry regarding phenolics remains largely unresolved. However, through the use of oenological principles and practices one can reduce the level of unpredictability in order to produce a product of high quality at affordable operation costs.

Ageing or maturation of red wines in wooden barrels is a normal practice during production and is essential for the production of premium quality wines. Export of South African wines has increased dramatically since 1993, the number of producers has increased significantly and the production of grapes and fine wines has increased substantially. Marketing trends indicate a growing demand for red wines world-wide and 52 million liters (45%) of the 113 million liters of natural wine exported in 1998 was red, compared to 98 million of the 266 million litres in 2004 (SAWIS, 2005).

Premium quality red wines have to be matured for at least 8 to 18 months in oak barrels. The cost of a new barrel is an important factor in the overall production costs of wine. A new 225L French oak barrel costs approximately R 6 500-00. The barrel may be used for 1-2 years before the wood extractives become too low to have a significant contribution towards wine quality. Oak maturation contributes R 16-00 to R 20-00/ liter (maturation overhead costs included) to the production costs of red wines. The South African wine industry spent R89 million during 1997 on the import
of cooperage related products (Figure 1.1). This value increased significantly to R189 million in 2003. All the barrels are imported from Europe, mostly from France and this means a significant loss in foreign exchange. Winemakers have a choice of wood of French, Russian or American origin, besides which they have to indicate their choice of toasting level for each barrel as well as several other factors. Various studies on the influence of wood on wine composition have been reported in the literature (Singleton, 1974; 1994). South African winemakers have used this knowledge in their methods of red wine production. During the last few years, especially with the increase in demand for wooded red wines, it has become evident that South African wine producers need scientific assessment of the influence of wood and cooperage related products for their unique situation. In this thesis the effects of different species of oak and oak elaborated products in the development of red wines are being discussed.

1.2 PROJECT AIMS

The conditions during maturation in South Africa differ from those in other wine producing countries. The cultivar Pinotage (which was crossed in S.A.) is extensively being cultivated in SA. Pinotage is becoming more important as a "niche" product for export. There is also pressure on winemakers to reduce the processing cost of wine; therefore the use of oak derived products such as oak blocks, chips, staves, balls etcetera has increased.

The specific aims of the research conducted thus were the following:

I. To evaluate the influence of different oak barrel types (French, American and Russian oak) on the colour and phenolic development during maturation of Pinotage and Shiraz from different regions within the Western Cape.
II. To evaluate the influence of oak and oak elaborated products on the colour and phenolic development of Pinotage.
III. To assess the quality of these wines by sensory evaluation.
1.3 LITERATURE CITED


2. LITERATURE REVIEW

2.1 INTRODUCTION

Numerous parameters influence the composition of the end product, but most important is the influence of the phenolic composition of the grapes (Macheix et al., 1991). The study of phenolics is truly one of the most complex sciences in the world. The years 1950-1990 found numerous scientists to start treading the maize of phenolic chemistry. Important research was done several decades ago which formed the basis of phenolic chemistry in plants.

Louis Pasteur (whose father was incidentally a farmer) said the following: "We are like dwarfs on the shoulders of giants, so that we can see more than they... not by virtue of any sharpness of sight on our part, or any physical distinction, but because we are carried high and rose up by their giant size."

These substances are known as phenolics and they are complex, their identification and quantification difficult and some are even not resolved (Boweyer, 2002). It is known however that phenolic substances are major wine constituents that are responsible for wine's colour and astringency. Wine's contribution in a healthy diet has attracted a lot of attention and research is currently been done regarding the "French paradox", that is the antioxidative capacity of red wine and their influence towards cardiovascular benefits. The phenolic composition of a wine is being influenced principally by four sets of parameters: the phenolic compounds in the grapes from which the wine is made, the influence of processing the grapes, juice extraction and different vinification techniques, the influence of oak barrels and/or oak derived products during and after fermentation and maturation, and finally a spectrum of physical and chemical reactions that take place during the maturation phase and that are influenced by the immediate environment of the wine and its characteristics (Macheix et al., 1991).

It can be said that fine wines are the result of total phenolic management from the vineyard to the bottle.

2.2 PHENOLIC COMPOSITION OF GRAPES AND WINE

2.2.1 Introduction

Wine contains a diverse selection of phenolic compounds derived from the basic structure of the phenol known as a hydroxybenzene. Total phenols are usually represented in arbitrary units of a phenolic standard, e.g. amount of gallic acid to produce the same analytical response (gallic acid equivalents or GAE). The phenolic content of red grapes is on average 5500 mg GAE/kg (Singleton and Esau, 1988) and is distributed in different quantities throughout the berry. The total amount of phenols range from approximately 1 to 5 g/L depending on the style of the wine ("light" versus "full bodied").
Flavanol concentrations fluctuate from 30 to 100 mg/L, with benzoic and cinnamic acid concentrations contributing from 100 to 200 mg/L in red wine (Price et al., 1995). The anthocyanin content alone can vary in younger wines from 100 mg/L in Pinot Noir to 1500 mg/L in varieties such as Shiraz and Cabernet Sauvignon (Somers, 1967; Singleton and Noble, 1976; Nagel, 1979). Phenolic compounds up to 300 mg GAE/L can be extracted from barrels into wine after one year of maturation. The anthocyanin content of red wines can drop to 50 mg/L or less during ageing due to polymerization and precipitation reactions.

The phenol composition of wines can differ considerably and is influenced by a number of variables. Cultivar variations however, are much greater than the influence of terroir or vintage (Bakker et al., 1986). Different species of the genus Vitis do not contain the same anthocyanins and corresponding characteristics reflected in the wines (Table 2.1). Thus, it is easy to differentiate between wines produced from Vitis vinifera and wines from hybrids (Ribereau-Gayon, 1974). Among the red grape vines, certain are able to synthesize anthocyanins in the diglucoside form and others in diglucoside and monoglucoside form (Ribereau-Gayon, 1974).

The phenol content are also being influenced by soil type, rootstock, canopy management techniques, state of maturity and state of health at harvest (Singleton and Noble, 1976).

The phenol content of wine is an indication of the phenol content of the grapes, although traditional oenological practises lead to a maximal extraction of up to 60%. Vinification practises influence the phenol contents of wines and have been the subject of much investigation (Timberlake et al., 1976). In this review, the phenol compounds of wine are discussed, as well as those originating from the grapes.

### 2.2.2 Non-flavanoid phenols

The non-flavanoid components, which are derivatives of hydroxycinnamic and hydroxybenzoic acids, arise principally from juice extraction and from the exposure of the wine to oak (Figure 2.1). It was concluded that this group of phenols occurs primarily in the juice and not in the solid parts of the berry and therefore are present in the wine to about equal extent (Ribéreau-Gayon, 1974). The tartaric esters of caffeic, p-coumaric and furanic acid, are the most prominent hydroxycinnamates in grapes.
Table 2.1 Distribution of anthocyanins among the different species of the Genus *Vitis*. Data are percentages of each pigment among total anthocyanin content. *Vitis vinefera* a = Muscat Hambourg and b = all other varieties studied. The difference in structure of 1 and 2 has not been explained yet. (Ribéreau-Gayon, 1959)

<table>
<thead>
<tr>
<th>Anthocyanin pigments</th>
<th>Spot no. (Fig.1)</th>
<th>rotundifolia</th>
<th>riparia</th>
<th>rupestris</th>
<th>Labrusca</th>
<th>Arizonica</th>
<th>Berlandieri</th>
<th>monticola</th>
<th>cordifolia</th>
<th>rubra</th>
<th>Lincecumii</th>
<th>aestivalis</th>
<th>coriacea</th>
<th>amurenensis</th>
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<td>Cyanidin</td>
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<td>Acylated monoglucoside</td>
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</tr>
</tbody>
</table>
Benzoic acid (a) | R₂ | R₃ | R₄ | R₅ | Cinnamic acid (b)
--- | --- | --- | --- | --- | ---
p-Hydroxybenzoic acid | H | H | OH | H | p-Coumaric acids
Protocatechuic acid | H | OH | OH | H | Caffeic acid
Vanillic acid | H | OCH₃ | OH | H | Ferulic acid
Gallic acid | H | OH | OH | OH |
Syringic acid | H | OCH₃ | OH | OCH₃ | Sinapic acid
Syllicyclic acid | OH | H | H | H |
Gentisic acid | OH | H | H | OH |

**Figure 2.1** Phenolic acids in grapes and wines (Ribereau-Gayon, 1974).

Numerous of these compounds are esterified to sugars, organic acids and alcohols. Hydroxycinnamic acyl groups, the hydrolysis products of anthocyanins, are the major source of non-flavanoid phenols (Singleton and Noble, 1976). Hydroxycinnamate derivatives comprise the majority of non-flavanoid phenols and are present in juice and wine as free acids and ethyl esters in the state of tartrate or tartrate-glucose esters. Post harvest hydrolysis, especially by pectin esterase, frees at least part of the hydroxycinnamates of grapes from their tartrate portion. The hydrobenzoic acids are primarily degradation products of oak wood of which gallic acid (Figure 2.1) is the most important one.

### 2.2.3 Flavanoids

Flavanoids are characterized as molecules containing two phenolic groups by a pyran (oxygen-containing) ring structure (Figure 2.2). Aglycones are the base structure of the flavanoids and consist of two aromatic rings joined through a pyran ring. Flavonoid phenols include all structures with variations in hydrogen, hydroxyl and ketone groups associated with carbons 2, 3 and 4 due to changes in the oxidation state.
The number and position of the hydroxyl- and methoxy groups on the skeleton of the structure will determine the phenolic class. Each structure can be variously substituted by glycosilation, acylation and esterification. Flavanoids exist free or in a polymerised state to sugars, other flavanoids or a combination of these. Acyl and glucoside derivatives refer to flavonoids esterified to non-flavonoids or sugars, respectively. Procyanidin polymers are being produced by the polymerisation of catechin and leucoanthocyanidin flavanoids. Polymerization of monomeric flavanoids produces dimeric and polymeric forms. Oxidative or non-oxidative polymerisation yields tannins and condensed tannins (Ribereau-Gayon et al., 2000) and can lead to precipitation under conditions of continued oxidation. Thus, the main flavanoid species important to chemical interactions, colour and the astringency of wines are the anthocyanins and the flavanols. These compounds are present in the skins, seeds and pulp of the grapes. Polymerisation and condensation reactions between members of the group, but also with non-flavanoids and sugars add to the diversity of this group.

2.2.3.1 Anthocyanins

The anthocyanins are red and blue pigments which are widely distributed in plants (Ribereau-Gayon, 1974). In certain species of *Vitis*, 5 to 17 anthocyanins have been identified, although many related anthocyanins are difficult to separate and identify. There are mainly 5 anthocyanins, also referred to as aglycanes, existing in different heterosidic forms or as anthocyanins: delphinidin, petunidin, malvinidin, cyanidin and peonidin (Figure 2.3).
Table 2.2 shows the occurrence of different anthocyanins in 15 different species of *Vitis*-grapes. It is evident that malvidin is the prominent pigment in many of the grapes varieties.

**Table 2.2** The results of the identification of the anthocyanins in the 15 species of *Vitis* (Ribéreau-Gayon, 1974).

<table>
<thead>
<tr>
<th>Glucoside</th>
<th>Aglycones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delphinidin</td>
</tr>
<tr>
<td>Diglucoside</td>
<td>1</td>
</tr>
<tr>
<td>Acylated diglucoside</td>
<td>6</td>
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<td>16</td>
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<tr>
<td>Acylated diglucoside</td>
<td>18</td>
</tr>
</tbody>
</table>

Malvidin-3-monoglycoside or oenin (Figure 2.3) is the main constituent of grape colour matter in *Vitis vinifera*, but does not represent the majority of pigments; it makes up almost 36% of the total pigment (Ribéreau-Gayon, 1974). Malvidin has also been found as esters of acetic, coumaric and caffeic acids. Other important anthocyanin derivatives include malvidin-3-O-(6-O-acetyl) glucoside and malvidin-3-O-(6-O-p-coumaroyl) glucoside (Burns *et al.*, 2003). 

Figure 2.3 Structure of anthocyanins in grapes and wine (Ribéreau-Gayon, 1959).
In the group of acylated anthocyanins, one molecule of cinnamic acid, often p-coumaric acid, is esterified with the hydroxyl group in the C6 position of a glucose molecule (Hrazdina and Franzese, 1974; Ribéreau-Gayon, 1974).

Anthocyanins occur in equilibrium in the medium and being amphotheric, their colour is primarily pH dependent as shown in Figure 2.4 (Ribéreau-Gayon et al., 2000).

![Diagram of anthocyanins in equilibrium](Stellenbosch University http://scholar.sun.ac.za)

**Figure 2.4 Anthocyanins in equilibrium** (Ribéreau-Gayon et al., 2000).

Sulphur addition can bleach the colour of anthocyanins. Carbon 4 is the site of sulphite binding, but also the reactive point were reactions with other pigments and tannin-pigment polymers occur. These complexes are more resistant to the bleaching effect of sulphur dioxide. Reactions of sulphur dioxide to the C4 position inhibits polymerisation in that the occupation of C4 makes co-polymerisation impossible between the latter and either C6 or C8 of the tannin to yield a red dimer. Polymeric pigments, however, show increased stability. The presence of acetaldehyde initiates rapid changes in colour via an acid-catalysed Bayer reaction (Singleton and Draper, 1964; Timberlake and Bridle, 1976).
The stability of the product is determined by the degree of polymerisation of the precursors and continued reaction with highly polymerised tannin may lead to precipitation. These reactions explain the increasing stability of wines matured in barrels. The formation of pH resistant polymers which involve anthocyanidins and acetaldehyde explains increased spectral colour results during maturation of red wines in barrels (Berg and Akiyoshi, 1975; Somers, 1978; Somers and Evans, 1986).

2.2.3.2 Flavanols

The primary flavanols are (+)-catechin and (-)-epicatechin and their concentration may reach up to 200 mg/L in red wines (Singleton and Esau, 1988). Different substitutions (-H or -OH) at carbons R1, R2 and R3 lead to the occurrence of four stereo isomers as shown in Figure 2.5.

<table>
<thead>
<tr>
<th>Flavanol</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
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<tr>
<td>(+)-catechin</td>
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<td>H</td>
</tr>
<tr>
<td>(+)-gallocatechin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>(-)-epicatechin</td>
<td>H</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>(-)-epigallocatechin</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
</tr>
</tbody>
</table>

Figure 2.5 Chemical structures of the flavanols (Ribéreau-Gayon, 1974).

They form dimers, trimers and various higher oligomers through interflavan C4-C6/C4-C8 linkages and are referred to as procyanidins or condensed tannins. The procyanidins consist of (epi) catechin units while prodelphinidins are based on (epi) gallocatechin-units. Some units are substituted by gallic acid on the 3-hydroxyl group. These phenols mainly occur in seeds and stalks.

The definition of tannin, according to Swain and Bate-Smith (1962) is as follows:

"It would seem more realistic to define tannins as all naturally occurring substances which have chemical and physical properties akin to those which are capable of making leather. They would be water soluble phenolic compounds, have molecular weights lying between 500 and 300, and, besides giving the usual phenolic reactions, have special properties such as the ability to precipitate alkaloids, gelatine and other proteins" (Haslam, 1998).
One can distinguish between hydrolysable tannin and condensed tannins, depending on their chemical structure. Hydrolyzable tannins are composed of a glucose molecule bound to different phenolic substances of which gallic acid and the lactone of its dimer, ellagic acid (Figure 2.6) are two. Hydrolyzable tannins are authorized by legislation to be added to wine and include commercial tannins (oak tannins). Condensed tannins are found naturally in grapes and wines and are the condensed forms of 3-flavanols and 3,4 flavandiols.

![Gallic and Ellagic Acids](image)

**Figure 2.6** Chemical structures of phenolic acids released by ellagitannins (Ribéreau-Gayon, 1974)

Dimeric procyanidins may be divided into two categories, identified by a letter of the alphabet and a number. Type-B procyanidins are dimers resulting from the condensation of two units of flavan-3-ols linked by a C4-C8 or C4-C6 bond. Type-A procyanidins are dimers that, in addition to the interflavan bond, also have an ether bond between the C5 or C7 carbons of the terminal unit and the C2 carbon of the upper units (Figure 2.7).

![Type-A Procyanidins](image)

**Figure 2.7** Type-A procyanidins have an ether bond between the C5 or C7 carbons of the terminal unit and the C2 carbon of the upper units (Vivas and Glories, 1996).
Trimeric procyanidins may also be divided into two categories: Type-C procyanidins are trimers with two interflavan bonds corresponding to those of type-B dimers. Type-D procyanidins are trimers with two interflavan bonds, one type A and one type B. Oligomeric procyanidins or condensed tannins correspond to polymers formed from three to ten flavanol units, linked by C-C bonds. An indefinite number of isomers are possible, which explains why it is so difficult to separate these molecules.

Catechin, epicatechin, epicatechin gallate and epigallocatechin are the major constitutive units of grape skin tannins (Souquet et al., 1996). Gallocatechin and epigallocatechin gallates have also been detected. The average degree of polymerization of the grape skin tannins is about 30 units with 15% galloylated and 30% being prodelphinidins (Moutounet, 1996), whereas in grape seeds galloylation varied from 13 to 30% as the mean degree of polymerization (mDP) increased from approximately 2 to 16 (Prieur et al., 1994). In summary, skin tannins differ from seed tannins by their lower amounts of galloylated derivatives and higher molecular weights. In young red wines, the procyanidins are primarily in the dimeric or trimeric form, whereas in older wines, polymers of eight to ten or more units are found (Ribéreau-Gayon, 1974). Authors noted the changes in condensation state during ageing by examining the average molecular weights of the tannins: 500-700 for young wines and 200-3000 for older wines (Ribéreau-Gayon, 1974).

Hathway and Seakins (1957) postulated another possibility for the condensation of flavan molecules. They noted that the oxidation of catechin by oxygen in the air or by polyphenoloxidase leads to a polymer of quinoidal structure, establishing a bond between C6 of the one molecule and C6 or C8 of the other possible. The polymers are yellow-brown in colour and deepen as the condensation increases. This could explain the browning of white wines. This oxidation reaction is further catalyzed by Fe$^{3+}$ ions (Ribéreau-Gayon, 1974).

The leucoanthocyanidins and their polymeric forms have the characteristics of transforming into red anthocyanins when heated in an acidic medium (Bockian et al., 1955). This characteristic differentiates them from the catechins. The reaction is not complete, but can undergo rapid condensation which leads to the phlobaphene which are insoluble brown-black products. Catechins convert entirely to phlobaphenes under the same conditions.

Flavan-3, 4-diols are different from catechins by an additional hydroxyl at Carbon 4. Eight possible isomers can occur due to the result of three sites of asymmetry (C2, C3 and C4). Leucoanthocyanadins are converted to coloured anthocyanidin forms and can serve as precursors to larger polymeric forms (Timberlake and Bridle, 1976). A possible covalent bond between C4 of the leucoanthocyanidin and either C6 or C8 of a second reactive leucoanthocyanadin can occur to produce a polymer. Several polymeric species are possible according (Ribéreau-Gayon, 1974). The condensation reaction may continue if C4 is available, yielding a polymer of 3-4 members (Somers and Evans, 1986).

One of the important characteristics of tannins is their ability to bind to proteins. This is directly related to the state of polymerization of the tannin molecule which contains 2-10 flavan molecules. These polymers are known as flavolans.
2.3 VARIABLES THAT INFLUENCE THE PHENOLIC COMPOSITION OF WINE

2.3.1 Introduction

Phenolic compounds are responsible for all the differences between red and white wines, especially the colour and flavour of red wines. The phenolic fraction of wine may range from 1-5 g/L (Somers, 1971) of which the concentration of anthocyanin may contribute 300-7500 mg/L (Burns et al., 2003). They have interesting “healthful” properties and bactericide, antioxidant and vitamin properties that enhance consumer’s cardiovascular abilities (Saint-Cricq de Gaulejac et al., 1998; Soleas et al., 1997; Vinson and Hontz, 1995). These phenolic substances came from various parts of the grape bunches, and to a lesser extent from oak products, and are extracted during the vinification process. The phenolic extractives are the most important variables that determine the resulting wine type, making it possible to produce distinctly different wines from the same harvest material (Somers and Ziemelis, 1985). Many variables influence the phenolic composition of grapes and their evolution in the resulting wine, and their structure varies a great deal when the wine ages in the barrel and the bottle.

2.3.2 Cultivar or Grape-species

Grape phenols have already been studied in depth (De Freitas et al., 2000). Different species of the genus Vitis do not contain the same anthocyanins and their corresponding characteristics are reflected in the resulting wines. It is thus possible to differentiate between wines produced from Vitis vinifera and wines from hybrids (Ribéreau-Gayon, 1974). Table 2.1 gives the distribution of anthocyanins among the different species of the genus Vitis.

Of the genus Vitis, 5 to 17 anthocyanins have been identified. Amongst the red varieties are species able to synthesize anthocyanins in their monoglucoside form (Ribéreau-Gayon, 1974). Authors noted anthocyanin diglucosides in American species, but not in Vitis vinifera. The presence of cyanidin and peonidin derivatives among the anthocyanins is common. They are found in all the species, although they are occurring in higher concentrations in only a few (Ribereau-Gayon, 1974). A practical method for differentiating red wines from Vitis vinifera and hybrids has been developed by Ribereau-Gayon in 1959 (Ribereau-Gayon, 1959), although research conducted by Burns et al., (2003) questioned it. Burns et al (2003) stated that the examination of the entire anthocyanin profile of a wine in parallel with the quantitative assessment of the proportions of mono-glucosides and acetylated anthocyanins to be a more reliable approach.

Several authors have documented differences in the anthocyanin pigments of several Vitis vinifera varieties (Albach et al., 1959; Rankine et al., 1958), with the most striking difference being between Pinot noir and the other varieties. It was hypothesized that the significantly lower amount of anthocyanins (compared to the
other *vinifera* varieties) and the absence of acylated anthocyanins were responsible for the low colour density of the resulting wines (Rankine *et al.*, 1958). This was confirmed by studies of Fong *et al.* (1971). Studies by Hrazdina and Franzese (1974) reported the structure and properties of the acylated anthocyanins from *Vitis* – *species*.

Mazza *et al.*, (1999) documented differences in phenolic composition between Cabernet franc, Merlot and Pinot noir to be transferred into the wines. De Freitas *et al.* (2000) documented Cabernet Sauvignon to have a higher amount of low molecular weight procyanidins than Merlot. They also documented the contribution of polyphenols extracted from the seeds of Cabernet Sauvignon to be higher in the resulting wine than the wine produced from the Merlot. Harbertson *et al.* (2002) documented seed tannin per seed to vary almost 40% between Cabernet Sauvignon, Pinot noir and Shiraz. They noted the number of seeds per berry, rather than the amount of tannin per seed to be the major factor that contributes to a difference in total seed tannin per berry.

### 2.3.3 Distribution of phenolics in grape bunches

The quality of red wines is closely related to the chemical composition of the grapes and is influenced by enological practices. During vinification, phenolic compounds are extracted from skins and seeds, which contribute to the organoleptical properties of the resulting wines. It is known that colour is dependent on the concentration of anthocyanins and polymerization reactions (Somers, 1978) and that phenolic compounds from seeds (such as monomeric flavan-3-ols and tannins) contribute to the mouth feel properties of the wine (Ristic *et al.*, 1998) and can affect the colour and ageing characteristics of a red wine (Ristic *et al.*, 1998).

Skins and seeds of the grapes, and to a lesser extent the flesh, contain the major part of phenolic compounds (Somers and Ziemelis, 1985). The only exception appears to be the anthocyanins present in the flesh and skin of the *tenturier*-varieties. These phenolics include principally catechins, dimeric and oligomeric procyanidins in the solid parts of the grapes. The anthocyanins and low levels of flavanols are located in the skins (Somers and Ziemelis, 1985).

Prieur *et al.* (1994) reported catechin, epicatechin and epicatechin-3-O-gallate to be the only monomers in grape seed, that is grape seed tannins to be procyanidins. The same authors documented the ratio of monomers (11%): oligomers (34%): polymers (55%) in grape seed extract. Research by Da Silva *et al.* (1991) identified for the first time procyanidin dimers (B5, B6 and B8), four procyanidin trimers and five galloyl procyanidins. Souquet *et al.*, (1996) reported catechins and epicatechins to dominate in Merlot skin extract, with the proportion of galloylated units contributing a mere 3-6%. Thus, grape skin tannins appear to be different from seed tannins primarily by the presence of prodelphinidins and significantly lower amounts of galloylated derivatives.

Catechins, epicatechins and epicatechin-gallate commonly occur as the terminal subunits in grape seeds, but catechin is the major subunit in grape skins (Romeyer *et
al., 1986; Souquet et al., 1996). Epigallocatechin subunits have only been reported in grape skin (Cheynier et al., 1997). The same author also documented significantly higher total tannin content in seeds than in skins, although the polymer length is generally significantly lower in the seeds.

Although grape seeds contain more tannins than the skins, skin tannins are more easily extracted during vinification (given their localization in the vacuolar liquid, bound to the vacuolar membrane and to the cell wall and the higher polarity of the prodelphinidins (Souquet et al., 1996). Processing and vinification dynamics should consequently modify the proanthocyanidin pool and thus influence wine quality.

2.3.4 Notions of ripeness, terroir and viticultural practices

Wine’s optimum quality also depends on the ripeness level at which the grapes are being harvested. It is therefore important to understand the significant parameters that may influence the phenolic composition of grapes. The development of phenolics during ripening has been studied by a number of authors (Somers, 1976; Ribéreau-Gayon, 1982; Romeyer et al., 1986; Ristic et al., 1998; Mazza et al., 1999; Downey et al., 2003; Geny et al., 2003). Ribéreau-Gayon (1982) noted the development of anthocyanins from the beginning of ripening to complete maturity (Table 2.3). Three phases were noted:

1) A rapid increase of phenolic substances
2) Increase in the rate of accumulation
3) Decrease towards the end of maturity

Table 2.3 Accumulation of anthocyanins and tannins during maturation of Cabernet Sauvignon in grams per 200 berries (Ribéreau-Gayon, 1982).

<table>
<thead>
<tr>
<th>Date</th>
<th>Anthocyanins (g)</th>
<th>Tannins (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 25, 1969</td>
<td>0.02</td>
<td>0.42</td>
</tr>
<tr>
<td>September 1, 1969</td>
<td>0.11</td>
<td>0.70</td>
</tr>
<tr>
<td>September 8, 1969</td>
<td>0.27</td>
<td>0.89</td>
</tr>
<tr>
<td>September 15, 1969</td>
<td>0.35</td>
<td>0.93</td>
</tr>
<tr>
<td>September 22, 1969</td>
<td>0.37</td>
<td>0.95</td>
</tr>
<tr>
<td>September 26, 1969</td>
<td>0.31</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Downey et al. (2003) documented the main period of proanthocyanidin accumulation in grape seeds to be immediately after fruit set reaching maximum levels around veraison. Proanthocyanidin accumulation in the skins occurred until 1-2 weeks after veraison. The proanthocyanidin subunits were different in the seeds and skins. The degree of polymerisation in the skins was higher than in the seeds through all the stages of berry development. The levels of the of proanthocyanidins decreased between veraison and harvest. Romeyer et al., (1986) reported a significant decrease in flavan-3-ols after veraison. The increase of dimeric proanthocyanidins during maturation is suggested to be synthesized from monomers with or without enzymatic control.
According to the results, prolonged maturation does not increase the anthocyanin and tannin content of the grapes. Water evaporation can, however, induce an increase of anthocyanin as a function of water loss. Somers (1976) reported a maximum volume of anthocyanins at 20-30 days after veraison. The levels of anthocyanin and tannin in grapes can almost double from year to year in the same vineyard (Ribéreau-Gayon, 1982).

Authors showed that the influence of the vintage may have a more significant effect on the phenolic composition than the variety, although Flanzy et al. (1972) showed that varietal differences play as important role as the vintage. They compared nine red grape varieties (Table 2.4) at maturation and measured the amount of anthocyanins in mg per 100 berries (Ribéreau-Gayon, 1982).

Table 2.4 The weight (milligrams) of anthocyanins in 100 berries of different varietals (Ribéreau-Gayon, 1982).

<table>
<thead>
<tr>
<th>Varietal</th>
<th>Anthocyanin weight (mg) per 100 berries.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinsaut</td>
<td>706</td>
</tr>
<tr>
<td>Grenache</td>
<td>1033</td>
</tr>
<tr>
<td>Tempranillo</td>
<td>1427</td>
</tr>
<tr>
<td>Carignan</td>
<td>1496</td>
</tr>
<tr>
<td>Mourverde</td>
<td>1316 and 1740</td>
</tr>
<tr>
<td>Syrah</td>
<td>2526</td>
</tr>
<tr>
<td>Alicante-Bouschet</td>
<td>4402</td>
</tr>
<tr>
<td>Seibel 8357</td>
<td>7815</td>
</tr>
<tr>
<td>Aramon</td>
<td>471</td>
</tr>
</tbody>
</table>

Light and temperature are the most important factors influencing anthocyanin biosynthesis. Authors noted less anthocyanin in grapes produced under conditions of reduced light than vines exposed to full light (Ribéreau-Gayon, 1982). Temperature, however, should not exceed certain levels for maximum anthocyanin biosynthesis. Kliewer (1970) found a higher anthocyanin content in Pinot noir grapes grown at lower temperatures (Ribéreau-Gayon, 1982). Grape varieties may also vary in their ability to tolerate higher temperatures and show less fluctuation in anthocyanin content (Ribéreau-Gayon, 1982).

Morrison and Noble (1990) found a significantly higher concentration of anthocyanins in sun exposed grape bunches than in the shaded bunches of Cabernet Sauvignon. Price et al., (1995) reported the same tendency in Pinot noir wines produced from highly exposed grape bunches which had 60% higher anthocyanin concentration than the shaded and 14% higher than the moderately exposed bunches.

Smart and Robinson (1991) compared the canopy surface area of different trellis systems and documented that wines produced from dense canopies had higher pH values, lower anthocyanin concentrations and total phenol concentrations and lower colour density.
Authors have shown that important practices like crop level management by cluster thinning, canopy management practices, irrigation and the influence of soils and climatic conditions (or terroir) may influence the phenolic composition of grapes and ultimately the quality of wine, significantly (Jackson and Lombard, 1993).

Petrie et al. (2003) documented wines produced from mechanically pruned vines to have significantly higher anthocyanin and total phenolic content than the other pruning techniques due to small berry size of the mechanically pruned vines. In experimental pruning trials the colour density and ionized anthocyanins were significantly lower on spur-pruned wines than the other treatments. The resulting wine also had a higher hue value, i.e. a greater proportion of brown pigments relative to red pigments (Ribéreau-Gayon, 1982). Peña-Neira et al., (2004) reported no clear relationship between the phenolic compounds during ripening in grape skins or seeds of Cabernet Sauvignon growing with low, medium or high vigour.

The use of fertilizers and the yield influence anthocyanin accumulation during grape maturation. It has been proven that excess nitrogen fertilizer in soils can reduce the colour of wines produced from those grapes by 30% (Ribereau-Gayon 1959).

Geny et al., (2003) documented the tannins from the cell walls of grape seeds to be more polymerised than those of the inner part of the seed, and becomes more significant as maturity progresses. The epicatechin gallate proportion was significantly higher in the cell wall fraction. The same authors concluded that there was no significant effects of water deficit on the degree of polymerisation of seed tannins.

Another proposed technique of increasing colour development in vineyards is by regulated deficit irrigation (De Garis, 2003). The principle is that reduced berry size and increasing sugar concentration are correlated with an increased anthocyanin concentration in the berries. Roby et al., (2004) documented an increase in the amount of skin tannin and anthocyanin per berry in addition to decreasing berry size as a result of water deficit. Farquhar (2004) documented rootstock to have a significant influence on the colour of Pinot noir. It is well documented that leafroll-type virus infections result in a reduction in the accumulation of anthocyanins (Farquhar, 2004).

Botrytis cinerea can also affect the anthocyanin content by inducing into the berry the production of an anthocyanase, which is an oxidase belonging to the laccase family. It can destroy anthocyanin both in grapes and later during vinification (Ribéreau-Gayon, 1982).

2.3.5 The influence of vinification practices

Peynaud (1987) noted “By applying different vinification methods to the same red grape harvest can make a white wine, a rosé, a light or a strong and full-bodied red wine, depending on the extent of the maturation and, as it were, on ones inclination. A red wine is an enduring record of the quality of cap management during fermentation.”
The phenolic composition of a red wine can be influenced significantly by the oenological practices in the cellar such as maceration techniques e.g. thermovinification (Burns et al., 2003), maceration carbonique (Kovac et al., 1992; Watson et al., 1995), length of maceration (Ribereau-Gayon, 1982), fermentation temperature (Gomez et al., 2000), effect of alcohol (Berg and Akiyoshi, 1975), the use of sulphur dioxide (Dallas and Laureano, 1994; Mazza, 1995), yeast (Bartowsky et al., 2004), lees contact (Vasserot et al., 1997), aeration (Ribereau-Gayon et al., 1983) and fermentation vessel and extraction technique. It can be said that a red wine is an enduring record of the quality of skin management during fermentation.

Different variables can influence phenolic substances during the course of fermentation, e.g. period of maceration, temperature, alcohol, SO₂, enzymes for colour extraction (Revilla and González-Sanjose, 2001; Clare et al., 2002) aeration and physical variables i.e. pump types and tank types. Authors noted small differences between different colour extraction techniques during alcoholic fermentation. Different extraction/maceration techniques have been developed for a number of reasons, e.g. thermovinification, carbonic maceration, cold soaking, prolonged skin contact after fermentation, short time heat maceration, punching down, pumping over, cell-cracking and saignée or must-bleeding (Singleton, 1972). The phenolic content of free-run juice is qualitatively different from wine made from macerated juice (Somers and Ziemelis, 1985).

In summary it can be said that an increase in total phenols correlates with ongoing skin contact, while anthocyanin and thus also the colour intensity of the wine will decrease due to polymerization after an initial increase during fermentation.

2.3.6 The use of oak

Oak barrels and oak derived products contribute to the phenolic composition of wines. Aiken and Noble (1984) reported significant sensory differences between Cabernet Sauvignon matured in oak barrels and glass-aged wines. The effect of oak barrels, oak derived alternatives (i.e. staves, blocks and chips) and maturation conditions will be discussed in section 2.5 of this chapter.

2.4 THE COMPOSITION OF OAK

2.4.1 Cell wall constituents

Wood cell walls contain primarily cellulose (40-45%), Hemicelluloses (25-30%) and lignins (20-23%) and 8-12% total tannins that are extractable with hot water (Haluk and Irmouli, 1998). It is these macromolecular components that contribute to the physical characteristics or advantages of oak being used for the production of barrels:

1. Good mechanical properties indispensable to the strength of the barrel.
2. Ease of splitting.
3. Ease of curving and bending the staves.
4. Good thermal insulation.
5. Slight porosity, favoring oxygenation that influences the colour.

2.4.1.1 Cellulose

Cellulose is the principle component of wood. It is a homopolysaccharide made up of \(\beta\-D\-\text{glucopyranose}\) units bound by glycosidic \(\beta\)-bonds. The structure can be presented as cellobiose (dimmer of \(\beta\)-glucose \((1\rightarrow4)\)) or a polymer of glucose with monomers linked by glycosidic \(\beta\)-bonds \((1\rightarrow4)\).

![Figure 2.8 The structure of cellulose (Haluk and Irmouli, 1997)](image)

The length of the native cellulose molecule is at least 5000 nm (Haluk and Irmouli, 1997). The cellulose chains form fibers known as microfibrils, which are 10-20 nm wide. They are combined into larger fibrils and lamellae. These fibers are deposited in different planes immersed in a matrix of hemicellulose and lignin. Disordered cellulose molecules, amorphous hemicellulose and lignin are located in the spaces between the microfibrils (Haluk and Irmouli, 1997).

Heated oak cellulose produces furanic aldehydes. Hexoces which form part of cellulose forms hydroxymethyl furfural and methyl-5-furfural which can contribute to the flavour of wine (Haluk and Irmouli, 1997).

2.4.1.2 Hemicellulose

Hemicellulose is a group of heterogeneous polysaccharides that act as binding substances, along with pectins, to hold cellulose and lignins together. Two constituents of hemicellulose are of significant importance, i.e glucuronoxylan (Figure 2.9) and glucomannan (Figure 2.10) bound with \((1\rightarrow4)\) and \((1\rightarrow2)\) glycosidic bonds.

![Figure 2.9 The simplified formula of glucuronoxylan (Haluk and Irmouli, 1997)](image)
These bonds are readily hydrolysable by the acidic conditions of wine and can release sugars and acetyl groups. New barrels can lead to an increase in volatile acidity in wines opposed to wines matured in clean used barrels. This is due to acetate estrifying the polysaccharides of the xylane-type (Haluk and Irmouli, 1997). In practice, a slightly higher increase in volatile acidity (0.1 to 0.15 g/L) occurs at moderate toasting levels, compared to strong toast levels (<0.1g/L) (Haluk and Irmouli, 1997). Heating also converts some sugars to furan aldehydes, e.g. furfural and 5-(hydroxymethyl)-2-furfuraldehyde.

### 2.4.1.3 Lignin

Lignin polymers of phenolic units are derived from the structure of phenylpropane. Certain aspects of the chemistry of lignin still remain unexplained, for example specific structural differences of lignin located in different morphological sections of ligneos xylem (Haluk and Irmouli, 1997). In oak, the phenylpropane units contain either hydroxyl or methoxyl groups and form respectively coniferyl alcohols and sinapyl alcohol. These respective alcohols can polymerize into guacol and syringyl lignins. Lignin impregnates the matrix of cell walls. Volatile phenolic compounds, phenolic aldehydes and the phenylacetones are products of the thermal degradation of lignin. Some of these molecules can influence the aroma of wines; especially the smokey, spicy and vanilla undertones. Untoasted barrels contain low quantities of volatile phenolic compounds, e.g. eugenol (Figure 2.11).

![Figure 2.11 The chemical structure of Eugenol (Haluk and Irmouli, 1997)](image)

These substances contribute favourably to wine in lower concentrations (Haluk and Irmouli, 1997). Aldehydes in oak include benzoic aldehydes (vanillin and syringaldehyde) and hydroxycinnamic aldehydes (coniferaldehyde and sinapeldehyde) (Figure 2.12). The heartwood of oak has high concentrations of syringyl units (Vivas et al., 1998).
These aldehydes are found in the extracts after moderate heat treatment. Their thermal degradation can produce phenolic acids via the oxidation of the corresponding aldehydes (Figure 2.13). Heating of wood also brings about the phenylcetones, e.g. acetovanillone and syringone. (Figure 2.15) (Haluk and Irmouli, 1997).

**Figure 2.12** Chemical structures of coniferaldehyde (a) and sinapaldehyde (b) (Haluk and Irmouli, 1997).

**Figure 2.13** Thermal degradation of phenolic acids via the oxidation of their corresponding aldehydes (Haluk and Irmouli, 1997).

**Figure 2.14** Chemical structures of the phenylcetones, acetovanillone (a) and syringone (b) (Haluk and Irmouli, 1997).
2.4.2. Cell lumen constituents

Only a central cavity (lumen) formed by the remaining cell wall is left after wood cells die. This is a result of the degradation of the cytoplasm. Different phenolic substances, mainly hydrolysable tannins, are deposited in the lumen. The tannin content of oak varies considerably and is a function of different variables, e.g. specie, terrier, age of tree and allocation in forest. Other phenolic substances extracted from the lumen are cinnamic acid derivatives, p-coumaric and ferulic acid. Yeasts and lactic acid bacteria can convert aromatic phenols such as 4-ethyl phenol, 4-vinyl guiacol and 4-ethyl guiacol (Dubois, 1989). Moutounet et al. (1989) suggested lyoniresinol as a derivative from oak. Other substances found in oak heartwood include resins, sterols, lactones and fat. These substances are modified during toasting and increase the concentration of cis-β-methyl-y-octalactone and octanone through octadecanoic fatty acids (Chatonnet, 1990).

2.4.3 Volatile substances

The solubility of oak substances and the by-products as a result of degradation and other chemical reactions varies significantly. Some of these extractives can influence the bouquet; while other may influence the mouth feel. Some substances, which are extracted in larger amounts, may influence all the organoleptic properties of wine. Alcohol is a good solvent for some of these reactions to happen. Many of these substances are not found in wines however, but in distilled beverages.

2.4.3.1 Oak lactones

The first lactones to be identified by Masuda and Nishimura were β-methyl-y-octalactone and 4-hydroxy-nonanic acid-y-lactone (Nishimura et al., 1983). These were found in various woods, including all the deciduous oaks used for cooperage, but not in the tested evergreen species (Singleton, 1995). Only cis (3S, 4S) and trans (3S, 4S) isomers occur in oak, although four isomers have been identified in pure solutions. These two isomers are also referred to as whisky lactones (Waterhouse and Towey, 1994) and are responsible for the oakiness of wines (Singleton, 1995). Figure 2.15 shows the chemical structure of the various isomers of β-methyl-y-octalactone. The cis-isomer (threshold 12 times lower than that of the trans-isomer) possesses odours of coconut, celery and sawdust (Masson et al., 1997) and is regarded as the most important of the volatile compounds in oak wood that are extracted into wine and spirits during barrel maturation (Wilkinson et al., 2004).

Authors observed an increased intensification of oak aromas after the removal of oak. This may cause a wine to become over-oaked thus decreasing the quality (Wilkinson et al., 2004).

The optimal concentration in wines is between 150-375 µg/L. Chattonet et al. (1991) also demonstrated that the quality of wine could be decreased at higher concentrations, usually associated with resinous, varnish and coconut-like aromas.
Figure 2.15 The chemical structure of various isomers of β-methyl-γ-octalactone of which the first three have been identified (Ribéreau-Gayon et al., 2000).

Maga (1989) and Chatonnet et al. (1998a) noted an increase of oak lactone as a result of increased toasting (Sefton, 1991), although Masson et al. (1997) documented a slight modification in the contents and the ratio of β-methyl-γ-octalactone enantiomers.

Guichard et al. (1995) recorded a significantly higher amount of the (3S, 4R) oak lactone in brandy which matured in American white oak than those matured in Quercus robur. The same authors also documented no modification of the enantiomeric ratios as a result of toasting and charring which contradicts the findings of Masson et al. (1997).

Staves, which have been naturally seasoned by air, result in wines with higher quantities of the β-methyl-γ-octalactone than wines matured in barrels produced from artificially seasoned staves (Masson et al., 1995). The White oaks, Quercus alba, contains significantly more β-methyl-γ-octalactone than the European species.

2.4.3.2 Volatile phenols

The greatest fraction of volatile oak phenols is based on the guaiacol or syringyl nucleus (Figure 2.16). These result as degradation products of lignin and can further be broken down by microbial metabolism, hydrolysis and pyrolysis (heat) (Sefton 1991). The guaiacol derivatives have lower thresholds than the syringyl derivatives and impact wine flavour significantly more. Guaiacol, 2, 6-dimethoxyphenol, catechol,
resorcinol and hydroquinone can form from pyrolysis of lignin (Singleton, 1995). These compounds have smoky and medicinal smells, but appear in too low concentrations in wine to have a significant effect (Singleton, 1995).

<table>
<thead>
<tr>
<th>R_4</th>
<th>Name</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH_2 - CH_3</td>
<td>Ethyl phenol</td>
<td>Red wine</td>
</tr>
<tr>
<td>CH = CH_2</td>
<td>Vinyl phenol</td>
<td>White wine</td>
</tr>
<tr>
<td>OH</td>
<td>Gaiacol</td>
<td>Wood</td>
</tr>
<tr>
<td>CH_3</td>
<td>Methyl gaiacol</td>
<td>Wood</td>
</tr>
<tr>
<td>CH_2 - CH_3</td>
<td>Ethyl gaiacol</td>
<td>Wood</td>
</tr>
<tr>
<td>CH = CH_2</td>
<td>Vinyl gaiacol</td>
<td>Red wine</td>
</tr>
<tr>
<td>CH_2 - CH_2 - CH_3</td>
<td>Propyl gaiacol</td>
<td>Wood</td>
</tr>
<tr>
<td>CH = CH - CH_3</td>
<td>Allyl gaiacol</td>
<td>Wood</td>
</tr>
<tr>
<td>H</td>
<td>Syringol</td>
<td>Wood</td>
</tr>
<tr>
<td>CH_3</td>
<td>Methyl syringol</td>
<td>Wood</td>
</tr>
</tbody>
</table>

**Figure 2.16** The chemical structure of volatile phenols that occur in wine; some originating from oak wood and others from the wine (Ribéreau-Gayon et al., 2000).

Vanillin (Figure 2.17) is a major component of oak extracts. It is the main active component in natural vanilla (Spillman et al., 1997; Sefton, 1991). Dubois (1989) who disputed the role of vanillin in the "vanilla oak" character contributes the aroma rather to other products formed during the toasting of the barrels. Singleton (1995) noted that other compounds in the group strengthen the aroma of vanillin. Naudin (1990) confirmed this finding. The vanilla aroma may be intensified by oak extracted ketone-phenyls like acetovanillone, propriovanillone and propiosyringone.

Levels of vanillin increase significantly with heating and toasting of barrels (Nishimura, 1983). Spillman et al. (1997) documented an increase of vanillin during wood ageing, but a decrease after long periods of wood ageing due to chemical changes.

Eugenol, has a low flavour threshold (Table 2.5) and has a spicy, clovey aroma. Eugenol originates from oak and can be readily extracted during oak maturation. Towey and Waterhouse (1996) reported a decrease in the extraction rates of volatile phenols in Chardonnay matured in older barrels compared to the wines matured in
new oak barrels. Eugenol levels can vary between the American and European-species (Singleton, 1995). Pérez-Coello et al. (1999) proposed quantitative ratios of eugenol, vanillin, syringaldehyde and oak lactones to be used to distinguish between different oak types.

![Figure 2.17 The chemical structure of vanillin (Ribéreau-Gayon et al., 2000).](image)

Table 2.5 A summary of the threshold values of certain volatile phenols in a water and alcohol solutions (Singleton, 1995).

<table>
<thead>
<tr>
<th>Aromatic aldehyde</th>
<th>Water (Threshold)</th>
<th>10% ethanol (Threshold)</th>
<th>10% ethanol (Threshold)</th>
<th>40% ethanol (Threshold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanillin</td>
<td>2 mg/L</td>
<td>0.5 mg/L</td>
<td>-</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>Syringaldehyde</td>
<td>-</td>
<td>25 mg/L</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sinapaldehyde</td>
<td>-</td>
<td>80 mg/L</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4-ethylguaiacol</td>
<td>20 µg/L</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4-vinylguaiacol</td>
<td>10 µg/L</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eugenol</td>
<td>-</td>
<td>11 µg/L</td>
<td>50 µg/L</td>
<td>-</td>
</tr>
</tbody>
</table>

*: not presented

Yeasts can reduce vanillin to less flavourful vanillic alcohol. 4-Ethylphenol (Figure 2.16) is described as "animally", powerful, woody and phenolic and has been recognized in red wine at 0.4 mg/L. 4-Ethylguaiacol (Figure 2.16) is described as smoky, spicy, clovey, burnt and medicinal and has been found in red wines at concentrations of 400 µg/L or more (Singleton, 1995). Both phenols were originally believed to be related to oak (Singleton, 1995). It has been postulated that malolactic fermentation can be a source of these two 4-ethyl phenols, because some bacteria can reduce the corresponding hydroxycinnamates and decarboxylate it. Chatonnet et al. (1990) found that **Brettanomyces** metabolism in wines can lead to an increase in 4-ethyl phenols (Singleton, 1995). Similarly, the 4-vinyl derivatives also originated from sources other than oak. 4-Vinylguaiacol (Figure 2.16) smells like spice and cloves and is formed from ferulic acid (Dubois, 1989). Some yeasts have the capacity to decarboxylate hydroxycinnamates to 4-vinyl components (Chatonnet et al., 1993).
2.4.3.3 Carbohydrate derived volatiles

Another class of important volatile flavour components are derived from the thermal degradation (pyrolysis) of cellulose and hemisellulose. Amongst these compounds are a group of furan aldehydes of which furfural (Figure 2.18a) is the most important (Sefton, 1991). These compounds are reduced enzymatically to the corresponding hydroxymethyl analogues, which have little or no influence on the flavour of the wine. Nishimura (1983) noted “sweet” and “toasty” aromas from toasted oak chips and attributed this to maltol (Figure 2.18e) and cyclotene (Figure 2.18d). Other important compounds in extracts of toasted oak include ethoxylactone “sweet” and “fruity” aromas), pyrazines, pyridines and isomaltol (Ribéreau-Gayon et al., 2000).

![Chemical structures of common carbohydrate derived volatiles](image)

Figures 2.19 The chemical structures of common carbohydrate derived volatiles, (a) furfural, (b) methyl-5-furfural, (c) hydroxymethyl-5-furfural, (d) cyclotene, (e) maltol and (f) isomaltol as a result of the heating process during cooperage that occurs in wines matured in barrels (Ribéreau-Gayon et al., 2000).

Chatonnet et al. (1991) more recently noted 2,3-dihydro-5-hydroxy-2-methyl-4(H)-pyran-4-one (“sweet vanilla”), 4-hydroxy-2,5-dimethyl-3(2H)-furan-3-one (“fruity-toasty”) and 2,3 dihydro-3,5-dihydroxy-2-methyl-4(H)-pyranone (“toasty”, “fruity” and “caramel”).

2.4.3.4 Terpenes

Thirty norisoprenoids were identified in extracts from Q. alba and Quercus species from Vosges (Sefton, 1991; Sefton et al., 1993). Sefton (1991) noted significantly higher concentrations of these compounds in Quercus alba. Bicyclic compounds and two isomers of trienone (Figure 2.19a) were significant in American oak, but were less significant in Vosges-oak extracts. β-ionone (figure 2.19b) and two isomers of the oxaspiro compound (Figure 2.19c) were only found in the Vosges oak extracts. Their role in wine flavour is yet to be established (Singleton, 1995). These terpene derivatives are very odorous with aromas of resins, violets, lemon and leather. A
combination of oak lactones, eugenol and terpenoids thus must be responsible for the "oakiness" of wines (Singleton, 1995).

![Chemical structures of terpenes](image)

**Figures 2.20a, b and c** The chemical structures of terpenes that occur in oak wood: trienone (a), β-ionone (b) and oxaspiro (c) (Sefton et al., 1993).

### 2.4.3.5 Volatile acids

A slight increase in several acids take place in spirits during maturation in barrels. Authors noted a drop in pH from 5 to 4 during the early stages of barrel maturation due to accumulation of acetic acid. The decrease in pH in brandy maturing in French oak was more significant than for the American oak samples (Aiken and Nobel, 1984). It was confirmed that a considerable part of the increase was derived from oak wood components that contain an acetic acid moiety (Nishimura, 1983). Vivas et al. (1995) documented an increase in volatile acidity due to two reasons: firstly from the acidity that may originate from oak as a result of the toasting process; secondly due to the metabolism of acetic acid bacteria in wine. Yeasts have the capacity to esterify the extracted acetic acid to ethyl acetate due to the large excess of ethyl alcohol (Onishi et al., 1977).

### 2.4.3.6 Other volatiles compounds

Singleton (1995) documented triglycerides of unsaturated C12 and C16 fatty acids, sterols and ferulic acid ester extracted from oak. The influence of these compounds on wine has not been documented yet. Trans-2-nonenal, the "sawdust" or "planky", odour in wines has been described by Chatonnet and Dubourdieu (1998b) and Ribereau-Gayon et al. (2000). Diethyl succinate has been reported as an extractive from oak and esterifies with ethyl alcohol to form diethyl succinate (Onishi et al., 1977).

### 2.4.4 Non-volatile substances

#### 2.4.4.1 Non-volatile phenols

The increase in phenolic compounds in wine is not solely the result of extraction, but also the result of the oxidation of aromatic aldehydes. Aromatic aldehydes are considered to be degradation products of lignin (Vivas et al., 1998; Jindra and Gallander, 1987). Two different forms of coumarins are extracted from oak. The one is a glycosylated form (esculine and scopoline) associated with "green wood" and the
other a ciclycane form (esculetine and scopoletin) found in naturally seasoned oak. They affect the mouth feel of wines differently, the first giving a bitter taste and the latter contributes to the astringency of the wine (Ribereau-Gayon et al., 2000).

### 2.4.4.2 Non-hydrolyzable tannins

The non-hydrolyzable tannins or condensed tannins are the main flavanoid substances in grapes (Singleton, 1995). Low amounts of these compounds are found in oak and their extraction from barrels into wine does not influence the matured wine significantly (Puech et al., 1999).

### 2.4.4.3 Hydrolyzable tannins

Five to fifteen percent of the dry weight of oak is compromised by the hydrolysable tannin (Quinn and Singleton, 1985). The quality and taste of wines and spirits matured in barrels can be influenced by ellagitannins (Zhentian et al., 1999). These tannins are easily hydrolyzed by enzymes or acid or base conditions (Puech et al., 1999) and can be classified as either gallotannins or ellagitannins, according to the type of acid formed (Puech et al., 1999). Bate-Smith (1972) proposed ellagitannins to be esters of glucose with hexahydroxydiphenic acid, which, after hydrolysis, yield ellagic acid. This reaction takes place as oak heartwood matures and is evident by the higher content of ellagic acid in the inner wood compared to the outer heartwood (Kiumpers, et al., 1994).

Quinn and Singleton (1985) recorded the extraction of ellagic tannins into wine through oak-chip treatment or barrel maturation. They postulated the importance of the contribution of ellagic tannins to the astringent taste or mouth feel of the wines. On the contrary, Somers (1990) suggested these compounds to be present at too low concentrations in wines to have a significant sensory effect. Quinn and Singleton (1985) recommended further research on the influence of ellagitannins and sensory profiles of wines.

Different authors have documented on the anabolism of these substances from their precursors, the complex pathways involved and the related compounds formed (Haslam, 1981; Vivas et al., 1995; Hervé et al., 1991a and b; Helm et al., 1997). The most common ellagitannins (Figure 2.20) in oak have been identified as vascalagin and castalagin (Mayer, 1967). An additional 6 ellagitannins, grandinin, and roburins A-E as derivatives of vascalagin and castalagin, were documented by Mayer (1967).
The solubility of tannins varies, and is influenced by their type, size and reactions with other compounds (Puech et al., 1999). Scalbert and Peng (1990) documented the modification of astringency due to polymerization reactions involving ellagitannins. Puech et al., (1990) recorded the presence of ellagic tannins in oak wood extracts and to a lesser extent in spirits. They reported a structural rearrangement of ellagic tannins during maturation in barrels as a result of oxidation, polymerisation and hydrolysis (Puech et al., 1990). Ellagic tannins reach their maximum concentration in the first year of barrel maturation (Viriot et al., 1993). According to Puech (1988), vascalagin has a lower stability than castalagin and is more susceptible to oxidation in alcoholic solutions. Vivas et al. (1996) reported an increase in the decline in ellagitannin concentration with greater oxygen availability. Pocock et al., (1994) documented an increase in colour density in red wines matured in barrels. Vivas and Glories (1996) documented ellagic tannin to have a "remarkable effect on the structure of phenolic compounds and on the colour of red wines". Ellagic tannins increase the condensation of procyanidins and inhibit the degradation of condensed tannin precipitation and anthocyanin destruction. Other important functions include antioxidant and free radical scavenging abilities (Hagerman and Riedl, 1998), antimicrobial properties (Scalbert, 1992) and their medicinal properties (Soleas et al., 1997).
2.5 VARIABLES THAT INFLUENCE THE CONTRIBUTION OF EXTRACTED OAK COMPOUNDS IN WINE

2.5.1 Introduction

Mosedale et al. (1999) noted, "One does not need to do anything, one must wait. The right time will come for everything. The alcohol now enters the oak, and the wood yields everything it has. It yields sun, it yields fragrance, and it yields colour."

The technique of barrel maturation was introduced in the 18th century and currently 10% of wines produced in the world today are aged in barrels. Barrel maturation is a phase in the development of the wine through which it acquires its capacity to age in the bottle.

"Oak-aged" therefore is a term that can be applied to a wine. It implies that the wine was matured in oak barrels, but can also mean exposure to oak alternatives. There are however, different factors influencing the phenolic composition of oak and their contribution to wine. Goldschmidt (1999) noted the influence of cooperers to have a more significant on the organoleptic profile of the wine, than the influence of the geographic source of the oak. There may also be other factors such as the alcohol content of the wine or spirits, external variables (humidity and temperature of maturation milieu) and microbiological dynamics (lees contact) which may alter the phenolic profile of a wine.

2.5.2 Species of oak – differences between European and American oak

Oak belongs to the botanical genus Quercus, represented throughout the world by little fewer than 300 species, which take form as a tree or a shrub. Eight species are found in France, of which two types are being used for the making of barrels, the pedunculate oaks (Quercus robur or Q. pedonculata) and sessile or durmast oaks (Quercus petraea or Q. sessilis) (Ribéreau-Gayon et al., 2000). The European species are quite similar and may hybridize (Singleton, 1974). Another species, the American Quercus alba, is of importance in the cooperage profession for the international wine industry. Singleton (1974) noted European oak to have more phenols and other extractables whereas American oak has more odourants. Other authors reported similar conclusions (Puech et al., 1999). Several physical and chemical differences (Table 2.6) were found between species in a study conducted by Chatonnet and Dubourdieu (1998a).
Table 2.6 The influence of botanical origin on the composition of oak wood (average of 7 to 10 different results) (Chatonnet and Dubourdieu, 1998a).

<table>
<thead>
<tr>
<th>Extraction by a model hydro alcoholic solution</th>
<th>Q. petraea</th>
<th>Q. robur</th>
<th>Q. alba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-volatile compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total extractives (mg/g)</td>
<td>90 (15)</td>
<td>140 (7)</td>
<td>57 (34)</td>
</tr>
<tr>
<td>Total polyphenols (OD 280)</td>
<td>22 (2.9)</td>
<td>30 (1.8)</td>
<td>17 (5.6)</td>
</tr>
<tr>
<td>Ellagic tannins (mg/g)</td>
<td>8 (1.4)</td>
<td>15 (1.5)</td>
<td>6 (2.4)</td>
</tr>
<tr>
<td>Catechic tannins (mg/g)</td>
<td>0.030 (0.001)</td>
<td>0.040 (0.008)</td>
<td>0.023 (0.011)</td>
</tr>
<tr>
<td>Volatile compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cis + trans Methylloctalactone (µg/g)</td>
<td>77 (24)</td>
<td>16 (15)</td>
<td>158 (27)</td>
</tr>
<tr>
<td>Eugenol (µg/g)</td>
<td>8 (1)</td>
<td>2 (1.5)</td>
<td>4 (1.5)</td>
</tr>
<tr>
<td>Vanillin (µg/g)</td>
<td>8 (3)</td>
<td>6 (2.5)</td>
<td>11 (5.5)</td>
</tr>
<tr>
<td>Oxo-retro-α-ionol</td>
<td>Traces/absent</td>
<td>absent</td>
<td>2 isomers</td>
</tr>
</tbody>
</table>

(): Standard deviation

Pedunculate oak has the highest amount of ellagic tannins, whereas sessile oak releases smaller quantities of polyphenols and American oak even less. The American species have significantly higher amounts of whisky lactones and vanillin as can be seen from table 2.6 (Chatonnet and Dubourdieu, 1998a). It is also the only oak that contains the two isomers of 3-oxo-retro-α-ionol. The physical differences between the species include the following:

- Growth rings of the American oak tended to be more variable (1-5 mm/year) than those of sessil oak (1-3 mm/year), but always finer than pedunculate oak (3-10 mm/year).
- Both American oak and sessile oak had two lines of large vessels (spring oak) compared to two or more for pedunculate oak.
- The vessels in American oak and sessile oak were comparable in size, but always smaller than those in pedunculate oak.
- American oak is slightly denser than the European species. The density is a function of the thyllae that acts as blocks in the open vessels of the sapwood.

All these differences in the physical and chemical composition in the different oak species can influence the style of the wine significantly. Aiken and Noble (1984) reported higher concentrations of total and non-flavanoid phenols in Cabernet Sauvignon that matured in French oak barrels compared to the American oak matured wines. This was confirmed by Chatonnet and Dubourdieu (1998). They
suggested that American oak may be identified easily by the high concentration of β-methyl-y-octalactone, the low quantity of extractable polyphenols, the presence of two isomers of 3-oxo-retro-α-ionol and its aromatic potential. Aiken and Noble (1984) documented a significant concentration of potassium in the American oak samples, higher than the French oak and glass matured samples.

Chatonnet and Dubourdieu (1998) recommended Pedunculate oak with its low aromatic potential and high ellagitannin content best suited for the maturation of spirits. Chatonnet (1995) reported a concentration of the trans-isomer of oak lactone higher in Tronçais oak than the flavour threshold in red wine of 320 µg/L (Sauvageot and Feuillat, 1999). They also reported French oak to contain lactone levels far from the average lactone difference between the European oak species and American oak. Oak lactones vary according to species (Sauvageot and Feuillat, 1999). It was also documented that the level of oak lactone in sessile oak is more than for pedunculate oak (Sauvageot and Feuillat, 1999). De Simón et al. (2003) concluded significant similarities in volatile and polyphenol compounds between French and Spanish oak species with respect to the American oak.

Several authors documented tasting panels to differentiate between American and French oak species (Rous and Alderson, 1983, Aiken and Noble, 1984; Pocock et al., 1994; De Simón et al., 2003).

2.5.3 Location in the forest

Opinion and test results have concluded that not only species, but also forest site as an important variable affecting the wine (Singleton, 1995). Although the origin of the oak is a determining factor when comparing amounts of extractable components, the most recent research has shown variability amongst individual trees to be higher than the variability between species, when comparing the European species (Miller et al., 1992; Marco et al., 1994; Masson et al., 1995; Mosedale, 1996; Feuillat and Keller, 1997; Mosedale et al., 1999 and Sauvageot and Feuillat, 1999).

Several authors reported significant differences, both physical and chemical, between individual trees (Feuillat and Keller, 1997; Feuillat and Moio, 1997; Masson et al., 1995). Mosedale (1996) reported these differences between trees as a "strong, genetic control".

2.5.4 Age of the tree

The age of a tree is another variable influencing the composition of the tree and is more important than the effect of sampling position (height and orientation) (Masson et al., 1995). On the contrary, Singleton (1995) found the more significant content of extractable compounds in the youngest heartwood nearest to the sapwood. Authors documented the number of phenolic compounds for young heartwood to be higher than for the older heartwood of the same tree (Singleton, 1995). In contrast, Masson et al. (1995) noted the influence of age to influence ellagic tannin content significantly, but no significant effect on the distribution of hexahydroxydiphenoyl
esters. Feuillat and Keller (1997) stated that the influence of forest origin was significantly lower than effect of the *specie*.

Because of the influence of all these parameters, the coopers find themselves with a heterogeneous raw product from which they have to compile a homogeneous product. This is very difficult to achieve.

### 2.5.5 The cooperage profession

#### 2.5.5.1 The notion of grain

The grain is defined as the average size and regularity of annual growth rings. One ring consists of spring wood (porous and rich in large vessels) and summer wood (dense and rich in fibre). It is accepted that oak with tight grain is a result of slow growth, and oak with wider grain is a result of faster growth (Vivas, 1995). Vivas (1995) documented wide grained oak to be richer in extractable compounds and ellagitannins, whilst poorer in aromatic compounds such as eugenol and whiskey-lactones. Pontallier *et al.* (1986) reported tight grained oak to result in a wine with more intense oaky aroma and less polyphenols.

The difference in flavour in resulting wines as a function of especially grain resulted in the initiation of a study group in 1989 to increase the understanding of variability in cooperage (Feuillat *et al.*, 1999). Feuillat *et al.* (1997) reported the effect of wood anatomy on the extraction of tannins and lactones to be less significant than the intrinsic differences in the levels of extractives found in the same wood tissue of different trees and *species*.

#### 2.5.5.2 The influence of oak seasoning

Seasoning is done in the open air for several years in a well air-circulated flat yard (Figure 2.21). The wood staves, after it has been processed, undergo a modification of its internal physio-chemical composition (Chatonnet, 1995). The stave stacks are torn down and restacked at least once a year to increase consistency of the process.
Figure 2.21 Seasoning takes place in the open air. At a rate of 10mm per year, intense dehydration takes place during the first ten months. The oak matures, thus improving its physical and aromatic qualities.

Chatonnet (1995) noted that it is evident that natural seasoning, because of greater climatic variations, is incapable of consistently providing the best conditions for the woods evolution. A large diversity of microbes, including moulds, yeasts and bacteria are present in the wood during natural seasoning. The result of the enzymatic action of these microbes permits them to easily assimilate these free sugars of the cellulose, hemicellulose and hydrolysable tannins, to degrade the ellagitannins, but to not greatly affect the lignins. Some microorganisms may induce the wood to rot (Chatonnet, 1995). Another alternative is to artificially season the staves in an oven in order to reduce the seasoning period and costs. Chatonnet (1995) compared the effects of natural seasoning to artificial seasoning in an oven (Table 2.7).
Table 2.7 The effects of the origin of oak wood and the seasoning method on the composition (Chatonnet, 1995)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Limousin Region</th>
<th>Central region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Natural drying</td>
<td>Artificial drying</td>
</tr>
<tr>
<td>Dry extracts (mg/g)</td>
<td>135 (7)</td>
<td>145 (7.5)</td>
</tr>
<tr>
<td>Total phenols (D 280)</td>
<td>30.4 (1.8)</td>
<td>31.2 (2.4)</td>
</tr>
<tr>
<td>Coloration (D 420)</td>
<td>0.04 (0.008)</td>
<td>0.038 (0.002)</td>
</tr>
<tr>
<td>Catechins (mg/g)</td>
<td>0.59 (0.08)</td>
<td>0.56 (0.065)</td>
</tr>
<tr>
<td>Ellagitannins (mg/g)</td>
<td>15.5 (1.5)</td>
<td>17.2 (6.5)</td>
</tr>
<tr>
<td>Methyl-octalactone(cis)</td>
<td>12</td>
<td>0.85</td>
</tr>
<tr>
<td>Methyl-octalactone(trans)</td>
<td>4.5</td>
<td>0.22</td>
</tr>
<tr>
<td>Eugenol (µg/L)</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Vanillin (µg/L)</td>
<td>11</td>
<td>0.5</td>
</tr>
</tbody>
</table>

(): Standard deviation

The artificially seasoned wood possesses a higher level of extractable phenolic compounds (tannins) and a higher amount of (trans) β-methyl-y-octalactone. A decrease in ellagitannins, more so in the upper (1-3 mm) layer, was reported. The oven dried wood has a higher content of astringent tannins and bitter coumarins. It also contains less eugenol, vanillin and (cis) methyl-octalactone.

The climate of the seasoning venue had a significant effect on the aroma and extracts of oak wood. Sefton et al. (1993) reported a decrease in eugenol concentration after 12 months of seasoning of oak wood sourced from France. The oak wood from Limousin and Tronçais showed similar trends, but the eugenol concentration in the Vosges sample showed a more erratic variation. There was a significant overall decrease in eugenol concentration. The samples seasoned in Australia however, had lower levels of eugenol than the samples seasoned in France. Vanillin showed no significant difference, while the oak-lactone concentration showed significant fluctuations (Sefton et al., 1993). Sefton (1991) noted a decrease in the cis-isomer as a result of seasoning of Tronçais and Limousin oak. He also noted a much greater decrease in wood seasoned in France than the wood seasoned in Australia with the greatest decrease in the Limousin oak. Interestingly enough, the cis-isomer in Vosges and American oak doubled during two years of seasoning in Australia opposed to seasoning in France. The reasons for this are yet to be determined (Sefton, 1991).
Interactions between seasoning and toasting have received very little attention (Hale et al., 1999). Results documented by these authors shows the strong influence of seasoning time on the changes that take place during toasting.

It is fortunate (for the sake of consistency) that a barrel requires more than 30 staves per barrel. Thus, staves should be randomly chosen due to the effect of tree-to-tree variation and climatic fluctuations during natural seasoning, to minimize the effect of inconsistency and barrel-to-barrel variation.

2.5.5.3 Thermal treatment (toasting) of oak

Several authors documented the influence of cooperage variables, for example toasting method, on the composition (and especially the aromatic composition) of the resulting wine (Cutzach et al., 1997; Matricardi and Waterhouse, 1999). The toasting phase is one of the critical phases influencing the composition of the oak, which will affect the wine. Pyrolysis (heat treatment) of oak leads to degradation of wood components, resulting in many aromatic compounds. Chatonnet (1995) documented the formation of furanic aldehydes such as furfural, methyl-5-furfural and hydroxymethylfurfural as a result of the thermal degradation of polysaccharides. He also documented the production of enolic compounds such as cyclotene, maltol and isomaltol derived from hexoses in the presence of nitrogenated substances. Volatile phenols have smoky, spicy odours and are a result of the thermal degradation of lignin. Other aromatic compounds include vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde and reach maximum concentrations at medium toast levels. The formation of isomers of methyl-octalactone is a result of the thermal degradation of certain lipids or fatty acids. This reaction increases in proportion to heating intensity. As toasting level progresses from light to heavy, the oak aroma becomes more complex. At heavy toast level though, the intensity of the oak aroma decreases and there is an emphasis on smoky and burnt aromas (Chatonnet, 1995).

Francis et al. (1992) documented a significant impact of oak wood after heat treatment on the aroma of wine and reported aromas of vanilla, caramel, butter, nuts, cedar and a decreased raisin character. The tasting panel could easily distinguish between American oak and the European samples.

Authors documented variations in the execution of the toasting procedure that may affect the final product (Matricardi and Waterhouse, 1999). This important step is, unfortunately, not controlled with much specificity and can lead to inconsistency in the resulting barrels. The convective movement of air during toasting may also lead to inconsistency in intensity of the fire (Matricardi and Waterhouse, 1999). Consequently, all these variables may influence the resulting barrel. Much research has shown modifications in the composition of wood as a result of toasting (Chatonnet, 1999a). Pyrolysis has been shown to result in degradation of polymers, such as hemicellulose, cellulose and lignin, and extractives such as tannins. This disruption in the physical state of the wood can increase the accessibility of solvents, their amount and the rate of extraction (Marticardi and Waterhouse, 1999). Intensity
of the toasting is an important variable influencing the occurrence of certain volatile compounds.

Non-hydrolyzable tannins are found in wines matured in barrels and may affect aroma and taste (Quinn and Singleton, 1985). Other authors questioned this assessment (Pocock et al., 1994). Sarni et al. (1990) documented a decrease in the amount of ellagitannins (castalin and castalagin) and a simultaneous increase in ellagic acid. Toasting results in the thermal degradation of the tannins and these thermal products are reported to be less astringent.

Although the different attributes of toasting techniques on the organoleptic composition of wine is of greater interest, the main point of focus was on the aromatic compounds rather than the influence on taste. The little research done on the contribution of oak wood (ellagitannins) to the phenolic composition and taste of wine was contradictory. Francis et al. (1992) concluded that heating of oak wood is the most important variable that may influence the sensory characteristics of oak wood extracts.

Little research has been done on the influence of different toasting methods on the colour and/or colour evolution of red wines.

2.5.6 Barrel-to-Barrel variations

Wines with different organoleptic profiles as a result of barrel to barrel differences are common (Personal experience and personal communications). Some winemakers even select the barrels which resulted in significantly better wines as the “Flagship-range” of the cellar with brand names such as “Private selection”, “Limited addition”, “Barrel Selection” and “Winemaker’s selection”. The lack of information on these differences is of particular significance for the planning and statistical evaluation of barrel experiments (Towey and Waterhouse, 1996a). They reported a significantly high variability in all oak derived compounds that were measured in four barrel lots of ten barrels each (two sets of both American and French oak), and proposed further research to provide more accurate detail to compare barrel lots in wineries and academic settings more effectively.

2.5.7 Barrel size and shape

The larger the container, the smaller the surface of the container per unit of contents (Boulton et al., 1996). Thus, the volume of the barrel not only influences the extraction of compounds from the oak, but also affects oxidation, evaporations and therefore also physio-chemical changes within the wine. Guyman and Crowell (1970) reported less evaporation an increase in barrel size. Research has shown that storage in smaller barrels (200L) seemed to improve the concentration of some phenolics, because of the bigger surface-to-volume ratio. A 200L barrel has 90 cm² of surface per litre of wine (Singleton, 1974).

Wine stored in smaller barrels showed lower concentrations of anthocyanins, (+)-catechins, (-)-epicatechins and polymerized phenolics than wines stored in bigger
barrels. This indicates a more rapid polymerization of monomeric phenols. It was also proven that wines stored in smaller barrels improved the colour density, while the colour hue was not influenced. (Castellani et al., 2001). The same author also reported a more significant perception of oak derived volatiles for example vanilla and spicy descriptors in wines matured in smaller oak barrels, although fruitiness was more pronounced in wines stored in the bigger barrels (Figure 2.22).

A sphere has the smallest surface of any geometrical shape for a given volume. Boulton et al. (1996) noted a cube to have about 124% of the surface of an equal volume sphere, a cylinder twice as tall as its diameter about 120%, and a barrel 108% calculated as an ellipsoid of which the ends were cut off. It is evident that the cube will result in higher extraction due to its bigger surface area, but the shape of barrels was chosen for a number of reasons. It is possible, however, to increase the surface area inside the barrels by addition of oak derived products such as chips, blocks or staves, or create u-shaped trenches lengthwise on the staves on the inside of the barrels.

Figure 2.22 The effect of barrel size on the aroma distribution of wines, (Castellani et al., 2001).
2.5.8 Age of the barrels

In a study conducted by Rous and Alderson (1983), three important points were made. The first was that there are strong sensory differences between French and American oak from first fill to the third fill; the second was that the curves drawn from the phenolic extraction data appeared to change from the curves defining diffusion kinetics in the first and second fills, to straight lines defining hydrolysis kinetics in the third fill. There was in other words a significant decrease in phenolics, measured in GAE/L, between the first and second fill barrels, and the third fill barrels. Thus, the older the barrels, the lower the amount of extractable phenols. Interestingly, Towey and Waterhouse (1996b) reported a significantly higher amount of β-methyl-γ-octalactone (particularly the cis-isomer) in wines matured in one-year old (used) barrels compared to wines matured in new barrels. The β-methyl-γ-octalactone levels, however, decreased significantly in two-year and older barrels. Towey and Waterhouse (1996b) reported wines to be less smoky and spicy in aroma if it was matured in older barrels. Singleton (1995) stated that wines matured in older barrels, should be shifted to less prestigious places in the market than wines matured in barrels which had less fills. Of course this would be a generalization as good results on wines produced from certain Rhone-varieties, which were matured in second, and third fill barrels were reported (unpublished data and personal conversations with leading winemakers). Older barrels may not yield significant amounts of gallic acid, which plays an important role as an oxidation catalyst. There is also a higher risk of unpleasant odours from older barrels (Chatonnet et al., 1991).

2.5.9 Barrel treatments before filling

Renewal of barrels consists of several rotations and actions. If barrel use surpasses 5 years, the wine will not benefit from further ageing and it is seen as a malpractice. Used barrels must be properly treated to reduce the risk of development of unwanted microorganisms like Brettanomyces. Used barrels retain 5 litres of wine and can therefore act as an ideal place for the development of these micro organisms and the appearance of defects like volatile acidity, ethyl acetate, 4-ethylphenol, trichloreanisole and guaiacol (Boidron, 1995). Used barrels should be completely cleaned, drip-dried and sulphured (Lavigne, 1994). The author also discouraged the use of chemical products, dry sulphuring (because of taste defects like sawdust, resinous and vegetable odours and an increase in cis-octalactone), and the shaving and re-toasting of barrels.

2.5.10 SO₂ - Additions

SO₂ is a preservative widely used for the conservation of wine. Its actions against oxidation and microbial spoilage as primary functions are well documented (Boulton et al., 1996). Secondary effects of SO₂ include the bleaching effect of anthocyanins and its association with carbonyl groups of many compounds which may affect the
extraction of different compounds from the oak (Boulton et al., 1996). SO$_2$ may bind with acetaldehyde generating ethane sulfonic acid resulting in a decrease in free acetaldehyde. Higher aldehydes may also react with SO$_2$; resulting in a decrease in fruitiness of certain white wines (Ribéreau-Gayon et al., 2001). SO$_2$ does not effect the extraction of oak wood compounds, although it was found to bind vanillin, syringaldehyde, coniferaldehyde and 5-hydroxymethylfurfural at different SO$_2$ concentrations (Ancín et al., 2004). Ancín et al. (2004) recommends oak selection, consequently, to be based on the combination of species and origin, rather than only on species.

2.5.11 Ethanol concentration

Ethanol acts as a co-solvent in wine and increases the solubility of non-polar volatiles. Guyman and Crowell (1970) reported 50-60% alcohol concentrations most effective in extracting oak components with maximum extraction taking place at 55%. Singleton (1995) reported different aroma profiles at different alcohol concentrations. Ramirez-Ramirez et al. (2004) observed an increase in ethanol concentration from 10% to 15%. The same authors documented no significant influence on the behaviour of aroma compounds by the addition of glycerol. In a study conducted by Cerdán et al., (2004), the highest accumulation of volatile compounds was found at 40% ethanol (compared to 0%, 10%, 20% and 60%). No or little research has been done on profiling aroma compounds at different concentrations of ethanol in wine.

2.5.12 The influence of the maturation period on wine

One would expect a longer maturation period to lead to a greater intensity of oak flavours in wine. Figure 2.24 shows an increase in cis-oak lactone, while, in contrast, there was no increase in the concentration of 4-methyl guaiacol beyond the 11-week period (Sefton et al., 1993). The authors also stated an intensification of aroma and flavour, and an alteration in the “oakiness” of the wine as a result of an extended maturation period. The wine may become dominated by compounds such as oak lactones with their woody and coconut-like odours, while in contrast may be less influenced by the smoky component of 4-methyl guaiacol and guauacol (Sefton et al., 1993).
Cerdán et al. (2002) reported lower concentrations of furfural, 5-methylfurfural, furfuryl alcohol, coniferaldehyde, acetovanillone and phenols in wines matured for 18 months in re-used barrels than after 10-12 months. They documented the ethylphenols to increase right up to 18 months of maturation (which may have a negative influence on the quality of the wine), whilst there were few changes in the concentration of esters, except for ethyl lactate, which reached a peak after 12 months of maturation and decreased thereafter.

2.5.13 Effects of cellar temperatures and humidity

Both water and ethanol can evaporate from barrels during the maturation period of distilled beverages in oak barrels (Guyman and Crowell, 1970). The same effect applies for wine although limited data is available (Singleton, 1995). Non-volatile constituents rise in concentration in proportion to the loss of volume (Singleton, 1995). Singleton (1995) hypothesized evaporation within the wood as a function of the relative humidity of the cellar, influencing the rate of maturation. If the cellar has a high humidity, the diffusion of water is limited, resulting in a decrease of alcohol due to the alcohol that escapes. Singleton (1995) noted a greater effect of temperature with increasing ethanol loss. Optimal conditions for maturation, i.e. temperatures at 14-17°C and humidity level of 65-80%, would be beneficial for the quality of wines, whereas fluctuating conditions can lead to accelerated aging resulting in poorer quality wines.
2.5.14 The influence of microbial dynamics and oak interaction on the composition of wine

Barrels may be filled with wine before or after alcoholic fermentation, although some winemakers do partial alcoholic fermentations in barrels and/or variations thereof. These actions may have a profound influence on the aromatic and phenolic status of the wine influencing the style hugely. There are contradictory statements about the role of vanillin in the experience of the “vanilla-oak” character of wines. The content of higher alcohols is significantly increased by fermentation in barrels, while the aroma contribution of fatty acids and their esters are negligible small (Chatonnet et al., 1991). More important is the influence of yeasts on the change of some oak extracts. Furan derivatives can be converted into their corresponding alcohols by the yeast, e.g. the appearance of 5-methylfurfurylthiopropionate, an ester of 5-methylfurfural alcohol, which has a coffee-like aroma (Chatonnet et al., 1991). The reduction of furan aldehydes also occurs in wines stored in barrels after fermentation but with less intensity. Wines fermented in oak are higher in soluble polysaccharides and originates from either the grapes or the yeast and can influence the sensory properties of the resulting wine. Fermentation in barrels leads to a faster integration of aromatic and phenolic substances of wines and oak (Chatonnet et al., 1991).

Lees contact (and autolysis) during maturation in a barrel can influence the organoleptic profile of a wine. Chatonnet et al. (1991) reported the limitation of the contribution of oak wood components on the aromatic profile of the wine during maturation on the lees.

Chatonnet et al. (1991) reported a decrease in yellow colouration and a significantly lower proportion of free and reactive tannins in wines stored in contact with the lees in barrels than the wines stored in tanks. The reason is the capacity of cellular walls and mannoproteins (released into the wine during autolysis) to fixate or bind oak wood tannins.

Malolactic fermentation (MLF) is a normal microbiological stage in production of red wines and can alter the quality of red wines positively or negatively (Vivas et al., 1995). Winemakers can either allow MLF in stainless steel tanks, or in barrels, or partial MLF in tanks and then in barrels. Research has shown that MLF in barrels compared to tanks, result in a more significant increase in astringency due to an increase in polymerization and a stabilized colour. Wines that underwent MLF in tanks were preferred to those, which underwent MLF in barrels. The authors also noted that the differences between the treatments remained the same after 3 years in the bottle (Vivas et al., 1995).

Van der Westhuizen and Loos (1981) concluded that different strains of lactic bacteria compared with each other did not have any significant influence on the colour parameters of the resulting wine and that the colour loss of the experimental wines were effected only by the SO₂ concentration.
2.5.15 Oak derived products

The challenge for most wineries is to lower the production cost of wines, whilst maintaining quality and consistency (Heraty et al., 1999). The oak fraction in the production cost of wine constitutes 50-65% when using barrels, opposed to 12-26% using oak alternatives. According to Singleton et al. (1971), ageing in small cooperage can give at least the equivalent of a treatment with 15.5 g oak chips per litre of wine.

Staves, blocks, chips and extracts however, has met some skepticism. Needham (2003) reported: "It has been cast as a battle between old world and new. With Australian wine exports soaring, local winemakers say they are being targeted by rivals in France, Italy and Spain seeking to put a cork in the rising cheer of antipodean plonk with young European consumers. The vigneron of Europe want to ban inexpensive Australian table wine by taking exception to the Australian practice of using oak chips, rather than pricy oak barrels, to flavour. The traditionalists also want more rigid definitions of labeling terms such as classic, vintage, reserve, hand-picked and traminer that could exclude Australian wine."

Needham (2003) also reported that "vigneron of Europe want to ban inexpensive Australian table wine by taking exception to the Australian practice of using oak derived products".

Investigations with regards to oak derived products and their influence on wine quality yielded certain disadvantages, e.g. no retrieval of complexity compared to oak barrels (Bertrand et al., 1997), occurrence of hydrogen sulfide on wines associated with fermentation on chips (Dennison, 1999), precipitation of yellow, semi-crystalline substance as a result of wines treated with sawdust or shavings because of extracted ellagic acid (Baldwin, 1993; Pocock et al., 1984) and the general lack of information concerning the influence of oxygen provided by oak derived products and the positive contribution towards wine (polymerization) (Wilker and Gallander, 1988).

There are, however, important advantages as well. Lefort (2004) noted the significant cost reduction in the use of oak derived products opposed to the use of barrels. Wilkinson et al. (2004) proposed the addition of oak staves, chips, shavings or powder as a more rapid and economical method of oak treatment. The same authors also stated that the increased surface area of these oak derived products to result in more significant rates of extraction. Martínez et al. (2001) noted that 5-6 hours of heat treatment is adequate to allow the decay of lignin, with the subsequent formation of aromatic aldehydes. The same authors proofed that chip particles with a diameter of 3-5 mm favour the extraction process. Pérez-Coello et al. (2000) documented the use of 7g/L of oak chips during white wine fermentation to increase the favourable perception of a tasting panel.

Ducournau et al. (1999) documented oak chips to be more adapted for consistent toasting, resulting in more homogeneous lots. The use of oak derived products in combination with micro-oxygenation result in wines with increased body, soft tannins, stable colour and enhancement of fruit and oak aroma integration (Zoecklein et al., 2002).
2.6 COLOUR EVOLUTION IN WINES: THE RESULT OF COMPLEX PHENOLIC INTERACTIONS

2.6.1 Introduction

As a red wine matures, certain organoleptic changes become evident. The colour changes from bright red to orange-brown that corresponds to a decrease in concentration of monomeric anthocyanin and other phenolic compounds, but an increase in polymeric content. The intensity of the wine initially increases, especially when it is matured in contact with wood, and decrease with time. Acetaldehyde has been known to intensify red wine colour.

Even other sensory parameters change. Wines become more palatable and are usually associated with a decrease in astringency, bitterness and/or harshness. A series of physical and chemical changes thus occurs, enhancing the quality of red wine. In fact, some wines require longer maturation periods than others, while some wine styles for example Gamay noir in the Beaujolais are drinkable very young. Some wines are still drinkable after decades while some loose its complexity after a few years of bottle maturation. All the wines have in common though, chemical and physical changes that occur naturally as an effect of oxygen and phenolics.

Well-known authors like Singleton (1995), Boulton (1996), Glories (1999), Ribéreau-Gayon (1974), Somers (1978) and Iland (2000) summarized the changes or evolution of the phenolic identity as follows:

- Changes of the purple-red colour hue of young wines to the orange/brown/tawny hue in older wines are attributed to the conversion of monomeric compounds to polymeric pigments.
- Evolution of non-coloured phenolic components to pigmented species.
- Decrease in colour intensity due to polymerization reactions and regenerative polymerization and colourloss due to precipitation.
- Reactions include tannin-anthocyanin additions, acid-catalyzed bond making and bond-braking processes and oxidation reactions.
- Direct tannin-anthocyanin polymerization reactions include orange generating xanthytium salts, or indirect, involving acetaldehyde leading to purple pigments.

2.6.2 The copigmentation phenomena

Anthocyanins consist of several forms, which are in equilibrium at a wine pH 5. Only less than 25% are in the flavylium or red form (Singleton, 1988). Co-pigmentation, defined as the enhancement of colour due to the formation of a complex between pigment (anthocyanins) and co-pigments, can influence the equilibrium of these anthocyanins. Authors suggested that anthocyanins require auxiliary molecules (co-pigments) for the full expression of colour. They also suggested that the colour of anthocyanins depend on concentration, pH, copigmentation and the presence of
metal ions (Haslam, 1998). Co-pigments responsible for this process include identical anthocyanin (self-association), one of its aromatic acyl group constituents (intramolecular copigmentation) or other molecules (intermolecular copigmentation).

Several authors documented evidence that anthocyanins (flavilium ion) may undergo self-association (Haslam, 1998). Molecular aggregation under conditions where there exists an equilibrium of the neutral quinonoidal anhydro-base, HA, and the anionic form A⁻ (pH 7), takes the form of an equilibrium between the self-association of the quinonoidal anhydro-base, HA and the hetero-association of the quinonoidal anhydro base, HA with its anionic form, A⁻ (Figure 2.24) (Haslam, 1998).

\[
[\text{HA}]. [\text{HA}] \leftrightarrow [\text{HA}]. [\text{A}^-] \leftrightarrow [\text{A}^-]. [\text{A}^-]
\]

**Figure 2.24** The neutral quinonoidal anhydro-base, HA, and the anionic form A⁻ (pH 7), takes the form of an equilibrium between the self-association of the quinonoidal anhydro-base, HA and the hetero-association of the quinonoidal anhydro base, HA with its anionic form, A⁻. (Haslam, 1998).

Anthocyanin molecular aggregation around pH 7.0, self-association of the quinonoidal anhydro base, HA, and hetero-association of the quinonoidal anhydro base, HA with its anionic form, A⁻.

This effect was strongly intensified as the concentration of the pigment was increased, indicating the enhanced formation of aggregates in the more concentrated solution. Authors suggested "head to head" stacking geometry based upon CD and HNMR measurements (Figure 2.25), but also indicated other stacking geometrics (Haslam, 1998).

**Figure 2.25** A model for the chiral stacking or self-association of anthocyanin molecules.

Anthocyanin chromophores form an extended, hydrophobic core and the hydrophilic glucose residues surround this domain and render the aggregate water-soluble. The sugar residues of anthocyanin are now believed to act as important building blocks in the copigmentation process whether these occur inter-or intramolecularly (Haslam,
Such aggregation result in a "super molecule" which leads to protection of the anthocyanin by water thus ensuring stabilization in stead of the loss of colour. Copigments have little or no visible colour themselves, but when added to an anthocyanin solution they significantly enhance the colour of that solution (Haslam, 1998).

Many compounds have been identified to act as potential copigments, but flavanoids (e.g. quercetin-3-β-D-galactoside) and hydroxycinnamoyl esters (e.g. chlorogenic acid) are most commonly found in nature. It is generally assumed that non-covalent complex formation between anthocyanins and copigments are responsible for the intensification in colour of the medium. Several authors proposed that the intermolecular copigmentation phenomenon originates by the vertical "hydrophobic stacking" of the aromatic nuclei of the anthocyanin and the copigment. They further hypothesized that the hydrophobic effects direct the anthocyanin and the copigment to form a π-π complex, which is resistant against hydration and thus decolorization. Figure 2.26 illustrates the most efficient π-π overlap and interactions would occur with a planar flavanol as compared to a hydroxycinnamoyl or galloyl ester, or the non-planar flavan-3-ols and the sterically constrained ellagitannins such as vescalagin and castalagin.

![Figure 2.26 Comparison of π-π overlap possibilities in anthocyanin copigmentation.](image-url)
Authors proposed that the inherent stability of acylated anthocyanins derived from the ability of aromatic acyl groups to form an "intramolecular sandwich" with anthocyanidins. This intramolecular π-π arises from the hydrophobic interactions between the two electron rich phenolic nuclei of the acyl groups and the electron deficient anthocyanidin thus protecting the anthocyanidin from attack by water. The intensity of the copigmentation effect may be influenced by substances like metallic ions or macrocycles which are capable of either reacting with the pigment or the copigment (Haslam, 1998).

Examples of molecules acting as copigments include malvin, phloroglucinol and tryptophan, whereas chlorogenic acid, caffeine and (+)-catechin interact more strongly with the anthocyanin. Rutin is the most efficient copigment yet known (Haslam, 1998).

It is evident that certain variables influence the copigmentation effect. The highest copigment effect is within the pH range of 3 to 5. Wine acidity range falls within the limits where the effect is at its highest (Haslam, 1998). Copigmentation is specific to aqueous media and a change in water content will also influence the copigmentation effect: A decrease in water content always decrease the effect. Alcoholic cosolvents like methanol, ethanol or ethylene glycol all reduce complexation with the largest reduction being obtained with ethanol (Brouillard and Dangles, 1994). An increase in temperature brings about partial dissociation of the copigment complexes. The released flavylium ions become subjected to covalent hydration and become colourless (Brouillard and Dangles, 1994).

Conditions during maturation of wine (relatively low temperatures, pH values between 3 and 4 and 1:9 ethanol to water ration media) are favorable for good interactions between grape anthocyanin and copigments.

2.6.3 Formation of monomeric wine pigments

Monomeric pigments in wine occur as a result of cyclo addition mechanisms between anthocyanins and various metabolites derived from yeasts. Several of these pigments have been identified e.g. orange pigments were shown to be condensation products of anthocyanins and vinyl phenol (Cameira-dos-Santos et al., 1996 and Francia-Aricha et al., 1997). Decarboxylation of p-coumaric acid by yeast decarboxylase results in vinyl phenol and may react with malvidin, either as a monoglucoside or as an acylated monoglucoside (p-coumarylglucoside) (Fulcrand et al., 1996; Fulcrand, 1998).

2.6.4 Anthocyanin breakdown reactions

The concentration of anthocyanin molecules decrease significantly during the first few months of barrel maturation because of their instability. The decrease is due to a contribution of different reactions with different compounds in wine, especially tannins (Ribéreau-Gayon et al., 2000). These breakdown reactions may result in chalcones
(Ribéreau-Gayon et al., 2000), malvones (Hrazdina, 1970), phenolic acids, coumarins and dihydroflavanols (Ribéreau-Gayon et al., 2000).

Authors studied three types of these breakdown reactions in detail (Ribéreau-Gayon et al., 2000):

- Thermal degradation of anthocyanins when heated to $100^\circ$C causes the colour to fade. It can be explained by a shift in the equilibrium towards the chalcone and colourless forms. Malvidin is very sensitive to heat. Thus, temperature should be taken into account during the maturation period in order to protect colour (Ribéreau-Gayon et al., 2000).
- Oxidative degradation of anthocyanins takes place as these anthocyanins in an acidified alcohol medium lose their colour after a few days of light exposure (Ribéreau-Gayon et al., 2000).
- Degradation of anthocyanins by acetone and the consequent generation of orange-coloured compounds are possible (Ribéreau-Gayon et al., 2000).

2.6.5 Interactions of tannin, protein and polysaccharides

Figure 2.27 (Ribéreau-Gayon et al., 2000) explains a model of which polyphenols are spread over the surface of the protein in a single layer, thus decreasing their hydrophilic character. The result is proteins clumping together which leads eventually to precipitation. In the case of high concentrations of proteins, phenolic compounds act as "ligands" crosslinking these various molecules. Reaction time, pH, temperature, solvents and ionic strengths influence the formation of these tannin-protein complexes.
Tannins are capable of forming stable combinations with proteins and polysaccharides via various types of interactions of which the mechanisms are not completely understood. The two main types include hydrophobic interactions and hydrogen bonds and covalent bonds (Figure 2.28).

Figure 2.28 Interaction between proteins and polyphenols (Ribéreau-Gayon et al., 2000).
2.6.6 Formation of carbocations from procyanidins

Condensed tannins (section in thesis) are polymers of flavan-3-ol with a defined bond between two carbons in the flavan units. The bond is between the C4 in the upper reaction and C6 or C8 in the lower part and is degradable by acid catalysis. Acid catalysis is proposed to cause the breakdown of the B8 dimer with the release of the catechin (flavan-3-ol) which originates from the lower part of the dimer and an activated catechin which originates from the upper part. The carbocation formed after the protonation has an electrophilic centre that could react with various nucleophilic compounds making it highly reactive to form procyanidins. According to the Bate-Smith reaction, heating a procyanidin in a strong inorganic acid medium (HCl) in the presence of oxygen produces red cyanidin (Ribéreau-Gayon et al., 2000).

2.6.7 Procyanidin oxidation Reactions

Phenols are highly oxidizable compounds (Ribéreau-Gayon et al., 2000). This created considerable interest in studies regarding the medical and nutritional attributes of phenols (De Beer et al., 2003). The oxidation reactions are very complex and are influenced by light, temperature, the presence of hydroperoxides and certain metals (Fe²⁺ and Cu⁺) which act as catalysts.

Molecular oxygen (O₂) may become a hydroperoxide radical HO₂⁻ and the superoxide anion O₂⁻ which can create a number of oxygenated free radicals. Phenolic compounds, especially tannins, take priority in the oxidation reactions thus acting as scavengers of free radicals. The oxidation of tannins is proposed to result in polymers and insoluble brown pigments known as phlobaphenes which may precipitate (Ribéreau-Gayon et al., 2000).

2.6.8 Procyanidin polymerization reactions

Procyanidins are capable of forming a carbocation in an acid medium without oxygen. This carbocation is likely to react with the negative points of another procyanidin increasing its degree of polymerization. When a procyanidin B2 for example is stored, it produces a C1 trimer, other polymers and (-)-epicatechins. Polymerized procyanidins are produced by a C4-C8 or C4-C6 organized polymerization (Ribéreau-Gayon et al., 2000).

Disorganized polymerization is a result of the formation of free radicals in a strongly oxidative medium. In media under conditions of controlled aeration, combined oxidation of the procyanidins lead to the formation of ethanal from ethanol. This reaction is much faster than organized polymerization and yields polymers which are likely to precipitate (Ribéreau-Gayon et al., 2000).
2.6.9 Reactions between compounds with polarized double bonds and anthocyanins

Cameira dos Santos et al. (1996) demonstrated the formation of arranged pigments which are stable in both pH and SO₂ variations. Vinyl phenol results from the decarboxylation of p-coumaric acid by yeast decarboxylases (Ribéreau-Gayon et al., 2000). This may react with malvidin either as a monoglucoside or in the form of an acylated monoglucoside. The double bond is added between the carbon 4 of the anthocyanin and the oxygen held by carbon 5. The result is a colourless compound (Ribéreau-Gayon et al., 2000). Another group of pigments in wine, which corresponds to the addition of piranic acid, was documented by Bakker and Timberlake (1997). They suggest that compounds such as enolic ethanol may also be involved in colour changes. These pigments occur in very small quantities in wine (Ribéreau-Gayon et al., 2000).

2.6.10 Anthocyanin and Tannin condensation reactions

According to Jurd (1969), maturation involves a slow, continous shift of pigments from the monomeric form to a polymeric form. Jurd (1969) also proposed the flavylium cation to react directly with various components such as anuric acids, phloroglucinol and catechin resulting in a colourless flav-2-ene substituted in C 4. The disappearance of anthocyanins has been found to correlate with colour that remains stabled or intensified (Ribéreau-Gayon et al., 2000). These pigments that were formed are stable in variations of pH and SO₂ variations. Three types of condensation reactions have been identified and the resulted polymers ranged from orange to mauve in colour. It was documented that an A-T reaction where anthocyanins act as cations (A⁺) on the negative nodes (6 or 8) of the procyanidins (P) forming a colourless flavene (A-P) (Figure 2.29) is possible. When anthocyanin solutions are kept in a reactive milieu, the colour may decrease. This may be reversed by aeration. In practice it is revered to as a wine picking up colour as it picks up oxygen during rackings (Ribéreau-Gayon et al., 2000). Figure 2.30 shows direct T-A condensation reactions where procyanidins form a carbocation after protonation and react with the nucleophilic sites, such as nodes 6 or 8 of an anthocyanin molecule. This complex is colourless and may turn to a reddish-orange colour on dehydration. This reaction takes place in reductive media and is enhanced by temperature (Ribéreau-Gayon et al., 2000).

The third condensation reaction is with an ethyl cross-bond. In an acid medium, like wine, ethanol forms a carbocation that reacts with the negative nodes (4 and 8) of flavanols and anthocyanins in the neutral form, i.e. carbinol base. The reaction depends on the pH of the medium and the proportion of flavanols and anthocyanins. This reaction takes place as a result of controlled oxygenation during barrel maturation. The result is a wine that becomes darker after a few months in the barrel.
Figure 2.29 Direct A-T type condensations of anthocyanins and tannins (Ribéreau-Gayon et al., 2000).
2.6.11 Astringency

Astringency is not a taste, but a sensation resulting from the reactions of the tannins on the glycoproteins in saliva which causes the saliva to disappear. A wine is more likely to be astringent when it is rich in reactive molecules (Glories, 1999). Astringency increases with an increase in the degree of polymerization up to a limit. Wines that matured for a short period of time in barrels may be high in little polymerized procyanidin; thus having an acidic character at higher pH and low acidity. As the structure of tannins are modified during maturation as a result of polymerization reactions, astringency decreases. The presence of bitter anthocyanins and ellagic tannins may accentuate the hardness of wine, while proteins and lipids may accentuate the reactions of tannins with proteins, thus making the wine to appear to be suppler (Glories, 1999).

The gelatine index is used as a measurement of the reactivity of tannins. An index value of >50 indicates a wine with highly reactive tannins which may be perceived as astringent and tannic. An index below <50 indicates the presence of less reactive
tannins. Wines with a lower gelatine index value generally appear to have less body. (Glories, 1999) Values obtained by this index must be interpreted with care and preferably in conjunction with an organoleptical evaluation.
2.7 LITERATURE CITED


Chapter 3

Research Results

The influence of different barrel origins (French oak, American oak & Russian oak) and oak derived products on the colour and phenolic evolution and the organoleptic profile and quality of Pinotage and Shiraz wines in South Africa.
3. RESEARCH RESULTS

The influence of different barrel origins (French oak, American oak and Russian oak) and oak derived products on the colour and phenolic evolution and organoleptic profiles and quality of Pinotage and Shiraz wines in South-Africa

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It has long been accepted that red wines undergo a range of complex colour changes during their conservation in barrels and other vessels. Red wines, especially premium quality red wines, require barrel maturation to incorporate oak aromas and increase the palatability. Barrels, however, are the most expensive part of the production chain and several methods and alternatives have been proposed to incorporate the oak flavour in the organoleptic profile of wines. Oak derived products, however, have been associated with "cooperage by-products" and limited quality control over their production as well as limited research has emphasized the importance of more intense research in order to establish/increase the confidence in using it in the production of quality wine. The aims of this study were to compare the effects of barrels of different origin regarding the colour and phenolic development during ageing of Shiraz and Pinotage, as well as the effect of different aged barrels and alternative oak treatments on the mentioned parameters in Pinotage. This study shows that, although new barrels had a more significant effect on wine colour than used barrels and oak derived products such as chips, blocks, dust and freeze dried tannins, the differences between barrels of different origin (French, American and Russian) and used barrels compared to oak derived products on the colour evolution of Shiraz and Pinotage wines were not significant. The effect of new barrels on the colour evolution of wines may be the result of a larger degree of oxygenation and a higher concentration of ellagic tannins readily available for polymerisation reactions. Colour density, percentage of red colour and SO\textsubscript{2} resistant pigment increased initially during barrel maturation, but decreased with total red pigment (the latter in the barrel as well) during bottle ageing. This tendency was also observed in the experiment with the alternative oak treatments. New barrels also gave higher oak and lower fruit aromas compared to older barrels and certain oak alternatives.
**3.1 INTRODUCTION**

Phenolic compounds constitute an important part of grapes, oak and wine composition and can profoundly influence the appearance, flavour and general quality of a wine. The main ones are phenolic acids and their derivates (ex cinnamic acids), flavonoids (ex kaempferol and quercitin), anthocyanins (ex malvidin-3-glucoside) and tannins, which consist of hydrolysable and condensed tannins. The latter consists more-or-less of complex polymers of flavan-3-ols or catechins and epicatechins originating from the grape, while hydrolysable tannins consist of gallo- and ellagitannins, originating from the oak barrels used to age wine in. The tannins in wine contribute to the mouth feel of the wine and can contribute to elicit an astringent or bitter taste in the wine. Anthocyanins are molecules responsible for the colour of red wine, in particular the flavylum cation form. Anthocyanins can however also occur in different other forms in wine, including the colourless carbinol base, the blue quinonic base and the yellowish chalcone form. The occurrence of these forms is influenced by, among others, pH, SO₂, age of the wine and storage temperatures (Ribéreau-Gayon et al., 2000).

Red wines are often matured in wooden barrels. During this maturation process, oxygen comes in contact with the wine, causing polymerization reactions between different phenolic compounds, which can influence the organoleptic quality of the wine. Tannins, originating from the grapes and oak, can react with anthocyanins to form other red pigments and this influence the stability and the appearance of the colour of red wine over time. Tannins can also react with other tannins, which can also contribute to reduce the astringent taste of a red wine. Certain compounds originating from the wood can also enhance the flavour of the wine, like oak lactones etcetera.

Several authors have recognized the importance of wine colour as a quality parameter. Several studies have been conducted in the past on the various aspects of colour. The correlation between wine colour and overall wine quality score have been investigated by several authors (Somers and Evans, 1974; Jackson et al., 1978; Tromp and Van Wyk, 1977).

It is widely accepted in the wine industry that colour density relates to quality, with deeper coloured red wines normally having a more intense flavour than the lighter coloured wines (Somers and Evans, 1974). Tromp and Van Wyk (1977) even documented that odour and taste was affected by the perception of the colour of the wine by the judges. They documented the colour of the wine to be the most important factor for the determination of red wine quality, on condition that the wine has minor defects with regards to odour and taste and variations thereof (Tromp and Van Wyk, 1977).

Berry colour is used by some wineries as an indicator of wine colour potential and thus quality. Simple methods are being utilized by winemakers to measure the colour of berries (Iland et al., 2000). Benefits include a system
which is objective and quantifiable and the information obtained can be utilized as a quality assessment parameter for the grapegrower as well as valuable insight with regards to better canopy management, improved wine balance, better irrigation scheduling and thus better turnover (Barnett, 2004). There are several proposed considerations for determination of wine quality, although some required significant capital outlay and logistical problems.

The determination of colour by spectrophotometry however, has been shown to meet the criteria for red grapes and red wines (Gischen et al., 1998). Colour analysis by spectrophotometry has long been popular to follow phenolic evolution. As wine maturation progresses, the absorbence 520nm decrease corresponding to an increase in the yellow brown colour at 420 nm. The blue component contributed to the quinoidal forms of free and combined anthocyanins must be taken into account when assessing these colours. Optical density measurements at 420, 520 and 620 nm may thus be more complete (Ribéreau-Gayon et al., 2000). The wine hue, shade or tint, measured at the ratio of absorbances A420/A520 typically increases from 0.5-0.6 in younger wines to 0.8-0.9 in mature red wines as they become more brown (Somers and Evans, 1974). This is an indication of the increased contribution of polymeric pigments to the wine colour. Iland et al. (2000) used a dilution of wine in 1M HCl to push the anthocyanin equilibrium to the flavylium form and break up any possible colour enhancement by copigmentation to measure the total amount of potential colour.

Copigmentation complexes are also bleached with SO2, which may result in a colourloss form (Levengood, 1996). As much as half of the total colour could be from copigmented forms in young wines (Boulton, 2001).

Various spectrophotometric and spectroscopic methods have been used to determine the total phenol content of wine. The Folin-Ciocalteu method has been a popular method (Ribereau-Gayon et al., 2000), usually with an arbitrary standard such as gallic acid. The Folin-Ciocaltelu determines total phenols (and other easily oxidised substances) by producing a blue colour from reducing yellow heteropolyphosphomolybdate-tungstate anions. Somers and Evans (1974) and Iland et al., (2000) used the expression OD280 - 4 as an index of total polyphenols in red wine.

The PVPP index is used to quantify phenolic polymers (pigmented and non-pigmented) based on the fact that free anthocyanins and other monomers can be washed off a PVPP column with dilute alcohol. The polymerised or bound anthocyanins will not be washed away thus giving an estimation of the bound anthocyanin fraction.

Other indices are used to estimate the characteristics of tannins, e.g. HCl, Dialysis and Gelatine index. The HCl index is based on the instability of procyanidins in a concentrated HCl medium, where the precipitation speed depends on the degree of polymerisation and therefore reflects the state of
polymerisation of tannins in the wine (Ribéreau-Gayon et al., 2000). The
dialysis index relates to the structure of the tannin with highly charged
molecules which pass through the pores of a dialysis membrane more slowly
than the smaller molecules with lower charges (Ribéreau-Gayon et al., 2000).
A high dialysis index indicates that there are big, generally polymerised
pigments. This will correlate with a higher HCl index value. The HCl index may
be low in wines with particularly high anthocyanin content, although the
dialysis index is high. The gelatin index is based on the capacity of tannins to
react with proteins, which results in stable combinations. Higher values will
correlate with highly reactive tannins which may be responsible for the
sensation of astringency when tasting red wine. Values may vary from 25 to
80, but is influenced by the origin of the wine and vinification practices
(Ribéreau-Gayon et al., 2000).

Haslam (1992) documented the reaction between tannins and proteins to
depend on the degree of polymerisation of the procyanidins. Lea (1978)
documented the capacity of polyphenols to bind proteins, increase with the
degree of polymerisation and galloylation. Cheynier (1992) noted that the
pattern of hydroxylation on either the A or B rings of condensed tannins
influences protein interaction, explaining the higher affinity of prodelphinidins
(3 hydroxyl groups) for proteins, than procyanidin (2 hydroxyl groups).
The isolation and separation of phenolic compounds in grapes and wine is
a complex science and methods for analysing the structures of the various
phenolic compounds are currently limited to dimeric, trimeric or, possibly,
tetrameric procyanidins (Fereira and Bekker, 1996). Such techniques include
the separation of procyanidins with HPLC (high performance liquid
chromatography) columns used coupled with UV-visible detectors (Lea, 1979;
Putman and Butler, 1989; Ricardo da Silva, 1990), preparative methods
based on the separation of procyanidins on a molecular weight basis (Revilla
et al., 1989; Shoji et al., 1999), ion exchange which has been effective in the
separation of monomeric anthocyanins and smaller pigment (Spagna and
Pifferi, 1992) and counter-current chromatography (CCC) which separates
phenolics according to their partition coefficients in two liquid phases (Marston
and Hostettmann, 1994).

The characterization and identification of grape and wine polyphenols
include electrospray ionisation mass spectrometry (ESI-MS) (De Freitas et al.,
1998) and $^1$H NMR and $^{13}$C NMR spectrometry (Laouenan et al., 1997) which
are the most popular methods used currently, although it can only be applied
to pure compounds, which are very difficult to obtain in sufficient amount.

The objectives of this study were to investigate the development of colour,
phenolics and aroma profiles of different South-African Shiraz and Pinotage
wines (1999 vintage) in French, American and Russian oak barrels on a
commercial scale and to compare the use of oak derived alternatives with
Pinotage (2003 vintage) matured in different age, used barrels. Some of these
wines were also evaluated and tested after 36 months of bottle maturation. It is the first time, according to our knowledge, in SA that a project on the colour evolution of Pinotage has been conducted and evaluated after 36 months in the bottle.

3.2 MATERIALS AND METHODS

3.2.1 PROJECT A

3.2.1.1 Project Outlay

Colour analysis trials were done on four different farms in four wines (all 1999 vintage) made on commercial scale: Bellingham Pinotage (Franschoek region), Kanonkop Pinotage (Stellenbosch region), Vergelegen Shiraz (Helderberg region) and Altydgedacht Shiraz (Durbanville region). Nine Seguin-Moreau barrels, which consisted of three French oak barrels, three Russian oak barrels and three American oak barrels, all medium plus toasting level, were filled with the abovementioned wines at each individual farm after the completion of malolactic fermentation. The wines underwent barrel maturation for twelve months, except the Altydgedacht Shiraz which was bottled after nine months. These wines were transferred in 20L canisters to the US where the free SO₂ was adjusted to 35 mg/L, filtered through a K700 filter and bottled in screw cap bottles. The wines were stored in the bottle maturation cellar of the US at 16°C. Colour analyses were conducted prior to transferring the wines to the barrels and then every three months in conjunction with a sensory tasting.

3.2.1.2 Phenolic analysis with spectrophotometry

Colour analysis was done mainly according to Iland et al. (2000). The following red wine colour and phenolic measures were conducted with a spectrophotometer:

i. Wine colour density / modified wine colour density (420 + 520nm)
ii. Wine colour hue / modified wine colour hue (420/520 nm)
iii. Total red pigments
iv. Total phenolics (280 nm)
v. Degree of red pigment colouration
vi. Modified degree of red pigment colouration.

The definition of the spectral measures for describing red wine colour and phenolics:
Wine colour density
- Describes the intensity of the colour. Generally values of 0 to 6 OD units would describe a light colour wine, between 6 to 10 OD units are described as medium red colour and > 10 OD units as deep red.
- $A_{420}$ relates to the concentration of red coloured pigments and $A_{520}$ to the concentration of yellow/brown pigments.
- Higher values thus indicate more intensely coloured wines.

Wine colour hue
- Express the hue, tint or shade of the colour rather than its intensity.
- Lower values indicate better colour (more red and less brown).
- Too high values directly after fermentation indicates poor potential for maturation

Degree of red pigment colouration
- The percentage of the total wine pigments that are coloured at actual wine pH and SO$_2$ levels, i.e. wine pigments which contribute to actual wine colour.
- Higher values will be better.

Estimate of SO$_2$ resistant pigments
- The percentage of red coloured pigments (as a result of polymerisation reactions) which are resistant to the bleaching effect of wine.

Modified degree of wine colour density, modified degree of wine colour hue and Modified degree of red pigment colouration.
- Describes the abovementioned measurement, but with the bleaching effect of SO$_2$ eliminated and at a uniform pH value (pH 3.5).

Total red pigment colour
- An estimate of the concentration of the total pigments (both anthocyanins and tannins) in the wine.
- Higher values indicate more intense (“heavy bodied”) wines.

Total phenolics
- An estimate of the total phenolic material in wine.
- Because of their chemical diversity, there is no method by which it can be precisely quantified.

Gelatine Index values were obtained after 36 months of bottle maturation (Ribéreau-Gayon et al., 2000). Ilband et al. (2000) was again used to determine the colour aspects. The technique of Stefano et al., (1989) as adapted by Nicolini and Valenti (2001) was used to determine the total flavanoids, total anthocyanins and total tannins of the wines during their conservation in the different barrels. All data presented is the average of the barrels in triplicate with error bars.
3.2.1.3 Sensory evaluation

Preference tastings were held with selected panels every three months during maturation in the barrels. A preference tasting where the panel was also asked to indicate on an intensity scale the intensity of certain predetermined organoleptic characteristics, was held after four months of bottle maturation and then again after 36 months of bottle maturation. The data was analysed statistically according to the method of Kahan et al. (1973) as adapted by Amerine and Roessler (1983).

3.2.2 PROJECT B

3.2.2.1 Project Outlay

Pinotage (vintage of 2003) from the Stellenbosch region was processed in the cellar of Distell and stored until after malolactic fermentation after which it underwent a bulk filtration. The wine was transported to the cellar of the University of Stellenbosch at Welgevallen where it was transferred to 225L French oak barrels (supplied by Radoux Cooperage) with the different treatments. The blocks and chips were also supplied by Radoux. The Oenotan was supplied by Columbit and the Oakmore oak dust by African Cork Suppliers. Two 1000L stainless steel tanks were also filled with the wine and treated with the same oak chips and blocks respectively. The project outlay is summarized in Table 3.1. The additions were done according to the supplier’s recommendations. The free SO₂ was adjusted to 35 mg/L and maintained at this level over the nine month period.
### Table 3.1 Treatments of the Pinotage wine at Welgevallen Cellar (US)

<table>
<thead>
<tr>
<th>Barrel Number</th>
<th>Treatment</th>
<th>Structure/Form</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 2*</td>
<td>Barrels</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3 – 5**</td>
<td>Barrels</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6 – 8***</td>
<td>Barrels</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9 – 11•</td>
<td>French oak blocks</td>
<td>30x5x100mm</td>
<td>6 g/L</td>
</tr>
<tr>
<td>12 – 14•</td>
<td>French oak chips</td>
<td>3-10mm shavings</td>
<td>6 g/L</td>
</tr>
<tr>
<td>15 – 17•</td>
<td>Oenotan</td>
<td>Freeze dried oak tannin</td>
<td>90 mg/L</td>
</tr>
<tr>
<td>18 – 20•</td>
<td>Oakmore</td>
<td>Coarse oak dust</td>
<td>6 g/L</td>
</tr>
<tr>
<td>T1 (tank) 1000L</td>
<td>Blocks</td>
<td>30x5x100mm</td>
<td>6 g/L</td>
</tr>
<tr>
<td>T2 (tank) 1000L</td>
<td>Chips</td>
<td>3-10mm shavings</td>
<td>6 g/L</td>
</tr>
</tbody>
</table>

* : new 225L barrel  
** : 2nd fill 225L barrel  
*** : 3rd fill 225L barrel  
• : 5th fill 225L barrel

#### 3.2.2.2 Phenolic analysis with spectophotometry

Colour analysis was done mainly according to Iland et al. (2000). The following red wine colour and phenolic measures were conducted with a spectrophotometer:

- Wine colour density / modified wine colour density
- Wine colour hue / modified wine colour hue
- Total red pigments
- Total Phenolics
- SO₂ resistant pigments
- Modified degree of SO₂ resistant pigments
- Degree of Red Pigment Colouration
- Modified Degree of Red Pigment Colouration.

#### 3.2.2.3 Sensory evaluation

A sensory evaluation as described in section 3.2.1.2 of this chapter, was conducted after nine months of maturation.
3.3 RESULTS AND DISCUSSION

3.3.1 PROJECT A

3.3.1.1 Colour Analysis

Authors have shown a positive correlation between wine colour density and quality ratings. In general wines with a higher colour density, higher total red pigment content and higher total phenolics can be regarded as full-bodied styles. In Figures 3.1 to 3.4 the colour density and modified colour density of Bellingham and Kanonkop Pinotage can be seen. The Vergelegen and Altydgedacht Shiraz wines show the same tendency (Figures 3.5 and 3.6). Very little, if any, difference between the different barrels (French vs. American vs. Russian) was observed. It is clear that an increase in colour intensity can be observed due to the storage of the wine in wooden barrels. A wine, which has a colour intensity lower than 6 is considered as lightly coloured, between 6 to 10 OD units medium coloured and > 10 OD units deeply red (Illand et al., 2000).

Figure 3.1 Colour density

Figures 3.1 and 3.2 Wine colour density and modified wine colour density of Bellingham Pinotage, respectively.

Figure 3.3 Colour density

Figures 3.3 and 3.4 Wine colour density and modified wine colour density of Kanonkop Pinotage, respectively.
Three of these wines, Bellingham Pinotage, Vergelegen Shiraz and Altydgedacht Shiraz, (Figure 3.1, Figure 3.5 and Figure 3.6, respectively) had values lower than 10 initially, but this increased in all of them to almost 12 after three months of maturation in the wooden barrels, reaching a maximum six months after barreling. This tendency was also observed in the Kanonkop Pinotage, although it had a higher initial value of 11 OD units (Figure 3.3).

Figure 3.5 Vergelegen Shiraz
Figures 3.5 and 3.6 Wine colour density of Vergelegen Shiraz and Altydgedacht Shiraz, respectively.

The same pattern can be seen with the modified colour density, in which the effect of SO₂ and pH differences has been eliminated. The difference between the initial colour density and modified colour density is also clear at the time of barreling, with a difference of almost 6 OD units between these two measurements in the Bellingham Pinotage for example. Ribéreau-Gayon et al. (1983) noted a red wine stored in a barrel to increase in colour and to decrease in the anthocyanin concentration. Figure 3.1 shows a sharp increase in colour density of almost 5 units after the first 3 months of barrel maturation. The other three wines showed the same tendency for both wine colour density and modified wine colour density with an increase of between 2-5 units during the first three months.

Figure 3.7 shows a decrease of the total anthocyanins for all the wines matured in French oak barrels as would be expected. The Kanonkop Pinotage showed the same tendency (results not shown). This correlates with the work of Ribéreau-Gayon et al. (1983)
Figure 3.7 The decrease of total anthocyanins in the wines from the 4 different estates which matured in 225L French oak barrels.

The decrease in the amount of the total anthocyanins might be due to polymerization reactions between tannins and anthocyanins. Figure 3.8 shows an initial increase in total tannins of almost 300 mg/L during the first three months of barrel maturation of Bellingham Pinotage. Again very little differences were observed between the different barrel types.

Tannins were extracted from the barrels and can take part in condensation reactions or Bayer reactions which increases the coloured form. An increase in wine colour density for all the wines during the first three months correlates with a small increase in tannins (due to extraction from the oak barrels) and decrease in free anthocyanins as an indication of polymerization.

Figure 3.8 The development of total tannins of Bellingham Pinotage during maturation in 225L oak barrels.
Less of the anthocyanins molecules are at this stage condensed to tannins than later in the maturation process. The flavylum cation in its free form is more prone to the bleaching effect of SO₂, which explain the larger difference between the colour density and the modified wine colour density of the young wines. As the wine mature however, more of the anthocyanine molecules are involved with condensation reactions with tannins, making these pigments more resistant to SO₂ bleaching which explains the smaller difference between the colour density and the modified colour density as the wine mature in the barrels. Ellagitannins, originating from wooden barrels can act as catalysts of this polymerization process.

Differences between the three types of barrels were, however, not significant. Figure 3.5 shows a decrease in both wine colour density and modified wine colour density after from 3 to 12 months of barrel maturation. This might be accounted for by the wine undergoing regenerative polymerization reactions which may lead to precipitation and loss of colour; thus a decrease in colour density. This tendency correlates with the decrease in total flavanoids, total tannins and total anthocyanins of Bellingham Pinotage (Figure 3.9).

![Figure 3.9](image.png)

**Figure 3.9** The development of total flavanoids, total tannins and total anthocyanins of Bellingham Pinotage matured in 225L French oak barrels.

There was a very small increase in the colour hue during this maturation period, both in the different types of barrels and different wines. An increase of almost 0.1 OD units was evident for all the wines (Figures 3.10 and 3.11), which represents the same tendency observed in all four wines. No significant differences occurred between the different types of barrels.
Figure 3.10 Colour hue

Figures 3.10 and 3.11 The development of Kanonkop Pinotage colour hue and modified wine colour hue, respectively, during its maturation in 225L barrels.

Somers and Evans (1986) documented a rapid decrease in total anthocyanins and an increasing contribution of polymeric pigments to wine colour during storage of the wine in barrels.

Figure 3.12 Bellingham Pinotage

Figures 3.12 and 3.13 show the degree of red pigment colouration of Bellingham Pinotage and Kanonkop Pinotage maturing in 225L barrels, respectively.

Figure 3.14 Vergelegen Shiraz

Figures 3.14, 3.15 show the degree of red pigment colouration of Vergelegen Shiraz and Altydgedacht Shiraz maturing in 225L barrels, respectively.
Figure 3.12 to 3.15 show the proportion of pigments that are actually in the coloured form of these wines. It is clear that more of the pigments are in the colourless form in a young, red wine. The condensation reaction between anthocyanins and tannins can form colourless flavenes, which can be transformed into the coloured form with subsequent oxygen pick up, as would happen in a wooden barrel. This pigment has a more intense colour than the free forms. Oxygen can also contribute to this by forming ethanal from the oxidation of ethanol, which is involved in ethyl cross bonds between anthocyanins and tannins. The resistance of the coloured pigments later in the maturation period to SO$_2$ bleaching is also clear when one considers the smaller difference in the degree of red pigment colour and the modified degree of red pigment colour later in the maturation process (Figures 3.1 to 3.4). For all the wines (Figures 3.12 to 3.15) an increase in the percentage of coloured pigments is about 11.5% to 14.5% due to maturation in oak wood barrels. If the bleaching effect of SO$_2$ and difference in pH has been eliminated, the percentage is 15% to 20% (results not shown). This correlates with the decrease in anthocyanin molecule concentrations of these wines (Figure 3.7) as more of these molecules are being transformed into the anthocyanin-tannin pigment. Anthocyanins concentration can be decreased from an initial concentration of up to 1500 mg/L after fermentation to 0 to 50 mg/L after prolonged ageing of the wine (Ribéreau-Gayon et al., 2000).

The total red pigment colour however showed a decline, probably due to precipitation of phenolic compounds, as can be seen in Figures 3.16 to 3.19. Differences in the values between the three different types of barrels are small for each wine, except in the Altydgedacht Shiraz six months after barreling. The transformation of a bigger part of the colourless form of the anthocyanins/colour pigments during ageing compensates, however, for this loss, resulting in a visible colour that did not decrease during ageing.
Figures 3.16 and 3.17 show the total red pigments of Bellingham Pinotage and Kanonkop Pinotage, respectively, after 36 months of bottle maturation.

Figures 3.18 and 3.19 show the total red pigments of Vergelegen Shiraz and Altydgedacht Shiraz, respectively, after 36 months of bottle maturation.

Total phenol concentration also decreased due to polymerization and precipitation (Figures 3.20 to 3.23). Again, no significant differences could be observed between the different barrel types. It is thus clear that the type of wood (French vs. American vs. Russian) did not play a significant role in the colour development of the wine during ageing in the barrels. The tasting trial also confirmed this. The maturation of the wine in oak barrels did, however, help to increase the colour of the wine and this increase reached its maximum after six months of maturation. This correlates with the research of Ribéreau-Gayon et al. (1983) who reported a more significant increase in colour density of wines matured in barrels compared to a wine stored in a concrete tank, although there were no significant differences in tannin levels. However, they reported that the decrease in anthocyanins and the increase in colour density was a result of mild conditions of oxygen in the barrels. It thus seems that the origin of the barrel does not play such a significant role concerning the colour development of the wine during barrel maturation under South-African conditions.
Figures 3.20 and 3.21 show the total phenolics of Bellingham Pinotage, Kanonkop Pinotage maturing in 225L barrels, respectively.

Figures 3.22 and 3.23 show the total phenolics of Vergelegen Shiraz and Altydgedacht Shiraz maturing in 225L barrels, respectively.

3.3.1.2 Sensory Evaluation

Results of tasting trials of the wine made on the four farms while maturing in the wooden barrels are being summarized in Tables 3.2 to 3.5. A sawdust character, which is probably being caused by (E)-2-nonenal was perceived at certain stages of the maturation process. Chatonnet and Dudourdieu (1998) documented the possibility of (E)-2-nonenal being responsible for the sawdust aroma in wines that matured in new barrels. General preference differences were also noted between the tasters in different wines as can be seen in these tables. A few significant preferences were documented. During the maturation period of Kanonkop, 5/6 judges preferred Russian oak after 3 months, although it changed drastically after a further 3 months when 1/9 judges preferred it. After 9 months of maturation at Bellingham, 0/6 judges preferred the French oak. At Vergelegen, 6/9 judges preferred the French oak barrels after 6 months. After 9 months, 5/6 judges preferred the American oak barrels of the Altydgedacht Shiraz. These preferences became non-significant towards the end of the maturation period.
A tasting was also held 4 months after bottling of the wine. There was no significant difference according to the overall quality average between the different wines matured in French, American and Russian oak barrels, as can be seen in Tables 3.6 to 3.9.

The organoleptic profiles of the wines after 36 months of bottle maturation can be seen in Figures 3.24 to 3.27. Interestingly enough, the average values for colour density obtained from the tasting panel indicate Russian oak to have the higher colour density. It is only the spectral colour measurements of the Vergelegen Shiraz and Altydgedacht Shiraz which could confirm this. The values, however, obtained were not significantly higher than the other wines.

Figure 3.24 Contribution of different types of oak barrels to the organoleptic profile of Bellingham Pinotage after 36 months of bottle maturation.
**Figure 3.25** Contribution of different types of oak barrels to the organoleptic profile of Kanonkop Pinotage after 36 months of bottle maturation.

**Figure 3.26** Contribution of different types of oak barrels to the organoleptic profile of Vergelegen Shiraz after 36 months of bottle maturation.
Figure 3.27: Contribution of different types of oak barrels to the organoleptic profile of Altydgedacht Shiraz after 36 months of bottle maturation.

It is also interesting to see that the average hue value for Russian oak is the highest. The spectral values however obtained could not confirm this. American oak proved to have had the most intense oak bouquet compared to French and Russian oak. The average value for oak intensity (nose) was the highest for all the estates except for Bellingham Pinotage where the differences between the values were very small. General comments of the panel showed that the intensity of oak and identification of American oak was still evident after 36 months in the bottle. This might be because of the higher concentration of whiskey-lactones as documented by Sefton et al. (1993). No significant differences in the perception of fruit intensity between the different wines could be detected, although the fruit intensity of the Kanonkop Pinotage in the Russian oak treatment was more intense than the French and American oak. This might be because the intense perception of oak may mask or dominate the perception of fruit of the wines. The average values for oak/fruit integration showed that Russian oak was preferred on both Vergelegen Shiraz and Altydgedacht Shiraz. It must, however be stressed that the differences were very small. There was no statistically significant preference differences between the judges on a 5% level of significance as analysed statistically according to the method of Kahan et al. (1973) as adapted by Amerine and Roessler (1983).
3.3.1.3 Colour analysis after 36 months of bottle maturation

Figure 3.28 shows the contribution of the different colour fractions to the colour of the different wines. The percentage of free anthocyanins decreased as a result of polymerisation reactions that occurred, as well as regenerative polymerisation in the bottle over the 36 month period. The amount of co-pigmented anthocyanin, however, is still relatively big in relation to the polymerised anthocyanins. Interestingly, there is a tendency that the wines matured in French oak (especially the Pinotage wines) had between 2 – 10% more free anthocyanins than the other wines.

Figure 3.28 The contribution of different colour fractions to the colour of Pinotage and Shiraz matured over a 12-month period in 225L French, American and Russian type oak barrel (Seguin-Moreau barrels) after 36 months bottle maturation.

Figure 3.29 shows the development of the colour density of the two Pinotage wines over 48 months. There is an increase in colour density up to 6 months.
and then a decrease of almost 20% due to precipitation reactions that continued in the bottle. The same tendency occurred for all the other wines. There were no significant differences between the plots, varietals or barrels. Figures 3.30 shows the development of the total phenolics of Pinotage over 48 months of maturation.

![Figure 3.29 The development of colour density in Pinotage over 48 months.](image)

The total red pigments (Figures 3.16 to 3.19) show a significant decrease as a result of polymerisation and precipitation reactions, which continued in the bottle. Altydgedacht Shiraz shows a significant decrease in total red pigments and had only 21% of its original red pigments left. Bellingham Pinotage, Kanonkop Pinotage and Vergelegen had, respectively, 32%, 33% and 35% of its original red pigments left after bottle maturation. This might be due to the longer barrel maturation time of the last three Estates, resulting in more tannin extraction for colour stabilization.

There is a very slight decrease in total phenolics (Figure 3.30) also a result of ongoing polymerisation reactions. No significant difference between the different barrel types regarding the total phenolics could be detected.
Figure 3.31 The Gelatine-index values of the wines after 36 months in the bottle. Blue: French, Maroon: American and Yellow: Russian oak respectively.

Figure 3.31 shows the gelatine index values for the different plots. Interestingly, the index values for both Bellingham and Kanonkop Pinotage matured in French oak is lower than for the other barrels. The Russian oak gelatine index value of Altydgedacht Shiraz is lower than the other barrels. Higher values indicate highly reactive tannins and should cause a significantly more astringent mouth feel (Ribéreau-Gayon et al., 2000). The integration of fruit and oak tannins (Figure 3.27) in the Russian oak Shiraz of Altydgedacht was better than for the other wines. Integration also refers to tannins which softened as a result of polymerisation reactions over the course of maturation. The Pinotage wines, however, showed no significant difference in mouthfeel (integration) between the different barrel types, although the integration in the French oak Pinotage was thought to be slightly softer.
3.3.2 PROJECT B

3.3.2.1 Colour analysis

The colour density and modified colour density for all the wines increased during the first 3 months. This may be the result of a steady increase of A420 + A520 ratio and correlates with the work of several authors (Ribéreau-Gayon, 1982; Bakker et al., 1986; Somers and Vérette, 1988). Figure 3.32 shows a more significant increase in both wine colour density and modified wine colour density for the new oak barrels. This would be expected as the new oak barrels has more ellagic tannins to be extracted which may take part in polymerisation reactions which intensifies the colour. The increase in colour density in new oak barrels was almost 7 OD units in the first three months. This correlates with the research of Vivas and Glories (1996).

![Graph of colour density and modified wine colour density](image1)

**Figures 3.32** The development of colour density and modified wine colour density (0x, 3x, 9x) of Pinotage in new, 2\(^{nd}\) fill and 3\(^{rd}\) fill 225L barrels over a 9 month maturation period.

![Graph of modified wine colour density](image2)

**Figure 3.33** The development of modified wine colour density of Pinotage in 5\(^{th}\) fill 225L barrels treated with oak derived products barrels over a 9 month maturation period.
The difference between the colour density and modified wine colour density might be due to larger degree of polymerisation between anthocyanins and tannins in the wines maturing in new oak barrels, thus leading to a smaller bleaching effect of SO₂.

Figure 3.33 shows a sharp increase in modified colour density for all the wines treated with oak elaborated products. The wines treated with oak chips shows a slightly higher increase in modified wine colour density than any of the other treatments. The reason for this is because of the bigger surface area of the chips in contact with the wine. The wines treated with blocks and oakmore did not show the same increase in colour density although the concentrations of the additions were the same.

Figures 3.32 and 3.33 shows a decrease in wine colour density and modified wine colour density as from month 3 to month 9 in all treatments. The reason for this decrease in colour density may be accounted for by the polymerization reactions which has taken place and which lead to precipitation and loss of colour. The wine matured in the third fill barrels showed a slower rate of decrease in colour density because of less tannins to take part in the polymerisation and presipitation reactions. The wine colour density in all the treatments increased from 5-7 OD units to 12-13 OD units in three months. A wine with a colour density of <10 is considered a lighter red wine (lland et al., 2000). Addition of oak elaborated products or maturation of wine in new and used barrels can thus increase the wine colour density and thus the quality of the red wine.

An increase in colour hue from month 3 to month 9 occurred (results not shown). The increase was very small and may be accounted for by a slight increase in brown polymers due to polymerisation reactions catalysed by acetaldehyde as a result of oxidation of ethanol (Ribéreau-Gayon et al., 2000) due to the oxygenation effect of the barrels and to a larger extent due to the oak elaborated products. The topping up of the barrels may also introduce oxygen to the maturing wine.

The third fill barrels showed a slightly higher increase in colour hue than the new oak barrels. The wine treated with the freeze dried tannin also slightly increased the colour hue more than the wines treated with the other oak elaborated products.

Both the degree of red pigment colour and modified degree of red pigment colour showed an increase for all the treatments. Figures 3.34 shows the degree of red pigment colour for the wines treated with chips, blocks, oenotan and oakmore.

An increase in degree of red pigment colour of almost 15 OD units was evident for both the treatments with chips and blocks after 9 months, with oenotan and especially oakmore showing increasing slightly.
The development of degree of red pigment colour and modified degree of red pigment colour of Pinotage in 5th fill 225l barrels treated with oak derived products barrels over a 9 month maturation period.

The wines treated with chips and blocks shows a higher degree of red pigment colouration (Figure 3.34). The reason for this may be because of a more significant oxygenation effect of the more porous blocks and staves which may lead to the formation of ethanal and the subsequent polymerisation of tannins and anthocyanins as confirmed by Timberlake and Bridle (1976) and Ribéreau-Gayon et al. (1983).

The total red pigment value for the barreled wines after 9 months, specifically the new and second fill barrels, were higher than the wines treated with oak derived products (almost 3 OD units).

The reason for the increase in degree of red pigment colour, as discussed in the results and discussion of Project A is because of the condensation reaction between anthocyanins and tannins resulting in colourless flavenes, which can be transformed into the coloured form with subsequent oxygen pick up, as would happen in a wooden barrel. This pigment has a more intense colour than the free forms. Oxygen, as discussed, can also contribute to this by forming ethanal from the oxidation of ethanol, which is involved in ethyl cross bonds between anthocyanins and tannins (the so-called Bayer reaction). The resistance of the coloured pigments later in the maturation period to SO$_2$ bleaching is also clear when one considers the smaller difference in the degree of red pigment colour and the modified degree of red pigment colour later in the maturation process (Ribéreau-Gayon et al., 2000).

Figure 3.35 and 3.36 shows the SO$_2$ resistant pigments. Both the treatments with blocks and chips had slightly higher concentrations of SO$_2$ resistant pigments.

As maturation progresses, polymerisation reactions between tannins and the free anthocyanin forms polymers which are resistant against the bleaching effect of SO$_2$. No significant differences could be detected between the new oak barrels, 2nd fill or 3rd fill barrels. The differences were more in the wines
treated with oak derived products. The wines treated with oak chips and oenotan show the most significant increase reaching a peak after 6 months. The wines treated with oakmore showed a less significant increase up to 6 months and decreased thereafter. All the treatments showed a decrease in SO₂ resistant pigments and the modified estimate of SO₂ resistant pigments after 9 months because of precipitation reactions.

**Figures 3.35 and 3.36** The development of the estimate of SO₂ resistant pigments and the modified estimate of SO₂ resistant pigments of Pinotage in new, 2nd fill, 3rd fill and 5th fill 225L barrels treated with oak derived products barrels over a 9 month maturation period.

After the first 3 months the total phenolics decreased as precipitation reactions progressed during maturation (results not shown). New barrels had more phenolics available for extraction and precipitation which occurred over time. This was the same for the oak derived products.

### 3.3.2.2 Sensory evaluation

A panel of 18 judges (all with relevant qualifications in oenology and viticulture) were asked to rate the wines on a 10-point scale for various organoleptic aspects. The average % values of the different characteristics were calculated and are presented in Figures 3.37 and 3.38.
It is evident that the new oak barrels had a significant impact on all the aspects of the wine as judged by the panel. The new barrels had a significantly higher score average for colour density. This can be confirmed by the higher value of the colour density (Figure 3.32), although the differences between measured the OD Units were small. The perception of "oakiness" of the new oak barrels was even more significant compared to the older barrels.
Common comments of the judges were that the "oakiness" of the wine could be detected. The fruit in the 2nd and 3rd fill barrels were also more intense, probably due to the masking effect of the higher whiskey lactone concentration in the wines matured in the new barrels. The wine matured in the new barrels also had higher tannin intensity on the palate. The wine treated with chips (both the tank and barrels) scored the lowest mark for fruit intensity, with an intense oak bouquet perceived in the wine which matured in barrels treated with chips. Both the wines treated with oakmore and oenotan scored the highest for fruitiness, with the latter scoring low in tannin intensity. It thus seems that the age of the barrel and type of oak derived product played a bigger role in aroma and taste of Pinotage under South-African conditions than on its colour development.

3.4 CONCLUSION

Wine consumers demand for red wines with different flavour profiles. Maturation of red wines in oak barrels lead to wines with more complex sensory properties. The modification of these red wines is attributed to the aroma constituents and phenols that were extracted from the oak. Processing costs involved in the use of oak barrels are extremely high and producers are constantly under pressure to reduce operational costs in wineries for maximal financial gain. The use of oak elaborated products such as chips, staves, blocks and dust have long been considered by-products and are associated with medium to low quality wines.

It appears that the heat gradient generated during the cooperage process results in a wide range of components which bring certain attributes to the wines which were matured there in. Oak derived products though, often produced through simpler processes, show poor chemical diversity and complexity and are often inconsistent in quality.

Nevertheless the experiments conducted showed homogenous tendencies for all the wines, whether they were treated with old or new barrels (French, American or Russian oak barrel types) or oak derived products such as chips, blocks, staves, dust or freeze dried tannin. All the wines showed an initial increase in colour density (because of polymerisation reactions), a slight increase in colour hue (because of limited oxygenation resulting in brown polymers), an increase in SO2 resistant pigments and a decrease in free anthocyanins. The gelatin index values differed, especially in the Pinotage wines after 36 months of bottle maturation. The new oak barrels had a more significant effect than the other treatments probably because of the higher amounts of ellagic tannins that were available for these reactions. Differences between different oak types were negligible small, although the style of red wine may be influenced by the oak type (American oak vs French or Russian) and was evident in the formal tasting after 36 months of bottle maturation.
The effect of terrior is thus more significant in the wines than barrel differences with regards to colour evolution. It should be stressed however, that the most expensive wine to make is a wine with too little extract obtained in the vineyard, to balance significant amounts of oak, whether it is a barrel, chips or blocks.

3.5 ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the following people for their financial, technical and intellectual support: Radoux, Columbit, L.P. Ellis, Prof. M. Lambrechts and Dr. J. Marais.
### Bellingham Pinotage

<table>
<thead>
<tr>
<th></th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>French oak</strong></td>
<td>• Pinotage character (fruitiness) more prominent, coffee-like character</td>
<td>• Wood and natural fruitiness of wine very well integrated</td>
<td>• Very subtle wood, well integrated</td>
<td>• Strong wood character, slightly overshadowing the fruit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Soft, subtle tannins</td>
<td>• Sawdust aroma, less prominent than (c)</td>
<td>• Full bodied</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Very complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>American oak</strong></td>
<td>• More subtle wood, green flavours</td>
<td>• Sweet, boiled sweets, berries, plum flavours</td>
<td>• Sweet caramel, vanilla, butterscotch</td>
<td>• Subtle wood; fruit and wood nicely balanced</td>
</tr>
<tr>
<td></td>
<td>• Typical acetone character of Pinotage prominent</td>
<td>• Wood not dominating</td>
<td>• Subtle fruit</td>
<td>• Slight vanilla overtones</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Subtle wood compliments Pinotage character</td>
<td></td>
</tr>
<tr>
<td><strong>Russian oak</strong></td>
<td>• Vanilla prominent</td>
<td>• Fruit less prominent</td>
<td>• Soft, subtle wood</td>
<td>• Delicate wood with coconut overtones</td>
</tr>
<tr>
<td></td>
<td>• Wood flavours detectable</td>
<td>• Slightly &quot;planky&quot;, sawdust and pencil shaving overtones</td>
<td>• Definite sawdust aroma</td>
<td>• Tannins not in balance with the delicate wood and subtle fruit flavours</td>
</tr>
<tr>
<td></td>
<td>• Dusty, complex flavours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>General preference</strong></td>
<td>French (2); American (0); Russian (4)</td>
<td>French (4); American (4); Russian (2)</td>
<td>French (0); American (4); Russian (2)</td>
<td>French (3); American (5); Russian (1)</td>
</tr>
<tr>
<td></td>
<td>Panel = 6</td>
<td>Panel = 10</td>
<td>Panel = 6</td>
<td>Panel = 9</td>
</tr>
</tbody>
</table>

Table 3.2 A summary of comments and general preferences given by the panel of tasters of Bellingham Pinotage.
## KANONKOP PINOTAGE

<table>
<thead>
<tr>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>French oak</strong></td>
<td>• Strong berry overtones</td>
<td>• Strong Pinotage-character</td>
<td>• Strong Pinotage-character</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Slightly “planky”; sawdust-aroma</td>
<td>• Strange, perfume character; “furniture polish”</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>American oak</strong></td>
<td>• Medicinal, barrel differences</td>
<td>• Fruity, berries, slightly medicinal overtones</td>
<td>• More subtle wood</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Vanilla wood overpowering</td>
<td>• Cultivar character prominent</td>
</tr>
<tr>
<td><strong>Russian oak</strong></td>
<td>• Vanilla, prominent wood and fruit</td>
<td>• Soft, subtle wood</td>
<td>• Soft, subtle wood</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Strong fruit</td>
<td>• Sawdust aroma (“old barrel wood character”)</td>
</tr>
<tr>
<td><strong>General preference</strong></td>
<td>French (1); American (0); Russian (5)</td>
<td>French (4); American (4); Russian (1)</td>
<td>French (2); American (3); Russian (1)</td>
</tr>
<tr>
<td></td>
<td>Panel = 6</td>
<td>Panel = 9</td>
<td>Panel = 6</td>
</tr>
</tbody>
</table>

Table 3.3 A summary of comments and general preferences given by the panel of tasters of Kanonkop Pinotage
<table>
<thead>
<tr>
<th></th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>French oak</td>
<td>• Berry, fruit, harsh tannins</td>
<td>• Intense, ripe berries and chocolate overtones</td>
<td>• Spicy, perfume, peppery flavours</td>
<td>• Barrel differences</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Slight &quot;green&quot; finish</td>
<td>• Harsh tannins</td>
<td>• Wood and fruit flavours well integrated; almost peppery</td>
</tr>
<tr>
<td>American oak</td>
<td>• Subtle wood, softness</td>
<td>• Strong vanilla, caramel, toffee flavours slightly dominating the fruit</td>
<td>• Subtle wood, slightly &quot;green&quot; flavours</td>
<td>• Strong vanilla</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Wood and subtle fruit not well integrated</td>
<td>• Wood not as well integrated as (c)</td>
<td>• Lacks Shiraz-character</td>
</tr>
<tr>
<td>Russian oak</td>
<td>• Most wood flavours, smoky, sweet</td>
<td>• Subtle wood; almost earthy organic</td>
<td>• Delicate wood, well integrated</td>
<td>• Shiraz-character (fruitiness) more prominent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Compliments the fruitiness of the wine</td>
<td>• Subtle wood, well balanced</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Light dustiness</td>
<td></td>
</tr>
<tr>
<td>General preference</td>
<td>Russian (4) French (2) American (0)</td>
<td>French (6) Russian (2) American (1)</td>
<td>Russian (4) French (1) American (1)</td>
<td>Russian (4) French (3) American (2)</td>
</tr>
<tr>
<td></td>
<td>Panel = 6</td>
<td>Panel = 9</td>
<td>Panel = 6</td>
<td>Panel = 9</td>
</tr>
</tbody>
</table>

Table 3.4 A summary of comments and general preferences given by the panel of tasters of Vergelegen Shiraz
## ALTYDGEDACHT SHIRAZ

<table>
<thead>
<tr>
<th></th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>French oak</strong></td>
<td>• Peppery, berry, good wood</td>
<td>• Prominent, ripe berries</td>
<td>• Slight green and &quot;jammy&quot; flavours</td>
<td>• Removed from barrel at this stage</td>
</tr>
<tr>
<td></td>
<td>• Wood not dominating, well integrated</td>
<td>• Wood not dominating, well integrated</td>
<td>• Wood and fruitiness well balanced</td>
<td></td>
</tr>
<tr>
<td><strong>American oak</strong></td>
<td>• Most prominent wood</td>
<td>• Vanilla, caramel-character dominates the fruit</td>
<td>• Coffee, chocolate with subtle sweet overtones</td>
<td>• Removed from barrel at this stage</td>
</tr>
<tr>
<td></td>
<td>• Slight &quot;plankiness&quot; or &quot;dustiness&quot;</td>
<td>• Slight &quot;plankiness&quot; or &quot;dustiness&quot;</td>
<td>• Wood compliments rather than dominates</td>
<td></td>
</tr>
<tr>
<td><strong>Russian oak</strong></td>
<td>• Sweet woody, vanilla and chocolate</td>
<td>• Wood very subtle, compliments ripe, berry</td>
<td>• Subtle wood; less complex wood flavours</td>
<td>• Removed from barrel at this stage</td>
</tr>
<tr>
<td></td>
<td>• Tannin structure not integrated</td>
<td>character</td>
<td>• Definite &quot;sawdust&quot;-aroma</td>
<td></td>
</tr>
<tr>
<td><strong>General preference</strong></td>
<td>French (2) American (2) Russian (2)</td>
<td>French (3) American (3) Russian (3)</td>
<td>American (5) Russian (1) French (0)</td>
<td>• Removed from barrel at this stage</td>
</tr>
<tr>
<td></td>
<td>Panel = 6</td>
<td>Panel = 9</td>
<td>Panel = 6</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.5 A summary of comments and general preferences given by the panel of tasters of Altydgedacht Shiraz
Table 3.6 A summary of comments and general preferences given by the panel of tasters of Bellingham Pinotage after 4 months of bottle maturation.

<table>
<thead>
<tr>
<th>Oak</th>
<th>After 4 months bottle maturation</th>
<th>Colour</th>
<th>Nose</th>
<th>Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>French</td>
<td>Light, ruby red</td>
<td>Smokey, vanilla, ripe bananas, subtle oak aromas.</td>
<td>Firm tannins, intense fruit.</td>
<td></td>
</tr>
<tr>
<td>American</td>
<td>Dark purple, most intense</td>
<td>“Sweet”, vanilla with strong spicy/licorice overtones.</td>
<td>Fruit follows through nicely, soft tannins. Bittery/Dryish/Planky aftertaste.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wine</th>
<th>Ranking (Preference*)</th>
<th>Overall quality average on 20 point scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colour</td>
<td>Nose</td>
</tr>
<tr>
<td>French</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>American</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Russian</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

*Tasting Panel consisted out of 12 judges
Table 3.7 A summary of comments and general preferences given by the panel of tasters of Kanonkop Pinotage after 4 months of bottle maturation.

<table>
<thead>
<tr>
<th>Oak</th>
<th>After 4 months bottle maturation</th>
<th>Colour</th>
<th>Nose</th>
<th>Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>French</td>
<td>Bright purple red, intense red robe</td>
<td>Black plums, berries, subtle oak flavours well integrated.</td>
<td>Oak prominent on taste, though good balance between oak and fruit.</td>
<td></td>
</tr>
<tr>
<td>American</td>
<td>Dark, plum with brick red rim - more intense than French oak</td>
<td>Vanilla (medium - strong intense), coconut</td>
<td>Bittery/dryish finish, almost walnut character.</td>
<td></td>
</tr>
<tr>
<td>Russian</td>
<td>Plum red colour</td>
<td>Spicy, peppery. Typical Pinotage character not dominated by oak. Oak derived flavours less intense.</td>
<td>Soft tannins, intense fruit.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wine</th>
<th>Ranking (Preference*)</th>
<th>Overall quality average on 20 point scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colour</td>
<td>Nose</td>
</tr>
<tr>
<td>French</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>American</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Russian</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

*Tasting Panel consisted out of 12 judges
Table 3.8 A summary of comments and general preferences given by the panel of tasters of Vergelegen Shiraz after 4 months of bottle maturation.

<table>
<thead>
<tr>
<th>Oak</th>
<th>After 4 months bottle maturation</th>
<th>Nose</th>
<th>Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>French</td>
<td>Ruby red with purple rim</td>
<td>Spiced fruit, subdued by oak undertones. Less complex than Russian oak.</td>
<td>Well-balanced, soft tasty oak aromas, more intense than Russian oak. Oak integration superb.</td>
</tr>
<tr>
<td>American</td>
<td>Darkest, ruby red</td>
<td>Vanilla prominent</td>
<td>Oak more prominent an aftertaste than Russian and French oak. Strong wood profile an palate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wine</th>
<th>Ranking (Preference*)</th>
<th>Overall quality average an 20 point scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>French</td>
<td>4 6 3</td>
<td>15.0</td>
</tr>
<tr>
<td>American</td>
<td>4 5 6</td>
<td>15.4</td>
</tr>
<tr>
<td>Russian</td>
<td>5 2 4</td>
<td>15.5</td>
</tr>
</tbody>
</table>

- Tasting Panel consisted out of 12 judges
Table 3.9 A summary of comments and general preferences given by the panel of tasters of Altydgedacht Shiraz after 4 months of bottle maturation.

<table>
<thead>
<tr>
<th>Oak</th>
<th>After 4 months bottle maturation</th>
<th>Colour</th>
<th>Nose</th>
<th>Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Smokey, black cherries. Slight herbaceous overtones</td>
<td>Slight planky, bitter aftertaste</td>
</tr>
<tr>
<td>French</td>
<td>Dark, ruby red colour.</td>
<td>French Ruby red colour</td>
<td>Sweet spice, vanilla, blackberry fruit, cassis.</td>
<td>Oak flavours on palate. Subtle, almost sweet, tannins. Good oak integration</td>
</tr>
<tr>
<td></td>
<td>Most intense</td>
<td></td>
<td>Intense ripe fruit with definite peppery overtones</td>
<td>Fruit intense on palate. Also spicy and peppery aftertaste. Most typical Shiraz-character of three.</td>
</tr>
<tr>
<td>Russian</td>
<td>Ruby red colour.</td>
<td>Russian Ruby red colour</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wine</th>
<th>Ranking (Preference*)</th>
<th>Overall quality average on 20 point scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colour</td>
<td>Nose</td>
</tr>
<tr>
<td>French</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>American</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Russian</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

* Tasting Panel consisted out of 12 judges
3.6 REFERENCES CITED


De Freitas, V.A. Characterisation of oligomeric and polymeric procyanidins from grape seeds by liquid secondary ion mass spectrometry. Phytochemistry. 49, 1435-1441.


Chapter 4

Research Results

The influence of lees contact and pH on the development of micro flora and colour of wine during conservation in barrels.
4. RESEARCH RESULTS

The influence of lees contact and pH on the development of microflora and colour of wine during conservation in barrels

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\textsuperscript{1}Department of Viticulture and Oenology, University of Stellenbosch, Z.A.-7600, Stellenbosch, South-Africa
\textsuperscript{2}Diemersfontein Wines, P.O.Box 41, Wellington, 7654

Organoleptic comparisons between red wines which underwent malolactic fermentation (MLF) in oak barrels and in stainless steel tanks often reveals the wines from the barrels to be better. MLF in barrels result in wines with appreciable colour and phenolic modifications. The colour density, SO\textsubscript{2} resistant pigments and degree of red pigment colouration increases as a result of polymerization reactions. Astringency decreases as a result of tannins increasing in their degree of polymerization. The oak aromas incorporate themselves in the aroma profile of the wines. These are all favourable modifications. This study showed that no correlation between the amount of acetic bacteria, lactic acid bacteria and yeast numbers and lees treatments could be detected during ageing of Shiraz in oak barrels. Higher pH must underwent a slower alcoholic fermentation rate which resulted in longer skin contact and thus a more intense colour. It was evident that free SO\textsubscript{2} concentration maintenance of 35 mg/L did not inhibit the survival of all the microbes during the conservation period of these wines in the barrels. Bulk-filtration after MLF did not have a significant effect on the microbial population or the colour development of the wines over a twelve month period, although the concentration of yellow brown pigments (OD 420 nm) was lower in the wines which matured on the lees and underwent MLF in the barrel.
4.1 INTRODUCTION

"Unfiltered", "no-fining" and "naturally fermented" are just some of the terms that may form part of a cellar's brand building exercise. It is believed that these terms will project a message of grapes grown with minimal intervention and the wine produced from it (by using no commercially produced yeasts, only gravity flow as a physical process to transfer the wine from vessel to vessel, and absolutely no fining or filtrations), will produce wine of superior quality. It is believed that physical actions on the wine that is finings, filtrations and transfers by means of pumps, may alter the composition, quality and finesse of the wine negatively. Keeping wines on lees enrich these wines with polysaccharides which resulted from the hydrolysis of yeast walls (autolysis). The effect is less significant in tanks because of the larger exchange surface than barrels. The process of sur lie or lees contact is generally used in the production of white wines and is less common in red wine production. Keeping red wines especially on bacterial lees after malolactic fermentation is seen as a "dangerous" process, because of the higher pH in wines and related implications.

The clarification and stabilization of wine is a delicate chemical and physical science and different techniques or principles regarding wine stability have evolved (Miller et al., 1985; Hsu et al., 1987; Peri et al., 1988; Ricardo-da-Silva et al., 1991; Sims et al., 1995; Iritani and Mukai, 1997; Riberéreau-Gayon et al., 2000). Limited research has been done on different filtrations and pump technology and its influence on the composition of wine. Research has been done on ultra filtration and its influence on enhancing the stability of wine (Flores et al., 1990) by removal of suspended particles, thus enhancing the clarity of the wine, removal of polymeric flavanoids, proteins and oxidizing enzymes, thus increasing the resistance of the wine against oxidative browning and enhancing the microbial stability of the wine by removal of micro-organisms (Cattaruzza et al, 1987). Cattaruzza et al. (1987) documented the removal of a high molecular weight compound proposed to be a polymeric flavanoid or a protein-flavanoid complex. They also stated a strong reduction in the soluble colloid content of the wine, which may affect the taste and tartrate stability of the wine. Cattaruzza et al. (1987) confirmed non-selective removal of phenolics by HPLC analysis. Their findings failed to confirm a decrease in the colour hue due to the reduction in brown polymers. Ellis (2002) documented that cross flow filtered wines were slightly lower in colour density compared to unfiltered wines; colour hue was unaffected and had a slightly lower total red pigment colour.
Puig Deu et al. (1996) found no modification in the chemical parameters of a wine treated with a rotary vacuum filter and different fining methods. They documented a decrease in some wine components e.g. nitrogenous substances, phenolic compounds and aroma according to these treatments, and significantly high volume losses where rotary vacuum filters and bentonite-finings were used. Several authors documented the dynamics regarding the growth during fermentation and maturation of wines (Costello et al., 1983; Lafon-Laforcade, 1983; Fleet et al., 1984; Heard and Fleet, 1985; Du Toit, 2000). Yeasts may be the cause of wine spoilage if natural fermentation is allowed (Bisson, 1999; Ribéreau-Gayon, et al., 2000). The occurrence and growth of lactic bacteria (Wibowo, et al., 1985), the oenological significance of lactic bacteria and their contribution towards the organoleptical profiles of wines (Davis, et al., 1985; Davis et al., 1988; Avedovech, et al., 1992; Costello, 1993; Sauvageot and Vivier, 1997) have been well documented, although it might also be responsible for off flavours (Boulton at al., 1996) and sluggish or stuck fermentation (Bisson, 1999). The role of acetic bacteria in the spoilage of wine has been well documented (Du Toit, 2000; Du Toit and Pretorius, 2002).

Relatively little research has been done on the effective removal of microorganisms by filtration. Little literature is available on the comparison of different filtration techniques and their ability to enhance the stability of a wine. Limited literature is available on the comparison of treated wines and its influence on certain quality parameters e.g. colour, mouthfeel variables e.g. integration of tannin from oak and grapes, weight (as a function of autolysis) and organoleptic intensity, longevity of the wine and general parameters i.e. volatile acidity, pH etc. The effect of long-term storage of red wine on lees is also relatively unknown and can be considered a dangerous operation.

One of the most important aspects regarding the quality of wine is pH. Several authors documented the importance of pH management in wines (Boulton et al., 1996) especially with regards to microbial stability. Several authors documented the influence of a high pH on the viability of microorganisms (Boulton et al., 1996). The purpose of this study was to look at the influence of maturation of red wine on bacterial lees (as a function of drum filtration) at different pH levels (high and low) on the viability of microorganisms and the influence on colour during the wine’s conservation in barrels and to compare the effect of malolactic fermentation in barrels with malolactic fermentation in tanks.
4.2 MATERIALS AND METHODS

4.2.1 Vinification and treatment of the wine

Shiraz grapes from the same block in the Stellenbosch Wine of Origin area were harvested, crushed and separated equally into two separate rotor fermentation tanks.

The pH of the one tank (R2) was adjusted to 3.5 by means of tartaric acid and the other tank remained at a pH 3.8. Both tanks underwent cold soaking for 5 days at a temperature of 12°C prior to inoculation. Both tanks were inoculated with the WE 372-strain (*Saccharomyces cerevisiae* – Anchor Yeast) at an inoculum level of 25g /hl DAP (diammonium phosphate) at a concentration of 45 g/L was added in three lots during fermentation. The fermentation temperature was controlled at 26°C to 28°C. Both the tanks were turned five times per day to mix the fermenting skins and must until alcoholic fermentation was completed. The tanks were pressed (Sutter E50 press) after alcoholic fermentation was completed. Press wine of up to a fraction of 0.8 bar pressure was added to both lots. Both batches were racked 48 hours after the press cycle to reduce the amount of gross lees. Malolactic fermentation (MLF) was then induced by addition of *Oenococcus oenos* (strain LOD 89004 - Darleon). The free SO₂ concentration was adjusted to 35 mg/L after completion of MLF.

A fraction of each tank was separated for malolactic fermentation in barrels, while the rest underwent MLF in stainless steel tanks. MLF was monitored weekly by the method of Iland *et al.* (2000). The barrel cellar had an average temperature of 18°C and the processing cellar an average temperature of 16°C. Of both tanks, the volume of wine for one barrel was filtered using a Cadalope bulk drum filter Celatom FP 3), after completion of malolactic fermentation and transferred into a barrel. Another barrel of each batch was left on the secondary fermentation lees for maturation on the lees in the barrel.

The lots that underwent malolactic fermentation in barrels were racked separately and treated the same, respectively (in other words, one filtered and one left on the lees). The free SO₂ level was measured every 6 weeks and maintained at 35 mg/L during the course of maturation. The barrels were all new 225L French oak (medium toast) barrels from the same batch supplied by Radoux Cooperage.
4.2.2 Analysis

4.2.2.1 Microbial analysis

Samples were taken directly after the processing of the grapes, after 5 days of cold soaking, alcoholic fermentation, MLF and every 3 months over a period of 9 months.

Acetic acid bacteria were isolated by plating 100 μl of a dilution series of juice or wine onto GYC [glucose (5% m/v), yeast extract (1% m/v), CaCO₃ (3% m/v) and agar (2% m/v)] and Mannitol [mannitol (2.5% m/v), yeast extract (1%) and agar (1.5% m/v)] media. Yeast and lactic bacteria growth were eliminated by the addition of 50 mg/L pimaricin (Delvocid, Gist-Brocades) and 50 mg/L nicin, respectively, to the media. Yeast strains were cultured on YPD medium containing 1% m/v yeast extract, 2% m/v bacto-peptone, 2% m/v glucose and 1.5% m/v agar. Lactic acid bacteria were plated out on MRS (Merck, Germany) and Acid Grape (AG) (Dicks et al., 1990) agar. Both media were supplemented with 50 mg/L Delvocid (Delvocid, Gist-Brocades) and 25 mg/L kanamycin sulphate (Boehringer Mannheim, Germany) for the inhibition of yeast and acetic acid bacteria. Plates were incubated for 6 to 8 days at 30°C after which representative colonies were isolated. Gram staining and catalase tests were performed on representative bacterial colonies.

4.2.2.2 Colour analysis

Spectral analysis was done according to the method of Iland et al. (2000) after 12 months of barrel maturation on the 8 barrels. The spectral measurements included the colour and modified colour density, hue and modified colour hue, degree and modified degree of red pigment colour, total phenolics and the total red pigment colour.

4.3 RESULTS AND DISCUSSION

4.3.1 Results on microbial counts

The high pH medium influenced the viability of the microorganisms favourably before MLF. All the media showed significantly higher amounts of
microorganisms, for both lactic bacteria-species, acetic bacteria-species and yeasts (results not shown). Higher levels of bacteria during initiation of alcoholic bacteria may lead to high-risk fermentations or even stuck fermentations. Many authors documented acetic acid bacterial growth as one of the reasons for stuck fermentation (Bisson, 1999; Du Toit et al., 2000). Other disadvantages include the production of acetic acid and acetoin compounds as a result of lactic bacterial growth, (Vivas et al., 1995; Ducruet, 1997), production of metabolites by yeast species that may inhibit growth of the inoculated species (Bisson, 1999) and depletion of nutrients in the fermentation medium by unfavorable yeasts (Bisson, 1999). This was evident in the slower onset of alcoholic fermentation in the tank with the higher pH (results not shown). The rate of alcoholic fermentation in tank R1 (pH>3.6) was slower than in tank R2 (pH<3.6) resulting in longer maceration time of approximately 36 hours.

Although there was not a significant difference in pH after malolactic fermentation, the tank with the higher pH underwent malolactic fermentation slightly faster (results not shown).

The rate of malolactic fermentation in the barrels (seven weeks) was significantly slower than in the tanks (four weeks). Saraiva (1983) was one of the first authors to document on the relations between lactic acid bacteria, yeasts and and phenolic compounds in wine (Vivas, 1995; Vivas et al., 2000). Vivas (1995) documented gallic acid and the free anthocyanins to have desirable effects on the viability of Oenococcus, while, on the contrary, the procyanidins to be strong inhibitors of bacterial viability. Vivas also noted extracts from oak wood to possess the same properties as the procyanidins. Ducruet (1997) documented a slight decrease in fermentation when it is performed in barrels, which confirmed the results of Vivas (1995). The average temperature in the maturation cellar was also lower than in the fermentation cellar, which may have contributed to slower malolactic fermentation. A lower level of yeast counts was found in R1, which resulted in a slower alcoholic fermentation rate. Alcoholic fermentation lasted approximately 36 hours longer than in R2 and had a significantly longer lag phase. It was evident that yeast cells can remain viable during the maturation phase (results not shown), even though all the wine underwent bulk filtration. This is a very important consideration that some wine defects can be contributed to spoilage by yeasts, for example Brettanomyces and the production of off-flavours 4-vinylphenol and 4-ethylguaiacol (Ribéreau-Gayon, et al., 2000).

A significant difference in the number of lactic acid bacteria cells is probably due to the difference in pH during cold maceration and alcoholic fermentation. Lactic acid bacteria numbers increased from about $1 \times 10^3$ cfu/mL...
to $1 \times 10^4$ and $3 \times 10^3$ cfu/mL in tanks R2 and R1 during cold soaking, respectively. This increased to $1.2 \times 10^4$ and $8 \times 10^3$ cfu/mL after alcoholic fermentation.

It is possible for malolactic fermentation to occur spontaneously prior to completion of alcoholic fermentation, resulting possibly in stuck or sluggish fermentation (Bisson, 1999) or the production of off odours related to the spontaneous growth of lactic acid bacteria (Bisson, 1999). It is thus an important aspect to consider inhibiting compounds such as SO₂ and lipozyme in the control of lactic bacterial numbers during skin contact prior to alcoholic fermentation.

Acetic acid bacterial numbers also increased during the cold soaking phase, especially in the tank with the higher pH must (R2) originally and during alcoholic fermentation. This correlates with results obtained by Du Toit and Lambrechts (2002) on acetic acid bacterial growth in high pH musts. There is an amount of viable cells present after malolactic fermentation and even further survival in the barrels as time progresses (Table 4.1).

A free SO₂ level of 35 – 40 mg/L did not have a significant effect on the number of viable cells during maturation. Barrels F and G even showed an increase during the last 6 months of maturation (Table 4.1). The topping up of the barrels during maturation might be responsible for this. Du Toit et al. (2004) documented the varying resistance of different strains of acetic bacteria against SO₂, although Acetobacter pasteurianus could survive under anaerobic conditions in wine in the presence of SO₂. Delfini and Morsiani (1992) documented varying levels of resistance of lactic acid bacteria against significant concentrations of SO₂ and the possibility of increasing their levels of resistance against SO₂ by adaptation. There was however no significant correlation between lees contact and lactic acid bacterial and yeast numbers during maturation (results not shown). The same can also be seen in Table 4.1 for acetic acid bacterial numbers during maturation.

Maturation of wine on secondary lees is, however, a vinification practice that needs close monitoring and good management. Aspects regarding SO₂ management, pH-management and maturation conditions needs close attention, especially maturation temperature. Vivas et al (1995) concluded that maturation
<table>
<thead>
<tr>
<th>Treatments of must</th>
<th>After crushing/destemming</th>
<th>After 5 days skin maceration</th>
<th>After alcoholic fermentation</th>
<th>After malolactic ferm</th>
<th>After 6 months in barrel</th>
<th>After 12 months in barrel</th>
<th>After 18 months in barrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 (pH 3.8)</td>
<td>1x10^2</td>
<td>5x10^7</td>
<td>7x10^7</td>
<td>6x10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2 (pH 3.5)</td>
<td>1x10^2</td>
<td>1x10^6</td>
<td>1x10^7</td>
<td>5x10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barrel malolactic ferm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barrel A *</td>
<td></td>
<td></td>
<td></td>
<td>1x10^6</td>
<td>5x10^6</td>
<td>2x10^4</td>
<td>0</td>
</tr>
<tr>
<td>Barrel B §</td>
<td></td>
<td></td>
<td></td>
<td>2x10^6</td>
<td>2x10^4</td>
<td>0</td>
<td>4x10^2</td>
</tr>
<tr>
<td>Barrel C *</td>
<td></td>
<td></td>
<td></td>
<td>2x10^5</td>
<td>2x10^4</td>
<td>5x10^3</td>
<td>5x10^3</td>
</tr>
<tr>
<td>Barrel D §</td>
<td></td>
<td></td>
<td></td>
<td>1x10^5</td>
<td>1x10^4</td>
<td>0</td>
<td>3x10^1</td>
</tr>
<tr>
<td>Tank malolactic fermentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barrel E *</td>
<td></td>
<td></td>
<td></td>
<td>5x10^3</td>
<td>5x10^6</td>
<td>1x10^3</td>
<td>1x10^1</td>
</tr>
<tr>
<td>Barrel F *</td>
<td></td>
<td></td>
<td></td>
<td>6x10^3</td>
<td>5x10^6</td>
<td>0</td>
<td>2x10^3</td>
</tr>
<tr>
<td>Barrel G §</td>
<td></td>
<td></td>
<td></td>
<td>5x10^3</td>
<td>4x10^4</td>
<td>0</td>
<td>3x10^3</td>
</tr>
<tr>
<td>Barrel H §</td>
<td></td>
<td></td>
<td></td>
<td>6x10^3</td>
<td>3x10^4</td>
<td>6x10^3</td>
<td>6x10^3</td>
</tr>
</tbody>
</table>

**Table 4.1** Acetic acid bacteria counts (cells/mL) during the vinification process with different treatments. *: Wine filtered after malolactic fermentation. §: Wine not filtered after malolactic fermentation.
of wine in excess of temperatures of 17 – 18°C may provoke an increase in volatile acidity.

4.3.2 Results on colour analysis

Ducruet (1997) documented better and faster evolution of the red colour of a wine, with an increase in colour stability, compact tannin structure, more rapid clarification and less astringent and condensed tannins if these wines were matured in oak barrels. Figure 4.1 shows the results of the colour density and modified wine colour density. From an organoleptic point of view, no significant differences in colour were observed. Interestingly, barrels E and F (wine with adjusted pH) showed lower colour density and modified colour density values (Figure 4.1).

![Figure 4.1 The colour density and modified wine colour density of Shiraz wine matured in new 225 L French oak barrels (Medium toast).](image)

This may be accounted for by the faster alcoholic fermentation rate initially, thus resulting in a shorter maceration time and MLF in tanks, with less extraction of grape and oak tannins which contributed to the stabilization of colour. There were little differences in colour density between the wines from the tanks and barrels that were filtered or left on the lees.

Table 4.2 shows the 420nm absorbance-values of the different treatments. Interestingly, the lowest 420-values correlate with the wines that underent MLF in the barrels and were kept on the lees during the course of maturation. The
420nm-absorbance value gives an estimate of the concentration of yellow brown pigments at normal wine conditions (Somers, 1978). This correlates with the findings of Chatonnet et al., 1991). The wines, which underwent MLF in the barrels, could thus have extracted more oak tannins that were absorbed by the lees.

Table 4.2 The OD420-Absorbance values of the different treatments.

<table>
<thead>
<tr>
<th>Barrel/Treatment</th>
<th>420 nm value (10 mm pathway)</th>
<th>MLF Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.1</td>
<td>TA</td>
</tr>
<tr>
<td>B</td>
<td>5.0</td>
<td>TA</td>
</tr>
<tr>
<td>C</td>
<td>5.5</td>
<td>BA</td>
</tr>
<tr>
<td>D</td>
<td>4.0</td>
<td>BA</td>
</tr>
<tr>
<td>E</td>
<td>3.7</td>
<td>TA</td>
</tr>
<tr>
<td>F</td>
<td>3.9</td>
<td>TA</td>
</tr>
<tr>
<td>G</td>
<td>5.9</td>
<td>BA</td>
</tr>
<tr>
<td>H</td>
<td>4.9</td>
<td>BA</td>
</tr>
</tbody>
</table>

*: All the barrels with wine maturing on the lees.
TA: Tank MLF
BA: Barrel MLF

The average total red pigment colour showed little differences, although barrels C, D, F and H showed values slightly higher than A, B, E and G.

The total phenolic and colour hue value shows very little differences. Figure 4.3 shows that Barrels C, G and H have values higher than those of the other barrels.

Figure 4.2 The total phenolics of Shiraz wine matured in new 225 L French oak barrels (Medium toast)
4.4 CONCLUSION

There is a reasonably good correlation between the different treatments and the results. It is evident that MLF does not evolve in tanks in the same way as in new barrels. The development of bacteria and the metabolism of malic acid are slightly delayed as a function of certain oak phenols. It was evident that microbial cells remain viable during the whole maturation process, even after a racking (to enhance the clarity of the wine) and filtration. Although benefits of maturation on the wine lees are known, it is seen as a dangerous practice in the production of red wines because of the high pH of the wines and related problems that may occur. It thus seems that microbial development during cold soaking, alcoholic fermentation and during MLF is rather dependant on pH. No correlation between acetic acid bacteria, lactic acid bacteria and yeast and maturation on the lees, however, were found during barrel maturation. Although slight differences could be spectrophotometrically detected after 12 months of barrel maturation, organoleptic differences in colour could not be detected. Three of the four wines that were kept in the barrels during MLF showed higher colour density and total phenolic concentration than the wines that underwent MLF in the tanks. The influence of filtration per se on colour parameters was negligibly small. Skin contact time, as well as the choice of the container for MLF (stainless steel tanks vs. oak barrels) thus seems to influence the colour of red wines more, even after 12 months of barrel maturation. Optimal management of certain processes in the cellar, i.e. pre-fermentation cold soaking, rackings, filtration processes, autolysis and general hygiene is very important as they can influence the wine quality.

4.5 ACKNOWLEDGEMENTS

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3.6 LITERATURE CITED


Chapter 5

General Discussion & Conclusions
5. GENERAL DISCUSSION AND CONCLUSIONS

5.1 CONCLUDING REMARKS AND OTHER PERSPECTIVES

Grape growers and wine makers have three challenges. The quality of red wines is closely related to the chemical and physiological composition of the grapes it's made from. Several primary processes influence the composition of grapes as from establishing the vineyards to the mature phase of the vineyards where it projects its harvest every year. "Winemaking starts in the vineyard" has grown to be more than simply a cliché used by the marketing department of every cellar. Thus, in depth knowledge of varietals, clones, rootstock, terroir, vine physiology, and all the dynamics in optimally nurturing the grapes (for example canopy management, irrigation, etcetera) is a crucial requirement in order to obtain healthy and premium quality grapes. This is the first challenge.

The second challenge is to harvest at the optimal ripeness level. Words like "optimal ripeness" or "physiological ripeness" have been the topic of much controversy. "Optimal ripeness" is probably the correct term to use as it refers to the delicate balance between the chemical parameters (sugar, acidity, pH, etcetera) and the status of polymerisation or ripeness of the phenolic compounds (anthocyanins and tannins) and aroma constituents of grapes. Several authors documented on the relationship between phenolics and grapes on wine quality (Timberlake and Bridle, 1976; Czochanska et al., 1979; Robichaud et al., 1990; Nicolini et al., 1998; Brossaud et al., 1999; Cheynier et al., 2000; De Freitas et al., 2000; Ristic et al., 2000; Sun et al., 2001).

The third challenge is the transfer of these optimally harvested grapes to premium quality wine. Processing and vinification practises have a significant influence on the quality of wine. The importance of optimum vinification has been documented by several authors, including Ough (1975) who has written his thesis, "Studies to improve wines and winery practices", on this subject. Barrel maturation is one of the important steps in the production of quality wines. The benefits of maturation in barrels were mentioned in Chapter 2 of this thesis. The influence of oak on the colour development of wines has been documented by several authors (Somers, 1974; 1983).

As discussed earlier in this thesis, cellars are under constant pressure to reduce the production cost of wine. Unfortunately it is the cost of barrel maturation, which is the most expensive component of the production costs of premium quality wine. There is, however, growing demands for wine with an oak dimension in their sensorial profile. Thus, the alternative is the use of oak
derived products for example chips, blocks, staves, dust, etcetera. Limited knowledge about the quality of these oak derived products and their use has kept winemakers from using it. This project has shown that these oak derived products have the same effect on colour development as the use of barrels. The Pinotage wine used in this study also showed the biggest difference in colour between the barrels of different ages compared to the difference in different oak derived products. The contribution of "oakiness" on the wines of new barrels was significantly more than used barrels and oak derived products. This study has also shown that the origin of the oak (French versus American versus Russian) does not influence the colour development of South-African Shiraz and Pinotage differently during both barrel and bottle maturation. The influence and importance of colour on the quality perception of wines have been documented by several authors (Ribéreau-Gayon, 1973; Somers and Evans, 1974; Singleton and Noble, 1976; Jackson et al., 1978; Lanthany, 1999; Parr et al., 2003; Bamett, 2004; Gischen et al., 2002).

Research must still be conducted on the use of different types of oak derived products with different types of seasoning (natural versus artificial), different types of oak (French, American, Russian and perhaps Chestnut) and different levels of toasting (light, medium and heavy) and their influence on the aromatic and phenolic profiles of wines during various production phases like malolactic fermentation, alcoholic fermentation, maturation, skin maceration, etcetera. Limited research has been done on the combination of these products with micro and macro-oxygenation under South-African conditions, especially on Pinotage.

Intensive market research must be done in association with oenological research. It is important that "identity profiles" for all the South-African brands competing in different price categories in different markets (locally and especially internationally) should be benchmarked against the "identity profiles" of competing countries (such as Chile, California, Argentina and Australia) as well as financially viable techniques in order to reduce operational costs to establish wines which suit these profiles to have maximum capital gain.

Ongoing research on oak barrels and oak derived products under South-African conditions can assist to achieve this goal.

"We are like dwarfs on the shoulders of giants, so that we can see more than they... not by virtue of any sharpness of sight on our part, or any physical distinction, but because we are carried high and rose up by their giant size."
5.2 LITERATURE CITED


