ANTIOXIDANT CAPACITY OF PINOTAGE WINE AS AFFECTED BY VITICULTURAL AND ENOLOGICAL PRACTICES

DALENE DE BEER

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Promotor: Dr E. Joubert
Co-promotors: Dr J. Marais
Dr M. Manley

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DECLARATION

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

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Dalene de Beer Date

ABSTRACT

The aim of the study was to provide the South African wine industry with guidelines for the production of Pinotage wines with optimal total antioxidant capacity (TAC), while retaining sensory quality. The contribution of individual phenolic compounds to the wine TAC is important in this regard. The wine TAC was measured with the 2,2'-azino-di(3-ethylbenzo-thiazoline-sulphonic acid radical cation) ($ABTS^{\bullet+}$) scavenging assay. The contributions of individual phenolic compounds to the wine TAC were calculated from their content in the wines and the Trolox equivalent antioxidant capacity (TEAC) of pure phenolic standards. The effects of climate region, vine structure, enological techniques (pre-fermentation maceration, juice/skin mixing, addition of commercial tannins, extended maceration) and maturation (oak barrels, alternative oak products, oxygenation) on the phenolic composition, TAC and sensory quality of Pinotage wines were also investigated.

The TEAC values of quercetin-3-galactoside, isorhamnetin and peonidin-3-glucoside were reported for the first time. TEAC values observed for most compounds were much lower than those reported previously, although TEAC values for gallic acid, caftaric acid, caffeic acid and kaempferol were consistent with some previous reports. Caftaric acid and malvidin-3-glucoside were the largest contributors to the wine TAC. The contents of monomeric phenolic compounds and procyanidin B1, however, only explained a small amount (between 11 and 24%) of the wine TAC, with the remaining TAC attributed to oligomeric and polymeric phenolic compounds and other unknown compounds. Some synergy between different monomeric phenolic compounds was also demonstrated.

All the viticultural and enological factors investigated affected the phenolic composition of Pinotage wines, while the wine TAC was only affected by some treatments. Changes in wine TAC could not always be explained by changes in phenolic composition as the contribution of oligomeric, polymeric and unknown compounds could not be assessed, but could play a large role. Differences in wine colour were also difficult to explain due to the large number of factors involved and the dark wine colour, which made objective measurements difficult. The concentration of vitisin A, an orange-red pyranoanthocyanin, was increased consistently as a result of pre-fermentation maceration treatments and affected the wine colour of oxygenated wines. Increased wine TAC was observed when cultivating Pinotage grapes on bush vines and in cooler climatic regions, compared to cultivation on trellised vines in warmer climatic regions. All the climatic regions and vine structure treatments, however, resulted in wines with good sensory quality. In terms of enological techniques, pumping-over, as opposed to punching-down and rotor treatments, is not recommended as a juice/skin mixing technique, due to reduced wine TAC, colour and sensory quality. Pre-fermentation maceration, addition of commercial tannin preparations, and oak maturation using traditional and alternative treatments, resulted in improved sensory quality, but with no change in wine TAC. However, optimisation of the tannin addition protocol may result in increased wine TAC if additions are made after fermentation or higher dosages are used. Oxygenation of Pinotage wine needs further investigation to optimise the protocol, as improvements to the wine colour and fullness were observed for some treatments, but loss of sensory quality and TAC were observed in most cases.

UITTREKSEL

Die doel van die studie was om riglyne aan die Suid-Afrikaanse wynbedryf te verskaf vir die produksie van Pinotage wyne met optimale totale antioksidantkapasiteit (TAK), maar met die behoud van sensoriese kwaliteit. Die bydrae wat individuele fenoliese verbindings tot die wyn-TAK lewer, is belangrik in hierdie opsig. Die wyn-TAK is gemeet met die 2,2'-asino-di(3-etielbensotiasoliensulfoonsuur)-radikaalkatioon ($ABTS^{\bullet+}$) blussingstoets. Die bydrae wat individuele fenoliese verbindings tot die wyn-TAK lewer, is bereken deur hul konsentrasies in die wyn en die Trolox ekwivalente antioksidantkapasiteit (TEAK) van suiwer fenoliese standaarde in ag te neem. Die effek van klimaatarea, stokontwikkeling, wynmaaktegnieke (dopkontak voor gisting, sap/dop vermenging, byvoeging van kommersiële tanniene, verlengde dopkontak) en veroudering (eikevate, alternatiewe eikeprodukte, oksigenasie) op die fenoliese samestelling, TAK en sensoriese kwaliteit van Pinotage wyne, is gevolglik ondersoek.

Die TEAK-waardes van kwersitien-3-galaktosied, isorhamnetien en peonidien-3-glukosied is vir die eerste keer bepaal. Die TEAK-waardes vir meeste verbindings was heelwat laer as gepubliseerde waardes, maar die TEAK-waardes vir gallussuur, kaftaarsuur, kafeësuur en kaempferol was soortgelyk aan dié wat voorheen gerapporteer is. Kaftaarsuur en malvidien-3-glukosied het die grootste bydraes tot die wyn-TAK gelewer. Die inhoud van monomeriese fenoliese verbindings en prosianidien B1 het egter slegs 'n klein hoeveelheid (tussen 11 en 24%) van die wyn-TAK verklaar, terwyl die oorblywende TAK aan oligomeriese en polimeriese fenoliese verbindings en onbekende verbindings toegeskryf kan word. 'n Mate van sinergie tussen verskillende monomeriese fenoliese verbindings is ook aangetoon.

Al die wingerd- en wynboukundige faktore wat ondersoek is, het die fenoliese samestelling van Pinotage wyne beïnvloed, terwyl die wyn-TAK slegs deur sommige behandelings beïnvloed is. Veranderinge in wyn-TAK kon nie altyd deur verskille in fenoliese samestelling verklaar word nie, omdat die bydrae van oligomeriese en polimeriese fenoliese verbindings en onbekende verbindings lewer nie bepaal kon word nie, maar 'n groot rol kan speel. Verskille in wynkleur was ook moeilik om te verklaar weens die groot aantal faktore wat 'n rol speel, en die donker wynkleur wat objektiewe meting bemoeilik. Vitisien A, 'n oranje-rooi pirano-antosianien, se vlakke is deurgaans verhoog deur middel van dopkontak voor gisting, en het die wynkleur van geoksigeneerde wyne beïnvloed. 'n Hoër wyn-TAK is waargeneem waar Pinotage druiwe op bosstokke in koeler klimaatsareas verbou is teenoor verbouing op opgeleide stokke in warmer areas. Al die klimaatsareas en stokontwikkelingsbehandelings het egter wyne met goeie sensoriese kwaliteit gelewer. Betreffende wynmaaktegnieke, word oorpomp- teenoor deurdruk- en rotorbehandelings, nie aanbeveel sap/dop mengtegniek nie, aangesien dit die TAK, kleur en sensoriese kwaliteit van die wyn verlaag het. Dopkontak voor gisting, byvoeging van kommersiële tanniene, en veroudering met behulp van eikehout (tradisionele en alternatiewe eikeprodukte) het wyne met verbeterde sensoriese kwaliteit gelewer, maar geen verandering in wyn-TAK is waargeneem nie. Optimering van die tannienbyvoegingsprotokol behoort verhoogde wyn-TAK tot gevolg te hê as die byvoegings na gisting of in hoër dosisse gemaak word. Die oksigenasieprotokol vir Pinotage wyn benodig verdere optimering, aangesien verbeterings in die wynkleur en –volheid vir sommige behandelings waargeneem is, maar 'n verlies aan sensoriese kwaliteit in meeste gevalle aangetref is.

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CONTENTS

The language and style in this dissertation are in accordance with the requirements of the Journal of Agricultural and Food Chemistry. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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Chapter 1: Introduction

The antioxidant capacity of foods and wine is receiving considerable scientific and commercial interest. As consumers have become more aware of the health benefits of antioxidants via the mainstream media, the food and beverage industries have recognised new marketing opportunities for their products. The antioxidant capacity of foods and beverages may therefore become an important quality parameter, especially in niche markets concerned with health benefits. In this regard, the South African wine industry by means of Winetech, their research funding organisation, has expressed interest in the potential of wine antioxidant capacity in marketing.

Red wine received prominent press coverage in recent years as a result of the "French Paradox". This phenomenon is the relatively low level of coronary heart disease incidence, despite the high intake of saturated fat observed in the French population. This phenomenon is associated with the consumption of red wine (Renaud & De Lorgeril, 1992). Phenolic compounds in red wine exhibit a broad spectrum of beneficial pharmacological properties, believed to be related to their antioxidative properties (Kinsella et al., 1993). Anti-atherogenic activity (Stocker & O'Halloran, 2004), anti-tumour activity (Clifford et al., 1996), anti-ulcer activity (Saito et al., 1998), regulation of platelet aggregation (Keevil et al., 2000) and anti-inflammatory activity (Estruch et al., 2004) have all been demonstrated by the consumption of red wine and/or red wine phenolic compounds. These properties are thought to contribute to the prevention or alleviation of coronary heart disease, cancer and ageing. French scientists recently produced an antioxidant-enriched Chardonnay wine, which showed antioxidant capacity levels much higher than that of other French white wines, and closer to that of French red wines (Landrault et al., 2003). The purpose of their study was to obtain a white wine with similar health benefits than those of red wines.

Enhancement of the phenolic content, and hence the antioxidant capacity, of South African wines, while retaining sensory quality, will provide local winemakers with an opportunity to ensure greater international market share (Beyers Truter, Beyerskloof Wines, personal communication, 2004). A first step in this direction was a preliminary study of the free radical scavenging and lipid peroxidation inhibitory activity of the major commercial South African red and white cultivar wines, was carried out to identify cultivar wines with the highest antioxidant potential (De Beer et al., 2003; 2005). The results demonstrated that the unique South African cultivar, Pinotage, produces wines with good free radical scavenging and lipid peroxidation inhibitory activity, with similar activity to Cabernet Sauvignon wines. Since Pinotage is the focus of increasing interest in international markets, further study of its antioxidant capacity and the role of different phenolic compounds in these wines, was merited.

Pinotage is a cross between *Vitis vinifera* L. cv. Pinot noir and Cinsaut (Hermitage), developed by Professor Abraham Perold in 1924 in South Africa. Wines of this cultivar are typically fruity, with berry, plum and banana characters, and can be made for early drinking or ageing. Pinotage grapes represented 20% of the total red grapes crushed for the production of South African red wine in 2004, along with roughly the same amount of Cabernet Sauvignon and Shiraz grapes (Anonymous, 2005). Research on Pinotage wine has mainly focused on determining cultivar impact aroma compounds (Van Wyk et al., 1979; Waldner & Marais, 2002) and improving grape and wine quality (Marais, 2003a, 2003b; Marais, 2004). The unique phenolic composition of Pinotage has received attention recently: Schwarz et al. (2003) isolated a new anthocyanin-derived pigment similar to vitisin A, which they named pinotin A (after the Pinotage cultivar). The high caffeic and/or caffeoyltartaric acid content of Pinotage wine further highlight its unique phenolic profile (Rossouw & Marais, 2003, 2004; Schwarz et al., 2004).

Many viticultural factors can affect the phenolic content of red wines. Macro-climatic factors, such as temperature, sunlight radiation, rainfall and wind, have a great effect on grapevine physiology and the biosynthesis of phenolic compounds (Mullins et al., 1992). In the vineyard, the micro-climate of the grapevine can be adjusted by canopy management techniques, which aim to optimise berry temperature and sunlight exposure (Smart & Robinson, 1991). The effect of macro-climatic factors and soil characteristics on phenolic content and quality of various wines has been studied (Kliewer, 1970; Mateus et al., 2001). Interactions between these factors are very complex culminating in the concept of terroir, which has been studied (e.g. Vivas de Gaulejac et al., 2001; Van Leewen et al., 2004), although many aspects still need further elucidation. In addition, the effects of grapevine training systems on Shiraz and Cabernet Sauvignon wines (Wolf et al., 2003; Vanden Heuvel et al., 2004) and micro-climate on Shiraz wines (Bergqvist et al., 2001; Downey et al., 2004) have also been studied. However, the effects of climatic factors and training systems on wine antioxidant capacity have not yet been studied to the best of the author's knowledge.

Many developments have taken place in terms of enological practices in recent years. Enological techniques such as enzyme treatments (Pardo et al., 1999; Bautista-Ortín et al., 2005), use of commercial tannins (Bautista-Ortín et al., 2005), use of alternative oak sources (Del Alamo Sanza & Domínguez, 2006), and micro-oxygenation (Castellari et al., 1998), have become commonplace in modern wineries. The use of some of these techniques is mainly based on trial-and–error, and more research is needed to clarify the effects of these treatments on the phenolic composition and quality of red wines. Although very few studies have addressed the effect of these treatments on the antioxidant capacity of red wines thus far, Del Álamo et al. (2006) observed an initial increase in the redox potential of red wines over the first three months of oak maturation, followed by a decrease for at least the next eight months.

Antioxidant activity can be measured by a large variety of assays. Radical scavenging activity is an important aspect of antioxidant activity, although the *in vitro* radical scavenging activity of wine components does not necessarily coincide with *in vivo* antioxidant activity, as bioavailability, metal chelating properties, lipid phase partitioning, and metabolism of individual wine components may differ considerably (Astley, 2003). On the other hand, for development purposes, an easy and rapid screening method is required. The 2,2'-azino-di(3-ethylbenzo-thiazoline-sulphonic acid radical cation ($ABTS^{\bullet+}$) scavenging assay is such a method (Re et al., 1999) and has already been used extensively for analysis of food, beverages and plant extracts. Furthermore, the use of the $ABTS^{\bullet+}$ scavenging assay will also permit comparison of results with those obtained in a previous study on South African wines, including Pinotage wines (De Beer et al., 2003). These reasons determined the choice of the $ABTS^{\bullet+}$ scavenging assay for use during this study. Prior et al. (2005) also proposed that the $ABTS^{\bullet+}$ scavenging assay be standardised for high-throughput screening of samples, along with the oxygen radical antioxidant capacity (ORAC) and Folin-Ciocalteau assays.

The first objective of this study was to investigate the contributions of individual phenolic compounds to the total antioxidant capacity of Pinotage wines. In this regard, Trolox equivalent antioxidant capacity values of individual phenolic compounds and the Pinotage wines were determined by the $ABTS^{\bullet+}$ scavenging assay. The phenolic composition of the wines was determined by HPLC. The second objective was to determine the effect of climatic region, vine structure, enological techniques (pre-fermentation maceration, juice/skin mixing, addition of commercial tannins, extended maceration) and maturation (oak barrels, alternative oak products, oxygenation) on the phenolic composition, total antioxidant capacity and sensory quality of Pinotage wines. The knowledge gained in this study will provide the South African wine industry with guidelines for the production of Pinotage wines with optimal total antioxidant capacity, while retaining sensory quality.

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Chapter 2: Phenolic Compounds in Red Wine - Role in Antioxidant Capacity and Quality, Factors Influencing Composition, and Quantitation

Introduction

Phenolic compounds in red wines are responsible for colour and contribute to astringency and mouthfeel. This very important group of compounds in wine also has excellent antioxidant properties. In recent years a myriad of studies on the possible health benefits has caused greater awareness of antioxidants in general and phenolic compounds in particular among consumers. The antioxidant capacity of foods and beverages, especially red wines, may become an important new quality parameter. In the light of this phenomenon, the South African wine industry is interested in research to enhance the phenolic content and subsequently the antioxidant capacity of wines with retention of sensory quality for greater competitiveness in the international and local market.

This review aims to give a overview of phenolic compounds found in red wines and their role in the antioxidant and quality characteristics of red wine. Methods for determining phenolic composition, antioxidant capacity and quality of red wines will be discussed. A discussion on the structure-activity relationships of the phenolic compounds in red wines using the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation scavenging assay will follow. Finally, factors affecting the phenolic composition and content of red wines, as investigated in this study, will be covered. Emphasis, in terms of viticultural aspects, will be on climatic conditions and viticultural treatments affecting the micro-climatic conditions. In terms of enological factors the focus will be on maceration techniques and factors affecting maceration, as well as maturation using various oak products and the effect of oxygen application.

Phenolic Compounds

Phenolic compounds occur in all fruits as a large and complex group of compounds with particular importance regarding sensory characteristics (colour, astringency, bitterness and aroma) and bio-activity (antimicrobial, anti-inflammatory and antioxidant activity) of red wines. An aromatic ring bearing one or more hydroxyl groups is a common structural feature of this group of compounds. The phenolic compounds in red wines are produced by yeast metabolism and extracted from the grape pulp, skin, seeds and stems during fermentation, as well as from oak cooperage after fermentation (Macheix et al., 1990). Two distinct groups of phenolic compounds occur in red grapes and wine, namely non-flavonoids and flavonoids. The major phenolic compounds occurring

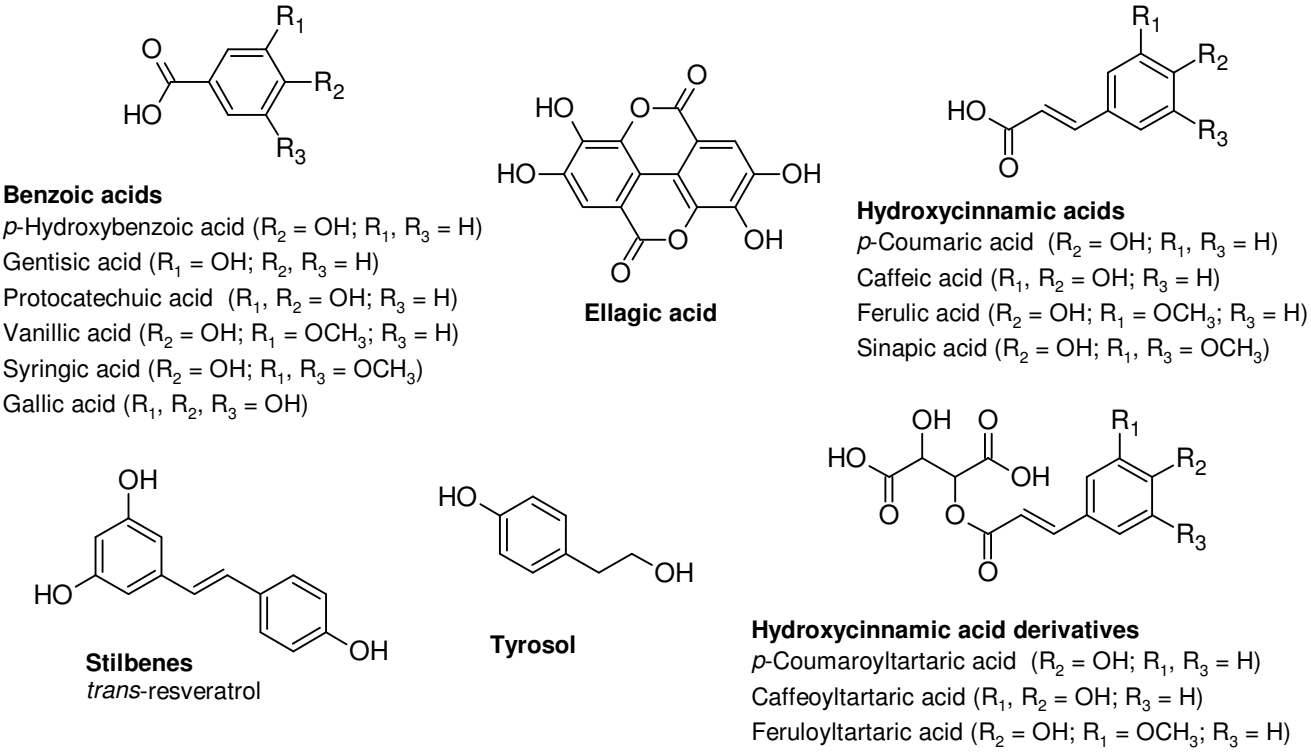
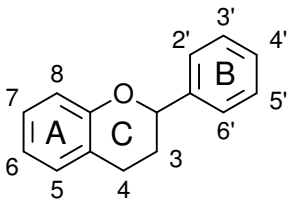


Figure 1. Structures of the most common non-flavonoids found in red grapes and wine.

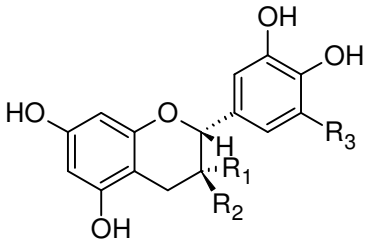
in grapes and wine will be discussed briefly, focussing specifically on those in red wine. A detailed review on this subject has been published recently (Monagas et al., 2005).

Non-flavonoids

The non-flavonoids occurring in red grapes and wines comprise the hydroxycinnamic acids, hydroxybenzoic acids, stilbenes, as well as various derivatives (**Figure 1**). The most common non-flavonoids in red wine are the *trans*-hydroxycinnamic acid derivatives, namely the *trans*-isomers of the tartaric acid esters of caffeic, ferulic and *p*-coumaric acid (Ribéreau-Gayon, 1965), which are extracted from the grape pulp and skins. The free forms of these acids generally do not occur in grapes, but can be released by enzymatic hydrolysis after crushing of the grapes (Macheix et al., 1990). The *cis*-forms of the hydroxycinnamic acids and their derivatives are only found in small quantities (Singleton et al., 1978). Benzoic acids usually occur in low quantities in red wine, except for gallic acid, which occurs in larger quantities. Of the benzoic acids, only gallic acid is extracted from the grape pulp. Other benzoic acids found in wines, namely *p*-hydroxybenzoic, protocatechuic, vanillic, syringic and gentisic acid (Macheix et al., 1990), are extracted from oak wood or formed during hydrolysis of oak wood hydrolysable tannins (Singleton et al., 1971; Puech, 1987). Ellagitannins extracted from oak wood are hydrolysable tannins consisting of ellagic acid and glucose moieties in polymerised form. The stilbenes, of which *trans*-resveratrol is the most common, also occur in dimeric and glycosylated forms and are extracted from the grape skins.

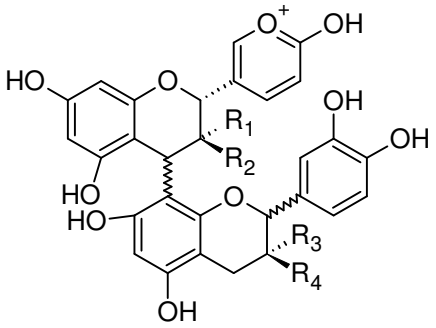


Basic flavonoid skeleton with numbering



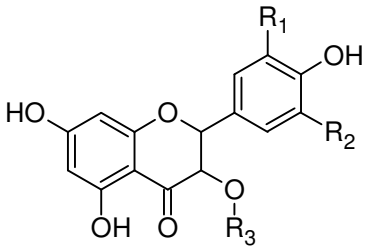
Flavan-3-ol monomers

(+)-Catechin (R_1, R_3 = H; R_2 = OH)
(-)-Epicatechin (R_1 = OH; R_2, R_3 = H)
(+)-Gallocatechin (R_1 = H; R_2, R_3 = OH)
(-)-Epigallocatechin (R_1, R_3 = OH; R_2 = H)



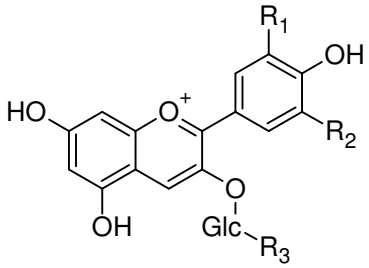
Flavan-3-ol dimers

Procyanidin B1 (R_1, R_4 = OH; R_2, R_3 = H)
Procyanidin B2 (R_1, R_3 = OH; R_2, R_4 = H)
Procyanidin B3 (R_2, R_4 = OH; R_1, R_3 = H)
Procyanidin B4 (R_2, R_3 = OH; R_1, R_4 = H)



Flavonols

Quercetin (R_1 = OH; R_2, R_3 = H)
Quercetin-3-glc (R_1 = OH; R_2 = H; R_3 = glc)
Quercetin-3-gal (R_1 = OH; R_2 = H; R_3 = gal)
Quercetin-3-rham (R_1 = OH; R_2 = H; R_3 = rham)
Myricetin (R_1, R_2 = OH; R_3 = H)
Kaempferol (R_1, R_2, R_3 = H)
Isorhamnetin (R_1 = OCH_3, R_2, R_3 = H)



Anthocyanins

Delphinidin -3-glc (R_1, R_2 = OH; R_3 = H)
Petunidin-3-glc (R_1 = OCH_3; R_2 = H; R_3 = H)
Peonidin-3-glc (R_1 = OCH_3; R_2 = H; R_3 = H)
Malvidin-3-glc (R_1, R_2 = OCH_3; R_3 = H)
For acetate esters and coumarate esters R_3 = acetyl and coumaroyl groups, respectively

Figure 2. Structures of the most common flavonoids found in red grapes and wine.

Tyrosol is an example of a yeast-derived non-flavonoid in red wine, which can contribute to bitterness (Thorngate, 1997).

Flavonoids

The flavonoids consist of two phenols bridged by a three-carbon chain (C_6-C_3-C_6 flavone skeleton). The three-carbon bridge is commonly cyclised with oxygen. The most common flavonoids in red wines comprise the anthocyanins, flavan-3-ols and flavonols, which are differentiated by the degree of unsaturation and oxidation of the three-carbon bridge (Macheix et al., 1990) (**Figure 2**). Flavonoids occur in free, polymerised, glycosylated or acylated forms. The

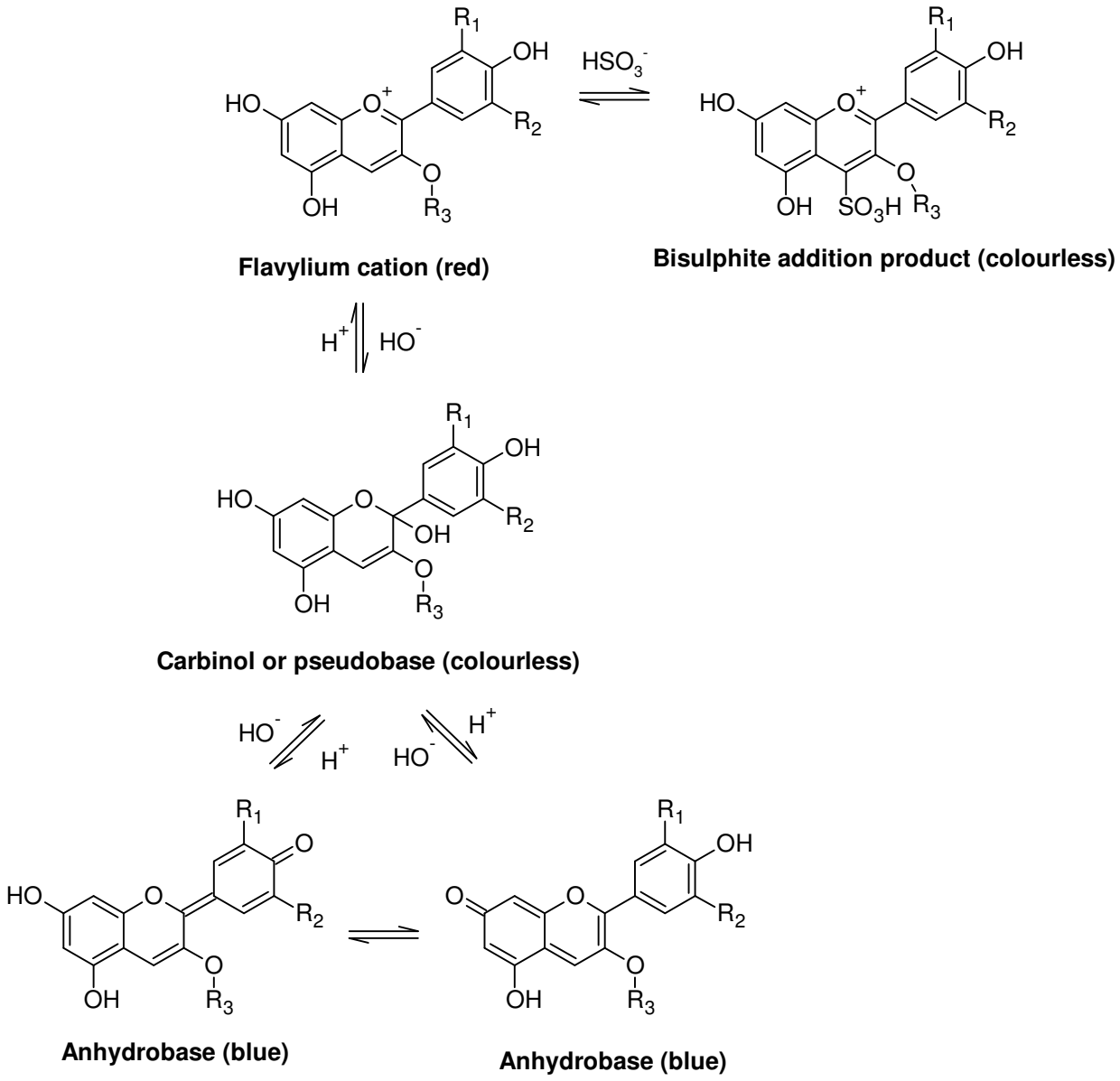


Figure 3. Equilibria among the various forms of anthocyanins in wine as affected by pH and sulphur dioxide (R_1, R_2 = H, OH or OCH_3; R_3 = H or glucosyl).

anthocyanins and flavonols occur primarily in the grape skins, while flavan-3-ols are mainly found in the grape seeds and stems. Oligomers and polymers consisting of flavan-3-ol and anthocyanin subunits are believed to be mainly formed after fermentation and will be discussed in more detail in the section dealing with phenolic reactions in red wine.

Anthocyanins are red pigments occurring in red grapes and wine mostly as 3-*O*-glucosides of delphinidin, petunidin, cyanidin, peonidin and malvidin, although 3,5-*O*-diglucosides are also found in non-*vinifera Vitis* species (Macheix et al., 1990). These basic anthocyanins can also be acylated in the C-6 position of the glucose molecule with acetic, caffeic or *p*-coumaric acid. Anthocyanins, in their flavylium form (**Figure 3**), are especially important for red wine colour. Flavan-3-ols

mainly occur in free forms, such as (+)-catechin, (-)-epicatechin, (+)-gallocatechin and (-)-epigallocatechin. Oligomers (proanthocyanidins) and polymers (condensed tannins) consisting of flavan-3-ol subunits are also abundant in red wines (Macheix et al., 1990). The most common oligomers are the dimers (procyanidin B1, B2, B3 and B4), trimers and tetramers of the B-type procyanidins. Flavonols are yellow pigments occurring in red grapes and wine mostly as 3-*O*-glycosides of quercetin, myricetin, kaempferol and isorhamnetin (Macheix et al., 1990). Glycosyl moieties include glucose, glucuronide, rhamnose and galactose. The aglycons most likely originate from hydrolysis of glycosides during vinification and maturation (Zou et al., 2002; Zafrilla et al., 2003). Anthocyanins and flavan-3-ols or other wine components can also react to form various anthocyanin-derived pigments as discussed in the next section.

Chemical Reactions of Phenolic Compounds in Red Wines

Phenolic compounds possess many structural features contributing to their reactivity (**Figure 4**) (Fulcrand et al., 2004). The *o*-diphenol structure seen in the B ring of many phenolic compounds acts as a powerful reducing agent. Once oxidised, it can participate in coupled oxidation or Michael-addition reactions as an electrophile, with the restoration of the *o*-diphenol structure. The flavonoid A ring has two nucleophilic sites due to three hydroxyl moieties, two in the *ortho*- and three in the *para*-positions, which can participate in aromatic substitution reactions. The C ring can have an electrophilic site, such as in the flavylium form of the anthocyanins or the carbocation produced by acid-catalysed bond cleavage from procyanidins. These species can undergo nucleophilic addition reactions. The C ring of anthocyanins can also participate in cycloaddition reactions. Given the structural diversity and multiple reactive sites of anthocyanins and other flavonoids a large number of products can be formed, which may undergo further reactions. The complexity of grape phenolic composition is therefore increased by numerous reactions taking place in wine. It is clear that the impact of these transformations occurring in wines is difficult to estimate. The major reactions involving mostly anthocyanins and flavan-3-ols will be discussed briefly, as a detailed review of this subject was published by Monagas et al. (2005).

Enzymatic and Non-enzymatic Oxidation Reactions

One of the most important chemical reactions involving phenolic compounds is oxidation. Enzymatic oxidation occurs in both red and white wines with reactions in white wines leading to browning. The first step is the enzymatic oxidation of caffeoyltartaric (caftaric) and *p*-coumaroyltartaric (coutaric) acids, major substrates of polyphenoloxidase (PPO), to *o*-quinones (Singleton et al., 1985). Caffeic acid (Cheynier & Moutounet, 1992) and (+)-catechin (Guyot et al., 1996) have also been demonstrated as substrates for PPO resulting in dimeric caffeic acid and

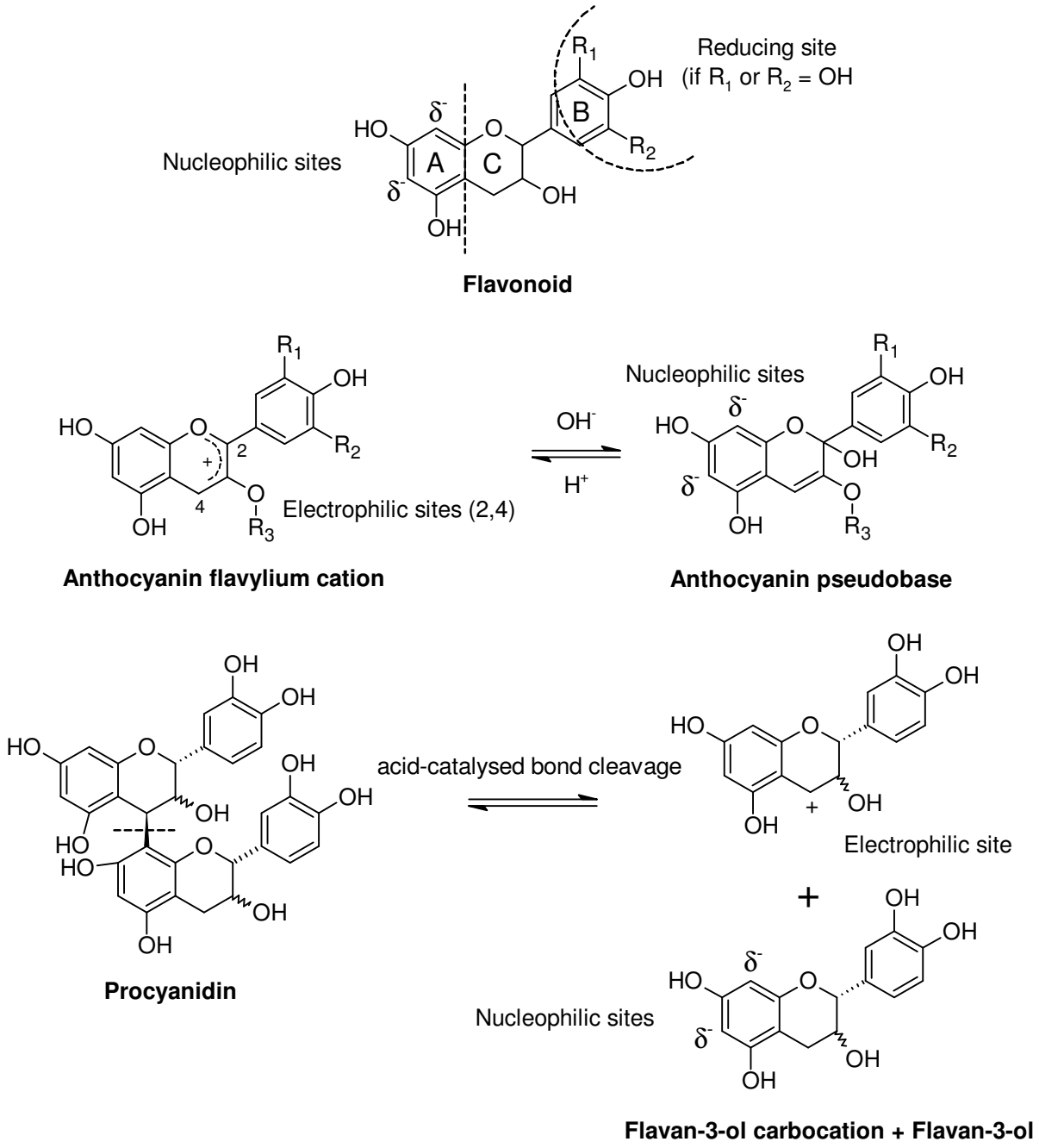


Figure 4. Flavonoid reactive sites (adapted from Fulcrand et al., 2004).

dimeric *o*-quinones, respectively. The quinone species formed by enzymatic oxidation are very reactive and rapidly oxidise other components of the grape must, such as glutathione (Singleton et al., 1985), other *o*-diphenols (Cheynier et al., 1988), ascorbic acid, sulphur dioxide (Rigaud et al., 1991) and ethanol (Wildenradt & Singleton, 1974), in a process called coupled oxidation. The most important product formed in grape must is 2-*S*-glutathionyl-caffeoyltartaric acid (also called grape reaction product) from the nucleophilic addition of glutathione to caffeoyltartaric *o*-quinone (Singleton et al., 1985; Cheynier et al., 1986) (**Figure 5**). Reactions of quinones with glutathione and ascorbic acid compete with the reactions with phenolic compounds, subsequently protecting the

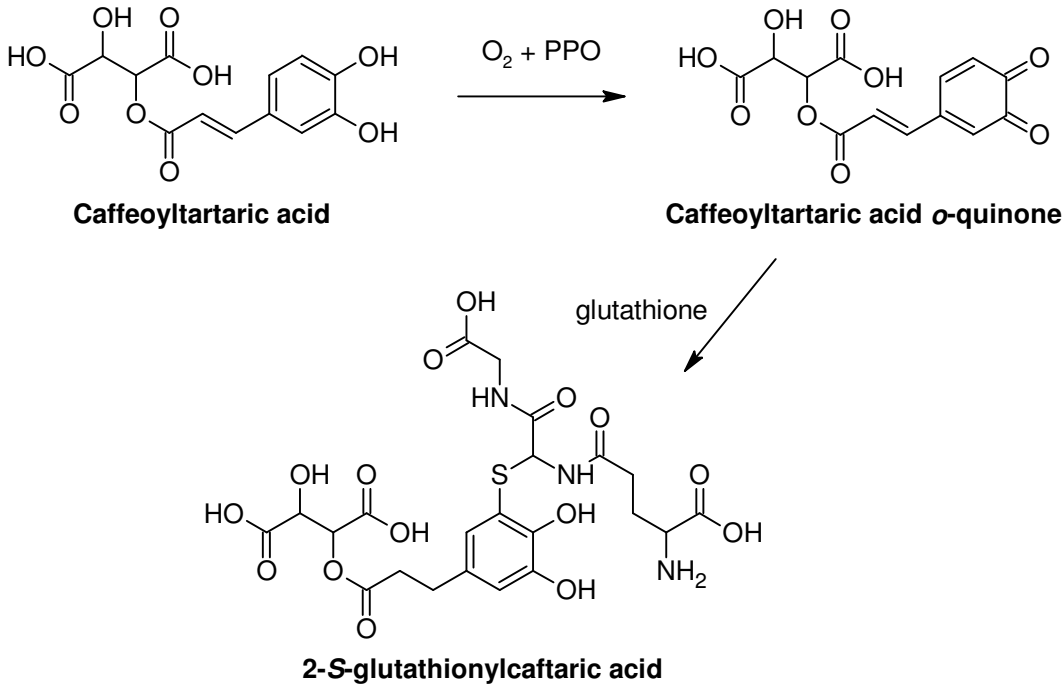


Figure 5. Coupled oxidation of caffeoyltartaric acid and glutathione producing 2-*S*-glutathionyl-caffeoyltartaric acid.

phenolic compounds, especially anthocyanins, from degradation when the oxygen supply is limited. However, in the case of oxidative conditions where grapes contain large amounts of hydroxycinnamates and low levels of glutathione, phenolic compounds are unprotected. In this case, the anthocyanins can be oxidised by the quinones leading to anthocyanin degradation (Raynal & Moutounet, 1989; Wesche-Ebeling & Montgomery, 1990) or can condense with the quinones producing an adduct (Sarni-Machado et al., 1997).

After enzymatic oxidation stops, due to the lack of oxygen and/or the inactivation of PPO, non-enzymatic oxidation can take place. Non-enzymatic oxidation is much slower than enzymatic oxidation and is catalysed by copper, iron, light and peroxide radicals that activate molecular oxygen. Many non-enzymatic oxidation reactions can take place in wine. Autoxidation of gallic acid produces ellagic acid (Tulyathan et al., 1989). Non-enzymatic oxidation of (+)-catechin (Guyot et al., 1996) and caffeic acid (Cheynier & Moutounet, 1992) generates the same products as enzymatic oxidation. In addition, products resulting from the oxidation of tartaric acid (glyoxylic acid) and ethanol (acetaldehyde) promote flavan-3-ol-flavan-3-ol and anthocyanin-flavan-3-ol condensation, respectively. Oxidation can therefore lead to polymeric pigments with more stable colour, although excessive oxidation can cause irreversible damage to a wine in terms of loss of sensory quality.

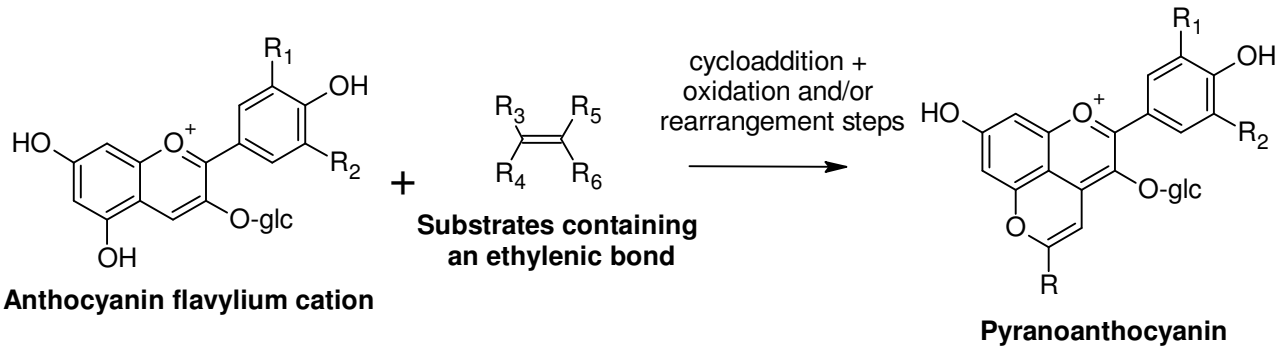


Figure 6. Formation of pyranoanthocyanins (R_1 and R_2 = H, OH or OCH_3; R = phenol, H, COOH or flavan-3-ol). Vitisin A: R_1 and R_2 = OCH_3, R_3 and R_4 = H, R_5 = OH, R_6 = COOH, R = COOH; Pinotin A: R_1 and R_2 = OCH_3, R_3 = catechol, R_4 = H, R_5 = OH, R_6 = COOH, R = catechol.

Formation of Pyranoanthocyanins

Pyranoanthocyanins are formed by the cycloaddition of anthocyanins with various grape must components such as vinylflavan-3-ols, hydroxycinnamic acids, as well as vinylphenols, pyruvic acid and other yeast metabolites, followed by subsequent oxidation and/or rearrangement steps (**Figure 6**).

4-Vinylphenol resulting from the decarboxylation of *p*-coumaric acid by yeast decarboxylases may react with anthocyanin-monoglucosides or anthocyanin-acetylmonoglucosides to form anthocyanin-vinylphenol adducts (Hayasaka & Asenstorfer, 2002; Vivar-Quintana et al., 2002). Other hydroxycinnamic acids may also be decarboxylated resulting in other vinylphenols (Chatonnet et al., 1993), which may in turn lead to the formation of pyranoanthocyanins (Hayasaka & Asenstorfer, 2002). A new mechanism for the formation of anthocyanin-vinylphenol adducts involving the direct reaction of free hydroxycinnamic acids with anthocyanins without enzymatic intervention has been reported recently (Schwarz et al., 2003). The fact that Pinotin A (malvidin-3-glc-vinylcatechol) continues to increase in Pinotage wines after fermentation provided a clue to this alternative mechanism. Anthocyanin-vinylflavan-3-ol adducts have also been reported (Mateus et al., 2002b), although the reaction mechanisms are still unclear. Anthocyanin-vinylphenol and –vinylflavan-3-ol adducts are generally orange-red in colour (Fulcrand et al., 1996; Vivar-Quintana et al., 2002), partially explaining the change in red wine colour from red to tawny during ageing. These pigments are also more stable and resistant to sulphite bleaching than anthocyanin-monoglucosides (Vivar-Quintana et al., 2002; Håkansson et al., 2003).

Pyruvic acid is an intermediate product in the glycolysis cycle of yeast metabolism during fermentation, ultimately leading to ethanol formation. For this reason the formation of pyruvic acid-anthocyanin adducts, such as vitisin A and acetylvitisin A (Bakker & Timberlake, 1997; Heier et

al., 2002), mostly occur during fermentation (Asenstorfer et al., 2003; Morata et al., 2003). Anthocyanin-pyruvic acid adducts are formed by cycloaddition followed by dehydration and re-aromatisation steps (Fulcrand et al., 1998). Other secondary metabolites of yeast metabolism, namely acetaldehyde, acetone, acetoin, oxalacetic acid and diacetyl among others, present keto-enol tautomerism. In their enolic form they may therefore also participate in cycloaddition reactions with anthocyanins via a similar mechanism as the reaction with pyruvic acid (Benabdeljalil et al., 2000). Some of these products have been identified in wine and port, such as vitisin B (Bakker & Timberlake, 1997; Heier et al., 2002), acetylvitisin B (Bakker & Timberlake, 1997; Heier et al., 2002), coumaroylvitisin B (Vivar-Quintana et al., 2002), anthocyanin-vinylmethyl adducts (Hayasaka & Asenstorfer, 2002) and castavinols (Castagnino & Vercauteren, 1996). Anthocyanin-adducts with yeast metabolites generally present orange-red colours with higher colour intensity than the original monoglucosides (Bakker & Timberlake, 1997), except for colourless castavinols (Castagnino & Vercauteren, 1996).

Aldehyde-mediated Condensation Reactions Involving Anthocyanins and Flavan-3-ols

Acetaldehyde is present in wine originating from yeast metabolism during fermentation (Romano et al., 1994) and from coupled oxidation of ethanol (Wildenradt & Singleton, 1974). Acetaldehyde in acidic medium can react with a flavan-3-ol (monomer, oligomer or polymer) or anthocyanin in position C-6 or C-8, whereafter the intermediate product reacts with the C-8 position of an anthocyanin or the C-6 or C-8 position of another flavan-3-ol (Timberlake & Bridle; 1976a) giving rise to various ethyl-linked flavan-3-ol-anthocyanin, anthocyanin-anthocyanin or flavan-3-ol-flavan-3-ol adducts (**Figure 7**). Many ethyl-linked condensation products have been identified in wines (Heier et al., 2002; Vivar-Quintana et al., 2002; Monagas et al., 2003). Other aldehydes such as glyoxylic acid, furfural and other furfural derivatives can also react in the same manner (Fulcrand et al., 1997; Es-Safi et al., 1999b; Es-Safi et al., 2002; Nonier et al., 2006). Glyoxylic acid is formed by the oxidation of tartaric acid in the presence of iron (Oszmianski et al., 1996) or copper ions (Clark & Scollary, 2002). Coloured aldehyde-mediated condensation products are more stable with regard to pH differences and bisulphite bleaching than the parent anthocyanins (Escribano-Bailon et al., 2001; Asenstorfer et al., 2006). Ethyl-linked pigments are, however, relatively unstable and can undergo rearrangement to form xanthylium pigments (Es-Safi et al., 2000; Del Alamo et al., 2000) or polymerise further (Es-Safi et al., 1999a).

Direct Condensation Reactions Involving Anthocyanins and Flavan-3-ols

Two mechanisms are responsible for direct condensation of anthocyanins and flavan-3-ols resulting in coloured pigments (**Figure 8**) (Remy et al., 2000). The first mechanism involves the nucleophilic addition of the flavan-3-ol (C-8 or C-6 position) to the electrophilic C-4 position of the

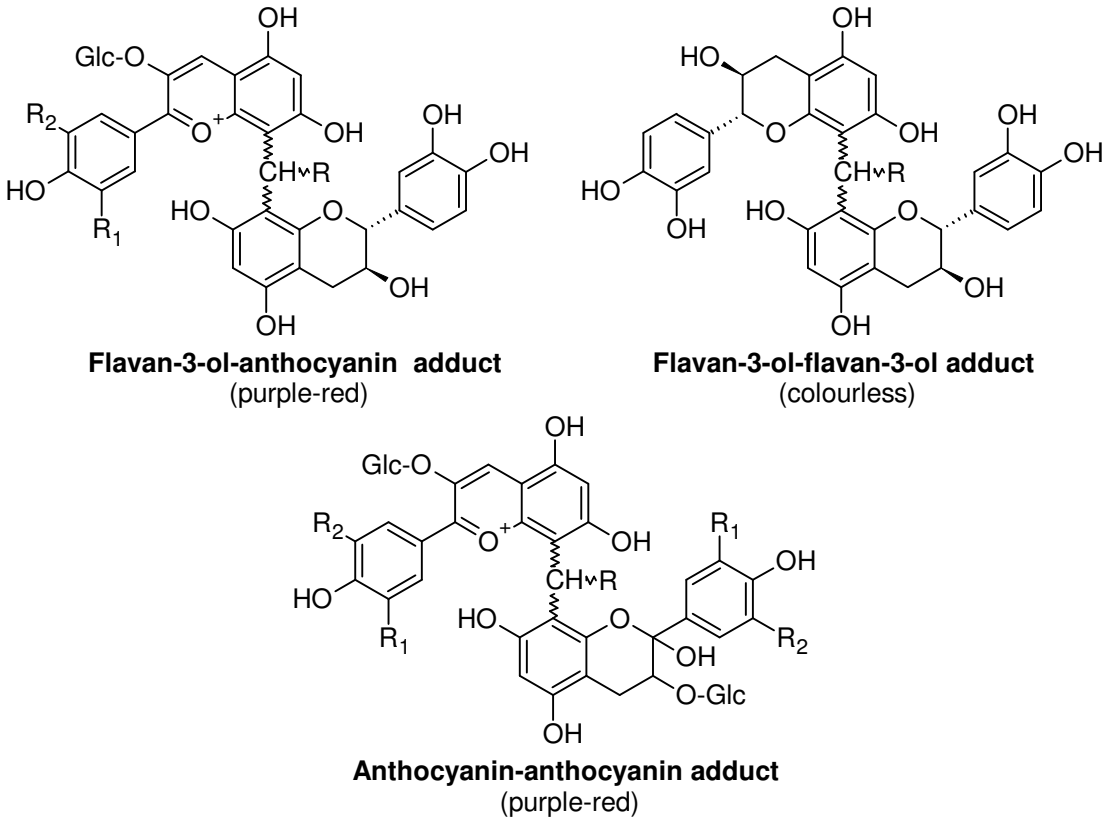


Figure 7. Various types of aldehyde-mediated condensation products (R_1 and R_2 = H, OH or OCH_3; R = CH_3, COOH, methine, furyl, hydroxymethylfuryl).

anthocyanin resulting in anthocyanin-flavan-3-ol adducts. The second mechanism involves the electrophilic C-4 position of a flavan-3-ol carbocation produced from the acid-catalysed interflavanic bond cleavage of procyanidins reacting with the nucleophilic C-6 or C-8 position of the anthocyanin resulting in flavan-3-ol-anthocyanin adducts. Anthocyanin-flavan-3-ol dimers can react further to form colourless adducts, namely anthocyanin-flavan-3-ol dimers in the flavene form or bicyclic anthocyanin-flavan-3-ol dimers (Remy-Tanneau et al., 2003). Condensation of two anthocyanins should also be possible (**Figure 8**) and condensation of pyranoanthocyanins with flavan-3-ols has also been reported (Mateus et al., 2003). Direct condensation products of anthocyanins and flavan-3-ol oligomers have also been identified up to octamer level (Hayasaka & Kennedy, 2003). Condensed pigments of these types are generally associated with reactions taking place during wine ageing, but they have been reported recently in fresh fruits, including grape skins (Vidal et al., 2004c; González-Paramás et al., 2006). Polymeric pigments of this type are generally resistant to pH changes and bisulphite bleaching (Somers & Evans, 1977), although some may only be partially resistant (Timberlake & Bridle, 1968).

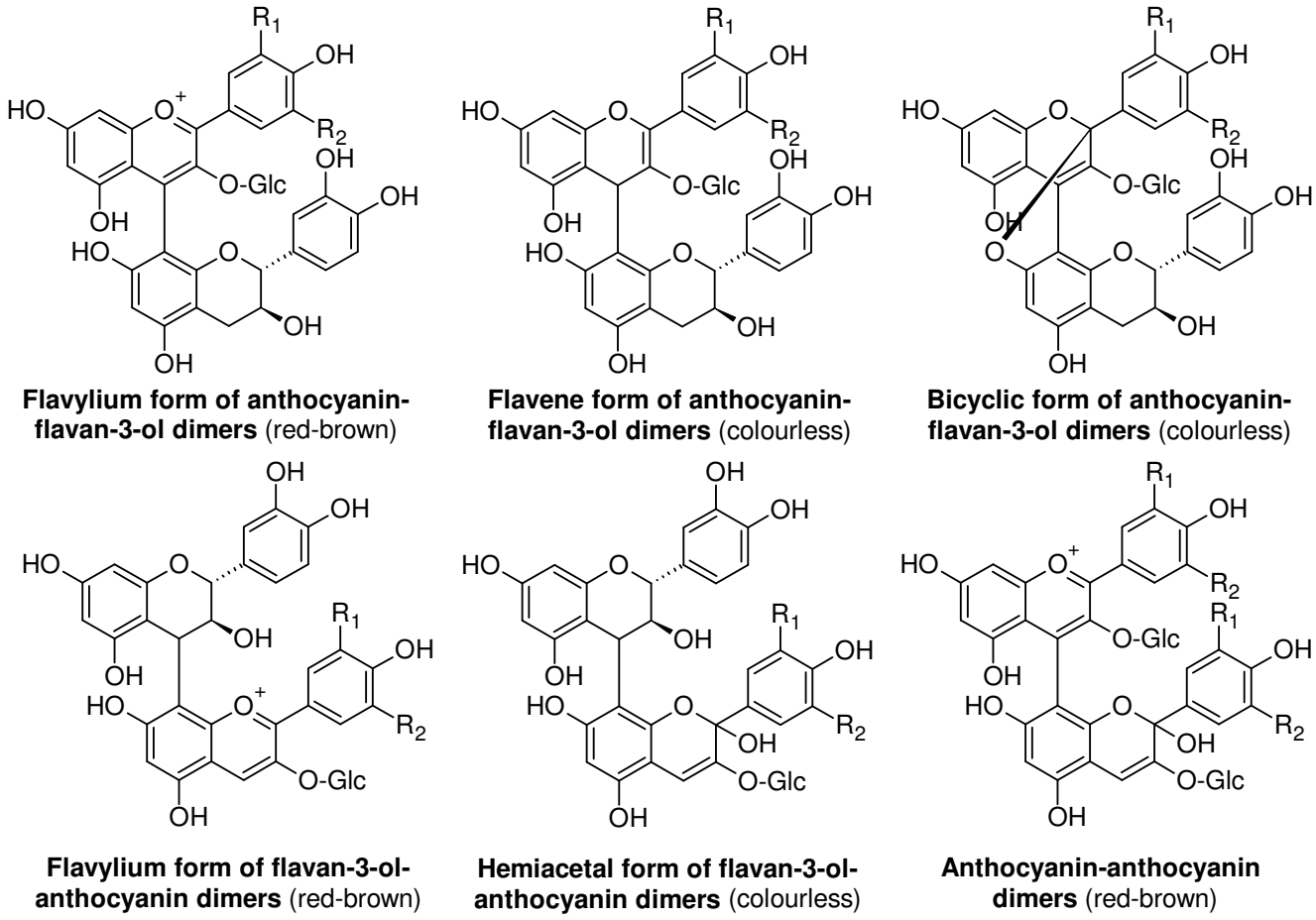


Figure 8. Various types of direct condensation products (R_1 and R_2 = H, OH or OCH_3).

Co-pigmentation

Co-pigmentation is the molecular association of pigments, such as anthocyanins in red wine, with other organic molecules, referred to as co-pigmentation factors or co-pigments, in solution. The colour intensity (also linked to chroma) of anthocyanin solutions are enhanced several-fold (hyperchromism) due to co-pigmentation and a shift in hue towards blue usually occurs (bathochromism) (Asen et al., 1972; Gonnet, 1999). Co-pigmentation in red wine is thought to occur by the hydrophobic planar stacking of the co-pigment molecule on the anthocyanin flavylium ion with some hydrogen-bonding possibly occurring (**Figure 9**). This partially prevents the nucleophilic attack of water and increases the stability of the anthocyanin. Colour enhancement therefore occurs by keeping a higher ratio of flavylium ions to colourless forms in solution (see **Figure 3** for anthocyanin equilibria). Colour effects depend on the nature of the pigment and co-pigment, pigment concentration, co-pigment concentration, co-pigment to pigment ratio, pH (Gonnet, 1999) and ethanol content (Dufour & Sauvaitre, 2000; Hermosin Guttiérez, 2003). Co-pigments in wine include phenolic acids (Asen et al., 1972; Dimitri□ Markovi□ et al., 2003a; 2003b), flavonols (Asen et al., 1972; Davies & Mazza, 1993), flavan-3-ols (Asen et al., 1972; Berké

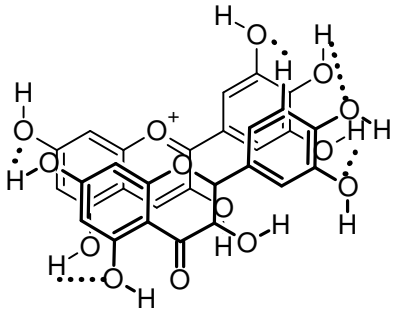


Figure 9. Schematic of co-pigmentation complex for delphinidin-3-glucoside and quercetin (dotted lines indicates possible hydrogen-bonding).

& De Freitas, 2005) and volatile phenols (Dufour & Sauvaitre, 2000). Generally, flavonols give the greatest co-pigmentation effects (Asen et al., 1972; Chen & Hrazdina, 1981).

In wine, co-pigmentation has been reported to account for 30 to 50% of the colour of young wines (Boulton, 2001). Hermosin Guttiérez et al. (2005) reported a decrease in co-pigmentation with ageing in three red wine cultivars with no or little co-pigmentation effect observed nine months after fermentation. Co-pigmentation complexes are disrupted by dilution, causing a non-linear relationship between colour and pigment concentration, e.g. when red wines are diluted (Somers & Evans, 1977). This phenomenon can be used to quantify the colour due to co-pigmentation and has been included recently in a spectrophotometric assay (Mazza et al., 1999; Boulton, 2001; Hermosin Guttiérez, 2003). Anthocyanins with acyl- or *p*-coumaroylgroups can also undergo intramolecular co-pigmentation in concentrated solutions (Dangles et al., 1993). Recently, Dimitri□Markovi□et al. (2003a; 2003b) have shown that co-pigmentation affects the oxidation potential of the anthocyanin and co-pigment molecules.

Role of Phenolic Compounds in Red Wine Quality

Colour

Colour has an important effect on the sensory perception and acceptability of foods and beverages (Clydesdale, 1993), including red wine (Parr et al., 2003). Objective measurement of red wine colour is therefore important for quality determination. Traditionally, colour is determined objectively using spectrophotometric measurements at different wavelengths. Sudraud (1958) suggested measuring the absorbances at 420 and 520 nm. These measurements take the yellow and red colour components into account, but may not accurately reflect the colour of young wines. Glories (1984) added a measurement at 620 nm to include the blue colour component, which is generally higher in young wines than in older wines. The colour intensity is then defined as the sum

of absorbances, while the yellow, red and blue contribution is calculated as a percentage of the colour intensity. The colour hue (or tonality or brown index) is defined as the ratio of absorbance at 420 nm to that at 520 nm (Sudraud, 1958; Glories, 1984).

The CIELab objective colour parameters are preferred as an accurate objective measurement of wine colour, since they permit better differentiation between wines than traditional measurements Bakker et al., 1986; Almela et al., 1995; Heredia et al., 1997; Pérez-Magariño & González-Sanjosé, 2002). The parameters defining the CIELab colour space are: a^* (red/green chromaticity), b^* (yellow/blue chromaticity) and L^* (lightness). These three basic parameters are used to calculate C^* (chroma) and h^* (hue angle), the psychophysical parameters correlating with the perception of colour by human observers (Hunt, 1978).

The colour of red wines is mainly attributed to the anthocyanin pigments, namely monomeric anthocyanins, as well as pigments derived from anthocyanins by various reactions as outlined in a previous section. Flavonols can also contribute to the yellow colour component (Macheix et al., 1990). Co-pigmentation of anthocyanins with phenolic acids, flavan-3-ols and flavonols can greatly affect wine colour, especially the chroma and the hue, as discussed in a previous section. Sulphur dioxide content also influences red wine colour by bleaching anthocyanins. The pigment concentration, however, does not always have a linear relationship to the colour attributes, especially in dark coloured wines, due to a phenomenon called inversion (Eagerman et al., 1973; Gonnet, 1999). This phenomenon occurs at low lightness values, such as in dark coloured solutions, due to the difficulty of photocells to adjust to low luminosity situations. Recently, it was shown that visual perception of red wine colour could not be predicted from the wine phenolic content due to saturation of visual perception (Preys et al., 2006).

Sensory Perception

The sensory properties important for evaluating red wine quality are colour, aroma, astringency, bitterness and fullness. The perception of colour has been discussed briefly in the previous section. The aroma of red wines is related to the concentrations of various aroma compounds such as alcohols, ketones, esters, aldehydes, volatile phenols and terpenes (Rapp, 1988). These aroma compounds are extracted from the grape, formed during yeast metabolism from grape precursors or extracted from oak wood during barrel maturation. Phenolic compounds can also interact with aroma compounds influencing their volatility and perceived intensity (Aronson & Ebeler, 2004). Astringency of wines is mostly caused by its tannin content (Vidal et al., 2004a), while compounds such as monomeric flavan-3-ols and gallic acid are the major contributors to bitterness (Robichaud & Noble, 1990; Kielhorn & Thorngate, 1999). Astringency is a tactile sensation due to binding of salivary proteins by tannins (Kallithraka et al., 1998), which increases with increasing degree of polymerisation (Vidal et al., 2003). However, very large polymers (>10

subunits) are likely to precipitate and therefore would not contribute to astringency. The structure of tannins also qualitatively and quantitatively affects the astringent sensation (Brossaud et al., 2001; De Freitas & Mateus, 2001; Vidal et al., 2003). The formation of anthocyanin-flavan-3-ol adducts are thought to contribute to the loss of astringency during maturation (Vidal et al., 2004a). Factors such as acidity and ethanol content can enhance the perception of astringency, while sweetness and viscosity tend to decrease it (Noble, 1998). The astringency of red wines has been predicted from their phenolic composition (Preys et al. 2006). The sensory perception of astringency and the vocabulary used by tasters to describe these sensations has been investigated by Gawel et al. (2000). The fullness of red wines is associated with the alcohol, glycerol and polyol content (Sponholz, 1988). Polysaccharides may also increase the fullness of red wines (Vidal et al., 2004b).

Influence of Viticultural Factors on the Phenolic Composition

Climatic Conditions

Traditionally, cooler viticultural areas are regarded as producing wines of higher quality (Jackson & Lombard, 1993). This would indicate a relationship between climate and wine quality. In practice, this is not so easy to determine as different geographical factors, soil conditions, as well as viticultural and enological practices, can also have an influence on wine quality. Defining a climate is also not easy as minimum, maximum and average temperatures, as well as sunlight radiation, rainfall, humidity and wind play a role. When considering all the climatic and geographical factors the concept of terroir is obtained, which refer to small geographical areas with similar viticultural potential (Laville, 1993). Terroir is also expected to influence the phenolic composition of wines (Brossaud et al., 1999).

The temperature during berry ripening is one of the most important factors affecting the biosynthesis of phenolic compounds in grape berries (Mullins et al., 1992). Gladstones (1992) suggested that a mean temperature range of 20 to 22 °C is optimal for physiological ripening of grapes and for colour, flavour and aroma compound biosynthesis. Specific climatological events, such as heat waves, low night temperatures, rain or wind storms during berry ripening can greatly affect the phenolic content of the berry at harvest (Mullins et al., 1992; Mori et al., 2005). Many systems to describe viticultural regions in terms of their climate have been described such as those of Amerine and Winkler (1944), Huglin (1986) and Smart and Dry (1980), which are based on temperature. A division of the Western Cape (South Africa) viticultural regions have been carried out by Le Roux (1974), according to the heat summation model of Amerine and Winkler (1944), as well as by De Villiers et al. (1996) (**Figure 10**), according to the mean temperature of the warmest month model of Smart and Dry (1980), using the mean February temperatures.

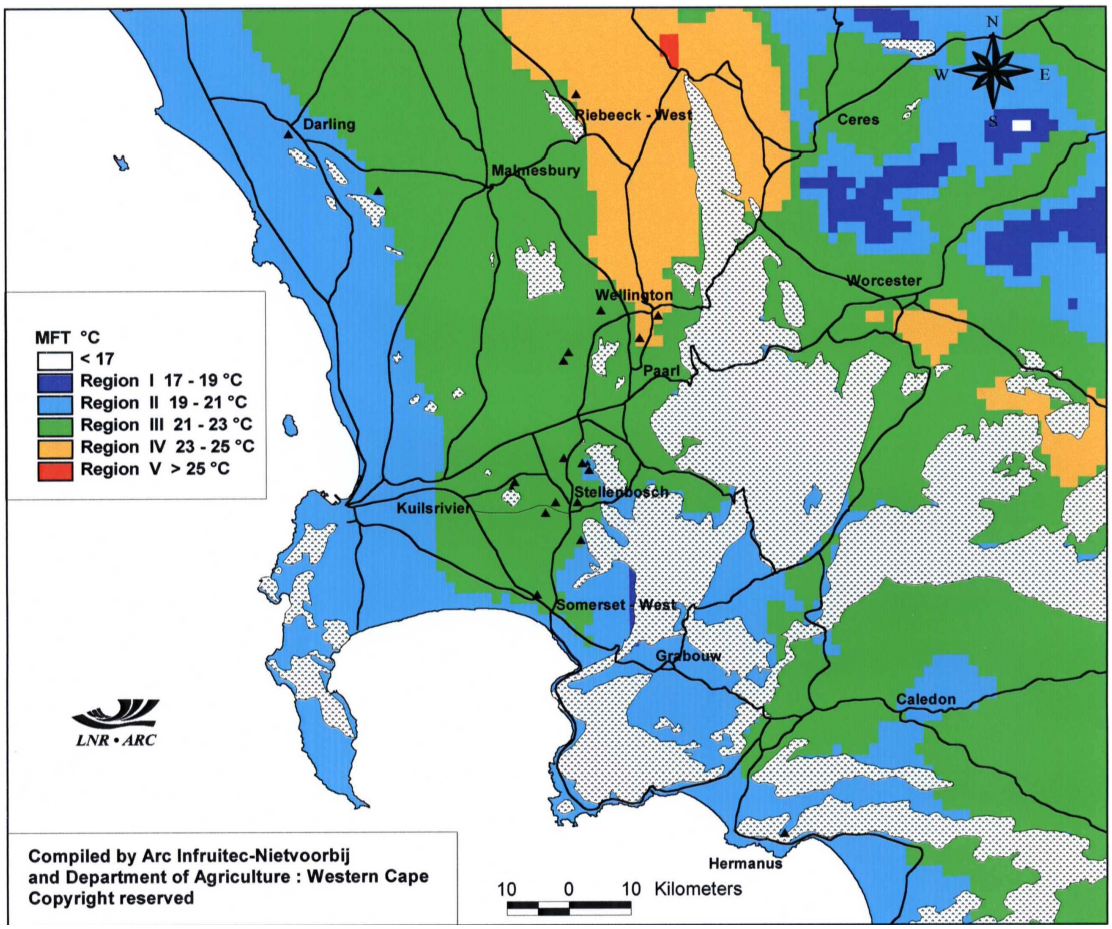


Figure 10. Division of Western Cape Pinotage cultivation areas into climatic regions on the basis of mean February temperatures (MFT) as described by De Villiers et al. (1996).

Grapes grown in high temperature areas have been reported to have lower anthocyanin (Kliewer, 1970; Bergqvist et al., 2001; Spayd et al., 2002; Miguel-Tabares et al., 2002) and total phenol (Bergqvist et al., 2001) contents, compared to grapes grown in cooler areas. In one study, the phenolic composition of both the grape berries, as well as the resulting Port wines, was affected by the mean ambient temperature, which was altered by vineyard altitude (Mateus et al., 2001; 2002a). The anthocyanin monoglucoside and acetylated anthocyanin contents of the grape berries and Port wines were higher for a cooler area compared to a warmer area, while the opposite trend was observed for the proanthocyanidin content.

Sunlight Exposure

In addition to temperature, light radiation intensity is an important factor influencing the metabolism of the grapevine and the biosynthesis of phenolic compounds (Mullins et al., 1992). The amount of incident light radiation on the grape berries is influenced by the vineyard latitude, season, time of day, cloud cover and shading in the canopy (Smart & Robinson, 1991). Sunlight

exposure can greatly increase the temperature of exposed berries compared to shaded berries (Smart & Sinclair, 1976; Spayd et al., 2002). Most studies investigated the differences between shaded and non-shaded clusters in the same vineyard, i.e. berry temperatures of the two treatments would differ considerably. Shading of grape clusters decreased the juice pH, as well as the total soluble solids, anthocyanin, flavonol and total phenol contents of the berry, while titratable acidity, malate content, and mass of the berry were increased (Kliewer, 1970; Crippen & Morrison, 1986; Reynolds et al., 1986; Downey et al., 2004). One study (Spayd et al., 2002) separated the effects of shading and berry temperature. Some shaded berries were heated to the same temperature as exposed berries, while some exposed berries were cooled to the same temperature as shaded berries. In this study, sunlight exposure increased the anthocyanin and flavonol content of berries, while higher berry temperature decreased the anthocyanin content of berries with no effect on their flavonol content.

Canopy Management

Canopy management includes a wide variety of practices that influence canopy characteristics, such as training system, pruning, shoot positioning, leaf removal, shoot removal and cluster thinning (Smart et al., 1990). The canopy characteristics are determined by the amount and distribution of leaf area in space (Smart et al., 1990). The most important micro-climatic factor controlled by the canopy characteristics is the light levels, but temperature, humidity and wind speed are also modified (Smart et al., 1990). The aim of canopy management is to improve the canopy micro-climate, especially in terms of light levels, for optimal wine grape growing conditions. Increased grape quality and/or grape yield is a result of optimal canopy management due to higher sunlight levels (Smart et al., 1985). Optimal sunlight interception occurs when the canopy has approximately three leaf layers from side to side (Smart & Robinson, 1991; Hunter, 1999).

Various training systems have been developed for use in a variety of climates. Most training systems can be classified by the bearing wood origin, i.e. head or cordon trained, and bearing wood length, i.e. spur or cane pruned (Jackson, 2000). Examples of training systems are Guyot, double Guyot, Goblet, Mosel Arch, Hudson River umbrella, umbrella Kniffin and Lyre (Jackson, 2000). In some of the newest training systems the canopy is divided to enhance fruit exposure to sunlight, e.g. Geneva double curtain, Ruakura Twin Two Tier and Scott-Henry (Smart & Robinson, 1991). In South Africa, bush (head-trained and spur-pruned) and trellised (trained to a bilateral horizontal cordon and spur-pruned with upward vertical shoot positioning) vines are most commonly used. Effects of different canopy management practices on berry composition, as well as wine composition and quality, are modulated by the degree of change in the canopy microclimate, especially the berry temperature and sunlight exposure. However, crop yield and vine vigour may also be affected by canopy management practices (Reynolds et al., 2004; Vanden Heuvel et al.,

2004), which in turn may affect the grape composition (Chapman et al., 2004; Pozo-Bayón et al., 2004; Cortell et al. 2005). Good canopy management practice with optimised canopy micro-climate not only increased grape yield for Riesling vines, but also improved the sensory quality of resulting wines (Reynolds et al., 2004). These effects may be cultivar-dependent.

Influence of Vinification Techniques on Phenolic Composition

The most common vinification processes are depicted in **Figure 11**. Many variations in vinification protocol are possible at every step, while some steps are optional. Any process that affects extraction or removal of phenolic compounds will alter the phenolic composition of the resulting wine and possibly its antioxidant capacity.

An important part of vinification is maceration, which begins as soon as the grapes are crushed (Ribéreau-Gayon et al., 1998). Maceration is the extraction of components from the grape solids, namely the skins and seeds. Phenolic compounds, which contribute to the wine colour and structure, are extracted and represent a large portion of the total phenolic content of the wine (Sun et al., 1999; 2001). The extractability of phenolic compounds from grape skins seems to be related to the skin cell-wall composition, especially with regard to pectin and cellulose (Ortega-Regules et al., 2006). A higher content of pectin and cellulose would make the cell walls more difficult to break, hampering extraction of phenolic compounds. Aroma compounds, aroma precursors, nitrogen compounds, polysaccharides and minerals are among the other compounds released into the wine during maceration (Ribéreau-Gayon et al., 1998).

Pre-fermentation Maceration at Low Temperatures

Pre-fermentation maceration, usually carried out under cool (15 °C) to cold (4 °C) conditions, is used for enhancing wine aroma in red, rosé and white wines via increased aqueous extraction of aroma precursors from the grape skins (Marais & Rapp, 1988; McMahon et al., 1999; Salinas et al., 2005; Esti & Tamborra et al., 2006). Marais (2003a) observed an increase in berry aroma intensity of Pinotage wines when pre-fermentation maceration was applied at 10 °C, but the levels of acetate and ethyl esters were reduced for pre-fermentation maceration at 15 °C compared to a control wine. Pre-fermentation maceration has been shown to improved aroma and complexity for Pinot Noir wines (Heatherbell et al., 1997). Pre-fermentation maceration has been shown to enhance the colour and anthocyanin contents of red (Heatherbell et al., 1997; Watson et al., 1997; Reynolds et al., 2001; Gómez-Míguez et al., 2006) and rosé (Salinas et al., 2005) wines. This technique is especially used in the case of cultivars or growing areas where colour extraction is problematic (Gómez-Míguez et al., 2006). Heatherbell et al. (1997), however, reported that differences in anthocyanin content between treated and untreated Pinot Noir wines were less noticeable after two years of

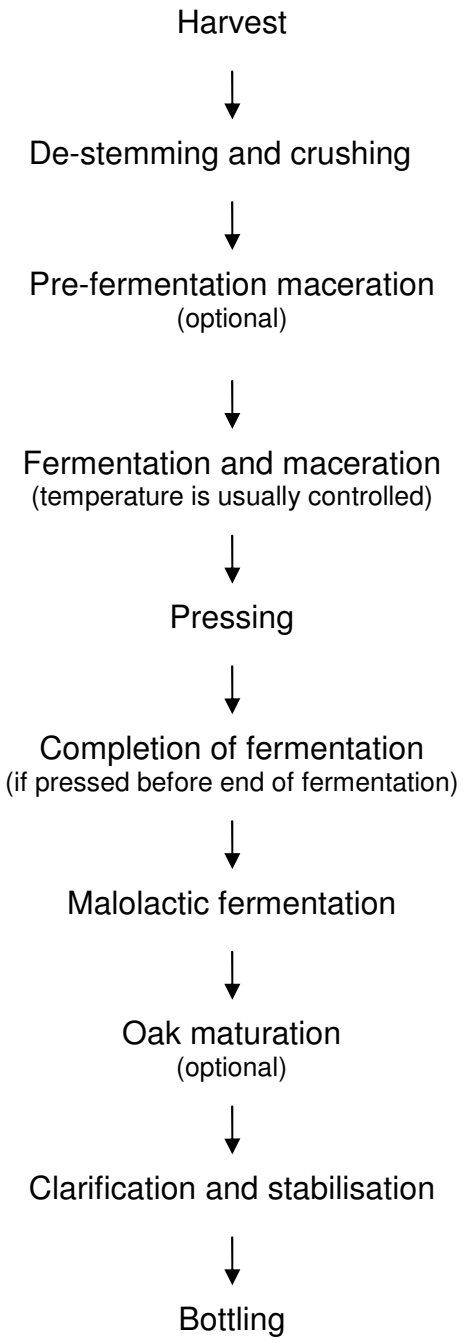


Figure 11. Flow diagram of the most common vinification processes in the production of red wine.

ageing than directly after production. Pre-fermentation at low temperatures can also increase the stilbene content of wines (Poussier et al., 2003; Clare et al., 2004). In the absence of ethanol, as is the case when pre-fermentation is carried out, flavan-3-ols are expected to have relatively low extraction rates compared to that of anthocyanins (González-Manzano et al., 2004).

A study, using liquid nitrogen or solid-state carbon dioxide to enable pre-fermentation at temperatures between -5 and 5 °C, reported increased levels of total phenol, monomeric anthocyanin and anthocyanin-tannin condensation products, as well as improved sensory quality for Sangiovese wines (Parenti et al., 2004). In this case, initial freezing induced formation of ice crystals inside grape cells rupturing them to release phenolic and aroma compounds into the must. Similar results were obtained for Monastrell wines when using dry ice for pre-fermentation maceration at 0 to 2 °C (Álvarez et al., 2006).

Juice/Skin Mixing Techniques During Maceration

Juice/skin mixing techniques are used during fermentation to facilitate contact between the juice and skin for extraction of aroma, flavour and phenolic compounds. The most common juice/skin mixing techniques are punching-down, pumping-over and rotor action. During fermentation, ethanol is present and phenolic compounds, such as monomeric and polymeric flavan-3-ols, are readily extracted from the grape skins and seeds. Wines with varying phenolic composition were obtained when different juice/skin mixing techniques, such as mechanical and manual punching-down, mechanical pumping-over or rotary fermenters, were used, although results depended on the cultivar (Leone et al., 1983; Fischer et al. 2000; Castillo-Sanchez et al., 2006). The use of manual punching-down, pumping-over and rotor juice/skin mixing techniques affected the volatile aroma compounds and sensory quality of Pinotage wines significantly, with very little effect observed for different mixing frequencies (Marais, 2003b). The effect of varying mixing frequencies on the antioxidant capacity has not been investigated to the best of the author's knowledge.

Other Factors and Techniques Affecting Extraction of Phenolic Compounds During Maceration

The extraction of compounds is quantitatively and qualitatively influenced by the absence (before fermentation) or presence (during fermentation) of ethanol (González-Manzano et al., 2004), as well as fermentation temperature (Girard et al., 2001; Reynolds et al., 2001), sulphur dioxide content (Bakker et al., 1998) and maceration time (Yokotsuka et al., 2000; Zou et al., 2002). At higher fermentation temperatures increased extraction of phenolic compounds is obtained, due to increased permeability of the grape skins and increased solubility of phenolic compounds in the wine. The presence of sulphur dioxide also leads to increased phenolic content, compared to its absence, by aiding extraction, as well as preserving phenolic compounds after extraction (Bakker et al., 1998). The anthocyanin, hydroxycinnamic acid and flavonol contents usually show a biphasic trend during fermentation with an increase in their content up to a point, whereafter the content decreases (Yokotsuka et al., 2000; Zou et al., 2002). On the other hand, gallic acid, flavan-3-ol and tannin contents increase throughout skin contact (Kovac et al., 1992; Yokotsuka et al., 2000; Zou et

al., 2002). The use of extended maceration after fermentation therefore generally results in higher flavan-3-ol and polymer content. Improved wine antioxidant capacity has been reported as a result of increased maceration time of several red cultivar wines (Echeverry et al., 2005; Villaño et al., 2006).

Other techniques, which are used to increase the extraction of phenolic compounds, are thermovinification, carbonic maceration and maceration using pectolytic enzymes. Thermovinification involves heating the grape skins separately from the must to a high temperature (60 or 70 °C) for a short time before fermentation. The grape skins are then added back to the must and fermentation proceeds as normal. This technique is used to facilitate anthocyanin extraction and to give improved wine colour (Gao et al., 1997). As no ethanol is present during the heating step, no increased extraction of polymeric compounds is expected. This technique has been reported to improve the antioxidant capacity of red wine (Netzel et al., 2003). Carbonic maceration is carried out by allowing a partial anaerobic fermentation of the whole berries for one to two weeks before pressing. The pressed juice is then fermented further to produce light fruity wines for early consumption. The effect of carbonic maceration on the phenolic content of wines has been variable with some investigators reporting increased (Lorincz et al., 1998) and others decreased phenolic content (Timberlake & Bridle, 1976b; Sun et al., 2001). It is possible that cultivar affects the results. Carbonic maceration has been reported to result in wines with total antioxidant capacity higher than that of aged wines (Pellegrini et al., 2000). Another technique aimed at breaking the grape skin cell walls, to release anthocyanins for colour improvement, is maceration with pectolytic enzymes. Improved colour, as well as increased flavour and phenolic compound content have been reported (Bakker et al., 1999; Gil & Vallés, 2001; Bautista-Ortín et al., 2005). Monomeric anthocyanin content did not increase due to incorporation into polymeric pigments (Parley et al., 2001). Improved antioxidant capacity has been reported for Tannat wines, when a colour extracting enzymatic preparation was used (Echeverry et al., 2005).

The use of commercial tannins, derived from grape seed or oak wood, to improve the tannin content of wine, is a recent development (Keulder et al., 2004; Bautísta-Ortin et al., 2005; Obradovic et al., 2005). This is generally done if the grapes do not have enough extractable tannin. These tannins can be added before or after fermentation and help to stabilise the wine colour (Keulder et al., 2004; Obradovic et al., 2005), as the tannin can react with grape anthocyanins to form polymeric pigments (Remy et al., 2000). Bautísta-Ortin et al. (2005), however, noted detrimental effects to wine colour and sensory quality when using commercial tannins. Addition of commercial tannins will presumably enhance antioxidant capacity.

Influence of Maturation on Phenolic Composition

Maturation is an important phase in the production of quality red wines. The colour stability, taste and quality of wine are improved with ageing (Somers & Pocock, 1990; Castellari et al., 2001). The changes in wine colour during maturation and ageing of red wines are attributed to the incorporation of grape anthocyanins into more stable polymeric pigments by various types of condensation reactions (Ribéreau-Gayon et al., 1983; Zimman & Waterhouse, 2004). These reactions also lead to reduced astringency (Vidal et al., 2004a). Traditionally, oak barrels are used for red wine maturation, but alternative oak sources such as chips, staves and oak extracts are receiving increasing attention from winemakers and researchers. Another relatively new technique, oxygenation, is also sometimes used with or without alternative oak products for red wine maturation.

Oak Maturation

Flavour compounds, mostly volatile phenols and aromatic aldehydes, are extracted from the oak wood promoting complexity of the wine flavour (Maga, 1989). The concentration of cinnamic and benzoic acid derivatives increase in wine during oak maturation, due to hydrolysis of oak wood hydrolysable tannins, namely ellagitannins (Laszlavik et al., 1995; Kadim & Mannheim, 1999; Del Alamo Sanza et al., 2004). The concentration of ellagitannins extracted from oak wood is not expected to influence the taste of wines as the level in matured wines is below the taste threshold (Pocock et al., 1994). Maturation of red wine in used barrels results in less extraction of ellagitannins and aroma compounds than when new barrels are used (Gómez-Plaza et al., 2004). Condensation reactions of anthocyanins with flavan-3-ols, forming oligomeric and polymeric phenolic compounds, result in stabilised colour (Singleton, 1987; Revilla & González-SanJosé, 2001).

During maturation some oxygen is present in the wine, presumably entering the barrel during topping up (Singleton, 1995). In the presence of oxygen, oak wood ellagitannins are oxidised producing peroxides, which in turn produce acetaldehyde via ethanol oxidation (Vivas & Glories, 1996). When no more ellagic acid is present, coupled oxidation of ethanol with other phenolic compounds can produce acetaldehyde. Consequently, acetaldehyde-mediated condensation reactions are promoted during oak barrel maturation. Oak barrels that are used more than once decrease in oxygen permeability so that the level of dissolved oxygen in wine matured in a third fill barrel will be approximately the same as in a stainless steel tank (Vivas, 1995). Ethyl-linked dimers are produced quite early during barrel maturation with a subsequent decrease in concentration (Alcalde-Eon et al., 2006), as they react further to form xanthylium pigments (Es-Safi et al., 2000; Del Alamo et al., 2000) or more polymerised pigments (Es-Safi et al., 1999a). Many of the

pyranoanthocyanins also require a polymerisation step to form. Consequently they also increase in concentration during the early part of barrel maturation (Castellari et al., 2004; Alcalde-Eon et al., 2006). The flavonol and hydroxycinnamic acid contents of red wine also decrease during oak maturation (Hernández et al., 2006).

Alternative oak treatments used by winemakers in recent years include oak chips, staves and extracts (Maga, 1989; Bertrand et al., 1997). Large quantities of oak chips or staves are introduced into the wine for a short time to accelerate the oak maturation process (Arapitsas et al., 2004; Del Alamo Sanza et al., 2006). The use of chips in stainless steel tanks resulted in a wine with similar content of volatile compounds from oak wood, compared to a wine matured in new barrels (Arapitsas et al., 2004). Reactions occurring during oak barrel maturation are also expected to occur during maturation with alternative oak products. Faster polymerisation reactions have been reported on the basis that the anthocyanin monomer content decrease faster for a wine matured with chips and staves in stainless steel tanks, compared to the same wine matured in oak barrels (Del Alamo Sanza et al., 2006). In another study, wine matured with oak chips in stainless steel tanks achieved similar characteristics than wine matured in oak barrels, while wine matured with oak staves in stainless steel tanks was different (Del Álamo et al., 2006). The differences were mainly a higher volatile acidity and lower ethanol content for wines matured with staves in stainless steel tanks, compared to the other wines. Alternative oak treatments can, however, also be used to simulate normal barrel maturation by introducing it into used barrels at lower dosages. A study on South African Cabernet Sauvignon showed good results in terms of colour and sensory quality after maturation, using alternative oak products in old barrels or stainless steel tanks (Van Rensburg & Joubert, 2002). In this study, staves gave the best sensory results when applied in old barrels, while oak extracts performed better when added to wine in stainless steel tanks. Bertrand et al. (1997), however, found that oak extracts did not add much wood aroma to a red wine. The advantages of using alternative oak products are their lower cost and ease of use, compared to oak barrels.

Many factors influence the composition of oak wood and therefore its effect on the wine composition when used to mature red wine. Several species of oak can be used for barrel production, most importantly the European species *Quercus robur* and *Q. sessilis* and the American species *Q. alba*. Studies on maturation of red wine using different oak species have pointed out that the oak species have a great influence on the wine sensory characteristics and phenolic composition (Lazlavik et al., 1995; Cadahia et al., 2003; Fernandez de Simon et al., 2003). Other sources of variation include geographical origin (Ancín et al., 2004), seasoning (Hale et al., 1999) and level of toasting (Martinez et al., 1996; Hale et al., 1999) of the oak wood. The size of barrels used to mature red wine will also affect extraction of compounds into the wine, due to differences in the surface to volume ratio (Pérez-Coello et al., 1999).

Very few studies on the effect of oak maturation on the antioxidant capacity of red wines are conducted. A wine matured in oak barrels for six months showed decreased TRAP and platelet inhibitory activity compared to the same wine stored in bottles (Baldi et al., 1997). Saint-Cricq de Gaulejac et al. (1998) showed that a white wine fermented in oak barrels had a much higher radical scavenging activity than the same wine fermented in stainless steel tanks. This was due to the presence of ellagitannins in the white wine fermented in oak barrels. On the other hand, ageing of red and white wines has been shown to decrease their total phenol content and antioxidant capacity (De Beer et al., 2005). The redox potential of red wines, aged in oak barrels and stainless steel tanks with chips and staves, was reported to increase during the first three months of maturation with a subsequent decrease up to 11 months of maturation, when the maturation experiment ended (Del Álamo et al., 2006). These authors reported very small differences in the redox potential of wine matured with the different systems. It is difficult to predict what the effect of oak maturation will be on the antioxidant capacity of red wines, as some new phenolic compounds are extracted into the wine from the oak, while monomeric compound concentrations decrease and polymeric compound concentrations increase.

Oxygenation of Wine

Oxygen can be applied in discrete doses or continuously at very low doses (micro-oxygenation) in the range of 1 – 5 mg O_2/L/month for up to six months during red wine maturation to accelerate colour stabilisation. Both oxygenation in discrete doses (Castellari et al., 2000) and in continuous doses (Atanasova et al., 2002; Du Toit & Groenewald, 2003) have been reported to positively affect the colour stability and sensory quality of red wine, although the total phenol content decreased. Micro-oxygenation has also been used to accelerate biological ageing of Sherry wines (Muñoz et al., 2005). Adding large doses of oxygen during fermentation (macro-oxygenation) in the range of 2 – 3 mg O_2/L/day for one to three days can be used to prevent stuck fermentations and remove reductive aromas (Sablayrolles et al., 1996).

In the presence of oxygen, acetaldehyde is produced via coupled oxidation of ethanol (Wildenradt & Singleton, 1974). Consequently, acetaldehyde-mediated condensation reactions are favoured above direct anthocyanin-tannin condensation reactions during oxygenation (Atanasova et al., 2002; Fulcrand et al., 2004). Pyranoanthocyanin formation is also favoured by the presence of oxygen as its formation requires an oxidation step. Castellari et al. (2004) reported that micro-oxygenation at 5 mL/L/month resulted in a dissolved oxygen content similar to that obtained by oak barrel maturation. As the oxygen capacity of wines differs due to differences in phenolic composition, the amount of oxygen needed for beneficial effects is difficult to determine. Wines should therefore be constantly monitored during the oxygenation process to prevent over-oxidation (Lemaire, 2003). Excess oxygen may also promote growth of undesirable spoilage bacteria.

Phenolic Composition Determination

Traditional techniques for determining the phenolic composition of wines rely on spectrophotometric measurements made directly on the wine, or after selective reaction with a reagent giving a coloured product. Distinctive spectral properties distinguish phenolic groups depending on their structures (**Table 1**). These spectrophotometric assays generally only give an estimation of a group of compounds. Identification and quantification of individual phenolic compounds are only possible using sophisticated chromatography techniques coupled to various detection techniques, such as HPLC or GC with UV-visible, fluorescence or mass-spectroscopic detection. The most popular HPLC technique for phenolic analysis is reversed-phase HPLC, although some normal-phase HPLC applications also exist.

Spectrophotometric Assays

The most popular method of measuring the total phenol content of wines is the Folin-Ciocalteau assay (Singleton & Rossi, 1965). The reagent, consisting of heteropoly phosphotungstate-molybdates in an aqueous acidic solution, oxidises monophenols and vicinal diphenols via an one-electron transfer to form blue products after addition of sodium bicarbonate. The absorbance at 765 nm is used against a standard curve of gallic acid to determine the total phenol content expressed as mg gallic acid equivalents/L. Other standards may also be used. Many possible interfering substances have been tested, including ascorbic acid, amines and sulphur dioxide (Singleton et al., 1999), but most of these occur in red wines in amounts too low to cause serious problems. Binding of sulphur dioxide with acetaldehyde before determination of total phenol content eliminates its interference, but this is only needed for wines with very high sulphur dioxide contents (Singleton et al., 1999). As the Folin-Ciocalteau assay is based on the potential of wine phenols to reduce the reagent, it can also be classified as a measurement of antioxidant activity (Huang et al., 2005). Other methods for determining total phenol content include reaction with potassium permanganate (Singleton & Esau, 1969), complexation with iron salts (Deshpande et al., 1984) and direct measurement of ultraviolet absorbance (Somers & Vérette, 1988). These methods are not, however, used routinely.

The anthocyanin content of wines can be estimated using absorbance measurement at different pH values (Ribéreau-Gayon & Stonestreet, 1965). Wines are diluted with buffers at pH <1 and pH 3.5 and their absorbance measured at 520 nm. At pH <1 anthocyanins are in their red flavylium form, allowing the determination of the total anthocyanins, while at the higher pH the monomeric anthocyanins are mainly in the colourless carbinol form with most of the absorbance attributable to polymeric anthocyanins. The difference in absorbance between pH <1 and the higher pH value is

Table 1. Spectral properties[a] of phenolic groups found in red grapes and wine.

Phenolic group	UV band II	UV band I	Visible band
Benzoic acids	270 – 280		
Hydroxycinnamic acids and derivatives	(290 – 300)[b]	305 – 330	
Anthocyanins	240 – 280	(315 – 325)[c]	450 – 560
Flavonols	250 – 270	(300)[b] 350 – 380	270 – 280
Flavan-3-ols	270 – 280		

[a] compounds in methanol, but methanol-hydrochloric acid used for anthocyanins; spectral properties depends on solvent composition; [b] shoulder; [c] in the case of acylation by hydroxycinnamic acids.

thus due to the monomeric anthocyanin content. Cabrita et al. (2000), however, has shown that the minimum absorbance for anthocyanins commonly occurring in wines is at pH 4.9. Using pH 4.9 instead of pH 3.5 should therefore give an improved estimate of monomeric and polymeric anthocyanin contents. Anthocyanins are quantified as mg malvidin-3-glucoside equivalents/L, the major anthocyanin in red wine, using its molar extinction coefficient $\varepsilon = 28000$ (Burns et al., 2000). Another popular method for determining the monomeric and polymeric anthocyanin contents spectrophotometrically is the bisulphite bleaching assay (Somers & Evans, 1977). This assay is based on the fact that monomeric anthocyanins are bleached by sulphur dioxide, while polymeric anthocyanins are resistant to bisulphite bleaching. Some monomeric anthocyanins of the pyranoanthocyanins group are, however, also resistant to bisulphite bleaching (Vivar-Quintana et al., 2002; Håkansson et al., 2003), while some oligomeric and polymeric anthocyanins formed during wine maturation may be partially bleached (Timberlake & Bridle, 1968; Escribano-Bailon et al., 2001).

The total flavan-3-ol content of wines has been measured spectrophotometrically using the reaction of flavan-3-ols with various aldehydes, such as vanillin (Goldstein & Swain, 1963; Sun et al., 1998) and dimethylaminocinnamaldehyde (DAC) (McMurrough & McDowell, 1978), in acidic solution. Interference by anthocyanins at the absorbance for measurement (500 nm) makes the vanillin assay problematic for use with red wines (Sun et al., 1998), while in the DAC assay no interference is present as measurement takes place at 640 nm (McMurrough & McDowell, 1978). The DAC assay is considered to be more sensitive and specific than the vanillin assay, although the timing is more critical (McMurrough & McDowell, 1978; Nagel & Glories, 1991). Reaction times between 2 and 4 min are generally preferred as the reaction is then complete, but no loss of colour should have occurred yet. (+)-Catechin is generally used as a standard and the results expressed as mg catechin equivalents/L. Flavan-3-ol monomers react most strongly with the DAC reagent and reactivity decreases as degree of polymerisation increases (McMurrough & McDowell, 1978).

HPLC Methods

Many reversed-phase HPLC methods have been developed to quantify individual phenolic compounds in wines. See **Table 2** for a selection of methods using UV/visible diode array detection. Individual compounds up to dimer level can generally be separated using reversed-phase HPLC, although separation of all monomeric compounds of interest remains a problem. Consequently a wide variety of methods are used depending on the compounds of interest. Fluorescence detection can greatly improve the sensitivity of detection of compounds such as flavan-3-ols, flavonols and stilbenes (Rodríguez-Delgado et al., 2001). Methods, using pre-treatment and/or different gradient programmes to separate different groups of compounds, can quantify more compounds due to elimination of interfering substances and improved selection. These methods, however, are very time-consuming and not viable when large numbers of samples need to be analysed. A method by Price et al. (1995), optimised for red wine by Peng et al. (2002), can be used to quantify a large range of monomeric and dimeric phenolic compounds. A broad peak consisting of polymeric material is detected at 280 and 520 nm for quantification of non-coloured and coloured polymers, respectively. This method also has much less baseline shift than other methods that also do not use sample pre-treatment. Methods to distinguish more effectively between monomeric, oligomeric and polymeric flavan-3-ols and pigments mostly involve normal-phase HPLC (Lazarus et al., 1999; Kennedy & Waterhouse, 2000). Recently the use of mass spectroscopic detection has greatly increased the number of compounds that can be identified and quantified in red wine (reviewed by Monagas et al., 2005).

Antioxidant Capacity Determination

Antioxidant Capacity Assays

Recently, several reviews comparing the wide range of antioxidant assays that are commonly used for plant extracts and compounds have been published Antolovich et al., 2002; Huang et al., 2005; Prior et al., 2005; Roginsky & Lissi, 2005). The most widely used assays include the $ABTS^{\bullet+}$ scavenging, 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging, oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays. The Folin-Ciocalteau assay has recently been accepted to also provide a measurement of antioxidant capacity (Huang et al., 2005; Prior et al., 2005). All antioxidant assays, however, have advantages and disadvantages and no single assay will be able to provide a complete picture of antioxidant capacity.

The $ABTS^{\bullet+}$ scavenging assay was chosen for use during this study, due to its ease of use and high-throughput capabilities. Prior et al. (2005) also recommended that the $ABTS^{\bullet+}$ scavenging, ORAC and Folin-Ciocalteau assays be standardised for high-throughput screening of samples. The

Table 2. HPLC methods for quantification of phenolic compounds in red wines using reversed-phase HPLC with UV/visible diode array detection.

Source	Compounds	Column	Solvents	Advantages	Disadvantages
Nagel & Wulf, 1979	phenolic acids	Zorbax ODS, 4.6 x 250 mm	Solvent = 0.5% aq. formic acid/CH_3CN (9/91)	1. Very good separation as conditions is adapted for each phenolic group	1. Requires several analyses to quantify all phenolic groups;
	anthocyanins	Lichrosorb ODS, 4.6 x 250 mm	Solvent A = 10% aq. formic acid; Solvent B = acetone/formic acid/water (25/10/65)		2. Long analysis time (~120 min.)
	ethyl acetate extractables	Zorbax ODS, 4.6 x 250 mm	Solvent A = 1% aq. acetic acid at pH 4; Solvent B = acetic acid/ CH_3CN/water (1/40/59) at pH 4		
Lamuela-Raventós & Waterhouse, 1994; Ritchey & Waterhouse, 1999; Hermosín Guttiérez et al., 2005	phenolic acids and derivatives; flavan-3-ols; flavonols; anthocyanins	Various C18 columns	Solvent A = 50 mM aq. $NH_4H_2PO_4$ at pH 2.6; Solvent B = 20% A + 80% CH_3CN; Solvent C = 0.2 M aq. H_3PO_4 at pH 1.5	1. Separates compounds from different groups; 2. No sample pre-treatment	1. Polymeric material cause baseline shift; 2. Fairly long analysis time (>60 min.)
Ibern-Gómez et al., 2002	noncarboxylic phenols; phenolic acids and derivatives; flavan-3-ols; flavonols; anthocyanins	Zorbax Stablebond C18, 4.6 x 30 mm, 3.5 μm particle size	Solvent A = 0.2% aq. trifluoroacetic acid; Solvent B = 0.2% trifluoroacetic acid in CH_3CN	1. Separates compounds from different groups; 2. No sample pre-treatment; 3. Very fast analysis time (~20 min.)	1. Polymeric material causes baseline shift
Price et al., 1995; Peng et al., 2002	phenolic acids and derivatives; flavan-3-ols; flavonols; anthocyanins; polymers	PLRP-S, 4.6 x 250 mm, 5 μm particle size, 100 Å pore size	Solvent A = 1.5% aq. H_3PO_4; Solvent B = 20% Solvent A + 80% CH_3CN	1. Separates compounds from different groups; 2. No sample pre-treatment; 3. Polymeric material elutes as a separate peak	1. Long analysis time (~100 min.)
Gil-Muñoz et al., 1999	phenolic acids	Hewlett-Packard C18, 0.4 x 250 mm, 0.5 μm particle size	Solvent A = 2% aq. acetic acid; Solvent B = CH_3CN	1. More compounds can be identified as interfering substances are removed	1. Fractionation of phenolic groups using SPE required.
	flavan-3-ols		Solvent A = 4.5% aq. formic acid; Solvent B = 4.5% aq. formic acid/ CH_3CN (90/10)	2. Very good separation as conditions is adapted for each phenolic group	2. Requires several runs to quantify all phenolic groups
	flavonols		Solvent A = 2% aq. acetic acid; Solvent B = CH_3CN	3. No baseline shift due to sample pre-treatment	3. Very long analysis time (pre-treatment + ~5 hrs)
	anthocyanins		Solvent A = 5% aq. formic acid; Solvent B = CH_3CN		

use of the $ABTS^{\bullet+}$ scavenging assay will also permit comparison of results with those obtained in a previous study on South African wine (De Beer et al., 2003).

Methodological Aspects of ABTS Radical Cation Scavenging Assay

The free radical scavenging assays involve an electron or a hydrogen atom transfer from the antioxidant to the oxidant. In the case of the $ABTS^{\bullet+}$ scavenging assay, a mixture of both mechanisms applies (Prior et al., 2005). In general, the oxidant, namely ABTS, is reacted with the antioxidant solution at 3 °C and the decrease in absorbance at 734 nm measured (Re et al., 1999). Trolox, a water-soluble vitamin E analogue, is used as reference antioxidant to quantify the results. Many protocols have been published for the $ABTS^{\bullet+}$ scavenging assay (see **Table 3** for some examples). Some of the methodological features that influence the assay will be discussed in this section.

Firstly, different radical generation strategies have been used, namely enzymatic, chemical and electrochemical generation. Enzymatic radical generation was first proposed by Miller and Rice-Evans (1993). Enzymes that have been used are metmyoglobin (Miller & Rice-Evans, 1993) and horseradish peroxidase (Cano et al., 1998). Among the chemical oxidants used for radical generation, MnO_2 (Miller et al., 1996), $K_2S_2O_8$ (Re et al., 1999), peroxyl radical generators (Van den Berg et al., 1999) and hydrogen peroxide (Erel, 2004) are the most common. Electrochemical radical generation is a relatively novel strategy (Ivekovi□et al., 2005). Radical generation usually occurs in the absence of the antioxidant molecules in recently described protocols, whereas the original assay employed enzymatic radical generation in the presence of antioxidant molecules. Over-estimation of the antioxidant capacity may be a problem in the latter case if the antioxidant molecules could inhibit enzyme activity (Miller & Rice-Evans, 1997b).

Several authors have investigated the effect of reaction medium on the assay. Labrinea and Georgiou (2004) using a number of phenolic acids and flavonoids, demonstrated that the scavenging activity generally increases with decreased pH in a buffer medium and with increased reaction time. A similar result was reported for hydroxyflavones (Lema□ska et al., 2001). Dissociation of phenolic acids was found to influence their antioxidant activity as TEAC values were lower when measured in an ethanolic medium compared to an aqueous medium at pH 7.4 (Nenadis et al., 2004).

The $ABTS^{\bullet+}$ scavenging activity can be measured at different time-points with reaction times varying from 0.1 s (Pannala et al., 2001) to 30 min (Awika et al., 2003), although most authors use a reaction time between 1 and 6 min (see **Table 3** for examples). It seems that most phenolic compounds of interest have completed the reaction with $ABTS^{\bullet+}$ after this time (Re et al., 1999; Villaño et al., 2005).

Table 3. ABTS radical scavenging assay protocols used for the determination of Trolox equivalent antioxidant capacity of phenolic compounds.

Source	Radical generation strategy	Solvent for $ABTS^{\bullet+}$ solution	Solvent for phenolic solution	Reaction time	Calculation method
1: Rice-Evans et al., 1995; **2**: 1996	metmyoglobin and H_2O_2 in presence of test compound	phosphate-buffered saline (pH 7.4)	ethanol, distilled water or 70% DMSO	6 min.	directly from calibration curve using average of 3 compound concentrations determined on 2 separate days
3: Miller & Rice-Evans, 1997a	metmyoglobin and H_2O_2 in presence of test compound	phosphate-buffered saline (pH 7.4)	not reported	not reported	directly from calibration curve using average of 3 compound concentrations determined on 2 separate days
4: Plumb et al., 1998	metmyoglobin and H_2O_2 in presence of test compound	phosphate-buffered saline (pH 7.4)	not reported	6 min.	directly from calibration curve using average of 3 compound concentrations determined on 3 separate days
5: Re et al., 1999	$K_2S_2O_8$ pre-generation	ethanol	ethanol	1, 4 or 6 min.	slopes of dose-response curves for Trolox (4 concentrations) and compound (3 concentrations)
6: Baderschneider & Winterhalter, 2000; **7**: 2001	metmyoglobin and H_2O_2 in presence of test compound	phosphate-buffered saline (pH 7.4)	not reported	6 min.	directly from calibration curve using one compound concentration
8: Pellegrini et al., 2003	$K_2S_2O_8$ pre-generation	ethanol	ethanol	1 min.	directly from calibration curve using average of 5 compound concentrations determined on 3 separate days
9: Garcia-Alonso et al., 2004	$K_2S_2O_8$ pre-generation	distilled water	dissolved in DMSO; diluted with distilled water	6 min.	slopes of dose-response curves for Trolox and compound (4 concentrations) on 3 separate days
10: Nenadis et al., 2004	$K_2S_2O_8$ pre-generation	ethanol	ethanol	6 min.	slopes of dose-response curves for Trolox (4 concentrations) and compound (3 concentrations)
11: Borkowski et al., 2005	microperoxidase-8 and H_2O_2 pre-generation	phosphate-buffered saline (pH 7.4)	0.01 M hydrochloric acid in methanol	6 min.	slopes of dose-response curves for Trolox and compound (unknown number of concentrations)
12: Ivekovi□et al., 2005	electrochemical pre-generation	phosphate-buffered saline (pH 7.4)	phosphate-buffered saline (pH 7.4) in 35% ethanol or distilled water	1 min.	directly from calibration curve using one compound concentration
13: Villaño et al., 2005	horseradish peroxidase and H_2O_2 pre-generation	glycine-HPLC buffer (pH 4.5)	15% ethanol	2 min.	directly from calibration curve using average of 5 – 6 compound concentrations

The total capacity of mixtures or wines to scavenge $ABTS^{\bullet+}$ is referred to as total antioxidant capacity (TAC) (Landrault et al., 2001; Serafini & Del Rio, 2004), expressed as Trolox equivalents, although the terms total antioxidant status (Soleas et al., 1997), total antioxidant activity (Miller et al., 1993) and total antioxidant potential (Simonetti et al., 1997) are also sometimes used. The concentration of Trolox (mM) with equivalent antioxidant capacity as a 1 mM solution of a compound is called the Trolox equivalent antioxidant capacity (TEAC) of that compound. Methods to calculate the TEAC values differ between authors (**Table 3**) with some using the response from one concentration of compound (Nenadis et al., 2004) and others a concentration series (Re et al., 1999; Labrinea & Georgiou, 2004) to calculate the TEAC. Other differences include the use of the ratio of slopes of the dose-response curves (Re et al., 1999; Villaño et al., 2005) or the EC_{50} concentrations (Lee et al., 2003) of the compounds to that of Trolox. All the above-mentioned methodological differences contributed to the large variation in TEAC values reported for phenolic compounds (**Table 4**), subsequently hampering comparisons between results from different reports.

Structure-activity Relationships of Phenolic Compounds

Several studies have investigated the structure-activity relationships of phenolic compounds in the $ABTS^{\bullet+}$ scavenging assay (Rice-Evans et al., 1996; Lien et al., 1999; Nenadis et al., 2003), although this approach has been criticised recently (Arts et al., 2003; Nenadis et al., 2004). The most important criticism raised by these authors is that the reaction products of the phenolic compounds also seems to react with the $ABTS^{\bullet+}$, leading to over-estimation of activity. Important structural features for the radical scavenging activity of phenolic acids in the $ABTS^{\bullet+}$ scavenging assay, as well as other radical scavenging assays, include the number and position of phenolic hydroxyl groups, as well as the presence and position of electron-donating or -withdrawing moieties on the phenolic ring (Miller & Rice-Evans, 1997a; Silva et al., 200; Nenadis et al., 2003). A 3'4'-dihydroxy moiety on the B ring, a 2,3-double bond in combination with a 4-keto group on the C ring or a combination of 3- and 5-hydroxyl groups on the C and A rings, respectively, with a 4-keto group on the C ring were found to be important structural features for flavonoid radical scavenging activity (Bors et al., 1990; Rice-Evans et al., 1996).

Conclusions

Phenolic compounds are important with regard to the colour and sensory quality of red wines. The effect of climatic, viticultural and enological factors on the colour, sensory quality and phenolic composition of red grapes and wines is a popular subject in wine research. The effects of these changes in phenolic composition on the antioxidant capacity of the wine are not certain. Limited studies on the effect of these factors on red wine antioxidant capacity have been carried out.

Table 4. Trolox equivalent antioxidant capacity (TEAC) values[a] of wine phenolic compounds.

Compounds	1[b]	2[b]	3[b]	4[b]	5[b]	6[b]	7[b]	8[b]	9[b]	10[b]	11[b]	12[b]	13[b]
Phenolic acids													
Gallic acid		3.01	3.01	2.91				2.45				4.26	1.98
Ellagic acid												4.69	
p-Coumaric acid		2.22	2.22		1.51[c]; 1.82[d]; 2.00[e]		1.4			2.00			
Coutaric acid							0.5						
Caffeic acid		1.26	1.26		0.99[c]; 0.98[d]; 0.98[e]		1.1	0.92		1.01		1.04	1.01
Caftaric acid							1.1						
Stilbenes													
trans-resveratrol		2.00				2.6							0.4
Flavan-3-ols													
(+)-Catechin	2.2	2.4		2.47			2.7					3.68	0.57
(-)-Epicatechin	2.5	2.5		2.23			3.0						0.99
Procyanidin B1				4.73			4.3						
Flavonols													
Quercetin-3-rham							2.5						
Quercetin	4.7	4.78		4.7	2.77[c]; 3.03[d]; 3.10[e]		3.8	2.49		1.85		4.33	1.14
Myricetin	3.1	3.72											1.02
Kaempferol	1.34	1.34			1.02[c]; 1.02[d]; 1.02[e]								0.79
Anthocyanins													
Delphinidin-3-glc									4.0		2.61		
Petunidin-3-glc									3.0				
Malvidin-3-glc		1.78							3.6		1.89		

[a] Trolox concentration (mM) with antioxidant capacity equivalent to that of 1 mM solution of compound; [b] numbers correspond to those of **Table 3**; [c] 1 min. reaction time; [d] 4 min. reaction time; [e] 6 min. reaction time.

However, studies of this nature are becoming increasingly more frequent. Comparison of results between reports is also problematic due to the many different assays and protocols used in antioxidant studies. Research into identification of new compounds, formed during the vinification and maturation processes, has made great advances, but the presence of many unknown reaction products are still postulated. More research is needed to elucidate the effects of viticultural and enological practices on the antioxidant capacity of red wines.

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Chapter 3: Unravelling the Total Antioxidant Capacity of Pinotage Wines - Contribution of Phenolic Compounds*

Abstract

The total antioxidant capacity (TAC) and phenolic composition of 139 Pinotage wines (2002 and 2003 vintages) were determined using the $ABTS^{\bullet+}$ scavenging assay and high-performance liquid chromatography (HPLC), respectively. The contribution of individually quantified phenolic compounds to the wine TAC was calculated using their concentrations and Trolox equivalent antioxidant capacity (TEAC) values. The TEAC values of quercetin-3-galactoside, isorhamnetin and peonidin-3-glucoside are reported for the first time. Between 11 and 24% of the measured TAC of Pinotage wines was explained by the sum of the calculated contributions of their quantified phenolic compounds comprising monomeric phenolic compounds and procyanidin B1. Ultrafiltration was carried out to attempt separation of monomeric and polymeric phenolic compounds. Analysis of ultrafiltration permeates and retentates enabled estimation of the TAC contribution of large molecular weight (MW) unknown compounds (46%) (> 50 kDa), including oligomeric and polymeric phenolic compounds, and small MW unknown compounds (34%) (< 50 kDa). Three mixtures, containing 12 phenolic compounds in typical concentrations expected in Pinotage wines, exhibited 16 – 23% synergistic antioxidant activity. This suggests that synergy between phenolic compounds does play a role in the wine TAC, but that it does not explain the large discrepancy between measured and calculated TAC values.

Introduction

Enhancement of red wine antioxidant capacity, while retaining sensory quality, is a challenge facing the wine industry. An increasing phenolic concentration will increase the antioxidant capacity of wines, but can also negatively affect their sensory qualities. A wide range of variables such as cultivar, viticultural practices, and vinification techniques, can affect the phenolic composition of red wines. A recent study reported that the unique South African cultivar wine, Pinotage (1998 vintage commercial wines), had an average total antioxidant capacity (TAC) of 15.3 mM Trolox equivalents (TE) as measured using the 2,2'-azino-di(3-ethylbenzo-thiazoline-sulphonic acid radical cation ($ABTS^{\bullet+}$) scavenging assay (De Beer et al., 2003). This was comparable with that of other commercial cultivar wines of the same vintage produced in South Africa. Other studies (Rossouw &

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Marais, 2003; 2004; Schwarz et al., 2003; 2004) highlighted the unique phenolic composition of Pinotage wines, especially with regard to very high caffeoyltartaric or caffeic acid levels.

The $ABTS^{\bullet+}$ scavenging assay offers an easy and rapid method to screen large numbers of samples. The contribution of individual compounds with regard to the TAC of antioxidant mixtures, such as wine, is important especially where optimisation of TAC is a goal. Previous studies on wine, estimated the importance of individual compounds by determining their correlation with the TAC (Soleas et al., 1997; Landrault et al., 2001; Minussi et al., 2003). Such an approach uses statistical correlations to indicate whether a compound has a relationship with the TAC. Although this gives valuable information, correlations do not prove a causal relationship between the content of a specific compound and the TAC, nor do they give an indication of the relative contributions of individual compounds to the TAC of a complex mixture. Soleas et al. (1997) reported that 96% of wine TAC could be predicted using only eight individual monomeric phenolic compounds based on a linear multiple regression model.

A different approach is to use the content and the antioxidant potency of individual compounds to calculate their contribution to the TAC (Chun et al., 2003; Miller & Rice-Evans, 1997c; Rice-Evans et al., 1996). Rice-Evans et al. (1996) found that only 25% of the TAC of a red wine could be estimated from 10 quantified phenolic compounds. The antioxidant potency of many wine phenolic compounds in terms of their Trolox equivalent antioxidant capacity (TEAC) values has been reported previously (Rice-Evans et al., 1995; Salah et al., 1995; Miller & Rice-Evans et al., 1997a; Plumb et al., 1998; Re et al., 1999; García-Alonso et al., 2004). Whereas these values are valuable in determining the relative importance of the respective compounds, published data cannot be used to calculate the contribution of individual compounds to the TAC of a specific wine. Differences in the protocols and calculation methods will lead to different TEAC values for the respective phenolic compounds. TEAC values of pure reference standards should, therefore, be measured using the same assay protocol as used for determining the TAC of the wines.

Polymeric phenolic compounds, present in wine at levels between 65 and 85% of the total phenolic content, when measured using normal phase high-performance liquid chromatography (HPLC), depending on its age and origin (De Beer et al., 2004), may represent a sizable portion of the TAC of wine. TEAC values for polymeric compounds in the wines can, however, not be determined, although proanthocyanidin oligomers up to six units have a higher antioxidant activity than their monomeric counterparts (Hagerman et al., 1998; Lotito et al., 2000; Ursini et al., 2001; Tsai et al., 2004). Ultrafiltration may be used to separate the monomeric and polymeric phenolic compounds in wine in order to estimate the TAC contribution of the large molecular weight (MW) compounds, including polymeric phenolic compounds. However, synergy between phenolic

compounds may also possibly influence the TAC of wines (Vivas et al., 1997; Jørgensen et al., 1999; Saucier & Waterhouse, 1999; Liao & Yin, 2000).

Knowledge of the antioxidant activity of wine phenolic compounds and their contribution to wine TAC is essential to evaluate which phenolic compounds to manipulate in order to achieve an increase in TAC without detrimental effects to the sensory quality. The aim of the study was to determine the relative contribution of individual phenolic compounds in Pinotage wine to its TAC. This information is needed to prepare guidelines for manipulating the phenolic composition of these wines to obtain increased TAC. The contribution of polymeric phenolic compounds was estimated and the possible role of synergy between phenolic compounds was investigated.

Materials and Methods

Wines

A series of 139 Pinotage wines (63 wines from the 2002 vintage and 76 wines from the 2003 vintage), made from grapes (*Vitis vinifera*) originating from different climatic areas in the Western Cape (South Africa), were selected. Grapes were harvested at ~24 °B. The wines were produced from 20 – 30 kg of grapes for each wine according to a standard procedure with no wood contact in the experimental cellar of ARC Infruitec-Nietvoorbij (Stellenbosch, South Africa): After crushing, di-ammoniumfosfate (50 g/HL), SO_2 (50 mg/L) and *Saccharomyces cerevisiae* strain VIN 13 (30 g/HL) were added. Fermentation was carried out at 25 °C, and the cap was punched down three times per day. The skins were separated from the juice using a pneumatic press as soon as the sugar content dropped to < 5 °B. Pressed juice was added to the free-run juice and fermented dry at 25 °C. After fermentation, the total SO_2 content was adjusted to 35 mg/L, and 50 g/HL bentonite (ProteaChem, Cape Town, South Africa) was added. The wines were cold-stabilized for 2 weeks at 0 ºC, filtered using diatomous earth filter sheets (ProteaChem), sterile-filtered using 0.45 µm nitrocellulose membrane filters (Millipore, Bedford, MA) and bottled in N_2-filled bottles at room temperature, after adjustment of the total SO_2 content to 40 mg/L. After bottling, the wines were stored at 15 ºC. Eight months after production, aliquots of each wine were frozen at -20 °C, to prevent further phenolic changes, until analyses were carried out. Samples were analysed immediately after defrosting.

Chemicals and Phenolic Reference Standards

2,2'-Azino-di-(3-ethylbenzo-thialozine-sulphonic acid) (ABTS) was obtained from Boehringer Mannheim GmbH (Mannheim, Germany) and HPLC grade acetonitrile and phosphoric acid were from Riedel-de Haën (Seelze, Germany) and Fluka (Buchs, Switzerland), respectively. Potassium persulphate ($K_2S_2O_8$) was obtained from Sigma Chemical Co. (St. Louis, MO) and 6-hydroxy-

2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox) was from Aldrich Chemical Co. (Gillingham, United Kingdom). Phenolic reference standards included gallic acid, (+)-catechin, (-)-epicatechin, quercetin-3-galactoside (gal) and quercetin-3-rhamnoside (rham) from Sigma; caffeoyltartaric acid from Chromadex (Santa Ana, CA); caffeic acid, quercetin and kaempferol from Fluka; procyanidin B1, quercetin-3-glucoside (glc) and myricetin from Extrasynthese (Genay, France); and delphinidin-3-glc, peonidin-3-glc, petunidin-3-glc and malvidin-3-glc from Polyphenols AS (Sandnes, Norway). Water used in the experiments was purified and deionised with a Modulab water purification system (Separations, Cape Town, South Africa), except for preparation of HPLC mobile phases where deionised water was further treated using a Milli-Q academic water purifier (Millipore).

HPLC Analysis of Phenolic Composition

The individual phenolic compounds were quantified in duplicate using a reversed-phase HPLC method modified from Peng et al. (2002). The HPLC apparatus used was a Waters LC Module I equipped with a Waters 2996 photodiode array detector using Millenium^{32} version 4.0 software (Waters, Milford, MA). Separation was achieved on a PRP1 column (250 mm x 4.1 mm, 100 Å pore size, 5 μm particle size) from Hamilton (Reno, NV). A guard cartridge (20 mm x 2.3 mm) packed with the same material and a PEEK PAT frit (5 μm) were used to protect the analytical column. Wines were filtered using 0.45 μm Millex-HV hydrophilic PVDF 33 mm syringe-tip filter devices (Millipore) before automated duplicate injections of 20 μL each. The column was held at 30 °C during the run and the flow rate was 0.9 mL/min. Data were obtained in the wavelength range of 250 - 600 nm. The mobile phases used were: 1.5% (v/v) aqueous phosphoric acid (A) and 1.5% (v/v) phosphoric acid in acetonitrile/water (80/20) (B). The gradient program was as follows: from 94 to 69% solvent A in the first 73 min, reduced from 69 to 38% from 73 to 78 min, held isocratic at 38% from 78 to 86 min, increased from 38 to 94% from 86 to 90 min, and equilibration at 94% for 20 min.

Compounds were identified by comparison of their retention times and spectral data to those of pure reference standards except in the following cases: The anthocyanin acetate esters, malvidin-3-*p*-coumaroylglucoside (glc-coum) and vitisin A were identified from their spectra, which are similar to that of malvidin-3-glc, and their retention times relative to the other anthocyanin compounds (Peng et al., 2002). *p*-Coumaroyltartaric acid was identified from its spectrum (Meyer et al., 1998), which is similar to that of caffeic acid, and its retention time relative to that of caffeic acid. (-)-Epicatechin and procyanidin B2 were not quantified due to coelution with peonidin-3-glc and malvidin-3-glc, respectively. Initially, calibration curves for each phenolic reference standard were set up. For each phenolic compound, the response ratio between it and the representative standard

for their phenolic group at the optimal wavelength (gallic acid for benzoic acids at 280 nm; (+)-catechin for flavan-3-ols at 280 nm; caffeic acid for hydroxycinnamic acids and their derivatives at 316 nm; rutin for flavonols at 360 nm; and malvidin-3-glc for anthocyanins at 520 nm) was calculated. For subsequent analyses, each group of analyses was started with calibration standards including gallic acid (5 – 50 mg/L), (+)-catechin (10 – 150 mg/L), caffeic acid (5 – 150 mg/L), rutin (3 – 100 mg/L) and malvidin-3-glc (10 – 400 mg/L). The response ratios were used to calculate the content of the phenolic compounds in mg/L in the wines. The anthocyanin acetate and coumarate esters were quantified as mg of the corresponding anthocyanin-3-glc equivalents/L, while *p*-coumaroyltartaric acid was quantified as mg *p*-coumaric acid equivalents/L.

The polymeric content, expressed as mg (+)-catechin equivalents/L, was quantified from the area of the broad peak eluting between 80 and 85 min. Peng et al. (2002) showed that this peak contains mainly polymeric compounds using ultrafiltration, protein binding, and SO_2 bleaching.

ABTS Radical Cation Scavenging Assay

The TAC of wines and the TEAC of phenolic reference standards were determined in triplicate using the $ABTS^{\bullet+}$ scavenging assay (Re et al., 1999). An ABTS solution (7 mM) in water was preincubated for at least 12 h with 2.45 mM (final concentration) $K_2S_2O_8$ to produce the radical cation. The $ABTS^{\bullet+}$ solution was then diluted with ethanol to an absorbance of ~0.7 (±0.02) at 734 nm. In the reaction mixture, 1 mL of $ABTS^{\bullet+}$ solution was added to 50 µL of wine sample (50 times diluted with 10% ethanol), standard Trolox solution (0 - 400 µM in ethanol), or 10% ethanol (control) and the absorbance was determined after exactly 4 min of incubation at 37 °C. Spectrophotometric measurements were made in disposable polystyrene 2.5 mL macro cuvettes (Brand Gmbh & Co Kg, Wertheim, Germany) with 1 cm path length using a Beckman DU-65 UV/vis spectrophotometer (Beckman Instruments Inc., Fullerton, CA). The concentration of $ABTS^{\bullet+}$ in the control and samples was calculated using the absorbance readings and the molar extinction coefficient of $ABTS^{\bullet+}$, $\varepsilon = 16\,000$ (Re et al., 1999). A plot of remaining $ABTS^{\bullet+}$ concentration against the Trolox concentration in the standard samples was used to calculate the TAC of the wines expressed as mM TE.

The TEAC value of a compound is the concentration of Trolox in mM needed to achieve the same amount of $ABTS^{\bullet+}$ scavenging as a 1 mM solution of that compound. The effect of the solvent composition on the TEAC values of phenolic compounds was tested (see **Addendum A**). As the solvent composition affected the TEAC values, a composition close to that of the wine was selected for determining the TEAC values of the phenolic compounds. A concentration range (4 – 8 concentrations) of each phenolic reference standard, dissolved and diluted in 10% ethanol, was analysed using the $ABTS^{\bullet+}$ scavenging assay on two separate days in order to determine their TEAC

values. Quercetin-3-gal, quercetin, myricetin, kaempferol, and isorhamnetin were dissolved in dimethyl sulfoxide and diluted with water and 10% ethanol to contain 90% water as for compounds dissolved in 10% ethanol. The slopes of the dose-response curves (concentration vs nmoles of $ABTS^{\bullet+}$ scavenged) of the test compounds were compared to that of Trolox to determine the TEAC values (mM):

$$\text{TEAC} = \frac{\text{slope (test compound)}}{\text{slope (Trolox)}} \qquad (1)$$

The TAC contribution (mM TE) of individual phenolic compounds to the wine TAC was calculated from their content (mg/L) and TEAC values (mM):

$$\text{TAC contribution} = [\text{compound}] \times \text{TEAC} \qquad (2)$$

Ultrafiltration of Wines

Two wines with a similar phenolic composition were selected for ultrafiltration. Ultrafiltration of each wine was performed in duplicate using Vivaspin 4 mL centrifuge devices (Vivascience, Hanover, Germany) with polyethersulphone membranes with nominal molecular weight cutoff of 10, 30, and 50 kDa. Three ultrafiltration protocols were tested (see **Addendum B**) and the best one, described here, was used. Centrifugal ultrafiltration of 2 mL of wine, diluted with 1 mL of 10% ethanol, was performed at a speed of 5000 rpm and a temperature of 20 °C, using a Sorvall RC-3B refrigerated centrifuge (Sorvall Instruments, Newtown, PA) until ~100 µL of retentate was left. Then 1 mL of 10% ethanol was added to the retentate and centrifugation was resumed until ~100 µL of retentate was left. Both the pooled permeate and the retentate were diluted to the total volume (4 mL) with 10% ethanol and stored at -20 °C until HPLC and antioxidant analyses. The original wine, diluted (1:1) with 10% ethanol, was also stored at -20 °C and analysed. The tannin content of ultrafiltration retentates was determined in duplicate using a protein precipitation assay (Harbertson et al., 2003), to confirm the presence or absence of polymeric phenolic compounds.

Synergy between Phenolic Compounds

Three mixtures containing 12 phenolic compounds in typical concentrations in the range as measured in Pinotage wines in this study, were prepared in 10% ethanol and analysed, using the $ABTS^{\bullet+}$ scavenging assay. The TAC of the mixtures was estimated by calculation using the concentration and TEAC values of the phenolic compounds ($TAC_{calculated}$ in mM) and by measurement with the $ABTS^{\bullet+}$ scavenging assay ($TAC_{measured}$ in mM). The percent synergy was calculated as follows:

$$\%\ \text{synergy} = \frac{TAC_{measured} - TAC_{calculated}}{TAC_{calculated}} \times 100 \qquad (3)$$

Statistical Analysis

Statistical analyses were carried out using the SAS version 8 software (SAS Institute Inc., Cary, NC). Analysis of variance analysis was performed on the means of duplicate ultrafiltration samples and means were compared statistically with the Student's *t*-LSD test ($P < 0.05$).

Results and Discussion

Phenolic Composition of Wines

The phenolic composition of a large selection of Pinotage wines, in terms of 23 monomeric compounds, procyanidin B1 (dimer) (see **Figure 1** for representative chromatograms at different wavelengths and **Figure 2** for compound structures) and their polymeric content are summarized in **Table 1**. Qualitative and quantitative differences were observed. Compounds such as quercetin-3-gal, myricetin, kaempferol, and isorhamnetin were only detected in some wines. Pinotin A, a reaction product of malvidin-3-glc and caffeic acid, which has recently been isolated and identified in Pinotage wines (Schwarz et al., 2003; 2004), was not detected in the wines. This is possibly due to relatively low levels of caffeic acid in these wines and the fact that they were very young. Several other monomeric phenolic compounds that have been shown to occur in red wine, such as (-)-epicatechin, flavan-3-ol gallate esters, *S*-glutathionylcaftaric acid, stilbenes, stilbene glucosides and pyranoanthocyanins other than vitisin A, have not been detected and/or quantified. These, and possibly others, could well be present in the Pinotage wines, although not detectable and/or quantifiable using the current HPLC methodology. On the other hand, the polymers quantified should not include other dimers; possibly even trimers may be excluded.

The most abundant phenolic compounds were malvidin-3-glc (115.9 – 297.9 mg/L) and caffeoyltartaric acid (109.3 – 260.1 mg/L). Other phenolic compounds occurring in average concentrations of > 15 mg/L were procyanidin B1, (+)-catechin, *p*-coumaroyltartaric acid, delphinidin-3-glc, petunidin-3-glc, malvidin-3-acetylglucoside (glc-ac), and malvidin-3-glc-coum. The polymers were present at an average content of 150.4 mg/L (21.5 – 274.8 mg/L). The total concentration of the quantified compounds was between 567.8 and 1174.1 mg/L (average = 820.1 mg/L). The polymer content observed was much lower than that reported previously using normal-phase HPLC (De Beer et al., 2004). The reversed phase method used in the present study only gives an estimation of the relative polymer content as only a 60% recovery of polymeric phenolic content from the column has been reported (Peng et al., 2002).

TEAC of Phenolic Reference Standards

The TEAC values of pure standard compounds were between 0.88 and 2.79 mM (**Table 1**) with kaempferol and gallic acid exhibiting the lowest and highest TEAC values, respectively. The

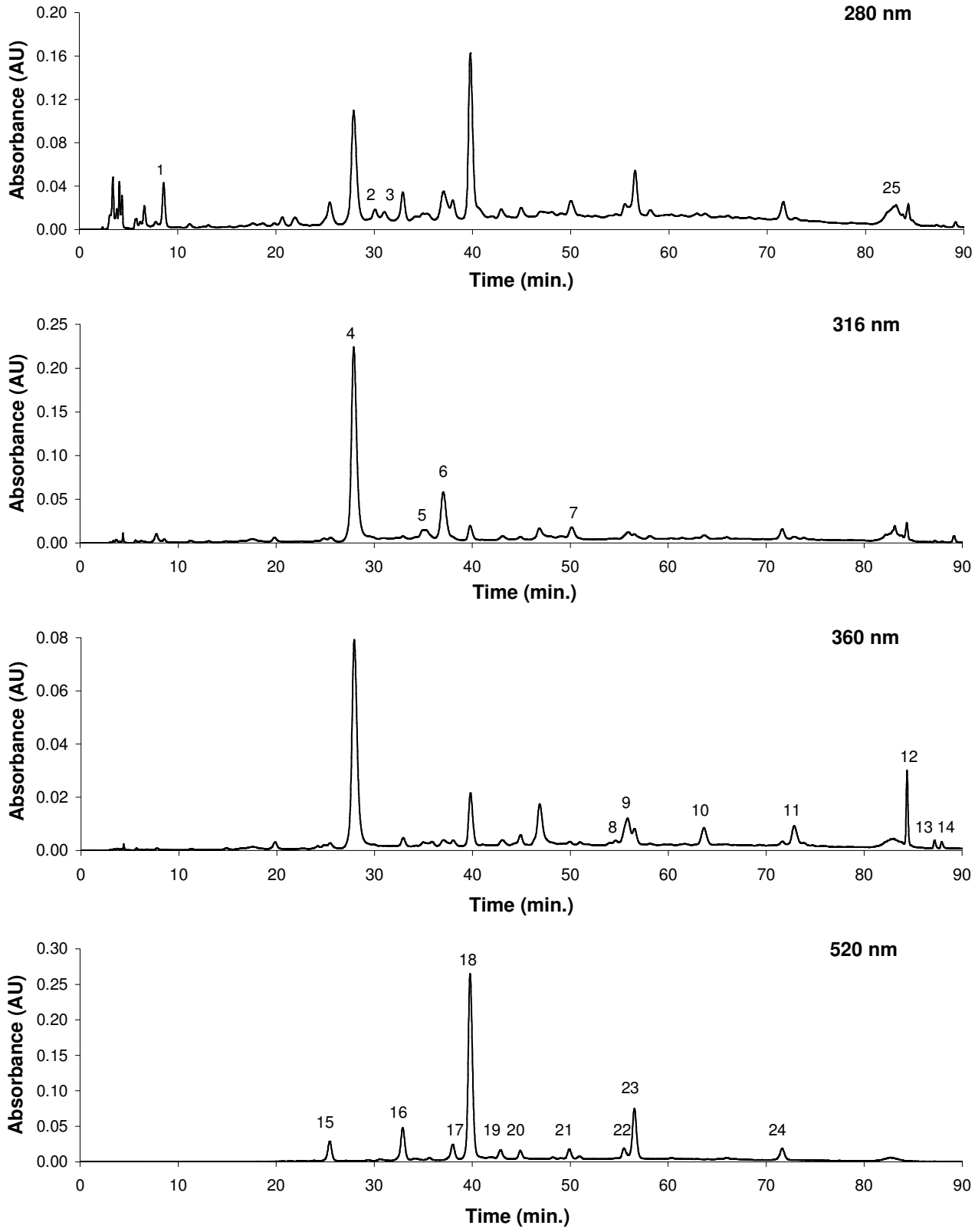
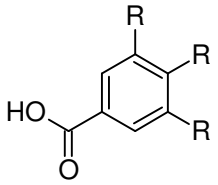
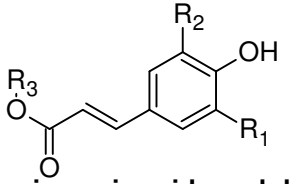


Figure 1. Typical HPLC chromatograms of Pinotage wine recorded at 280, 316, 360 and 520 nm (see **Table 1** for peak identification).



Benzoic acids

Gallic acid (R = OH)



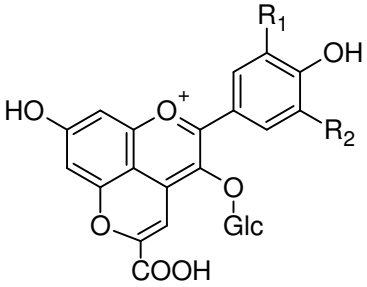
Hydroxycinnamic acids and derivatives

Caffeic acid (R_1 = OH; R_2, R_3 = H)

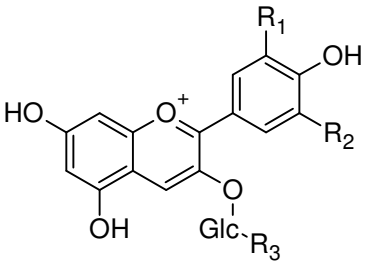
p-Coumaric acid (R_1, R_2, R_3 = H)

Caffeoyltartaric acid (R_1 = OH; R_2, R_3 = tartrate)

p-Coumaroyltartaric acid (R_1, R_2, R_3 = tartrate)



Vitisin A



Anthocyanins

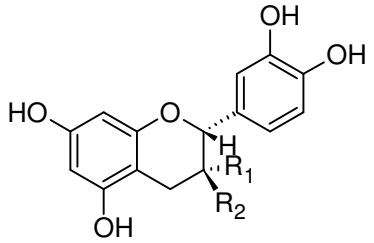
Delphinidin -3-glc (R_1, R_2 = OH; R_3 = H)

Petunidin-3-glc (R_1 = OCH_3; R_2 = H; R_3 = H)

Peonidin-3-glc (R_1 = OCH_3; R_2 = H; R_3 = H)

Malvidin-3-glc (R_1, R_2 = OCH_3; R_3 = H)

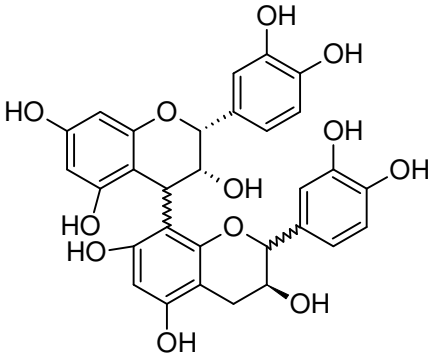
For acetate esters and coumarate esters R_3 = acetyl and *p*-coumaroyl groups, respectively



Flavan-3-ols

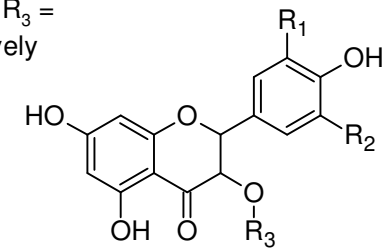
(+)-Catechin (R_1 = H; R_2 = OH)

(-)-Epicatechin (R_1 = OH; R_2 = H)



Procyanidin B1

[(-)-epicatechin 4β → 8 (+)-catechin]



Flavonols

Quercetin (R_1 = OH; R_2, R_3 = H);

Quercetin-3-glc (R_1 = OH; R_2 = H; R_3 = glc);

Quercetin-3-gal (R_1 = OH; R_2 = H; R_3 = gal);

Quercetin-3-rham (R_1 = OH; R_2 = H; R_3 = rham);

Myricetin (R_1, R_2 = OH; R_3 = H);

Kaempferol (R_1, R_2, R_3 = H);

Isorhamnetin (R_1 = OCH_3, R_2, R_3 = H)

Figure 2. Structures of phenolic compounds identified and quantified in Pinotage wines.

Table 1. Content and total antioxidant capacity contribution of individual phenolic compounds in Pinotage wines (n = 139).

Compound	M_r[a]	TEAC[b]	content[c]				TAC contribution[d]			
			min	max	avg	SD	min	max	avg	SD
1. Gallic acid	170.1	2.79	5.2	43.0	11.9	5.4	0.09	0.70	0.20	0.09
2. Procyanidin B1	578.5	2.30	8.5	59.4	21.6	11.3	0.03	0.24	0.09	0.05
3. (+)-Catechin	290.3	0.96	5.4	39.6	15.1	8.0	0.02	0.13	0.05	0.03
4. Caffeoyltartaric acid	312.2	0.90	109.3	260.1	178.2	31.4	0.32	0.75	0.51	0.09
5. Caffeic acid	180.2	0.98	nd	10.2	3.4	2.6	0.00	0.06	0.02	0.01
6. *p*-Coumaroyltartaric acid[e]	296.2	na	10.5	27.6	17.2	3.5	na	na	na	na
7. *p*-Coumaric acid	164.25	< 0.01	0.3	5.7	1.7	1.1	[f]	[f]	[f]	[f]
8. Quercetin-3-gal	464.4	0.96	nd	4.8	2.9	0.9	0.00	0.01	0.01	0.00
9. Quercetin-3-glc	464.4	0.92	5.8	38.3	14.2	5.4	0.01	0.08	0.03	0.01
10. Quercetin-3-rham	448.4	0.91	3.9	16.2	8.8	2.4	0.01	0.03	0.02	0.01
11. Myricetin	318.2	2.67	nd	8.2	2.6	1.7	0.00	0.07	0.02	0.01
12. Quercetin	302.2	1.75	0.6	10.7	3.9	1.9	0.00	0.06	0.02	0.01
13. Kaempferol	286.2	0.88	nd	2.4	0.8	0.4	0.00	0.01	0.00	0.00
14. Isorhamnetin	316.3	0.95	nd	1.3	0.6	0.3	0.00	0.00	0.00	0.00
15. Delphinidin-3-glc	465.4	2.40	5.1	27.3	15.0	5.0	0.02	0.13	0.07	0.02
16. Petunidin-3-glc	479.4	2.06	11.3	35.6	22.6	5.2	0.05	0.14	0.09	0.02
17. Peonidin-3-glc	463.4	1.49	0.9	17.1	7.5	3.5	0.00	0.05	0.02	0.01
18. Malvidin-3-glc	493.4	1.46	115.9	297.9	221.3	31.5	0.32	0.82	0.61	0.09
19. Delphinidin-3-glc-ac[h]	-	na	1.9	10.1	5.3	1.7	0.01	0.05	0.03	0.01
20. Vitisin A[h]	-	na	0.5	14.7	5.7	3.0	0.00	0.04	0.02	0.01
21. Petunidin-3-glc-ac[h]	-	na	2.0	10.2	5.5	1.7	0.01	0.04	0.02	0.01
22. Peonidin-3-glc-ac[h]	-	na	2.3	9.4	5.1	1.7	0.01	0.03	0.02	0.01
23. Malvidin-3-glc-ac[h]	-	na	20.5	100.9	59.4	15.2	0.06	0.28	0.16	0.04
24. Malvidin-3-glc-coum[h]	-	na	6.9	41.4	21.2	6.9	0.02	0.11	0.06	0.02
25. Polymers[i]	na	na	21.5	274.8	150.4	54.1	na	na	na	na
Total			567.8	1174.1	820.1	97.6	1.59	2.79	2.04	0.20
TAC measured[c]							9.04	18.89	13.15	1.98
% TAC accounted[j]							11.1	23.7	16.1	2.4

[a] molecular weight in g/mole; [b] Trolox equivalent antioxidant capacity in mM ± SD; [c] mg/L except where otherwise noted; [d] Total antioxidant capacity (TAC) expressed as mM Trolox equivalents; [e] mg *p*-coumaric acid equivalents/L; [f] neglible; [g] mg rutin equivalents/L; [h] mg corresponding anthocyanin-3-glc equivalents/L; [i] mg (+)-catechin equivalents/L; [j] % TAC accounted for = TAC calculated × 100/TAC measured; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; nd = not detected; na = not available; rham = rhamnoside.

average relative SDs for TEAC values was 4% (data not shown). *p*-Coumaric acid exhibited no $ABTS^{\bullet+}$ scavenging activity (TEAC < 0.01 mM). The TEAC values of quercetin-3-gal (0.96 mM), isorhamnetin (0.95 mM) and peonidin-3-glc (1.49 mM), measured using the $ABTS^{\bullet+}$ scavenging assay, are reported here for the first time to the best of the authors' knowledge. Gallic acid (2.79 mM) exhibited the highest TEAC value of the phenolic acids due to its vicinal tri-hydroxyl group. Esterification of caffeic acid with tartaric acid, i.e., caffeoyltartaric acid, caused a slight decrease in TEAC value from 0.98 to 0.90 mM. Among the nonglycosylated flavonols, myricetin (2.67 mM) had the highest TEAC value due to the vicinal tri-hydroxylation of the B ring. The glycosides of quercetin, namely, quercetin-3-glc (0.92 mM), quercetin-3-rham (0.91 mM), and quercetin-3-gal (0.96 mM) had substantially lower TEAC values than the aglycone (1.75 mM). When considering the anthocyanins, delphinidin-3-glc (2.40 mM) had the highest TEAC value due to its three hydroxyl groups on the B ring. Malvidin-3-glc (1.46 mM), on the other hand, had the lowest TEAC value of the anthocyanins due to having only one hydroxyl-group and two electron-withdrawing methoxyl groups on the B ring. The procyanidin dimer B1 [(-)-epicatechin 4β → 8 (+)-catechin] exhibited a TEAC value (2.30 mM) slightly higher than would be expected from the doubling in available hydroxyl groups as in (-)-epicatechin (1.25 ± 0.02 mM; data not shown) and (+)-catechin (0.96 mM). Oligomers (2 – 6 units) have been reported to have higher antioxidant activity in a variety of antioxidant assays than their corresponding monomeric phenols (Hagerman et al., 1998; Lotito et al., 2000; Ursini et al., 2001; Tsai et al., 2004). Some authors (Plumb et al., 1998; Counet et al., 2003) reported that the antioxidant activity of oligomers per monomer subunit is even higher than that of the respective monomer subunits in the $ABTS^{\bullet+}$ assay. This phenomenon is ascribed to larger areas available for charge delocalisation. However, when the degree of polymerisation exceeds a critical value, the increased molecular complexity is likely to promote a decrease in antioxidant activity due to steric hindrance reducing the availability of hydroxyl groups (Plumb et al., 1998).

TEAC values observed for gallic acid, caftaric acid, caffeic acid and kaempferol are consistent with values reported by Re et al. (1999) and Baderschneider and Winterhalter (2001), while TEAC values observed for other compounds are much lower than those reported previously (Rice-Evans et al., 1995; Miller & Rice-Evans, 1997a; Plumb et al., 1998; Re et al., 1999; Baderschneider & Winterhalter, 2001; García-Alonso et al., 2004). It is important to note that published TEAC values also differ between sources. Differences in values observed can be due to differences in radical generation in the presence or absence of the antioxidant molecules (Miller & Rice-Evans et al., 1997b), reaction time (Labrinea & Georgiou, 2004), and reaction medium (Lema□ska et al., 2001; Labrinea & Georgiou, 2004; Nenadis et al., 2004). Differences in calculation methods between

authors can also affect the TEAC values. For this reason the same protocol was used to analyse the wine and phenolic compounds.

Contribution of Individual Phenolic Compounds to TAC of Wine

The measured TAC values for the series of 139 experimental wines varied between 9.04 and 18.89 mM TE (average = 12.84 mM TE) (**Table 1**), which were similar to TAC values [average = 15.29 mM TE; standard deviation (SD) = 2.96 mM TE] previously obtained for commercial Pinotage wines (De Beer et al., 2003). The SD for TAC of individual wines was between 0.02 and 0.29 mM TE (average = 0.11 mM TE) corresponding to relative SDs of less than 3% in all cases. The sum of TAC contributions (calculated TAC) for the quantified individual phenolic compounds was only between 1.59 and 2.79 mM TE (average = 2.04 mM TE), accounting for between 11 and 24% (average = 16%) of the measured TAC of the experimental wines. The calculated TAC obtained here is somewhat lower than that estimated by Rice-Evans et al. (1996) from the average TAC of several red wines and the phenolic composition of a red wine as reported by Frankel et al. (1995).

The TAC contribution of individual phenolic compounds varied according to their TEAC values and concentration in the wines (**Table 1**). The largest TAC contributions were observed for malvidin-3-glc (0.32 – 0.82 mM TE; average = 0.61 mM TE) and caffeoyltartaric acid (0.32 – 0.75 mM TE; average = 0.51 mM TE). Although these two compounds had relatively low TEAC values, they were the highest contributors to the TAC due to their very high concentration in the wines. Other important TAC contributors (> 0.05 mM TE) were gallic acid (average = 0.20 mM TE), procyanidin B1 (average = 0.09 mM TE), (+)-catechin (average = 0.05 mM TE), delphinidin-3-glc (average = 0.07 mM TE), petunidin-3-glc (average = 0.09 mM TE), malvidin-3-glc-ac (average = 0.16 mM TE), and malvidin-3-glc-coum (average = 0.06 mM TE). In the case of gallic acid and procyanidin B1, their high TEAC values gave rise to the high TAC contributions, although they were present in relatively modest amounts. Quercetin-3-gal, kaempferol and isorhamnetin contributed the least to the TAC of Pinotage wines (< 0.01 mM TE), due to their very low concentrations.

Ultrafiltration

Ultrafiltration was carried out to attempt separation of monomeric and polymeric compounds in order to determine their respective contributions to the TAC of the wine. For this reason, membranes with a range of nominal MW cutoffs (10, 30 and 50 kDa) were tested to determine at which cutoff this separation occurs. Results for the two wines exhibited similar trends (**Tables 2, 3**), and the average phenolic composition and TAC of the wines and ultrafiltration permeates and retentates are presented in **Table 4**.

Table 2. Phenolic composition[a] and TAC values of ultrafiltration permeates and retentates of Pinotage wine A.

	Wine	10 kDa membrane		30 kDa membrane		50 kDa membrane	
		Permeate	Retentate	Permeate	Retentate	Permeate	Retentate
Gallic acid	15.2 a[b]	14.7 b (97%)[c]	2.0 d (13%)	14.5 b (95%)	1.6 e (11%)	13.4 c (88%)	0.0 f (0%)
Procyanidin B1	22.7 a	6.4 d (28%)	9.5 c (42%)	9.5 c (42%)	7.0 d (31%)	14.8 b (65%)	0.0 e (0%)
(+)-Catechin	15.5 a	12.0 b (77%)	0.0 d (0%)	11.9 b (77%)	0.0 d (0%)	8.0 c (52%)	0.0 d (0%)
Caffeoyltartaric acid	283.3 a	224.1 d (79%)	51.8 e (18%)	239.9 (85%)	35.0 f (12%)	251.2 bc (89%)	17.8 g (6%)
Caffeic acid	8.8 a	6.2 c (70%)	0.0 d (0%)	7.4 b (84%)	0.0 d (0%)	6.7 bc (76%)	0.0 d (0%)
Quercetin-3-glc	19.4 a	7.5 de (39%)	8.4 cd (43%)	8.6 c (44%)	6.8 e (35%)	11.6 b (60%)	4.3 f (22%)
Quercetin-3-rham	14.7 a	5.4 d (37%)	5.0 d (34%)	6.4 c (44%)	4.1 e (28%)	7.7 b (52%)	2.8 f (19%)
Delphinidin-3-glc	12.8 a	6.4 d (50%)	6.6 d (52%)	7.8 c (61%)	5.5 e (43%)	10.1 b (79%)	3.4 f (27%)
Petunidin-3-glc	16.5 a	7.5 cd (45%)	8.3 de (50%)	9.3 c (56%)	6.5 e (39%)	12.0 b (73%)	3.8 f (23%)
Peonidin-3-glc	7.6 a	3.9 de (51%)	4.0 d (53%)	4.7 c (62%)	3.5 e (46%)	5.7 b (75%)	2.1 f (28%)
Malvidin-3-glc	162.6 a	70.4 de (43%)	78.5 cd (48%)	89.8 c (55%)	61.0 e (38%)	116.9 b (72%)	34.4 f (21%)
Delphinidin-3-glc-ac[d]	4.3 a	0.0 c (0%)	2.9 ab (67%)	1.4 b (33%)	2.7 ab (63%)	3.1 ab (72%)	0.0 c (0%)
Vitisin A[d]	8.2 a	3.3 d (40%)	6.4 b (78%)	3.7 d (45%)	5.8 b (71%)	4.7 c (57%)	4.6 c (56%)
Petunidin-3-glc-ac[d]	3.7 a	2.3 d (62%)	2.3 d (62%)	2.5 c (68%)	0.0 e (0%)	2.8 b (76%)	0.0 e (0%)
Peonidin-3-glc-ac[d]	2.6 a	0.0 d (0%)	0.0 d (0%)	1.9 c (73%)	0.0 d (0%)	2.0 b (77%)	0.0 d (0%)
Malvidin-3-glc-ac[d]	39.9 a	16.9 d (42%)	20.1 c (50%)	21.4 c (54%)	16.0 d (40%)	27.4 b (69%)	9.9 e (25%)
Malvidin-3-glc-coum[d]	17.9 a	4.0 d (22%)	8.5 b (47%)	5.7 c (32%)	7.5 b (42%)	7.4 b (41%)	5.3 c (30%)
Total monomers and procyanidin B1[e]	660.3 a	391.1 d (59%)	214.2 e (32%)	446.5 c (68%)	162.9 f (25%)	505.3 b (77%)	88.4 g (13%)
Polymers[f]	93.7 a	0.0 d (0%)	20.6 b (22%)	0.0 d (0%)	17.1 b (18%)	0.0 d (0%)	11.3 c (12%)
Tannin[g]		nd		nd		nd	
TAC calculated[h]	2.17 a	1.35 d (62%)	0.66 e (30%)	1.52 c (70%)	0.50 f (23%)	1.68 b (77%)	0.25 g (12%)
TAC measured[h]	18.57 a	3.62 g (19%)	13.92 b (75%)	4.72 f (25%)	12.75 c (69%)	7.33 e (39%)	9.26 d (50%)

[a] content in mg/L; [b] different lower case letters in a row indicate significant ($P < 0.05$) differences; [c] % of original content; [d] content in mg corresponding anthocyanin-3-glc equivalents/L; [e] sum of monomeric phenolic compounds quantified and procyanidin B1; [f] mg (+)-catechin equivalents/L; [g] mg (+)-catechin equivalents/L measured using the tannin assay; [h] total antioxidant capacity in mM Trolox equivalents; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; nd = not detected; rham = rhamnoside.

Table 3. Phenolic composition[a] and TAC values of ultrafiltration permeates and retentates of Pinotage wine B.

	Wine	10 kDa membrane		30 kDa membrane		50 kDa membrane	
		Permeate	Retentate	Permeate	Retentate	Permeate	Retentate
Gallic acid	15.3 a[b]	14.5 b (95%)[c]	0.0 d (0%)	14.5 b (95%)	0.0 d (0%)	13.4 c (88%)	0.0 d (0%)
Procyanidin B1	17.1 a	7.2 c (42%)	0.0 d (0%)	9.5 b (56%)	0.0 d (0%)	10.7 b (63%)	0.0 d (0%)
(+)-Catechin	14.1 a	10.4 b (74%)	0.0 d (0%)	10.7 b (76%)	0.0 d (0%)	7.4 c (52%)	0.0 d (0%)
Caffeoyltartaric acid	276.9 a	237.4 c (86%)	31.0 d (11%)	247.1 bc (89%)	20.9 de (18%)	252.5 b (91%)	9.2 e (3%)
Caffeic acid	8.5 a	7.6 ab (89%)	0.0 c (0%)	7.6 ab (89%)	0.0 c (0%)	6.6 b (76%)	0.0 c (0%)
Quercetin-3-glc	17.0 a	7.4 d (44%)	5.9 e (35%)	9.0 c (53%)	4.8 e (28%)	11.5 b (68%)	2.6 f (15%)
Quercetin-3-rham	13.5 a	5.6 d (41%)	3.9 e (29%)	6.7 c (50%)	3.2 f (24%)	8.6 b (64%)	0.0 g (0%)
Delphinidin-3-glc	9.4 a	6.0 c (64%)	3.8 d (40%)	6.8 c (72%)	3.3 d (35%)	7.9 b (84%)	0.0 e (0%)
Petunidin-3-glc	11.6 a	6.8 d (59%)	4.3 e (37%)	8.0 c (69%)	3.7 e (32%)	9.3 b (80%)	2.4 f (21%)
Peonidin-3-glc	5.8 a	3.6 c (62%)	2.4 d (41%)	4.0 c (69%)	0.0 e (0%)	4.8 b (83%)	0.0 e (0%)
Malvidin-3-glc	110.2 a	62.7 c (57%)	38.4 d (35%)	73.0 c (66%)	31.4 d (28%)	88.4 b (80%)	14.7 e (13%)
Delphinidin-3-glc-ac[d]	3.6 a	1.2 c (33%)	1.2 bc (33%)	0.0 c (0%)	0.0 c (0%)	3.0 ab (83%)	0.0 c (0%)
Vitisin A[d]	10.8 a	4.7 d (44%)	7.4 b (69%)	5.2 d (48%)	6.6 c (61%)	7.1 bc (66%)	4.7 d (44%)
Petunidin-3-glc-ac[d]	2.8 a	2.2 c (79%)	2.3 b (82%)	2.2 c (79%)	2.2 c (79%)	2.3 b (82%)	0.0 d (0%)
Peonidin-3-glc-ac[d]	2.1 a	0.0 b (0%)	0.0 b (0%)	0.0 b (0%)	0.0 b (0%)	0.0 b (0%)	0.0 b (0%)
Malvidin-3-glc-ac[d]	27.2 a	14.8 c (54%)	10.7 d (39%)	17.3 c (64%)	9.1 d (33%)	20.9 b (77%)	5.1 e (19%)
Malvidin-3-glc-coum[d]	10.0 a	0.0 d (0%)	4.6 bc (46%)	3.9 c (39%)	4.2 c (42%)	5.3 b (53%)	0.0 d (0%)
Total monomers and procyanidin B1[e]	558.1 a	392.0 c (70%)	115.9 d (21%)	425.6 bc (76%)	89.5 d (16%)	459.9 b (82%)	38.6 e (7%)
Polymers[f]	74.0 a	0.0 d (0%)	11.3 bc (15%)	0.0 d 0(%)	12.3 b (17%)	0.0 d (0%)	6.7 c (9%)
Tannin[g]		nd		nd		nd	
TAC calculated[h]	1.86 a	1.36 c (73%)	0.33 d (18%)	1.46 bc (78%)	0.26 d (14%)	1.54 b (83%)	0.11 e (6%)
TAC measured[h]	16.24a	4.03 d (25%)	11.40 b (70%)	5.04 d (31%)	10.33 b (64%)	7.77 c (48%)	6.90 c (42%)

[a] content in mg/L; [b] different lower case letters in a row indicate significant ($P < 0.05$) differences; [c] % of original content; [d] content in mg corresponding anthocyanin-3-glc equivalents/L; [e] sum of monomeric phenolic compounds quantified and procyanidin B1; [f] mg (+)-catechin equivalents/L; [g] mg (+)-catechin equivalents/L measured using the tannin assay; [h] total antioxidant capacity in mM Trolox equivalents; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; nd = not detected; rham = rhamnoside.

Table 4. Average phenolic composition[a] and total antioxidant capacity of ultrafiltration permeates and retentates of two Pinotage wines.

	Wine	10 kDa membrane		30 kDa membrane		50 kDa membrane	
		Permeate	Retentate	Permeate	Retentate	Permeate	Retentate
Gallic acid	15.3 a[b]	14.6 a (96%)[c]	1.0 c (7%)	14.5 a (95%)	0.8 cd (5%)	13.4 b (88%)	0.0 d (0%)
Procyanidin B1	19.9 a	6.8 cd (34%)	4.8 d (24%)	9.5 bc (48%)	3.5 de (18%)	12.7 b (64%)	0.0 e (0%)
(+)-Catechin	14.8 a	11.2 b (76%)	0.0 d (0%)	11.3 b (77%)	0.0 d (0%)	7.7 c (52%)	0.0 d (0%)
Caffeoyltartaric acid	280.1 a	230.8 c (82%)	41.4 d (15%)	243.5 b (87%)	28.0 e (10%)	251.9 b (90%)	13.5 f (5%)
Caffeic acid	8.6 a	6.9 bc (80%)	0.0 d (0%)	7.5 b (87%)	0.0 d (0%)	6.6 c (77%)	0.0 d (0%)
Quercetin-3-glc	18.2 a	7.5 cd (41%)	7.1 de (39%)	8.8 c (48%)	5.8 e (32%)	11.5 b (64%)	3.5 f (19%)
Quercetin-3-rham	14.1 a	5.5 cd (39%)	4.4 de (31%)	6.6 c (47%)	3.7 e (26%)	8.2 b (58%)	1.4 f (10%)
Delphinidin-3-glc	11.1 a	6.2 cd (56%)	5.2 d (47%)	7.3 bc (66%)	4.4 d (40%)	9.0 b (81%)	1.7 e (15%)
Petunidin-3-glc	14.0 a	7.1 cd (51%)	6.3 cd (45%)	8.6 bc (62%)	5.1 de (36%)	10.7 b (76%)	3.1 e (22%)
Peonidin-3-glc	6.7 a	3.8 bc (56%)	3.2 cd (48%)	4.4 bc (65%)	1.7 de (26%)	5.3 ab (79%)	1.0 e (16%)
Malvidin-3-glc	136.4 a	66.6 cd (49%)	58.5 cd (43%)	81.4 bc (60%)	46.2 de (34%)	102.7 b (75%)	24.5 e (18%)
Delphinidin-3-glc-ac[d]	3.9 a	0.6 cd (15%)	2.1 bc (53%)	0.7 cd (18%)	1.3 cd (34%)	3.0 ab (77%)	0.0 e (0%)
Vitisin A[d]	9.5 a	4.0 d (42%)	6.9 b (73%)	4.5 cd (47%)	6.2 b (65%)	5.9 bc (62%)	4.7 cd (49%)
Petunidin-3-glc-ac[d]	3.3 a	2.2 b (68%)	2.3 b (71%)	2.3 b (71%)	1.1 c (34%)	2.6 ab (78%)	0.0 d (0%)
Peonidin-3-glc-ac[d]	2.4 a	0.0 c (0%)	0.0 c (0%)	1.0 b (40%)	0.0 c (0%)	1.0 b (42%)	0.0 c (0%)
Malvidin-3-glc-ac[d]	33.6 a	15.9 cd (47%)	15.4 cd (46%)	19.3 bc (58%)	12.5 de (37%)	24.1 b (72%)	7.5 e (22%)
Malvidin-3-glc-coum[d]	14.0 a	2.0 e (14%)	6.5 b (47%)	4.8 cd (35%)	5.8 bcd (42%)	6.4 bc (46%)	2.6 cd (19%)
Total monomers and procyanidin B1[e]	609.2 a	391.5 c (64%)	165.1 d (27%)	436.0 b (72%)	126.2 e (21%)	482.6 b (79%)	63.5 f (10%)
Polymers[f]	83.9 a	0.0 c (0%)	16.0 b (19%)	0.0 c (0%)	14.7 b (18%)	0.0 c (0%)	9.0 b (11%)
Tannin[g]		nd		nd		nd	
TAC calculated[h]	2.02 a	1.36 d (67%)	0.50 e (25%)	1.49 c (74%)	0.38 f (19%)	1.61 b (80%)	0.18 g (9%)
TAC measured[h]	17.35 a	3.82 f (22%)	12.66 b (73%)	4.88 e (28%)	11.54 c (67%)	7.55 d (44%)	8.08 d (47%)

[a] content in mg/L; [b] different lower case letters in a row indicate significant ($P < 0.05$) differences; [c] % of original content; [d] content in mg corresponding anthocyanin-3-glc equivalents/L; [e] sum of monomeric phenolic compounds quantified and procyanidin B1; [f] mg (+)-catechin equivalents/L; [g] mg (+)-catechin equivalents/L measured using the tannin assay; [h] total antioxidant capacity in mM Trolox equivalents; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; nd = not detected; rham = rhamnoside.

Recovery of some phenolic compounds after ultrafiltration was not quantitative (**Table 4**). The low recovery of procyanidin B1, (+)-catechin, quercetin-3-rham, and malvidin-3-glc-coum in the permeates and retentates was possibly due to adsorption on the membrane, while that of delphinidin-3-glc-ac and peonidin-3-glc-ac can be ascribed to their low quantities in the original wine, making quantification of even lower concentrations in the permeates and retentates difficult. Myricetin and quercetin were present in very low quantities in the original wine and were not detected in any of the ultrafiltration permeates or retentates (data not shown). Recovery of polymers in the retentates was very low. Adsorption of polymers on the ultrafiltration membrane is a likely source of polymer loss during ultrafiltration. Furthermore, recovery of the retentate from the ultrafiltration device was also not entirely quantitative.

Permeation increased with increasing membrane pore size for most phenolic compounds. Permeation of nearly all of the gallic acid from the original wine was achieved using the 10 kDa membrane (**Table 4**). Other compounds with good permeation through the 10 kDa membrane were (+)-catechin, caffeoyltartaric acid, and caffeic acid, with > 70% of the original content detected in the 10 kDa permeate. Most of the phenolic compounds reached levels of 60% of the original level or higher in the 50 kDa permeate. Exceptions were quercetin-3-rham, peonidin-3-glc-ac, and malvidin-3-glc-coum due to low recovery values. The low amount of (+)-catechin observed in the 50 kDa permeate was unexpected, as 76 and 77% of the original (+)-catechin content were observed in the 10 and 30 kDa permeates, respectively. The 50 kDa permeate contained 79% of the total monomer and procyanidin B1 content of the original wine, while no polymers were detected in the 50 kDa permeate, by HPLC or a protein precipitation assay. Many of the individual phenolic compounds, namely, gallic acid, procyanidin B1, (+)-catechin, caffeic acid, delphinidin-3-glc-ac, petunidin-3-glc-ac, and peonidin-3-glc-ac, were not detected in the 50 kDa retentate, while the others were present in very low concentrations. On the basis of these data, it is clear that the 50 kDa ultrafiltration membrane was the most effective for separation of the monomeric and polymeric phenolic compounds. The calculated TAC for the ultrafiltration permeates was relatively low as compared to the measured TAC as observed for the original wines. The relative contribution of the quantified phenolic content of the permeate to its measured TAC increased with a decrease in membrane pore size, which was 21, 31, and 36% for the 50, 30, and 10 kDa permeates, respectively. This may suggest that more unknown compounds are retained with the smaller membrane size. Another possible explanation may be that as more of the proteins and peptides are excluded with decreasing membrane size, less masking of antioxidant activity of the phenolic compounds occurred. Masking of the antioxidant activity of phenolic compounds by proteins has been reported previously (Arts et al., 2001; 2002).

The TAC of the original wine can be divided into different portions based on the ultrafiltration data using the 50 kDa membrane, if TAC contributions of different compounds and classes of compounds are additive. The contribution of monomeric phenolic compounds and procyanidin B1 to the measured TAC of the original wine, as calculated from their content and TEAC values, was only 12% with 88% of the wine TAC contributed by unidentified compounds and/or factors such as masking or synergy (**Figure 3**). Using data from the ultrafiltration experiment, the low MW fraction (50 kDa permeate) and high MW fraction (50 kDa retentate) accounted for 44 and 47% of the wine TAC, while 10% of the TAC is lost during ultrafiltration. Some of the TAC not recovered during ultrafiltration is due to loss of monomeric compounds and procyanidin B1 (1% of the original wine TAC). The rest is attributed to loss of unknown compounds and polymers, as well as possible effects of masking and synergy that changed with the modification of the matrix due to physical separation of compounds. The fact that recovery of polymers was very low during ultrafiltration suggests that most of the TAC loss is due to polymers. If ~9% of the original wine TAC is due to polymers, which is a significant contribution as compared to that of the monomers, a large amount of the TAC is not accounted for by the quantified monomers, procyanidin B1 and polymers. After subtracting the amount of TAC due to quantified phenolic compounds in the 50 kDa permeate and the 50 kDa retentate, 34% and 46% of the wine TAC from the low MW and high MW fraction were unexplained, respectively. This is ascribed to low MW unknowns and high MW unknowns, respectively. The high MW unknown fraction includes the polymeric phenolic compounds although other high MW compounds such as proteins, peptides or polysaccharides could also be present. Fernández-Pachón et al. (2004) reported that phenolic polymers retained on a C18 SPE cartridge after elution with acetonitrile and ethyl acetate contributed 51% of the TAC of red wines.

Synergy and Unknown Compounds Affecting TAC

Because synergy between compounds was considered to contribute to the TAC of wine, this effect was tested, using mixtures of some phenolic compounds in typical concentrations as found in Pinotage wines (**Table 5**). Synergy of between 16 and 23% was observed. This suggests that some, but not all, of the discrepancy between measured and calculated TAC values can be explained by synergy between the phenolic compounds. The situation is, however, more complex as synergy between the phenolic compounds and other wine constituents cannot be ruled out.

Sulphur dioxide has the ability to regenerate phenolic compounds from their phenoxyl radicals, causing a synergistic increase in antioxidant activity (Saucier & Waterhouse, 1999). However, at the concentrations normally present in wines, it does not contribute significantly to the free radical scavenging activity of wines against ABTS, DPPH, DMPD or superoxide radicals (Manzocco et al., 1998; Fogliano et al., 1999; De Beer, 2002). Using the same $ABTS^{\bullet+}$ scavenging assay protocol as

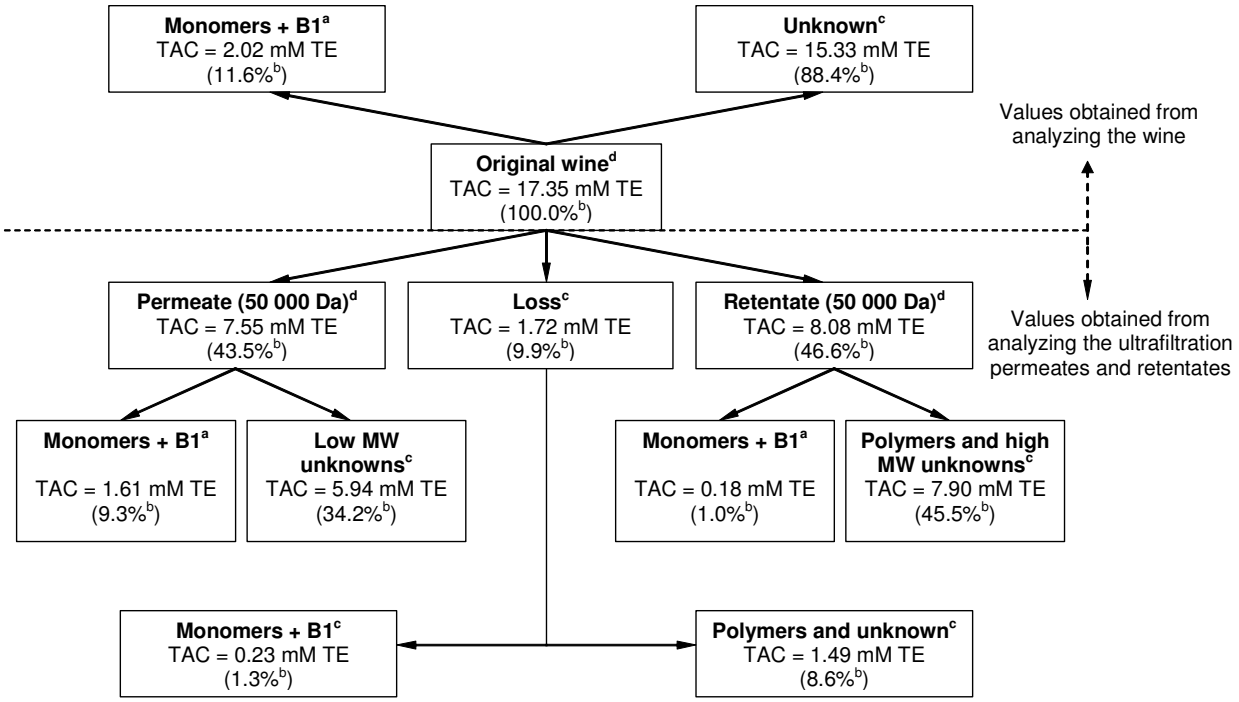


Figure 3. Scheme of total antioxidant capacity (TAC) contribution of different wine fractions to the total antioxidant capacity (TAC) of wine [[a] calculated from phenolic composition and TEAC values (mM); [b] TAC as % of original wine TAC; [c] calculated by difference; [d] Measured; B1 = procyanidin B1; MW = molecular weight].

Table 5. Mixtures of phenolic compounds[a] in typical concentrations found in Pinotage wines tested for synergy.

	Mixture 1	**Mixture 2**	**Mixture 3**
Gallic acid	9.92	29.75	11.90
Procyanidin B1	32.34	19.60	19.60
(+)-Catechin	22.75	14.48	14.48
Caffeoyltartaric acid	180.96	109.04	120.64
Caffeic acid	5.78	9.64	7.71
Quercetin-3-glc	12.36	8.24	20.60
Quercetin-3-rham	7.83	7.83	8.70
Quercetin	3.94	3.94	4.92
Delphinidin-3-glc	18.08	20.34	9.04
Petunidin-3-glc	25.62	25.62	14.64
Peonidin-3-glc	9.30	14.88	9.30
Malvidin-3-glc	212.16	254.40	149.76
TAC calculated[b]	1.78	1.98	1.34
TAC measured[b]	2.18	2.30	1.60
% Synergy[c]	22.5%	16.2%	19.4%

[a] content in mg/L; [b] total antioxidant capacity in mM Trolox equivalents; [c] % synergy = (TAC measured – TAC calculated) x 100 / TAC calculated.

used in the present study, the addition of sulphur dioxide up to 150 mg/L did not affect the TAC of Pinotage wine (De Beer, 2002). Phenolic antioxidants are able to recycle ascorbic acid and α-tocopherol in a lipid peroxidation assay (Liao & Yin, 2000). One study presented data on the regeneration of phenoxyl radicals by phenolic compounds, indicating that (+)-catechin is able to regenerate quercetin from its phenoxyl radical (Jørgensen et al., 1999). This may be a mechanism for the synergistic effect observed for mixtures. Regeneration of phenoxyl radicals will depend on competing reactions such as disproportionation and dimerisation of the phenoxyl radicals, as well as further oxidation of the phenoxyl radical to form a quinone.

Other possibilities include unidentified low MW phenolic compounds of high potency present in concentrations that were too low to quantify or other monomeric phenolic compounds not detected with the current HPLC methodology. Possible candidates include (-)-epicatechin, flavan-3-ol gallate esters, *S*-glutathionylcaftaric acid, stilbenes, stilbene glucosides, and pyranoanthocyanins other than vitisin A. As procyanidin B1 was detected in the ultrafiltration permeates, it is reasonable to expect other dimers such as the procyanidin dimers and anthocyanin-flavan-3-ol condensation products to be present also. Other oligomers (trimers and tetramers) may also be divided between the permeate and the retentate and thereby contributes to the unknown portions of these fractions. These were, however, not expected to be responsible for the 80 – 90% of unexplained TAC. Other compounds of low or high MW such as proteins (Rice-Evan et al., 1995; Arts et al., 2001; 2002), peptides, polysaccharides, and possibly others could also conceivably contribute to the TAC of Pinotage wines.

Conclusions

The present study showed that only a small amount of the TAC of Pinotage wines is contributed by their content of monomeric phenolic compounds and procyanidin B1, with oligomeric and polymeric phenolic compounds, as well as other unknown compounds, largely contributing to the remaining TAC. Simple addition of TAC contributions as calculated in this study may, however, not be appropriate, as synergy between phenolic compounds and possibly other wine constituents and even masking of antioxidant capacity by proteins cannot be ruled out. These findings suggest that by manipulating the monomeric phenolic composition of Pinotage wines the TAC is not likely to be increased substantially as was previously supposed, although some improvement may be possible. More detailed characterisation and quantification of the phenolic content of red wines, e.g., using LC-MS techniques, are needed to obtain a clearer picture of the contribution of various types of phenolic compounds. Radical scavenging activity is an important aspect of antioxidant activity, although the *in vitro* radical scavenging activity of wine components does not necessarily coincide with *in vivo* antioxidant activity, as bioavailability, metal chelating

properties, lipid phase partitioning, and metabolism of individual wine components may differ considerably. Knowledge of the metabolites and their antioxidant activity is required to better evaluate their relative importance. Unabsorbed compounds may also exert protective effects in the gastrointestinal system. These issues need more attention before firm recommendations can be made with regard to which phenolic compounds need to be manipulated in order to obtain a red wine with optimal health benefits.

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Chapter 4: Climatic Region and Vine Structure - Effect on Pinotage Wine Phenolic Composition, Total Antioxidant Capacity and Colour*

Abstract

The phenolic composition, total antioxidant capacity (TAC) and colour of Pinotage wines of the 2001, 2002 and 2003 vintages were investigated, using spectrophotometric, high-performance liquid chromatography (HPLC), free radical scavenging and objective colour analyses. Grapes were harvested from grapevines in three climatic regions ranging from cool to warm with bush (20 and 30 cm trunk height) and trellised (30 and 60 cm trunk heights) vine treatments on several vineyard sites in each climatic area. Climatic region had a significant effect on the content of many phenolic compounds with concentrations of anthocyanin monoglucosides, flavonols, flavan-3-ols and tartaric acid esters of hydroxycinnamic acids generally increasing as the climatic region becomes cooler, while concentrations of acylated derivatives and free hydroxycinnamic acids decreased. Wines made from bush vines contained higher concentrations of flavonols, gallic acid and flavan-3-ols than those from trellised vines, but lower concentrations of some anthocyanin monoglucosides and acylated derivatives, as well as non-coloured polymers. These trends resulted in differences in TAC and objective colour parameters, although the different vintages did not produce the same trends in all cases. More vintages should, therefore, be investigated to clarify effects. Wines from the cool climatic regions and from bush vines were generally darker coloured with higher TAC. High TAC, therefore, coincided with higher colour quality. Variations in TAC were partly explained by trends for individual phenolic compounds, although unknown compounds also played a role.

Introduction

Grape phenolic composition is greatly affected by climatic conditions and vine management practices (Jackson & Lombard, 1993). A wide variety of systems has been developed to describe the viticultural potential of a climatic region, many of them based on temperature (*inter alia* Amerine & Winkler, 1944; Huglin, 1986; Smart & Dry, 1980), since it is one of the most important parameters affecting the grapevine, especially during the final month of berry maturation (Jackson & Lombard, 1993). In the South African context, the Western Cape viticultural regions have been divided according to the heat summation model of Amerine and Winkler (1944) by Le Roux (1974), as well as, the mean temperature of the warmest month model of Smart and Dry (1980), using the mean February temperatures by De Villiers et al. (1996). High temperatures have been reported to

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result in lower anthocyanin (Kliewer, 1970; Bergqvist et al., 2001; Spayd et al., 2002) and total phenol (Bergqvist et al., 2001) berry content compared to lower temperatures. Van Leeuwen et al. (2004) postulated that the effects of climate and soil on the fruit composition are due to the effects thereof on the vine water status, as most parameters measured correlated with the intensity of water stress.

Vine management practices, such as training, pruning, shoot positioning, cluster thinning and leaf thinning, modify the canopy microclimate in order to control sunlight exposure and fruit temperature during berry maturation. Sunlight exposure generally results in higher juice pH, total soluble solids, anthocyanin, flavonol and phenolic contents, while titratable acidity, malate content, and berry mass are lower (Kliewer, 1970; Crippen & Morrison, 1986; Reynolds et al., 1986; Spayd et al., 2002; Downey et al., 2004). In warm climates, however, a high degree of sunlight exposure negatively affects the anthocyanin content of red grapes (Haselgrove et al., 2000). Tamborra et al. (2003) reported a significant difference in berry skin phenolic acid content depending on whether bush vine or trellis training systems were used. Vine management practices can also lead to differences in crop yield with spur-pruned training systems generally lower yielding than cane-pruned training systems (Vanden Heuvel et al., 2004), which have been shown to affect the phenolic composition of sparkling wines (Pozo-Bayón et al., 2004). Vine vigour has also been shown to influence the phenolic composition of red wines (Cortell et al., 2005).

Generally, Pinotage vines grown in South Africa are head-trained and spur-pruned (bush vines) or trained to a bilateral horizontal cordon and spur-pruned with upward vertical shoot positioning (trellised vines). Winemakers and producers speculate whether bush vines or trellised vines are preferable for making high quality Pinotage wines (D. van Schalkwyk, ARC Infruitec-Nietvoorbij, personal communication, 2005). It is also not clear whether cultivation of Pinotage under cool or warm climatic conditions is optimal for obtaining high quality wine. It is expected that these factors will also affect the antioxidant capacity of Pinotage wines. No research to show the effect of climatic region or vine management practices on the antioxidant capacity of red wines has been reported. Consequently, the aim of this project was to determine the effect of vine structure (training system and cordon height), as well as climatic region, on the phenolic composition, total antioxidant capacity (TAC) and colour of Pinotage wines from the Western Cape.

Materials and Methods

Viticultural Treatments and Wine-making Procedure

Vineyard sites were located in three climatic regions of the coastal region (Western Cape, South Africa), differentiated according to average February temperatures using macro climatic

weather station data as described by De Villiers et al. (1996) (see **Figure 10** in **Chapter 2**): region II (av February temperature = 19.0 – 20.9 °C), region III (av February temperature = 21.0 – 22.9 °C) and region IV (av February temperature = 23.0 – 24.9 °C). Temperature data taken during February 2004 and 2005 using mini data loggers (Tinytag Plus TGP-1500, Gemini Data Loggers (UK) Ltd., Chichester, UK) at individual vineyards were used to confirm allocation of vineyard sites on the border between regions to a specific region (D. van Schalkwyk, ARC Infruitec-Nietvoorbij, personal communication, 2005). The seven vineyard sites in climatic region II were located in the Darling (1 site), Stellenbosch (higher than 300 m above sea level) (4 sites), Faure (1 site) and Hemel and Aarde Valley (Hermanus) (1 site) regions. In climatic region III the six vineyard sites were located in the Kuilsriver (2 sites), Stellenbosch (lower than 300 m above sea level) (3 sites) and Vlottenburg (1 site) regions. In climatic region IV the experimental sites were located in the Darling (1 site), Riebeeck-Wes (1 site), Wellington/Paarl (2 sites) and Agter-Paarl (3 sites) regions. All vines were Pinotage clone PI 48 grafted onto 99 Richter rootstock. Vine distances and row orientation were not standardised. Vine structure treatments were bush (head-trained and spur-pruned) and trellised (trained to a bilateral horizontal cordon and spur-pruned with upward vertical shoot positioning) vines with main cordon heights of 20 or 30 cm for bush vines and 30 or 60 cm for trellised vines. Canopy management was applied for all vines, namely suckering to two bearer shoots per bearer, suckering between bearers and leaf removal at berry set to three leaf layers to obtain an optimal canopy density (Smart & Robinson, 1991; Hunter, 1999). All combinations of these treatments were carried out on each of the vineyard sites during the 2000/2001, 2001/2002 and 2002/2003 growing seasons.

The sugar content of the grapes, when harvested, ranged between 24 and 26 °B with 14%, 14% and 16% of the treatments harvested outside of this range during 2001, 2002 and 2003, respectively. Different vineyard sites in the same climatic region represented repetitions. Wines were produced from 20 – 30 kg of grapes per treatment at the experimental cellar of ARC Infruitec-Nietvoorbij (South Africa) according to the standard wine-making protocol with no wood contact (described in **Chapter 3**). After bottling, the wines were stored at 15 ºC. Eight months after production, aliquots of each wine were frozen at -20 °C, to prevent further phenolic changes, until analyses were carried out. Samples were analysed immediately after defrosting.

Chemicals and Phenolic Reference Standards

Chemicals and phenolic reference standards used for high-performance liquid chromatography (HPLC) analysis and the $ABTS^{\bullet+}$ scavenging assay were described in **Chapter 3**. The following additional chemicals were used: Folin-Ciocalteau's phenol reagent (Merck, Darmstadt, Germany); 4-dimethylamino-cinnamaldehyde (DAC) (Fluka AG, Buchs, Switzerland); methanol (AR),

concentrated hydrochloric acid (AR), sodium chloride (AnalAR) and sodium hydroxide (AnalAR) (SaarChem, Midrand, South Africa); glacial acetic acid (Riedel-de Haën, Seelze-Hanover, Germany).

Spectrophotometric Analysis of Phenolic Composition

Pinotage wines from all vintages were subjected to spectrophotometric analysis of the major phenolic groups described below.

The total phenol content of wines was determined at 765 nm after reaction of wine samples (40 times diluted with 10% ethanol) with the Folin-Ciocalteau reagent (Singleton & Rossi, 1965). The original method was scaled down to a final reaction volume of 5 mL. Gallic acid was used as standard and results were expressed as mg gallic acid equivalents/L.

The anthocyanin content of wines was estimated using a pH shift method modified from Ribéreau-Gayon and Stonestreet (1965). Two test tubes were set up, each containing 100 - 200 µL of wine depending on the anthocyanin content and 500 µL of 0.1% HCl in 95% ethanol. Five millilitres of 2% HCl (pH 0.6) were added to one tube to decrease the pH to <1 and 5 mL of pH 4.9 buffer (solution containing 200 mM acetic acid and 170 mM NaCl, adjusted to pH 4.9 with NaOH) to the other. Absorbance was read at 700 nm to allow for correction of the haze and then at 520 nm for anthocyanin determination. Anthocyanins were quantified as mg malvidin-3-glucoside equivalents/L, the major anthocyanin in red wine, using the molar extinction coefficient $\varepsilon = 28000$. The total anthocyanin content (pH shift) was calculated from the absorbance at pH <1 as all anthocyanins are in their red flavylium form at this pH. The polymeric anthocyanin content (pH shift) was calculated from the absorbance at pH 4.9, as the monomeric anthocyanins' absorbance is at a minimum at this pH (Cabrita et al., 2000). The difference in absorbance between pH <1 and pH 4.9 is, therefore, used to calculate the monomeric anthocyanin content (pH shift).

The total flavan-3-ol content (DAC) of wines was measured at 640 nm after reaction of diluted wine samples (40 times diluted in 10% ethanol) with DAC reagent (2.9 mM DAC in a mixture of 25% (v/v) concentrated HCl and 75% (v/v) methanol) for 2 min. at room temperature (McMurrough & McDowell, 1978). (+)-Catechin was used as a standard and the results expressed as mg catechin equivalents/L.

Spectrophotometric measurements were made in triplicate using disposable polystyrene 2.5 mL macro cuvettes with 1 cm path length (Brand Gmbh & Co Kg, Wertheim, Germany) using a Beckman DU-65 UV/Vis spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA).

HPLC Analysis of Phenolic Composition

Individual phenolic compounds, as well as coloured and non-coloured polymers detected at 520 and 280 nm, respectively, were quantified in duplicate in Pinotage wines from the 2002 and 2003

vintages using an HPLC method (Peng et al., 2002), modified and described in **Chapter 3**. Polymers included polymeric phenolic compounds with 5 or more subunits, consisting of anthocyanins and flavan-3-ols for coloured polymers, and only flavan-3-ols for non-coloured polymers.

ABTS Radical Cation Scavenging Assay

The total antioxidant capacity (TAC) of Pinotage wines from all vintages was measured (TAC_M) in triplicate using the $ABTS^{\bullet+}$ scavenging assay (Re et al., 1999). The content of individual phenolic compounds, measured by HPLC, and their experimental Trolox equivalent antioxidant capacity (TEAC) values (reported in **Chapter 3**) were used to calculate the theoretical TAC (TAC_{CAL}). The remaining TAC (TAC_R) is the difference between TAC_M and TAC_{CAL}. Analysis and calculations were carried out as described in **Chapter 3**.

Objective Colour Parameters

A Colorgard System 2000 Colorimeter (BYK-Gardner, Geretsried, Germany) was used to obtain the objective colour parameters of the undiluted Pinotage wines from all vintages in transmittance mode with a 5 mm fixed path length optical cell. The colorimeter was calibrated before use with a non-diffusing black reflectance standard (BYK-Gardner, Geretsried, Germany) to obtain a zero calibration. Duplicate objective colour measurements were taken <1 h after opening of a wine bottle to minimise colour changes. The CIELab parameters, namely a^* (red/green chromaticity), b^* (yellow/blue chromaticity) and L^* (lightness), were measured using the CIE 1931 standard colorimetric observer under illuminant C (geometry is 45° illumination and 0° viewing). The h^* (hue angle; °) and C^* (chroma) were calculated as follows:

$$h^* = \tan^{-1}(b^*/a^*) \qquad (1)$$

$$C^* = \left[(a^*)^2 + (b^*)^2\right]^{1/2} \qquad (2)$$

Names for hues were adapted from Gonnet (1999) based on the h^* values. Hue angle values of 0°, 7.5°, 15°, 22.5°, 30°, 37.5° and 45° correspond to magenta, red-magenta, magenta-red, red, orange-red, red-orange and orange, respectively.

Statistical Analysis

Analysis of variance was performed on the means for climatic regions and vine structure treatments to determine whether significant differences occurred. The Student *t*-LSD test ($P < 0.05$) was used to determine the statistical differences between means. Covariance analysis was also performed with grape sugar content (°B) as covariate. Analysis of variance, difference testing and covariance analysis was done using the SAS version 8 software package (SAS Institute Inc., Cary, NC). In cases where the covariate had a significant ($P < 0.05$) effect, the adjusted means were

compared. Where no interactions between different factors were observed or where treatments did not differ significantly, data were pooled. Canonical discriminant analysis of data obtained for wines produced during 2002 and 2003, using forward stepwise variable selection, was performed to distinguish between climatic regions and vine structure treatments. Pearson product moment correlation coefficients between parameters and their P-values were calculated. Canonical discriminant analysis and calculation of correlation coefficients were done using the STATISTICA 6 software package (StatSoft, Inc., Tulsa, OK).

Results

Vintage-related Variations

Some vintage-related variations were observed in terms of the phenolic composition and TAC of Pinotage wines (**Tables 1, 2**). The average grape sugar content did not differ significantly between the vintages (**Table 1**). The climatic region and vine structure treatments had varying effects depending on vintage. For data on individual wines, see **Addendum C**.

Spectrophotometric determination of phenolic content showed significant differences between wines from different vintages (**Table 1**). Wines of the 2001 vintage had the highest total phenol content, as well as the highest monomeric, polymeric and total anthocyanin content (pH shift). The 2002 wines had the lowest polymeric and total anthocyanin content (pH shift), while the 2003 wines had the lowest total flavan-3-ol content (DAC).

The individual phenolic compounds were quantified for the 2002 and the 2003 wines only (**Table 2**). Some flavonol compounds, namely quercetin-3-galactoside (gal), myricetin, kaempferol and isorhamnetin, were only detected in some wines. Of the 63 wines produced during 2002, 18, 38 and 47 contained measurable amounts of quercetin-3-gal, kaempferol and isorhamnetin, respectively, while 23, 25 and 36 of the 77 wines produced during 2003 contained measurable amounts of quercetin-3-gal, myricetin and isorhamnetin, respectively. Values for these compounds in the respective vintages will not be reported as statistical analysis was not possible. The total flavonol content, however, refers to the sum of all flavonols including quercetin-3-gal, myricetin, kaempferol and isorhamnetin where they could be quantified.

Large vintage-related variations were found for the contents of individual phenolic compounds (**Table 2**). The 2002 wines had significantly higher concentrations of most phenolic compounds compared to the 2003 wines, except for vitisin A, malvidin-3-*p*-coumaroylglucoside (glc-coum), quercetin-3-glucoside (glc), gallic acid, caftaric acid and non-coloured polymers which did not differ significantly and malvidin-3-glc, peonidin-3-acetylglucoside (glc-ac), malvidin-3-glc-ac,

Table 1. Vintage-related variation in sugar content of grapes, as well as the phenolic composition (measured spectrophotometrically), antioxidant capacity and objective colour parameters of the 2001, 2002 and 2003 Pinotage wines.

	2001[a]	**2002**[a]	**2003**[a]
Sugar content[b]	25.0 a[c] (± 0.1)[d]	24.9 a (± 0.1)	25.0 a (± 0.1)
Phenolic composition			
Total phenols[e]	2347.1 a (± 57.6)	1743.2 c (± 32.2)	1879.4 b (± 32.9)
Monomeric anthocyanins[f]	494.3 a (± 8.2)	443.5 b (± 7.4)	462.5 b (± 7.4)
Polymeric anthocyanins[f]	130.6 a (± 3.4)	54.1 c (± 1.5)	64.7 b (± 2.0)
Total anthocyanins[f]	624.9 a (± 11.0)	497.5 c (± 8.4)	527.2 b (± 9.2)
Total flavan-3-ols[g]	153.2 a (± 5.2)	144.1 a (± 4.3)	182.6 b (± 3.4)
Antioxidant capacity			
TAC_M[h]	11.84 c (± 0.28)	14.87 a (± 0.28)	13.36 b (± 0.24)
TAC_{CAL}[i]	na	2.13 a (± 0.03)	1.97 b (± 0.02)
TAC_R[j]	na	12.84 a (± 0.27)	11.35 b (± 0.23)
Objective colour parameters			
C^*[k]	59.88 b (± 0.36)	61.81 a (± 0.33)	60.75 b (± 0.40)
h^*[l]	14.05 a (± 0.28)	14.09 a (± 0.36)	13.62 a (± 0.27)
L^*[m]	29.16 b (± 0.87)	33.07 a (± 0.72)	31.94 a (± 0.72)
a^*[n]	58.03 b (± 0.32)	59.87 a (± 0.29)	59.30 a (± 0.23)
b^*[o]	14.58 a (± 0.34)	15.08 a (± 0.41)	14.38 a (± 0.33)

[a] means taken over all climatic regions and vine structure treatments for a specific vintage; [b] °B; [c] different letters in a row denote significant differences ($P < 0.05$); [d] standard error of mean; [e] mg gallic acid equivalents/L; [f] mg malvidin-3-glucoside equivalents/L; [g] mg (+)-catechin equivalents/L; [h] total antioxidant capacity in mM Trolox equivalents as measured; [i] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [j] $TAC_R = TAC_M - TAC_{CAL}$; [k] chroma; [l] hue angle (°); [m] lightness; [n] red/green chromaticity; [o] yellow/blue chromaticity; na = not available.

coloured polymer (HPLC), an unknown flavonol and quercetin-3-rhamnoside (rham), which were significantly lower.

The TAC of the wines varied significantly between vintages, with the TAC_M highest during 2002 and lowest during 2001 (**Table 1**). The TAC_{CAL} and TAC_R were lower for the 2003 wines than the 2002 wines. For each vintage, the total phenol content correlated well ($P < 0.001$) with the TAC_M values of the wines of that particular vintage, while a weaker, but still significant correlation ($P < 0.001$) was observed when data of the three vintages were pooled (**Figure 1; Table 3**). Similar trends were observed for the correlations ($P < 0.001$) between the total flavan-3-ol content (DAC) and the TAC_M values for the different vintages, although the correlation for the pooled flavan-3-ol content (DAC) of all three vintages with the TAC_M were better than for the total phenol content. A very weak correlation ($P < 0.05$) was observed for the total monomer content (HPLC) with the TAC_M when data of the 2003 vintage were considered, where no correlation ($P \square 0.05$) was obtained for the 2002 data, although when data of the 2002 and 2003 vintages were pooled, a weak,

Table 2. Vintage-related variation in phenolic composition[a] (measured by HPLC) of the 2002 and 2003 Pinotage wines.

Compound/Phenolic group	2002	2003
Anthocyanins		
Delphinidin-3-glc	16.82 a[b] (± 0.60)[c]	13.50 b (± 0.54)
Petunidin-3-glc	24.30 a (± 0.60)	21.21 b (± 0.58)
Peonidin-3-glc	9.70 a (± 0.39)	5.71 b (± 0.32)
Malvidin-3-glc	211.21 b (± 4.30)	228.88 a (± 3.10)
Delphinidin-3-glc-ac[d]	6.20 a (± 0.18)	4.59 b (± 0.18)
Vitisin A[d]	6.29 a (± 0.38)	5.30 a (± 0.39)
Petunidin-3-glc-ac[d]	6.26 a (± 0.17)	5.15 b (± 0.35)
Peonidin-3-glc-ac[d]	4.07 b (± 0.14)	6.04 a (± 0.17)
Malvidin-3-glc-ac[d]	49.47 b (± 1.50)	67.61 a (± 1.47)
Malvidin-3-glc-coum[d]	20.78 a (± 0.86)	21.48 a (± 0.79)
Total monomeric anthocyanins[e]	355.12 b (± 6.74)	379.46 a (± 5.11)
Coloured polymers[f]	8.21 b (± 0.47)	13.96 a (± 0.43)
Flavonols		
Unknown flavonol[g]	18.96 b (± 0.89)	24.64 a (± 0.99)
Quercetin-3-gal	data not shown[h]	data not shown[h]
Quercetin-3-glc	13.65 a (± 0.49)	14.70 a (± 0.75)
Quercetin-3-rham	8.31 b (± 0.27)	9.25 a (± 0.29)
Myricetin	3.25 (± 0.18)	data not shown[h]
Quercetin	4.38 a (± 0.30)	3.37 b (± 0.14)
Kaempferol	data not shown[h]	0.67 (± 0.05)
Isorhamnetin	data not shown[h]	data not shown[h]
Total flavonols[f]	50.54 a (± 1.91)	53.78 a (± 1.99)
Phenolic acids		
Gallic acid	12.75 a (± 0.66)	11.27 a (± 0.63)
Caftaric acid	180.78 a (± 4.49)	175.92 a (± 3.22)
Caffeic acid	5.60 a (± 0.21)	0.84 b (± 0.08)
Coutaric acid[i]	18.45 a (± 0.52)	16.08 b (± 0.29)
p-Coumaric acid	2.10 a (± 0.14)	1.40 b (± 0.10)
Total phenolic acids[f]	219.69 a (± 4.92)	205.51 b (± 3.51)
Flavan-3-ols		
(+)-Catechin	22.63 a (± 0.74)	8.95 b (± 0.26)
Procyanidin B1	32.04 a (± 1.13)	13.01 b (± 0.26)
Non-coloured polymers[j]	119.77 a (± 5.30)	125.17 a (± 6.03)
Total monomers[k]	680.01 a (± 10.02)	660.71 a (± 6.13)

[a] mg/L unless otherwise noted and means taken over all climatic regions and vine structure treatments for a specific vintage; [b] different letters in a row denote significant differences ($P < 0.05$); [c] standard error of mean; [d] mg corresponding anthocyanin-3-glucoside equivalents/L; [e] mg malvidin-3-glucoside equivalents/L; [f] sum of phenolic group content; [g] mg rutin equivalents/L; [h] data not shown due to large number of wines without detectable amounts of compound; [i] mg *p*-coumaric acid equivalents/L; [j] mg (+)-catechin equivalents/L; [k] sum of all quantified monomeric phenolic compounds; gal = galactoside; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; rham = rhamnoside.

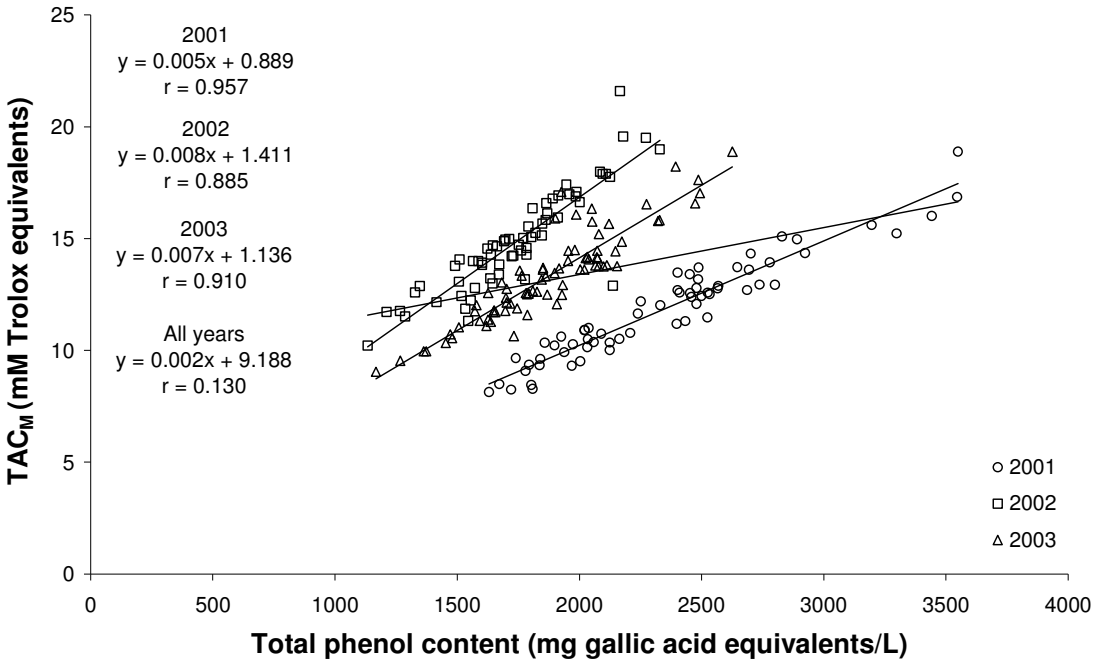

Figure 1. Correlation of total phenol content with measured total antioxidant capacity (TAC_M) for Pinotage wines.

Table 3. Correlations between phenolic group content and total antioxidant capacity of the 2001, 2002 and 2003 Pinotage wines.

Phenolic group	All vintages (pooled)	2001	2002	2003
Spectrophotometric assay				
Total phenols[a]	0.361[b] ***	0.958 ***	0.885 ***	0.910 ***
Total anthocyanins (pH shift)[c]	0.131 ns	0.633 ***	0.285 *	0.633 ***
Total flavan-3-ols (DAC)[d]	0.650 ***	0.926 ***	0.819 ***	0.892 ***
HPLC				
Total monomers[e]	0.271 ***	na	0.242 ns	0.236 *
Total anthocyanins[f]	-0.315 ***	na	-0.240 ns	-0.262 *
Total flavonols[f]	0.325 ***	na	0.430 ***	0.363 ***
Total phenolic acids[f]	0.497 ***	na	0.429 ***	0.506 ***

[a] mg gallic acid equivalents/L; [b] correlation coefficient for correlation between phenolic group and the total antioxidant capacity; [c] mg malvidin-3-glucoside equivalents/L; [d] mg (+)-catechin equivalents/L; [e] sum of all quantified monomeric phenolic compounds; [f] sum of phenolic group content; na = not available; ns = not significant (P □0.05); * P < 0.05; ** P < 0.01; *** P < 0.001.

but significant ($P < 0.001$) correlation was observed. The TAC_M had a significant moderate positive correlation ($P < 0.001$) with the total anthocyanin content (pH shift) for the 2001 and 2003 vintages only, while the 2002 vintage showed a weak, but significant positive correlation ($P < 0.05$). On the other hand, the monomeric anthocyanin content (HPLC) showed weak negative correlations ($P < 0.05$) for the pooled data of the 2002 and 2003 vintages, as well as for the 2003 data separately. The total phenolic acid and total flavonol contents (HPLC) correlated weakly, but significantly ($P < 0.001$) with the TAC_M when data for the 2002 and 2003 vintages were considered separately or pooled.

The objective colour parameters, C^*, L^* and a^*, of the wines were significantly affected by vintage, but no significant differences were observed for h^* and b^* (**Table 1**). The 2002 wines had higher C^* values, and the 2001 wines lower L^* and a^* values than the wines from other years. A plot of L^* values against C^* values revealed an interesting phenomenon (**Figure 2**). As L^* decreased, C^* increased up to a point, where after an inversion occurs with a further decrease in L^* corresponding to a decrease in C^*. This inversion also occurs for both a^* and b^*.

Climatic Region x Vine Structure Treatment Interaction

Only a small number of interactions between climatic region and vine structure treatment were observed for the wines (**Table 4**).

During 2002, the climatic region affected the malvidin-3-Glc content of wines only for the trellised vine treatments, with region III wines having a higher content than region II wines (**Table 4**). Significant differences between wine produced from bush and trellised vines were only observed for region III, with the trellised vine treatments resulting in a higher malvidin-3-glc content compared to the bush vine treatments. A similar trend, although not significant, was observed for the malvidin-3-glc content of region II and IV wines. The monomeric anthocyanin content (HPLC) during 2002 followed the same trend as the malvidin-3-glc content.

Different results were obtained for the anthocyanin content of the 2003 wines, than that observed for the 2002 wines (**Table 4**). The malvidin-3-glc-ac content of wines produced from bush vines was lower than that of trellised vines in region IV only. The trend for climatic region, however, was similar for both bush and trellised vines, with region IV wines having a significantly higher content than region III wines. The malvidin-3-glc-coum content of the wines produced from bush vines was lower than that from trellised vines for all the climatic regions during 2003. Significant differences between climatic regions were obtained for trellised vines with region IV resulting in wines with a higher content than region II and III.

For both 2002 and 2003, bush vines in region IV gave wines with a significantly higher *p*-coumaric acid content compared to trellised vines (**Table 4**). Furthermore, the *p*-coumaric acid

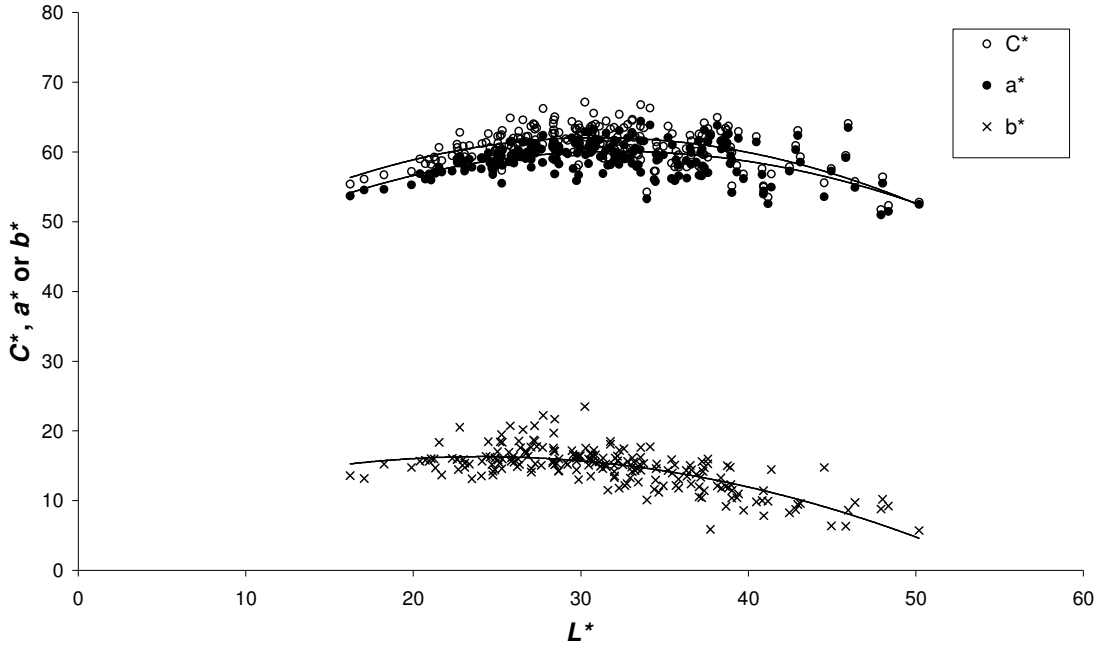

Figure 2. Cartesian plot of *L** values against *C** (chroma), *a** (red/green) and *b** (yellow/blue) values for all Pinotage wines.

Table 4. Interaction of climatic region and vine structure system with regard to phenolic composition[a] of the 2002 and 2003 Pinotage wines.

		2002			2003		
Climatic region	**Vine structure treatment**	**Mv-3-glc**	**Monomeric anthocyanins[b]**	***p*-Coumaric acid**	**Mv-3-glc-ac**	**Mv-3-glc-coum**	***p*-Coumaric acid**
Region II	Bush vines	194.20 c[c] (± 7.03)[d]	334.81 c (± 10.15)	1.73 b (± 0.25)	57.97 d (± 1.88)	15.65 d (± 0.75)	1.00 c (± 0.19)
	Trellised vines	202.88 bc (± 11.47)	351.81 bc (± 17.56)	1.72 b (± 0.38)	60.77 cd (± 1.93)	20.59 bc (± 0.80)	1.43 abc (± 0.29)
Region III	Bush vines	188.17 c (± 10.54)	311.93 c (± 16.48)	1.98 b (± 0.21)	59.90 cd (± 2.18)	18.21 cd (± 1.42)	1.65 ab (± 0.28)
	Trellised vines	244.31 a (± 13.18)	404.40 a (± 21.27)	2.07 b (± 0.29)	66.11 bc (± 2.06)	23.66 b (± 1.59)	1.34 abc (± 0.18)
Region IV	Bush vines	204.82 bc (± 5.73)	341.46 bc (± 9.07)	3.24 a (± 0.38)	71.37 b (± 2.20)	19.17 cd (± 1.18)	1.85 a (± 0.29)
	Trellised vines	227.34 ab (± 5.46)	380.84 ab (± 8.35)	1.52 b (± 0.20)	86.61 a (± 2.81)	31.24 a (± 1.30)	1.18 bc (± 0.16)

[a] mg/L unless otherwise noted; [b] sum of phenolic group content; [c] different letters in a column denote significant differences ($P < 0.05$); [d] standard error of mean; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Mv = malvidin.

content of wines from region IV bush vines in 2002 was substantially higher than that of all the other vintages, climatic region and vine structure treatment combinations. The overall lowest *p*-coumaric acid content was observed for wines made from region II bush vines in 2003. In the case of trellised vines, the climatic region did not affect the *p*-coumaric acid content, irrespective of vintage.

No interactions between climatic region and vine structure treatment were observed for any of the antioxidant capacity or objective colour parameters of the wines.

Climatic Region: Grape Sugar Content

The grape sugar content did not differ significantly between climatic regions for any of the vintages (**Table 5**).

Climatic Region: Effect on Phenolic Composition

In most cases, the climatic region where grapevines were cultivated had a significant impact on the phenolic composition of the wines as measured by spectrophotometric assays (**Table 5**). This was confirmed by HPLC analysis of individual phenolic compounds (**Tables 6-8**).

The total phenol content of the 2001 wines was lower for wines from region IV (warmest) compared to the other regions, while for the 2002 vintage the total phenol content of the wines from the warmest region was significantly lower than that of region II (coolest) (**Table 5**). For the 2003 vintage, however, the total phenol content of region II and III wines did not differ significantly, but region II wines had higher total phenol content than region IV wines.

The monomeric, polymeric and total anthocyanin contents (pH shift) of the wines were lower for the warmest climatic region during 2001 compared to the other regions (**Table 5**). However, these parameters, as well as the monomeric anthocyanin content (HPLC) of the wines, did not differ significantly between wines of different climatic regions for the 2002 vintage (**Tables 5, 6**). The polymeric anthocyanin content (pH shift) of the 2003 wines was significantly lower for the wines from region IV compared to those of region III, while no significant differences between wines from different climatic regions were observed for the monomeric and total anthocyanin content (pH shift), as well as the monomeric anthocyanin content (HPLC) for the 2003 vintage. The coloured polymer content (HPLC) was not affected by climatic region for either of the 2002 and 2003 vintages. During both 2002 and 2003, a decrease in some individual anthocyanin contents of the wines, namely delphinidin-3-glc, petunidin-3-glc and peonidin-3-glc, was observed from the coolest to the warmest climatic region, while the opposite trend was observed for other anthocyanins, namely vitisin A in 2002, and malvidin-3-glc-ac and malvidin-3-coum in 2003 **(Table 6)**. The malvidin-3-glc, delphinidin-3-glc-ac, petunidin-3-glc-ac and peonidin-3-glc-ac contents of the wines, on the other hand, were not affected by climatic region of either of the vintages.

Table 5. Sugar content of grapes and phenolic composition[a] (measured spectrophotometrically) of the 2001, 2002 and 2003 Pinotage wines.

	Sugar content[b]	Total phenols[c]	Monomeric anthocyanins[d]	Polymeric anthocyanins[d]	Total anthocyanins[d]	Total flavan-3-ols[e]
2001: Climatic region[f]						
Region II	25.5 a[g] (± 0.2)[h]	2618.0 a (± 134.7)	540.4 a (± 21.9)	150.7 a (± 7.2)	691.1 a (± 27.4)	173.8 a (± 12.9)
Region III	24.8 a (± 0.2)	2578.5 a (± 93.27)	508.0 a (± 15.1)	138.1 a (± 5.0)	646.1 a (± 19.0)	178.3 a (± 8.9)
Region IV	25.0 a (± 0.2)	2032.6 b (± 84.0)	462.9 b (± 13.6)	115.4 b (± 4.5)	578.4 b (± 17.1)	122.8 b (± 8.1)
2002: Climatic region[f]						
Region II	24.5 a (± 0.2)	1954.6 a (± 70.0)	452.3 a (± 17.8)	54.9 a (± 3.6)	507.1 a (± 20.2)	172.7 a (± 9.0)
Region III	24.5 a (± 0.2)	1771.4 ab (± 65.4)	439.3 a (± 16.6)	53.4 a (± 3.4)	492.7 a (± 18.9)	151.3 a (± 8.4)
Region IV	25.3 a (± 0.2)	1586.0 b (± 62.6)	441.9 a (± 15.9)	54.2 a (± 3.2)	496.1 a (± 18.0)	119.9 b (± 8.0)
2003: Climatic region[f]						
Region II	25.1 a (± 0.2)	1854.9 ab (± 66.3)	465.8 a (± 14.2)	66.1 ab (± 3.8)	532.0 a (± 17.5)	180.6 ab (± 7.5)
Region III	24.6 a (± 0.2)	1987.4 a (± 77.1)	471.7 a (± 16.5)	70.2 a (± 4.4)	541.9 a (± 20.3)	197.9 a (± 8.8)
Region IV	25.2 a (± 0.2)	1777.7 b (± 63.1)	449.3 a (± 13.5)	58.1 b (± 3.6)	507.4 a (± 16.6)	168.5 b (± 7.2)
2001: Vine structure treatment[i]						
Bush vines	24.9 a (± 0.2)	2449.1 a (± 112.1)	503.5 a (± 15.6)	136.9 a (± 5.9)	640.4 a (± 20.6)	165.1 a (± 10.7)
Trellised vines	25.1 a (± 0.2)	2370.2 a (± 113.9)	504.0 a (± 15.9)	132.6 a (± 6.0)	636.6 a (± 21.0)	151.5 a (± 10.8)
2002: Vine structure treatment[i]						
Bush vines	24.7 a (± 0.1)	1812.0 a (± 62.4)	441.5 a (± 13.3)	58.2 a (± 2.5)	499.7 a (± 15.1)	155.3 a (± 8.2)
Trellised vines	25.0 a (± 0.3)	1729.3 a (± 59.2)	447.5 a (± 12.7)	50.1 b (± 2.3)	497.7 a (± 14.3)	140.6 a (± 7.7)
2003: Vine structure treatment[i]						
Bush vines	25.1 a (± 0.2)	1963.6 a (± 56.2)	461.0 a (± 12.1)	66.9 a (± 3.4)	527.9 a (± 15.2)	194.8 a (± 6.4)
Trellised vines	24.9 a (± 0.2)	1783.0 b (± 56.2)	463.5 a (± 12.1)	62.7 a (± 3.4)	526.3 a (± 15.2)	169.9 b (± 6.4)

[a] all phenolic composition means were adjusted for grape sugar content using covariate analysis; [b] °B; [c] mg gallic acid equivalents/L; [d] mg malvidin-3-glucoside equivalents/L; [e] mg (+)-catechin equivalents/L; [f] means taken over all vine structure treatments for a specific vintage, climatic regions as described in materials and methods; [g] different letters in a group in a column denote significant differences ($P < 0.05$); [h] standard error of mean; [i] means taken over all climatic regions and cordon heights for a specific vintage.

Table 6. Anthocyanin content[a] of the 2002 and 2003 Pinotage wines.

	Monomeric anthocyanins											Coloured polymers[d]
	Dp-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Dp-3-glc-ac[b]	Vitisin A[b]	Pt-3-glc-ac[b]	Pn-3-glc-ac[b]	Mv-3-glc-ac[b]	Mv-3-glc-coum[b]	Total[c]	
2002: Climatic region[e]												
Region II	20.98 a[f] (± 1.00)[g]	26.99 a (± 0.83)	12.12 a (± 0.85)	200.67 a (± 10.90)	6.55 a (± 0.44)	4.71 b (± 0.82)	6.45 a (± 0.43)	4.28 a (± 0.28)	44.17 a (± 3.56)	19.22 a (± 1.53)	345.47 a (± 17.39)	6.96 a (± 0.96)
Region III	16.68 b (± 0.74)	24.73 ab (± 1.01)	9.38 b (± 0.76)	217.55 a (± 9.79)	6.03 a (± 0.40)	5.74 ab (± 0.74)	6.11 a (± 0.39)	4.30 a (± 0.29)	48.67 a (± 3.20)	20.96 a (± 1.65)	359.93 a (± 15.63)	7.67 a (± 0.87)
Region IV	14.11 c (± 0.82)	22.14 b (± 0.36)	8.30 b (± 0.75)	214.36 a (± 9.66)	6.02 a (± 0.39)	7.69 a (± 0.73)	6.18 a (± 0.38)	3.74 a (± 0.18)	53.79 a (± 3.16)	21.71 a (± 1.32)	358.78 a (± 15.41)	9.36 a (± 0.85)
2003: Climatic region[e]												
Region II	15.54 a (± 1.04)	23.35 a (± 1.16)	6.89 a (± 0.66)	224.71 a (± 4.88)	4.91 a (± 0.40)	4.41 a (± 0.87)	6.14 a (± 0.92)	6.50 a (± 0.40)	59.26 b (± 1.35)	18.19 b (± 1.67)	370.49 a (± 7.15)	13.17 a (± 0.79)
Region III	14.48 a (± 1.20)	21.91 ab (± 1.35)	5.89 ab (± 0.77)	229.15 a (± 5.60)	4.73 a (± 0.47)	6.50 a (± 1.01)	4.61 a (± 0.34)	5.78 a (± 0.46)	63.01 b (± 1.61)	20.72 ab (± 1.95)	375.79 a (± 9.43)	15.08 a (± 0.91)
Region IV	10.81 b (± 0.99)	18.66 b (± 1.10)	4.24 b (± 0.63)	232.53 a (± 5.64)	4.21 a (± 0.38)	5.37 a (± 0.83)	4.65 a (± 0.30)	5.81 a (± 0.38)	78.99 a (± 2.28)	25.46 a (± 1.60)	390.68 a (± 9.55)	13.93 a (± 0.75)
2002: Vine structure treatment[h]												
Bush vines	15.73 b (± 0.76)	22.96 b (± 0.71)	10.32 a (± 0.76)	197.01 b (± 7.20)	5.90 a (± 0.33)	6.62 a (± 0.65)	6.02 a (± 0.32)	4.40 a (± 0.19)	44.62 b (± 2.57)	16.58 b (± 0.67)	331.08 b (± 10.93)	7.29 a (± 0.70)
Trellised vines	19.92 a (± 0.91)	25.64 a (± 0.92)	9.55 a (± 0.73)	224.71 a (± 6.96)	6.49 a (± 0.32)	5.48 a (± 0.63)	6.50 a (± 0.31)	3.74 b (± 0.20)	53.13 a (± 2.49)	24.99 a (± 1.17)	378.37 a (± 10.57)	8.70 a (± 0.67)
2003: Vine structure treatment[h]												
Bush vines	14.50 a (± 1.01)	21.89 a (± 1.10)	6.88 a (± 0.56)	216.17 b (± 3.24)	4.88 a (± 0.34)	5.55 a (± 0.74)	5.62 a (± 0.64)	6.65 a (± 0.30)	63.32 b (± 1.53)	17.89 b (± 1.28)	362.22 b (± 5.61)	12.86 b (± 0.57)
Trellised vines	12.72 a (± 1.01)	20.72 a (± 1.10)	4.47 b (± 0.56)	242.27 a (± 4.45)	4.36 a (± 0.34)	5.29 a (± 0.74)	4.65 a (± 0.25)	5.41 b (± 0.30)	72.14 a (± 2.34)	25.02 a (± 1.28)	397.64 a (± 7.68)	15.26 a (± 0.57)

[a] mg/L unless otherwise noted, most means were adjusted for grape sugar content using covariate analysis except for Dp-3-glc, Pt-3-glc, Pn-3-glc-ac and Mv-3-glc-coum content in 2002 and Mv-3-glc, Pt-3-glc-ac, Mv-3-glc-ac and total monomeric anthocyanin content in 2003; [b] mg corresponding anthocyanin-3-glc equivalents/L; [c] sum of phenolic group content; [d] mg malvidin-3-glc equivalents/L; [e] means taken over all vine structure treatments for a specific vintage, climatic regions as described in materials and methods; [f] different letters within a group in a column denote significant differences ($P < 0.05$); [g] standard error of mean; [h] means taken over all climatic regions and cordon heights for a specific vintage; Dp = delphinidin; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Pt = petunidin; Pn = peonidin; Mv = malvidin.

Table 7. Flavonol content[a] of the 2002 and 2003 Pinotage wines.

	Unknown[b]	Q-3-glc	Q-3-rham	Myricetin	Quercetin	Kaempferol	Total[c]
2002: Climatic region[d]							
Region II	23.29 a[e] (± 1.89)[f]	14.82 a (± 1.17)	9.66 a (± 0.69)	2.91 a (± 0.71)	6.44 a (± 0.53)	data not shown[g]	60.73 a (± 4.13)
Region III	16.09 b (± 1.00)	11.57 b (± 0.79)	7.69 b (± 0.62)	3.31 a (± 0.27)	3.68 b (± 0.49)	data not shown[g]	43.84 b (± 2.61)
Region IV	18.31 b (± 1.39)	14.53 a (± 0.52)	7.90 ab (± 0.61)	3.44 a (± 0.33)	3.54 b (± 0.34)	data not shown[g]	48.97 b (± 2.55)
2003: Climatic region[d]							
Region II	24.95 a (± 1.99)	15.92 a (± 1.58)	9.84 a (± 0.59)	data not shown[g]	3.52 a (± 0.29)	0.74 a (± 0.11)	56.27 a (± 4.07)
Region III	22.97 a (± 2.32)	12.07 a (± 1.84)	8.33 a (± 0.69)	data not shown[g]	3.39 a (± 0.33)	0.62 a (± 0.12)	48.46 a (± 4.74)
Region IV	25.50 a (± 1.90)	15.18 a (± 1.50)	9.22 a (± 0.56)	data not shown[g]	3.20 a (± 0.27)	0.63 a (± 0.10)	54.61 a (± 3.88)
2002: Vine structure treatment[h]							
Bush vines	17.73 a (± 1.07)	14.57 a (± 0.59)	8.62 a (± 0.54)	3.26 a (± 0.28)	4.51 a (± 0.45)	data not shown[g]	50.95 a (± 2.70)
Trellised vines	20.19 a (± 1.41)	12.73 a (± 0.75)	8.22 a (± 0.52)	3.25 a (± 0.22)	4.24 a (± 0.39)	data not shown[g]	50.13 a (± 2.75)
2003: Vine structure treatment[h]							
Bush vines	25.45 a (± 1.43)	16.05 a (± 1.29)	9.90 a (± 0.47)	data not shown[g]	3.73 a (± 0.23)	0.75 a (± 0.09)	56.95 a (± 3.30)
Trellised vines	23.79 a (± 0.85)	12.73 a (± 1.29)	8.36 b (± 0.47)	data not shown[g]	3.01 b (± 0.23)	0.57 a (± 0.09)	49.27 a (± 3.29)

[a] mg/L unless otherwise noted, most means were adjusted for grape sugar content using covariate analysis except for unknown flavonol, Q-3-glc, myricetin, quercetin and total flavonol contents in 2002; [b] mg rutin equivalents/L; [c] sum of phenolic group content; [d] means taken over all vine structure treatments for a specific vintage, climatic regions as described in materials and methods; [e] different letters within a group in a column denote significant differences ($P < 0.05$); [f] standard error of mean; g data not shown due to large number of wines without detectable amounts of compound; [h] means taken over all climatic regions and cordon heights for a specific vintage; glc = glucoside; Q = quercetin; rham = rhamnoside.

Table 8. Phenolic acid, flavan-3-ol and polymer content[a] of the 2002 and 2003 Pinotage wines.

	Phenolic acids						Flavan-3-ols			Total monomers[e]
	Gallic acid	Caftaric acid	Caffeic acid	Coutaric acid[b]	*p*-Coumaric acid	Total[c]	(+)-Catechin	Procyanidin B1	Non-coloured polymers[d]	
2002: Climatic region[f]										
Region II	13.87 ab[g] (± 1.61)[h]	209.45 a (± 5.72)	5.51 a (± 0.27)	22.31 a (± 0.73)	1.80 a (± 0.37)	252.37 a (± 6.52)	26.78 a (± 1.58)	37.83 a (± 2.39)	116.97 a (± 10.21)	721.66 a (± 19.62)
Region III	14.90 a (± 1.45)	171.49 b (± 8.03)	5.68 a (± 0.35)	17.92 b (± 0.82)	2.11 a (± 0.34)	211.59 b (± 8.01)	23.17 ab (± 1.42)	32.00 ab (± 2.15)	122.29 a (± 10.35)	668.87 b (± 19.42)
Region IV	10.12 b (± 1.43)	168.72 b (± 6.26)	5.61 a (± 0.42)	16.24 b (± 0.64)	2.21 a (± 0.33)	203.94 b (± 7.07)	19.41 b (± 1.40)	28.13 b (± 2.12)	119.66 a (± 7.83)	660.61 b (± 12.26)
2003: Climatic region[f]										
Region II	10.64 a (± 0.59)	176.40 a (± 5.88)	0.99 a (± 0.15)	16.61 a (± 0.62)	1.20 a (± 0.17)	205.84 a (± 6.59)	9.28 a (± 0.44)	12.33 a (± 0.57)	111.44 b (± 10.59)	656.63 a (± 11.80)
Region III	12.44 a (± 1.26)	177.13 a (± 7.12)	0.78 a (± 0.13)	16.16 a (± 0.58)	1.50 a (± 0.17)	208.00 a (± 7.65)	8.89 a (± 0.48)	14.09 a (± 0.66)	144.92 a (± 12.31)	657.66 a (± 13.72)
Region IV	10.93 a (± 1.30)	174.53 a (± 4.15)	0.76 a (± 0.12)	15.52 a (± 0.33)	1.51 a (± 0.17)	203.26 a (± 4.41)	8.69 a (± 0.42)	12.61 a (± 0.54)	122.76 ab (± 10.09)	671.66 a (± 11.24)
2002: Vine structure treatment[i]										
Bush vines	15.49 a (± 1.08)	171.47 b (± 5.97)	5.91 a (± 0.34)	17.49 b (± 0.71)	2.36 a (± 0.26)	212.25 a (± 6.67)	25.23 a (± 1.24)	35.06 a (± 1.87)	102.15 b (± 6.88)	652.08 b (± 13.26)
Trellised vines	10.44 b (± 1.04)	190.10 a (± 6.37)	5.30 a (± 0.25)	19.40 a (± 0.73)	1.72 a (± 0.25)	227.13 a (± 7.10)	21.01 b (± 1.20)	30.25 a (± 1.80)	137.39 a (± 6.79)	707.95 a (± 13.43)
2003: Vine structure treatment[i]										
Bush vines	13.29 a (± 1.06)	177.46 a (± 4.88)	0.93 a (± 0.11)	16.35 a (± 0.44)	1.49 a (± 0.16)	209.51 a (± 5.22)	10.13 a (± 0.33)	13.70 a (± 0.47)	120.31 a (± 9.20)	657.44 a (± 9.55)
Trellised vines	9.13 b (± 0.47)	174.30 a (± 4.20)	0.75 a (± 0.11)	15.80 a (± 0.39)	1.31 a (± 0.12)	201.29 a (± 4.64)	7.70 b (± 0.27)	12.31 b (± 0.47)	132.43 a (± 9.19)	666.53 a (± 9.55)

[a] mg/L unless otherwise noted, most means were adjusted for grape sugar content using covariate analysis except for caftaric, caffeic, coutaric and total phenolic acid contents in 2002 and 2003, non-coloured polymers and total monomers content in 2002 and gallic acid, *p*-coumaric acid and (+)-catechin content in 2003; [b] mg *p*-coumaric acid equivalents/L; [c] sum of phenolic group content; [d] mg (+)-catechin equivalents/L; [e] sum of all quantified monomeric phenolic compounds; [f] means taken over all vine structure treatments for a specific vintage, climatic regions as described in materials and methods; [g] different letters within a group in a column denote significant differences ($P < 0.05$); [h] standard error of mean; [i] means taken over all climatic regions and cordon heights for a specific vintage.

The total flavonols, quercetin and the unknown flavonol were significantly more abundant in region II wines, compared to region III and IV wines of the 2002 vintage (**Table 7**). The climatic regions had no significant effect on the flavonol content of wines from different climatic regions during 2003. Quercetin-3-glc were significantly less abundant in region III wines, compared to region II and IV wines of the 2002 vintage, while quercetin-3-Rham content of region III wines were lower than that of region II only.

The climatic regions did not affect the phenolic acid content of the 2003 wines, but total phenolic acid content and some individual phenolic acids of the 2002 wines were affected (**Table 8**). Region II gave wines with a higher total phenolic acid content than the other regions. These wines also contained significantly higher caftaric and coutaric acid contents. Wines produced from region III grapes had a higher gallic acid content than those from IV grapes.

Trends for the flavan-3-ol content of wines from different climatic regions also differed for the three vintages investigated (**Tables 5, 8**). In 2001 and 2002, the warmest region produced wine containing a lower total flavan-3-ol content (DAC) than wines from the other regions. In the case of the 2003 wines, the total flavan-3-ol content (DAC) did not differ significantly between region II and III wines, but region III wines had a significantly higher total flavan-3-ol content (DAC) than region IV wines. The non-coloured polymer content of the 2002 wines was not affected by climatic region, while the 2003 wines from region II had significantly less non-coloured polymers than the wines from region III. Climatic region only had an effect on the (+)-catechin and procyanidin B1 contents in 2002. (+)-Catechin and procyanidin B1 concentrations were higher for wines from the coolest region compared to wines from the warmest region in 2002.

The total monomer content (HPLC) was affected only in 2002, with wines produced from the coolest region having a higher content (**Table 8**).

Climatic Region: Effect on Antioxidant Capacity

The TAC_M of the wines was affected by the climatic region for only the 2001 and 2002 vintages (**Table 9**). Region II and III produced wines with significantly higher TAC_M values, compared to that of region IV for both the 2001 and 2002 vintages. No TAC_{CAL} or TAC_R data is available for the 2001 wines as the phenolic content of these wines were not analysed using HPLC. The TAC_{CAL} of the wines from region II was significantly higher than that of regions III and IV during 2002, while no significant difference was observed during 2003. The TAC_R comprised between 80 and 90% of the TAC_M and followed similar trends. The phenolic acid and anthocyanin content contributed the most to the TAC_{CAL} of the 2002 and the 2003 wines (**Figure 3**). The contributions of phenolic acids and flavonols to the TAC_{CAL} were higher for region II wines

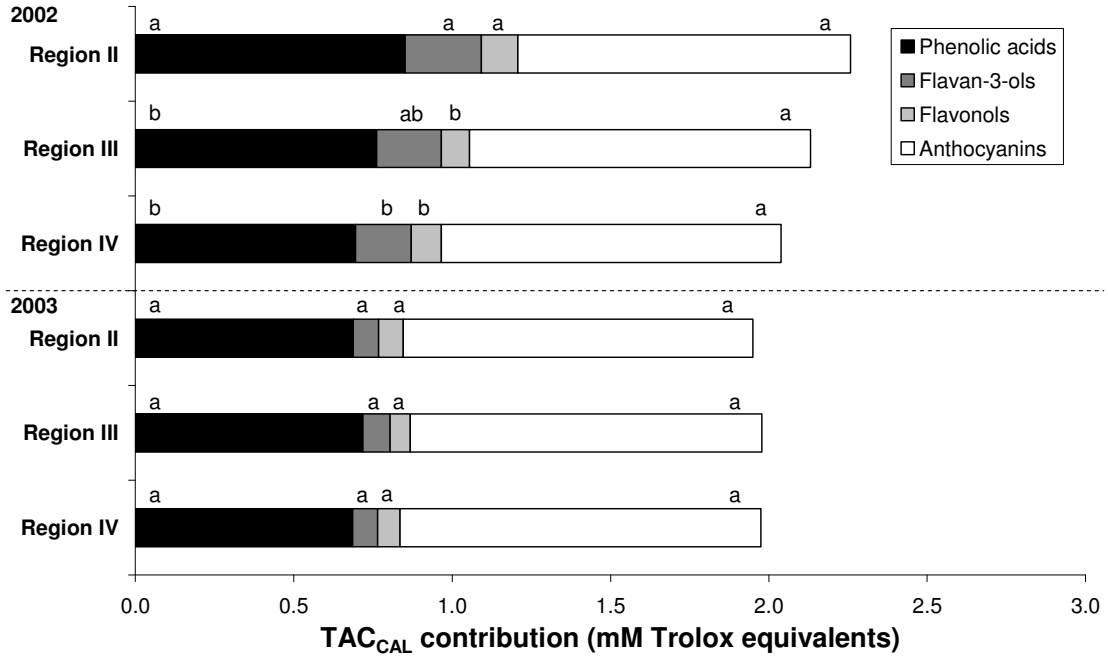

Figure 3. Phenolic group contributions to the calculated total antioxidant capacity (TAC_{CAL}) of wines from different climatic regions (as described in the materials and methods) [different letters for the contribution of a specific phenolic group in the same year denote significant differences ($P < 0.05$)].

compared to wines from the other regions during 2002, while the TAC contribution from flavan-3-ols was higher for wines from region II compared to wines from region IV. During 2003, the TAC_{CAL} contribution of flavonols of the region II wines was not significantly different from that of the region IV wines, but significantly higher than that of the region III wines. The TAC_{CAL} contributions of anthocyanins in 2002 and phenolic acids, flavan-3-ols and anthocyanins in 2003 were not affected by climatic region.

Climatic Region: Effect on Objective Colour Parameters

The objective colour parameters of the wines were only affected by climatic region for the 2001 and 2002 vintages with wines from the 2001 vintage most affected (**Table 9**). Wines from region IV had higher *L** and lower *C** and *b** values than wine from the other regions of the 2001 vintage. The *a** values of region III wines were significantly higher than that of region IV wines, while *h** values of region II wines were significantly higher than wine from the other regions for the 2001 vintage. In the case of the 2002 wines, only *C**, *a** and *b** values were affected by climatic region. The *C** and *a** values of region II wines were significantly higher than wines from region III and IV, while the *b** values of region II wines were significantly higher than that of region III wines. Wines from the 2003 vintage also showed a slightly higher *C** when produced from the cooler climate, although the difference was not statistically significant.

Table 9. Antioxidant capacity and objective colour parameters of the 2001, 2002 and 2003 Pinotage wines.

	Antioxidant capacity[a]			Objective colour parameters				
	TAC_M[b]	TAC_{CAL}[c]	TAC_R[d]	C^*[e]	h^*[f]	L^*[g]	a^*[h]	b^*[i]
2001: Climatic region[j]								
Region II	12.77 a[k] (± 0.67)[l]	na	na	60.85 a (± 0.76)	15.50 a (± 0.61)	25.62 b (± 1.85)	58.49 ab (± 0.65)	16.66 a (± 0.72)
Region III	13.02 a (± 0.47)	na	na	61.12 a (± 0.50)	14.52 b (± 0.42)	26.76 b (± 1.28)	59.17 a (± 0.47)	15.14 a (± 0.51)
Region IV	10.44 b (± 0.42)	na	na	58.41 b (± 0.52)	13.02 b (± 0.38)	32.74 a (± 1.15)	56.86 b (± 0.48)	13.20 b (± 0.42)
2002: Climatic region[j]								
Region II	16.11 a (± 0.62)	2.26 a (± 0.05)	14.02 a (± 0.61)	63.44 a (± 0.70)	14.83 a (± 0.71)	30.89 a (± 1.73)	61.23 a (± 0.63)	16.34 a (± 0.81)
Region III	15.17 a (± 0.46)	2.13 b (± 0.04)	13.04 a (± 0.46)	61.02 b (± 0.66)	13.52 a (± 0.57)	34.58 a (± 1.62)	59.26 b (± 0.58)	14.36 b (± 0.64)
Region IV	13.77 b (± 0.31)	2.04 b (± 0.03)	11.87 b (± 0.26)	61.24 b (± 0.63)	14.02 a (± 0.57)	33.34 a (± 1.55)	59.35 b (± 0.56)	14.77 ab (± 0.65)
2003: Climatic region[j]								
Region II	13.32 a (± 0.50)	1.95 a (± 0.04)	11.37 a (± 0.48)	61.55 a (± 0.87)	14.17 a (± 0.53)	31.64 a (± 1.33)	59.63 a (± 0.21)	15.09 a (± 0.65)
Region III	14.02 a (± 0.58)	1.98 a (± 0.04)	12.04 a (± 0.46)	60.08 a (± 1.02)	13.40 a (± 0.62)	31.16 a (± 1.55)	59.32 a (± 0.44)	14.01 a (± 0.75)
Region IV	12.69 a (± 0.47)	1.98 a (± 0.03)	10.71 a (± 0.46)	60.57 a (± 0.83)	13.19 a (± 0.51)	33.35 a (± 1.27)	58.97 a (± 0.41)	13.90 a (± 0.62)
2001: Vine structure treatment[m]								
Bush vines	12.44 a (± 0.52)	na	na	59.51 a (± 0.54)	14.51 a (± 0.46)	27.82 a (± 1.42)	57.64 a (± 0.48)	14.66 a (± 0.44)
Trellised vines	11.71 a (± 0.53)	na	na	60.25 a (± 0.48)	14.19 a (± 0.46)	28.93 a (± 1.44)	58.43 a (± 0.43)	14.49 a (± 0.52)
2002: Vine structure treatment[m]								
Bush vines	15.41 a (± 0.45)	2.08 a (± 0.04)	13.50 a (± 0.41)	61.86 a (± 0.61)	14.96 a (± 0.24)	31.06 b (± 1.26)	59.74 a (± 0.53)	15.98 a (± 0.28)
Trellised vines	14.32 a (± 0.31)	2.17 a (± 0.04)	12.18 b (± 0.31)	61.93 a (± 0.58)	13.23 b (± 0.64)	34.81 a (± 1.19)	60.15 a (± 0.50)	14.18 b (± 0.74)
2003: Vine structure treatment[m]								
Bush vines	14.16 a (± 0.40)	1.99 a (± 0.03)	12.17 a (± 0.39)	61.31 a (± 0.73)	14.28 a (± 0.43)	31.10 a (± 1.15)	59.42 a (± 0.25)	15.13 a (± 0.53)
Trellised vines	12.53 b (± 0.40)	1.95 a (± 0.03)	10.58 b (± 0.39)	60.16 a (± 0.73)	12.90 b (± 0.43)	33.00 a (± 1.15)	59.17 a (± 0.40)	13.54 b (± 0.53)

[a] antioxidant capacity values for 2003 were adjusted for grape sugar content using covariate analysis; [b] total antioxidant capacity in mM Trolox equivalents as measured; [c] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [d] $TAC_R = TAC_M - TAC_{CAL}$; [e] chroma; [f] hue angle (°); [g] lightness; [h] red/green chromaticity; [i] yellow/blue chromaticity; [j] means taken over all vine structure treatments for a specific vintage, climatic regions as described in materials and methods; [k] different letters within a group in a column denote significant differences ($P < 0.05$); [l] standard error of mean; [m] means taken over all climatic regions and cordon heights for a specific vintage; na = not available.

Climatic Region: Discriminant Analysis

Canonical discriminant analysis was performed to attempt discrimination between the wines from different climatic regions with regard to variables relating to phenolic composition. Forward stepwise variable selection was applied to obtain variables with the highest discriminating power for climatic region for each of the 2002 and 2003 vintages. Sixteen and 18 variables were selected for the 2002 and 2003 vintages, respectively (**Figures 4, 5**). Region II and III wines can easily be discriminated from region IV wines by the first discriminant function in both vintages, while region II and III wines are separated by the second discriminant function with minor overlapping. More overlapping between region II and III wines occurs during 2003. During 2002, the caftaric acid, malvidin-3-glc-ac and coloured polymer (HPLC) contents had the highest positive correlations to the first discriminant function, while the coutaric acid, *p*-coumaric acid and malvidin-3-glc contents contributed greatly in the negative direction of the first discriminant function. The second discriminant function for the 2002 wines was mostly controlled by the caftaric acid and malvidin-3-glc-ac contents in the positive direction and by the malvidin-3-glc content in the negative direction. Among the variables contributing most to the first discriminant function for the 2003 wines were the positively-correlated delphinidin-3-glc-ac, malvidin-3-glc and coutaric acid contents and the negatively-correlated malvidin-3-glc-ac and delphinidin-3-glc contents. The coutaric acid, (+)-catechin and delphinidin-3-glc-ac contents made the greatest positive contribution to the second discriminant function for the 2003 wines, while the greatest negative contributions were made by the caftaric acid, procyanidin B1 and vitisin A contents.

Vine Structure: Grape Sugar Content

The grape sugar content did not differ significantly between vine structure treatments for any of the vintages (**Table 5**).

Vine Structure: Effect on Phenolic Composition

Cordon height had a significant effect on the phenolic composition of the wines in a small number of cases only (**Table 10**). Bush vines with cordon height of 20 cm produced wines with a higher total phenol content than the 30 cm treatment for the 2002 vintage. Individual phenolic composition was affected by cordon height of wines produced in 2003, with the 20 cm bush vine treatment resulting in wines with significantly lower delphinidin-3-glc content and significantly higher caffeic and *p*-coumaric acid contents compared to the 30 cm bush vine treatment. For the trellised vines, only the coloured polymer content (HPLC) of the 2003 wines was affected with a higher content for wines from the 60 cm treatment compared to the 30 cm treatment. Due to the relatively minor influence of cordon height on the phenolic composition of the wines, data for wines produced from bush and trellised vines with averages taken over the different cordon height

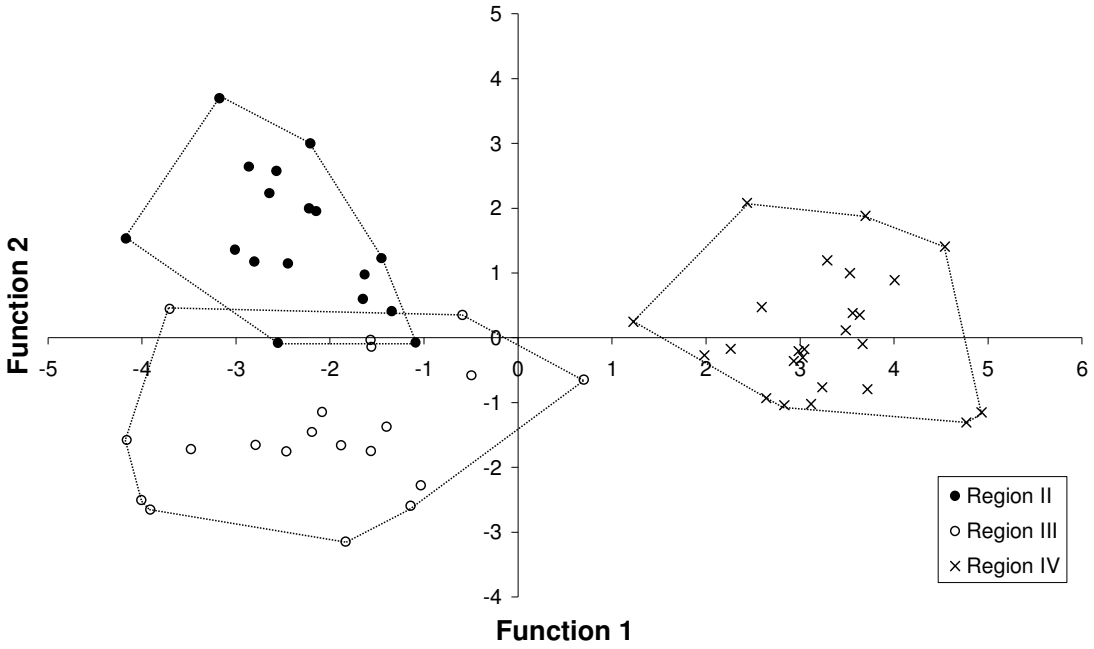

Figure 4. Distribution of the 2002 Pinotage wines in the plane defined by the first two discriminant functions according to climatic regions (as described in the materials and methods) [variables selected = petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, vitisin A, malvidin-3-acetylglucoside, coloured polymer (HPLC), quercetin-3-glucoside, kaempferol, isorhamnetin, gallic acid, caftaric acid, caffeic acid, coutaric acid, *p*-coumaric acid, (+)-catechin and non-coloured polymer contents].

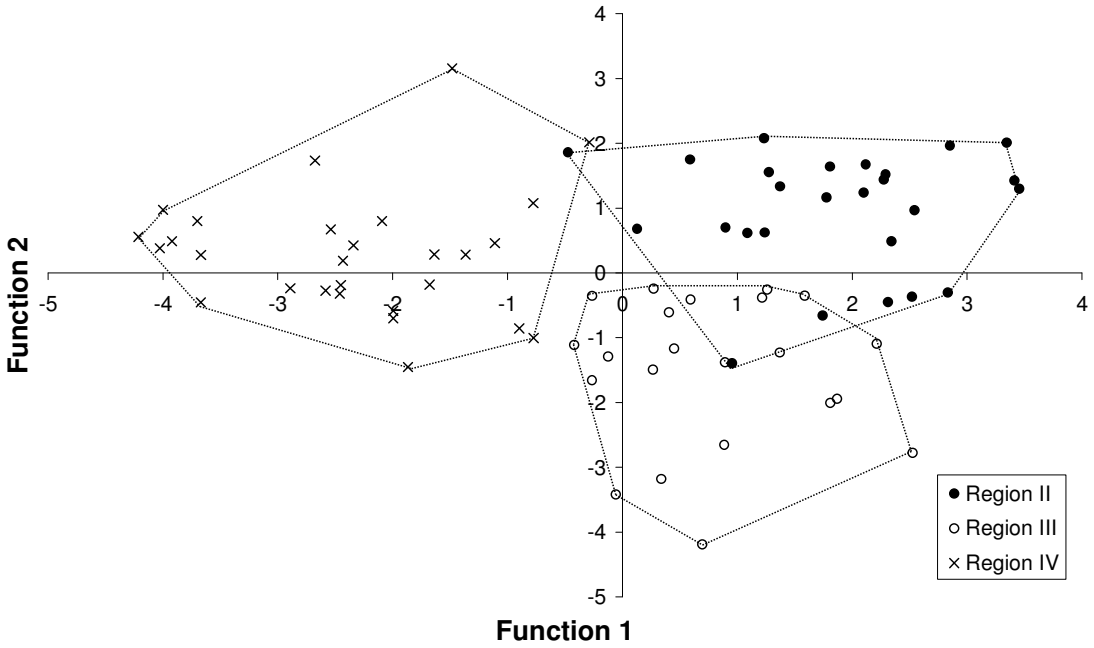

Figure 5. Distribution of the 2003 Pinotage wines in the plane defined by the first two discriminant functions according to climatic regions (as described in the materials and methods) [variables selected = delphinidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, delphinidin-3-acetylglucoside, vitisin A, peonidin-3-acetylglucoside, malvidin-3-acetylglucoside, malvidin-3-*p*-coumaroylglucoside, coloured polymer (HPLC), unknown flavonol, quercetin-3-glucoside, quercetin-3-rhamnoside, gallic acid, caftaric acid, caffeic acid, coutaric acid, (+)-catechin and procyanidin B1 contents].

Table 10. Effect of cordon height on the phenolic composition[a] of the 2002 and 2003 Pinotage wines.

Vine structure treatment	Cordon height	2002	2003			
		Total phenols[b]	Dp-3-glc-ac	Coloured polymers[c]	Caffeic acid	*p*-Coumaric acid
Bush vines	20 cm	1843.5 a (± 60.7)	12.83 b (± 1.12)	12.00 b (± 0.54)	1.21 a (± 0.16)	1.89 a (± 0.24)
	30 cm	1671.3 b (± 82.9)	15.59 a (± 0.94)	13.43 b (± 0.67)	0.66 b (± 0.12)	1.11 b (± 0.17)
Trellised vines	30 cm	1709.6 ab (± 55.28)	11.64 b (± 0.92)	13.87 b (± 0.76)	0.68 b (± 0.13)	1.36 b (± 0.19)
	60 cm	1748.5 ab (± 52.18)	13.83 ab (± 1.24)	16.70 a (± 1.07)	0.83 ab (± 0.18)	1.26 b (± 0.15)

[a] mg/L unless otherwise noted; [b] mg gallic acid equivalents/L; [c] mg malvidin-3-glc equivalents/L; [d] different letters in a column denote significant differences ($P < 0.05$); [e] standard error of mean; Dp = delphinidin; glc = glucoside; glc-ac acetylglucoside.

treatments are presented in **Table 5** to **9**.

The total phenol content was lower for wines from trellised vines than from bush vines for all the vintages, although this trend was only significant for the 2003 vintage (**Table 5**).

Vine structure treatment had little effect on the anthocyanin content (pH shift) of the wines (**Table 5**). Only the polymeric anthocyanin content (pH shift) of the 2002 wines was affected, with trellised vines resulting in wines with a lower content. Considering individual anthocyanins, vine structure treatment did not affect peonidin-3-glc, delphinidin-3-glc-ac, vitisin A and petunidin-3-glc-ac contents of the 2002 wines, and the delphinidin-3-glc, petunidin-3-glc, delphinidin-3-glc-ac, vitisin A and petunidin-3-glc-ac contents of the 2003 wines (**Table 6**). Apart from peonidin-3-glc-ac (2002 and 2003 wines) and peonidin-3-glc (2003 wines) that were increased in the wine by using grapes from bush vines, the contents of other anthocyanins, monomeric anthocyanins (HPLC) and coloured polymers (HPLC) (2003 only) were higher in trellised vine wines.

The flavonol content of the 2002 wines was not affected by the vine structure treatment, while only the quercetin-3-rham and quercetin contents of the 2003 wines from bush vines were significantly higher than those from trellised vines (**Table 7**).

The vine structure treatment did not affect the total phenolic acid, caffeic acid or *p*-coumaric acid content of wines, for either of the vintages (**Table 8**). The gallic acid content of wines produced from bush vines was significantly higher than wines produced from trellised vines for both vintages. The caftaric and coutaric acid contents, on the other hand, were lower for wines from bush vines compared to trellised vines for the 2002 vintage, with no effect observed for the 2003 wines.

Bush vines resulted in wines with a higher total flavan-3-ol content (DAC) than trellised vines for the 2003 vintage only (**Table 5**). When using HPLC analysis, this trend was confirmed for the

2003 wines, and the same trend was also observed for the 2002 wines (**Table 8**). In addition, bush vines gave wines with higher (+)-catechin contents for both vintages and procyanidin B1 contents for the 2003 vintage. On the other hand, the 2002 wines produced from bush vines had a lower non-coloured polymer content compared to wines produced from trellised vines, but no effect was observed for the 2003 wines.

The total monomer content (HPLC) was higher for wines from trellised vines than from bush vines of the 2002 vintage, with no effect observed for the 2003 vintage (**Table 8**).

Vine Structure: Effect on Antioxidant Capacity

Cordon height did not affect the TAC_M, TAC_{CAL} or TAC_R of the wines (data not shown). Wines produced from bush vines had higher TAC_M values than those produced from trellised vines (all vintages), although this trend was only significant for the 2003 wines (**Table 9**). The TAC_{CAL}, however, was not affected by the different vine structure treatments for either the 2002 or 2003 vintages, but the TAC_R of bush vine wines was higher than that of wines produced from trellised vines of both vintages. For the 2002 wines, the lower anthocyanin contribution to the TAC_{CAL} of the bush vine wines was balanced out by the higher contribution of flavan-3-ol content, while for the 2003 wines the lower anthocyanin contribution was cancelled out by the higher contributions of phenolic acids, flavan-3-ols and flavonols (**Figure 6**).

Vine Structure: Effect on Objective Colour Parameters

Cordon height did not significantly affect any of the objective colour parameters of the wines (data not shown). No significant differences in objective colour parameters between wines from bush and trellised vines were observed for the 2001 vintage (**Table 9**). The 2002 and 2003 wines, however, showed significantly higher h^* and b^* values for wines from bush vines, compared to wines from trellised vines, while the a^* and C^* values were not significantly affected by vine structure treatment. For the 2002 vintage, the L^* value of wine from trellised vines was significantly higher than that of wines from bush vines.

Vine Structure: Discriminant Analysis

Canonical discriminant analysis with forward stepwise variable selection was also performed to attempt discrimination between the wines produced from different vine structure treatments using variables relating to phenolic composition. Fifteen and 16 variables were selected in the 2002 and 2003 vintage, respectively (**Figures 7, 8**). Wines from vines with different cordon heights could not be discriminated for either of the two vintages. When the first two discriminant functions arising in the canonical discriminant analysis of the 2002 vintage are plotted for bush and trellised vine wines, there is very little overlapping. This indicates fairly good discrimination between the vine structure treatments. For the first discriminant function of the 2002 vintage data, the malvidin-3-glc and

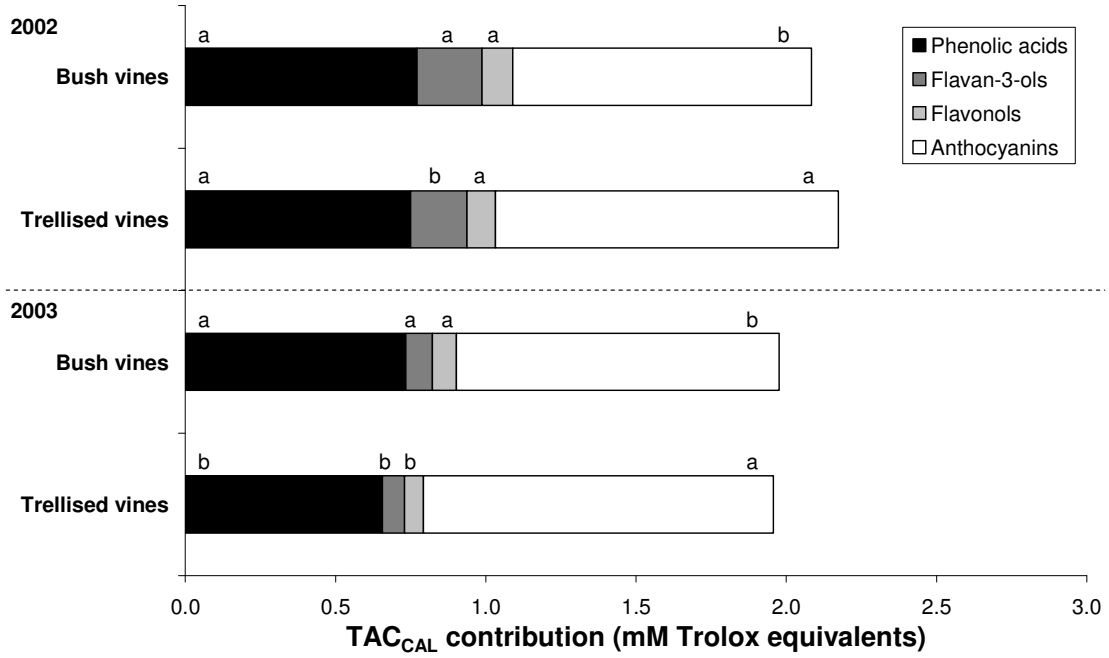

Figure 6. Phenolic group contributions to the calculated total antioxidant capacity (TAC_{CAL}) of wines from different vine structure treatments [different letters for the contribution of a specific phenolic group in the same year denote significant differences ($P < 0.05$)].

gallic acid contents were highly positively correlated, while the malvidin-3-glc-coum, non-coloured polymer and delphinidin-3-glc-ac contents were highly negatively correlated. The second discriminant function was highly influenced in the positive direction by the quercetin and petunidin-3-glc contents, while the delphinidin-3-glc-ac and kaempferol contents were the highest contributors in the negative direction. For data from the 2003 vintage, the discrimination between wines from bush and trellised vines is less pronounced with more overlapping. Variables with high correlation to the first discriminant function in the 2003 vintage were the coloured polymer (HPLC) (positive), peonidin-3-glc (negative) and malvidin-3-glc-ac (negative) contents, while for the second discriminant function the malvidin-3-glc-coum and malvidin-3-glc contents were the greatest contributors in the positive and negative direction, respectively.

Discussion

Pinotage wines from the first vintage (2001) were analysed for phenolic composition using spectrophotometric assays, antioxidant capacity and objective colour parameters. Since this preliminary study showed that climatic region and vine structure treatment significantly affected wine properties, wines prepared during the subsequent two vintages were analysed more extensively. In addition to spectrophotometric analyses, HPLC analysis of the 2002 and 2003 wines

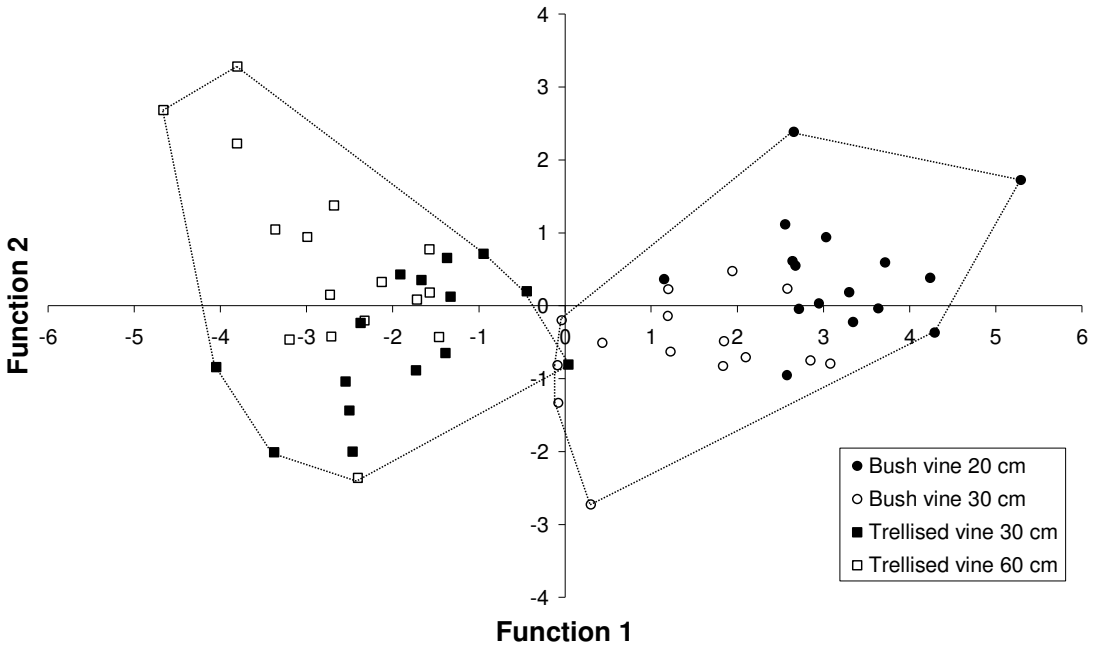

Figure 7. Distribution of the 2002 Pinotage wines in the plane defined by the first two discriminant functions according to vine structure treatment [variables selected = delphinidin-3-glucoside, malvidin-3-glucoside, delphinidin-3-acetylglucoside, petunidin-3-acetylglucoside, peonidin-3-acetylglucoside, malvidin-3-*p*-coumaroylglucoside, coloured polymer (HPLC), quercetin-3-glucoside, myricetin, quercetin, kaempferol, gallic acid, caftaric acid, (+)-catechin and non-coloured polymer contents].

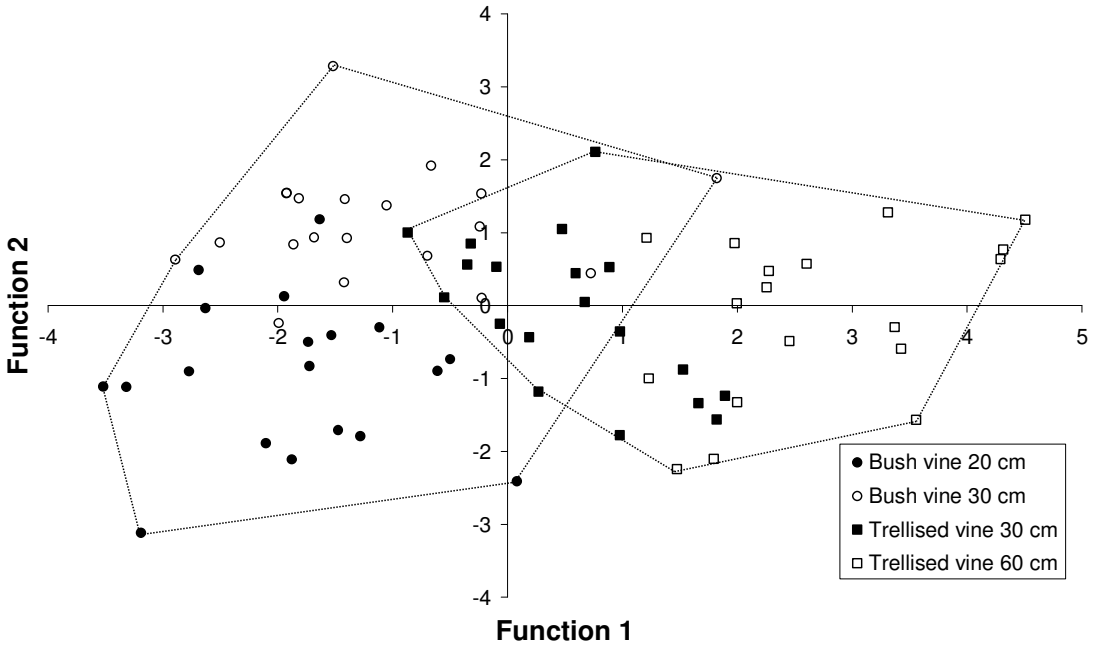

Figure 8. Distribution of the 2003 Pinotage wines in the plane defined by the first two discriminant functions according to vine structure treatment [variables selected = peonidin-3-glucoside, malvidin-3-glucoside, vitisin A, petunidin-3-acetylglucoside, peonidin-3-glucoside, malvidin-3-acetylglucoside, malvidin-3-*p*-coumaroylglucoside, coloured polymer (HPLC), quercetin-3-glucoside, quercetin-3-rhamnoside, myricetin, isorhamnetin, caffeic acid, *p*-coumaric acid, (+)-catechin and procyanidin B1 contents].

was thus performed to identify trends for individual phenolic compound content and used to explain trends in antioxidant capacity and objective colour parameters.

Effect of Grape Maturity

Grape composition, including sugar content, changes during ripening (Kennedy et al., 2000; Downey et al., 2004) and this may affect the composition and characteristics of the resulting wines. As grape sugar content is generally used as a ripeness indicator, grapes for all treatments should be picked at the same sugar content to be able to compare the Pinotage wine characteristics between different treatments. In this study, it was attempted to harvest grapes within a window of 2 °B, i.e. between 24 and 26 °B. To enable harvesting at the same grape sugar content, the grapes were sampled and the sugar concentrations monitored regularly as grape development took place over the ripening season. Problems such as widely dispersed vineyard sites and the dependence of ripeness development near the critical level on local daily weather phenomena, such as heat waves and rain, hampered the harvesting of grapes at the same grape sugar content. In this study, ~15% of treatments were harvested too early or too late, i.e. with a grape sugar content <24 °B or >26 °B. The grape sugar content did not, however, differ significantly between the vintages or between climatic regions and vine structure treatments in the respective vintages. For this reason, data for wines made from grape sugar content <24 °B or >26 °B were not removed from the dataset. Covariance analysis with grape sugar content as covariate was, however, performed and the means for affected variables were adjusted.

Vintage-related Variations

Vintage-related variations in terms of phenolic composition and TAC are presumably due to variation in weather patterns for the respective years. Individual climatological events in a specific vintage could also have a great impact on the vine physiology and therefore the biosynthesis of phenolic compounds (Mullins et al., 1992). Cooler night temperatures during the berry ripening period in 2003 caused lower average February temperatures in the respective climatic regions, compared to 2002 (D. van Schalkwyk, ARC Infruitec-Nietvoorbij, personal communication, 2005). These variations resulted in the contribution of monomeric phenolic compounds to the TAC_M, represented by TAC_{CAL}, being lower for the 2003 wines compared to the 2002 wines. This trend is especially due to lower contents of high potency compounds (**Chapter 3**) such as (+)-catechin, procyanidin B1, and all the anthocyanin monoglucosides, except malvidin-3-glc. The opposite was true for some acylated anthocyanins and quercetin-3-rham. Cool night temperatures during berry ripening, as observed for 2003, caused lower anthocyanin monoglucoside concentrations in the resulting wines. This is in agreement with findings of Mori et al. (2005) showing that anthocyanins concentrations in Pinot noir berry skins were decreased by lower night temperatures. The TAC_R

was also lower for the 2003 wines compared to the 2002 wines, due to changes in unknown compounds or oligomers with less than 5 subunits as the non-coloured polymer content (polymers with 5 subunits or larger) showed no significant differences between vintage wines. The lower TAC_M of the 2003 wines is, therefore, mostly due to decreased antioxidant capacity of monomeric phenolic compounds and unknown compounds.

The 2001 wines were darker (lower L^*), with lower a^* values than the 2002 and 2003 wines (**Table 1**). Their higher monomeric, polymeric and total anthocyanin content, all determined using the pH shift assay, explains their darker colour. The average wine hues for the different vintages were a similar magenta-red hue, although hues of individual wines in each vintage ranged from red-magenta through magenta-red to pure red (**Addendum C**). The lower a^* values of the 2001 wines were unexpected as the higher anthocyanin content should lead to increased C^* and a^* values and decreased L^* values. However, inversion was observed with C^* and a^* values, especially at lower L^* values corresponding to very dark wines. This phenomenon has been reported previously for dark-coloured beverages (Eagerman et al., 1973), port (Bakker et al., 1986), young red wines (Almela et al., 1995) and dark-coloured anthocyanin solutions (Gonnet, 1999) and is related to the difficulty of photocells to adjust to low luminosity situations such as when L^* is low. In the present study, a 5 mm cell was used instead of the longer pathlength cells available in an attempt to prevent this from happening. However, it seems that for very dark coloured wines, such as the wines in this study, the use of even shorter pathlength cells would be advisable. L^* values for the 2001 wines were generally lower than for the 2002 wines, with more wines having L^* values where inversion occurred, explaining this discrepancy. The higher colour saturation (higher C^*) of the 2002 wines could possibly be due to higher contents of anthocyanin monoglucosides with high specific absorptivity, such as petunidin-3-glc (Cabrita et al., 2000). Lower contents of some acylated anthocyanins, which generally have lower absorptivity (Giusti et al., 1999), should affect the colour saturation to a lesser extent. The higher phenolic acid and flavan-3-ol contents observed for the 2002 wines could also have increased the colour saturation due to an enhanced co-pigmentation effect with anthocyanins (Gonnet, 1999). The relationship between phenolic content and objective colour parameters is not always easy to interpret as many factors play a role, such as concentration of various pigments and co-pigments, as well as the co-pigment to pigment ratios. The anthocyanin species present in wine have different hues with differences in degree of ionisation at a specific pH, playing a role especially in colour saturation and lightness (Heredia et al., 1998). Scale inversion also played a role, while pH and sulphur dioxide content could differ between wines. Sulphur dioxide is not expected to have an important role in anthocyanin bleaching in this case as all wines were prepared and stored using a standard protocol.

Effect of Climatic Region

In a study on two Port wine cultivars (Mateus et al., 2002), both the anthocyanin monoglucoside and acetylated anthocyanin contents of the berries, as well as those of the resulting Port wines, were higher for a cooler area compared to a warmer area. Similar results were obtained in terms of anthocyanin monoglucoside contents in the present study, while the acylated anthocyanin contents mostly did not change or the opposite effect was observed. Using a pH shift method, Miguel-Tabares et al. (2002) found higher monomeric, polymeric and total anthocyanin contents for Listan Negro and Ruby Cabernet grapes grown in a cool climatic region, as was found in the present study for Pinotage wines in 2001. Lower berry temperature has been shown to increase the monomeric anthocyanin content in berry skins of various red cultivars (Bergqvist et al., 2001; Spayd et al., 2002). Factors other than air temperature, such as degree of light radiation, airflow through the grapevine canopy and humidity could, however, also affect berry temperature.

Mateus et al. (2001) reported that vineyard sites with higher average temperatures during ripening produced berries and resulting Port wines with higher flavan-3-ol content in terms of both monomers and dimers. The opposite trend was observed in the present study during 2002, particularly for the monomer, (+)-catechin, and the dimer, procyanidin B1.

Previous reports on flavonol accumulation in grape berries focussed on the effect of sunlight exposure (Haselgrove et al., 2000; Downey et al., 2004), which does not necessarily coincide with the effect of temperature. In the case of anthocyanins, increased concentrations are observed in grapes with sunlight exposure (Kliewer, 1970; Crippen & Morrison, 1986; Spayd et al., 2002), but higher average temperatures decreased concentrations (Kliewer, 1970; Spayd et al., 2002). As sunlight exposure could differ between climatic regions, vineyard sites and individual canopies, this effect can not be separated from other climatic factors in the present study.

Previous studies did not include the effect of climate on the phenolic acids content. In the present study, different results were obtained for the two vintages, suggesting that more vintages are needed to clarify effects. Canonical discriminant analysis confirmed the results of analysis of variance for the phenolic composition of wines from different climatic regions. Good separation between regions was obtained with slight overlapping between region II and III, as was the case for some individual phenolic compound contents.

The TAC_M values for wines from different climatic regions can be explained by their total phenol content. A relationship between TAC and total phenol content of wines, as measured by the Folin-Ciocalteau assay, has previously been shown (Landrault et al., 2001; De Beer et al., 2003). The trend for TAC_{CAL} of the 2002 wines could mainly be explained by a higher contribution of phenolic acids, flavan-3-ols and flavonols, due to higher concentrations of specifically caftaric acid,

(+)-catechin, procyanidin B1 and quercetin for region II wines, compared to wines from the other regions. (+)-Catechin, procyanidin B1 and quercetin are high potency antioxidant compounds (**Chapter 3**). The caftaric acid content was much higher for wines from the cooler climatic region, thus increasing its relative contribution to the TAC_{CAL} substantially despite its relatively low antioxidant potency (**Chapter 3**). The antioxidant contributions of both monomeric phenolic compounds (TAC_{CAL}) and unknown compounds (TAC_R) were higher for wines from cooler regions compared to warmer regions, as no significant differences in non-coloured polymer content were observed. The coloured polymer content (HPLC) was higher for region IV wines compared to the wines from region II and III and would thus negate some of the effect of the decreasing concentrations of other compounds in these wines. During 2003, however, no significant difference in TAC_{CAL} was observed between wines from different climatic regions, due to the non-significant differences in contributions by phenolic acids, flavan-3-ols or anthocyanins. During both 2002 and 2003, the content of some anthocyanin monoglucosides increased in wines as the climatic region became progressively cooler, while the opposite was true for some acylated anthocyanins. For the 2002 vintage the total contribution of anthocyanins to the TAC_{CAL} for the wines from the cooler climatic region was higher than for the wines from the warmer climatic region. For the 2003 vintage, on the other hand, this was similar for all climatic regions due to much lower acylated anthocyanin contents for wines from the cooler climatic region and similar differences in anthocyanin monoglucoside contents. The phenolic acid and flavan-3-ol contents, on the other hand, showed very little difference between wines from the different climatic regions.

Phenolic compounds contributing to the TAC_R would include (-)-epicatechin, flavan-3-ol gallate esters, *S*-glutathionylcaftaric acid, stilbenes, stilbene glucosides, pyranoanthocyanins other than vitisin A, procyanidin dimers other than procyanidin B1, as well as anthocyanin-flavan-3-ol dimers (**Chapter 3**). The flavan-3-ol gallate esters and procyanidin dimers are especially high potency antioxidant compounds (Plumb et al., 1998). Especially the flavan-3-ol contribution is, therefore, underestimated. Other compounds of low or high MW such as proteins, peptides, polysaccharides and possibly others could also contribute to the TAC of Pinotage wines as discussed in **Chapter 3**.

The cooler climatic region produced wines that were generally darker (lower L^*) with a higher colour saturation (higher C^*) and a higher h^*, namely a magenta-red hue closer to pure red, due to a higher b^* value compared to wines from the warmer regions during 2001. However, in 2002 the region II wines had higher colour saturation (higher C^*) due to higher a^* and lower b^* values, and no significant difference in lightness (L^*). The average hue for the 2002 wines from all the climatic regions was in the magenta-red range. The higher L^* and C^* values observed for the 2001 wines from the cool climatic region is attributed to higher monomeric anthocyanin contents. On the other

hand, the higher *C** for the 2002 wines from the cooler region can be attributed to increasing anthocyanin monoglucoside contents. The higher co-pigment factor contents, e.g. phenolic acids, flavonols and/or flavan-3-ols, for wines from cool regions, compared to warm region wines for the 2001 and 2002 vintage would further explain the higher *C** values for these wines (Gonnet, 1999). On the other hand, the higher co-pigment factor contents in the 2001 wines from region II caused a higher *h** compared to wines from region III and IV, while the opposite was true for the 2002 wines. This apparent discrepancy is similar to both "blueing" (higher *h**) and "yellowing" (lower *h**) effects for solutions containing the anthocyanin pigment, cyanin, and the co-pigment, rutin, with increasing co-pigment content as described by Gonnet (1999). At very low pigment concentrations "yellowing" mostly occurred, although factors such as co-pigment to pigment ratio and pH also had an effect. During 2003, no significant differences in total phenolic acid, total flavan-3-ol and monomeric anthocyanin contents for wines from different climatic regions were observed, while only differences in some flavonols occurred. These results explain the lack of significant differences between objective colour parameters of wines from different climatic regions. The average hue for the 2003 wines from the different climatic regions was in the magenta-red range.

Cool climatic regions, producing wines with high TAC_M, also resulted in the highest overall sensory quality and colour acceptability when the same wines were evaluated eight months after production (D. van Schalkwyk, ARC Infruitec-Nietvoorbij, personal communication, 2005). On the other hand, wines from the warmest region, especially in the 2002 vintage, displayed more aromas which were not typical of Pinotage wines, than wines from the cooler regions (D. van Schalkwyk, ARC Infruitec-Nietvoorbij, personal communication, 2005). Non-typical aromas may result in a decrease in overall wine quality. (+)-Catechin and procyanidin B1, observed in higher concentrations in wines from the cool climatic region, could increase bitterness and astringency (Robichaud & Noble, 1990; Kielhorn & Thorngate, 1999). Despite non-typical aromas for wines from the warm climatic region and the higher flavan-3-ol content for wines from the cool climatic region, the overall wine quality of all wines was high. Variations observed in hue and colour saturation did not negatively impact the colour acceptability of wines, indicating that a range of hues and colour saturation levels are deemed acceptable for Pinotage wines by expert wine judges.

Effect of Vine Structure

Vine structure treatments had contrasting effects on the polymeric anthocyanin content (pH shift) and the coloured polymer content (HPLC). Several reasons could explain this phenomenon. The polymer peak in the HPLC chromatograms contains only polymers of 5 or greater subunits (Peng et al., 2002), whereas polymeric anthocyanins of less than 5 subunits are also less dependent

on pH changes than monomeric anthocyanins and will be included in the pH shift assay measurement. Furthermore, the monomeric anthocyanins will contribute a small amount to the polymeric pigment content due to residual absorbance at pH 4.9 in the pH shift assay (Cabrita et al., 2000). No differences in individual anthocyanin contents in the grape skins of two Italian grape varieties, Uva di Troia and Bombino Nero, were observed for berries from bush vines compared to trellised vines (Tamborra et al., 2003), while Wolf et al. (2003) found no differences between several different trellis systems for berry skin total anthocyanin content measured using the bisulphite bleaching method. In contrast to these two reports, the present study showed significant differences in the concentrations of several individual anthocyanins in Pinotage wines, especially peonidin-3-glc-ac which was higher for wines from bush vines and malvidin-3-glc, malvidin-3-glc-ac and malvidin-3-glc-coum which were higher for wines from trellised vines, as well as the monomeric anthocyanin content (HPLC), although the monomeric, polymeric and total anthocyanin contents measured using the pH shift assay did not differ.

Similarly, Tamborra et al. (2003) found no significant differences in the individual flavonol contents of Shiraz grape skins from bush and trellised vines, while the present study showed significant differences in the concentrations of some individual flavonols, namely quercetin and quercetin-3-rham, although only for the 2003 wines. Higher flavonol concentrations for wines from bush vines should, however, not be due to differences in sunlight exposure between vine structure treatments, as both bush and trellised vines had optimal canopy management to ensure similar canopy densities in terms of leaf layers.

On the other hand, Tamborra et al. (2003) showed a similar trend for phenolic acid content, namely lower *cis*- and *trans*-caftaric acid and lower *cis*- and *trans*-coutaric acid in grape skins for grapes from bush vines compared to trellised vines, as observed in the present study for the 2002 wines.

Neither of these two studies (Tamborra et al., 2003; Wolf et al., 2003) considered the effect of vine structure on the flavan-3-ol content of grapes or wines. As wines from bush vines were found to have higher (+)-catechin and procyanidin B1 concentrations, but a lower non-coloured polymer content, the astringency of wines could be affected as the monomeric to polymeric flavan-3-ol ratios are affected. The results of the analysis of variance were supported by the canonical discriminant analysis taking a number of phenolic content parameters into account. Wines from bush and trellised vines were separated with minor overlapping, while cordon heights could not be separated.

The differences in TAC_M for the vine structure treatments can be explained by their difference in total phenol content as was observed for different climatic regions, with both parameters only significant for the 2003 vintage. For the 2002 vintage, the lower anthocyanin contribution to the

TAC_{CAL} of the bush vine wines, which is especially due to a much lower content of anthocyanin monoglucosides and acylated malvidin derivatives, was balanced out by the higher contribution of the high antioxidant potency flavan-3-ols, specifically (+)-catechin (**Chapter 3**). In 2003 the lower anthocyanin contribution, mostly due to substantially lower contents of malvidin-3-glc and its acylated derivatives, was cancelled out by higher contributions by phenolic acids, flavan-3-ols and flavonols, especially due to higher contents of the high potency antioxidant compounds, gallic acid, (+)-catechin and procyanidin B1 (**Chapter 3**). The increased TAC_M of wines from bush vines compared to those from trellised vines in 2002 and 2003 are thus mostly due to unknown compounds, especially since both the coloured and non-coloured polymer contents were lower for wines from bush vines.

Results for the objective colour parameters of the 2002 and 2003 wines indicate that wines produced from bush vines generally had a less magenta hue (higher *h**) than those produced from trellised vines, due to a higher *b** value, although hues for wines from both treatments were in the magenta-red range. Higher *h** and *b** values for the 2002 wines from bush vines are attributed to their higher co-pigment content (gallic acid and (+)-catechin) and lower monomeric anthocyanin content than those from trellised vines, which changes the co-pigment to pigment ratio (Gonnet, 1999). These results, as well as their higher polymeric anthocyanin content (pH shift), explain their darker colour (lower *L**). The higher polymeric anthocyanin (pH shift) content for the 2002 wines from bush vines did not, however, affect the *a** values. Inversion could be a factor in this case. The higher quercetin and quercetin-3-rham contents in the 2003 wines would result in higher *b** values, explaining the higher *h** and *b** values for wines from bush vines compared to wines from trellised vines. Higher gallic acid, flavan-3-ol and flavonol contents cancelled out the negative effect of lower monomeric anthocyanin content on the *L** values of the 2003 wines from bush vines.

The higher total phenol content of wines from bush vines, compared to wines from trellised vines, resulting in higher TAC values, was not detrimental to their sensory characteristics, as the overall wine quality and colour acceptability of all wines were high (D. van Schalkwyk, ARC Infruitec-Nietvoorbij, personal communication, 2005). The higher gallic acid, (+)-catechin and procyanidin B1 contents of wines from bush vines, which could lead to higher levels of bitterness and astringency, only resulted in decreased overall wine quality for the 2003 wines (D. van Schalkwyk, ARC Infruitec-Nietvoorbij, personal communication, 2005). In fact, the 2002 wines from bush vines had higher overall sensory quality and colour acceptability scores than wines from trellised vines (D. van Schalkwyk, ARC Infruitec-Nietvoorbij, personal communication, 2005). Wines from bush vines from the 2002 vintage were also shown to be darker and more orange-red, when considering their objective colour parameters, indicating that these characteristics are important for sensory colour acceptability. The overall quality scores for wines generally followed

similar trends as wine colour acceptability scores indicating the important effect of colour on wine quality.

Conclusions

The phenolic composition, antioxidant capacity and objective colour parameters of Pinotage wines were significantly affected by climatic region and vine structure treatments, although the cordon height of the vine had few significant effects. In general, the warmer climatic regions produced lighter coloured wines with lower TAC, mainly due to lower total phenol content, although sensory quality and colour were acceptable. This trend could be partly explained by the variation in phenolic composition among wines from the different climatic regions, although unknown compounds are mostly responsible for differences in TAC. Wines from bush vines were generally darker resulting in higher colour acceptability scores with slightly higher TAC than wines from trellised vines, although the latter wines had higher monomeric anthocyanin concentrations. This was also due to increased total phenol content, although some phenolic compounds were present in lower quantities. Once again, unknown compounds contributed significantly to the differences in TAC between wines from different vine structure treatments. Canonical discriminant analysis confirmed the observations from analysis of variance for individual parameters by enabling discrimination between wines from different climatic regions and vine structure treatments (bush versus trellised vines) based on a large number of parameters. Cultivation of Pinotage bush vines in cooler climatic regions not only increased the TAC of the resulting wines, but also improved their colour and sensory quality and is therefore recommended when higher TAC is a requirement. Some vintage-related variation was observed, which indicates that wines from more vintages needs to be analysed in order to verify the results obtained in the present study.

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Chapter 5: Maceration Before and During Fermentation - Effect on Pinotage Wine Phenolic Composition, Total Antioxidant Capacity and Objective Colour Parameters*

Abstract

Low-temperature maceration treatments (1, 2 and 4 days at 10 and 15 °C) before fermentation and juice/skin mixing treatments (punching-down, pumping-over and rotor action every hour and every 3 hours) during fermentation were investigated in terms of their effects on Pinotage wine phenolic composition, total antioxidant capacity (TAC) and colour over three vintages (2000 to 2002). Results for pre-fermentation maceration were not consistent between vintages. Very few significant differences in the phenolic content, TAC and objective colour parameters were observed between the control wines and wines subjected to different pre-fermentation maceration treatments. Pre-fermentation maceration increased the vitisin A content, especially for treatments at 15 °C. Improvement of wine quality when using pre-fermentation maceration treatments at 10 °C were noted previously, while no detrimental effect on the wine TAC was observed. The pumping-over treatment yielded wines with lower TAC and phenol content, as well as less favourable objective colour values, indicating that the punching-down or rotor treatment would be preferred. Although mixing at hourly intervals yielded a higher content of some phenolic compounds compared to the 3 hour interval mixing, mixing frequency did not affect the TAC of the wine. The objective colour parameters, h^* and b^*, were slightly lower at the higher mixing frequency in 2002 indicating a shift in the direction of a magenta hue.

Introduction

Maceration, i.e. extraction of components from grape solids, is one of the most important processes that give red wine its characteristic taste and flavour profile differentiating it from white wine (Ribéreau-Gayon et al., 1999). Primarily, phenolic compounds are extracted which contribute to wine colour and structure. Other components released into the wine during maceration include aroma compounds, aroma precursors, nitrogen compounds, polysaccharides and minerals. Control of the maceration process is essential to ensure a good balance between the wine components. As a large proportion of the phenolic compounds found in red wines originated from the skins and seeds

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(Sun et al., 1999; Sun et al., 2001), maceration has a substantial effect on the antioxidant capacity of red wines (Villaño et al., 2006). The absence (before fermentation) and presence (during fermentation) of ethanol during maceration greatly affects the compounds that are preferentially extracted (González-Manzano et al., 2004).

Pre-fermentation maceration is used to enhance wine aroma in red, rosé and white wines through the increased aqueous extraction of aroma precursors from the grape skins (Marais & Rapp, 1988; McMahon et al., 1999; Salinas et al., 2005). However, decreased concentrations of acetate and ethyl esters have been reported for pre-fermentation low-temperature skin contact at 15 °C for 2 and 4 days compared to a control fermented immediately after crushing (Marais, 2003a). Pre-fermentation skin contact at 10 °C increased the sensory quality of Pinotage wines compared to the control wine for three separate vintages (Marais, 2003a). Pre-fermentation maceration at 10 and 15 °C has been shown to enhance the colour and anthocyanin content of red (Heatherbell et al., 1997; Watson et al., 1997; Reynolds et al., 2001; Gómez-Míguez et al., 2006) and rosé (Salinas et al., 2003; Salinas et al., 2005) wines. Differences in anthocyanin content between treated and untreated wines, however, decreased with ageing (Heatherbell et al., 1997). The extraction of flavan-3-ols and tannins during the pre-fermentation maceration in the absence of ethanol is expected to be relatively low (González-Manzano et al., 2004). The stilbene content of wines, made with pre-fermentation low-temperature maceration, was shown to be higher compared to those made without pre-fermentation maceration (Poussier et al., 2003; Clare et al., 2004).

Juice/skin mixing techniques such as punching-down, pumping-over and rotor action are used during fermentation to ensure good contact between the juice and skins for optimum extraction of aroma, flavour and phenolic compounds. In the presence of ethanol, phenolic compounds such as monomeric and polymeric flavan-3-ols are extracted from the grape skins and seeds (González-Manzano et al., 2004). Punching-down and rotor treatments resulted in wines with higher sensory quality scores than wines produced according to the punching-down protocol for three separate vintages (Marais, 2003b). Mechanical pumping-over in closed tanks gave Pinot Noir, Dornfelder and Portugieser wines with a different phenolic composition than a mechanical punch-down action or rotor action in closed tanks (depending on the cultivar) (Fischer et al., 2000). Similar results were also obtained by Leone et al. (1983) for Sangiovese, Primitivo and Negramaro wines using mechanical pumping-over and punching-down treatments. An hourly pumping-over and twice daily rotary action resulted in Vinhão wines with differences in colour, anthocyanin content and colour stability (Castillo-Sánchez et al., 2006). The type of mixing action can therefore also be expected to affect the antioxidant capacity of red wines.

The aim of the study was to investigate the effect of different maceration techniques before and during fermentation on the total antioxidant capacity and colour of Pinotage wines. The effect of the

treatments on the phenolic composition of wines was determined to explain differences in total antioxidant capacity and colour. The results were related to the sensory results obtained in a parallel study on the same wines.

Materials and Methods

Pre-fermentation Maceration

Grapes were harvested from the one vineyard at the Nietvoorbij farm (Stellenbosch, South Africa) at ~23 to 24 °B and pH 3.2 to 3.3 in 2000, 2001 and 2002. Pre-fermentation treatments and wine-making were done as described by Marais (2003a). Briefly, the grapes were divided into two equal representative batches and stored overnight at 10 and 15 °C, respectively. After storage, each batch was divided into three equal, representative lots for the three different pre-fermentation treatments. The treatments (in triplicate) were 1 day, 2 days and 4 days skin contact, respectively. Each treatment was carried out with 60 kg of grapes after destemming, crushing and addition of 80 mg SO_2/L. After the pre-fermentation maceration treatments were completed, the wines were produced according to the standard wine-making protocol with no wood contact (described in **Chapter 3**) at the experimental cellar of ARC Infruitec-Nietvoorbij (Stellenbosch, South Africa). Fermentation was completed after ~4 days. A control wine was prepared with the same standard winemaking protocol, without a pre-fermentation maceration step. After bottling, the wines were stored at 15 ºC. Eight months after production, aliquots of each wine were frozen at -20 °C, to prevent further phenolic changes, until analyses could be carried out. Samples were analysed immediately after defrosting.

Juice/Skin Mixing during Maceration

Grapes were harvested from the same vineyard, as for the pre-fermentation maceration treatments, at the Nietvoorbij farm (Stellenbosch, South Africa) at ~23 to 24 °B and pH 3.2 to 3.3 in 2000, 2001 and 2002. Juice/skin mixing treatments and wine-making were done as described by Marais (2003b). Briefly, wine-making was carried out according to the standard wine-making protocol with no wood contact (described in **Chapter 3**) at the experimental cellar of ARC Infruitec-Nietvoorbij (Stellenbosch, South Africa). During fermentation three mixing treatments were conducted, i.e. manual punching-down of the cap, pumping-over of the juice (imitated by racking the juice into a bucket and pouring it over the skins) and rotor action (imitated by rolling a closed drum filled with the crushed grapes, once to and fro by hand over a 5 m distance). Each treatment was conducted at two frequencies, i.e. every hour and every 3 hours (in triplicate), between 7:00 and 19:00 until completion of fermentation after ~4 days. Each treatment was carried out with 60 kg of grapes. After bottling, the wines were stored at 15 ºC. Eight months after

production, aliquots of each wine were frozen at -20 °C, to prevent further phenolic changes, until analyses could be carried out. Samples were analysed immediately after defrosting.

Chemicals and Phenolic Reference Standards

Chemicals and phenolic reference standards used for phenolic and antioxidant analyses were described in **Chapters 3** and **4**.

Spectrophotometric Analysis of Phenolic Content

Pinotage wines from all vintages and treatments were subjected to spectrophotometric analysis of the major phenolic groups. Total phenol, total flavan-3-ol, as well as, monomeric, polymeric and total anthocyanin contents were determined in triplicate using the Folin-Ciocalteau (Singleton & Rossi, 1965), dimethylaminocinnamaldehyde (DAC) (McMurrough & McDowell, 1978) and pH shift (Ribéreau-Gayon & Stonestreet, 1965) assays, respectively, as modified and described in **Chapter 4**.

HPLC Analysis of Phenolic Composition

Individual phenolic compounds, as well as coloured and non-coloured polymers detected at 520 and 280 nm, respectively, were quantified in duplicate in Pinotage wines from the 2001 and 2002 vintages using an HPLC method (Peng et al., 2002), modified and described in **Chapter 3**. Polymers included polymeric phenolic compounds with 5 or more subunits, consisting of anthocyanins and flavan-3-ols for coloured polymers, and only flavan-3-ols for non-coloured polymers.

ABTS Radical Cation Scavenging Assay

The total antioxidant capacity (TAC) of Pinotage wines from all vintages and treatments was measured (TAC_M) in triplicate using the $ABTS^{\bullet+}$ scavenging assay (Re et al., 1999). The content of individual phenolic compounds, measured by HPLC, and their experimental TEAC values (reported in **Chapter 3**) were used to calculate the theoretical TAC (TAC_{CAL}). The remaining TAC (TAC_R) is the difference between TAC_M and TAC_{CAL}. Analysis and calculations were carried out as described in **Chapter 3**.

Objective Colour Parameters

The objective colour parameters of Pinotage wines from all vintages and treatments were measured in duplicate on the CIELab scale, namely a^* (red/green chromaticity), b^* (yellow/blue chromaticity) and L^* (lightness), and the C^* (chroma) and h^* (hue angle), calculated as described in **Chapter 4**. Names for hues were adapted from Gonnet (1999) based on the h^* values and are described in **Chapter 4**.

Statistical Analysis

Analysis of variance was performed on the means for different treatment combinations to determine whether significant differences occurred. The Student *t*-LSD test ($P < 0.05$) was used to determine the statistical differences between means. Analysis of variance and difference testing was done using the SAS version 8 software package (SAS Institute Inc., Cary, NC). Pearson product moment correlation coefficients between parameters and their P-values were calculated using the STATISTICA 6 software package (StatSoft, Inc., Tulsa, OK).

Results

The actual values for all determinations (pre-fermentation maceration trial), as well as data not shown here (pre-fermentation maceration and juice/skin mixing trial), are reported in **Addendum D**.

Vintage-related Variation

Vintage-related variation was observed in terms of phenolic composition, antioxidant capacity and objective colour parameters with different trends for the pre-fermentation maceration and juice/skin mixing trials, except for the total phenol, monomeric and polymeric anthocyanin (pH shift) contents (**Tables 1, 2**). Data for each trial are thus presented separately for each vintage.

The 2000 wines had the lowest monomeric and total anthocyanin contents (pH shift), the 2001 wines the highest polymeric anthocyanin (pH shift), total flavan-3-ol (DAC) and total phenol contents, and the 2002 wines the highest monomeric anthocyanin and lowest polymeric anthocyanin contents (pH shift) (**Table 1**). This was true for both trials. The flavan-3-ol content was the lowest in the 2000 and 2002 wines, prepared in the juice/skin mixing and pre-fermentation maceration trials, respectively. The individual phenolic compounds were only quantified for the 2001 and 2002 wines (**Table 2**). Of the anthocyanins, only four showed significant differences between the vintages in the pre-fermentation maceration trial, i.e. vitisin A and malvidin-3-acetylglucoside (glc-ac) were present in higher and peonidin-3-glucoside (glc) and peonidin-3-glc-ac in lower concentrations in the 2001 wines. In the juice/skin mixing trial all the mono-glucosides and some of the acylated anthocyanins (peonidin-3-glc-ac, malvidin-3-glc-ac, malvidin-3-*p*-coumaroylglucoside (glc-coum)) were higher in the 2002 wines compared to the 2001 wines. An opposite trend was observed for the vitisin A content. The 2001 wines, however, had a higher monomeric anthocyanin content (HPLC; pH shift) than the 2002 wines.

The remaining phenolic compounds, or groups of compounds, were largely in higher concentrations in the 2001 wines, with wines from the pre-fermentation maceration trial more

Table 1. Vintage-related variation in phenolic composition (measured spectrophotometrically), antioxidant capacity and objective colour parameters of the 2000, 2001 and 2002 Pinotage wines[a].

Parameter	Pre-fermentation maceration treatments			Juice/skin mixing treatments		
	2000	2001	2002	2000	2001	2002
Phenolic composition						
Total phenols[b]	2252.4 b[c] (± 147.5)[d]	2698.0 a (± 194.3)	2334.1 b (± 151.8)	2056.3 b (± 196.0)	2698.7 a (± 148.9)	2113.3 b (± 187.4)
Monomeric anthocyanins[e]	207.0 c (± 20.0)	358.5 b (± 29.8)	389.3 a (± 32.7)	185.0 c (± 18.3)	345.5 b (± 21.8)	368.6 a (± 37.1)
Polymeric anthocyanins[e]	75.0 b (± 11.1)	94.9 a (± 9.8)	52.5 c (± 8.3)	77.7 b (± 11.0)	97.3 a (± 10.7)	44.1 c (± 8.4)
Total anthocyanins[e]	282.0 b (± 27.3)	453.2 a (± 35.1)	441.5 a (± 39.2)	257.7 c (± 27.8)	442.8 a (± 29.5)	412.7 b (± 43.1)
Total flavan-3-ols[f]	168.6 b (± 14.1)	193.0 a (± 17.1)	150.9 c (± 10.4)	158.6 c (± 20.9)	207.0 a (± 15.4)	170.1 b (± 18.6)
Antioxidant capacity						
TAC_M[g]	13.61 b (± 1.25)	15.59 a (± 1.51)	15.26 a (± 0.95)	11.87 c (± 1.45)	15.69 b (± 0.75)	16.85 a (± 1.46)
TAC_{CAL}[h]	na	2.01 a (± 0.21)	1.80 b (± 0.11)	na	1.62 b (± 0.11)	1.80 a (± 0.12)
TAC_R[i]	na	13.58 a (± 1.46)	13.42 a (± 0.89)	na	14.07 b (± 0.69)	15.05 a (± 1.39)
Objective colour parameters						
C^*[j]	58.05 c (± 2.71)	61.59 b (± 1.69)	79.56 a (± 3.14)	57.77 c (± 3.58)	62.49 a (± 1.33)	59.79 b (± 3.54)
h^*[k]	16.23 b (± 1.66)	14.05 c (± 1.63)	28.19 a (± 1.47)	17.16 a (± 1.46)	14.48 b (± 1.71)	12.03 c (± 1.75)
L^*[l]	41.27 b (± 4.36)	36.70 c (± 3.78)	52.46 a (± 4.29)	41.00 a (± 5.29)	35.13 b (± 4.29)	41.85 a (± 5.00)
a^*[m]	55.70 c (± 2.23)	59.72 b (± 1.34)	70.74 a (± 2.09)	55.17 c (± 3.27)	60.47 a (± 0.94)	58.44 b (± 3.21)
b^*[n]	16.28 b (± 2.29)	14.98 c (± 2.02)	37.52 a (± 0.80)	17.07 a (± 2.06)	15.64 b (± 2.09)	12.53 c (± 2.34)

[a] means taken over all treatment combinations for a specific vintage; [b] mg gallic acid equivalents/L; [c] different letters in a row pertaining to a specific trial denote significant differences ($P < 0.05$); [d] standard deviation; [e] mg malvidin-3-glucoside equivalents/L; [f] mg (+)-catechin equivalents/L; [g] total antioxidant capacity in mM Trolox equivalents; [h] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [i] $TAC_R = TAC_M - TAC_{CAL}$; [j] chroma; [k] hue angle (°); [l] lightness; [m] red/green chromaticity; [n] yellow/blue chromaticity; na = not available.

Table 2. Vintage-related variation in phenolic composition (measured by HPLC) of the 2001 and 2002 Pinotage wines[a].

Compound/ Phenolic group	Pre-fermentation maceration treatments		Juice/skin mixing treatments	
	2001	2002	2001	2002
Anthocyanins				
Dp-3-glc	10.35 a[b] (± 1.55)[c]	10.21 a (± 1.20)	6.59 b (± 1.12)	8.31 a (± 1.26)
Pt-3-glc	14.97 a (± 2.13)	14.78 a (± 1.38)	10.33 b (± 1.43)	12.62 a (± 1.52)
Pn-3-glc	5.09 b (± 0.75)	5.66 a (± 0.72)	3.25 b (± 0.64)	4.50 a (± 0.85)
Mv-3-glc	188.91 a (± 22.84)	187.93 a (± 13.94)	145.50 b (± 18.43)	171.42 a (± 16.41)
Dp-3-glc-ac[d]	3.92 a (± 0.41)	3.78 a (± 0.30)	2.27 a (± 0.34)	2.32 a (± 0.28)
Vitisin A[d]	11.31 a (± 2.71)	8.50 b (± 2.21)	8.84 a (± 3.17)	6.03 b (± 1.75)
Pt-3-glc-ac[d]	3.29 a (± 0.60)	3.24 a (± 0.25)	1.92 a (± 0.29)	2.02 a (± 0.23)
Pn-3-glc-ac[d]	4.48 b (± 0.40)	4.86 a (± 0.36)	2.96 b (± 0.31)	3.60 a (± 0.41)
Mv-3-glc-ac[d]	60.83 a (± 7.50)	55.84 b (± 4.65)	47.07 b (± 5.89)	51.21 a (± 4.65)
Mv-3-glc-coum[d]	28.40 a (± 5.90)	27.86 a (± 3.39)	19.34 b (± 3.26)	24.87 a (± 3.51)
Total monomeric anthocyanins[e]	331.57 a (± 39.43)	322.66 a (± 23.63)	248.06 b (± 27.77)	286.89 a (± 27.26)
Coloured polymers[f]	14.07 a (± 3.38)	10.65 b (± 2.03)	6.79 a (± 2.25)	7.98 a (± 1.75)
Flavonols				
Unknown flavonol[g]	21.11 a (± 3.30)	10.33 b (± 1.92)	17.51 a (± 2.32)	9.78 b (± 2.23)
Q-3-glc	17.42 a (± 2.77)	7.49 b (± 0.85)	15.55 a (± 2.18)	7.51 b (± 0.98)
Q-3-rham	11.66 a (± 1.47)	9.30 b (± 0.82)	10.05 a (± 1.25)	8.70 b (± 0.94)
Quercetin	2.57 a (± 0.90)	3.10 a (± 0.75)	1.84 b (± 0.32)	3.05 a (± 0.90)
Kaempferol	0.72 (± 0.25)	data not shown[h]	0.50 a (± 0.09)	0.23 b (± 014)
Isorhamnetin	data not shown[h]	data not shown[h]	data not shown[h]	0.30 (± 0.15)
Total flavonols[e]	32.37 a (± 4.78)	20.12 b (± 2.38)	45.45 a (± 5.52)	29.56 b (± 4.65)
Phenolic acids				
Gallic acid	14.04 a (± 1.74)	12.82 b (± 1.25)	13.45 a (± 1.10)	12.96 a (± 1.49)
Caftaric acid	212.88 a (± 44.84)	173.92 b (± 22.31)	176.75 b (± 25.43)	207.34 a (± 30.42)
Caffeic acid	0.90 a (± 0.23)	0.67 b (± 0.14)	1.25 a (± 0.13)	1.36 a (± 0.75)
Coutaric acid[i]	24.01 a (± 5.20)	16.64 b (± 2.07)	20.80 a (± 2.78)	20.21 a (± 2.70)
p-Coumaric acid	1.33 a (± 0.51)	1.18 b (± 0.31)	2.01 a (± 0.56)	2.04 a (± 0.42)
Total phenolic acids[e]	227.82 a (± 44.72)	187.41 b (± 22.00)	214.27 b (± 28.73)	243.92 a (± 32.87)
Flavan-3-ols				
(+)-Catechin	12.21 a (± 0.99)	9.19 b (± 0.53)	10.29 a (± 0.54)	10.07 a (± 0.70)
Procyanidin B1	19.45 a (± 1.59)	16.20 b (± 1.54)	17.26 a (± 1.50)	16.81 a (± 1.34)
Non-coloured polymers[j]	213.01 a (± 43.05)	178.04 b (± 23.90)	174.30 a (± 28.57)	178.89 a (± 28.59)
Total monomers[k]	669.86 a (± 79.28)	584.17 b (± 42.63)	535.33 b (± 39.96)	587.23 a (± 43.15)

[a] mg/L unless otherwise noted and means taken over all treatments for a specific vintage; [b] different letters in a row pertaining to a specific trial denote significant differences ($P < 0.05$); [c] standard deviation; [d] mg corresponding anthocyanin-3-glc equivalents/L; [e] sum of phenolic group content; [f] mg malvidin-3-glc equivalents/L; [g] mg rutin equivalents/L; [h] data not shown due to large number of wines without detectable amounts of compound; [i] mg *p*-coumaric acid equivalents/L; [j] mg (+)-catechin equivalents/L; k sum of all quantified monomeric phenolic compounds; Dp = delphinidin; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Pt = petunidin; Pn = peonidin; Mv = malvidin; Q = quercetin; rham = rhamnoside.

affected by vintage than wines from the juice/skin mixing trial (**Table 2**). Exceptions were quercetin, which was not affected by vintage (pre-fermentation maceration trial) or was present in a higher concentration in the 2002 wines (juice/skin mixing trial). Similarly, the caffeoyltartaric (caftaric) acid, total phenolic acid and total monomer contents were higher in the 2002 wines (juice/skin mixing trial). Only a few of the 2002 wines from the pre-fermentation maceration trial contained kaempferol compared to the 2001 wines which all had measurable concentrations. All the 2002 wines from the juice/skin mixing trial contained kaempferol, but its content was less than that of the 2001 wines. Isorhamnetin was only detected in the 2002 wines from the juice/skin mixing trial. The phenolic acids, caffeic acid, *p*-coumaroyltartaric (coutaric) acid and *p*-coumaric acid, flavan-3-ols, and polymers were not affected by vintage in the juice/skin mixing trial.

The TAC_M of the 2000 wines from both trials was lower than that of the other vintages (**Table 1**). The TAC_{CAL} was higher for the 2001 wines compared to the 2002 wines (pre-fermentation maceration trial), while the TAC_R showed no significant differences. In the juice/skin mixing trial, the TAC_M, TAC_{CAL} and TAC_R of the 2002 wines had higher values compared to those of the 2001 wines.

All objective colour parameters had markedly higher values for the 2002 wines of the pre-fermentation maceration trial (**Table 1**). The lowest h^*, L^* and b^* values were observed for the 2001 wines, while the 2000 wines had the lowest C^* and a^* values in this trial. In the juice/skin mixing trial, the 2000 wines also had the lowest C^* and a^* values, but the highest h^* and b^* values. The lowest h^* and b^* values were observed for the 2002 wines, while the 2001 wines had the highest C^* and a^* values. Similar to the wines of the pre-fermentation trial, the 2001 wines had lower L^* values compared to the 2000 and 2002 wines.

Pre-fermentation Maceration: Effect on Phenolic Composition

The treatments that either significantly increased or decreased the phenolic content of the Pinotage wines are considered. The 10 °C/2 days treatment in 2000 and the 15 °C/2 days treatment in 2001 resulted in wines with increased total phenol content (**Figure 1**). The same treatments also increased the total flavan-3-ol (DAC) content of the wine. The (+)-catechin content of the 2001 wines was increased by the 15 °C/2 days treatment (**Figure 2**). On the other hand, the 10 °C/4 days and 15 °C/1 day treatments decreased the total flavan-3-ol content (DAC) of the 2001 wines. In 2002, the (+)-catechin and procyanidin B1 contents were decreased by the 15 °C/1 day treatment, but the total flavan-3-ol content (DAC) was not affected by any of the treatments. Treatments also had little effect on the non-coloured polymer content of the wine. Only the 10 °C/4 days treatment in 2002 resulted in wines with a higher non-coloured polymer content relative to that of the control wine. Furthermore, pre-fermentation treatments also did not affect the individual flavonol content of

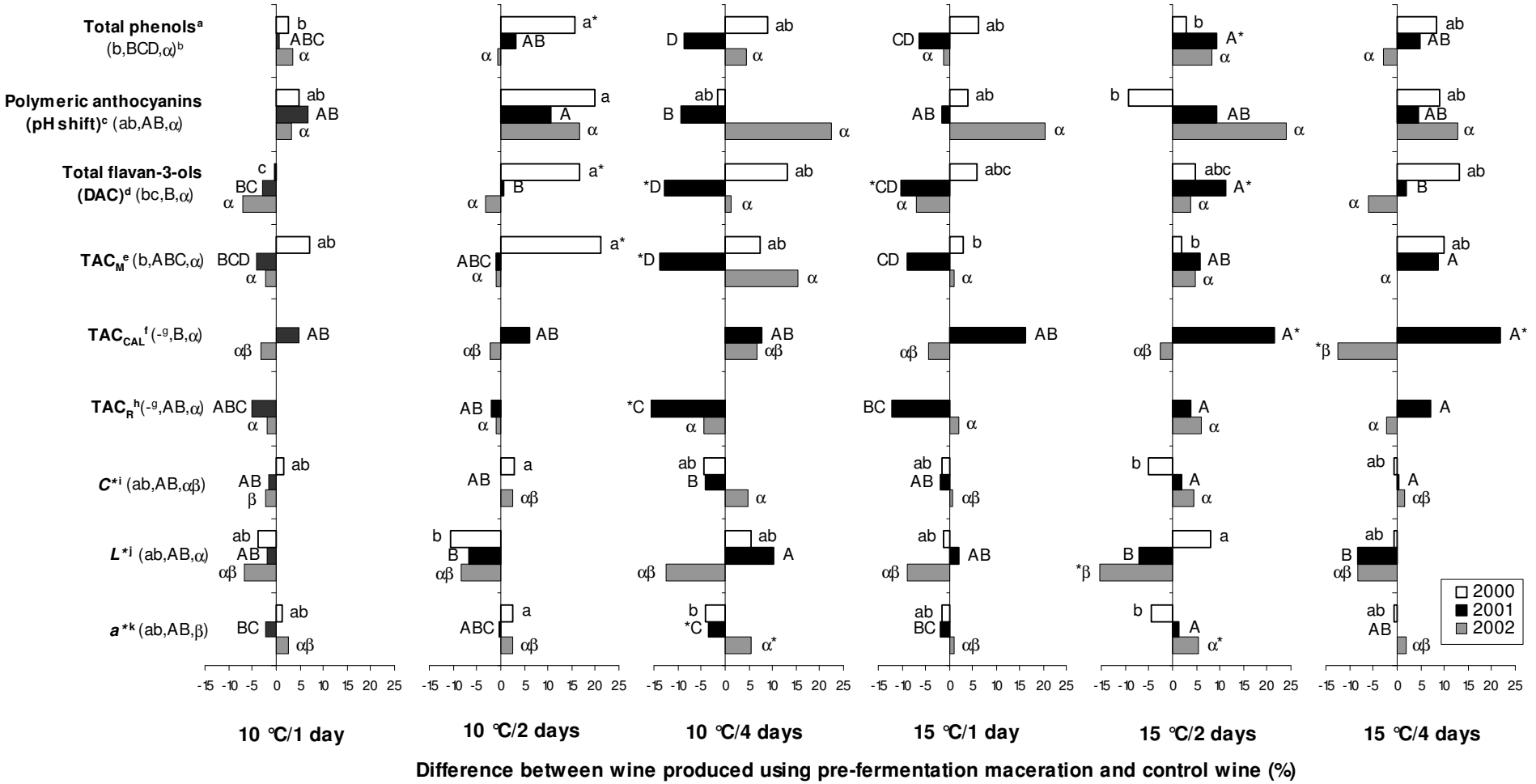

Figure 1. The percentage differences in phenolic content as measured spectrophotometrically, total antioxidant capacity and objective colour between the 2000, 2001 and 2002 Pinotage wines produced according to different pre-fermentation maceration protocols compared to the control wine [[a] measured using the Folin-Ciocalteau assay; [b] different lowercase Roman (2000), uppercase Roman (2001) and Greek (2002) alphabet letters on the bars for the 2000, 2001 and 2002 wines and the control, indicated next to the parameter name, denote significant differences ($P < 0.05$); [c] measured using the DAC assay; [d] measured using the pH shift assay; [e] total antioxidant capacity in mM Trolox equivalents; [f] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [g] no values for 2000 wines; [h] $TAC_R = TAC_M - TAC_{CAL}$; [i] chroma; [j] lightness; [k] red/green chromaticity; * indicate significant ($P < 0.05$) differences from control wines].

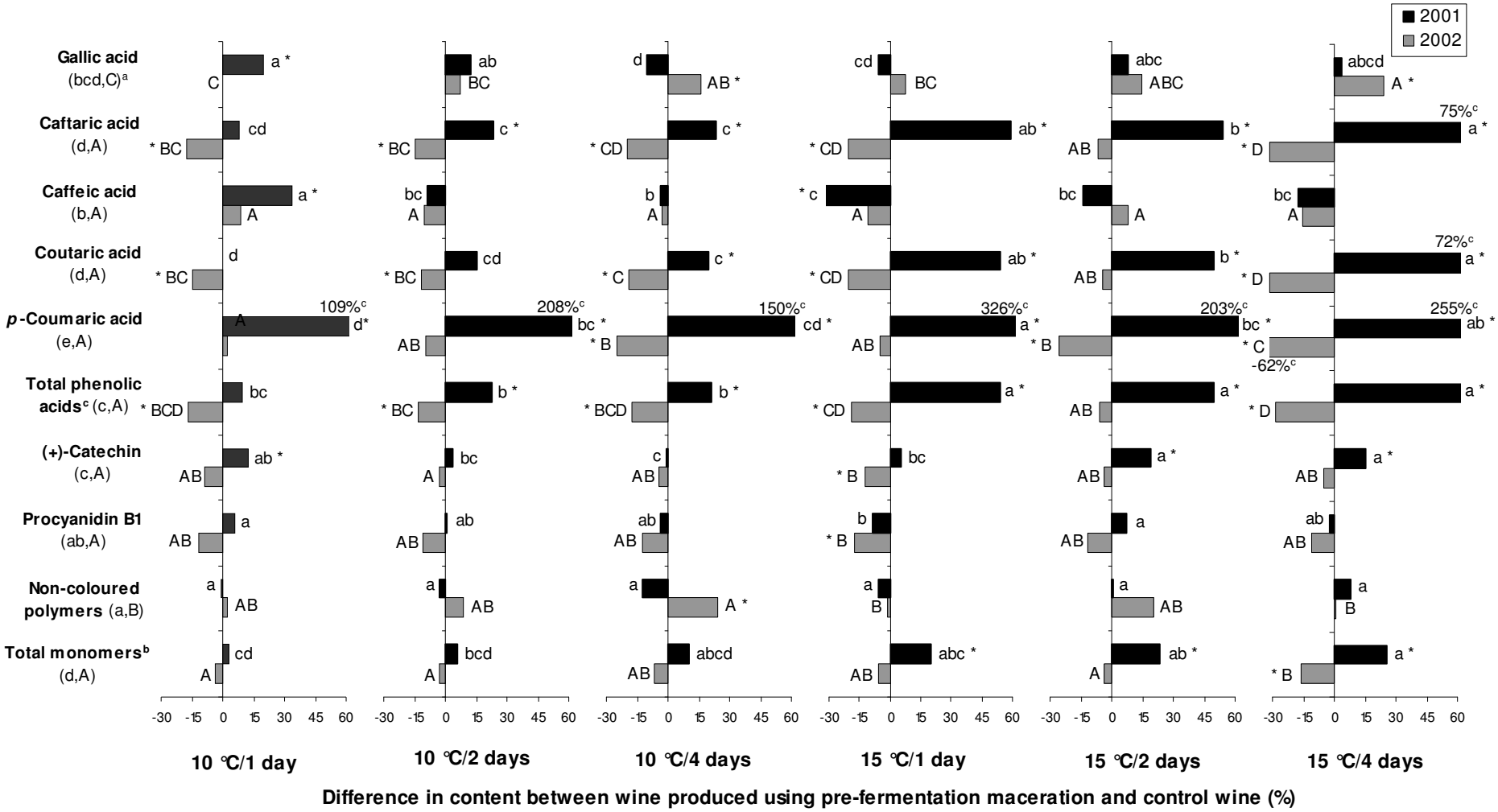

Figure 2. The percentage differences in phenolic acid, flavan-3-ol, non-coloured polymer and total monomer content between the 2001 and 2002 Pinotage wines produced according to different pre-fermentation maceration protocols and the control wine [[a] different lowercase (2001) and uppercase (2002) letters on the bars for the 2001 and 2002 wines and the control, indicated next to the parameter name, denote significant differences ($P < 0.05$); [b] sum of phenolic group content; [c] values exceed the scale; * indicate significant ($P < 0.05$) differences from control wines].

the wines (data not shown), except for the 15 °C/4 days treatment in 2001 resulting in wines with a higher unknown flavonol content than the control wines (**Figure 3**).

The monomeric and total anthocyanin contents (pH shift), as well as, peonidin-3-glc, peonidin-3-glc-ac and coloured polymer (HPLC) contents of the wines were not significantly affected by any of the treatments, irrespective of vintage (data not shown). Concerning individual anthocyanins, the delphinidin-3-glc-ac and vitisin A contents of the wine were increased by pre-fermentation maceration treatments. The 15 °C/1 day and 15 °C/2 days treatments increased the delphinidin-3-glc-ac content of the 2001 wines (**Figure 3**). The vitisin A content of the 2001 wines was increased by all the treatments, except for the 10 °C/4 days treatment, which had no effect. However, in 2002 the 10 °C/4 days treatment, as well as the 15 °C/2 days treatment, increased the vitisin A content of the wines.

In 2001, the individual phenolic acid contents were significantly increased by the pre-fermentation maceration treatments, especially when using the higher temperature. Substantial increases were obtained for caftaric, coutaric and *p*-coumaric acid contents, and thus the total phenolic acid content (**Figure 2**). However, the opposite trend was observed for the 2002 wines. The caffeic acid content of the 2001 wines was affected by the 10 °C/1 day and 15 °C/1 day treatments, giving higher and lower values than that of the control, respectively, while no significant differences were observed for the 2002 wines. Considering gallic acid, the 10 °C/1 day treatment in 2001 and the 10 °C/4 days and 15 °C/4 days treatments in 2002 resulted in wines with higher contents than the control.

The total monomer content (HPLC) of the 2001 wines treated at 15 °C was higher than that of the control wines (**Figure 2**). In 2002, the pre-fermentation maceration treatments had no effect, except for the 15 °C/4 days treatment, giving wines with a lower total monomer content (HPLC) than the control.

Pre-fermentation Maceration: Effect on Antioxidant Capacity

The effect of pre-fermentation maceration treatments on TAC_M was only significant for the 10 °C/2 days treatment in 2000 (**Figure 1**). This treatment gave wines with higher TAC_M values than the control. In 2001 and 2002, the pre-fermentation maceration treatments had no effect on the TAC_M of the wines. Taking the individual phenolic content into account, the 15 °C/2 days and 15 °C/4 days treatments resulted in wines with higher TAC_{CAL} values in 2001 than that of the control wine, while the 15 °C/4 days treatment had the opposite effect in 2002. In both 2001 and 2002, the pre-fermentation maceration treatments did not affect the TAC_R of wines compared to that of the control. The contribution of different phenolic groups to the TAC_{CAL} of the 2001 and

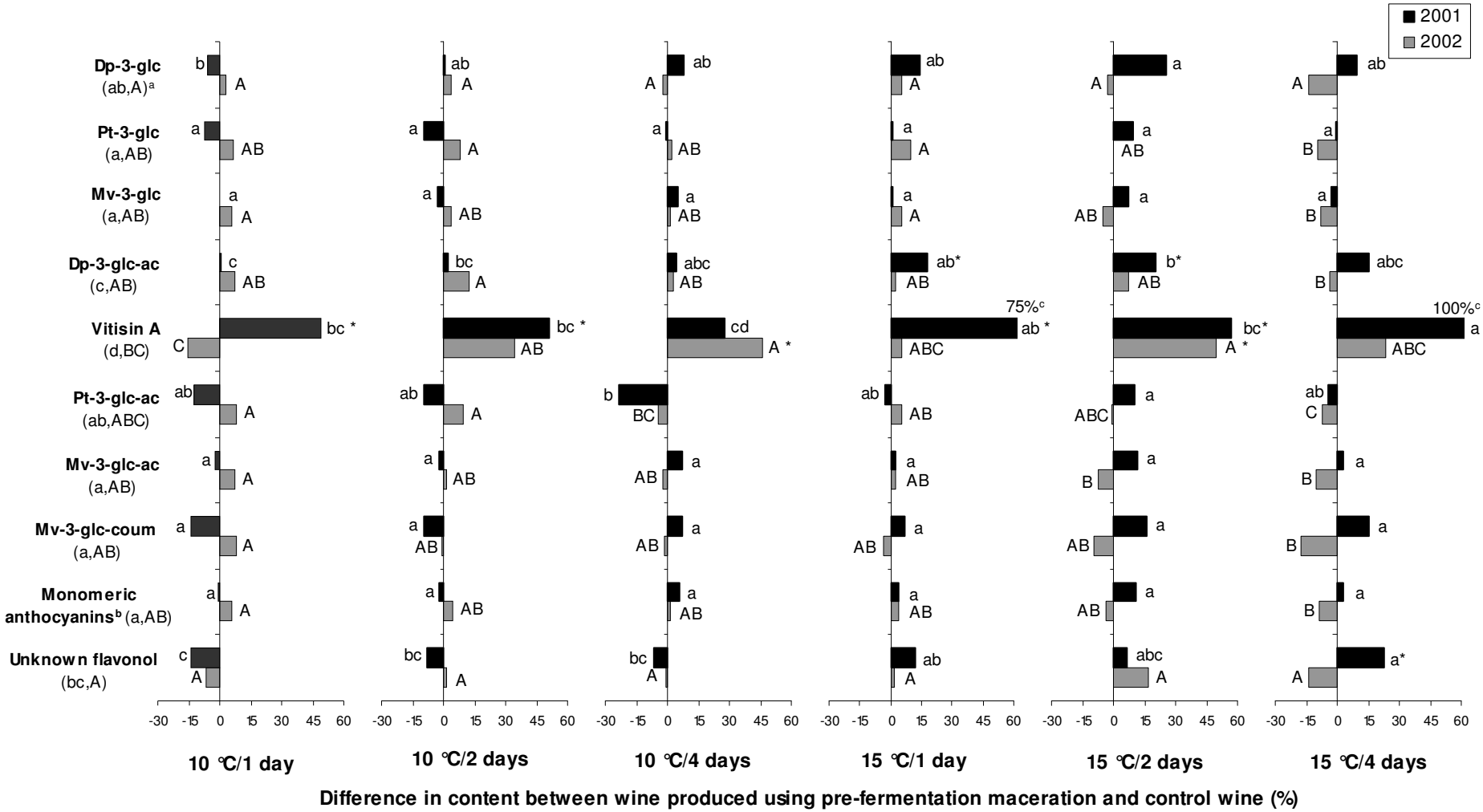

Figure 3. The percentage differences in monomeric anthocyanin and flavonol content between the 2001 and 2002 Pinotage wines produced according to different pre-fermentation maceration protocols and the control wine [[a] different lowercase (2001) and uppercase (2002) letters on the bars for the 2001 and 2002 wines and the control, indicated next to the parameter name, denote significant differences ($P < 0.05$); [b] sum of phenolic group content; [c] values exceed the scale; * indicate significant ($P < 0.05$) differences from control wines ;Dp = delphinidin; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Pt = petunidin; Pn = peonidin; Mv = malvidin].

2002 wines is depicted in **Figures 4** and **5**. Both the flavan-3-ols and flavonols had a small contribution to the TAC_{CAL}, and none of the treatments increased the contribution of these phenolic groups relative to that of the control. The 15 °C/1 day treatment decreased the contribution of flavan-3-ols to the TAC_{CAL} of the 2002 wines in comparison to the control. In 2001, several of the treatments, most notably the treatments at 15 °C, increased the contribution of total phenolic acids to the $TAC_{CAL.}$ In 2002, the 10 °C/1 day, 15 °C/1 day and 15 °C/4 days treatments decreased the contribution of phenolic acids to TAC_{CAL}, compared to that of the control wines.

Pre-fermentation Maceration: Effect on Objective Colour Parameters

Variation in objective colour parameters between vintages was large, similar to the results for phenolic composition and antioxidant capacity. None of the objective colour parameters of the 2000 wines were affected by the pre-fermentation maceration treatments compared to that of the control wine (**Figure 1**). Furthermore, the pre-fermentation maceration treatments had no effect on the h^* or b^* values of wines of any of the vintages (data not shown). The 10 °C/4 days treatment lowered the a^* value of the 2001 wines, compared to the control wines. However, in 2002, the same treatment increased the a^* value. The only other treatment that had a significant effect on the objective colour parameters, was the 15 °C/2 days treatment of 2002, which lowered L^* and increased a^*.

Juice/Skin Mixing during Maceration: Effect on Phenolic Composition

Considering the juice/skin mixing techniques used during maceration, the pumping-over action in general was less effective in extracting phenolic compounds than the punching-down and rotor actions (**Tables 3-5**). The latter treatments gave wines with similar phenolic content. These trends were most notable for the 2000 and 2002 wines. In 2000, pumping-over treatment resulted in wine with lower total phenol, polymeric anthocyanin (pH shift) and flavan-3-ol contents when the other mixing techniques were used (**Table 3**). The rotor treatment gave wines with the highest flavan-3-ol content (DAC). According to spectrophotometric data, mixing treatment had no effect on the phenolic content of the 2001 wines. In 2002, the pumping-over treatment gave wines with lower total phenol and flavan-3-ol (DAC) contents, as well as monomeric, polymeric and total anthocyanin contents (pH shift), than the other treatments.

None of the individual anthocyanin and coloured polymer (HPLC) contents of the 2001 wines were affected by mixing technique. Furthermore, no differences in delphinidin-3-glc, petunidin-3-glc, peonidin-3-glc, delphinidin-3-glc-ac and petunidin-3-glc-ac contents were observed for the 2002 wines (data not shown). The mixing techniques did, however, affect the remaining individual anthocyanin and the coloured polymer (HPLC) contents of the 2002 wines, with pumping-over giving lower values than both the punching-down and rotor treatments (**Table 4**).

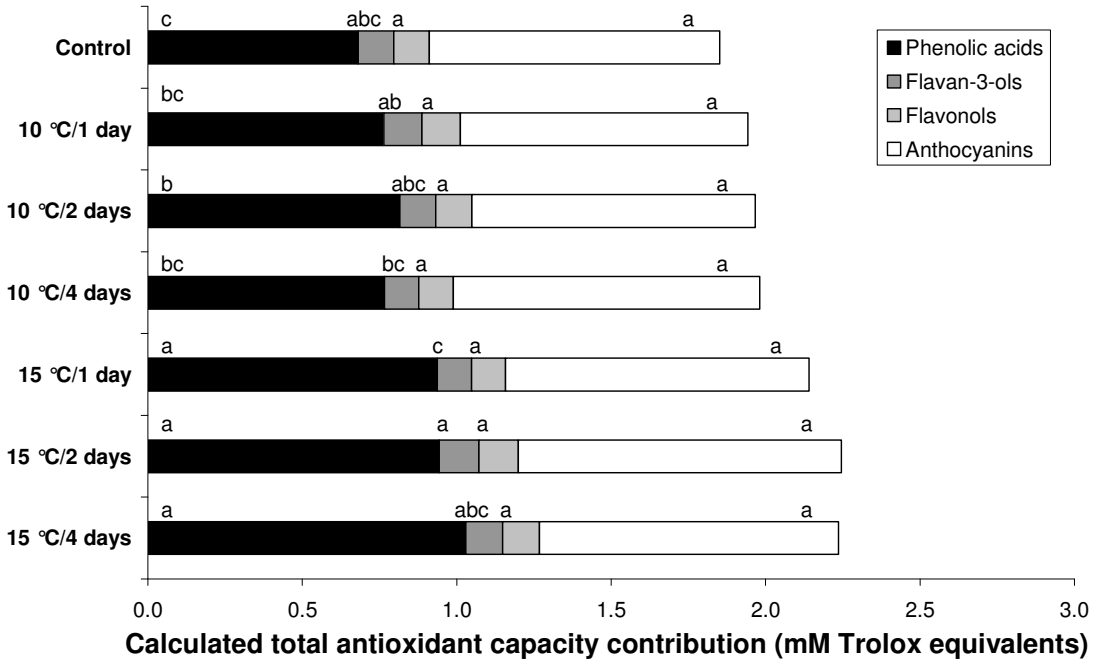

Figure 4. Calculated total antioxidant capacity contributions of phenolic groups for the 2001 Pinotage wines produced according to different pre-fermentation maceration protocols [different letters for the contribution of a specific phenolic group denote significant differences ($P < 0.05$)].

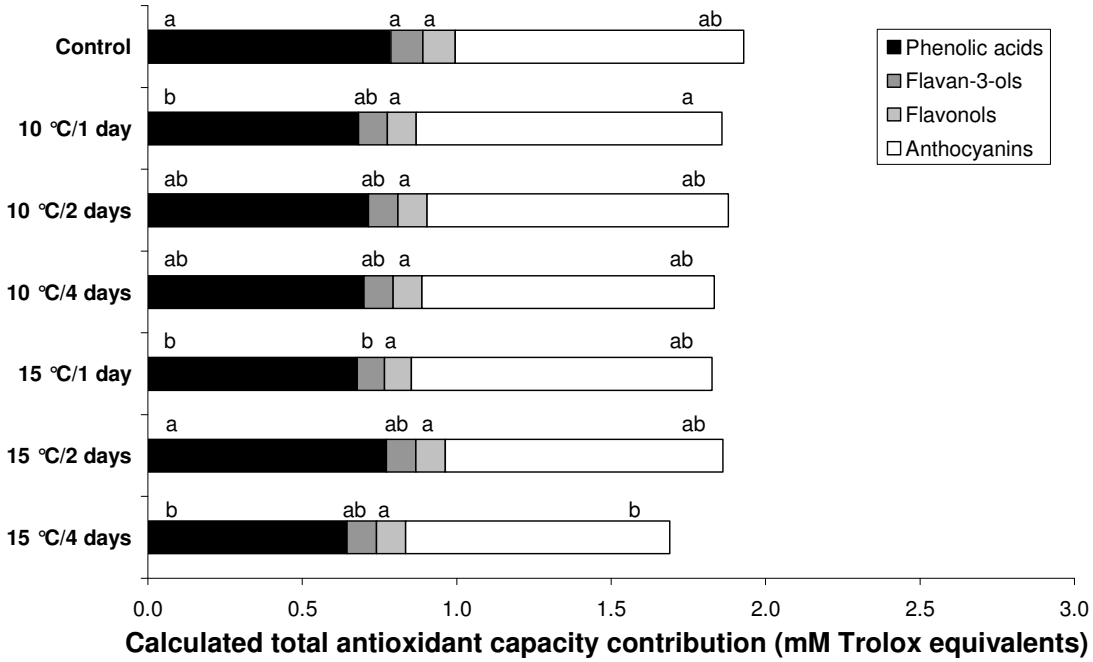

Figure 5. Calculated total antioxidant capacity contributions of phenolic groups for the 2002 Pinotage wines produced according to different pre-fermentation maceration protocols [different letters for the contribution of a specific phenolic group denote significant differences ($P < 0.05$)].

Table 3. Phenolic composition (measured spectrophotometrically) of the 2000, 2001 and 2002 Pinotage wines produced according to different juice/skin mixing protocols and mixing frequencies.

Year	Treatment/ Frequency	Total phenols[a]	Monomeric anthocyanins[b]	Polymeric anthocyanins[b]	Total anthocyanins[b]	Total flavan-3-ols[c]
2000	Punching-down	2109.7 a[d] (± 140.5)[e]	192.0 a (± 15.3)	82.0 a (± 5.6)	274.0 a (± 22.9)	159.4 b (± 12.4)
	Pumping-over	1851.4 b (± 79.7)	173.9 a (± 18.0)	62.2 b (± 7.0)	236.1 b (± 23.9)	140.6 c (± 9.9)
	Rotor	2207.8 a (± 152.2)	189.3 a (± 18.7)	73.8 a (± 7.3)	263.1 ab (± 24.9)	175.9 a (± 21.8)
2001	Punching-down	2774.1 a (± 119.2)	354.8 a (± 29.0)	100.3 a (± 11.3)	455.2 a (± 38.9)	211.5 a (± 14.7)
	Pumping-over	2681.7 a (± 210.6)	334.0 a (± 12.4)	91.8 a (± 11.7)	425.8 a (± 20.3)	210.1 a (± 21.3)
	Rotor	2640.4 a (± 74.7)	347.7 a (± 18.7)	99.9 a (± 8.1)	447.6 a (± 21.6)	199.4 a (± 5.6)
2002	Punching-down	2154.4 a (± 163.4)	383.0 a (± 26.5)	49.6 a (± 9.2)	432.6 a (± 28.6)	177.5 a (± 11.6)
	Pumping-over	1931.9 b (± 131.00)	336.3 b (± 43.1)	36.8 b (± 6.5)	373.1 b (± 49.4)	149.8 b (± 9.9)
	Rotor	2253.7 a (± 102.2)	386.6 a (± 15.4)	46.0 a (± 2.5)	432.5 a (± 15.6)	183.1 a (± 13.3)
2000	Every hour	2066.8 a (± 181.1)	186.1 a (± 19.7)	72.3 a (± 10.0)	258.4 a (± 28.7)	158.9 a (± 25.9)
	Every 3 hours	2045.8 a (± 220.5)	184.0 a (± 17.9)	73.0 a (± 12.6)	257.0 a (± 28.5)	158.4 a (± 15.9)
2001	Every hour	2698.0 a (± 195.7)	336.5 a (± 19.2)	97.1 a (± 8.4)	433.7 a (± 25.4)	203.4 a (± 12.7)
	Every 3 hours	2699.4 a (± 93.9)	354.5 a (± 21.4)	97.6 a (± 13.0)	452.0 a (± 31.8)	210.6 a (± 17.7)
2002	Every hour	2174.7 a (± 182.0)	375.7 a (± 24.9)	47.9 a (± 8.7)	423.5 a (± 30.4)	176.3 a (± 18.4)
	Every 3 hours	2051.9 a (± 181.8)	361.6 a (± 46.9)	40.4 b (± 6.5)	402.0 a (± 52.6)	164.0 b (± 17.6)

[a] mg gallic acid equivalents/L; [b] mg malvidin-3-glucoside equivalents/L; [c] mg (+)-catechin equivalents/L; [d] different letters within a group in a column denote significant differences ($P < 0.05$); [e] standard deviation

Table 4. Anthocyanin and flavonol content[a] of the 2001 and 2002 Pinotage wines produced according to different juice/skin mixing protocols and mixing frequencies.

Year	Treatment/	Monomeric anthocyanins						Coloured	Flavonols					
	Frequency	Mv-3-glc	Vitisin A[b]	Pn-3-glc-ac[b]	Mv-3-glc-ac[b]	Mv-3-glc-coum[b]	Total[c]	polymers[d]	Unknown[e]	Q-3-glc	Q-3-rham	Querce-tin	Kaemp-ferol	Total[c]
2001	Punching-down	152.07 a[f] (± 14.30)[g]	8.79 a (± 1.57)	3.16 a (± 0.34)	49.99 a (± 4.17)	20.16 a (± 1.91)	260.40 a (± 25.75)	7.19 a (± 2.41)	18.90 a (± 2.18)	16.22 a (± 2.25)	10.48 a (± 1.25)	1.99 a (± 0.29)	0.52 ab (± 0.08)	48.11 a (± 5.18)
	Pumping-over	145.95 a (± 23.57)	7.89 a (± 3.98)	2.89a (± 0.24)	46.49 a (± 6.61)	20.41 a (± 4.32)	247.68 a (± 31.65)	6.83 a (± 2.99)	16.94 a (± 2.74)	16.19 a (± 1.97)	10.22 a (± 0.91)	1.95 a (± 0.24)	0.54 a (± 0.04)	45.83 a (± 5.51)
	Rotor	138.46 a (± 16.82)	9.83 a (± 3.67)	2.84 a (± 0.27)	44.74 a (± 6.30)	17.44 a (± 2.71)	236.11 a (± 25.55)	6.34 a (± 1.41)	16.71 a (± 1.59)	14.23 a (± 2.01)	9.46 a (± 1.48)	1.57 b (± 0.29)	0.43 b (± 0.10)	42.41 a (± 5.16)
2002	Punching-down	181.10 a (± 20.24)	6.39 a (± 1.58)	3.87 a (± 0.48)	53.09 a (± 6.07)	24.93 ab (± 5.37)	299.12 a (± 34.43)	9.08 a (± 2.06)	10.14 a (± 1.02)	8.07 a (± 0.41)	9.41 a (± 0.56)	3.08 ab (± 0.84)	0.23 ab (± 0.10)	31.33 a (± 2.14)
	Pumping-over	162.53 b (± 16.27)	4.41 b (± 1.42)	3.40 b (± 0.44)	47.75 b (± 3.13)	23.13 b (± 1.87)	269.59 a (± 26.15)	7.08 b (± 0.90)	7.95 b (± 2.52)	6.54 b (± 0.93)	7.98 b (± 0.78)	2.41 b (± 0.87)	0.11 b (± 0.13)	25.14 b (± 4.35)
	Rotor	170.62 ab (± 5.73)	7.27 a (± 0.89)	3.51 ab (± 0.10)	52.78 a (± 2.30)	26.55 a (± 1.60)	291.95 a (± 9.40)	7.77 ab (± 1.71)	11.24 a (± 1.66)	7.91 a (± 0.71)	8.71 a (± 0.94)	3.65 a (± 0.58)	0.34 a (± 0.10)	32.21 a (± 3.80)
2001	Every hour	139.09 a (± 10.69)	10.42 a (± 2.15)	2.87 a (± 0.23)	45.60 a (± 4.06)	18.89 a (± 2.18)	241.20 a (± 17.63)	6.80 a (± 2.57)	17.56 a (± 2.06)	15.88 a (± 2.57)	10.14 a (± 1.47)	1.89 a (± 0.37)	0.49 a (± 0.11)	45.96 a (± 3.17)
	Every 3 hours	151.91 a (± 22.69)	7.25 b (± 3.33)	3.05 a (± 0.35)	48.55 a (± 7.23)	19.79 a (± 4.17)	254.93 a (± 34.96)	6.77 a (± 2.03)	17.47 a (± 2.68)	15.22 a (± 1.79)	9.97 a (± 1.05)	1.79 a (± 0.29)	0.80 a (± 0.06)	44.94 a (± 5.11)
2002	Every hour	173.79 a (± 9.25)	6.38 a (± 1.57)	3.63 a (± 0.25)	51.12 a (± 3.53)	24.41 a (± 3.06)	289.27 a (± 16.23)	8.53 a (± 2.07)	10.61 a (± 2.12)	7.91 a (± 0.78)	9.18 a (± 0.74)	3.07 a (± 0.89)	0.23 a (± 0.17)	31.29 a (± 4.08)
	Every 3 hours	169.05 a (± 21.76)	5.67 a (± 1.66)	3.56 a (± 0.54)	51.29 a (± 5.78)	25.33 a (± 4.04)	284.51 a (± 36.10)	7.42 a (± 1.25)	8.94 a (± 2.12)	7.10 b (± 1.02)	8.22 b (± 0.91)	3.03 a (± 0.95)	0.22 a (± 0.17)	27.82 b (± 4.75)

[a] mg/L unless otherwise noted; [b] mg corresponding anthocyanin-3-glc equivalents/L; [c] sum of phenolic group content; [d] mg malvidin-3-glc equivalents/L; [e] mg rutin equivalents/L; [f] different letters within a group in a column denote significant differences ($P < 0.05$); [g] standard deviation; Dp = delphinidin; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Pt = petunidin; Pn = peonidin; Mv = malvidin; Q = quercetin; rham = rhamnoside.

Table 5. Phenolic acid, flavan-3-ol, non-coloured polymer and total monomer contents[a] of the 2001 and 2002 Pinotage wines produced according to different juice/skin mixing protocols and mixing frequencies.

Year	Treatment/ Frequency	Phenolic acids						Flavan-3-ols			Total monomers[e]
		Gallic acid	Caftaric acid	Caffeic acid	Coutaric acid[b]	*p*-Coumaric acid	Total[c]	(+)-Catechin	Procyanidin B1	Non-coloured polymers[d]	
2001	Punching-down	13.99 a[f] (± 0.57)[g]	196.87 a (± 22.27)	1.24 a (± 0.10)	22.45 a (± 2.63)	2.27 a (± 0.58)	236.81 a (± 25.03)	10.49 a (± 0.44)	17.13 a (± 0.94)	175.18 a (± 20.75)	572.95 a (± 40.30)
	Pumping-over	13.27 a (± 1.23)	161.14 b (± 26.26)	1.31 a (± 0.12)	19.47 b (± 3.09)	1.70 a (± 0.58)	196.89 b (± 30.53)	10.48 a (± 0.55)	18.24 a (± 1.04)	179.92 a (± 46.14)	519.14 b (± 20.13)
	Rotor	13.10 a (± 1.31)	172.23 b (± 14.51)	1.21 a (± 0.16)	20.48 ab (± 2.06)	2.08 a (± 0.44)	209.09 b (± 15.59)	9.89 b (± 0.47)	16.42 a (± 1.92)	167.80 a (± 11.21)	513.91 b (± 28.94)
2002	Punching-down	13.35 a (± 1.82)	176.62 c (± 18.25)	1.94 a (± 1.12)	17.48 c (± 1.08)	1.69 b (± 0.21)	211.10 c (± 17.17)	9.90 b (± 0.49)	17.33 a (± 1.14)	195.86 a (± 25.09)	568.77 b (± 20.08)
	Pumping-over	11.76 b (± 0.82)	209.51 b (± 23.39)	1.05 b (± 0.17)	20.20 b (± 2.21)	2.15 a (± 0.43)	244.66 b (± 26.22)	9.67 b (± 0.51)	17.05 a (± 1.68)	151.61 b (± 17.36)	566.11 b (± 26.37)
	Rotor	13.77 a (± 0.89)	235.90 a (± 12.26)	1.07 b (± 0.14)	22.96 a (± 0.86)	2.29 a (± 0.38)	275.99 a (± 12.76)	10.63 a (± 0.77)	16.05 a (± 0.94)	189.22 a (± 21.92)	626.83 a (± 17.35)
2001	Every hour	12.98 a (± 0.90)	190.95 a (± 22.63)	1.26 a (± 0.14)	22.46 a (± 2.35)	2.24 a (± 0.49)	229.89 a (± 25.58)	10.56 a (± 0.36)	17.15 a (± 1.33)	178.45 a (± 34.68)	544.76 a (± 43.34)
	Every 3 hours	13.92 a (± 1.11)	162.54 b (± 20.19)	1.25 a (± 0.12)	19.14 b (± 2.17)	1.79 a (± 0.56)	198.64 b (± 23.45)	10.01 b (± 0.57)	17.38 a (± 1.74)	170.15 a (± 22.20)	525.90 a (± 36.26)
2002	Every hour	13.70 a (± 1.71)	195.34 b (± 32.59)	1.69 a (± 0.97)	19.26 b (± 2.83)	1.82 b (± 0.31)	231.82 b (± 35.10)	10.27 a (± 0.84)	17.27 a (± 1.11)	191.14 a (± 28.12)	579.92 a (± 45.06)
	Every 3 hours	12.23 b (± 0.73)	219.34 a (± 24.11)	1.02 b (± 0.14)	21.16 a (± 2.33)	2.26 a (± 0.42)	256.01 a (± 27.10)	9.87 a (± 0.51)	16.34 a (± 1.44)	166.65 b (± 24.67)	594.55 a (± 42.49)

[a] mg/L unless otherwise noted; [b] mg *p*-coumaric acid equivalents/L; [c] sum of quantified compounds in group; [d] mg (+)-catechin equivalents/L; [e] sum of all quantified monomeric phenolic compounds; [f] different letters in a column within a group indicate significant differences ($P < 0.05$); [g] standard deviation.

Similarly to the individual anthocyanins, the mixing technique did not affect the total or individual flavonol contents of the 2001 wines, except for a lower kaempferol content as a result of the rotor action (**Table 4**). Pumping-over gave wines with lower quercetin-3-glc, quercetin-3-rham and the unknown flavonol contents in 2002 than those obtained with either the punching-down or rotor treatments. It also gave wines with a lower content of the two aglycons, quercetin and kaempferol, than the rotor treatment.

The total phenolic acid content of the 2001 and 2002 wines was affected by the mixing technique, but whereas punching-down gave the highest total phenolic acid content in 2001, the lowest content was obtained in 2002 with the same technique (**Table 5**). Only the caftaric and coutaric acid contents of the 2001 wines were affected by the mixing technique, with punching-down giving better extraction of caftaric acid than both the pumping-over and rotor treatments, and its extraction of coutaric acid was better than with the pumping-over treatment. All the respective phenolic acids' contents of the 2002 wines were affected by mixing technique, although not in the same manner. Punching-down gave wines with the highest caffeic acid content in 2002, but the lowest caftaric and coutaric acid contents. The pumping-over and rotor treatments gave wines with a higher *p*-coumaric acid content than the punching-down treatment. For extraction of gallic acid, pumping-over was the least effective.

Considering individual flavan-3-ols, extraction of the dimer, procyanidin B1, was not affected by the mixing technique (**Table 5**). However, the (+)-catechin content was affected, with the rotor treatment resulting in wines with the lowest content in 2001, and the highest in 2002. The non-coloured polymer content was also affected by the mixing technique in 2002, with the pumping-over treatment giving wines with the lowest content.

Adding up all the individual monomeric phenolic compounds, quantified using HPLC, the punching-down and rotor treatments resulted in wines with higher total monomer content (HPLC) than the pumping-over treatment in 2001 and 2002, respectively (**Table 5**).

Mixing frequency had a limited effect on the phenolic composition of the 2001 wines. Their vitisin A, caftaric acid, coutaric acid, total phenolic acid and (+)-catechin contents were increased by the higher mixing frequency (**Tables 4, 5**). On the other hand, the caftaric, coutaric, *p*-coumaric and total phenolic acid contents of the 2002 wines decreased with the higher mixing frequency. The polymeric anthocyanin (pH shift), total flavan-3-ol (DAC), non-coloured polymer, quercetin-3-glc, quercetin-3-rham and total flavonol contents, as well as gallic and caffeic acid contents, of the wines were increased when hourly mixing was used during maceration (**Tables 3-5**).

Juice/Skin Mixing during Maceration: Effect on Antioxidant Capacity

The effect of the different juice/skin mixing techniques during maceration on the antioxidant capacity of Pinotage wines is summarised in **Table 6**. The pumping-over and rotor treatments yielded wines in 2000 with the lowest and highest TAC_M values, respectively.

The TAC_{CAL} (**Table 6**) of the 2001 wines, as well as the contribution of phenolic acids to the TAC_{CAL} (**Figure 6**), was higher when the punching-down treatment rather than the other mixing techniques was used. The TAC_M and TAC_R of the 2001 wines were, however, not affected. Also for this vintage, the pumping-over treatment gave wines with a higher flavan-3-ol contribution the wine TAC_{CAL} than the rotor treatment. The contributions by flavonols and anthocyanins to the TAC_{CAL} were not affected by the mixing technique.

In 2002, the TAC_M and TAC_R of the wines were lower when the pumping-over treatment instead of the other mixing techniques was used (**Table 6**). The TAC_{CAL} and the contributions of phenolic acids and flavonols to the TAC_{CAL} (**Figure 6**) were higher when the rotor treatment, instead of the pumping-over treatment was used. The contribution of phenolic acids to the TAC_{CAL} was also higher than that obtained with the punching-down treatment. The rotor and punching-down treatments, however, resulted in wines with higher flavonol contributions than the pumping-over treatment. No significant differences were observed between the mixing techniques in terms of flavan-3-ol and anthocyanin contributions. In all cases the contribution of flavan-3-ols and flavonols to the TAC_{CAL} was small.

The mixing frequency had no effect on the antioxidant capacity of the 2000 and 2001 wines (**Table 6**). The higher mixing frequency increased the TAC_M and TAC_R of the 2002 wines. Only the contribution of phenolic acids to the TAC_{CAL} was affected by mixing frequency. However, opposite effects for the 2001 and 2002 wines were obtained (**Figure 7**).

Juice/Skin Mixing during Maceration: Objective Colour Parameters

Similar to the phenolic composition and antioxidant capacity, mixing techniques did not significantly affect the objective colour parameters of the 2001 wines (**Table 6**). The pumping-over treatment resulted in wines having lower C^*, a^* and b^* values and higher L^* values in 2000 and 2002 than the other mixing techniques. Considering the 2002 wines, h^* was lower when the pumping-over treatment was used. The rotor treatment gave wines with the highest C^* and a^* values. Mixing frequencies did not significantly affect the objective colour parameters of the 2000 and 2001 wines. Hourly mixing gave wines with higher h^* and b^* values in 2001 than 3-hourly mixing.

Table 6. Antioxidant capacity and objective colour parameters of the 2000, 2001 and 2002 Pinotage wines produced according to different juice/skin mixing protocols and mixing frequencies.

Year	Treatment/	Antioxidant capacity			Objective colour parameters				
	Frequency	TAC_M[a]	TAC_{CAL}[b]	TAC_R[c]	C^*[d]	h^*[e]	L^*[f]	a^*[g]	b^*[h]
2000	Punching-down	11.83 b[i] (± 0.93)[j]	na	na	60.29 a (± 1.28)	18.04 a (± 0.69)	36.39 b (± 2.64)	57.32 a (± 1.15)	18.67 a (± 0.93)
	Pumping-over	10.58 c (± 0.54)	na	na	53.72 b (± 3.25)	16.68 a (± 1.56)	46.19 a (± 4.20)	51.44 b (± 2.99)	15.44 b (± 1.94)
	Rotor	13.22 a (± 1.34)	na	na	59.30 a (± 1.06)	16.76 a (± 1.71)	40.41 b (± 3.50)	56.76 a (± 0.85)	17.10 ab (± 1.88)
2001	Punching-down	16.00 a (± 1.04)	1.73 a (± 0.11)	14.28 a (± 0.99)	62.15 a (± 0.58)	14.31 a (± 1.35)	33.70 a (± 4.01)	60.21 a (± 0.39)	15.37 a (± 1.52)
	Pumping-over	15.51 a (± 0.59)	1.58 b (± 0.05)	13.93 a (± 0.55)	62.28 a (± 1.88)	14.12 a (± 2.19)	36.85 a (± 5.30)	60.35 a (± 1.39)	15.23 a (± 2.68)
	Rotor	15.55 a (± 0.56)	1.56 b (± 0.09)	14.00 a (± 0.52)	63.03 a (± 1.28)	15.00 a (± 1.70)	34.82 a (± 3.50)	60.85 a (± 0.80)	16.33 a (± 2.11)
2002	Punching-down	17.20 a (± 1.22)	1.76 b (± 0.13)	15.44 a (± 1.21)	60.19 ab (± 2.13)	12.69 a (± 2.27)	40.48 b (± 4.08)	58.67 ab (± 1.66)	13.27 a (± 2.73)
	Pumping-over	15.37 b (± 1.06)	1.73 b (± 0.08)	13.65 b (± 1.01)	57.11 b (± 4.64)	10.77 b (± 1.21)	46.05 a (± 5.70)	56.08 b (± 4.36)	10.74 b (± 2.00)
	Rotor	17.99 a (± 0.60)	1.91 a (± 0.05)	16.07 a (± 0.56)	62.09 a (± 1.19)	12.63 a (± 0.96)	39.03 b (± 1.68)	60.58 a (± 1.04)	13.59 a (± 1.18)
2000	Every hour	11.66 a (± 1.82)	na	na	57.84 a (± 2.99)	17.40 a (± 1.35)	40.05 a (± 4.70)	55.18 a (± 2.86)	17.29 a (± 1.62)
	Every 3 hours	12.09 a (± 1.04)	na	na	57.70 a (± 4.27)	16.91 a (± 1.61)	41.95 a (± 5.95)	55.17 a (± 3.81)	16.85 a (± 2.51)
2001	Every hour	15.80 a (± 0.87)	1.64 a (± 0.12)	14.17 a (± 0.81)	62.85 a (± 1.23)	14.23 a (± 2.02)	35.00 a (± 2.91)	60.77 a (± 0.89)	15.99 a (± 1.77)
	Every 3 hours	15.57 a (± 0.64)	1.60 a (± 0.11)	13.97 a (± 0.58)	62.12 a (± 1.40)	14.72 a (± 1.42)	35.25 a (± 5.53)	60.17 a (± 0.94)	15.29 a (± 2.42)
2002	Every hour	17.42 a (± 1.32)	1.79 a (± 0.12)	15.64 a (± 1.26)	60.95 a (± 2.54)	12.98 a (± 1.81)	40.05 a (± 4.32)	59.36 a (± 2.20)	13.73 a (± 2.29)
	Every 3 hours	16.28 b (± 1.43)	1.81 a (± 0.13)	14.47 b (± 1.33)	58.64 a (± 4.15)	11.08 b (± 1.09)	43.66 a (± 5.20)	57.53 a (± 3.90)	11.33 b (± 1.78)

[a] total antioxidant capacity in mM Trolox equivalents; [b] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [c] $TAC_R = TAC_M - TAC_{CAL}$; [d] chroma; [e] hue angle (°); [f] lightness; [g] red/green chromaticity; [h] yellow/blue chromaticity; [i] different letters within a group in a column denote significant differences ($P < 0.05$); [j] standard deviation; na = not available.

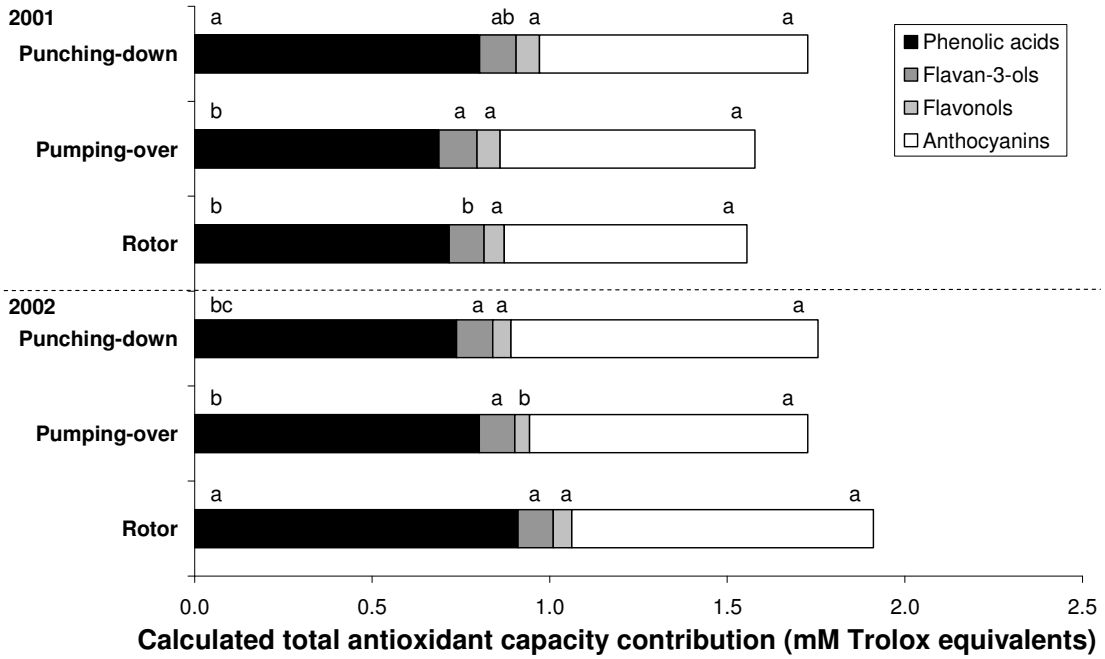

Figure 6. Calculated total antioxidant capacity contributions of phenolic groups for the 2001 and 2002 Pinotage wines produced according to different juice/skin mixing protocols [different letters for the contribution of a specific phenolic group in the same year denote significant differences ($P < 0.05$)].

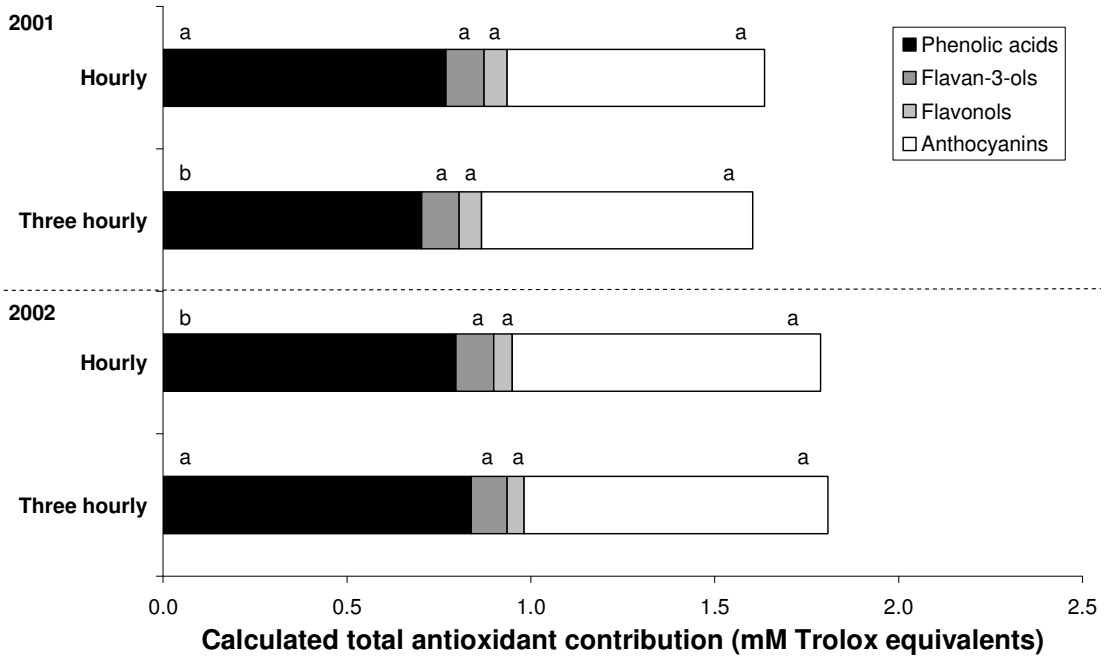

Figure 7. Calculated total antioxidant capacity contributions of phenolic groups for the 2001 and 2002 Pinotage wines produced with different juice/skin mixing treatment frequencies [different letters for the contribution of a specific phenolic group in the same year denote significant differences ($P < 0.05$)].

Discussion

Vintage-related Variation

Vintage-related variation, in terms of phenolic composition, total antioxidant capacity and colour, as were also observed in **Chapter 4**, is presumably due to variation in weather patterns during the respective vintages. Important factors include average minimum and maximum temperatures, rainfall and sunlight radiation. Timing of specific weather events (rainfall, heavy winds and temperature extremes), especially during the ripening season, will also have an effect on grape composition. Furthermore, the trends between the vintages were not consistent for the two trials, indicating that other factors also contributed to the vintage-related variation. The grapes for both trials were harvested on the same day from the same vineyard block and wine-making commenced simultaneously. Due to this, the effect of vintage will be discussed separately for the pre-fermentation maceration and juice/skin mixing trials.

In terms of antioxidant capacity, the difference in phenolic composition between the 2001 and 2002 wines of the pre-fermentation maceration trial did not manifest in significant differences in TAC_M. However, the increased TAC_{CAL}, which is due to increased monomeric anthocyanin content, and increased TAC_R explained the higher TAC_M for the 2002 wines, compared to the 2001 wines of the juice/skin mixing trial. The low TAC_M of the 2000 wines is presumably due to weather conditions influencing the grape composition of this vintage.

The wines from the 2002 vintage for the pre-fermentation maceration trial had substantially higher chromaticity values than wines of the other vintages. The higher a^* and b^* values were not supported by compositional data, since the individual monomeric anthocyanin (mainly red pigments) and flavonol (mainly yellow pigments) contents of the 2002 wines were either not significantly different from or substantially lower than that of the 2001 wines. Not only is pigment concentration important, but other factors such as pH, sulphur dioxide content and co-pigmentation effects can also influence chromaticity (Gonnet, 1999). Sulphur dioxide is, however, not expected to play an important role in anthocyanin bleaching, in this case, as all juice/skin mixing trial wines were prepared using the same sulphur dioxide content and stored under the same conditions. As the average pH of the berries also did not differ between vintages (Johann Marais, ARC Infruitec-Nietvoorbij, 2005), pigment content and co-pigmentation effects are considered to be of importance in this case.

Flavonol glycosides are among the most efficient co-pigments (Asen et al., 1972; Chen & Hrazdina, 1981). Co-pigmentation has a very prominent effect on colour at pH 3.5 (Gonnet, 1999), and would therefore play a large role in wine colour. Generally, lower L^* and higher C^* values accompanied by bathochromic shifts (blueing) occur with increasing co-pigment concentration

(Gonnet, 1999). However, the *C** values did not follow the expected pattern, even when taking other less efficient co-pigments, such as flavan-3-ols and phenolic acids, into account. Inversion (Eagerman et al., 1973) as a function of apparatus and measuring conditions, as discussed in **Chapter 4**, could also help explain this atypical behaviour. Inversion occurs especially in dark coloured beverages (Eagerman et al., 1973), which is the case for young red wines such as the ones investigated in the present study. Based on hue angle values, the 2000 and 2001 wines had magenta-red hues, while the major shift in hue of the 2002 wines indicated yellowing towards an orange-red hue.

Concerning wines from the juice/skin mixing trial, the 2001 wines displayed the darkest (lower *L**), most saturated (higher *C**) colour with higher *a** values, attributed to their high polymeric (pH shift) and flavonol (co-pigment) contents. The 2001 wines therefore had the best colour as high values for colour saturation are associated with high red wine quality. The 2000 wines, on the other hand, had the least saturated (lower *C**) colour due to substantially lower *a** values and moderately higher *b** values. The low monomeric (pH shift) and total flavan-3-ol (DAC) (co-pigments) contents of the 2000 wines contributed to their low *C** and *a** values. The hues of the 2000, 2001 and 2002 wines were all in the magenta-red range, but changed progressively from closer to pure red towards closer to red-magenta hues. These differences in hue are difficult to explain as the 2000 and 2002 wines both had lower total flavan-3-ol contents (co-pigments) than the 2001 wines, while the 2002 wines had lower flavonol contents (co-pigments) than the 2001 wines with no data available on the individual phenolic content of the 2000 wines. Inversion is most probably a confounding factor in this case as indicated earlier.

Pre-fermentation Maceration

The results showed that pre-fermentation maceration affected the phenolic composition of the wine showing significantly varying trends from vintage to vintage due to differences in initial grape composition, especially with regard to phenolic acid content. Pre-fermentation maceration was expected to possibly increase the anthocyanin content (Heatherbell et al., 1997; Watson et al., 1997; Reynolds et al., 2001; Gómez-Míguez et al., 2006), without affecting the flavan-3-ol content as pre-fermentation maceration occurs in the absence of ethanol (González-Manzano et al., 2004), in contrast to results given by Salinas et al. (2005). In the present study, however, only in some cases, pre-fermentation maceration resulted in higher delphinidin-3-glc and vitisin A contents with most wines having a similar anthocyanin content to that of the control wine. Only one treatment in 2000, and another in 2001, caused an increase in the total flavan-3-ol content (DAC) of the wine, while two treatments caused decreased concentrations in the 2002 wines. Similar trends were observed for (+)-catechin and procyanidin B1 content in the 2001 and 2002 wines.

No trends were observed for increased pre-fermentation duration at the same temperature, indicating the complexity of factors influencing the final phenolic content of the wines. Increased duration of pre-fermentation maceration may increase the concentrations of some compounds, but complex reactions between phenolic compounds taking place during, as well as after fermentation, also affect the phenolic composition as measured in the final wine (8 months after fermentation), contributing to conflicting results obtained in some cases. For this reason, this discussion will concentrate on individual treatments, explaining their effects on the antioxidant capacity and colour in terms of phenolic composition.

In spite of differences in phenolic composition, the overall effect on TAC_M was minor. The higher TAC_M of the wine produced according to the 10 °C/2 days protocol in 2000 compared to the control wine, is attributed to its higher total phenol and total flavan-3-ol (DAC) contents. The lower TAC_M of the wine produced according to the 10 °C/4 days protocol in 2001, compared to the control wine, is mostly due to lower contributions of unknown flavan-3-ols, as indicated by a lower total flavan-3-ol content (DAC), despite no differences in (+)-catechin and procyanidin B1 contents, and other unknown compounds, indicated by the TAC_R. The phenolic acids and anthocyanins contributed most to the TAC_{CAL} in both the 2001 and 2002 vintages. Higher contributions of total phenolic acids and the high antioxidant potency compounds, (+)-catechin and procyanidin B1 (**Chapter 3**), mostly explain the higher TAC_{CAL} of the 2001 wines produced according to the 15 °C/2 days and 15 °C/4 days protocols. The 15 °C/4 days treatment resulted in wines with a substantially decreased caftaric acid content and a lower content of most individual anthocyanins in 2002, except peonidin-3-glc, vitisin A and peonidin-3-glc-ac, which contributed to the decreased TAC_{CAL} of these wines. These changes in TAC_{CAL} of wines as a result of the pre-fermentation maceration treatments were relatively small, subsequently not affecting the TAC_M in comparison to the control wine.

The pre-fermentation maceration treatments did not result in any wine hue (h^*) changes compared to the control wine, although previously changes in hue towards purple-red were reported for Pinot Noir wines produced according to a pre-fermentation maceration protocol carried out at 4 or 10 °C (Heatherbell et al., 1994; Watson et al., 1994). The wine hues were in the magenta-red range for the 2000 and 2001 wines, while the 2002 wines had orange-red hues. None of the treatments improved or impaired the colour saturation of wines compared to the control wines. Small differences in a^* obtained for the 2001 and 2002 wines, produced according to the 10 °C/4 days protocol, did not affect the colour saturation or lightness in comparison to the control wines. The relative increase in a^* which in turn resulted in lower L^* for the 2002 wines produced according to the 15 °C/2 days protocol, is attributed to the increase in vitisin A content of the wines. A pre-fermentation treatment lasting 7 days at 15 °C resulted in Syrah wines with lower L^* values

than a wine made without pre-fermentation maceration (Gómez-Míguez et al., 2006). This increase in vitisin A content did not, however, influenced the wine hue, despite the fact that vitisin A has an orange-red hue (Bakker & Timberlake, 1997). In these cases, as was seen for the vintage-related variation in chromaticity, the anthocyanin and co-pigment content could not explain the variation in colour.

The pre-fermentation maceration treatments at 10 °C and the 15 °C/1 day treatment have been shown to increase the overall wine quality (Marais, 2003a). All treatments at 10 °C also increased the berry/plum intensity of wines, although no increase in acetate ester concentrations were observed. Some pre-fermentation maceration treatments at 15 °C decreased the acetate ester concentration of the wines without detrimental effects to the overall wine quality and berry/plum intensity.

Juice/Skin Mixing during Maceration

Campbell (1991) found that a commercial rotary fermenter made wine with a higher total phenol and total anthocyanin content compared to either punching-down or pumping-over treatments. In the present study, however, the rotor and punching-down treatments mostly resulted in wines with a similar phenolic composition. The pumping-over treatment is a less aggressive mixing action and was therefore the least effective in extracting phenolic compounds from the pomace as also found by Marais (2003b).

A factor influencing the phenolic composition of the wines is the greater degree of oxygen contact for the wines produced according to the pumping-over protocol compared to the punching-down and rotor protocols. The pumping-over treatment will also introduce more oxygen into the fermenting must than the punching-down treatment. Addition of oxygen can be beneficial to the yeast growth and metabolism (Sablayrolles & Barre, 1986), but could also cause oxidation and subsequent polymerisation of phenolic compounds which could precipitate (Ribéreau-Gayon, 1986). This phenomenon probably contributed to the reduced concentrations of some individual anthocyanins and flavan-3-ols, as well as the monomeric and polymeric anthocyanin contents (pH shift), observed for pumping-over wines compared to the other wines in 2000 and 2002. The same trend was not observed for the 2001 wines.

Increased mixing frequency is expected to increase the extraction of phenolic compounds due to better contact between the fermenting grape must and the pomace. Overall only small differences between the mixing frequencies were observed as was also noted by Marais (2003b). Only the extraction of flavonols was consistently favoured by the higher treatment frequency, while increased extraction of some flavan-3-ols was obtained, depending on the vintage. The phenolic acid content was affected differently for the 2001 and 2002 vintages with more frequent mixing,

either increasing extraction of tartaric acid esters of hydroxycinnamic acids (caftaric and coutaric acid), or leading to a breakdown of these derivatives to caffeic and *p*-coumaric acid, respectively. The extraction of monomeric anthocyanins was not affected by mixing frequency.

The pumping-over treatment was the least effective in obtaining a wine with a high antioxidant capacity. This could be expected from their lower phenolic content, especially in terms of anthocyanin, flavan-3-ol and flavonol content. Additionally, the low polymeric anthocyanin (pH shift), coloured and non-coloured polymer (HPLC) contents of these wines also contributed to their low TAC_M, as indicated by their low TAC_R. The high TAC_M of the 2000 wines, produced according to the rotor protocol, could be explained by their high total flavan-3-ol content (DAC). Phenolic acids and anthocyanins are the major contributors to the TAC_{CAL} of wines, irrespective of the mixing treatment, with flavan-3-ols and flavonols playing a minor role. However, the contribution of flavan-3-ols was underestimated, as only (+)-catechin and procyanidin B1 are taken into account.

Mixing frequency did not affect the TAC_M of the 2000 and 2001 wines, which is explained by no significant differences in total phenol content. Mixing frequency also did not affect the TAC_{CAL} of the 2001 and 2002 wines, despite the antioxidant capacity contribution of phenolic acids being higher and lower for the wines made with mixing every hour in 2001 and 2002, respectively. The differences in phenolic acid contribution to the TAC_{CAL} is attributed to differences in caftaric and coutaric acid content. Caftaric acid has a relatively low antioxidant potency, while coutaric acid did not show any antioxidant effect (**Chapter 3**). The mixing frequency also did not affect the TAC_R of the 2001 wines. The higher TAC_M of the 2002 wines, made with hourly mixing was due to higher non-coloured polymer and unknown compound content as no significant difference in TAC_{CAL} was observed.

The pumping-over treatment not only produced wines with lower phenolic content and antioxidant capacity; their colour was also affected. Their colour was lighter (higher L^*) with less saturation (lower C^*), largely due to lower a^* and lower b^* values, although this was only true for the 2000 and 2002 wines. This is mainly attributed to their lower polymeric and total anthocyanin contents (pH shift). In terms of individual anthocyanins, the malvidin-3-glc, vitisin A, peonidin-3-glc, malvidin-3-glc-ac and malvidin-3-glc-coum contents were lower in the 2002 wines. Decreased co-pigmentation due to their lower flavan-3-ol and flavonol contents, would also contribute to their lower C^* values. The hue of the 2002 wines, produced according to the pumping-over protocol, was red-magenta (lower h^*), while the hue obtained when using the punching-down and rotor treatments were shifted to magenta-red.

By increasing the mixing frequency of the juice and pomace during maceration, the hue of the final wine was less “blue”, than when 3-hourly mixing was used. No change in colour saturation was observed. A higher degree of co-pigmentation, due to a higher co-pigment content as

represented by flavan-3-ol (DAC) and the individual flavonols, quercetin-3-glc and quercetin-3-rham contents, together with a monomeric anthocyanin content (HPLC) that was not affected by mixing frequency, caused this yellowing shift in hue.

Previously, Marais (2003b), analysing the same wines as in the present study, reported that the rotor action resulted in wines with higher concentrations of acetate esters than a punching-down or pumping-over action. This caused lower overall sensory quality and berry/plum intensity. Differences in mixing frequency did not significantly affect the acetate ester content, overall quality or berry/plum intensity of the wines.

Conclusions

Pre-fermentation maceration shows potential to improve the colour of Pinotage wines, but does not affect the TAC or phenolic content in a consistent manner, with most treatments resulting in no difference in TAC compared to the control wines. This technique, could, however, be used to increase the quality of Pinotage wines (shown previously), with no detrimental effects to the TAC. On the other hand, both punching-down and rotor juice/skin techniques resulted in wines with higher TAC and phenolic content than the pumping-over treatment in two out of the three vintages investigated, although trends for phenolic composition were different between vintages. The pumping-over wines also exhibited less favourable objective colour parameters. However, all the mixing techniques were suitable for producing high quality wines when taking all the objective quality parameters into account, while high sensory quality was demonstrated previously. The frequency of mixing increased the measured TAC in only one vintage, while most phenolic contents were not affected. The pumping-over treatment during maceration should therefore be avoided when production of high TAC wines is the aim, while increased mixing frequency may increase phenolic extraction depending on the grape berry composition.

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Chapter 6: Use of Oak and Oxygenation during Maturation - Effect on Pinotage Wine Phenolic Composition, Total Antioxidant Capacity and Colour

Abstract

The effects of oak contact and oxygenation during maturation on the phenolic composition, total antioxidant capacity (TAC) and colour of Pinotage wines were investigated. Sensory analysis of oxygenated wines was also conducted. Oak maturation included traditional treatments, such as new barrels, second-fill barrels and third fill barrels, as well as alternative treatments (oak chips, staves, extract and dust) applied in old barrels over a period of 28 weeks. Oxygenation was carried out in discrete monthly doses at two oxygen concentrations (2.5 or 5.0 mg O_2/L/month) for each of 0, 2, 4 and 6 months. Oak maturation using traditional and alternative treatments, as well as oxygenation at the lower dosage for 2 months, had beneficial effects on Pinotage wine colour and sensory quality. The higher oxygen dosage and longer times had a substantial detrimental effect on the sensory quality of the Pinotage wine. Losses in TAC induced by decreased concentrations of monomeric phenolic compounds (most anthocyanins, flavan-3-ols, flavonols and hydroxycinnamic acids) during oak maturation were negated by increased concentrations of gallic acid extracted from the oak and new oligomeric and polymeric pigments formed. During oxygenation, however, there was a loss of wine TAC observed for all oxygenation levels and periods, despite increased concentrations of gallic acid. Oxygen addition at shorter intervals or delivery on a continuous basis may be less detrimental to the wine TAC and provide improved sensory quality. Oak maturation can be used for the production of Pinotage wine when retention of TAC is a high priority.

Introduction

Maturation is an important phase in the production of quality red wines. Matured red wines are characterised by increased stability (especially regarding colour) and improved taste and quality (Somers & Pocock, 1990; Castellari et al., 2001). Maturation generally occurs in oak barrels, but recently the use of alternative oak sources and oxygenation has increased the options available to the winemaker.

The main compounds extracted from oak during maturation are cinnamic and benzoic acid derivatives from the oak wood hydrolysable tannins, as well as furaldehydes from sugar degradation during the oak toasting process (Laszlavik et al., 1995; Kadim & Mannheim, 1999; Del Alamo Sanza et al., 2004). Phenolic compounds extracted from oak wood are not expected to influence the taste of wines as the amounts extracted are below the taste threshold (Pocock et al., 1994). Other

important changes in phenolic composition during maturation in oak barrels involve condensation reactions of anthocyanins with flavan-3-ols to form oligomeric and polymeric phenolic compounds leading to stabilised colour (Timberlake & Bridle, 1976; Singleton, 1987). Oxidation of ellagitannins from oak wood produces peroxides, which in turn oxidise ethanol to acetaldehyde (Vivas & Glories, 1996). Therefore, acetaldehyde-mediated condensation reactions involving anthocyanins and flavan-3-ols are especially important. Baldi et al. (1997) showed decreased the total radical-trapping antioxidant parameter (TRAP) and platelet inhibitory activity for a wine matured in oak barrels for 6 months, compared to the same wine stored in bottles. The evolution of wine redox potential during maturation using oak barrels, as well as oak chips and staves in stainless steel tanks, was reported by Del Álamo et al. (2006). An initial increase in redox potential was observed from 0 to 3 months of maturation with a subsequent decrease up to the end of maturation (11 months total maturation time). This trend is also expected for the antioxidant capacity as the redox potential of wine is related to its antioxidant capacity.

Alternative oak treatments used by winemakers in recent years include chips, staves and extracts (Rieger, 1993; Maga, 1989; Spillman, 1999; Rogers, 2002). By introducing large quantities of oak chips or staves for a short time, the oak maturation process is thought to be accelerated (Rieger, 1993; Rogers, 2002). Alternative oak treatments can, however, also be used to simulate normal barrel maturation by introducing it into used barrels at lower dosages. A study on South African Cabernet Sauvignon wines showed good results in terms of colour and sensory quality for alternative oak products (Van Rensburg & Joubert, 2002). It was found that staves gave the best sensory results when applied in old barrels, while oak extracts performed better when added to wine in stainless steel tanks.

The main aim of oxygenation of red wine during the maturation phase is to accelerate colour stabilisation. Castellari et al. (2004) showed that micro-oxygenation at 5 mL/L/month resulted in a dissolved oxygen content similar to that obtained by oak barrel maturation. In the presence of oxygen, ethanol is oxidised to acetaldehyde (Wildenradt & Singleton, 1974), which contributes to the formation of ethyl-linked anthocyanin-flavan-3-ol condensation products (Atanasova et al., 2002). Ingress of small amounts of oxygen also contributes to this phenomenon during maturation in oak barrels (Singleton, 1995; Castellari et al., 2004).

Maturation in some form or another is nearly always part of the process when high quality red wines are produced. Since both oak maturation and oxygenation affect the wine phenolic composition, especially with regard to polymerisation, it is possible that its total antioxidant capacity (TAC) will also be affected. A change in TAC during maturation is most likely to be negative. Some reactions of phenolic compounds during pre-bottling maturation are expected to be similar to those that occur during bottle maturation, which has been shown to decrease the TAC of

Pinotage and Cabernet Sauvignon wines (De Beer et al., 2005). To date no reports have been published on the effects of oxygenation on the antioxidant capacity of red wines. In order to produce wines with optimal TAC, the effect of oak maturation and oxygenation on their TAC should be taken into account. The aim of this study was to investigate the effect of traditional and alternative oak products, as well as oxygenation, during maturation on the phenolic composition, sensory quality and TAC of Pinotage wines.

Materials and Methods

Oak Maturation Treatments

A Pinotage wine was produced from grapes harvested at ~24 °B in February 2002 at Nietvoorbij (Stellenbosch, South Africa). Wine-making was carried out according to standard commercial wine-making procedures in a closed stainless steel fermenter at Distell (Stellenbosch, South Africa). After malolactic fermentation, bulk filtration was performed and free SO_2 adjusted to 35 mg/L before transference to oak barrels for maturation. Free SO_2 concentrations were maintained at 35 mg/L during the oak maturation period.

Treatments consisted of new barrels, second fill barrels, third fill barrels, as well as, old barrels (fifth fill) with oak chips (3 – 10 mm shavings at 6 g/L), oak staves (30 x 5 x 100 mm at 6 g/L), oak extract (freeze-dried French oak extract at 110 mg/L; Radoux Cooperage, South Africa) and oak dust (granular American oak dust at 6 g/L; African Cork Supplies, South Africa). All new and old barrels (225 L), oak chips and oak staves were from Radoux Cooperage (Stellenbosch, South Africa) and were produced from French oak. Wine was matured in triplicate for each treatment, except the new barrel treatment (duplicate) from May 2002 for 28 weeks. A sample (~200 mL) was taken at 0, 6, 15 and 28 weeks from each barrel. The original wine stored in stainless steel tanks was used to fill up barrels once a month to compensate for the volume of wine removed during sampling. Directly after sampling, aliquots of each sample were frozen at -20 °C, to prevent further phenolic changes, until analyses could be carried out. Samples were analysed immediately after defrosting.

Oxygenation Treatments

A Pinotage wine was produced from grapes harvested at ~24 °B at Nietvoorbij (Stellenbosch, South Africa) during March 2003. Wine-making was carried out according to the standard wine-making protocol with no wood contact, as described in **Chapter 3**, at the experimental cellar of ARC Infruitec-Nietvoorbij (Stellenbosch, South Africa). After filtering, instead of bottling, the wine was divided into 30 closed stainless steel containers (20 L), with three containers for each of the treatments (**Figure 1**). The oxygenation treatments consisted of a control (wine before

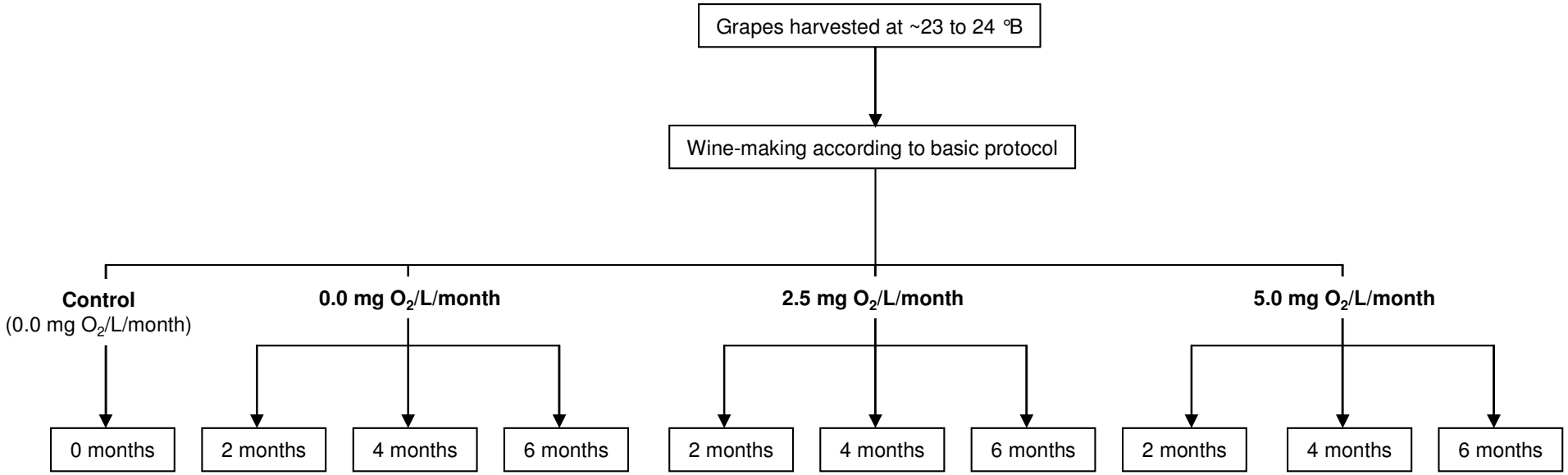

Figure 1. Scheme of oxygenation treatments of Pinotage wine.

oxygenation), no oxygenation (0 mg O_2/L/month), low level oxygenation (2.5 mg O_2/L/month) and high level oxygenation (5.0 mg O_2/L/month) for 2, 4 and 6 months. During the oxygenation treatments, wines were stored at 15 ºC. The control wines were bottled when the oxygenation treatments commenced. Oxygenation, carried out at monthly intervals, consisted of introducing compressed medical air (Afrox, Johannesburg, South Africa) into the wine using a gas diffuser until wine oxygen concentrations reached the desired concentration. The oxygen concentration was measured using an Oxi 330 Set oxygen analyser with a CellOx 325 probe (WTW, Weilheim, Germany). One week after each oxygenation treatment the SO_2 concentrations were adjusted to 25 mg/L free SO_2. Two weeks after completion of each oxygenation treatment, the wines were bottled. When all the treatments were completed, aliquots from each treatment and time combination were frozen at -20 °C, to prevent further phenolic changes, until analyses could be carried out. Samples were analysed immediately after defrosting.

Chemicals and Phenolic Reference Standards

Chemicals and phenolic reference standards used for phenolic and antioxidant analyses were described in **Chapters 3** and **4**.

Spectrophotometric Analysis of Phenolic Content

Wines were subjected to spectrophotometric analysis of the major phenolic groups. Total phenol, total flavan-3-ol, as well as, monomeric, polymeric and total anthocyanin contents were determined in triplicate using the Folin-Ciocalteau (Singleton & Rossi, 1965), dimethylamino-cinnamaldehyde (DAC) (McMurrough & McDowell, 1978) and pH shift (Ribéreau-Gayon & Stonestreet, 1965) assays, respectively, as modified and described in **Chapter 4**.

HPLC Analysis of Phenolic Composition

Individual phenolic compounds, as well as coloured and non-coloured polymers detected at 520 and 280 nm, respectively, were quantified in duplicate using an HPLC method (Peng et al., 2002), modified and described in **Chapter 3**. Polymers included polymeric phenolic compounds with 5 or more subunits, consisting of anthocyanins and flavan-3-ols for coloured polymers, and only flavan-3-ols for non-coloured polymers.

ABTS Radical Cation Scavenging Assay

The total antioxidant capacity (TAC) of the wines was measured (TAC_M) in triplicate using the $ABTS^{\bullet+}$ scavenging assay (Re et al., 1999). The content of individual phenolic compounds, measured by HPLC, and their experimental TEAC values (reported in **Chapter 3**) were used to calculate the theoretical TAC (TAC_{CAL}). The remaining TAC (TAC_R) is the difference between TAC_M and TAC_{CAL}. Analysis and calculations were carried out as described in **Chapter 3**.

Objective Colour Parameters

The objective colour parameters of the wines were measured in duplicate on the CIELab scale, namely *a** (red/green chromaticity), *b** (yellow/blue chromaticity) and *L** (lightness), and the *C** (chroma) and *h** (hue angle), calculated as described in **Chapter 4**. Names for hues were adapted from Gonnet (1999) based on the *h** values and are described in **Chapter 4**.

Sensory Analysis

The Pinotage wines from the oxygenation trial were evaluated eight months after production for colour, berry/plum intensity, astringency, fullness and overall wine quality by a panel of six experienced judges, comprising wine-makers from industry. Evaluation was done by making a mark on an unstructured 10 cm line scale. The scales were anchored at both ends by the terms, "unacceptable" and "excellent" for colour and overall wine quality, "low" and "high" for berry/plum intensity and astringency, and "thin" and "full" for fullness.

Statistical Analysis

Analysis of variance was performed on the means for triplicate or duplicate samples of each oak maturation and oxygenation treatment and time combination to determine whether significant differences occurred. The Student *t*-LSD test ($P < 0.05$) was used to determine the statistical differences between means. Analysis of variance and difference testing were done using the SAS version 8 software package (SAS Institute Inc., Cary, NC). Pearson product moment correlation coefficients between parameters and their P-values were calculated using the STATISTICA 6 software package (StatSoft, Inc., Tulsa, OK).

Results

The actual values for all determinations (oxygenation trial), as well as data not shown here (oak maturation and oxygenation trials), are reported in **Addendum E**. Only significant ($P < 0.05$) differences between values will be discussed unless otherwise noted.

Oak Maturation: Effect on Phenolic Composition

Oak maturation caused a decrease in the contents of all the individual monomeric anthocyanin, except for vitisin A (data not shown), as well as the total monomeric anthocyanin (pH shift; HPLC) contents of the Pinotage wine compared to the control wine (0 weeks) after completion of maturation (**Figure 2**). The polymeric anthocyanin (pH shift) and coloured polymer (HPLC) contents remained stable over the 28 week maturation period (data not shown).

All the treatments, except the staves treatment, produced the same trends for the monomeric anthocyanin (pH shift; HPLC), malvidin-3-glucoside (glc) and malvidin-3-acetylglucoside (glc-ac)

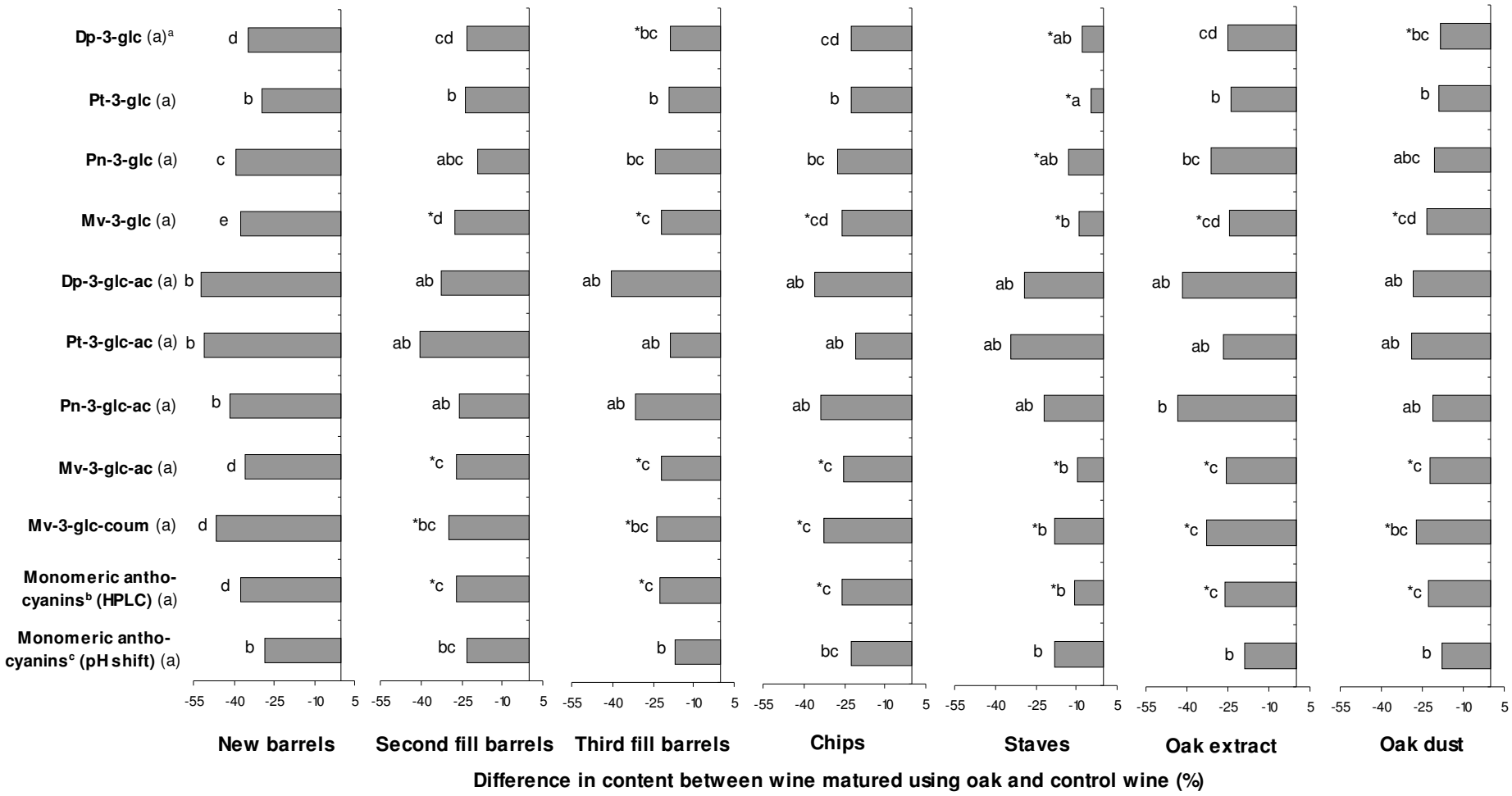

Figure 2. The percentage differences in monomeric anthocyanin content between Pinotage wines matured for 28 weeks using different oak treatments and the control wine [[a] different letters on the bars for the oak matured wines and the control wine (0 weeks), indicated next to the parameter name, denote significant differences ($P < 0.05$); [b] sum of phenolic group content measured by HPLC; [c] measured using the pH shift assay; * indicate significant ($P < 0.05$) differences from new barrel matured wines; Dp = delphinidin; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Mv = malvidin; Pn = peonidin; Pt = petunidin].

contents of the wine and only the monomeric anthocyanin content (HPLC) of the wines is thus shown (**Figure 3**). The staves treatment caused a decrease in the monomeric anthocyanin content (HPLC) of the wine, at the same rate as that of the other treatments, up to 15 weeks maturation whereafter it stabilised. The other treatments, on the other hand, caused a continuous decrease throughout the 28 week maturation period. After 15 weeks and at completion of maturation, the new barrel treated wine had the lowest monomeric anthocyanin content (HPLC) (**Figure 3**), as well as the lowest monomeric anthocyanin content (pH shift), malvidin-3-glc, malvidin-3-glc-ac and malvidin-3-*p*-coumaroylglucoside (glc-coum) contents (data not shown). The staves treatment, on the other hand, gave wine with higher delphinidin-3-glc, petunidin-3-glc, malvidin-3-glc, malvidin-3-ac and monomeric anthocyanin (pH shift; HPLC) contents than the other treatments after completion of maturation, but not the control wine (0 weeks).

The delphinidin-3-glc and petunidin-3-glc contents of the wine decreased for all treatments over the maturation period compared to the control wine (0 weeks), except for the wine treated with staves, which showed no change (**Figure 2**). No change was observed for the peonidin-3-glc content of the wine treated with second fill barrels, staves and oak dust, but the other treatments resulted in a lower content than the control wine (0 weeks). Only the wine matured in new barrels for 28 weeks had lower delphinidin-3-glc-ac and petunidin-3-glc-ac contents than the control wine (0 weeks). The peonidin-3-glc-ac content of the wine was decreased only in the case of the new barrel and oak extract treatments. The monomeric anthocyanin content (pH shift) showed a decrease for the new barrel, third fill barrel, staves, oak extract and oak dust treatments.

Oak maturation, irrespective of treatment, resulted in similar trends for the unknown flavonol, quercetin-3-rhamnoside (rham) and total flavonol contents of the wine with lower concentrations than the control wine (0 weeks) after completion of maturation (**Figure 4**). Only the oak extract treatment resulted in a decrease in quercetin-3-glc content of the wines. The quercetin content of the wines matured with third fill barrels, staves and oak extract did not change over the maturation period. The new barrel matured wine showed the lowest quercetin content after 28 weeks of maturation, although it differed significantly only from the control wine (0 weeks) and wine matured using oak dust.

All the wines matured in oak for 28 weeks had higher gallic acid content than the control wine (0 weeks) (**Figure 4**). The new barrel treatment had the greatest effect. The gallic acid content of these wines was also higher than that of the staves and oak dust treated wines after maturation for 28 weeks, but not the second fill, third fill, chips and oak extract treated wines.

Oak maturation decreased the caftaric acid and caffeic acid contents of the wine for all oak treatments to similar concentrations (**Figure 4**). The *p*-coumaric acid content of the wine was not significantly different from the control wine (0 weeks) after 28 weeks of oak maturation (data not

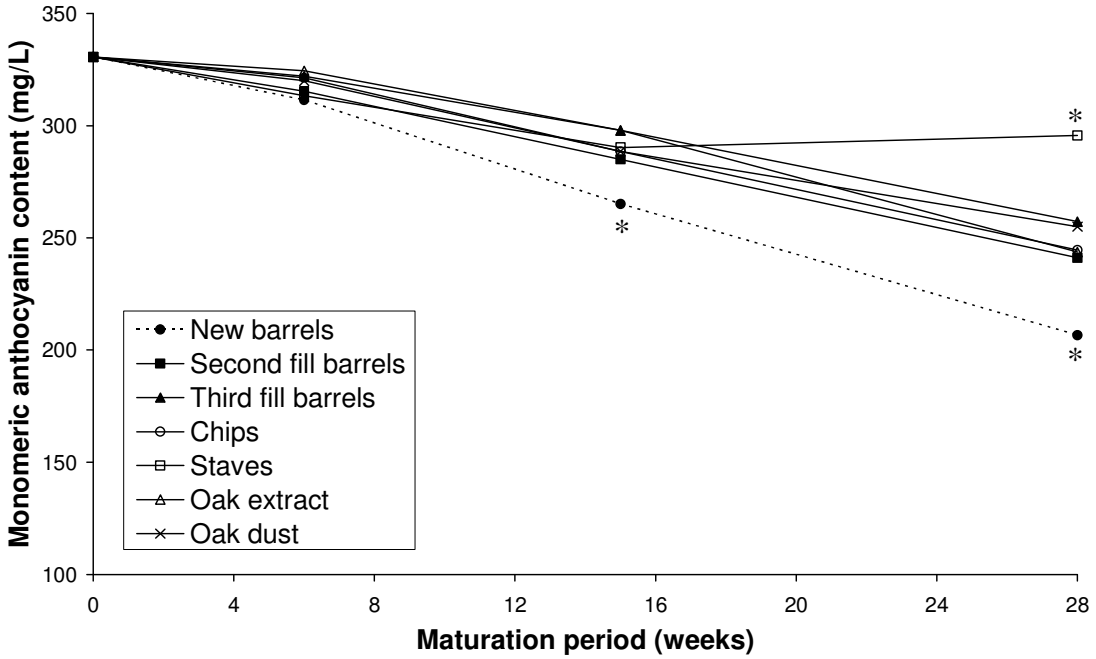

Figure 3. Effect of oak maturation on the monomeric anthocyanin content (HPLC) of Pinotage wines [* = significantly different from other treatments for the same maturation period ($P < 0.05$)].

shown). Only the second fill barrel, staves and oak extract treatments decreased the *p*-coumaroyltartaric (coutaric) acid content of the wine. The chips and oak dust treatments did not affect the total phenolic acid content of the wine, but the other treatments resulted in wines with lower concentrations after 28 weeks than the control wine (0 weeks).

Maturation decreased the total flavan-3-ol (DAC) content of the wine (**Figure 5**). Only the new barrel, second fill barrel and oak dust treated wines showed a lower content of (+)-catechin after maturation, while the same was true only for the procyanidin B1 content of the new barrel treated wine. No significant change in the non-coloured polymer content after maturation was observed for the individual treatments, except for the new barrel treated wine which had a much lower content after completion of maturation compared to the control wine (0 weeks).

After completion of maturation, only the new barrels and chips treated wines had a lower total phenol content than the control wine (0 weeks) (**Figure 5**). All the oak treatments resulted in wines with lower total monomer content (HPLC) than the control wine (0 weeks) after 28 weeks and the total monomer content (HPLC) of the wines matured with new barrels was lower than that of the other wines after completion of maturation.

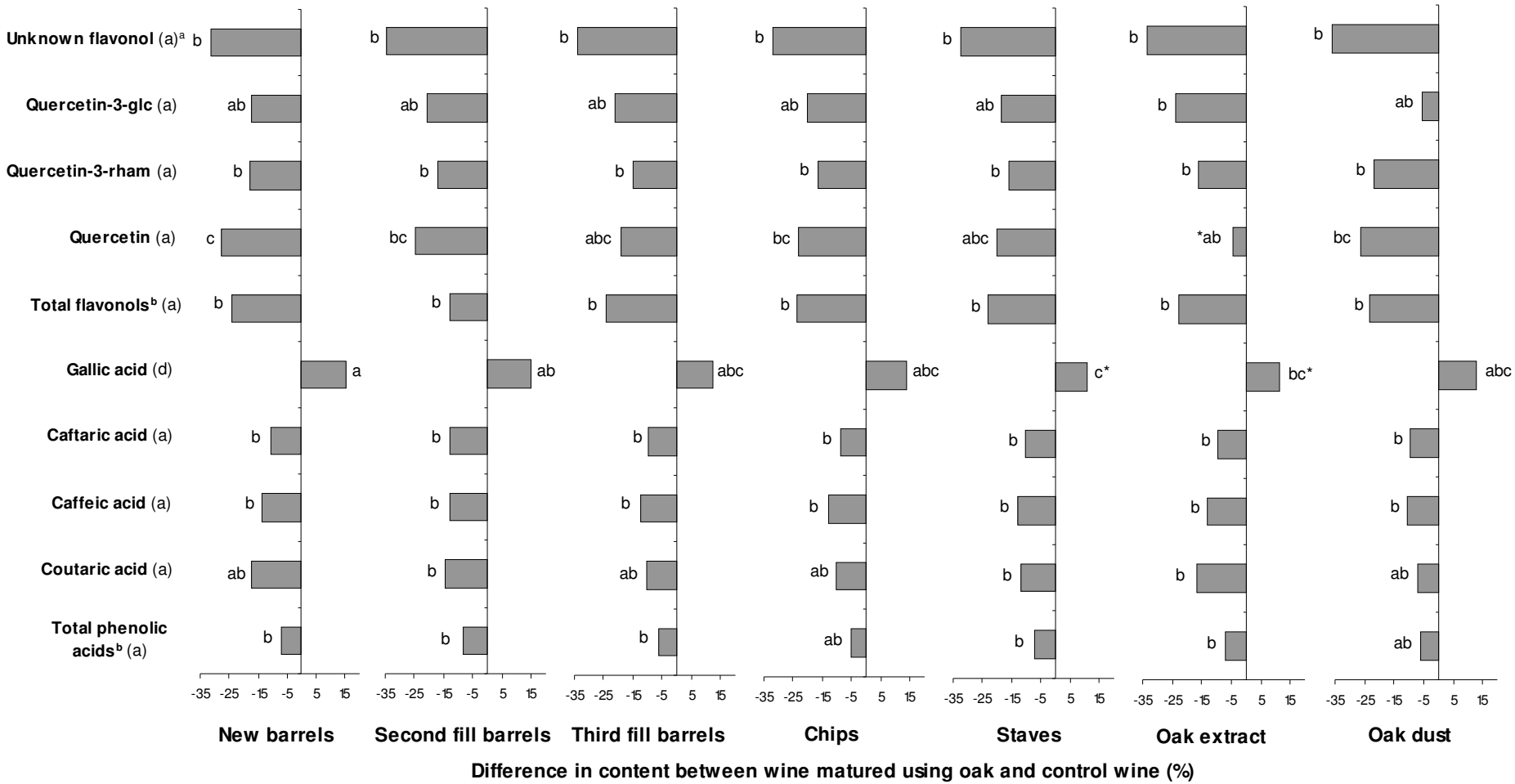

Figure 4. The percentage differences in flavonol and phenolic acid content between Pinotage wines matured for 28 weeks using different oak treatments and the control wine [[a] different letters on the bars for the oak matured wines and the control wine (0 weeks), indicated next to the parameter name, denote significant differences ($P < 0.05$); [b] sum of phenolic group content measured by HPLC; * indicate significant ($P < 0.05$) differences from new barrel matured wines; glc = glucoside; rham = rhamnoside].

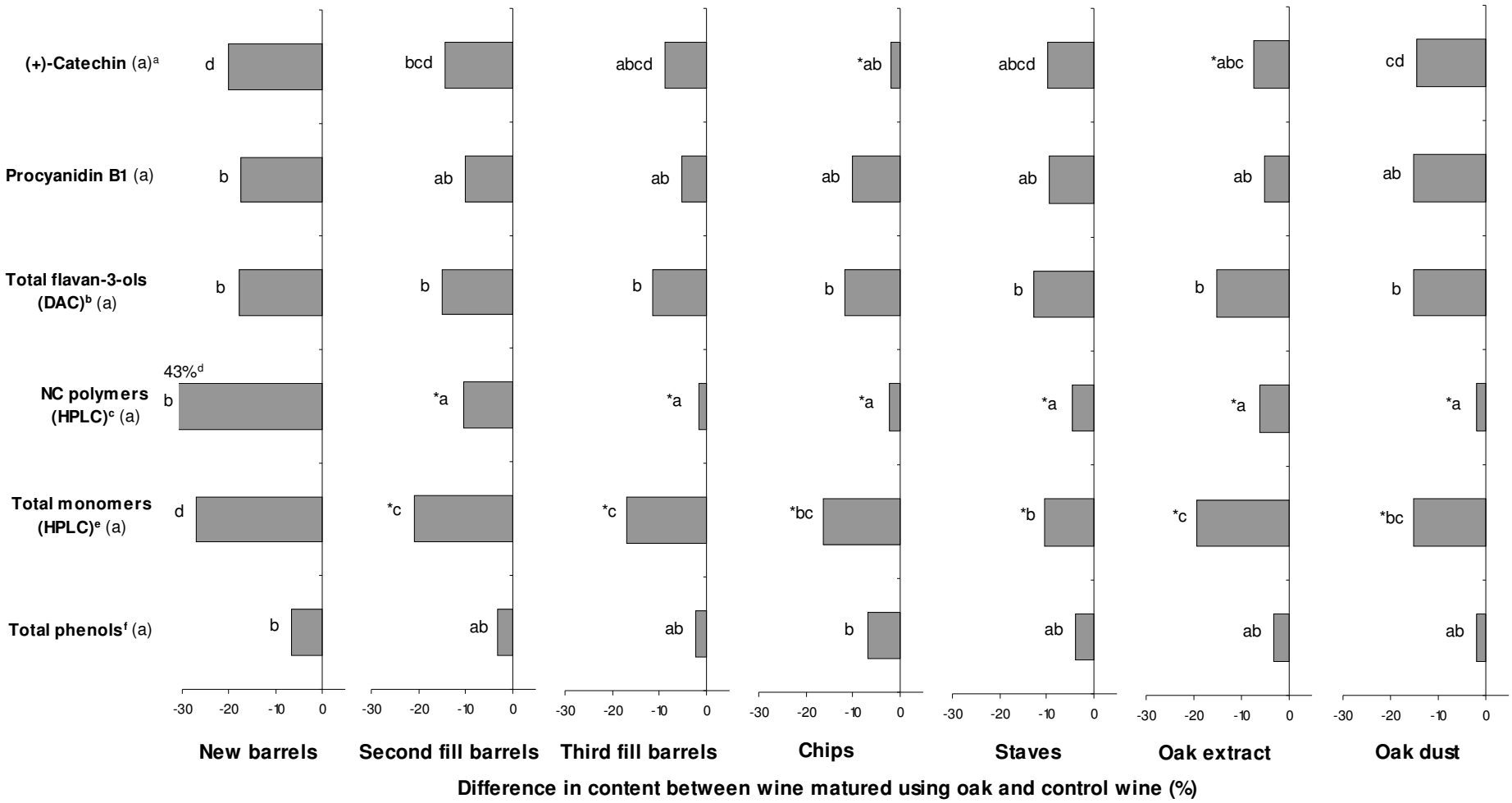

Figure 5. The percentage differences in flavan-3-ol, total monomer and total phenol content between Pinotage wines matured for 28 weeks using different oak treatments and the control wine [[a] different letters on the bars for the oak matured wines and the control wine (0 weeks), indicated next to the parameter name, denote significant differences ($P < 0.05$); [b] measured using the DAC assay; [c] non-coloured polymers measured by HPLC; [d] values exceed scale; [e] sum of phenolic group content measured by HPLC; [f] measured using the Folin-Ciocalteau assay; * indicate significant ($P < 0.05$) differences from new barrel matured wines].

Oak Maturation: Effect on Antioxidant Capacity

The trends for the TAC_M of the individual treatments differed (**Figure 6**). The new barrel and oak extract treated wines had higher TAC_M values than the control wine (0 weeks) after 6 weeks maturation, but thereafter, their TAC_M values decreased. Subsequently, the TAC_M values of new barrel and oak extract treated wine were not significantly different from the control wine after 15 and 28 weeks maturation (**Figure 7**). The oak dust treated wine showed higher TAC_M than the control wine (0 weeks) after 15 weeks maturation. However, the TAC_M of none of the oak treated wines after completion of maturation were significant different to that of the control wine (0 weeks). All oak treated wines had a lower TAC_{CAL} than the control wine (0 weeks) after completion of maturation, with the new barrel wine and the staves treated wine having the lowest and highest TAC_{CAL} of the oak treated wines, respectively. Decreases in the contributions of anthocyanins, hydroxycinnamic acids and flavonols to the TAC_{CAL} were observed for all oak treatments after completion of maturation, compared to the control wine (0 weeks) (**Figure 8**). The contribution of flavan-3-ols to the TAC_{CAL} after 28 weeks was only decreased for the new barrel, second fill barrel and oak dust matured wines. On the other hand, the gallic acid contribution to the TAC_{CAL} of all oak treated wines after 28 weeks was higher than that of the control wine (0 weeks) (**Figure 8**). After completion of maturation, the TAC_R, i.e. the difference between TAC_M and TAC_{CAL}, was higher than that of the control wine (0 weeks) only for the third-fill barrel and oak dust treated wine (**Figure 7**).

Oak Maturation: Effect on Objective Colour Parameters

The trends for the C^* and a^* values of the wine over the maturation period were very similar (**Figures 9, 10**). Oak maturation caused an increase in the C^* and a^* values of the wine from 0 weeks to 6 weeks, whereafter a decrease was observed. After 28 weeks of maturation, the C^* and a^* values of all oak treated wines were lower than that of the control wine (0 weeks), except for the C^* values of the wines treated with chips, staves and oak dust (**Figure 7**). Maturation using staves resulted in wine with the highest C^* and a^* values after 28 weeks of maturation.

Oak maturation initially caused a decrease in wine h^*, whereafter an increase was observed (**Figure 11**), but this was only significant for the chips, oak extract and oak dust treatments. No initial decrease in wine h^* was observed when other treatments were used. Maturation using new barrels resulted in an increase in h^* only after completion of maturation (**Figure 7**). All oak treatments resulted in wine with higher h^* and b^* values than that of the control wine (0 weeks) after completion of maturation. The least change in h^* and b^* value was observed for the new barrel treated wine, resulting in lower values than the other oak treatments. Progressively lower h^* values

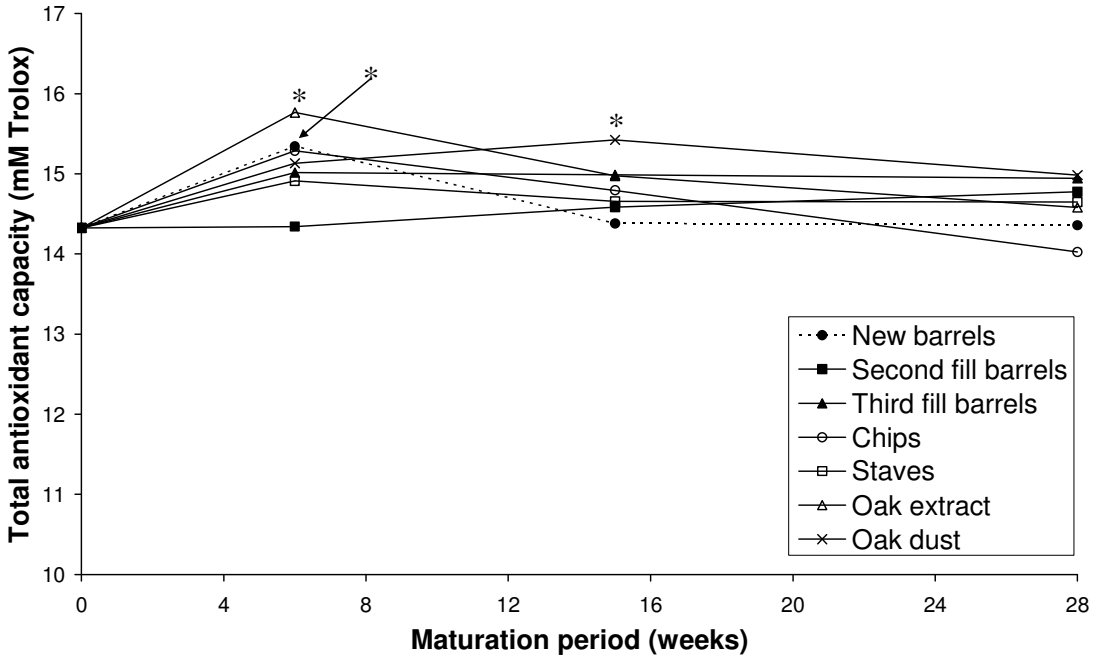

Figure 6. Effect of oak maturation on the measured total antioxidant capacity (TAC_M) of Pinotage wines [* = significantly different from control wine (0 weeks) ($P < 0.05$)].

were observed for wines treated with third fill barrels, alternative oak products, second fill barrels and new barrels after 28 weeks of maturation.

The *L** values of the wine decreased during oak maturation (**Figure 12**). The trends for the *L** values of the individual treatments were similar, with the new barrel treated wine showing a much more pronounced decrease than the other wines. After completion of maturation, the *L** values for all the wines were lower than that of the control wine (0 weeks) (**Figure 7**). The new barrel treatment resulted in wine with the lowest *L** value, while the least change in *L** occurred for the third fill barrel treatment. Progressively lower *L** values were obtained after 28 weeks of maturation for wines treated with third fill barrels, alternative oak products (no significant differences between treatments), second fill barrels and new barrels.

Oxygenation: Effect on Phenolic Composition

Non-oxygenated wine did not change much with regard to their phenolic composition during the 6 month period (**Figure 13**). Only the total monomer content (HPLC) was slightly higher for non-oxygenated wines after 6 months, compared to the control wine.

Oxygenation caused a decrease in the monomeric anthocyanin content (pH shift; HPLC) of the wine over the treatment period for both oxygenation levels, but the loss of anthocyanins was more pronounced for oxygenation at 5.0 mg O_2/L/month (**Figure 13**). The same trend was observed for

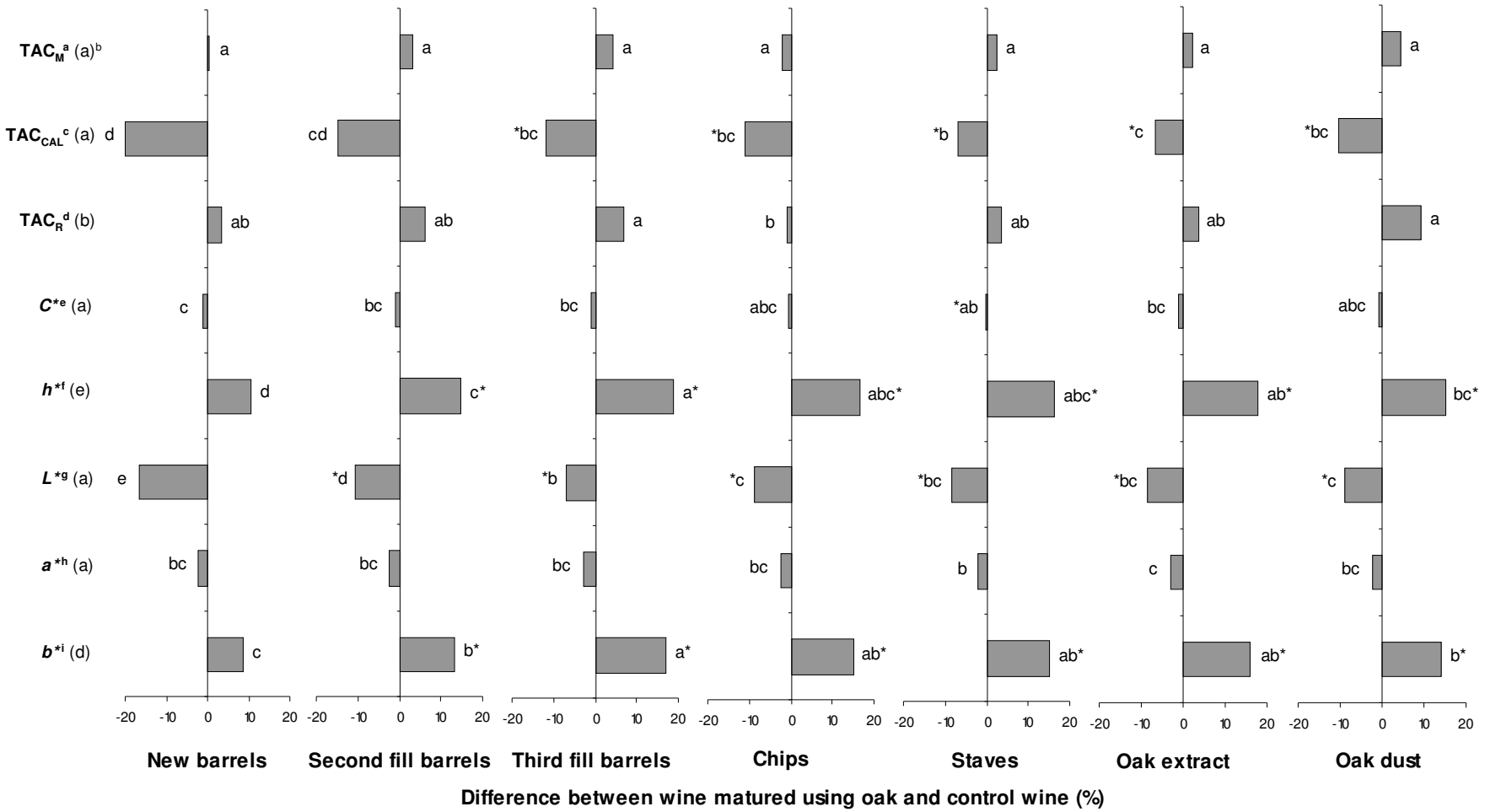

Figure 7. The percentage differences in total antioxidant capacity and colour, between Pinotage wines matured for 28 weeks using different oak treatments and the control wine [[a] total antioxidant capacity as measured; [b] different letters on the bars for the oak matured wines and the control wine (0 weeks), indicated next to the parameter name, denote significant differences ($P < 0.05$); [c] total antioxidant capacity as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [d] $TAC_R = TAC_M - TAC_{CAL}$; [e] chroma; [f] hue angle (°); [g] lightness; [h] red/green chromaticity; [i] yellow/blue chromaticity; * indicate significant ($P < 0.05$) differences from new barrel matured wines].

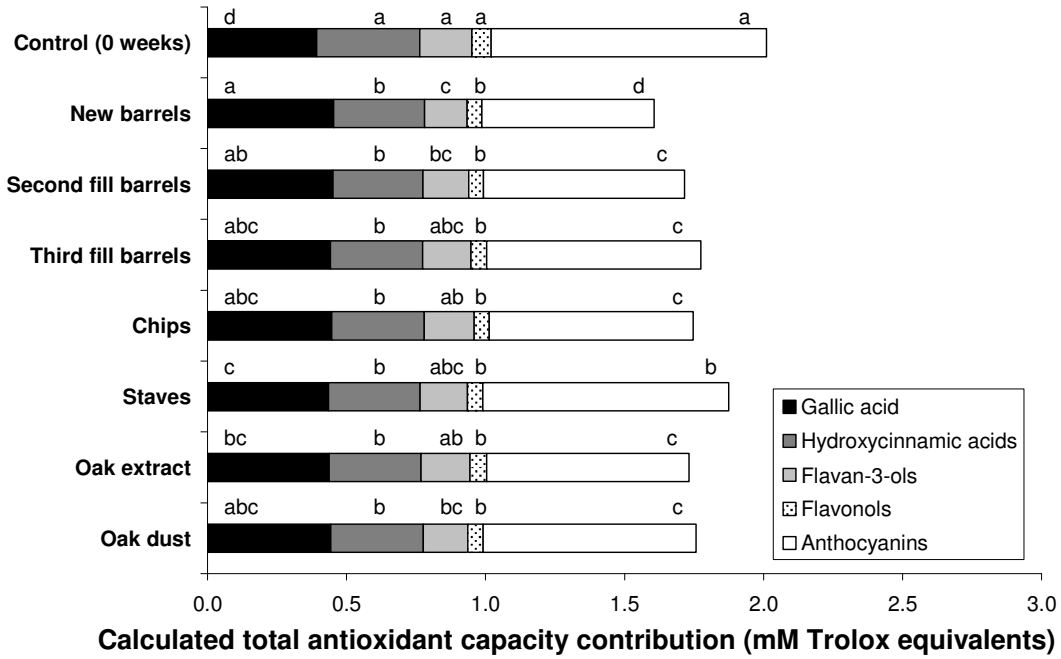

Figure 8. Calculated total antioxidant capacity contributions of phenolic groups for different oak maturation treatments [different letters for the contribution of a specific phenolic group, denote significant differences ($P < 0.05$)].

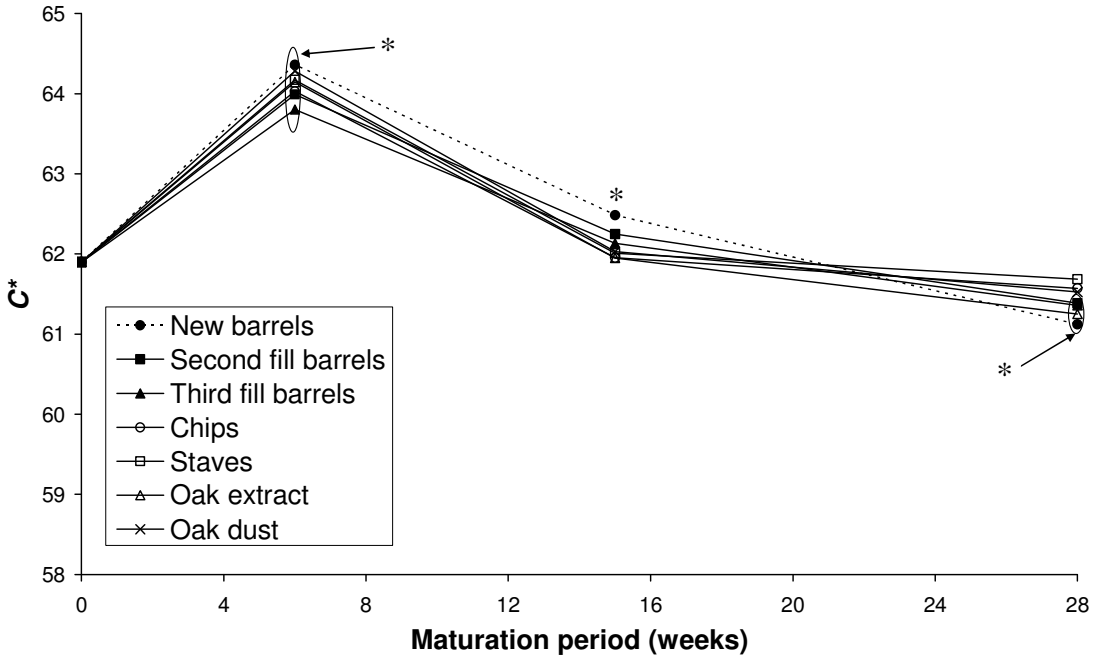

Figure 9. Effect of oak maturation on the chroma (C^*) of Pinotage wines [* = significantly different from the control wine (0 weeks) ($P < 0.05$)].

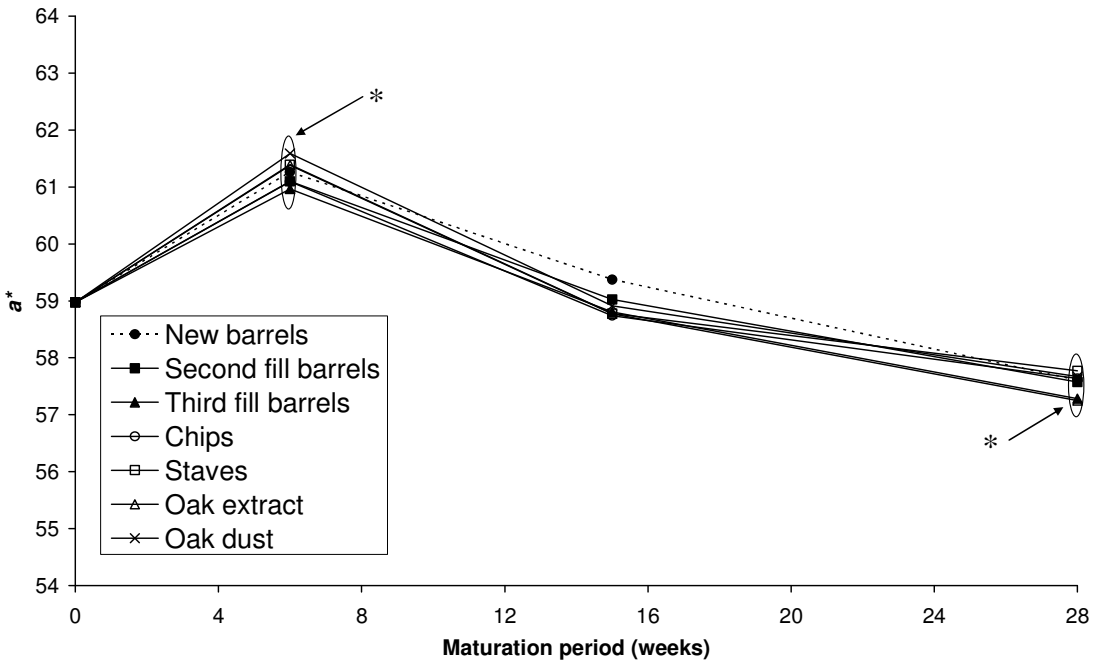

Figure 10. Effect of oak maturation on the *a** (red/green chromaticity) of Pinotage wines [* = significantly different from the control wine (0 weeks) (P < 0.05)].

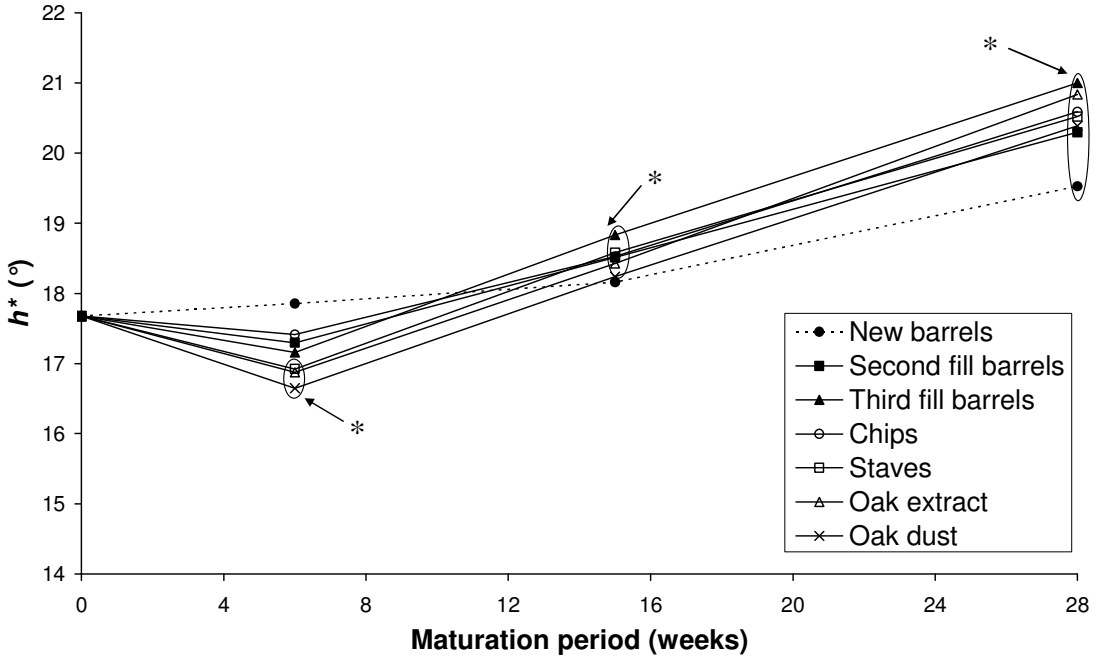

Figure 11. Effect of oak maturation on the hue angle (*h**) of Pinotage wines [* = significantly different from the control wine (0 weeks) (P < 0.05)].

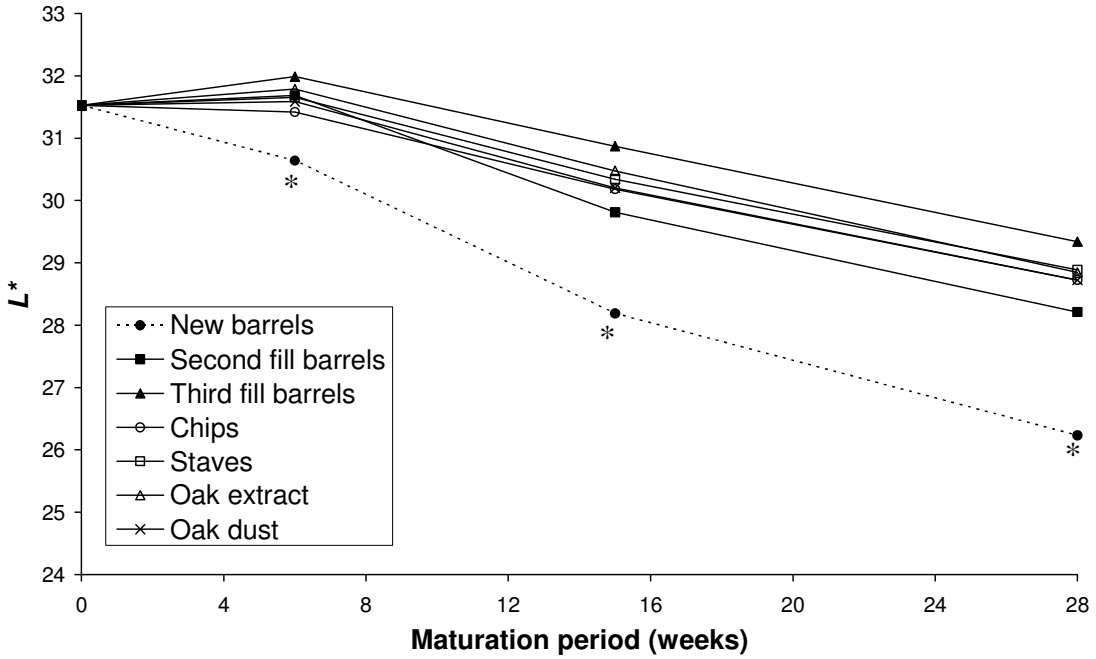

Figure 12. Effect of oak maturation on the lightness (L^*) value of Pinotage wines [* = values significantly different from other treatments for the same maturation period ($P < 0.05$)].

all the individual anthocyanin contents, except for the vitisin A content. The vitisin A content was increased by oxygenation at 2.5 mg O_2/L/month for 2 months and oxygenation at 5.0 mg O_2/L/month for 2 and 4 months, but after 6 months its content at both oxygenation levels was similar to that of the control. Several of the anthocyanins, namely peonidin-3-glc, delphinidin-3-glc-ac, petunidin-3-glc-ac and malvidin-3-glc-coum, could not be detected in the wine treated for 6 months with 5.0 mg O_2/L/month. The polymeric anthocyanin content (pH shift) exhibited increased concentrations when 5.0 mg O_2/L/month was applied, for all time intervals, as well as for when 2.5 mg O_2/L/month were applied for 4 and 6 months. However, only the 4 and 6 month treatments at 5.0 mg O_2/L/month caused an increase in coloured polymer content (HPLC).

Several of the flavonols, namely an unknown flavonol, quercetin-3-glc, quercetin-3-rham, isorhamnetin, as well as the total flavonol, contents decreased during application of oxygen, irrespective of the concentration (**Figure 13**). The application of 5.0 mg O_2/L/month also decreased the quercetin-3-gal, quercetin and kaempferol contents, with the wine treated for 6 months having lower contents than that of the control wine.

All oxygenated wines, except wines oxygenated at 2.5 mg O_2/L/month for 2 months, had higher gallic acid contents than the control (**Figure 13**). At both oxygenation levels the gallic acid content increased with oxygenation time with the highest gallic acid content observed when 5.0 mg O_2/L/month were applied for 6 months. The caftaric acid, coutaric acid (data not shown) and

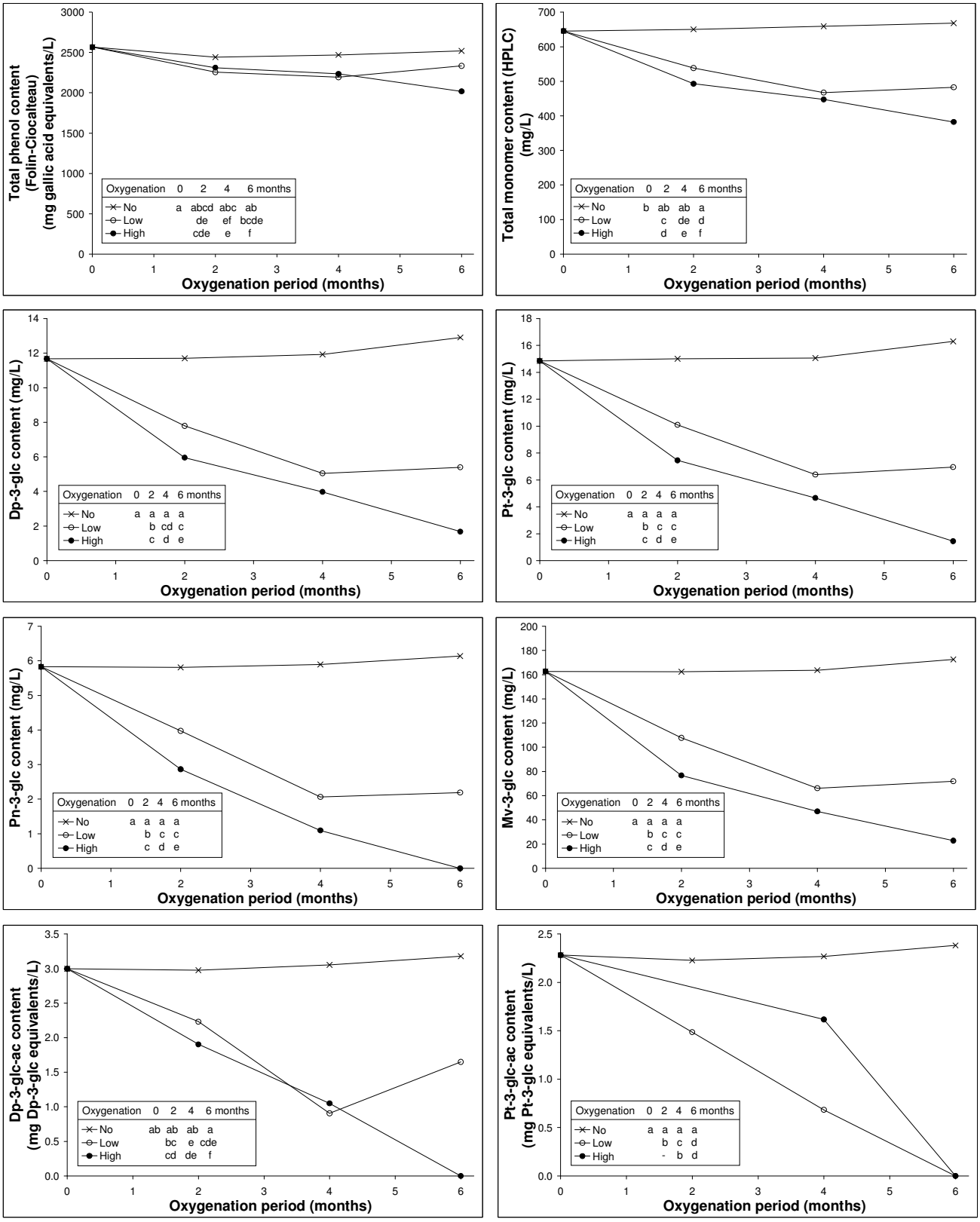

Figure 13. Effect of oxygenation on the phenolic composition of Pinotage wine, measured with spectrophotometric assays and HPLC [description of figure legends: no = application of 0.0 mg O_2/L/month; low = application of 2.5 mg O_2/L/month; high = application of 5.0 mg O_2/L/month; different letters denote significant differences ($P < 0.05$); gal = galactoside; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; rham = rhamnoside].

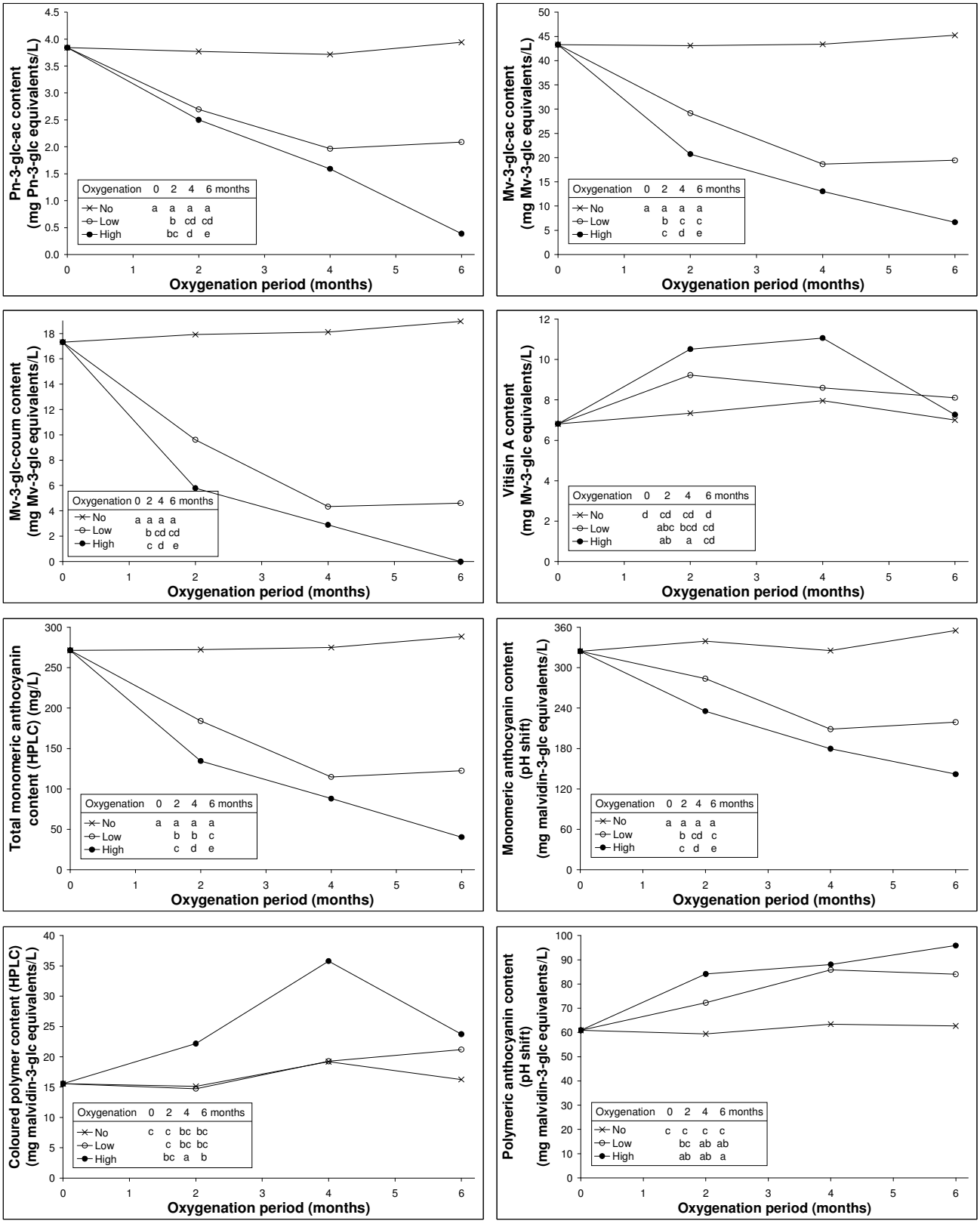

Figure 13. (continued)

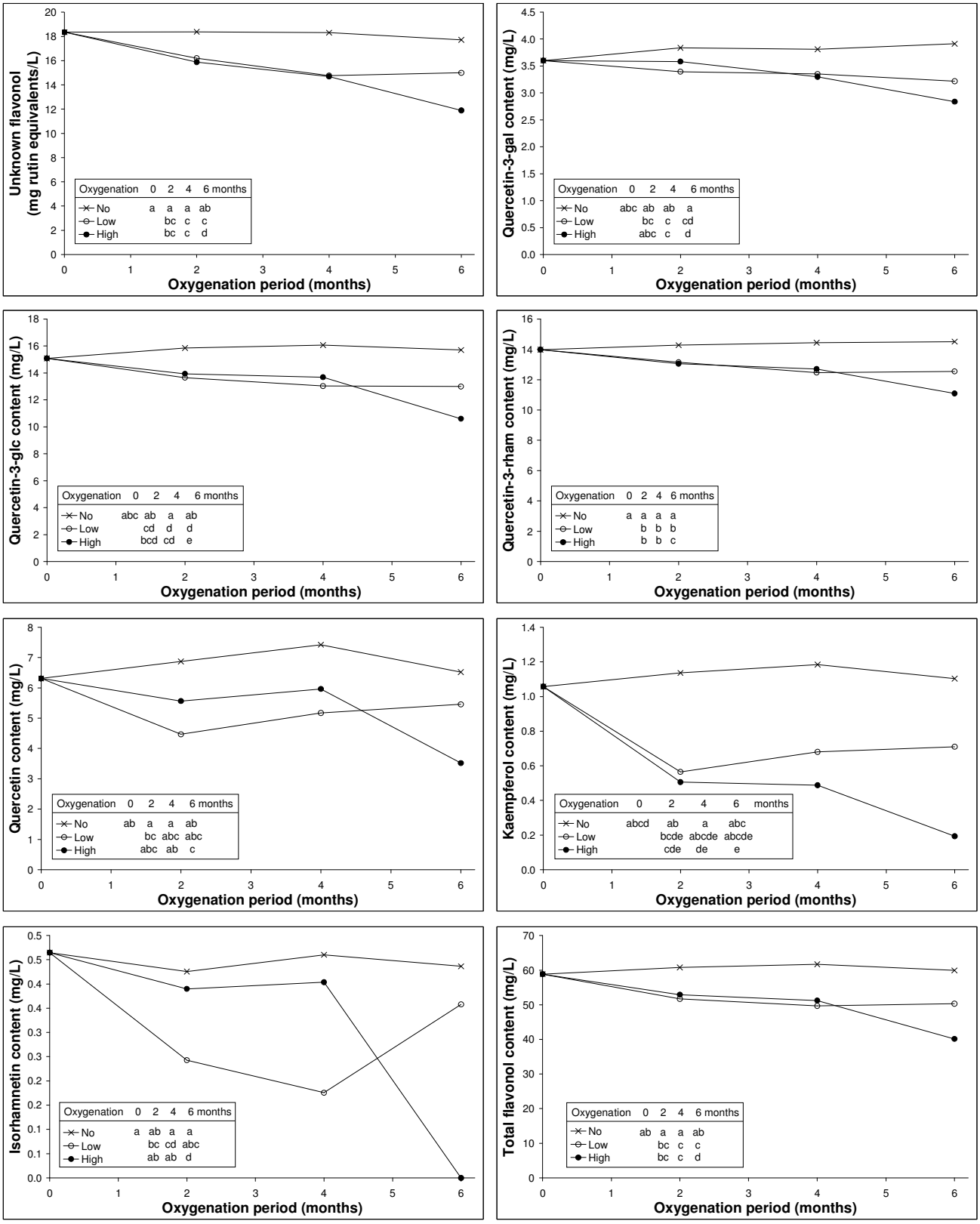

Figure 13. (continued)

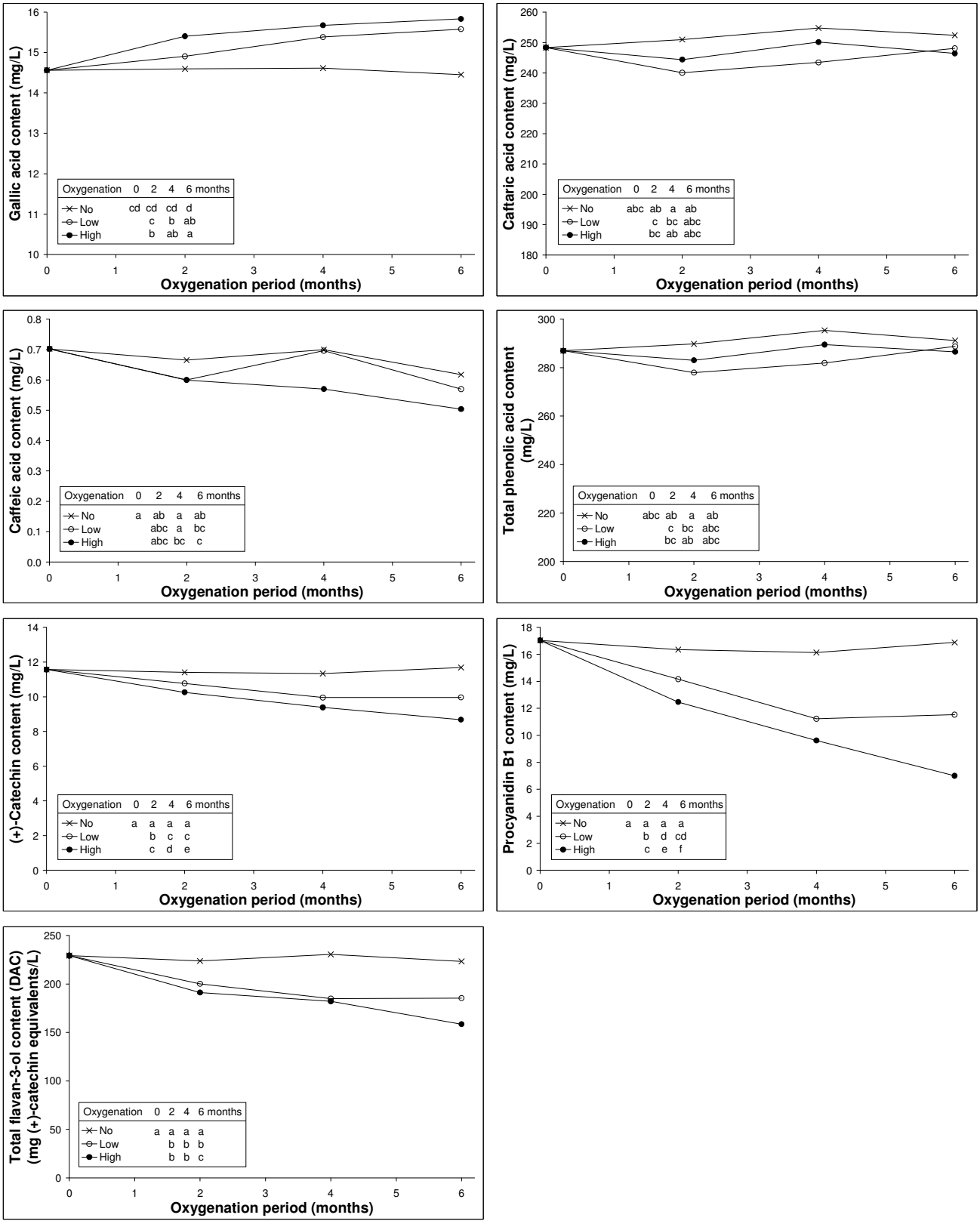

Figure 13. (continued)

p-coumaric acid (data not shown) contents, as well as the total phenolic acid content, showed similar trends, i.e. no significant change in content over the 6 month period. The caffeic acid content of the wine, on the other had, decreased with application of 2.5 mg O_2/L/month for 6 months and 5.0 mg O_2/L/month for 4 or 6 months.

For both oxygenation levels, the (+)-catechin and procyanidin B1, as well as total flavan-3-ol (DAC), contents of wine decreased over time, with a higher loss at 5.0 mg O_2/L/month (**Figure 13**). The non-coloured polymer content of the wines did not change significantly during oxygenation, irrespective of the dosage (data not shown).

Oxygenation caused a decrease in the total phenol and total monomer (HPLC) contents of the wine (**Figure 13**). Higher losses were observed at 5.0 mg O_2/L/month than at 2.5 mg O_2/L/month.

Oxygenation: Effect on Antioxidant Capacity

All wines treated with oxygen had lower TAC_M, TAC_{CAL} and TAC_R than the non-oxygenated wines, although the period of oxygenation did not have a significant effect on the TAC_M or TAC_R (**Figure 14**). The decrease in TAC_{CAL} was, however, more pronounced for longer oxygenation periods. The TAC_M, TAC_{CAL} and TAC_R of the non-oxygenated wines did not change during the oxygenation period. Substantial decreases in the contribution of flavan-3-ols, flavonols and especially anthocyanins to the TAC_{CAL} were observed for oxygenated wines. An increase in contribution to the TAC_{CAL} was observed only in the case of gallic acid (**Figure 15**).

Oxygenation: Effect on Objective Colour Parameters and Sensory Quality

The colour parameters of oxygenated and non-oxygenated wines are depicted in **Figure 14**. The C^* and a^* values decreased during the treatment time for both the non-oxygenated and oxygenated Pinotage wines (**Figure 14**). The decreases in C^* and a^* values were more pronounced for an application of 5.0 mg O_2/L/month than for 2.5 mg O_2/L/month. Oxygenation initially increased the h^* and b^* values of the wines, whereafter these values decreased. The final h^* of wines at both oxygenation levels was, however, still higher than that of the control wine. The L^* value of oxygenated wines, but not non-oxygenated wines, decreased during the 6 month period.

Non-oxygenated wines retained their sensory characteristics throughout the 6 month period (**Figure 16**). The sensory colour acceptability scores of the oxygenated wines increased, irrespective of the oxygen concentration. Berry/plum intensity scores decreased with oxygenation, and this loss was more pronounced when 5.0 mg O_2/L/month was applied. No significant changes in astringency scores of wines were observed during oxygenation. Fullness scores were higher for all wines oxygenated with 5.0 mg O_2/L/month than for the non-oxygenated wines, while oxygenation at 2.5 mg O_2/L/month increased fullness scores only for the 6 month treatment period. Considering the overall quality of the wine, oxygenation decreased the overall quality scores of the

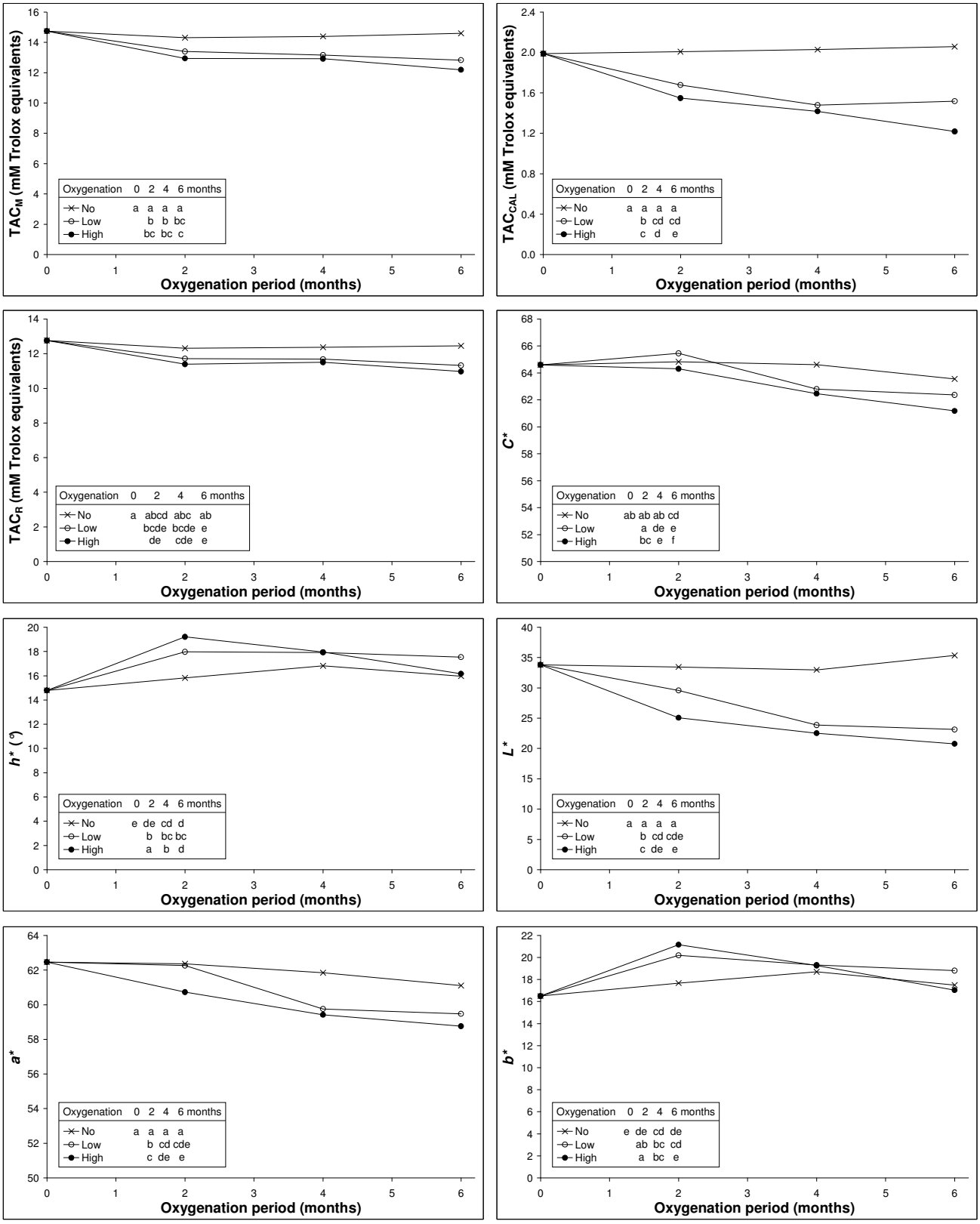

Figure 14. Effect of oxygenation on the total antioxidant capacity and colour of Pinotage wine [description of figure legends: no = application of 0.0 mg O_2/L/month; low = application of 2.5 mg O_2/L/month; high = application of 5.0 mg O_2/L/month; different letters denote significant differences ($P < 0.05$); C^* = chroma; h^* = hue angle (°); L^* = lightness; a^* = red/green chromaticity; b^* = yellow/blue chromaticity].

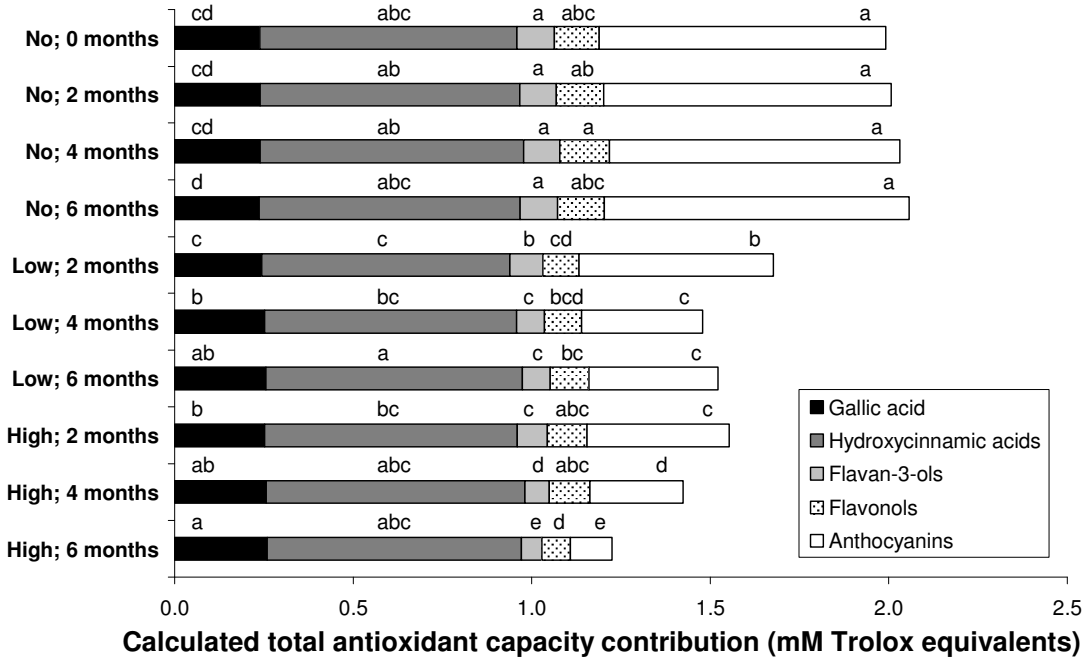

Figure 15. Calculated total antioxidant capacity contributions of phenolic groups for different oxygenation treatments [no = application of 0.0 mg O_2/L/month; low = application of 2.5 mg O_2/L/month; high = application of 5.0 mg O_2/L/month; different letters denote significant differences ($P < 0.05$)].

wine depending on the level and period of oxygenation. Wines receiving the 2.5 mg O_2/L/month treatment only gave decreased overall quality scores after 6 months. Application of 5.0 mg O_2/L/month, irrespective of the period of oxygenation, displayed low overall quality scores.

Discussion

Oak Maturation

Monomeric anthocyanins are increasingly incorporated into oligomeric and polymeric structures forming polymeric pigments during maturation, a process which starts with oak maturation. Maturation is a very important step during the production of high quality red wines. Direct and acetaldehyde-mediated condensation of anthocyanins and flavan-3-ols lead to oligomeric and eventually polymeric pigments with greater colour stability than the original pigments (Fulcrand et al., 2004). In the present study, the content of all individual monomeric anthocyanins, as well as the (+)-catechin and procyanidin B1 content of Pinotage wine decreased, but the polymeric anthocyanin (pH shift) and coloured polymer (HPLC) content unexpectedly did not increase. A possible explanation is that during the short maturation period of 6 months only coloured oligomers are formed, which are not detected in the coloured polymer HPLC peak (only 5 or more subunits) (Peng et al., 2002). Some of these oligomeric pigments, especially ethyl-linked

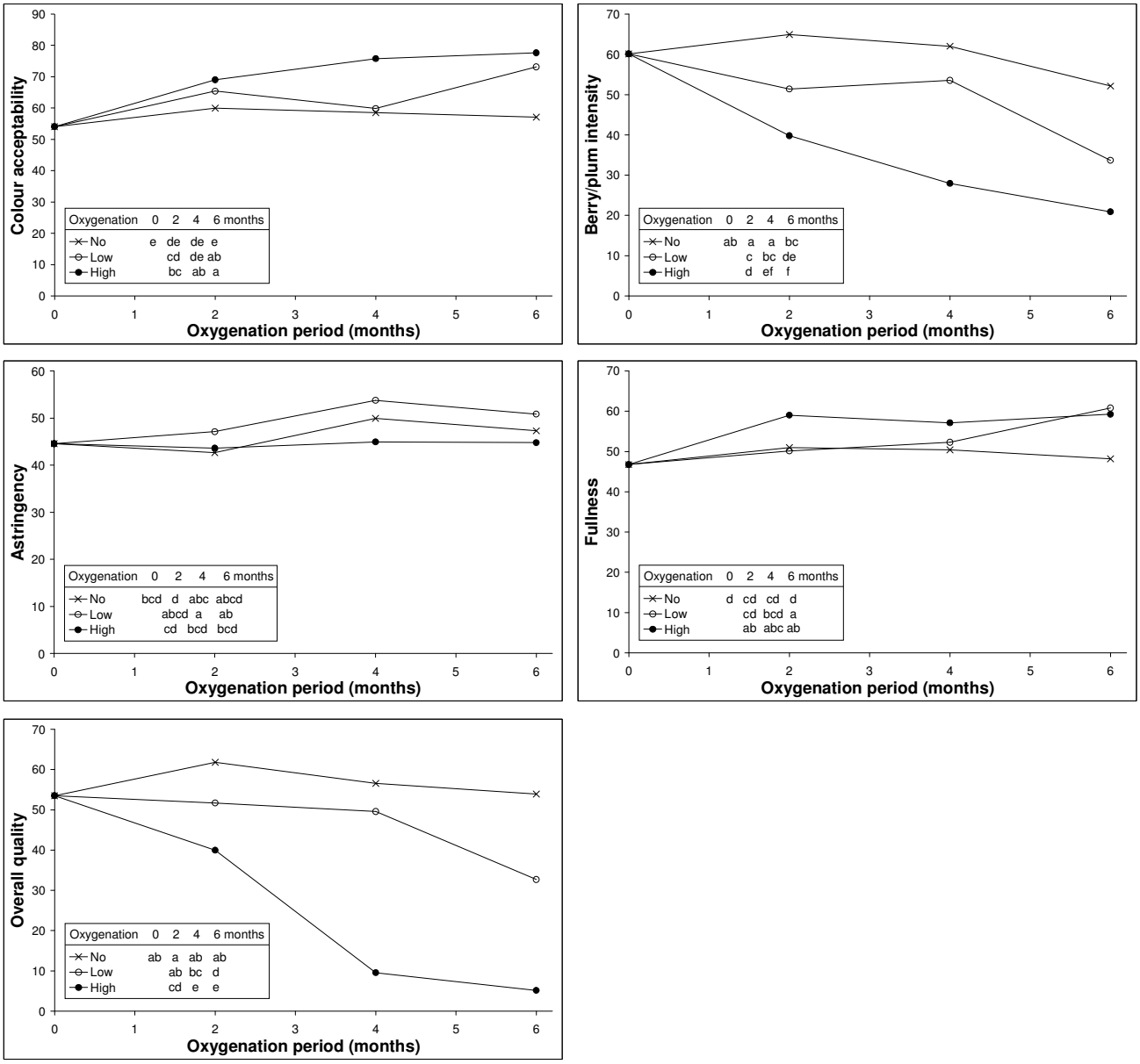

Figure 16. Effect of oxygenation on the sensory quality of Pinotage wine [description of figure legends: no = application of 0.0 mg O_2/L/month; low = application of 2.5 mg O_2/L/month; high = application of 5.0 mg O_2/L/month; different letters denote significant differences ($P < 0.05$)].

anthocyanin-flavan-3-ol condensation products, are also pH dependent, although to a lesser extent than anthocyanin monomers (Escribano-Bailón et al., 2001; Dueñas et al., 2006). This would cause an underestimation and overestimation of the polymeric and monomeric anthocyanin content (pH shift), respectively. This could also explain why the monomeric anthocyanin content (pH shift) of the wine decreased to a lesser extent (14%) than the monomeric anthocyanin content (HPLC) (24%). Some oxidative degradation of monomeric anthocyanins may also take place. The decrease in flavonol (unknown flavonol, quercetin-3-rham and quercetin) and hydroxycinnamic acid (caftaric, caffeic and coutaric acid) content in the wine is also attributed to oxidative degradation as a result of oak maturation. Similar results were obtained by Hernández et al. (2006). Products of oxidative degradation of *o*-diphenols include *o*-quinones, which can react further to form brown polymers (Cheynier et al., 1988), or adducts with glutathione and sulphur dioxide (Singleton et al., 1985; Rigaud et al., 1991).

The individual anthocyanins generally followed the same trend for oak maturation, although some anthocyanins, such as delphinidin-3-glc-ac, petunidin-3-glc-ac and peonidin-3-glc-ac, showed less distinct differences due to their low content, which makes quantification less accurate. A higher dissolved oxygen content was reported for wine in new barrels compared to used barrels (Castellari et al., 2004), which could increase the acetaldehyde content of the wine. The greater decrease in monomeric anthocyanin and flavan-3-ol content observed for new barrel matured wines is therefore presumably due to a higher rate of the acetaldehyde-mediated condensation reactions. The trend for stave treated wines is less clear. Reactions involving monomeric anthocyanins were clearly slowed down or stopped after 15 weeks of maturation as indicated by the stabilisation of the monomeric anthocyanin content, although the reasons for this are not known. Similar results would be expected for maturation of wine using staves and chips in old barrels. Different trends could be the result of the extraction rate of oak wood components as affected by the difference in surface to volume ratio.

Increased gallic acid content of wine with oak maturation as observed for Pinotage wine in the present study supports previous reports on maturation in oak barrels, as well as in stainless steel tanks with oak chips (Jindra & Gallander, 1987; Wilker & Gallander, 1988). This phenomenon can be ascribed to gallic acid formation by hydrolysis of ellagitannins from oak wood in a hydroalcoholic medium such as wine (Quinn & Singleton, 1985) or by hydrolysis of galloylated flavan-3-ols extracted from grape seeds during fermentation (Singleton & Trousdale, 1983).

Oak maturation caused a slight decrease in total phenol content of Pinotage wine similar to that found for in-bottle ageing for one year of Pinotage and Cabernet Sauvignon wines (De Beer et al., 2005). This was accompanied by a pronounced decrease in total monomer (HPLC) content. The total number of hydroxyl groups does not change much during direct and acetaldehyde-mediated condensation of anthocyanins with flavan-3-ols (Monagas et al., 2005). Despite no changes in the

amount of hydroxyl groups, they may be less available for reaction with the Folin-Ciocalteau reagent due to steric hindrance (Shahidi & Wanasundara, 1992). New pigments formed during maturation will be included in the total phenol content as they react with the Folin-Ciocalteau reagent, but not in the total monomer content (HPLC). This explains why the total monomer content (HPLC) decreased much more than the total phenol content.

Oak maturation using traditional, as well as alternative oak treatments applied in old barrels, were not detrimental to the TAC_M of Pinotage wine despite the fact that the TAC_{CAL} decreased due to the decrease in many individual monomeric phenolic compounds. The increase in TAC_R of the wine, which can be ascribed to the formation of new anthocyanin-derived compounds retaining some or all of the antioxidant capacity of the original compounds, counteracted the decrease in TAC_{CAL}. The same principles as described for the reaction of phenolic compounds with the Folin-Ciocalteau reagent apply for their reaction with $ABTS^{\bullet+}$. Although no differences in coloured and non-coloured polymer contents (HPLC) were observed, smaller polymers not detected using the current HPLC method are likely to increase in content, contributing to the increased TAC_R. Ellagitannins, which were not measured in the present study, are also likely to contribute to the increased TAC_R during oak maturation due to their extraction from the oak wood. Ellagitannins have been shown to have high radical scavenging activity (Saint-Cricq de Gaulejac et al., 1998), while the hydrolysis products of ellagitannins, namely ellagic (Ivekovi□et al., 2005) and gallic acid (**Chapter 3**) are also potent antioxidants due to many available hydroxyl groups. Changes in other unknown compounds, which are not necessarily phenolic in nature and shown to have a large contribution to the wine TAC (**Chapter 3**), cannot be estimated, but contribute to the TAC_R of the wine.

An initial increase in TAC_M observed for wines treated with new barrels, oak extract and oak dust can be ascribed to compounds extracted from the new oak wood or present in the oak preparations before substantial losses of wine phenolic compounds have occurred as discussed. A similar result was obtained by Del Álamo et al. (2006) when measuring the redox potential of wine matured in new barrels and stainless steel tanks, with chips and staves added. Although no detrimental effect to the wine TAC was observed in the present study, maturation over a longer period or in the presence of higher oxygen concentrations may negatively impact on the wine TAC. The maturation of Pinotage and Cabernet Sauvignon wines, which were not matured in oak, resulted in decreased wine TAC over a one year bottle ageing period (De Beer et al., 2005).

The Pinotage wine colour saturation (C^*) and a^* values increased initially followed by a decrease after 6 weeks of oak maturation. Using the same wines, a similar trend was observed by Fourie (2005) for the modified colour density ($OD_{520} + OD_{420}$ in the presence of acetaldehyde at pH 3.5) of the wine, while the modified degree of red pigment (OD_{520} in the presence of acetaldehyde

at pH 3.5 x 100/OD_{520} at low pH) showed an increase over the whole maturation period. Both these parameters were measured using the method described by Iland et al. (2000). Pomar and Gonzalez-Mendoza (2001) also observed an initial increase in colour density up to 3 months followed by a decrease during oak maturation, while only decreases in colour intensity after 8 and 12 months of oak maturation have been reported by others (Gómez-Cordovés & González-SanJosé, 1995; Perez-Magariño & Gonzalez-San José, 2006). It is important to note that the evolution of wine colour will depend on the initial composition of the wine, especially the anthocyanin content. The initial increase observed for C^* and a^* in the present study could be related to a decrease in pH, which would increase the proportion of anthocyanins in the red flavylium form, as observed by Pomar and Gonzalez-Mendoza (2001). A decrease in the monomeric pigment content partly explains the reduced colour saturation and a^* value of matured wines, compared to the control wine (0 weeks). Reduced co-pigment content (flavonols, phenolic acids and flavan-3-ols) also contributes to this trend (Gonnet, 1999). On the other hand, monomeric anthocyanins become part of colour-stable oligomeric and polymeric compounds counteracting the decrease in C^*, which is the reason for only a modest decrease in C^* despite substantial decreases in monomeric pigment and co-pigment contents.

The present study confirms the finding of Fourie (2005) for wine hue, namely an increase in modified wine hue (OD_{420} in the presence of acetaldehyde at pH 3.5/OD_{520} at low pH) during oak maturation, using the method described by Iland et al. (2000). The increase in h^* observed in the present study indicates a change from magenta-red hues in the direction of orange-red hues, due to decreased a^* values and increased b^* values, although the wine hues after 28 weeks of maturation were still in the pure red range. Similar trends were also obtained by Rivas et al. (2006). Some treatments, namely the staves, oak extract and oak dust treatments, initially caused slight hue changes towards magenta-red. The initial decrease in hue can be ascribed to formation of purple acetaldehyde-mediated condensation products (Timberlake & Bridle, 1976; Rivas-Gonzalo et al., 1995). The subsequent increase in hue is due to formation of orange-red pyranoanthocyanins (Fulcrand et al., 1996; Fulcrand et al., 1998) or further reaction of ethyl-linked pigments to form larger brown polymers (Es-Safi et al., 1999b) or yellow xanthylium pigments (Es-Safi et al., 1999a; Del Alamo et al., 2000). Alcalde-Eon et al. (2006) reported an increase in pyranoanthocyanin content in Tempranillo wine during oak maturation (6 months) and the subsequent bottle ageing period. No changes in vitisin A content were, however, observed in the present study, although the content of other pyranoanthocyanins, which was not detected using HPLC, could have increased. The decrease in ethyl-linked pigments observed previously (Alcalde-Eon et al., 2006) supports the unstable nature of these pigments.

The wines also became darker (lower L^*) after oak maturation in contrast to the finding of Rivas et al. (2006). Generally, the C^* and L^* values of an anthocyanin solution would increase and decrease, respectively, with increased pigment content and/or co-pigmentation. The L^* values, however, showed the opposite trend, namely decreasing as the C^* values increased. This trend could not be explained by the decrease in monomeric pigment and co-pigment contents. In the same way as for C^*, the increase in oligomeric and polymeric pigments would contribute to a decrease in L^*. It seems in this case, that the effect of polymerisation on the C^* and L^* values differ. This is possibly due to the formation of brown polymers during maturation contributing to a decrease in L^* without increasing C^*.

The new barrel treatment had the greatest effect on the objective colour parameters (C^*, h^*, L^* and b^*) of the wine with few significant differences between the other treatments after 28 weeks of maturation. This result is similar to trends observed by Fourie (2005) and Van Rensburg and Joubert (2002). The pronounced effect of new barrel treatment on Pinotage wine is explained by the fact that the pigment content, as well as the co-pigment content, of new barrel treated wines showed more pronounced changes after maturation than the other treatments.

The sensory characteristics of the wines were also evaluated by Fourie (2005). Wine treated with new barrels was significantly different from the other treatments with higher sensory colour density relating to the modified colour density and L^* value results. Higher oak bouquet and tannin intensity scores and lower fruit aroma intensity scores were also observed for the new barrel treated wine. The chips treatment resulted in wine with an intense oak bouquet with very low fruit aroma intensity which reflects negatively on the sensory quality. Similar negative sensory descriptions were obtained for a Cabernet Sauvignon wine matured in old barrels or stainless steel tanks with oak shavings (Van Rensburg & Joubert, 2002). The other alternative oak products, however, gave wines with sensory scores between those of the new and third fill barrel treated wines which indicate good sensory quality (Fourie, 2005). The oak dust treatment especially resulted in wine with good oak bouquet intensity, colour density and tannin intensity without loss of fruit intensity.

Oxygenation

Oxygenation is expected to facilitate direct and acetaldehyde-mediated anthocyanin-flavan-3-ol condensation reactions as is the case for oak maturation. Pyranoanthocyanins may also be a product when oxygen is present, as the formation of most of these compounds requires an oxidation step (Monagas et al., 2005).

The formation of anthocyanin-derived pigments would therefore explain the substantial decrease in the content of all monomeric anthocyanins, (+)-catechin and procyanidin B1 of the wines after oxygenation. More pronounced changes occurred at the higher oxygenation level as

would be expected. At the same time, the polymeric anthocyanin content (pH shift) increased moderately, but the coloured polymer content (HPLC) showed a slightly different trend with an increase only at the higher oxygenation level. As was described for oak maturation, the difference in trends is probably due to the analytical methods used. An increased concentration of sulphur dioxide resistant pigments, i.e polymers, and loss of monomeric anthocyanins have been shown previously when micro-oxygenation was used at different stages in the vinification process (Atanasova et al., 2002; Castellari et al., 1998; Castellari et al., 2000; Du Toit & Groenewald, 2003). Oxidative degradation of monomeric anthocyanins may also occur, especially at the high oxygenation level, as was the case for oak maturation.

Flavonols and hydroxycinnamic acids have also been shown previously to decrease when Sangiovese wines were oxygenated to saturation every month for 6 months (Castellari et al., 2000). In the present study, similar evidence of oxidative degradation was observed for all flavonols measured, as well as caffeic acid. Oxygenation increased the gallic acid content of wine over time, due to hydrolysis of galloylated flavan-3-ols releasing gallic acid (Singleton & Trousdale, 1983). Castellari et al. (2000), however, observed a decrease in gallic acid when a Sangiovese wine was oxygenated to saturation every month for 6 months. As was the case for oak maturation, the total phenol content decreased only slightly contrary to the HPLC quantified monomers, which is attributed to reaction products still having reactivity in the Folin-Ciocalteau assay. A decrease in total phenol content was also observed after oxygenation of a Sangiovese red wine (Castellari et al., 2000).

Oxygenation was detrimental to the TAC_M of the wines at all oxygenation levels and time periods. Both monomeric compounds (represented by TAC_{CAL}) and unknown compounds (represented by TAC_R) contributed to the decrease in TAC_M. The decreased TAC_{CAL} of oxygenated wines was more pronounced for longer oxygenation periods or the higher oxygen dosage due to greater losses of most monomeric phenolic compounds, despite the increased concentration of gallic acid. The formation of anthocyanin-derived pigments and their contribution to the TAC_M do not seem to compensate for losses of monomeric compounds in oxygenated wine in contrast to the trends observed for oak maturation, since the TAC_R of wines also decreased. Higher degradative losses could have occurred for oxygenation than oak maturation as much more oxygen is applied. Decreased concentrations of unknown antioxidant compounds could also play a role.

The decrease in colour saturation (C^*) and a^* values of the wine with oxygenation is attributed to a decrease in monomeric anthocyanin content, especially since only a small increase in polymeric anthocyanin content was observed for some treatments. Decreased concentrations of co-pigments factors, such as (+)-catechin, procyanidin B1 and all individual flavonols, would also have contributed to the decrease in wine C^* and a^* values (Gonnet, 1999). Atanasova et al. (2002)

reported a decrease in colour density (sum of absorbances at 420, 520 and 620 nm) over time, although this was less severe for a micro-oxygenated wine than for the control wine. Some authors (Castellari et al., 2000; Du Toit & Groenewald, 2003), however, observed an increase in colour density with continuous micro-oxygenation or oxygenation in discrete doses.

Oxygenation resulted in changes in the wine hue (h^*) from an initial magenta-red to pure red in the direction of orange-red, with a subsequent change back to magenta-red in contrast to the trends observed for oak maturation. These hue changes follow the changes in b^* values of the wines. On the basis of these hue and b^* value changes, it seems that the first phase of oxygenation is characterised by formation of orange-red pyranoanthocyanins (Fulcrand et al., 1996; Fulcrand et al., 1998), while the second phase corresponds to formation of purple acetaldehyde-mediated anthocyanin-flavan-3-ol condensation products (Timberlake & Bridle, 1976; Rivas-Gonzalo et al., 1995). The trend for vitisin A content (a pyranoanthocyanin) supports this conclusion. The formation of brown polymers during oxidative degradation of flavonoids would contribute to a hue change towards orange-red, but in this case the formation of purple-red ethyl-linked pigments seems to dominate. A similar trend to that observed in this study was also noted for the hue of a micro-oxygenated Cabernet Sauvignon wine (1.5 mg O_2/L/month and 3.0 mg O_2/L/month) over 15 weeks (Du Toit & Groenewald, 2003).

The wine colour also became darker (lower L^*) with oxygenation, which resulted in higher colour acceptability scores during sensory evaluation. Similar to oak maturation, this trend cannot be fully explained by the changes in phenolic composition due to a variety of confounding factors. Complex changes in the pigment content and composition took place during oxygenation. Brown polymers, for instance, contribute less to the wine chroma than the original anthocyanins, but contribute to the darkening of the wine (lower L^*). This is the case, especially where wines were exposed to large quantities of oxygen.

It is clear that 5.0 mg O_2/L/month is severely detrimental to Pinotage wine quality, especially with regard to berry/plum intensity and overall quality. However, a lower dosage given for a short time can be beneficial in terms of increased colour acceptability and fullness. Sensory astringency scores, mainly associated with the polymer content (Vidal et al., 2004), did not change during oxygenation despite modestly increased coloured polymer (HPLC) and polymeric anthocyanin (pH shift) contents for some treatments. The method of oxygen application can possibly affect the sensory quality. Continuous application of oxygen at very low quantities may have better results than application in discrete doses, although good results were obtained by Castellari et al. (2000) for oxygenation using discrete doses. In a previous study (Du Toit & Groenewald, 2003), continuous micro-oxygenation at levels of 1.5 mg O_2/L/month and 3.0 mg O_2/L/month for 15 weeks produced Cabernet Sauvignon wines which were preferred over the control treatments by a sensory panel. It

is very important to note that the optimal oxygenation rate and time will be subject to the initial composition of the specific wine, especially in terms of tannins and anthocyanins, and the desired outcome. Monitoring of the dissolved oxygen, free sulphur dioxide, monomeric anthocyanins, colour and sensory properties of wine during the oxygenation period is advocated to avoid over-oxygenation and to achieve the desired effect (Lemaire, 2003).

Conclusions

Both alternative oak products and oxygenation showed potential for producing Pinotage wines with good colour and high sensory quality. The use of alternative oak products in old barrels, as well as traditional oak barrels, during maturation did not have a detrimental effect on the TAC of Pinotage wine, but also did not achieve increased levels. In the case of oxygenation, care should be taken not to over-oxidise the wine as detrimental effects to sensory quality, phenolic content and TAC of Pinotage wines were observed for some treatments. A low oxygen dose/short time protocol, however, improved the colour of the wine, although some loss of TAC was still observed. Oak maturation is therefore a good technique for improving wine quality, without a negative impact on the wine TAC. Oxygenation should be investigated further to establish more favourable protocols that will allow improved sensory attributes, while retaining the wine TAC.

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Chapter 7: Effect of Various Treatments During and After Fermentation on Pinotage Wine Phenolic Composition, Total Antioxidant Capacity, Objective Colour and Sensory Quality

Abstract

Selected treatments from previous research were repeated or modified to assess their effects on the phenolic composition, total antioxidant capacity (TAC), colour and sensory quality parameters of Pinotage wine. The pumping-over treatment was repeated and previous results confirmed, namely wine with a reduced phenolic content, TAC and sensory quality compared to the control wine (punching-down treatment). Some objective colour differences were observed, resulting in reduced colour acceptability. Addition of a commercial oak tannin preparation during maturation, previously caused an initial increase in wine TAC, leading to the investigation of commercial oak and grape tannin addition during fermentation. Added oak and grape tannin resulted in wines with increased gallic acid and flavan-3-ol contents, respectively, but the differences from the control wine were too small to have a significant effect on the wine TAC. Addition of commercial tannin preparations did not significantly affect the wine colour and sensory quality. Extended maceration increased the wine flavan-3-ol content, but decreased its phenolic acid, flavonol and anthocyanin content. Despite these changes in phenolic composition, no change in TAC was observed. The extended maceration treatment also resulted in wines with decreased sensory quality. In contrast to a previous investigation, a modified oxygenation treatment (lower oxygen concentration for only 2 months) did not have much effect on the wine phenolic composition, nor was it detrimental to the wine TAC. However, a small decrease in sensory quality scores was observed. Despite the latter decrease in some instances, all the treatments investigated still produced wines with acceptable sensory quality scores. Addition of commercial tannin preparations showed the most promise for use as a technique to increase Pinotage wine TAC without loss of sensory quality.

Introduction

Previous research has shown that various enological techniques applied before, during or after fermentation affect the phenolic composition of red wines (**Chapters 5, 6**). Pre-fermentation maceration, juice/skin mixing techniques, oak maturation and oxygenation have been studied. Pre-fermentation treatments at 10 and 15 °C for 1, 2 and 4 days did not give consistent results over three vintages and did not significantly affect the total antioxidant capacity (TAC) of Pinotage wine (**Chapter 5**). The juice/skin mixing techniques showed relatively small differences in wine TAC

with the punching-down and rotor treatments giving wines with higher values than the pumping-over treatment (**Chapter 5**). Maturation using an oak extract added in old barrels gave an initial increase in TAC (**Chapter 6**). Oxygen applied to Pinotage wine in discrete doses (2.5 or 5.0 mg O_2/L) every month after fermentation and cold-stabilisation was mostly detrimental to its sensory quality and TAC (**Chapter 6**). These trends suggested that a refinement of the oxygenation treatment, i.e. applying lower oxygen doses for a short time only, may be necessary to prevent over-oxygenation. Based on these results, it was deemed necessary to repeat selected treatments that showed promise, either for improving wine TAC while retaining sensory quality, or for retaining TAC while improving sensory quality. The pumping-over treatment was repeated to confirm results obtained previously as this is a mixing technique commonly used for commercial vinification. This also allowed for direct comparison of treatments as grapes from the same vineyard and harvest date were used.

Commercial tannin preparations of different origin and composition can be used during or after fermentation to stabilise the wine colour and to protect wine from oxidation. Two groups of commercial tannins are commonly used, namely grape tannins consisting mainly of condensed tannins (oligomeric and polymeric proanthocyanidins) (Vivas et al., 1996) and oak tannins consisting of both condensed tannins and hydrolysable tannins (gallotannins and ellagitannins) (Puech et al., 1999). Grape skin and seed tannins added to wine before or after fermentation have been reported to improve wine colour, as well as to increase the polymeric pigment and tannin content (Obradovic et al., 2005). Similar results were reported for a preliminary study using various commercial tannins (Keulder et al., 2004). On the other hand, Bautista-Ortín et al. (2005) reported that gallotannins and condensed tannins were detrimental to sensory quality and colour. Condensed tannins can react with anthocyanins producing polymeric pigments with more stable colour than monomeric anthocyanins (Remy et al., 2000). Ellagitannins and their hydrolysis products are powerful oxidation regulators facilitating acetaldehyde-mediated condensation reactions between anthocyanins and proanthocyanidins which result in more stable polymeric pigments (Vivas & Glories, 1996), while the hydrolysis product of gallotannins (gallic acid) can participate in co-pigmentation (Singleton & Trousdale, 1983). By affecting the phenolic composition of red wines commercial tannins can therefore also have an impact on their total antioxidant capacity.

Maceration is an important part of red wine production as phenolic compounds such as flavan-3-ols, flavonols and anthocyanins are extracted from the grape skins and seeds (Ribéreau-Gayon et al., 1999). The anthocyanins, hydroxycinnamic acids and flavonols usually show a biphasic trend during fermentation with increased extraction up to a point whereafter decreased concentrations are observed (Yokotsuka et al., 2000; Zou et al., 2002), while gallic acid, flavan-3-ols and tannins only show increased concentrations during fermentation (Kovac et al., 1992; Yokotsuka et al., 2000; Zou

et al., 2002). Extended maceration is generally used to produce wines more suitable for maturation purposes, i.e. wines with high concentrations of oligomeric and polymeric flavan-3-ols, as well as total phenols (Yokotsuka et al., 2000; Gómez-Plaza et al., 2001; Vrhosek et al., 2002; Zimman et al., 2002; Zou et al., 2002). The optimal time of extended maceration will depend on the grape cultivar and grape phenolic composition. Extended maceration has been shown to improve the antioxidant capacity of various red wines (Echeverry et al., 2005; Villaño et al., 2006).

The aim of the study is to evaluate the effect of pumping-over as juice/skin mixing technique, addition of two types of commercial tannins, oxygenation and extended maceration on the phenolic composition, total antioxidant capacity, colour and sensory quality of Pinotage wine in comparison to wine produced according to the standard vinification protocol. The standard vinification protocol excludes the use of added tannins and oxygenation, use punching-down as juice/skin mixing technique and subjects wine to a short maceration period.

Materials and Methods

Wine-making Procedures

Pinotage grapes were harvested from the same vineyard at the Nietvoorbij farm (Stellenbosch, South Africa) at ~23 to 24 °B during the 2004 vintage and wines produced in the experimental cellar of the ARC Infruitec-Nietvoorbij (Stellenbosch, South Africa) according to the standard wine-making protocol with no wood contact as described in **Chapter 3**. **Figure 1** outlines the differences between the wine-making processes for the control and other treatments. The control treatment followed the standard protocol, which uses punching-down mixing. The pumping-over treatment (imitated by racking the wine into buckets and pouring the juice over the cap) was carried out three times daily. Commercial tannin extracts from French oak (Laffort Oenologie & Cie, Bordeaux, France) and from grape seeds (Laffort Oenologie & Cie) were added at 300 mg/L after crushing of the grapes as recommended by the manufacturer. Extended maceration was carried out by leaving the wine on the skins and seeds after completion of fermentation for a total of 15 days before pressing. The other treatments, including the control, were pressed at ~0 °B after 5 days of fermentation. Wine for the oxygenation treatment was produced as for the standard protocol, but after cold-stabilisation, the wine was filtered into 20 L capacity closed stainless steel canisters. Oxygenation was carried every two weeks for two months, when compressed medical air (Afrox, Johannesburg, South Africa) was delivered using a gas diffuser until wine oxygen concentrations reached 1 mg/L. Oxygen concentration was measured using an Oxi 330 Set oxygen analyzer with a CellOx 325 probe (WTW, Weilheim, Germany). Each treatment was carried out with 20 – 30 kg of grapes in triplicate. Eight months after production, aliquots of each wine were frozen at -20 °C, to

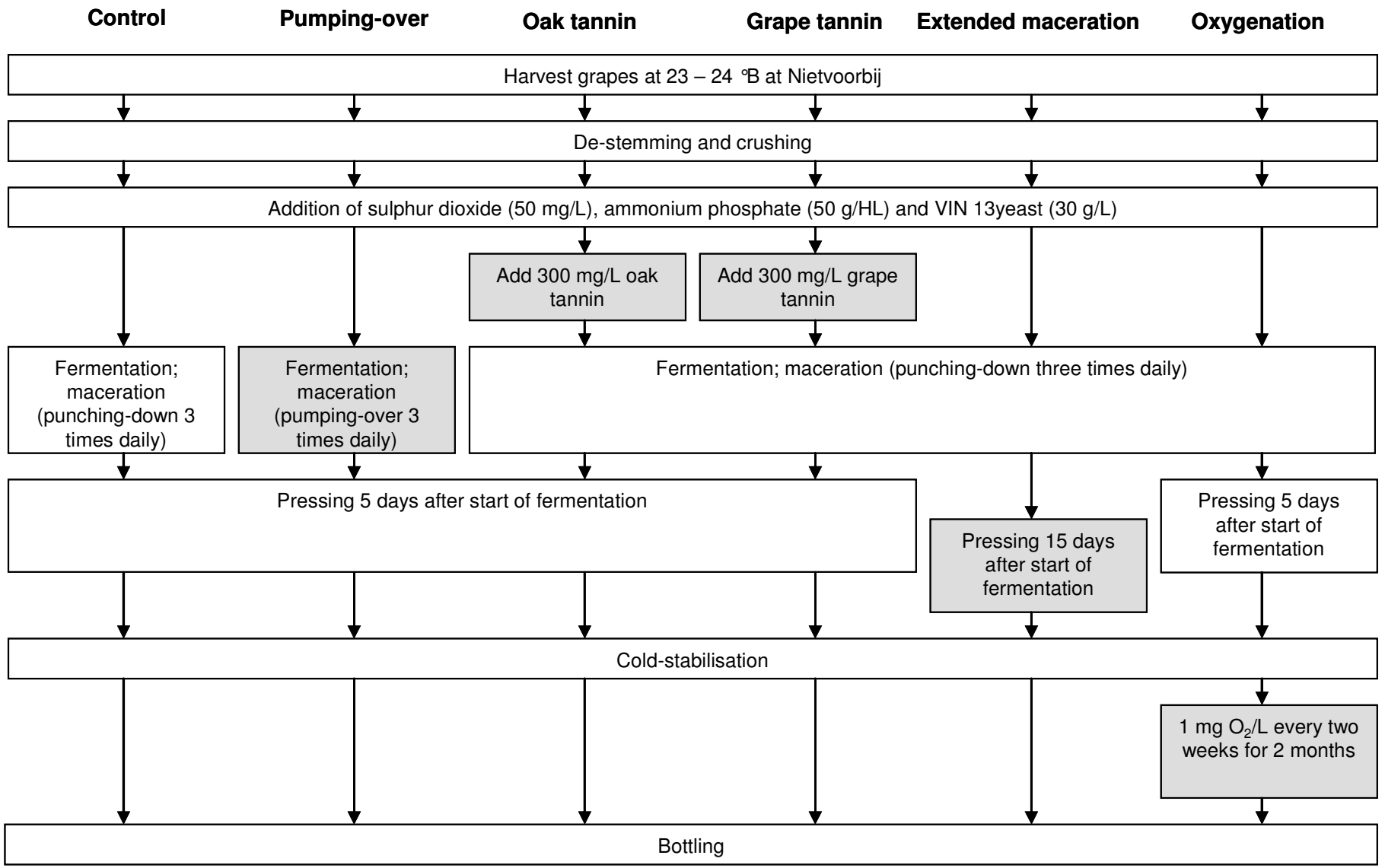

Figure 1. Diagram of enological treatments applied during and after fermentation (operations in grey blocks indicate where treatments differ from the control).

prevent further phenolic changes, until analyses could be carried out. Samples were analysed immediately after defrosting.

Chemicals and Phenolic Reference Standards

Chemicals and phenolic reference standards used for phenolic and antioxidant analyses were described in **Chapters 3** and **4**.

Spectrophotometric Analysis of Phenolic Content

Wines were subjected to spectrophotometric analysis of the major phenolic groups. Total phenol, total flavan-3-ol, as well as, monomeric, polymeric and total anthocyanin contents were determined in triplicate using the Folin-Ciocalteau (Singleton & Rossi, 1965), dimethylamino-cinnamaldehyde (DAC) (McMurrough & McDowell, 1978) and pH shift (Ribéreau-Gayon & Stonestreet, 1965) assays, respectively, as modified and described in **Chapter 4**.

HPLC Analysis of Phenolic Composition

Individual wine phenolic compounds, as well as coloured and non-coloured polymers detected at 520 and 280 nm, respectively, were quantified in duplicate using an HPLC method (Peng et al., 2002), modified and described in **Chapter 3**. Polymers included polymeric phenolic compounds with 5 or more subunits, consisting of anthocyanins and flavan-3-ols for coloured polymers, and only flavan-3-ols for non-coloured polymers.

ABTS Radical Cation Scavenging Assay

The total antioxidant capacity (TAC) of the wines and the commercial tannin preparations was measured (TAC_M) in triplicate using the $ABTS^{\bullet+}$ scavenging assay (Re et al., 1999). The content of individual phenolic compounds, measured by HPLC, and their experimental TEAC values (reported in **Chapter 3**) were used to calculate the theoretical TAC (TAC_{CAL}) of the wines. The remaining TAC (TAC_R) is the difference between TAC_M and TAC_{CAL}. Analysis and calculations were carried out as described in **Chapter 3**.

Objective Colour Parameters

The objective colour parameters of wines were measured in duplicate on the CIELab scale, namely a^* (red/green chromaticity), b^* (yellow/blue chromaticity) and L^* (lightness), and the C^* (chroma) and h^* (hue angle), calculated as described in **Chapter 4**. Names for hues were adapted from Gonnet (1999) based on the h^* values and are described in **Chapter 4**.

Sensory Analysis

Sensory analysis of the wines was performed as described in **Chapter 6**.

Statistical Analysis

Analysis of variance was performed on the means for different treatment combinations to determine whether significant differences occurred. The Student *t*-LSD test ($P < 0.05$) was used to determine the statistical differences between means. Analysis of variance and difference testing was done using the SAS version 8 software package (SAS Institute Inc., Cary, NC).

Results

The actual values for all determinations, as well as data not shown here, are reported in **Addendum F**. Only significant ($P < 0.05$) differences between values will be discussed.

Phenolic Composition

The phenolic composition of the wines, produced according to different treatment protocols, expressed as a percentage difference from the control wine is given in **Figures 2** and **3**. The respective treatments had little effect on the anthocyanin content of the wine. The coloured polymer content (HPLC) was affected only by the pumping-over treatment, resulting in wine with a lower content than the control. In terms of individual anthocyanins, the extended maceration treatment gave wine with lower delphinidin-3-glucoside (glc), peonidin-3-glc and vitisin A contents than the control wine. No significant differences between treatments were observed for the malvidin-3-*p*-coumaroylglucoside (glc-coum) content of the wines (data not shown).

Extended maceration also lowered the unknown flavonol, quercetin-3-glc and total flavonol contents of the wine compared to the control wine (**Figure 3**). The pumping-over treatment resulted in wine with a lower quercetin-3-rhamnoside (rham) content than the control wine. The quercetin content of the wine was not affected by the treatments (data not shown).

Both the oak tannin addition and extended maceration treatments resulted in wines with a higher gallic acid content than the control wine, with the extended maceration treatment resulting in the highest content (**Figure 3**). The caffeoyltartaric (caftaric), *p*-coumaroyltartaric (coutaric) and total phenolic acid contents of the wine were increased by the pumping-over and decreased by the extended maceration treatments, compared to the control treatment. No significant differences in caffeic or *p*-coumaric acid contents were observed between the wines (data not shown).

The total flavan-3-ol content (DAC) of the wine subjected to the pumping-over treatment was lower than that of the control wine, but its (+)-catechin content was higher (**Figure 3**). The (+)-catechin and procyanidin B1 contents of the wine made with added grape tannin and extended maceration were higher than that of the control wine. The pumping-over treatment also resulted in wines with a lower non-coloured polymer content and total phenol content than the control wine.

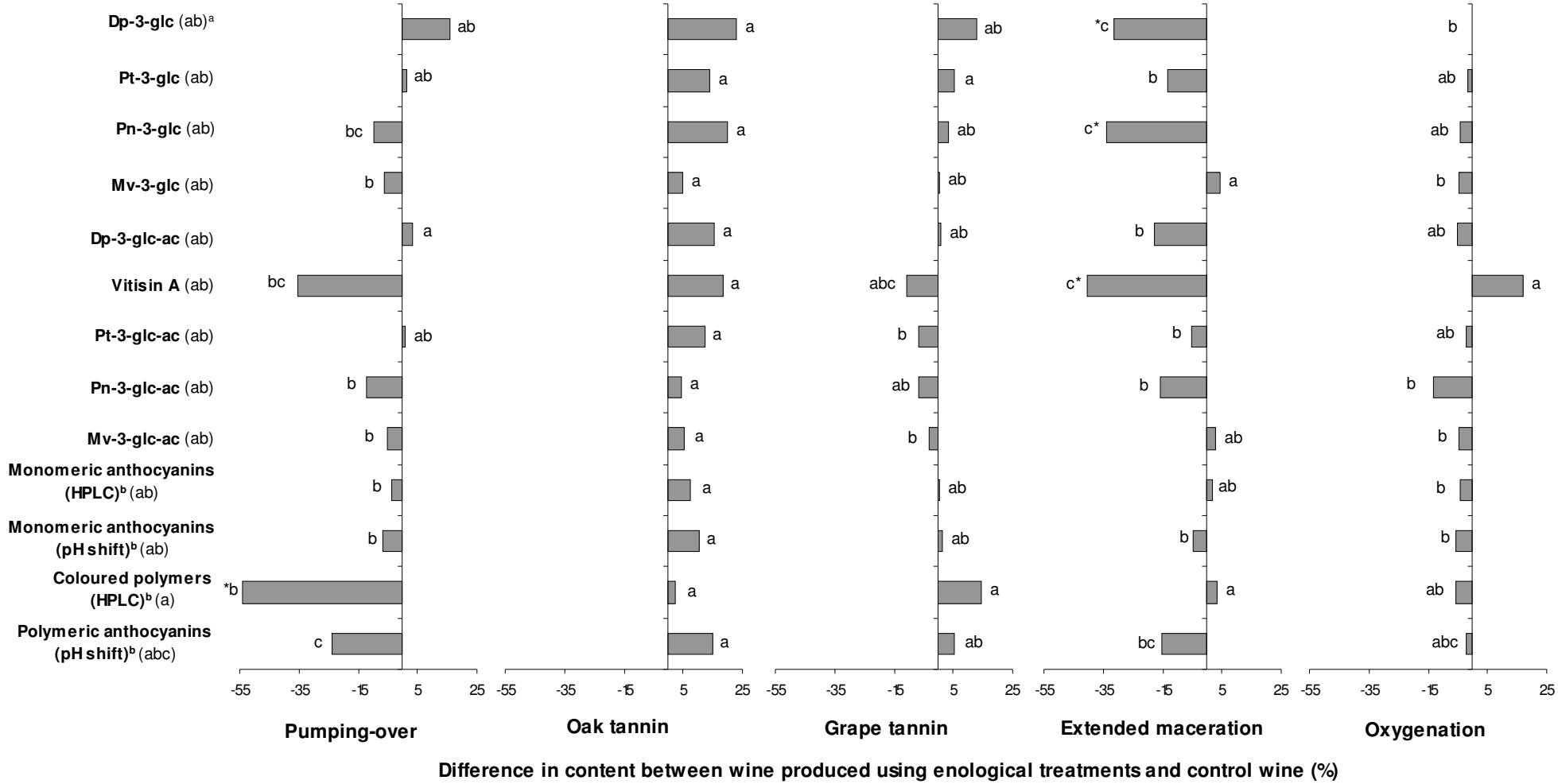

Figure 2. The percentage differences in anthocyanin content between Pinotage wines produced according to different enological protocols compared to the control wine [[a] different letters on the bars for the treated wines and the control wine, indicated next to the parameter name, denote significant differences ($P < 0.05$); [b] sum of phenolic group content measured by HPLC; [c] measured using the pH shift assay; [d] measured using HPLC; * indicate significant ($P < 0.05$) differences from control wines; Dp = delphinidin; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Mv = malvidin; Pn = peonidin; Pt = petunidin].

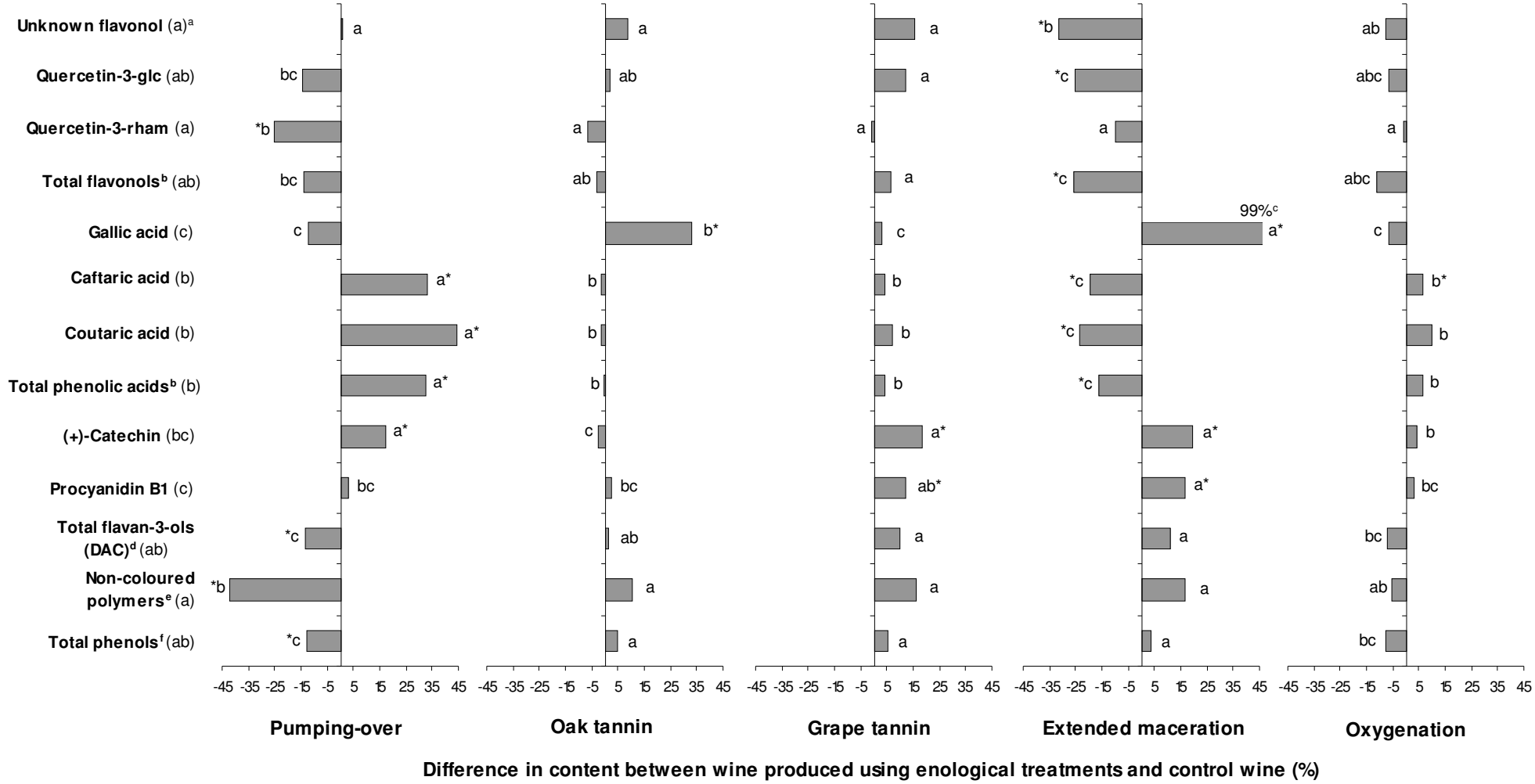

Figure 3. The percentage differences in flavonol, flavan-3-ol, polymer and total phenol content between Pinotage wines produced according to different enological protocols compared to the control wine [[a] different letters on the bars for the treated wines and the control wine, indicated next to the parameter name, denote significant differences ($P < 0.05$); [b] sum of phenolic group content measured by HPLC; [c] values exceed scale; [d] measured using the DAC assay; [e] non-coloured polymers measured by HPLC; [f] measured using the Folin-Ciocalteau assay; * indicate significant ($P < 0.05$) differences from control wines].

Table 1. Total antioxidant capacity (TAC) and total phenol content (TP) of oak and grape tannin extract and their respective theoretical contribution to the wine TAC and TP content.

Commercial tannin	TAC[a]	Contribution to wine TAC[b]	TP[c]	Contribution to wine TP[d]
Oak tannin	5.08 (± 0.04)[e]	1.52 (± 0.01)	683.9 (± 7.2)	205.2 (± 2.2)
Grape tannin	4.80 (± 0.12)	1.44 (± 0.04)	593.5 (± 6.3)	178.1 (± 1.9)

[a] TAC expressed as mmoles Trolox/g tannin extract; [b] amount of TAC in mM Trolox theoretically contributed to the wine TAC by adding 300 mg/L tannin extract to ~20 L of must; [c] TP content expressed as mg gallic acid equivalents/g tannin extract; [b] amount of TP content in mg gallic acid equivalents/L theoretically contributed to the wine TP content by adding 300 mg/L tannin extract to ~20 L of must; [e] SD.

No significant differences between treatments were observed for the total monomer content (HPLC) of the wines (data not shown). The oak and grape tannin had similar total phenol contents (**Table 1**).

Antioxidant Capacity

The pumping-over wine resulted in wine with lower TAC_M and TAC_R values than the control wine (**Figure 4**). The oak and grape tannin extracts had similar antioxidant potency (**Table 1**). The pumping-over treatment resulted in wine with higher phenolic acid contribution to the TAC_{CAL}, while grape tannin addition and extended maceration resulted in wines with higher flavan-3-ol contribution to the TAC_{CAL} (**Figure 5**). These differences in the contribution of different phenolic groups to the TAC_{CAL} between wines did not result in differences in TAC_{CAL}.

Objective Colour Parameters and Sensory Quality

The extended maceration treatment resulted in wine with lower C^* and a^* and higher L^* values than the control wine (**Figure 4**). The oxygenation and pumping-over treatments gave wines with higher and lower h^* and b^* values than the control wine, respectively.

In terms of sensory quality, the colour acceptability, berry/plum intensity and overall quality scores, the pumping-over treatment resulted in wine with lower scores than that of the control wine. In addition to these characteristics, the fullness scores of wines, was lowered when using the extended maceration and oxygenation treatments (**Figure 4**). The astringency scores of the treated wines did not differ from that of the control wine. None of the wines had unacceptably low scores for any of the sensory quality parameters (data not shown).

Discussion

In **Chapter 5**, the pumping-over and punching-down treatments showed varying trends, in terms of the phenolic composition of wines, depending on the vintage. This would also affect the complex reactions taking place during vinification. The trends obtained here for the 2004 wine

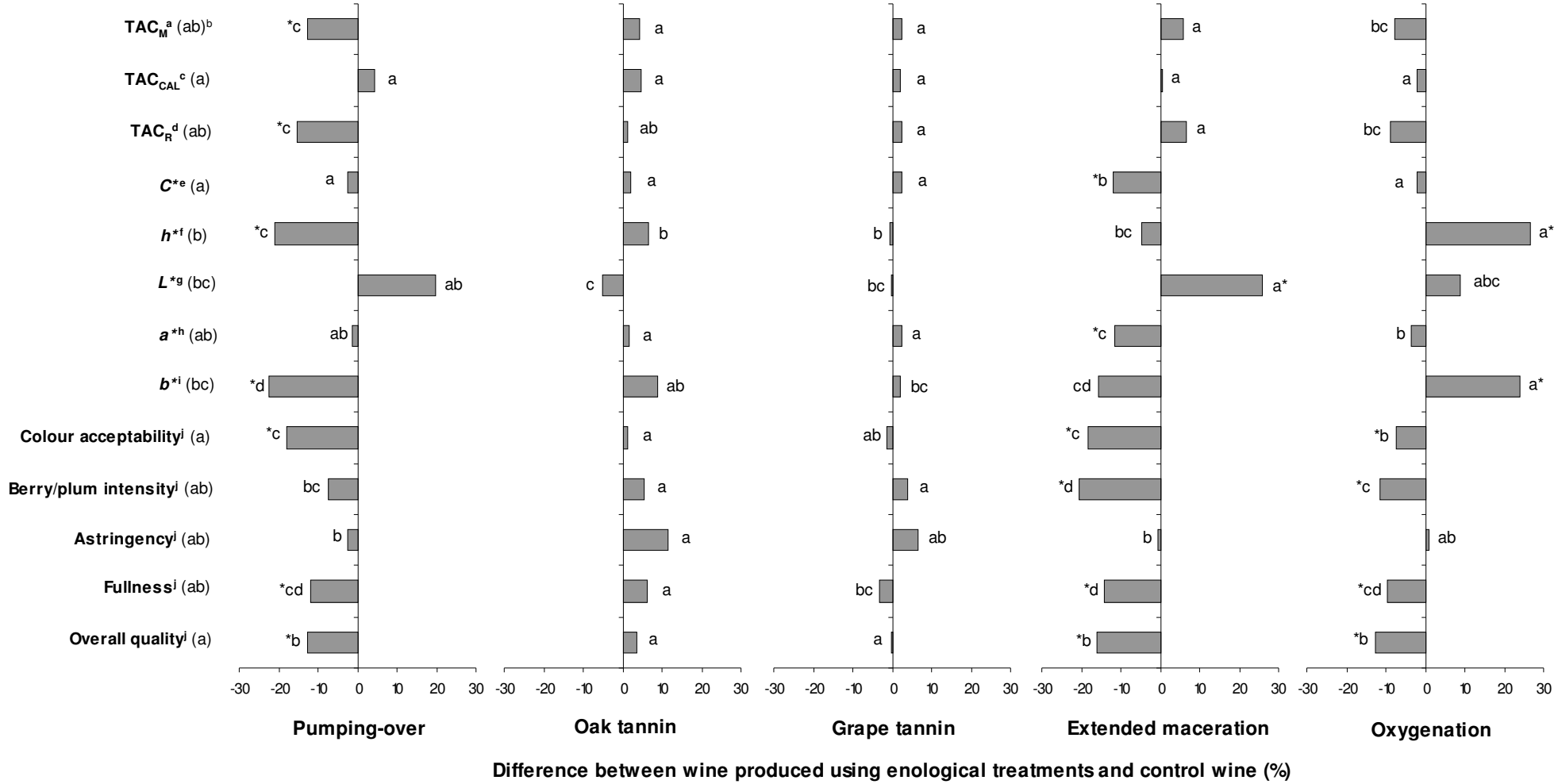

Figure 4. The percentage differences in total antioxidant capacity, colour and sensory quality between Pinotage wines produced according to different enological protocols compared to the control wine [[a] total antioxidant capacity as measured; [b] different letters on the bars for the treated wines and the control wine, indicated next to the parameter name, denote significant differences ($P < 0.05$); [c] total antioxidant capacity as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [d] $TAC_R = TAC_M - TAC_{CAL}$; [e] chroma (colour saturation); [f] hue angle (°); [g] lightness; [h] red/green chromaticity; [i] yellow/blue chromaticity; [j] determined using sensory analysis; * indicate significant ($P < 0.05$) differences from control wines].

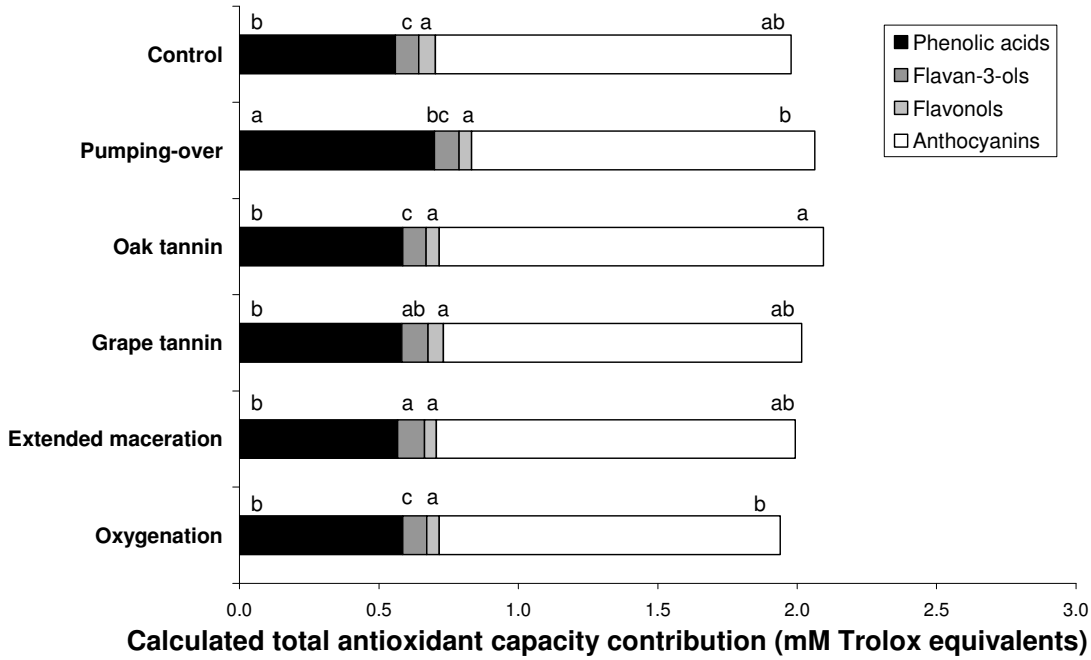

Figure 5. Calculated total antioxidant capacity contributions of phenolic groups for different enological treatments [different letters for the contribution of a specific phenolic group denote significant differences ($P < 0.05$)].

produced according to the pumping-over protocol, i.e. lower concentrations of coloured and non-coloured polymers, quercetin-3-rham, total flavan-3-ols (DAC) and total phenols than the control wine (punching-down treatment), are similar to that obtained for the 2002 vintage (**Chapter 5**). The less aggressive mixing action obtained by pumping-over compared to punching-down explains the resulting lower phenolic content, further contributing to the lower TAC_M. The total phenol content and TAC_M values showed similar trends. The low TAC_M of pumping-over wines were mostly due to a lower polymer content, including coloured polymers (HPLC) and non-coloured polymers, and possibly unknown compounds as the TAC_R (but not the TAC_{CAL}) of pumping-over wines were lower than that of the control wine. Pumping-over also resulted in wines with lower h^* than the control wine, due to lower b^*. The lower concentrations of total flavan-3-ols (DAC) and quercetin-3-rham, together with the unaffected monomeric anthocyanin content, would explain the lower b^* values for wine produced according to the pumping-over protocol. The control wine had a magenta-red hue, while the pumping-over treatment resulted in a red-magenta wine. The low colour acceptability scores also observed for these wines could be related to these objective colour characteristics. Results obtained for the sensory quality parameters confirmed those reported by Marais (2003).

Addition of tannin preparations did not affect the monomeric or polymeric anthocyanin contents of Pinotage wine, despite previous reports that commercial tannins can stabilise colour by facilitating polymerisation of anthocyanins (Vivas & Glories, 1996; Remy et al., 2000; Keulder et al., 2004; Obradovic et al., 2005). The increased gallic acid content of the wine with added oak tannin can be explained by hydrolysis of galloylated flavan-3-ols (Singleton & Trousdale, 1983) from the oak wood extract (Puech et al., 1999). The grape tannin preparation consists mostly of condensed tannins, such as procyanidin B1, which would explain the increased procyanidin B1 content of the wine. Both (+)-catechin and procyanidin B1 content of the wine increased when grape tannin was added, possibly due to self-dissociation of oligomeric proanthocyanidins (Haslam, 1980). However, addition of grape tannin did not result in higher total flavan-3-ol content (DAC) of the wine, compared to the control wine. The addition of oak tannin, on the other hand, had no effect, possibly due to a lower concentration of condensed tannins in the extract (Puech et al., 1999).

No significant difference in TAC_{CAL} was observed between wines with added grape tannin compared to the control wine, although addition of grape tannin increased the contribution of flavan-3-ols to the TAC_{CAL}. Both tannin treatments resulted in wines with slightly higher TAC_M than the control wine, but it was not significant, despite the increased concentrations of some phenolic compounds. Similar trends were also obtained for the total phenol content of the wines. From the TAC of the oak and grape tannin extracts and the amount added to the wine, an increase in TAC_M of the wines of 1.52 and 1.44 mM Trolox, respectively, would have been realistic if all the phenolic compounds were present in the wine unchanged. Similarly an increase in total phenol content of 205.2 and 178.1 mg gallic acid equivalents/L should be expected for addition of oak and grape tannin, respectively, while only a small non-significant increase in total phenol content of the wines was observed. The TAC_M and total phenol content of the control wine were 13.33 mM Trolox and 1808.3 mg gallic acid equivalents/L, respectively (data not shown). However, as the tannin extract was added before fermentation, some of the phenolic compounds may have been adsorbed onto the grape solids and were subsequently removed during pressing. A similar effect was seen for malvidin-3-glc added to fermenting grape must, showing a marked decrease in solution 24 h after addition (Zimman & Waterhouse, 2004). The possibility of degradation of added tannins can not be ruled out.

Both tannin treatments gave wines with favourable objective colour parameters and sensory quality, although not significantly different from the control. The higher concentrations of gallic acid and the flavan-3-ols, (+)-catechin and procyanidin B1, in wines with added oak and grape tannin, respectively, were therefore not enough to significantly affect co-pigmentation and influence colour perception.

Previous reports suggested that extended maceration should produce wines with increased gallic acid, flavan-3-ols and tannin contents (Kovac et al., 1992; Yokotsuka et al., 2000; Zou et al., 2002), while monomeric anthocyanin, flavonol and hydroxycinnamic acid contents may be decreased (Yokotsuka et al., 2000; Zou et al., 2002). In the present study, extended maceration had similar effects for gallic acid, (+)-catechin, and procyanidin B1, as well as some flavonols and other phenolic acids, compared to the control wine. However, only the delphinidin-3-glc, peonidin-3-glc and vitisin A contents of wine decreased as a result of extended maceration. Despite the phenolic compositional differences between these wines, their effect on TAC_M or TAC_{CAL} was not substantial enough to result in significantly lower TAC values for the wines subjected to extended maceration. This is in contrast to previous reports (Echeverry et al., 2005; Villaño et al., 2006) showing increased antioxidant capacity for red wines made with extended maceration compared.

The objective and sensory colour of wines deteriorated as a result of extended maceration, with wines being lighter (higher L^*), with less colour saturation (lower C^*) and lower a^* values. These trends are the result of the lower delphinidin-3-glc, peonidin-3-glc and vitisin A contents. Extended maceration also resulted in wine with lower contents of some co-pigments, namely some flavonols and phenolic acids, but higher levels of gallic acid, (+)-catechin and procyanidin B1. As opposite effects were obtained for co-pigments, and the individual effect of the compound will depend on its structure and concentration, it is not possible to predict whether co-pigmentation will increase or decrease. Extended maceration resulted in wine produced with a similar magenta-red hue as the control wine. However, since extended maceration was also detrimental to other sensory attributes, such as berry/plum intensity, fullness and overall quality, this practice as performed under the conditions of the present study is not beneficial for producing Pinotage wines of high quality and antioxidant capacity. Shorter extended maceration periods may, however, be beneficial.

Results obtained for the 2004 oxygenated wine (1 mg O_2/L every two weeks for 2 months) is in contrast to the trends observed for the 2003 wines, produced with a modified oxygenation protocol, when higher oxygen doses were used (2.5 mg O_2/L/month and 5 mg O_2/L/month every month for 2, 4 or 6 months) (**Chapter 6**). As a result of the phenolic composition of the 2004 oxygenated wine being similar to that of the control wine, no significant differences in TAC_M, TAC_{CAL} or the contribution of any phenolic group to the TAC_{CAL} were observed between the oxygenated and control wines. The oxygenation protocol that was carried out in 2003, on the other hand, resulted in decreased monomeric anthocyanin and flavan-3-ols contents, as well as lower contents of some flavonols, leading to decreased TAC_M and TAC_{CAL} even for the lowest oxygen concentration (2.5 mg O_2/L/month) for the shortest time (2 months), compared to the control wine. The oxygenated wine presented a magenta-red colour closer to red than the hue of the control wine due to higher b^* as was also observed for all oxygenated wines in 2003. These objective colour parameter trends

resulted in lower colour acceptability scores for oxygenated wines, compared to the control wine. In contrast, increased colour acceptability was obtained for all oxygenated wines in 2003, compared to the control wine, although the oxygenated wines of 2003 also had a darker colour (lower *L**) and less red (lower *a**) than the control wine. The oxygenation protocol followed in 2004 was less detrimental to the phenolic content of wines than that used in 2003 with no loss of TAC, but lower sensory quality scores (berry/plum intensity, fullness and overall quality) were still observed. It seems that a small difference in oxygenation dose can have a large effect as was observed for the results obtained by using 2.5 mg O_2/L/month or 2.0 mg O_2/L/month in 2003 and 2004, respectively.

Conclusions

None of the treatments investigated, increased the TAC of Pinotage wines, while the pumping-over treatment decreased the TAC when compared to the control. Tannin addition treatments, however, showed promise with increased concentrations of flavan-3-ols and good colour and sensory quality. Higher doses or addition of tannins after fermentation may therefore be more advantageous in terms of increasing the phenolic content, TAC, colour and sensory quality parameters of Pinotage wine. The period of extended maceration used, on the other hand, may have been too long resulting in losses of some phenolic compounds and sensory quality. Optimising the extended maceration period may lead to wines with increased phenolic content and TAC, while the sensory characteristics should be carefully monitored to ensure quality. The oxygenation protocol used was better than the previous protocols investigated as phenolic content and TAC were not lost. The decrease in sensory quality scores, however, remains a problem. More research is needed to find the optimal oxygenation protocol and to the retention of sensory quality, while increasing TAC.

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Chapter 8: General Discussion and Conclusions

Growing consumer awareness of the potential health benefits of dietary antioxidants suggests that antioxidant capacity may become an important quality parameter for foods and beverages. Enhancement of the antioxidant capacity of specific wines is an opportunity to ensure greater local and international market shares for such wines. Consequently, the aim of this study was to provide guidelines for the production of Pinotage wines with optimal total antioxidant capacity, while retaining sensory quality. The first objective was to elucidate the contribution of individual phenolic compounds to the total antioxidant capacity (TAC) of Pinotage wines. The second objective was to determine the effect of climatic region, vine structure and various enological practices on the phenolic composition, antioxidant capacity and colour of Pinotage wines. Enological techniques investigated included pre-fermentation maceration, juice/skin mixing techniques, addition of commercial tannins and extended maceration, as well as different maturation treatments, namely maturation in oak barrels, the use of alternative oak products in old barrels and oxygenation in stainless steel canisters. To ensure that conventional wine quality as perceived by the consumer, remains acceptable, the sensory quality of the wines was also taken into account. Many studies have reported the effects of various viticultural and enological techniques on the phenolic composition and quality of red wines, but few considered the effect on wine antioxidant capacity. This is also the first study specifically focussing on the effect of these processes on the antioxidant capacity of Pinotage wine, a major South African cultivar wine.

The 2,2'-azino-di(3-ethylbenzo-thiazoline-sulphonic acid radical cation ($ABTS^{\bullet+}$) scavenging assay and HPLC was chosen to measure the TAC and phenolic composition (in terms of individual phenolic compounds) of a series of young Pinotage wines, respectively. The contribution of the quantified compounds to the wine TAC was calculated using their measured Trolox Equivalent Antioxidant Capacity (TEAC) values and their content in the wines. The TEAC values of quercetin-3-galactoside, isorhamnetin and peonidin-3-glucoside were reported for the first time. TEAC values observed for most compounds were much lower than previously reported (Re et al., 1999; Baderschneider & Winterhalter, 2001). Only the TEAC values observed for gallic acid, caftaric acid, caffeic acid and kaempferol were consistent with values reported by Re et al. (1999) and Baderschneider and Winterhalter (2001). These differences in observed values are attributed to differences in assay protocols and highlight the need for standardised assay protocols, especially when used for product specifications and in marketing. For this reason, the same protocol was used to analyse the wine and the phenolic compounds.

The individual compounds with the largest contributions to the wine TAC were caftaric acid and malvidin-3-glucoside, due to their high concentrations in the wines. The content of monomeric phenolic compounds and procyanidin B1 in Pinotage wines, however, only explained a small amount (between 11 and 24%) of the wine TAC. The remaining TAC was attributed to oligomeric phenolic, polymeric phenolic and unknown compounds. Some synergy was also demonstrated in the present study when three mixtures of monomeric phenolic compounds (in concentrations typically expected in Pinotage wine) were analysed. Simple addition of TAC contributions as calculated for the wine may therefore not be appropriate. Masking of antioxidant capacity by proteins is another possible problem with this method.

The climatic region and vine structure treatments affected the phenolic composition, antioxidant capacity and objective colour parameters of Pinotage wines, while cordon height of the vine had few significant effects. The warmer climatic regions produced lighter coloured wines with lower TAC than the cooler regions. The concentrations of relatively potent antioxidants, i.e. anthocyanin monoglucosides, flavonols and flavan-3-ols, as well as the tartaric acid esters of hydroxycinnamic acids with lower potency, generally increased as the climatic region became cooler. On the other hand, the concentrations of acylated derivatives and free hydroxycinnamic acids decreased, contributing to lower TAC values. The decrease in the concentration of these compounds only partly explained the decrease in TAC, since unknown compounds are mostly responsible for the variation in the wine TAC. The sensory quality and colour of the wines produced from the warmer region were still acceptable, although an increase in aromas not typical of Pinotage wines were observed (Danie van Schalwyk, ARC Infruitec-Nietvoorbij, personal communication, 2005). From these results it is clear, that different climatic regions produce wines with significantly different characteristics. Canonical discriminant analysis of the data also confirmed this observation. The effect of climate on the phenolic acids content have not been reported, while the accumulation of flavonols in grape berries was mostly investigated in terms of the effects of sunlight exposure (Crippen & Morrison, 1986; Spayd et al., 2002). This is the first time that the effect of climatic region on the TAC of wines has been reported.

Wines from bush vines were darker resulting in higher colour acceptability scores. This is attributed to higher concentrations of co-pigments, i.e. flavonols, gallic acid and flavan-3-ols, in wines made from bush vines as opposed to those from trellised vines, although lower concentrations of some anthocyanin monoglucosides and acylated derivatives, as well as non-coloured polymers, were observed. Co-pigmentation thus negated the loss in colour due to lower concentrations of these anthocyanins. An increase in total phenol content of wines from bush vines compared to wines from trellised vines and a slightly higher TAC was the result of changes in individual phenolic compound contents. Unknown compounds contributed significantly to the differences in

TAC between wines from different vine structure treatments as was the case with different climatic regions. Once again, canonical discriminant analysis confirmed the observations. The effect of vine structure on the TAC of wines has not been reported previously.

Pre-fermentation maceration at 10 and 15 °C for 1, 2 and 4 days showed potential to improve the colour of Pinotage wines, but it did not affect the TAC or phenolic content in a consistent manner, with most treatments resulting in no differences compared to the control wines. The redness value (a^*) of the wine was slightly, but not significantly, increased for some wines made with pre-fermentation maceration compared to the control wine. Pre-fermentation maceration increases the colour and anthocyanin content of red wines (Heatherbell et al., 1997; Reynolds et al., 2001; Gómez-Míguez et al., 2006), but the effect of pre-fermentation maceration on wine TAC has not been reported to date. Since this technique is not detrimental to wine TAC and could be used to increase the quality of Pinotage wines (Marais, 2003a), it could be worthwhile for winemakers to introduce pre-fermentation maceration as part of their standard winemaking protocol.

Both punching-down and rotor juice/skin mixing techniques resulted in wines with higher TAC and phenolic content than the pumping-over treatment in two out of the three vintages investigated, although trends for phenolic composition were different between vintages. Pumping-over also resulted in wines with lower sensory quality scores (Marais, 2003b), as well as less favourable values for objective colour parameters. However, all the mixing techniques were suitable for producing high quality wines when taking all the objective quality parameters into account, while high sensory quality scores were demonstrated previously (Marais, 2003b). This technique is therefore not recommended if the winemaker aims at producing a wine with enhanced TAC. The effects of juice/skin mixing technique on wine composition may be dependent on cultivar (Leone et al., 1983; Fischer et al., 2000). The frequency of mixing increased the measured TAC of only one vintage, while the content of most phenolic compounds was not affected. This is the first time that the effect of juice/skin mixing techniques on wine TAC has been reported.

Oak maturation using traditional (new, second fill and third fill barrels) and alternative (chips, staves, oak extract and oak dust) oak treatments had improved Pinotage wine colour. All the traditional and alternative oak treatments resulted in wines with good sensory quality (Fourie, 2005). Losses in TAC induced by decreased concentrations of monomeric phenolic compounds (anthocyanins, flavan-3-ols, flavonols and hydroxycinnamic acids) during oak maturation were negated by increased concentrations of gallic acid and new oligomeric and polymeric pigments formed. The increased gallic acid content of wines after oak maturation can be explained by hydrolysis of ellagitannins from oak wood in the hydroalcoholic medium, or the hydrolysis of galloylated flavan-3-ols extracted from grape seeds during fermentation. New oligomeric and polymeric pigments are formed from direct or acetaldehyde-mediated condensation of monomeric

anthocyanins and flavan-3-ols during oak maturation. Oak maturation caused slight changes in the hue of the wine towards magenta-red, during the initial period for some treatments, which is attributed to the formation of purple acetaldehyde-mediated condensation products. The subsequent reversal towards orange-red is attributed to formation of orange-red pyranoanthocyanins or further reaction of ethyl-linked pigments to form larger brown polymers or yellow xanthylium pigments. The use of alternative oak products in old barrels, as well as traditional oak barrels, during maturation did not have a detrimental effect on the TAC of Pinotage wine, but also did not achieve increased levels.

Oxygenation of Pinotage wine was investigated at doses of 2.5 mg O_2/L and 5.0 mg O_2/L delivered at monthly intervals for 2, 4 and 6 months. Oxygenation at the lower dosage for 2 months had improved Pinotage wine colour and sensory quality, but resulted in some loss of TAC. Care should, however, be taken not to over-oxidise the wine, as the treatments with the higher oxygen dosage and longer duration not only reduced the phenolic content and TAC of the wines, but were also severely detrimental to the sensory quality. A loss of wine TAC was observed for all oxygenation levels and periods, despite increased concentrations of gallic acid. Oxygenation is expected to facilitate direct and acetaldehyde-mediated anthocyanin-flavan-3-ol condensation reactions, as is the case for oak maturation. However, the TAC contribution of oligomeric and polymeric phenolic compounds and unknown compounds decreased during oxygenation. Degradation of phenolic compounds due to over-oxidation is possibly the cause of this phenomenon. Oxygenation resulted in increased wine chroma (C^*) corresponding to an increase in wine colour density (Atanasova et al., 2002), while decreased wine colour density have also been observed (Castellari et al., 2000; Du Toit & Groenewald, 2003). Changes in wine hue were initially from magenta-red to pure red with a subsequent change back to magenta-red similar to previous results (Du Toit and Groenewald, 2003). It thus seems that the first phase of oxygenation is characterised by the formation of orange-red pyranoanthocyanins, while the second phase is characterised by the formation of purple-red acetaldehyde-mediated anthocyanin-flavan-3-ol condensation products. This is in contrast to the trend observed for the changes in wine hue during oak maturation. In a follow-up trial, oxygen addition at a dose of 1 mg O_2/L delivered every two weeks for 2 months was less detrimental to the wine phenolic composition and TAC than all previous oxygenation treatments. Notwithstanding this, a decrease in sensory quality scores was still observed, clearly indicating that more research is needed to find an optimal oxygenation protocol which will preserve sensory quality and wine TAC, while improving wine colour.

Addition of commercial tannins (from oak and grape origin) before fermentation, at the dosage recommended by the manufacturer, increased the gallic acid and flavan-3-ol concentrations, although the levels obtained were still well within the normal range for Pinotage wines observed in

this study. The wine TAC was not increased despite these changes in phenolic composition. No effect in terms of colour and anthocyanin composition of the wine was observed, despite claims by the manufacturer that the tannin preparations are to be used to stabilise wine colour. Commercial tannins stabilise wine colour by facilitating polymerisation of anthocyanins (Vivas & Glories, 1996; Remy et al., 2000; Keulder et al., 2004; Obradovic et al., 2005). It may be worthwhile in future to investigate the use of higher doses, or addition of tannins after fermentation, which may be more advantageous in terms of increasing the phenolic content, TAC, colour and sensory quality parameters of Pinotage wine.

Extended maceration for 11 days after completion of fermentation was also investigated as a potential vinification technique to increase phenolic extraction and thus the wine TAC. Extended maceration produces wines with increased gallic acid, flavan-3-ols and tannin contents (Kovac et al., 1992; Yokotsuka et al., 2000; Zou et al., 2002), while monomeric anthocyanin, flavonol and hydroxycinnamic acid contents are decreased (Yokotsuka et al., 2000; Zou et al., 2002). None of these studies related the change in phenolic content with changes in the wine antioxidant capacity. Based on these reports an increase in wine TAC might be expected as gallic acid and the monomeric and oligomeric flavan-3-ols have very high antioxidant potency, while the hydroxycinnamic acids have relatively low antioxidant potency. However, such predictions are tentative as the monomeric anthocyanins also have high antioxidant potency. The effect on the wine TAC will depend on the extent of changes in the phenolic composition. In the present study, the gallic acid, flavan-3-ol and flavonol contents increased, while a decrease in anthocyanin contents was observed. These changes in phenolic composition did not, however, result in any change in wine TAC. The sensory quality and colour of the wine were impaired as a result of extended maceration. The period of extended maceration might therefore have been too long. Optimising the extended maceration period may lead to wines with increased phenolic content and TAC, while the sensory characteristics should be carefully monitored to ensure retention of sensory quality.

Vintage-related variation in the wine phenolic composition, TAC and objective colour parameters were observed for trials incorporating vine structure, pre-fermentation maceration, juice/skin mixing and oxygenation treatments, as well as climatic regions. In the pre-fermentation maceration trial, no overall trends could be observed, as trends for the same treatment differed between vintages. In the other trials, trends for most parameters were similar with variation between vintages for other parameters. Vintage-related variation may be due to differences in the phenolic composition of grapes used for vinification.

Some interesting observations were made in terms of the vitisin A content of Pinotage wines. Climatic region had varying effects on the vitisin A content of the wines, with no effect for different vine structure treatments. Pre-fermentation maceration treatments, however, especially treatments at

15 °C, increased the vitisin A content of the wines. This is despite inconsistent changes observed for other anthocyanin contents. The reason for this phenomenon is not clear. During oxygenation, the vitisin A content increased initially with a subsequent decrease to similar levels as for the wine before oxygenation. These changes in vitisin A content matched the changes in wine hue. These changes were not, however, observed for oak maturation. Vitisin A was the only pyranoanthocyanin quantified, but similar trends may occur for the content of other pyranoanthocyanins in Pinotage wine.

Some difficulties concerning the analytical methods used were encountered during the study. The use of objective colour parameters, such as CIELab parameters, is preferred as an accurate objective measurement of wine colour since they permit better differentiation between wines than traditional measurements (Bakker et al., 1986; Almela et al., 1995). The Pinotage wines analysed in the present study, were all very young and darkly coloured. Some inversion was observed, i.e. the pigment content did not always correlate with the objective colour measurements. This phenomenon is observed for dark coloured beverages and anthocyanin solutions (Eagerman et al., 1973; Bakker et al., 1986; Almela et al., 1995) due to the difficulty of photocells to adjust to low luminosity situations. A possible solution is to use sample cells with a shorter path length for objective colour measurements of wines.

The findings of this study suggest that manipulating the monomeric phenolic composition of Pinotage wines is not likely to increase the TAC substantially, as was previously supposed. Some improvement may, however, be possible. In order to explain a greater proportion of the wine TAC, the content and TEAC values of more individual phenolic compounds are needed. More detailed characterisation and quantification of the phenolic content of red wines is necessary. This can be accomplished by using several HPLC analyses, each concentrating on a subset of phenolic compounds, sample pre-treatment to remove non-antioxidant compounds interfering with the determination or more sensitive detection techniques, such as mass spectroscopy or fluorescence, especially for flavan-3-ols and their derivatives. However, the lack of availability of pure standard compounds is still a major problem in terms of measuring the TEAC values of wine compounds. Implementation of on-line antioxidant capacity techniques in conjunction with HPLC will be helpful in this regard. The issue of synergy between different phenolic compounds and possibly between phenolic compounds and other wine constituents should also be investigated in more detail. These factors make it difficult to recommend which phenolic compounds should be manipulated in order to obtain a red wine with optimal TAC.

Guidelines for the phenolic composition needed to give a wine with optimal health benefits is impossible at this stage and was not the object of this study. It should be stressed, however, that health properties of individual phenolic compounds will eventually, when the relevant information

becomes available in future, add another dimension to the data obtained. Since the TAC only measures the *in vitro* radical scavenging activity, the TAC of wine components does not necessarily coincide with *in vivo* antioxidant activity, as bioavailability, metal chelating properties, lipid phase partitioning and metabolism of individual wine components may differ considerably. Knowledge of the metabolites and their antioxidant activity is required to evaluate their relative importance with regard to health benefits. Unabsorbed compounds may also exert protective effects in the gastrointestinal system.

The viticultural and enological techniques investigated are only a subset of possible techniques affecting the phenolic composition and hence the antioxidant capacity of Pinotage wines. Many other trellising systems have been shown previously to affect grape and wine composition and could be investigated in future. Viticultural aspects such as irrigation and soil type are also important. Techniques affecting the extraction of phenolic compounds during fermentation are very important and only a few, namely pre-fermentation maceration, juice/skin mixing technique and extended maceration, have been investigated in the present study. Enzyme maceration, carbonic maceration and thermovinification deserve attention in future. The present study considered the effects of oak and oxygenation treatments separately, but in many instances oxygenation is used in conjunction with alternative oak treatments in commercial winemaking. Combinations of these should also be investigated in future. Treatments recommended for winemaking to produce Pinotage wines with optimal TAC should also be tested on commercial scale to confirm the effects of treatments on the phenolic composition, TAC, colour and sensory quality of Pinotage wines, as all trials in the present study were carried out on experimental scale.

Some general recommendations can be made for producing Pinotage wines with optimal TAC, while retaining sensory quality. Cultivation of Pinotage grapes on bush vines or in cooler climatic regions compared to cultivation on trellised vines in warmer climatic regions can be recommended in terms of higher TAC and sensory quality. In terms of enological techniques, pumping-over, as opposed to punching-down and rotor treatments, is not recommended as a juice/skin mixing technique as reduced wine TAC, colour and sensory quality were obtained. Pre-fermentation maceration, addition of commercial tannin preparations, and oak maturation using traditional and alternative treatments, can be recommended in terms of improved sensory quality, although no change in wine TAC should be expected. Further, optimisation of the tannin addition protocol may result in increased wine TAC if additions are made after fermentation or if higher dosages are used. Optimisation of an oxygenation protocol for Pinotage wines is still needed, since loss of sensory quality and TAC were observed even at low levels of oxygenation. Some improvement of colour and fullness were, however, observed for some treatments indicating that this is a worthwhile technique to investigate further.

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Addendum A: Trolox Equivalent Antioxidant Capacity (TEAC) of Phenolic Compounds - Effect of Solvent Composition

Aim

The aim of the experiment was to investigate the effect of the test solution solvent composition on the Trolox equivalent antioxidant capacity (TEAC) of phenolic compounds found in Pinotage wines.

Materials and Methods

The TEAC values of phenolic compounds were determined using the $ABTS^{\bullet+}$ scavenging assay (Re et al., 1999), and using chemicals and phenolic reference standards also described in **Chapter 3**.

Trolox were dissolved in ethanol, 10% ethanol and 10% dimethylsulfoxide (DMSO) to obtain stock solutions. For the preparation of dose-response curves, the stock solutions were diluted using the same solvent, which were then added to the $ABTS^{\bullet+}$ reagent (in ethanol) in triplicate. The slopes of the dose-response curves obtained were used to determine whether the test solution solvent composition affects the $ABTS^{\bullet+}$ scavenging activity of Trolox.

Phenolic compounds were dissolved in ethanol or DMSO (stock solutions) and diluted with ethanol to obtain test solutions, that were added to the $ABTS^{\bullet+}$ reagent (in ethanol) in triplicate on two separate days to obtain duplicate dose-response curves for each phenolic compound. The TEAC (EtOH) values were calculated from the slope of the dose-response curve for each phenolic compound in ethanol using the dose-response curve for Trolox in ethanol as standard. For determination of the TEAC (10% EtOH) values, the stock and test solutions were prepared in 10% ethanol, except for the quercetin-3-rhamnoside (rham) and quercetin-3-glucoside (glc) stock solutions which were in ethanol, and the quercetin-3-galactoside (gal), quercetin, myricetin, kaempferol and isorhamnetin stock solutions which were in DMSO. Stock solutions in ethanol or DMSO were diluted with distilled water and 10% ethanol to obtain test solutions containing 10% ethanol and DMSO combined, i.e. 90% water, for determining TEAC (10% EtOH) values. The TEAC (10% EtOH) values were also calculated using the dose-response curves for each phenolic compound in 90% water against that of Trolox in ethanol to determine whether the test solution solvent composition has an effect on the TEAC value. Trolox (in ethanol) dose-response curves were prepared every day and for each phenolic compound a dose-response curve was prepared on two separate days. Introducing test solutions in either 100% ethanol or 90% water into the $ABTS^{\bullet+}$

reaction mixture (50 μL solution added to 1 mL $ABTS^{\bullet+}$ solution in ethanol) would give reaction mixtures with 0% or 4.3% water, respectively.

Results and Discussion

Dose-response curves of Trolox in different solvents are presented in **Figure 1**. From the slopes of the dose-response curves, it is clear that the test solution composition does not affect the $ABTS^{\bullet+}$ scavenging activity of Trolox. Since preparing the Trolox standard solutions in ethanol is the most convenient, this dose-response curve was used for calculating the TEAC values of the phenolic compounds. To determine whether the test solution solvent composition affects the $ABTS^{\bullet+}$ scavenging activity of phenolic compounds, gallic acid test solutions were prepared using different solvents as for Trolox. Test solutions in all three solvents containing ~90% water gave similar dose-response curve slopes with higher $ABTS^{\bullet+}$ scavenging activity than gallic acid test solutions prepared in ethanol only (**Figure 2**). As Pinotage wines contain ~12 to 14% ethanol and test solutions for determining their total antioxidant capacity (TAC) are obtained by diluting wine with 10% ethanol prior to addition to the $ABTS^{\bullet+}$ reaction mixture, the phenolic compounds test solutions should be prepared in a similar manner to obtain TEAC values for estimating their TAC contribution to the wines. The exact composition of the organic part of the reaction medium seems to be unimportant as substituting ethanol for DMSO when preparing the test solutions gives the same TEAC values, but the water content has a significant effect on the reaction. This can be ascribed to changes in polarity of the reaction medium that affect hydrogen bonding between the solvent molecules and the phenolic OH-groups (Litwinienko & Ingold, 2003; Nenadis et al., 2004). This phenomenon will influence the ease of hydrogen or electron abstraction causing a change in $ABTS^{\bullet+}$ scavenging activity. Similar changes in $ABTS^{\bullet+}$ scavenging activity is observed with changes in reaction medium pH (Lema□ska et al., 2001; Labrinea & Georgiou, 2004).

TEAC values for the phenolic compounds found in Pinotage wines were determined by preparing stock solutions in ethanol or DMSO and diluting with ethanol to obtain test solutions [TEAC (EtOH)] or by preparing stock solutions in 10% ethanol, ethanol or DMSO and diluting to obtain test solution containing 90% water [TEAC (10% EtOH)]. Only (+)-catechin, caffeic acid, quercetin-3-rham and isorhamnetin had similar TEAC (EtOH) and TEAC (10% EtOH) values (**Table 1**). The TEAC (10% EtOH) was higher than the TEAC (EtOH) for gallic acid, (-)-epicatechin, caffeoyltartaric acid, quercetin-3-gal, quercetin-3-glc, myricetin, quercetin, delphinidin-3-glc, petunidin-3-glc, peonidin-3-glc and malvidin-3-glc, while the TEAC (10% EtOH) values for procyanidin B1 and kaempferol were lower.

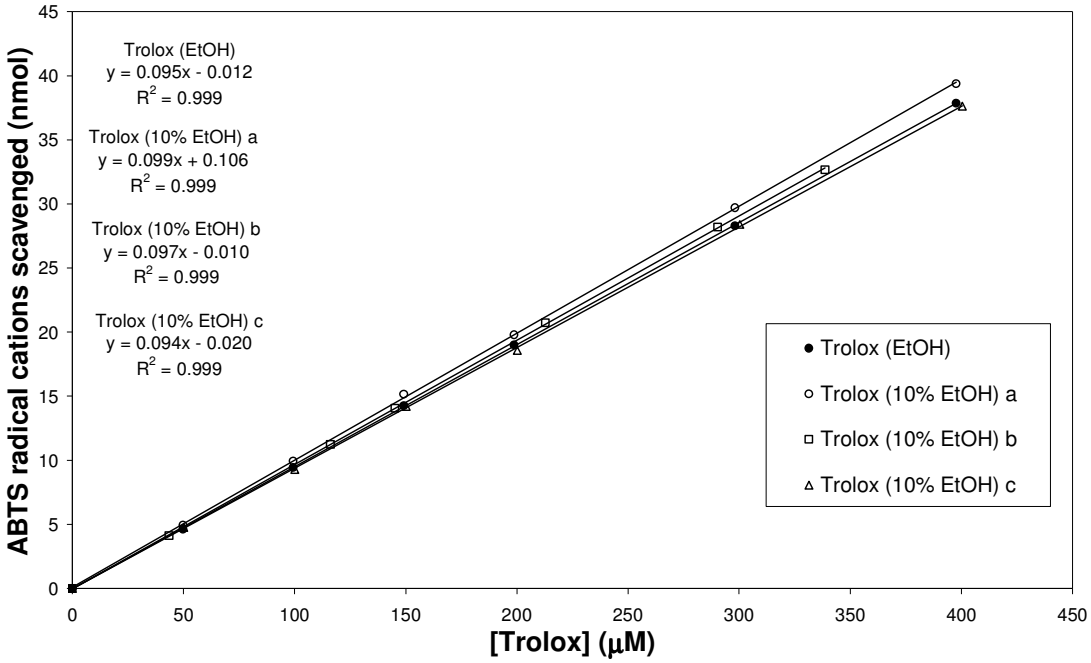

Figure 1. Dose-response curves of ABTS radical cation scavenging activity of Trolox test solutions in different solvents [Trolox (EtOH) = Trolox stock and test solution in ethanol; Trolox (10% EtOH) a = Trolox stock solution in ethanol diluted with 10% ethanol; Trolox (10% EtOH) b = Trolox stock and test solutions in 10% ethanol; Trolox (10% EtOH) c = Trolox stock solution in 10% dimethylsulfoxide diluted with 10% ethanol].

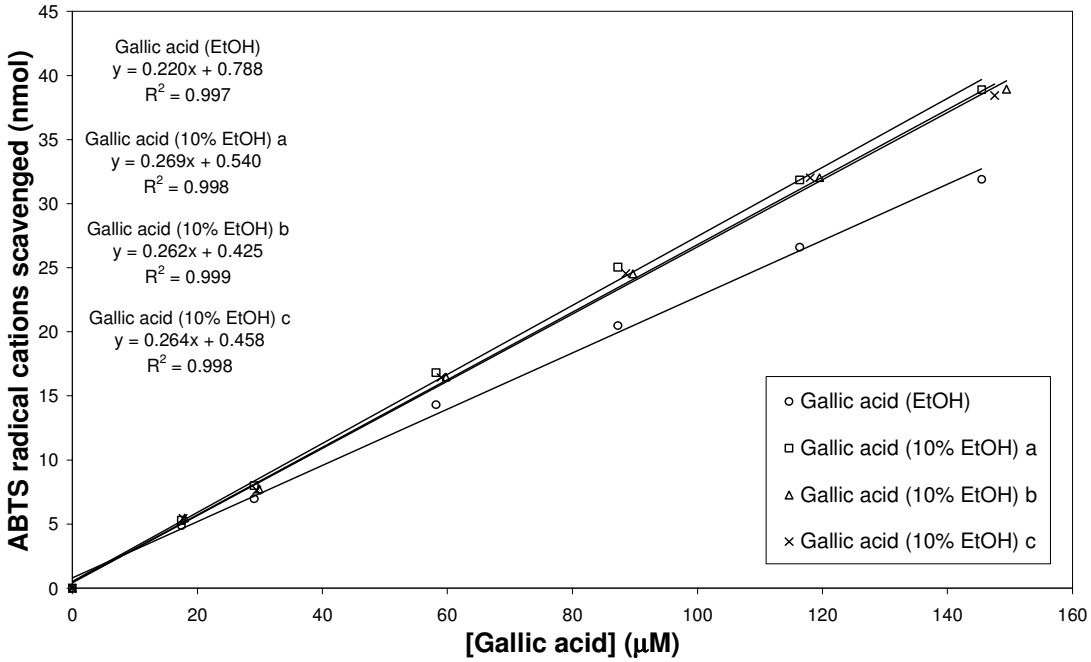

Figure 2. Dose-response curves of ABTS radical cation scavenging activity of gallic acid test solutions in different solvents [Gallic acid (EtOH) = gallic acid stock and test solutions in ethanol; Gallic acid (10% EtOH) a = gallic acid stock solution in ethanol diluted with 10% ethanol; Gallic acid (10% EtOH) b = gallic acid stock and test solutions in 10% ethanol; Gallic acid (10% EtOH) c = gallic acid stock solution in 10% dimethylsulfoxide diluted with 10% ethanol].

Table 1. Trolox equivalent antioxidant capacity values of phenolic compounds with test solution prepared using different solvents.

Phenolic compound	**TEAC (EtOH)[a]**	**TEAC (10% EtOH)[b]**
Gallic acid	2.23 (2.10; 2.36)[c]	2.78 (2.76; 2.81)
Procyanidin B1	2.57 (2.52; 2.62)	2.30 (2.26; 2.34)
(+)-Catechin	1.01 (1.00; 1.01)	0.96 (0.92; 1.00)
(-)-Epicatechin	1.00 (0.98; 1.01)	1.26 (1.28; 1.25)
Caffeoyltartaric acid	0.74 (0.75; 0.72)	0.90 (0.88; 0.92)
Caffeic acid	0.96 (0.96; 0.96)	0.98 (0.93; 1.03)
p-Coumaric acid	<0.01 (<0.01; <0.01)	<0.01 (<0.01; <0.01)
Quercetin-3-gal[d]	0.66 (0.64; 0.68)	0.96 (0.97; 0.96)
Quercetin-3-glc[e]	0.76 (0.75; 0.77)	0.91 (0.94; 0.89)
Quercetin-3-rham[e]	0.83 (0.81; 0.85)	0.91 (0.94; 0.89)
Myricetin[d]	2.03 (2.00; 2.06)	2.67 (2.82; 2.51)
Quercetin[d]	1.59 (1.56; 1.61)	1.75 (1.72; 1.78)
Kaempferol[d]	1.01 (1.01; 1.02)	0.88 (0.83; 0.93)
Isorhamnetin[d]	0.96 (1.01; 0.92)	0.95 (0.94; 0.97)
Delphinidin-3-glc	1.55 (1.52; 1.58)	2.40 (2.42; 2.39)
Petunidin-3-glc	1.38 (1.39; 1.38)	2.06 (2.22; 1.90)
Peonidin-3-glc	1.01 (0.96; 1.05)	1.49 (1.43; 1.55)
Malvidin-3-glc	0.72 (0.73; 0.71)	1.46 (1.44; 1.48)

[a] Trolox equivalent antioxidant capacity (TEAC) measured using the $ABTS^{\bullet+}$ scavenging assay; test solutions prepared to contain 100% ethanol; [b] TEAC measured using the $ABTS^{\bullet+}$ scavenging assay; test solutions prepared to contain 90% water; [c] duplicate values obtained on separate days; [d] compounds dissolved in ethanol; [e] compounds dissolved in dimethylsulfoxide; gal = galactoside; glc = glucoside; rham = rhamnoside.

Conclusions

The solvent used for preparing test solutions of Trolox did not affect its $ABTS^{\bullet+}$ scavenging activity, but the activity of most phenolic compounds found in Pinotage wines were substantially affected. The same water content in the final reaction medium should thus be ensured when determining the TAC of the wines and the TEAC values of the phenolic compounds to obtain a reasonable estimation of the total antioxidant contributions of phenolic compounds to the wine TAC.

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Addendum B: Ultrafiltration Protocol Testing

Aim

The aim of the experiment was to find an optimal protocol for the ultrafiltration of Pinotage wine for separating the monomeric and polymeric phenolic compounds.

Materials and Methods

Ultrafiltration of a Pinotage wine (2002 vintage; sample was frozen 8 months after vinification) was performed using Vivaspin 4 mL centrifuge devices (Vivascience, Hanover, Germany) having polyethersulphone membranes with membrane pore sizes of 10, 30 and 50 kDa. Centrifugal ultrafiltration were performed at a speed of 5 000 rpm and a temperature of 20°C using a Sorvall RC-3B refrigerated centrifuge (Sorvall Instruments, Newtown, USA). Three protocols were investigated, namely ultrafiltration of 4 mL wine (protocol 1), 2 mL wine diluted with 1 mL 10% ethanol (protocol 2) and 2 mL wine diluted with 1 mL 10% ethanol followed by a washing step using 1 mL 10% ethanol (protocol 3). Centrifugation was continued until ~100 µL of retentate were left. Ultrafiltration permeates and retentates were diluted to the original volume and kept in a freezer at -20 °C until analysis to prevent changes in phenolic composition. The permeates and retentates were analysed for phenolic composition using HPLC, and tannin content using the tannin precipitation assay (see **Chapter 3** for experimental details of analyses).

Results and Discussion

Dilution of wine (protocol 2) led to a greater recovery of phenolic acids and flavonols in the permeate of the respective ultrafiltration membranes (**Tables 1** and **2**). However, recovery of flavan-3-ols and monomeric anthocyanins was not affected by dilution. For total monomers, a higher recovery was achieved without dilution (protocol 1) than with dilution (protocol 2). Dilution with an added washing step (protocol 3) resulted in a greater recovery of phenolic acids and flavan-3-ols in the ultrafiltration permeate than dilution without a washing step (**Tables 2** and **3**). In the case of 30 and 50 kDa membranes, dilution with an added washing step (protocol 3) produced greater recovery of flavan-3-ols and monomeric anthocyanins than for the other two protocols (**Tables 1** to **3**). For all the membranes, the recovery of total monomers was higher for dilution and washing (protocol 3) than for the other two protocols. The use of protocol 3 (dilution of wine and a washing step) is therefore recommended for optimal recovery of monomeric phenolic compounds in the ultrafiltration permeate.

Table 1. Phenolic composition and tannin content of ultrafiltration permeates and retentates using protocol 1.

	Wine	10 kDa		30 kDa		50 kDa	
		Permeate	**Retentate**	**Permeate**	**Retentate**	**Permeate**	**Retentate**
Total phenolic acids[a]	304.46	176.47	132.50	197.15	100.28	241.04	62.85
Total flavan-3-ols[b]	34.88	11.24	11.74	10.14	10.10	20.73	7.92
Total flavonols[c]	34.09	7.91	21.15	9.76	19.52	16.16	16.27
Monomeric anthocyanins[d]	204.71	44.85	152.81	63.21	132.17	111.52	106.69
Total monomers[e]	578.14	240.47	318.20	280.25	262.07	389.46	193.73
Polymers[b]	122.84	23.59	81.22	26.71	84.00	29.14	101.83
Tannin[b]	308.04	nd	369.75	nd	350.64	nd	394.02

[a] mg gallic acid equivalents/L; [b] mg (+)-catechin equivalents/L; [c] mg rutin equivalents/L; [d] mg malvidin-3-glucoside equivalents/L; [e] sum of all quantified monomeric compounds; nd = not detected.

Table 2. Phenolic composition and tannin content of ultrafiltration permeates and retentates using protocol 2.

	Wine	10 kDa		30 kDa		50 kDa	
		Permeate	**Retentate**	**Permeate**	**Retentate**	**Permeate**	**Retentate**
Total phenolic acids[a]	304.46	198.29	93.71	213.91	83.15	244.81	38.72
Total flavan-3-ols[b]	34.88	9.52	9.44	17.76	9.47	19.93	nd
Total flavonols[c]	34.09	9.83	15.33	11.17	14.59	15.97	9.79
Monomeric anthocyanins[d]	204.71	66.63	118.89	76.55	114.13	118.97	69.55
Total monomers[e]	578.14	284.27	237.38	319.40	221.34	399.68	118.06
Polymers[b]	122.84	25.30	64.18	28.09	56.89	30.73	45.93
Tannin[b]	308.04	nd	358.24	nd	350.64	nd	312.89

[a] mg gallic acid equivalents/L; [b] mg (+)-catechin equivalents/L; [c] mg rutin equivalents/L; [d] mg malvidin-3-glucoside equivalents/L; [e] sum of all quantified monomeric compounds; nd = not detected.

Table 3. Phenolic composition and tannin content of ultrafiltration permeates and retentates using protocol 3.

	wine	10 kDa		30 kDa		50 kDa	
		Permeate	**Retentate**	**Permeate**	**Retentate**	**Permeate**	**Retentate**
Total phenolic acids[a]	304.46	222.65	71.05	255.61	41.14	275.48	11.87
Total flavan-3-ols[b]	34.88	nd	11.11	17.50	7.88	22.42	nd
Total flavonols[c]	34.09	11.08	12.91	15.03	11.61	19.77	5.72
Monomeric anthocyanins[d]	204.71	67.17	112.47	96.39	91.67	149.62	37.61
Total monomers[e]	578.14	300.90	207.54	384.53	152.29	467.30	55.20
Polymers[b]	122.84	25.87	51.21	28.59	56.11	33.30	40.34
Tannin[b]	308.04	nd	352.60	nd	345.25	nd	257.01

[a] mg gallic acid equivalents/L; [b] mg (+)-catechin equivalents/L; [c] mg rutin equivalents/L; [d] mg malvidin-3-glucoside equivalents/L; [e] sum of all quantified monomeric compounds; nd = not detected.

Very low amounts of polymers and no tannins were detected in ultrafiltration permeates, irrespective of the protocol used. Polymeric phenolic compounds are therefore not able to readily diffuse through the ultrafiltration membranes.

Conclusions

Ultrafiltration of diluted wine with a washing step is the optimal ultrafiltration protocol for the passage of monomeric phenolic compounds through the ultrafiltration membrane.

Addendum C: Chapter 4 Data

Aim

The aim is to list the phenolic composition, antioxidant and objective colour parameters for the individual Pinotage wines described in **Chapter 4**.

Table 1. Phenolic composition (measured using spectrophotometric assays), antioxidant capacity (measured) and objective colour parameters of the 2001, 2002 and 2003 individual Pinotage wines from different climatic regions and vine structure treatments.

Wine	Vintage	Region	Vine[a]	Trunk[b]	Site[c]	TP (F-C)[d]	TA (pH shift)[e]	MA (pH shift)[f]	PA (pH shift)[g]	TF (DAC)[h]	TAC_M[i]	*C**[j]	*h**[k]	*L**[l]	*a**[m]	*b**[n]
1	2001	II	Bush	20 cm	b	2737.0	655.6	512.3	143.4	209.9	12.94	64.62	18.19	26.55	61.39	20.17
2	2001	II	Bush	20 cm	c	2478.8	587.4	466.1	121.3	177.1	12.08	63.20	14.76	29.75	61.12	16.10
3	2001	II	Bush	20 cm	d	2890.2	825.5	636.1	189.3	167.3	14.97	56.72	15.57	18.25	54.64	15.23
4	2001	II	Bush	30 cm	b	3441.7	786.8	593.0	193.8	230.7	16.01	56.10	13.60	17.07	54.53	13.19
5	2001	II	Bush	30 cm	c	2701.3	572.9	431.5	141.4	191.2	14.32	62.98	17.14	26.30	60.18	18.56
6	2001	II	Bush	30 cm	d	2003.8	692.2	558.7	133.5	112.1	9.51	61.64	14.25	29.21	59.74	15.17
7	2001	II	Trellised	30 cm	b	3196.5	703.1	530.1	173.1	219.0	15.60	60.66	17.65	21.54	57.80	18.39
8	2001	II	Trellised	30 cm	c	2566.1	712.0	548.5	163.5	168.8	12.78	59.46	15.68	22.32	57.25	16.07
9	2001	II	Trellised	30 cm	d	2533.2	799.5	644.8	154.8	153.7	12.52	59.15	13.27	24.06	57.57	13.58
10	2001	II	Trellised	60 cm	b	2800.5	679.4	525.1	154.3	191.5	12.93	62.63	17.14	24.47	59.85	18.46
11	2001	II	Trellised	60 cm	c	2524.1	669.0	509.8	159.2	158.1	11.47	62.81	19.06	22.77	59.37	20.51
12	2001	II	Trellised	60 cm	d	2402.6	743.7	591.4	152.3	151.9	12.70	60.20	13.88	24.88	58.44	14.44
13	2001	III	Bush	20 cm	j	2478.8	667.8	513.6	154.3	161.4	12.79	59.30	15.04	23.06	57.27	15.39
14	2001	III	Bush	20 cm	h	2038.4	622.3	504.2	118.2	136.3	11.00	63.68	14.13	31.10	61.75	15.54
15	2001	III	Bush	20 cm	k	2779.3	758.0	562.2	195.8	189.4	13.93	55.38	14.20	16.23	53.69	13.59
16	2001	III	Bush	20 cm	i	2459.1	565.7	431.2	134.5	152.9	12.39	58.53	14.05	24.75	56.78	14.21
17	2001	III	Bush	20 cm	l	2829.3	628.9	506.0	123.0	224.6	15.09	62.77	15.21	29.97	60.57	16.47
18	2001	III	Bush	20 cm	m	2569.2	541.9	427.2	114.7	202.7	12.88	60.51	12.66	32.94	59.04	13.26
19	2001	III	Bush	30 cm	j	2034.3	629.4	510.4	119.0	130.4	10.50	61.89	14.43	29.10	59.94	15.42
20	2001	III	Bush	30 cm	h	2410.2	639.7	505.1	134.6	170.1	12.58	64.88	18.63	25.79	61.48	20.73
21	2001	III	Bush	30 cm	k	2922.9	756.5	588.7	167.8	210.7	14.36	58.23	15.98	21.06	55.98	16.03
22	2001	III	Bush	30 cm	i	2499.9	617.1	494.8	122.3	167.6	12.43	61.70	13.32	28.66	60.04	14.22
23	2001	III	Bush	30 cm	l	3549.3	705.4	530.1	175.4	272.4	18.88	58.98	15.38	20.40	56.87	15.64
24	2001	III	Bush	30 cm	m	2525.3	596.3	478.6	117.7	198.5	12.58	61.51	12.52	31.99	60.05	13.34
25	2001	III	Trellised	30 cm	j	2398.3	669.5	527.7	141.7	161.7	11.18	66.22	19.61	27.75	62.38	22.23
26	2001	III	Trellised	30 cm	h	1809.1	488.8	396.5	92.3	107.5	8.27	62.93	10.05	39.41	61.96	10.98
27	2001	III	Trellised	30 cm	k	2486.4	696.7	565.2	131.5	168.4	13.18	61.43	12.69	30.59	59.93	13.50
28	2001	III	Trellised	30 cm	i	2163.3	629.4	502.2	127.2	132.4	10.51	63.18	14.37	29.75	61.20	15.68
29	2001	III	Trellised	30 cm	l	3546.0	660.1	506.5	153.6	262.4	16.86	60.91	14.52	23.35	58.96	15.27
30	2001	III	Trellised	30 cm	m	2251.7	561.0	458.8	102.2	152.9	12.19	57.92	11.93	37.06	56.67	11.97
31	2001	III	Trellised	60 cm	j	2331.1	697.5	564.3	133.2	161.1	12.02	60.89	14.60	28.55	58.92	15.35
32	2001	III	Trellised	60 cm	h	2433.4	666.5	528.4	138.1	140.5	11.30	61.39	15.46	24.34	59.17	16.36
33	2001	III	Trellised	60 cm	k	2646.4	748.1	591.9	156.2	184.5	13.72	59.71	14.32	23.06	57.85	14.77
34	2001	III	Trellised	60 cm	i	2058.2	518.4	423.3	95.1	145.8	10.36	64.17	10.29	37.37	63.14	11.46
35	2001	III	Trellised	60 cm	l	3298.1	692.2	542.2	150.0	230.7	15.23	60.62	15.23	22.62	58.49	15.92
36	2001	III	Trellised	60 cm	m	2451.4	607.2	466.1	141.1	166.3	13.40	60.12	15.54	24.88	57.92	16.11
37	2001	IV	Bush	20 cm	p	1973.8	567.6	459.8	107.8	126.6	10.27	57.78	11.79	35.84	56.56	11.81
38	2001	IV	Bush	20 cm	q	2021.6	498.7	397.9	100.9	132.6	10.91	58.38	15.81	35.42	56.17	15.90
39	2001	IV	Bush	20 cm	o	2239.6	582.0	448.5	133.5	141.2	11.65	57.38	14.73	25.28	55.49	14.59
40	2001	IV	Bush	20 cm	m	1794.0	620.5	493.6	126.9	100.3	9.36	59.96	16.08	29.54	57.61	16.61
41	2001	IV	Bush	20 cm	s	2402.6	717.3	578.8	138.4	147.3	13.48	58.94	13.41	24.75	57.33	13.67
42	2001	IV	Bush	20 cm	t	1837.5	523.5	432.3	91.1	117.5	9.33	53.52	10.69	41.17	52.59	9.93

[a] vine structure treatment (bush or trellised vines); [b] trunk height; [c] Darling (a,n), Stellenbosch higher than 300 m above sea-level (b-e), Faure (f), Hemel and Aarde Valley (Hermanus) (g), Kuilsriver (h,i), Stellenbosch lower than 300 m above sea-level (j-l), Vlottenburg (m), Riebeeck-Wes (o), Wellington/Paarl (p,q), Agter-Paarl (r-t); [d] total phenol content measured using the Folin-Ciocalteau assay in mg gallic acid equivalents/L; [e] total anthocyanin content measured using the pH shift assay in mg malvidin-3-glc equivalents/L; [f] monomeric anthocyanin content measured using the pH shift assay in mg malvidin-3-glc equivalents/L; [g] polymeric anthocyanin content measured using the pH shift assay in mg malvidin-3-glc equivalents/L; [h] total flavan-3-ol content measured using the DAC assay in mg (+)-catechin equivalents/L; [i] total antioxidant capacity measured using the $ABTS^{\bullet+}$ scavenging assay in mM Trolox equivalents; [j] chroma; [k] hue angle (°); [l] lightness; [m] red/green chromaticity; [n] yellow/blue chromaticity.

Table 1. Continued …

Wine	Vintage	Region	Vine[a]	Trunk[b]	Site[c]	TP (F-C)[d]	TA (pH shift)[e]	MA (pH shift)[f]	PA (pH shift)[g]	TF (DAC)[h]	TAC_M[i]	*C**	*h**[j]	*L**	*a**	*b**
43	2001	IV	Bush	20 cm	n	2487.9	690.6	538.6	152.0	157.0	13.70	59.29	12.82	23.50	57.81	13.16
44	2001	IV	Bush	30 cm	p	2453.1	673.4	545.9	127.6	153.5	12.56	59.49	13.73	27.04	57.79	14.12
45	2001	IV	Bush	30 cm	q	1739.7	414.0	334.2	79.8	118.9	9.65	52.31	10.14	48.36	51.49	9.21
46	2001	IV	Bush	30 cm	o	2032.5	592.8	468.2	124.6	120.4	10.15	58.85	14.93	28.44	56.86	15.16
47	2001	IV	Bush	30 cm	m	1630.7	559.7	458.5	101.2	99.4	8.13	59.61	14.31	37.37	57.76	14.73
48	2001	IV	Bush	30 cm	s	1925.6	648.1	514.9	133.2	120.7	10.62	61.01	14.63	28.32	59.03	15.41
49	2001	IV	Bush	30 cm	t	1780.0	514.7	420.4	94.3	117.4	9.08	55.12	11.99	40.90	53.92	11.45
50	2001	IV	Bush	30 cm	n	2207.9	642.8	525.4	117.3	147.9	10.78	60.04	10.74	34.65	58.99	11.19
51	2001	IV	Trellised	30 cm	p	1969.0	603.7	487.4	116.4	102.1	9.31	58.15	12.93	29.86	56.68	13.01
52	2001	IV	Trellised	30 cm	q	2125.0	463.8	359.0	104.8	130.3	10.35	58.87	14.13	33.58	57.09	14.37
53	2001	IV	Trellised	30 cm	o	2020.1	548.8	436.4	112.4	104.9	10.90	57.68	14.48	29.75	55.85	14.42
54	2001	IV	Trellised	30 cm	m	1721.1	582.5	461.2	121.3	91.9	8.24	62.92	15.16	30.69	60.73	16.46
55	2001	IV	Trellised	30 cm	s	2125.0	597.0	468.4	128.6	125.1	10.01	60.71	13.91	27.04	58.93	14.60
56	2001	IV	Trellised	30 cm	t	1898.2	615.1	510.9	104.2	109.5	10.22	57.54	10.42	37.22	56.59	10.41
57	2001	IV	Trellised	30 cm	n	2686.6	659.1	513.1	146.0	156.7	12.69	58.75	13.46	21.70	57.14	13.68
58	2001	IV	Trellised	60 cm	p	2089.9	529.4	422.9	106.5	124.9	10.75	57.24	11.72	34.39	56.05	11.63
59	2001	IV	Trellised	60 cm	q	1803.0	413.1	337.8	75.2	98.1	8.45	51.74	9.78	47.92	50.99	8.79
60	2001	IV	Trellised	60 cm	o	1937.8	591.9	474.8	117.0	104.3	9.92	59.41	11.45	32.28	58.23	11.79
61	2001	IV	Trellised	60 cm	m	1840.5	644.8	523.5	121.3	106.7	9.62	63.44	14.01	33.76	61.55	15.36
62	2001	IV	Trellised	60 cm	s	1673.0	477.3	391.6	85.7	114.4	8.49	62.21	9.10	40.48	61.43	9.84
63	2001	IV	Trellised	60 cm	t	1858.6	591.9	485.1	106.8	115.3	10.34	57.84	13.51	36.33	56.24	13.51
64	2001	IV	Trellised	60 cm	n	2693.6	638.5	476.6	161.8	155.3	13.61	57.22	14.95	19.89	55.28	14.76
65	2002	II	Bush	20 cm	a	2178.6	601.1	515.7	85.4	171.9	19.56	58.30	15.71	20.72	56.12	15.79
66	2002	II	Bush	20 cm	b	2125.1	443.9	397.3	46.6	208.9	17.75	65.38	15.29	32.30	63.07	17.25
67	2002	II	Bush	20 cm	d	2095.9	582.0	514.5	67.5	162.5	17.90	62.25	15.25	26.85	60.06	16.37
68	2002	II	Bush	20 cm	f	2108.4	557.6	495.1	62.5	186.3	17.90	62.81	14.76	30.46	60.74	16.00
69	2002	II	Bush	30 cm	a	1984.7	530.8	472.9	57.9	173.6	16.88	64.03	15.88	28.36	61.59	17.53
70	2002	II	Bush	30 cm	b	2166.1	452.3	409.3	43.0	245.8	21.59	66.78	15.32	33.58	64.41	17.64
71	2002	II	Bush	30 cm	d	1959.1	509.1	445.9	63.2	165.8	16.97	63.45	16.25	27.37	60.92	17.75
72	2002	II	Bush	30 cm	f	1691.5	487.6	443.0	44.6	148.7	14.88	63.36	13.99	37.59	61.48	15.32
73	2002	II	Trellised	30 cm	a	1869.0	520.1	467.2	53.0	164.0	16.12	63.76	15.06	30.67	61.57	16.57
74	2002	II	Trellised	30 cm	b	2137.2	498.2	459.6	38.6	206.4	12.89	64.95	10.77	38.15	63.81	12.14
75	2002	II	Trellised	30 cm	c	1730.1	454.3	405.7	48.6	160.0	14.23	67.13	20.48	30.24	62.89	23.49
76	2002	II	Trellised	30 cm	d	1791.4	505.2	439.3	65.9	141.0	15.54	62.70	15.80	28.44	60.33	17.08
77	2002	II	Trellised	30 cm	f	1635.5	498.0	458.7	39.2	134.3	13.22	63.69	10.73	38.82	62.58	11.86
78	2002	II	Trellised	60 cm	a	1846.1	448.1	394.7	53.4	162.0	15.14	64.60	17.77	28.39	61.52	19.72
79	2002	II	Trellised	60 cm	b	2329.6	565.4	513.7	51.7	211.1	18.99	64.44	13.34	31.51	62.70	14.87
80	2002	II	Trellised	60 cm	c	1671.2	443.4	385.8	57.6	137.3	13.39	65.02	19.49	28.44	61.30	21.69
81	2002	II	Trellised	60 cm	d	1848.7	568.3	518.2	50.1	150.7	15.67	62.21	13.32	33.25	60.54	14.33
82	2002	II	Trellised	60 cm	f	1544.6	360.3	335.7	24.6	130.7	11.31	64.08	7.74	45.96	63.50	8.64
83	2002	III	Bush	20 cm	j	1623.9	551.1	482.6	68.5	123.8	14.55	61.84	16.54	26.39	59.28	17.61
84	2002	III	Bush	20 cm	h	1761.7	481.3	431.9	49.4	144.4	14.46	64.51	13.31	33.05	62.78	14.85
85	2002	III	Bush	20 cm	k	1946.7	301.8	261.4	40.4	209.6	17.41	55.58	15.40	44.53	53.58	14.76
86	2002	III	Bush	20 cm	i	2084.5	572.9	492.0	80.9	171.9	17.99	60.76	15.42	25.85	58.58	16.16
87	2002	III	Bush	20 cm	m	1864.7	489.6	435.2	54.3	204.7	16.58	62.23	14.11	33.54	60.35	15.18
88	2002	III	Bush	30 cm	j	1266.4	487.3	444.3	43.0	94.9	11.76	61.34	12.61	37.15	59.87	13.39
89	2002	III	Bush	30 cm	h	1783.6	474.9	426.4	48.6	158.5	14.58	66.27	15.49	34.14	63.87	17.70
90	2002	III	Bush	30 cm	k	1491.4	371.2	324.8	46.3	135.7	13.77	60.19	14.22	38.92	58.35	14.79

Table 1. Continued …

Wine	Vintage	Region	Vine[a]	Trunk[b]	Site[c]	TP (F-C)[d]	TA (pH shift)[e]	MA (pH shift)[f]	PA (pH shift)[g]	TF (DAC)[h]	TAC_M[i]	*C**	*h**[j]	*L**	*a**	*b**
91	2002	III	Bush	30 cm	i	1914.3	491.5	437.0	54.5	169.7	16.93	63.25	14.57	30.62	61.22	15.91
92	2002	III	Bush	30 cm	m	2271.9	505.5	446.5	59.0	206.6	19.50	62.80	14.76	28.70	60.73	16.00
93	2002	III	Trellised	30 cm	j	1415.1	562.4	516.0	46.3	98.7	12.15	60.71	11.72	38.95	59.44	12.33
94	2002	III	Trellised	30 cm	k	1697.4	446.3	395.8	50.6	138.9	14.95	59.24	15.67	37.59	57.04	16.00
95	2002	III	Trellised	30 cm	i	1672.3	536.6	484.2	52.3	141.4	13.84	63.70	14.73	33.56	61.61	16.20
96	2002	III	Trellised	30 cm	l	1988.1	423.9	368.5	55.4	176.8	17.08	63.48	15.94	32.58	61.04	17.44
97	2002	III	Trellised	30 cm	m	1912.7	603.1	534.8	68.3	126.6	15.93	60.02	13.83	28.70	58.28	14.35
98	2002	III	Trellised	60 cm	j	1646.2	597.6	537.3	60.3	114.1	14.70	60.05	13.66	33.06	58.35	14.18
99	2002	III	Trellised	60 cm	k	1517.8	526.4	486.4	39.9	121.3	12.44	59.34	9.33	43.11	58.55	9.62
100	2002	III	Trellised	60 cm	i	1723.8	418.6	386.7	31.9	162.2	14.21	59.50	6.13	45.82	59.16	6.36
101	2002	III	Trellised	60 cm	l	2002.2	431.0	379.6	51.4	180.0	16.63	63.71	13.01	35.21	62.08	14.34
102	2002	III	Trellised	60 cm	m	1586.4	517.0	472.7	44.3	121.9	13.98	57.64	9.99	40.82	56.77	10.00
103	2002	IV	Bush	20 cm	p	1890.9	567.7	497.9	69.8	152.8	16.79	61.97	15.31	25.97	59.77	16.37
104	2002	IV	Bush	20 cm	q	1783.6	513.3	449.4	63.9	133.4	14.27	62.18	14.56	30.70	60.18	15.64
105	2002	IV	Bush	20 cm	o	1751.6	433.0	383.3	49.7	134.6	14.72	59.58	12.70	35.84	58.12	13.10
106	2002	IV	Bush	20 cm	r	1509.9	522.1	457.6	64.5	111.1	14.07	63.26	17.07	27.26	60.48	18.58
107	2002	IV	Bush	20 cm	s	1327.7	394.7	342.1	52.5	99.8	12.59	57.87	15.17	35.62	55.86	15.14
108	2002	IV	Bush	20 cm	t	1638.6	567.6	515.5	52.1	122.5	14.28	61.44	13.76	34.51	59.68	14.62
109	2002	IV	Bush	20 cm	n	1804.3	580.7	501.1	79.6	129.8	15.07	61.26	14.98	25.05	59.18	15.84
110	2002	IV	Bush	30 cm	p	1770.6	609.3	536.8	72.5	134.9	15.03	61.50	16.17	26.70	59.07	17.13
111	2002	IV	Bush	30 cm	q	1507.3	506.8	449.9	57.0	107.3	13.07	61.47	13.34	32.00	59.81	14.18
112	2002	IV	Bush	30 cm	o	1347.4	440.1	398.6	41.5	107.7	12.87	60.09	11.70	35.69	58.85	12.19
113	2002	IV	Bush	30 cm	r	1286.7	471.4	422.2	49.2	93.4	11.52	63.30	16.61	31.78	60.66	18.10
114	2002	IV	Bush	30 cm	s	1134.1	431.9	388.0	43.9	78.2	10.21	56.82	14.73	41.37	54.96	14.45
115	2002	IV	Bush	30 cm	t	1564.2	439.0	379.4	59.6	123.4	14.00	61.04	17.62	31.78	58.18	18.48
116	2002	IV	Bush	30 cm	n	1602.0	494.2	430.0	64.2	124.2	13.82	63.66	16.11	27.03	61.16	17.67
117	2002	IV	Trellised	30 cm	q	1555.7	429.4	390.6	38.8	130.2	12.23	60.94	8.25	42.83	60.31	8.74
118	2002	IV	Trellised	30 cm	o	1644.6	427.0	384.2	42.8	113.1	12.98	56.82	8.74	39.71	56.16	8.63
119	2002	IV	Trellised	30 cm	r	1712.9	563.2	499.8	63.4	130.9	14.98	62.48	18.14	25.27	59.38	19.46
120	2002	IV	Trellised	30 cm	s	1210.6	460.0	421.2	38.8	95.4	11.72	54.23	10.74	33.94	53.29	10.11
121	2002	IV	Trellised	30 cm	t	1573.2	486.4	442.1	44.3	113.6	12.78	58.07	10.35	39.32	57.13	10.43
122	2002	IV	Trellised	30 cm	n	1808.6	584.2	510.8	73.4	143.4	16.36	61.53	14.73	25.84	59.51	15.64
123	2002	IV	Trellised	60 cm	q	1533.3	376.0	343.9	32.1	131.7	11.86	63.07	8.61	42.95	62.36	9.45
124	2002	IV	Trellised	60 cm	o	1861.7	519.7	461.4	58.3	137.3	15.83	59.52	12.30	33.43	58.16	12.68
125	2002	IV	Trellised	60 cm	r	1777.9	569.7	501.0	68.7	116.6	13.17	63.90	18.95	27.24	60.44	20.75
126	2002	IV	Trellised	60 cm	s	1602.5	524.8	467.2	57.6	121.5	13.92	62.10	15.31	32.18	59.90	16.40
127	2002	IV	Trellised	60 cm	t	1662.9	562.9	508.4	54.5	125.7	14.66	61.40	14.26	32.51	59.51	15.13
128	2002	IV	Trellised	60 cm	n	1820.6	550.7	482.9	67.8	145.1	15.26	61.34	14.32	26.34	59.44	15.17
129	2003	II	Bush	20 cm	a	2099.3	532.7	460.6	72.0	203.0	13.75	60.25	14.51	30.46	58.33	15.10
130	2003	II	Bush	20 cm	b	1702.7	502.0	449.5	52.4	181.3	12.14	62.60	12.89	35.46	61.03	13.96
131	2003	II	Bush	20 cm	d	2393.8	675.9	584.9	91.0	253.0	18.22	60.98	16.15	25.71	58.57	16.97
132	2003	II	Bush	20 cm	g	1702.7	448.2	395.3	52.8	183.8	12.75	62.39	14.47	37.24	60.41	15.59
133	2003	II	Bush	20 cm	e	1925.8	593.6	503.0	90.6	216.1	17.09	60.23	15.40	25.31	58.07	16.00
134	2003	II	Bush	20 cm	c	1699.8	483.9	433.1	50.9	179.9	12.35	62.98	12.17	38.35	61.57	13.28
135	2003	II	Bush	20 cm	f	2051.2	569.0	503.8	65.2	201.0	16.33	62.10	16.39	30.29	59.58	17.52
136	2003	II	Bush	30 cm	a	1626.9	522.9	460.4	62.5	148.6	12.57	61.60	15.17	32.06	59.46	16.13
137	2003	II	Bush	30 cm	b	1787.3	493.2	440.2	53.0	188.2	11.58	63.38	10.94	38.65	62.23	12.03
138	2003	II	Bush	30 cm	d	1987.0	398.4	355.2	43.2	208.7	16.08	62.39	13.97	36.53	60.54	15.07

Table 1. Continued …

Wine	Vintage	Region	Vine[a]	Trunk[b]	Site[c]	TP (F-C)[d]	TA (pH shift)[e]	MA (pH shift)[f]	PA (pH shift)[g]	TF (DAC)[h]	TAC_M[i]	*C**	*h**[j]	*L**	*a**	*b**
139	2003	II	Bush	30 cm	g	1696.9	431.0	384.9	46.1	171.9	11.77	63.12	13.75	38.73	61.31	15.00
140	2003	II	Bush	30 cm	e	2328.2	624.3	531.4	93.0	217.0	15.82	61.12	15.11	22.62	59.01	15.94
141	2003	II	Bush	30 cm	c	2065.8	582.2	501.2	81.1	209.7	13.79	63.07	16.92	25.31	60.34	18.36
142	2003	II	Bush	30 cm	f	1792.7	539.6	472.6	67.0	177.6	12.54	64.04	16.96	27.17	61.26	18.68
143	2003	II	Trellised	30 cm	a	1917.1	546.2	473.1	73.1	156.9	13.66	61.17	14.10	26.20	59.33	14.90
144	2003	II	Trellised	30 cm	b	1908.4	535.6	468.0	67.5	173.5	12.07	62.67	14.47	28.32	60.69	15.66
145	2003	II	Trellised	30 cm	d	2322.2	664.9	561.0	103.8	201.2	15.78	58.83	14.47	21.32	56.96	14.70
146	2003	II	Trellised	30 cm	g	1363.0	412.2	371.6	40.5	130.9	9.96	55.75	10.07	46.37	54.89	9.75
147	2003	II	Trellised	30 cm	e	1980.8	596.5	506.7	89.8	213.7	14.49	61.21	14.85	24.11	59.16	15.69
148	2003	II	Trellised	30 cm	f	1648.0	516.9	463.1	53.8	159.5	11.72	64.67	14.44	33.07	62.63	16.13
149	2003	II	Trellised	60 cm	a	2077.4	628.0	540.4	87.7	177.6	13.77	60.62	15.56	24.59	58.40	16.26
150	2003	II	Trellised	60 cm	b	1745.0	576.9	508.0	68.9	172.2	11.88	63.42	14.98	29.66	61.27	16.39
151	2003	II	Trellised	60 cm	d	2173.7	663.5	577.2	86.4	200.1	14.87	60.73	14.65	25.16	58.76	15.36
152	2003	II	Trellised	60 cm	g	1167.5	386.2	351.2	35.0	105.5	9.04	56.45	10.42	48.02	55.52	10.21
153	2003	II	Trellised	60 cm	e	1931.6	518.1	451.4	66.8	169.7	12.92	62.18	15.67	26.73	59.87	16.79
154	2003	II	Trellised	60 cm	f	1845.6	545.1	477.1	68.1	179.6	13.17	63.51	15.27	30.56	61.27	16.72
155	2003	III	Bush	20 cm	l	2121.2	468.0	405.0	63.0	225.6	15.65	59.52	12.02	36.59	58.22	12.40
156	2003	III	Bush	20 cm	h	2020.6	504.2	445.1	59.1	192.4	13.61	62.68	14.29	30.86	60.74	15.47
157	2003	III	Bush	20 cm	k	2493.0	736.4	621.7	114.7	260.7	17.03	58.95	15.43	20.94	56.83	15.68
158	2003	III	Bush	20 cm	i	1851.4	528.4	462.0	66.5	169.8	13.69	60.33	12.98	32.02	58.79	13.55
159	2003	III	Bush	20 cm	m	1785.8	437.9	388.6	49.3	186.5	12.51	59.96	11.04	39.03	58.85	11.48
160	2003	III	Bush	30 cm	l	2274.5	509.9	447.9	62.0	227.4	16.54	60.11	13.26	36.89	58.51	13.79
161	2003	III	Bush	30 cm	j	1572.8	480.8	424.3	56.4	158.0	11.75	59.67	13.75	37.28	57.97	14.18
162	2003	III	Bush	30 cm	k	2626.0	668.0	551.0	117.1	276.8	18.89	59.27	15.65	21.24	57.08	15.99
163	2003	III	Bush	30 cm	i	2073.4	553.3	481.0	72.3	186.4	14.46	61.73	14.34	27.28	59.81	15.29
164	2003	III	Bush	30 cm	m	2487.1	560.8	480.8	80.0	237.1	17.62	60.82	15.17	22.98	58.70	15.92
165	2003	III	Bush	30 cm	h	1928.7	510.2	451.9	58.3	194.2	12.49	63.98	15.01	30.87	61.80	16.57
166	2003	III	Trellised	30 cm	l	1953.3	432.0	379.8	52.2	199.6	14.01	61.59	11.06	38.39	60.45	11.82
167	2003	III	Trellised	30 cm	h	1901.2	637.0	544.6	92.4	197.6	15.90	60.14	13.91	22.69	58.38	14.46
168	2003	III	Trellised	30 cm	k	1954.7	579.3	502.5	76.8	202.1	14.46	62.28	17.22	25.18	59.49	18.44
169	2003	III	Trellised	30 cm	i	1619.1	495.1	439.4	55.6	158.6	11.09	62.55	9.70	37.05	61.66	10.54
170	2003	III	Trellised	30 cm	m	2038.6	585.1	519.4	65.7	210.1	14.07	62.05	14.22	31.48	60.15	15.25
171	2003	III	Trellised	30 cm	j	1267.5	291.6	266.7	24.9	142.3	9.54	38.19	8.86	37.74	62.31	5.89
172	2003	III	Trellised	60 cm	l	2474.0	504.1	439.2	64.9	231.0	16.57	62.25	14.00	27.64	60.40	15.06
173	2003	III	Trellised	60 cm	h	2154.7	623.8	544.3	79.5	211.1	13.77	62.04	14.93	26.26	59.95	15.98
174	2003	III	Trellised	60 cm	k	1861.6	686.3	595.2	91.1	167.7	13.30	62.27	15.87	25.03	59.90	17.03
175	2003	III	Trellised	60 cm	i	1656.0	429.1	385.1	44.0	159.7	11.71	61.30	8.63	38.66	60.61	9.20
176	2003	III	Trellised	60 cm	m	2147.4	584.9	514.4	70.5	206.9	14.43	63.45	14.99	29.92	61.29	16.42
177	2003	III	Trellised	60 cm	j	1373.1	388.1	354.9	33.1	143.2	9.96	52.79	6.23	50.20	52.48	5.73
178	2003	IV	Bush	20 cm	p	2080.3	629.1	542.7	86.4	198.1	15.20	60.40	14.89	26.01	58.38	15.52
179	2003	IV	Bush	20 cm	q	2026.4	536.7	463.3	73.4	191.7	14.13	63.16	15.79	28.39	60.78	17.19
180	2003	IV	Bush	20 cm	o	1826.6	424.9	365.5	59.3	168.5	12.62	57.25	13.09	34.46	55.77	12.97
181	2003	IV	Bush	20 cm	r	1717.3	519.2	460.6	58.5	154.1	12.10	60.78	14.55	34.53	58.83	15.27
182	2003	IV	Bush	20 cm	s	1763.9	487.9	426.2	61.7	162.8	13.34	61.10	16.76	27.72	58.50	17.62
183	2003	IV	Bush	20 cm	t	2074.5	522.6	452.2	70.5	212.8	14.10	61.17	14.11	31.17	59.32	14.92
184	2003	IV	Bush	20 cm	n	2052.6	606.3	517.1	89.3	192.9	15.76	59.64	14.76	24.69	57.67	15.20
185	2003	IV	Bush	30 cm	p	1850.5	530.8	471.2	59.6	170.2	13.61	60.41	11.90	32.75	59.11	12.46
186	2003	IV	Bush	30 cm	q	2112.4	438.6	389.9	48.7	199.2	13.81	65.57	15.24	30.76	63.27	17.24

Table 1. Continued …

Wine	Vintage	Region	Vine[a]	Trunk[b]	Site[c]	TP (F-C)[d]	TA (pH shift)[e]	MA (pH shift)[f]	PA (pH shift)[g]	TF (DAC)[h]	TAC_M[i]	*C**	*h**[j]	*L**	*a**	*b**
187	2003	IV	Bush	30 cm	o	1682.7	484.5	427.8	56.7	162.3	13.03	59.24	11.20	31.61	58.11	11.51
188	2003	IV	Bush	30 cm	r	1578.6	580.1	512.8	67.3	164.5	12.02	62.05	14.53	33.16	60.06	15.57
189	2003	IV	Bush	30 cm	s	1810.0	492.9	440.0	53.0	171.5	12.66	61.45	14.81	32.19	59.41	15.71
190	2003	IV	Bush	30 cm	t	1898.3	502.2	441.3	60.9	192.6	13.46	61.22	13.78	31.31	59.46	14.59
191	2003	IV	Bush	30 cm	n	2003.1	560.2	494.5	65.7	192.4	13.62	62.03	15.26	29.80	59.84	16.32
192	2003	IV	Trellised	30 cm	p	1453.3	386.7	345.9	40.8	149.0	10.33	57.86	8.19	42.45	57.27	8.25
193	2003	IV	Trellised	30 cm	q	1731.8	480.0	422.2	57.7	162.3	10.64	63.97	14.11	32.80	62.04	15.59
194	2003	IV	Trellised	30 cm	o	1651.6	474.9	432.0	42.9	143.4	11.79	54.96	8.19	40.91	54.40	7.83
195	2003	IV	Trellised	30 cm	r	1781.4	534.3	477.9	56.4	160.0	12.56	59.71	14.21	33.33	57.88	14.66
196	2003	IV	Trellised	30 cm	s	1591.9	589.6	516.8	72.8	138.5	11.32	59.13	15.81	31.33	56.89	16.11
197	2003	IV	Trellised	30 cm	t	1756.6	535.3	483.4	51.9	150.7	13.57	60.25	11.64	32.62	59.01	12.16
198	2003	IV	Trellised	30 cm	n	2071.9	546.5	485.8	60.7	199.3	14.17	64.37	15.49	29.47	62.03	17.19
199	2003	IV	Trellised	60 cm	p	1472.3	380.6	342.8	37.9	147.3	10.71	57.61	6.36	44.95	57.26	6.39
200	2003	IV	Trellised	60 cm	q	1868.9	556.3	491.9	64.4	180.6	12.49	63.51	13.71	31.99	61.70	15.05
201	2003	IV	Trellised	60 cm	o	1507.3	468.3	418.8	49.5	132.1	11.03	55.14	10.84	39.02	54.16	10.37
202	2003	IV	Trellised	60 cm	r	1479.6	499.0	445.0	54.0	136.1	10.54	61.62	13.55	36.60	59.91	14.44
203	2003	IV	Trellised	60 cm	s	1637.1	534.5	482.1	52.4	151.8	11.28	60.47	13.71	36.29	58.75	14.33
204	2003	IV	Trellised	60 cm	t	1626.9	547.5	491.4	56.2	143.7	11.38	60.80	11.51	34.93	59.58	12.14
205	2003	IV	Trellised	60 cm	n	2032.2	562.9	497.7	65.2	205.0	14.18	63.88	14.81	29.86	61.76	16.33

Table 2. Anthocyanin and flavonol content[a] (measured using HPLC) of the 2001, 2002 and 2003 individual Pinotage wines from different climatic regions and vine structure treatments.

Wine[b]	Dp-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Dp-3-glc-ac[c]	Vitisin A[c]	Pt-3-glc-ac[c]	Pn-3-glc-ac[c]	Mv-3-glc-ac[c]	Mv-3-glc-coum[c]	TA (HPLC)[d]	CP[e]	UF[f]	Q-3-gal	Q-3-glc	Q-3-rham	M	Q	K	Total flavonols[g]
65	17.42	25.06	9.00	180.42	6.07	10.46	5.90	3.15	35.78	10.40	303.64	9.67	12.33	0.00	10.03	6.01	2.72	4.72	1.17	37.57
66	17.91	22.57	13.30	168.52	5.11	2.27	5.21	4.56	35.08	16.42	290.93	7.56	24.44	3.22	17.41	11.61	1.84	7.39	1.12	67.79
67	22.38	30.30	14.39	209.83	8.03	4.65	8.07	6.26	47.96	14.65	366.52	5.72	26.43	4.51	19.11	10.28	2.40	7.76	0.84	72.23
68	16.55	24.79	11.76	221.32	5.39	3.72	5.78	5.30	50.84	22.11	367.57	9.32	23.89	2.52	16.00	9.64	2.64	3.71	0.00	59.10
69	27.31	32.35	15.27	190.17	8.22	4.90	8.17	4.90	38.48	13.50	343.28	4.28	21.37	1.93	12.87	8.16	3.30	6.13	1.09	55.60
70	23.52	26.78	17.10	181.62	6.25	1.95	6.09	5.13	37.99	19.48	325.91	3.81	26.36	4.82	18.39	14.02	2.49	10.69	2.24	80.04
71	21.09	27.73	11.34	182.50	7.81	6.51	7.00	4.61	40.02	14.66	323.25	10.31	25.54	2.62	17.54	8.92	5.20	8.81	1.21	70.95
72	16.50	23.81	10.62	219.25	5.16	3.41	5.35	3.60	45.83	23.82	357.35	4.00	21.40	0.00	15.05	8.61	1.85	5.36	0.58	53.56
73	21.81	28.92	9.59	217.47	6.66	5.43	6.58	2.59	46.31	21.34	366.71	10.99	32.05	0.00	13.59	7.74	2.05	3.66	0.64	60.31
74	22.57	28.63	13.21	233.97	6.19	3.00	7.25	3.71	50.31	29.66	398.49	4.54	33.11	4.14	24.55	12.91	1.53	7.24	1.91	86.04
75	26.36	28.46	15.86	162.12	7.27	2.79	6.64	4.47	30.48	13.77	298.22	4.66	10.61	0.00	7.11	7.52	4.59	5.47	0.84	36.91
76	15.47	22.15	9.31	176.61	6.97	7.88	6.34	4.55	43.36	13.01	305.66	8.44	17.91	0.00	8.92	7.76	3.64	4.63	0.72	44.42
77	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
78	24.80	27.75	12.32	170.64	6.41	4.01	5.95	2.71	32.15	17.19	303.93	9.74	22.30	0.00	12.06	8.75	4.29	9.01	1.36	58.67
79	27.03	33.73	16.66	255.23	7.57	3.97	7.53	6.17	58.22	31.07	447.19	11.00	39.32	4.38	22.60	16.05	1.82	9.61	2.38	97.18
80	23.13	27.66	14.72	166.14	7.54	4.08	7.16	5.01	35.18	14.65	305.27	8.88	9.96	0.00	8.57	8.65	5.04	4.57	0.52	38.04
81	16.92	26.79	6.46	228.90	8.83	5.90	7.76	2.91	60.57	22.14	387.17	6.12	27.49	0.00	15.01	7.09	2.44	4.33	0.78	57.74
82	15.96	21.29	9.38	214.81	4.54	2.02	4.72	3.11	48.96	28.87	353.65	0.00	21.46	2.42	13.15	10.23	1.62	6.34	1.01	56.23
83	14.66	23.41	8.47	187.24	6.76	7.83	6.97	4.44	45.30	13.46	318.54	6.78	12.68	0.00	12.76	7.40	3.05	3.27	0.00	39.71
84	17.69	24.47	11.45	207.40	4.86	1.85	6.01	4.10	46.01	19.77	343.61	4.65	15.94	2.95	16.07	10.66	3.51	5.24	0.52	55.86
85	12.29	16.30	8.15	115.85	4.42	3.43	3.76	8.15	20.54	6.91	199.80	6.02	6.56	0.00	6.47	4.56	1.48	0.95	0.00	20.02
86	8.95	17.75	6.34	191.88	4.50	10.56	4.22	4.08	45.82	17.46	311.56	11.36	19.38	0.00	15.37	8.17	4.31	4.70	0.40	53.38
87	16.39	24.07	11.26	200.95	5.19	5.66	5.50	4.39	27.33	16.03	316.78	4.79	22.92	2.02	14.73	9.65	1.08	3.33	0.44	54.77
88	15.84	25.27	6.54	231.56	6.46	6.42	6.81	2.83	53.12	20.01	374.87	4.88	15.85	0.00	13.14	6.32	2.21	2.42	0.00	39.95
89	21.89	26.30	15.31	210.27	5.82	2.14	6.31	4.41	48.23	19.77	360.47	3.87	15.04	2.84	16.45	8.49	3.25	4.41	0.39	51.68
90	14.29	19.53	8.39	147.65	5.20	6.08	4.58	3.81	27.33	10.64	247.52	4.10	9.18	0.00	8.11	5.35	2.36	0.60	0.00	25.59
91	13.40	20.84	9.25	202.19	4.68	5.19	4.75	3.84	46.59	18.19	328.92	6.31	15.23	0.00	14.72	9.13	4.64	3.26	0.00	47.84
92	18.79	24.84	12.38	186.70	5.68	6.02	5.68	3.66	37.08	16.44	317.26	5.56	22.68	3.99	15.93	11.40	5.18	8.70	1.38	70.26
93	22.43	34.60	8.82	294.26	10.10	6.10	10.17	5.94	74.01	26.97	493.39	5.57	14.90	0.00	8.39	7.56	4.26	4.18	0.57	40.58
94	18.65	26.64	10.03	200.61	7.23	8.22	6.61	5.31	38.68	16.34	338.30	11.15	14.43	0.00	6.90	6.20	3.74	0.97	0.00	32.24
95	20.58	28.62	12.26	253.36	6.64	4.23	7.09	3.60	51.81	23.70	411.89	10.86	11.21	0.00	11.23	5.73	4.20	1.12	0.00	33.50
96	15.45	20.97	9.30	169.16	5.68	5.70	5.17	4.20	39.69	16.99	292.31	11.19	14.91	0.00	8.81	7.50	5.14	5.11	0.57	42.98
97	16.90	25.11	8.21	251.54	5.71	3.08	5.88	2.79	60.07	29.81	409.09	4.99	24.61	0.00	13.82	8.64	2.19	3.16	0.00	52.93
98	17.83	30.82	7.27	291.35	8.41	9.58	8.54	4.79	74.88	33.17	486.65	13.82	18.26	0.00	9.00	7.60	4.03	7.06	1.00	47.81
99	20.97	31.30	10.79	271.90	8.09	3.80	8.55	5.85	61.75	26.26	449.25	4.14	19.19	0.00	6.82	5.40	1.96	0.94	0.00	34.30
100	15.20	25.71	8.59	238.55	4.66	2.52	4.74	2.94	54.82	30.25	387.98	6.07	18.22	0.00	13.16	8.87	2.01	4.54	0.54	48.05
101	15.87	22.03	9.83	202.22	4.80	4.38	4.47	2.77	43.29	22.36	332.03	11.87	16.12	0.00	12.45	8.27	3.66	5.99	0.66	48.10
102	15.58	25.95	7.39	270.10	6.21	7.23	6.65	4.11	65.23	34.70	443.15	9.91	14.40	0.00	6.98	7.23	3.94	3.62	0.46	37.33
103	13.84	23.59	6.79	225.66	6.99	14.61	6.46	3.46	60.15	22.06	383.60	16.53	16.66	0.00	14.85	8.38	9.16	5.28	0.39	55.48
104	12.11	20.21	9.15	195.66	4.52	6.69	5.65	4.44	49.48	15.99	323.88	8.03	17.75	0.00	14.81	7.84	3.11	1.86	0.00	45.93
105	11.52	18.29	6.94	181.36	4.97	7.40	5.35	3.17	49.36	18.17	306.54	4.79	23.90	3.29	21.38	12.66	2.58	8.64	1.45	74.81
106	14.31	14.70	9.39	186.29	6.59	9.06	6.70	5.11	46.30	14.25	312.72	7.10	10.98	0.00	12.03	7.00	4.17	2.81	0.00	37.46
107	10.79	19.55	6.55	205.41	5.58	10.18	5.85	4.77	54.84	14.98	338.50	6.32	11.27	0.00	13.88	7.69	2.76	1.98	0.00	37.58

[a] mg/L unless otherwise noted; [b] wine numbers correspond to those in **Table 1**; [c] mg corresponding anthocyanin-monoglucoside equivalents/L; [d] sum of individual anthocyanin content; [e] mg malvidin-3-glc equivalents/L; [f] mg rutin equivalents/L; [g] sum of individual flavonol content; ac = acetate; coum = coumarate; Dp = delphinidin; glc = glucoside; K = kaempferol; M = myricetin; Mv = malvidin; na = not available; Pn = peonidin; Pt = petunidin; Q = quercetin.

Table 2. Continued …

Wine[b]	Dp-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Dp-3-glc-ac[c]	Vitisin A[c]	Pt-3-glc-ac[c]	Pn-3-glc-ac[c]	Mv-3-glc-ac[c]	Mv-3-glc-coum[c]	TA (HPLC)[d]	CP[e]	UF[f]	Q-3-gal	Q-3-glc	Q-3-rham	M	Q	K	Total flavonols[g]
108	16.73	26.71	10.05	237.02	7.75	6.40	8.93	5.51	66.83	19.29	405.22	3.58	16.29	0.00	17.32	7.14	3.75	3.69	0.40	49.20
109	14.37	23.36	10.99	215.47	5.63	8.97	5.93	5.13	48.14	16.11	354.11	10.01	18.53	0.00	14.42	8.29	5.47	4.94	0.56	53.12
110	13.43	23.64	7.19	225.37	6.31	14.15	5.92	3.07	51.00	15.96	366.04	13.27	12.42	0.00	11.88	6.66	3.26	3.16	0.00	37.38
111	9.92	18.50	7.27	229.60	4.17	6.40	4.67	3.60	57.78	19.39	361.30	8.87	15.64	0.00	13.73	7.60	2.89	1.33	0.00	41.18
112	10.84	19.92	6.56	208.36	5.76	6.53	6.82	4.14	62.09	21.19	352.21	4.14	28.80	0.00	20.63	10.08	2.31	5.50	0.87	68.81
113	15.72	24.24	9.05	191.25	6.98	6.69	7.41	4.00	44.50	14.86	324.70	6.62	10.74	0.00	12.37	7.19	2.71	2.30	0.00	35.31
114	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
115	11.47	19.51	7.34	172.04	5.94	14.17	4.71	3.58	38.32	13.38	290.46	10.38	9.52	0.00	10.36	6.88	1.47	2.43	0.00	30.67
116	15.74	23.34	12.83	189.15	5.92	6.20	6.27	5.25	40.43	14.62	319.75	10.30	19.96	4.82	13.95	7.93	3.80	4.55	0.55	56.46
117	9.90	16.48	6.69	238.95	3.36	2.44	3.07	2.25	62.97	36.49	382.60	6.48	14.90	0.00	13.61	7.14	1.59	2.48	0.00	39.73
118	9.16	16.35	4.27	186.32	4.84	7.97	5.02	2.64	58.03	27.62	322.21	8.59	29.60	1.80	16.78	10.62	1.86	4.90	1.10	67.24
119	23.27	29.88	11.32	211.42	7.61	6.87	7.65	3.23	48.24	17.81	367.30	11.70	16.65	0.00	13.08	6.08	4.52	2.84	0.44	44.25
120	10.23	18.71	4.74	244.54	4.81	8.10	5.09	2.45	65.82	30.55	395.04	4.82	13.56	0.00	13.47	5.77	2.31	1.32	0.00	36.44
121	10.01	19.06	4.77	239.73	6.13	8.56	6.39	3.46	72.63	29.80	400.55	9.11	17.39	0.00	15.72	7.10	4.32	3.76	0.00	48.99
122	18.32	28.12	12.17	240.04	6.57	8.34	7.07	4.46	50.33	20.30	395.72	12.01	21.77	0.00	13.13	7.87	3.84	3.01	0.00	50.29
123	8.54	13.61	4.84	200.66	2.89	2.95	3.62	3.89	58.38	30.97	330.35	6.54	13.64	0.00	11.35	7.43	1.18	2.30	0.00	35.91
124	15.56	24.66	6.15	237.27	6.99	7.67	7.20	3.87	67.25	33.40	410.03	17.20	38.90	0.00	15.69	10.35	2.10	4.05	0.96	72.62
125	23.91	30.77	12.10	227.40	7.89	7.88	7.37	3.52	50.42	23.83	395.10	16.08	12.52	0.00	12.80	6.09	4.69	3.53	0.00	40.26
126	16.20	23.89	7.01	219.60	6.08	8.05	5.99	2.63	53.04	24.81	367.29	12.82	21.02	0.00	14.29	6.71	4.50	2.71	0.00	49.74
127	17.28	27.30	6.80	242.82	7.74	9.17	8.16	2.83	61.66	24.83	408.58	11.31	19.61	0.00	14.87	6.92	3.55	2.89	0.00	48.37
128	19.52	29.01	9.91	239.37	7.50	7.55	7.13	3.14	50.22	21.98	395.33	15.79	25.71	2.81	16.76	9.74	4.16	6.26	0.58	66.94
129	12.15	19.57	7.54	193.81	3.96	3.34	5.04	8.22	65.27	13.27	332.17	12.01	35.31	0.00	38.11	13.89	0.00	2.23	0.62	90.17
130	21.44	29.11	9.51	244.99	6.50	2.02	7.41	7.84	65.23	17.87	411.92	10.06	28.92	3.93	20.78	11.40	0.00	3.80	1.17	70.00
131	21.64	28.76	10.90	213.75	7.16	3.77	8.06	8.95	61.27	12.80	377.05	12.01	35.57	3.99	19.55	10.41	0.54	4.65	1.07	76.27
132	12.45	18.30	6.34	185.53	2.26	2.41	2.39	4.58	45.60	16.37	296.25	7.91	11.87	0.00	7.85	7.46	0.50	2.86	0.25	30.91
133	14.85	22.89	8.61	193.48	4.81	6.19	5.23	6.94	50.59	12.68	326.29	13.69	30.51	0.00	25.10	14.88	0.00	5.69	1.63	78.25
134	19.93	27.36	7.69	237.12	6.79	1.43	8.52	6.65	69.57	14.36	399.40	8.83	22.82	0.00	15.15	7.18	0.00	2.38	0.66	48.19
135	13.68	22.51	6.14	228.45	5.32	6.12	5.04	6.11	60.51	17.61	371.50	11.38	16.44	0.00	7.85	5.86	0.95	2.50	0.15	33.81
136	18.83	30.05	10.91	243.40	7.30	4.70	8.36	9.41	59.61	12.43	405.00	11.49	14.69	2.20	10.46	8.05	0.93	2.65	0.00	39.47
137	20.84	27.57	7.60	238.27	5.95	1.54	27.57	5.77	63.68	19.69	418.47	8.95	29.40	0.00	26.60	11.71	0.00	3.40	1.26	72.36
138	16.22	21.52	9.41	177.79	5.02	3.15	5.39	7.43	47.55	11.61	305.11	9.50	22.56	2.93	15.20	8.03	0.00	4.26	0.69	54.04
139	18.76	24.78	8.61	215.49	4.43	3.58	4.07	6.26	53.13	19.06	358.16	9.03	15.25	2.86	9.89	9.86	1.10	4.52	0.87	44.98
140	19.46	25.33	7.45	222.16	5.12	4.64	4.94	6.14	54.81	18.62	368.66	18.31	44.06	0.00	27.27	16.20	0.00	6.00	1.96	95.82
141	21.18	26.23	9.58	201.68	6.39	2.95	7.34	6.46	56.41	14.82	353.03	16.04	34.86	0.00	22.82	10.00	0.00	2.31	0.63	70.62
142	17.67	25.73	9.94	240.46	5.32	4.85	6.25	7.41	58.33	17.86	393.81	15.10	19.38	2.17	10.72	8.62	0.00	3.77	0.53	45.56
143	6.29	13.75	2.28	258.10	2.49	8.69	2.30	4.22	70.87	26.61	395.60	16.66	15.77	0.00	9.25	8.85	0.00	3.63	0.90	38.83
144	14.53	22.05	6.05	229.08	4.39	4.42	4.38	5.68	55.74	17.91	364.23	13.47	31.39	0.00	18.07	10.78	0.00	3.89	1.17	65.29
145	14.45	22.56	7.02	198.89	6.79	10.83	6.00	8.16	59.97	12.74	347.42	18.51	39.12	0.00	19.99	10.52	0.00	5.68	1.04	76.80
146	12.90	21.67	4.61	229.59	3.99	2.70	4.18	4.88	60.65	23.79	368.93	8.72	7.80	0.00	7.80	9.67	0.00	2.62	0.00	27.88
147	15.51	22.21	6.71	204.54	4.74	5.04	4.81	5.90	52.31	16.48	338.25	18.12	38.30	0.00	25.45	14.96	0.00	5.69	1.64	86.38
148	14.65	23.45	6.60	266.25	3.93	2.43	4.67	6.21	70.48	27.47	426.14	14.39	21.43	2.18	11.67	10.16	0.00	2.78	0.26	48.48
149	11.43	20.72	3.81	225.36	4.03	8.21	3.56	5.06	54.93	16.56	353.68	17.24	15.27	0.00	8.89	7.32	1.56	4.26	0.68	38.09
150	19.04	27.04	6.60	257.50	5.18	4.25	5.12	6.78	62.06	20.42	414.01	13.92	34.49	3.06	16.98	9.84	0.00	3.57	1.12	69.05
151	16.07	25.03	6.61	227.52	6.30	5.18	6.94	7.61	67.54	15.24	384.05	15.19	38.69	0.00	18.90	8.10	0.00	3.04	0.48	69.23
152	10.01	18.89	3.27	227.77	3.25	3.66	3.40	5.61	60.45	24.48	360.79	9.27	11.08	0.00	6.74	7.57	0.49	1.38	0.00	27.27
153	13.48	21.34	5.41	208.02	4.15	4.56	4.24	5.25	50.35	15.07	331.88	14.01	29.96	0.00	16.24	8.88	0.00	2.81	0.57	58.46

Table 2. Continued …

Wine[b]	Dp-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Dp-3-glc-ac[c]	Vitisin A[c]	Pt-3-glc-ac[c]	Pn-3-glc-ac[c]	Mv-3-glc-ac[c]	Mv-3-glc-coum[c]	TA (HPLC)[d]	CP[e]	UF[f]	Q-3-gal	Q-3-glc	Q-3-rham	M	Q	K	Total flavonols[g]
154	15.07	24.04	6.48	273.77	3.52	3.75	4.43	5.56	63.88	30.32	430.81	23.57	15.88	0.00	10.98	11.37	0.00	2.51	0.00	41.13
155	9.21	14.80	5.66	201.15	3.19	4.10	2.91	5.97	59.31	20.33	326.62	11.16	19.24	0.00	12.66	9.49	0.00	4.51	0.74	47.04
156	15.56	23.89	7.49	224.29	4.67	3.55	5.10	6.26	59.66	16.89	367.36	10.70	23.29	3.29	12.64	9.96	0.64	5.11	1.05	56.55
157	18.74	28.22	10.56	230.26	7.25	13.00	6.47	8.13	60.31	12.59	395.53	16.78	16.03	0.00	8.78	6.42	1.81	3.06	0.50	37.17
158	5.88	13.56	1.48	225.65	2.58	6.37	2.50	4.26	70.72	21.76	354.76	12.32	31.94	0.00	16.11	9.27	0.09	3.09	0.76	61.25
159	10.50	18.43	4.71	216.65	3.11	3.36	3.22	4.69	55.97	16.17	336.81	9.55	16.19	1.36	11.30	8.04	0.00	2.75	0.40	40.05
160	14.69	21.58	8.41	230.20	4.02	3.27	4.18	7.12	61.36	22.13	376.96	11.61	17.49	2.43	11.40	9.39	0.00	3.12	0.42	44.81
161	10.44	18.38	3.45	219.10	4.23	8.34	3.90	5.61	68.53	24.34	366.31	12.60	12.53	0.00	7.68	6.00	0.00	2.14	0.00	28.35
162	14.48	21.97	7.72	191.72	5.96	13.80	4.33	6.82	48.42	10.15	325.36	16.47	9.48	0.00	6.77	6.51	0.00	2.80	0.23	26.05
163	14.82	21.89	5.74	233.67	4.35	4.00	5.22	6.32	67.02	23.40	386.45	16.55	35.85	2.86	17.97	11.68	0.00	5.04	1.34	75.10
164	18.70	24.21	9.34	197.17	5.64	9.91	5.06	6.69	48.41	13.50	338.64	19.45	30.24	3.20	15.29	11.71	0.31	4.48	0.89	66.45
165	21.89	29.07	8.82	238.51	6.36	3.92	6.83	6.81	59.20	19.06	400.47	13.77	27.78	0.00	15.19	9.45	0.00	4.87	1.19	58.75
166	11.24	17.62	3.26	209.02	3.37	2.30	3.60	4.03	60.76	20.70	335.90	9.54	17.94	0.00	9.38	7.13	0.00	3.26	0.43	38.13
167	14.31	22.13	6.65	218.00	4.77	7.00	4.22	6.26	57.69	17.25	358.27	17.68	31.98	3.49	16.94	12.06	0.33	5.36	1.17	71.74
168	17.16	26.96	7.60	221.92	8.08	16.50	6.42	7.39	57.84	15.09	384.96	16.26	14.44	0.00	7.54	4.97	0.00	2.32	0.00	29.27
169	9.07	16.23	2.11	238.54	2.49	1.87	2.84	3.84	73.89	29.70	380.58	12.82	23.90	1.28	11.39	8.06	0.00	2.73	0.53	47.90
170	16.54	24.64	5.54	274.66	4.90	5.54	5.51	5.59	70.72	25.16	438.80	14.16	28.25	0.00	12.88	8.87	0.00	2.65	0.79	53.44
171	5.89	11.30	1.34	191.67	1.94	0.54	3.42	2.65	63.39	28.04	310.19	6.01	9.93	0.00	5.84	6.05	0.50	2.54	0.50	25.36
172	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
173	22.23	29.41	8.22	269.81	6.04	5.80	5.78	6.66	68.23	27.08	449.27	26.45	33.86	0.00	16.07	10.54	0.93	6.82	1.30	70.08
174	22.68	35.63	9.20	297.91	8.55	10.75	8.80	8.45	75.68	20.17	497.82	21.29	21.55	0.00	10.12	5.85	0.54	2.01	0.25	40.33
175	8.68	16.28	2.64	230.64	2.88	1.99	3.22	4.28	66.07	25.44	362.10	13.85	23.55	0.00	11.71	7.67	0.00	2.55	0.67	46.16
176	20.64	27.27	6.90	246.75	5.69	5.49	5.39	5.47	58.88	20.23	402.69	16.16	27.92	0.00	12.69	8.08	0.00	3.18	0.79	52.65
177	7.26	13.82	1.31	233.97	2.45	1.69	2.62	3.01	74.05	31.41	371.58	10.40	12.09	0.00	5.86	3.90	0.00	1.10	0.04	22.98
178	8.70	16.48	2.31	207.53	2.96	5.03	3.09	4.84	71.40	18.18	340.53	14.37	44.73	0.00	22.01	12.79	0.00	4.28	0.87	84.83
179	9.37	15.45	3.72	199.03	2.34	2.45	2.64	4.43	68.88	23.38	331.70	14.96	31.55	2.38	15.67	11.57	0.00	3.99	0.75	66.06
180	5.09	11.77	2.97	181.74	2.30	9.00	2.02	4.82	59.25	16.85	295.82	13.71	25.10	2.95	18.83	14.37	0.64	5.35	0.97	68.82
181	12.85	21.97	5.11	233.97	5.94	6.85	6.10	6.87	85.02	21.00	405.67	9.17	21.92	2.59	12.33	8.39	0.70	3.67	0.68	50.28
182	8.99	16.05	4.95	181.75	4.04	10.31	3.33	6.41	61.94	13.78	311.53	14.17	23.75	0.00	12.15	7.19	0.41	3.09	0.27	46.86
183	13.01	20.54	8.58	201.58	4.73	4.23	4.69	8.39	68.60	13.86	348.21	11.12	36.76	0.00	39.55	14.82	0.00	2.48	0.88	94.49
184	9.75	18.95	6.55	221.57	3.01	5.64	3.80	6.69	60.73	14.00	350.69	14.19	19.72	1.79	11.66	9.95	0.63	3.82	0.32	48.20
185	10.01	18.67	2.61	225.38	4.49	6.24	5.59	5.35	82.05	27.03	387.40	15.53	37.27	0.00	19.73	10.11	0.00	3.66	0.95	71.72
186	11.67	17.19	4.56	208.93	2.74	2.27	3.86	4.35	66.42	26.93	348.93	12.89	29.85	0.00	17.97	11.83	0.00	3.62	1.08	64.36
187	7.24	15.16	3.30	220.33	3.59	6.37	4.06	6.22	79.85	20.74	366.88	13.75	25.30	0.00	16.33	11.08	0.00	4.18	0.89	58.14
188	14.59	23.59	6.42	234.70	6.66	7.13	7.66	8.89	82.07	20.23	411.95	10.71	16.85	0.00	11.19	7.23	0.71	3.39	0.37	40.08
189	11.58	18.94	6.46	208.47	5.22	8.61	5.30	7.88	72.06	17.19	361.69	12.01	25.11	0.00	15.65	9.68	0.00	4.02	0.80	55.65
190	11.16	18.44	6.15	200.47	4.22	4.71	4.71	7.30	68.49	15.69	341.34	11.08	32.83	0.00	27.16	11.72	0.00	3.08	1.01	75.80
191	17.53	26.27	10.40	260.51	5.80	3.60	7.08	8.81	72.40	19.59	431.98	13.77	19.97	2.44	12.69	10.12	0.40	4.53	0.48	51.19
192	6.23	12.13	1.34	201.77	2.02	3.03	2.33	3.49	77.36	28.48	338.17	11.43	22.25	0.00	12.73	7.98	0.00	2.10	0.58	45.64
193	9.82	15.84	3.35	239.02	2.77	1.13	3.02	4.40	76.17	28.58	384.10	15.24	20.64	0.00	11.21	7.45	0.85	2.14	0.47	42.76
194	5.54	14.34	1.00	255.59	3.41	4.77	5.16	4.43	100.94	41.38	436.56	13.76	27.49	0.00	19.05	11.36	0.00	3.36	1.02	62.28
195	11.80	20.12	2.82	262.80	5.08	4.89	4.89	5.81	94.69	30.32	443.21	13.82	18.93	0.00	8.22	6.16	0.00	1.80	0.26	35.37
196	8.34	19.81	3.89	269.28	5.58	16.49	6.24	7.50	92.03	25.60	454.77	16.98	15.50	0.00	9.34	6.33	1.17	2.38	0.41	35.13
197	9.81	18.31	1.47	252.68	4.01	3.69	4.25	4.15	95.76	30.46	424.60	12.94	31.62	0.00	16.19	7.78	0.00	1.42	0.27	57.29
198	17.15	23.36	8.31	244.86	4.14	2.14	4.59	6.49	68.36	24.71	404.11	13.01	26.78	2.19	11.89	8.93	0.00	3.43	0.68	54.30
199	6.54	12.16	1.68	211.75	2.67	3.22	2.48	3.56	80.86	32.85	357.76	12.37	22.89	0.00	14.57	6.88	0.00	2.82	0.70	47.85

Table 2. Continued …

Wine[b]	Dp-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Dp-3-glc-ac[c]	Vitisin A[c]	Pt-3-glc-ac[c]	Pn-3-glc-ac[c]	Mv-3-glc-ac[c]	Mv-3-glc-coum[c]	TA (HPLC)[d]	CP[e]	UF[f]	Q-3-gal	Q-3-glc	Q-3-rham	M	Q	K	Total flavonols[g]
200	12.82	21.33	3.22	274.22	4.67	2.61	4.83	5.03	86.20	38.81	453.75	21.85	27.38	0.00	12.97	8.66	0.00	3.68	0.65	53.61
201	5.33	12.96	0.94	234.27	3.57	11.61	3.48	3.98	92.42	33.54	402.10	19.59	25.97	0.00	14.68	8.79	0.00	1.75	0.46	51.63
202	11.84	19.92	2.38	259.83	4.55	5.08	5.00	4.89	86.19	34.87	434.54	18.03	18.27	0.00	9.27	6.30	0.00	0.95	0.00	34.79
203	14.05	24.44	4.28	288.42	6.45	7.49	7.06	6.86	91.08	29.55	479.68	14.63	20.02	0.00	10.79	5.88	0.00	2.87	0.47	40.03
204	13.71	22.86	3.74	279.70	5.86	5.02	8.10	5.94	100.03	32.70	477.68	18.51	29.70	0.00	16.27	8.87	0.00	2.16	0.55	57.55
205	17.97	24.97	7.44	250.65	4.36	1.55	4.92	5.80	70.51	25.49	413.67	14.25	28.10	1.96	12.30	8.44	0.24	4.05	0.59	55.78

Table 3. Phenolic acid, flavan-3-ol and total monomer content[a] (measured using HPLC), and antioxidant capacity (calculated and remaining) of the 2001, 2002 and 2003 individual Pinotage wines from different climatic regions and vine structure treatments.

Wine[b]	Gallic acid	Caftaric acid	Caffeic acid	Coutaric acid[c]	*p*-Coumaric acid	Total phenolic acids[d]	(+)-Catechin	Procyanidin B1	Total flavan-3-ols (HPLC)[e]	Non-coloured polymers[f]	Total monomers[g]	TAC_{CAL}[h]	TAC_R[i]
65	20.90	198.69	3.66	19.13	2.42	244.81	33.64	23.03	56.67	113.93	642.69	2.16	17.40
66	17.25	212.97	6.84	24.11	1.54	262.71	52.91	37.13	90.04	88.58	711.47	2.28	15.48
67	12.24	185.60	6.06	20.86	1.69	226.45	36.07	24.43	60.51	117.20	725.70	2.25	15.65
68	20.34	193.00	5.31	19.72	1.50	239.89	38.65	30.40	69.05	143.62	735.60	2.37	15.52
69	14.94	188.08	4.21	20.67	1.14	229.03	43.22	29.64	72.85	33.01	700.77	2.26	14.61
70	17.15	233.62	6.76	26.15	1.22	284.90	59.36	39.56	98.92	76.12	789.77	2.52	19.06
71	11.52	218.90	7.21	23.80	3.15	264.58	29.44	21.13	50.57	161.85	709.35	2.20	14.78
72	11.27	195.05	3.45	17.86	1.17	228.80	33.77	24.36	58.13	110.30	697.84	2.14	12.74
73	10.51	227.42	4.75	24.21	0.82	267.71	36.09	23.97	60.07	137.37	754.80	2.27	13.85
74	15.09	247.69	5.25	26.08	0.74	294.85	45.80	31.53	77.33	137.80	856.71	2.62	10.27
75	10.23	184.53	5.36	18.92	1.83	220.87	40.07	28.51	68.58	92.05	624.57	2.02	12.21
76	12.02	172.19	6.62	18.41	3.34	212.58	29.42	23.08	52.50	105.66	615.15	1.94	13.60
77	na	na	na	na	na	na	na	na	na	na	na	na	na
78	9.56	209.49	4.49	23.48	0.80	247.81	26.91	19.90	46.82	138.22	657.22	2.04	13.10
79	16.46	248.28	6.34	27.56	1.11	299.75	39.77	29.54	69.30	226.80	913.42	2.80	16.19
80	11.16	186.81	5.34	20.15	1.88	225.34	36.00	24.28	60.28	120.05	628.93	2.03	11.36
81	8.13	231.40	6.01	24.45	3.82	273.81	33.54	21.14	54.68	112.42	773.40	2.29	13.38
82	8.61	226.90	5.98	23.74	1.15	266.38	31.43	23.04	54.47	73.51	730.73	2.18	9.13
83	11.88	180.12	3.98	18.70	1.97	216.64	24.56	20.11	44.67	85.14	619.57	1.95	12.61
84	13.67	155.34	5.06	16.27	1.50	191.84	32.59	19.79	52.39	91.31	643.70	2.05	12.41
85	40.79	113.03	10.16	10.66	3.34	177.99	36.03	38.96	74.99	114.31	472.79	1.97	15.44
86	19.89	162.31	6.03	17.56	2.89	208.69	28.31	24.24	52.55	158.95	626.18	2.05	15.94
87	15.44	192.97	5.94	20.82	1.42	236.58	45.83	31.53	77.37	59.80	685.50	2.17	14.41
88	5.49	178.75	4.39	19.28	1.66	209.57	23.86	17.33	41.19	80.67	665.58	1.97	9.79
89	14.16	139.37	4.25	15.57	1.63	174.98	43.33	23.81	67.15	48.24	654.27	2.11	12.46
90	20.32	122.93	6.34	13.09	2.17	164.85	33.39	24.55	57.95	87.73	495.90	1.74	12.03
91	15.86	187.42	6.30	19.50	1.81	230.90	38.88	29.67	68.56	115.18	676.22	2.17	14.75
92	14.99	222.33	5.76	23.90	1.43	268.41	45.92	26.79	72.71	146.59	728.64	2.31	17.19
93	6.80	203.13	4.20	21.07	2.37	237.57	19.74	17.41	37.15	97.28	808.69	2.43	9.72
94	21.43	109.28	6.57	11.66	2.92	151.85	27.69	24.71	52.41	172.79	574.79	1.99	12.96
95	13.74	120.17	3.66	12.33	1.53	151.43	39.26	23.55	62.80	130.38	659.62	2.14	11.70
96	14.04	215.11	8.08	21.40	2.48	261.11	31.67	24.74	56.40	185.99	652.80	2.09	14.99
97	8.98	203.44	4.83	19.90	1.06	238.21	29.44	19.39	48.83	96.73	749.05	2.24	13.70
98	9.38	196.98	5.59	19.98	3.79	235.72	20.57	14.13	34.71	190.99	804.89	2.44	12.27
99	8.95	145.08	4.06	15.63	1.80	175.52	26.64	20.51	47.16	82.46	706.23	2.16	10.28
100	10.93	174.51	5.26	19.59	1.02	211.31	32.95	21.05	54.00	133.59	701.34	2.15	12.06
101	13.35	198.01	6.30	20.76	1.19	239.60	39.65	25.93	65.58	215.68	685.31	2.17	14.46
102	9.50	209.57	6.81	20.65	2.55	249.09	21.94	14.81	36.75	152.07	766.31	2.33	11.66
103	16.22	200.32	8.56	19.74	4.40	249.23	32.22	25.81	58.03	182.08	746.34	2.40	14.39
104	13.86	141.63	4.63	13.40	1.77	175.29	35.85	22.81	58.66	111.37	603.76	1.92	12.35
105	13.91	206.58	9.96	18.69	3.72	252.85	26.26	22.05	48.31	111.57	682.51	2.11	12.61
106	12.13	142.81	4.18	14.23	2.62	175.97	26.29	18.73	45.02	48.46	571.17	1.82	12.25
107	13.27	142.19	8.74	12.96	4.18	181.35	17.47	16.68	34.16	82.20	591.58	1.88	10.71

[a] mg/L unless otherwise noted; [b] wine numbers correspond to those in **Table 1**; [c] mg *p*-coumaric acid equivalents/L; [d] sum of individual phenolic acid content; [e] sum of individual flavan-3-ol content; [f] mg (+)-catechin equivalents/L; [g] sum of individual monomeric phenolic compound content; [h] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [i] $TAC_R = TAC_M - TAC_{CAL}$; na = not available.

Table 3. Continued …

Wine[b]	Gallic acid	Caftaric acid	Caffeic acid	Coutaric acid[c]	*p*-Coumaric acid	Total phenolic acids[d]	(+)-Catechin	Procyanidin B1	Total flavan-3-ols (HPLC)[e]	Non-coloured polymers[f]	Total monomers[g]	TAC_{CAL}[h]	TAC_R[i]
108	10.78	187.69	7.13	17.03	3.42	226.04	34.85	23.03	57.88	28.11	738.33	2.29	11.99
109	10.47	125.85	2.94	12.65	2.33	154.23	31.31	23.76	55.07	117.93	616.52	1.93	13.14
110	16.01	168.15	9.00	15.66	5.67	214.50	38.98	23.02	62.00	154.41	679.93	2.20	12.83
111	12.92	130.44	4.87	12.53	2.38	163.13	28.05	15.91	43.97	115.57	609.59	1.91	11.16
112	8.98	172.95	7.32	17.39	2.77	209.41	25.32	24.80	50.12	76.37	680.55	2.02	10.85
113	9.28	130.10	4.31	14.17	1.60	159.47	18.49	14.24	32.73	91.73	552.21	1.73	9.79
114	na	na	na	na	na	na	na	na	na	na	na	na	na
115	17.17	159.88	5.86	13.47	5.50	201.88	36.19	23.23	59.42	138.79	582.43	1.92	10.22
116	9.81	126.50	3.95	12.79	1.77	154.82	27.32	19.64	46.96	75.60	577.98	1.80	10.97
117	8.75	165.54	5.70	15.86	1.00	196.85	32.80	18.16	50.96	128.73	670.15	2.02	12.91
118	8.70	227.43	7.29	22.11	2.15	267.66	18.39	15.07	33.46	118.05	690.57	2.01	9.76
119	8.70	196.09	4.38	20.54	0.74	230.44	18.54	13.06	31.59	137.15	673.59	2.07	10.69
120	7.25	154.93	4.51	14.46	2.19	183.34	23.21	17.53	40.74	89.95	655.56	1.96	14.23
121	8.81	173.07	6.59	15.60	2.85	206.92	21.67	15.51	37.18	130.00	693.63	2.09	9.95
122	10.94	140.26	3.37	14.01	1.12	169.70	40.67	24.97	65.64	140.50	681.35	2.13	13.53
123	8.69	178.54	5.26	17.40	1.02	210.91	36.05	19.86	55.91	120.54	633.08	1.91	11.12
124	7.88	241.22	6.74	24.30	2.19	282.33	19.52	14.32	33.84	184.07	798.82	2.30	11.84
125	8.96	168.39	2.11	15.61	0.98	196.04	16.75	12.53	29.28	169.26	660.68	2.05	12.42
126	8.69	193.52	4.97	19.41	1.18	227.77	24.22	18.11	42.32	138.09	687.12	2.08	13.06
127	10.23	190.80	5.65	18.28	1.89	226.86	28.38	18.41	46.79	127.62	730.60	2.24	10.22
128	11.39	153.20	2.20	13.86	0.96	181.61	39.36	24.06	63.42	173.32	707.30	2.20	10.97
129	12.02	146.20	2.35	14.46	1.58	176.61	13.64	12.67	26.32	126.95	625.27	1.83	11.93
130	8.75	171.61	0.95	16.20	0.99	198.51	13.20	11.75	24.95	34.54	705.38	2.07	10.07
131	14.41	220.44	1.80	22.91	0.91	260.48	14.86	12.23	27.09	57.00	740.89	2.23	15.99
132	9.45	153.53	1.60	15.06	1.69	181.34	8.79	7.51	16.30	104.38	524.80	1.59	11.16
133	13.79	185.01	1.62	15.92	0.67	217.00	13.96	13.90	27.86	156.93	649.40	1.96	15.12
134	6.89	171.25	0.73	16.28	0.61	195.75	14.86	11.37	26.24	30.75	669.59	1.97	10.38
135	13.24	173.73	0.00	16.81	2.77	206.55	13.30	8.63	21.93	140.55	633.79	1.95	14.38
136	12.57	148.11	0.77	14.54	1.75	177.73	12.11	9.15	21.26	36.23	643.46	2.00	10.56
137	5.78	178.57	1.61	17.31	0.00	203.27	13.08	10.24	23.33	37.83	717.43	2.08	9.50
138	13.79	213.79	1.92	21.30	0.67	251.46	13.42	11.90	25.32	113.79	635.93	1.94	14.13
139	9.18	172.90	0.77	16.04	0.93	199.81	9.62	8.02	17.64	40.43	620.59	1.87	9.90
140	10.24	224.91	1.21	20.08	0.67	257.10	11.90	9.98	21.89	184.31	743.47	2.13	13.69
141	8.10	193.76	0.99	18.73	0.32	221.90	14.31	11.07	25.38	132.75	670.92	1.94	11.85
142	11.99	130.79	0.45	12.17	0.50	155.89	15.54	9.87	25.41	147.27	620.68	1.91	10.63
143	12.92	219.74	0.00	20.75	3.51	256.92	14.20	6.92	21.12	187.11	712.47	2.11	11.55
144	8.65	160.72	1.06	14.43	0.45	185.31	11.96	8.21	20.17	139.59	634.99	1.84	10.22
145	14.97	215.23	1.04	20.02	0.90	252.16	12.66	10.57	23.23	170.82	699.60	2.09	13.69
146	12.34	130.34	0.00	10.49	1.20	154.37	9.48	5.91	15.40	112.01	566.58	1.77	8.19
147	10.41	212.57	1.17	19.40	0.41	243.97	12.95	10.22	23.17	168.41	691.77	2.00	12.49
148	11.45	140.16	0.00	12.98	0.92	165.50	12.66	7.65	20.30	144.90	660.42	1.98	9.74
149	11.46	200.25	0.00	18.48	3.26	233.45	10.33	6.12	16.45	172.31	641.66	1.93	11.83
150	5.17	190.97	1.30	18.15	0.72	216.31	10.32	7.31	17.63	44.79	717.00	2.02	9.85
151	10.01	184.41	2.18	18.69	1.76	217.06	11.42	8.58	20.00	149.89	690.33	2.00	12.87
152	6.17	165.66	0.00	14.87	1.35	188.05	8.51	5.50	14.02	28.95	590.13	1.72	7.32
153	6.72	148.42	2.14	14.05	1.39	172.72	10.89	8.22	19.11	140.59	582.17	1.67	11.25

Table 3. Continued …

Wine[b]	Gallic acid	Caftaric acid	Caffeic acid	Coutaric acid[c]	*p*-Coumaric acid	Total phenolic acids[d]	(+)-Catechin	Procyanidin B1	Total flavan-3-ols (HPLC)[e]	Non-coloured polymers[f]	Total monomers[g]	TAC_{CAL}[h]	TAC_R[i]
154	16.24	133.27	0.00	11.77	1.26	162.54	13.87	7.68	21.55	234.32	656.03	2.05	11.11
155	14.07	201.75	0.48	18.84	1.21	236.34	16.91	11.49	28.40	151.28	638.41	1.94	13.71
156	12.62	159.44	1.48	13.90	0.96	188.39	14.59	8.62	23.21	48.54	635.51	1.94	11.66
157	24.74	156.13	0.00	14.98	2.89	198.74	19.57	12.87	32.45	64.37	663.88	2.23	14.80
158	9.34	176.05	1.60	18.52	2.20	207.70	13.04	7.23	20.27	141.54	643.99	1.83	11.86
159	16.47	120.76	1.48	11.54	1.90	152.15	14.48	7.91	22.39	123.56	551.40	1.75	10.76
160	11.54	260.10	0.39	22.58	1.43	296.04	16.97	12.84	29.81	151.57	747.63	2.23	14.31
161	10.45	175.63	0.00	15.10	1.15	202.33	11.39	7.03	18.42	127.73	615.41	1.85	9.90
162	30.69	159.75	0.00	14.45	3.64	208.53	20.40	13.04	33.44	184.21	593.38	2.10	16.78
163	12.57	198.18	0.58	17.46	0.75	229.53	14.03	7.82	21.86	171.05	712.94	2.10	12.36
164	18.12	220.42	0.00	19.99	1.47	260.00	14.96	10.93	25.89	202.89	690.98	2.14	15.48
165	11.36	155.93	1.28	13.88	0.58	183.03	13.16	8.31	21.46	150.73	663.72	2.01	10.47
166	7.34	215.51	0.62	17.47	0.63	241.57	16.13	9.45	25.58	123.76	641.18	1.87	12.13
167	11.91	198.46	1.10	17.17	1.18	229.82	11.37	7.42	18.78	172.67	678.62	2.01	13.90
168	13.80	188.43	0.00	17.76	2.73	222.71	16.44	11.57	28.01	162.43	664.95	2.07	12.39
169	6.90	139.76	1.64	13.81	1.80	163.91	10.88	5.36	16.24	140.31	608.62	1.74	9.35
170	8.33	186.44	0.67	17.71	1.29	214.44	13.30	8.04	21.34	61.01	728.03	2.11	11.96
171	8.32	161.54	1.55	15.02	1.25	187.66	14.34	8.72	23.07	21.54	546.28	1.63	7.91
172	na	na	na	na	na	na	na	na	na	na	na	na	na
173	13.17	208.14	0.51	18.24	0.86	240.92	12.58	8.06	20.63	258.56	780.90	2.34	11.43
174	10.17	123.81	0.56	12.13	1.35	148.02	13.97	8.60	22.57	162.35	708.73	2.15	11.15
175	7.20	159.14	0.56	14.48	0.89	182.28	11.97	5.67	17.63	147.32	608.17	1.75	9.96
176	8.60	185.09	0.64	16.96	0.96	212.26	12.31	7.76	20.06	171.81	687.67	2.02	12.41
177	5.90	146.32	1.92	13.60	1.81	169.56	11.68	6.77	18.45	103.70	582.57	1.69	8.27
178	11.39	213.33	1.56	18.49	2.63	247.40	11.04	8.05	19.08	153.81	691.84	1.96	13.23
179	10.43	186.42	1.56	16.76	1.63	216.79	13.09	7.99	21.08	171.90	635.63	1.84	12.29
180	42.95	174.37	2.20	13.50	4.25	237.26	12.98	12.29	25.26	50.04	627.17	2.27	10.35
181	9.21	175.17	0.72	16.05	1.90	203.05	12.22	7.96	20.18	37.11	679.18	2.00	10.09
182	11.43	159.89	0.00	14.07	3.82	189.20	10.05	9.80	19.84	132.76	567.44	1.69	11.65
183	12.28	168.04	1.42	15.77	0.82	198.34	14.73	13.21	27.93	43.71	668.97	1.95	12.15
184	15.23	119.94	1.49	11.75	2.42	150.84	11.86	9.03	20.89	152.70	570.62	1.78	13.98
185	8.73	215.87	0.53	18.06	1.22	244.41	11.76	7.84	19.59	145.50	723.13	2.05	11.56
186	9.84	216.03	0.59	18.87	0.71	246.04	14.34	8.77	23.12	160.06	682.43	1.97	11.83
187	14.71	156.52	0.00	13.80	1.42	186.45	14.72	9.42	24.14	129.67	635.60	1.92	11.11
188	9.45	180.80	0.46	16.83	1.65	209.19	12.19	7.85	20.04	116.06	681.26	2.04	9.98
189	10.93	179.10	0.66	16.49	1.20	208.37	13.89	11.78	25.67	122.45	651.38	1.93	10.73
190	14.29	172.73	0.61	15.26	0.82	203.72	15.26	12.84	28.10	126.01	648.96	1.94	11.52
191	15.26	134.00	0.37	12.77	1.37	163.77	13.23	11.96	25.18	139.29	672.12	2.10	11.53
192	6.77	180.17	0.58	16.22	1.39	205.14	12.52	6.88	19.40	44.29	608.35	1.73	8.60
193	5.52	158.75	0.68	13.94	0.59	179.48	15.12	7.70	22.82	51.82	629.15	1.80	8.84
194	9.50	190.40	0.58	16.14	1.10	217.71	12.01	7.73	19.74	134.63	736.30	2.11	9.68
195	7.57	180.06	0.00	15.66	1.29	204.58	11.77	6.14	17.91	121.04	701.07	2.04	10.53
196	8.13	153.81	0.00	13.37	2.79	178.10	10.15	7.71	17.86	50.33	685.87	2.01	9.30
197	5.91	173.68	1.52	15.19	1.54	197.83	9.80	6.02	15.82	125.20	695.53	1.95	11.62
198	12.18	157.75	0.66	15.11	0.77	186.47	15.25	11.22	26.47	146.99	671.34	2.02	12.15
199	6.04	185.25	0.47	16.47	0.80	209.03	12.25	6.40	18.65	130.70	633.29	1.79	8.92

Table 3. Continued …

Wine[b]	Gallic acid	Caftaric acid	Caffeic acid	Coutaric acid[c]	*p*-Coumaric acid	Total phenolic acids[d]	(+)-Catechin	Procyanidin B1	Total flavan-3-ols (HPLC)[e]	Non-coloured polymers[f]	Total monomers[g]	TAC_{CAL}[h]	TAC_R[i]
200	8.55	192.38	0.40	17.48	0.58	219.39	15.89	7.73	23.61	205.10	750.36	2.17	10.32
201	7.33	194.58	0.44	16.79	1.04	220.18	9.68	5.86	15.54	149.89	689.46	1.95	9.08
202	6.43	176.53	0.56	15.84	1.21	200.58	10.74	5.49	16.23	140.75	686.13	1.98	8.56
203	8.48	170.33	0.71	15.59	1.56	196.66	11.46	8.05	19.51	142.03	735.88	2.16	9.11
204	7.35	169.18	0.48	13.35	0.42	190.77	11.17	6.66	17.84	155.21	743.84	2.14	9.24
205	10.02	151.85	2.13	14.97	1.46	180.43	13.40	10.92	24.32	153.48	674.20	2.00	12.18

Addendum D: Chapter 5 Data

Aim

The aim is to tabulate all the actual values for phenolic composition and objective quality (total antioxidant capacity and colour) parameters, including data not shown, for Pinotage wines produced according to pre-fermentation maceration protocols described in **Chapter 5**. Data not shown for Pinotage wines produced according to different juice/skin mixing protocols (as described in **Chapter 5**) are also tabulated.

Table 1. Phenolic composition (measured spectrophotometrically) of the 2000, 2001 and 2002 Pinotage wines produced according to different pre-fermentation maceration protocols.

Year	Treatment	Total phenols[a]	Monomeric anthocyanins[b]	Polymeric anthocyanins[b]	Total anthocyanins[b]	Total flavan-3-ols[c]
2000	Control	2118.3 b[d] (± 72.7)[e]	197.2 a (± 9.1)	72.2 ab (± 5.0)	269.4 a (± 11.0)	156.6 bc (± 4.4)
	10 °C/1 day	2172.0 b (± 95.2)	206.6 a (± 4.9)	75.8 ab (± 4.0)	282.5 a (± 8.1)	155.9 c (± 13.6)
	10 °C/2 days	2448.6 a (± 181.3)	218.7 a (± 24.4)	86.6 a (± 17.5)	305.3 a (± 40.9)	183.0 a (± 12.5)
	10 °C/4 days	2305.9 ab (± 76.9)	195.9 a (± 11.1)	71.1 ab (± 16.0)	267.0 a (± 26.9)	177.0 ab (± 8.2)
	15 °C/1 day	2250.4 ab (± 163.5)	203.7 a (± 31.0)	75.2 ab (± 7.9)	278.9 a (± 32.9)	166.1 abc (± 15.7)
	15 °C/2 days	2177.7 b (± 124.4)	200.8 a (± 32.5)	65.6 b (± 5.8)	266.5 a (± 33.1)	164.4 abc (± 14.9)
	15 °C/4 days	2294.0 ab (± 111.3)	255.9 a (± 1.2)	78.6 ab (± 11.5)	304.5 a (± 12.2)	177.2 ab (± 9.0)
2001	Control	2684.4 bcd (± 165.3)	363.5 a (± 30.2)	92.2 ab (± 8.7)	454.7 a (± 42.3)	196.2 b (± 8.7)
	10 °C/1 day	2704.6 abc (± 154.2)	343.0 a (± 53.7)	98.5 ab (± 13.8)	441.5 a (± 60.9)	190.8 bc (± 8.7)
	10 °C/2 days	2775.3 ab (± 171.6)	351.7 a (± 32.4)	102.1 a (± 16.2)	453.8 a (± 45.8)	197.3 b (± 5.5)
	10 °C/4 days	2454.9 d (± 103.3)	349.7 a (± 31.3)	83.8 b (± 1.7)	433.5 a (± 32.7)	171.4 d (± 8.1)
	15 °C/1 day	2518.0 cd (± 122.2)	358.1 a (± 13.6)	91.0 ab (± 6.2)	449.1 a (± 19.7)	176.3 cd (± 2.8)
	15 °C/2 days	2935.2 a (± 131.2)	393.9 a (± 19.5)	100.0 ab (± 2.7)	494.7 a (± 16.2)	218.5 a (± 19.9)
	15 °C/4 days	2813.7 ab (± 57.0)	363.2 a (± 19.1)	96.5 ab (± 4.4)	459.6 a (± 15.2)	200.2 b (± 6.7)
2002	Control	2296.2 a (± 132.2)	359.9 a (± 11.2)	46.0 a (± 5.7)	405.9 a (± 16.8)	155.0 a (± 10.1)
	10 °C/1 day	2380.1 a (± 230.6)	373.8 a (± 55.4)	47.4 a (± 16.3)	421.3 a (± 70.1)	143.9 a (± 12.1)
	10 °C/2 days	2286.0 a (± 118.6)	401.5 a (± 9.6)	53.7 a (± 8.4)	455.2 a (± 9.8)	149.9 a (± 5.6)
	10 °C/4 days	2403.3 a (± 182.8)	411.9 a (± 37.3)	56.3 a (± 6.4)	468.2 a (± 43.8)	157.0 a (± 6.6)
	15 °C/1 day	2273.1 a (± 185.2)	403.2 a (± 31.1)	55.4 a (± 2.3)	458.6 a (± 33.2)	144.1 a (± 13.1)
	15 °C/2 days	2484.5 a (± 135.1)	392.3 a (± 18.3)	57.0 a (± 7.8)	446.9 a (± 19.1)	160.9 a (± 4.1)
	15 °C/4 days	2231.1 a (± 66.3)	382.5 a (± 41.4)	51.9 a (± 6.5)	434.4 a (± 47.7)	145.6 a (± 12.3)

[a] mg gallic acid equivalents/L; [b] mg malvidin-3-glc equivalents/L; [c] mg (+)-catechin equivalents/L; [d] different letters within a group in a column denote significant ($P < 0.05$) differences; [e] SD.

Table 2. Anthocyanin content[a] of the 2001 and 2002 Pinotage wines produced according to different pre-fermentation maceration protocols.

	Treatment	Monomeric anthocyanins											Coloured polymers[b]
		Dp-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Dp-3-glc-ac[b]	Vitisin A[b]	Pt-3-glc-ac[b]	Pn-3-glc-ac[b]	Mv-3-glc-ac[b]	Mv-3-glc-coum[b]	Total[c]	
2001	Control	9.63 ab[e] (± 1.38)[f]	15.16 a (± 2.93)	4.48 a (± 0.49)	187.02 a (± 33.03)	3.61 c (± 0.41)	7.47 d (± 3.64)	3.50 ab (± 0.67)	4.58 a (± 0.61)	59.11 a (± 9.40)	27.54 a (± 5.88)	322.10 a (± 51.24)	12.70 a (± 4.23)
	10 °C/ 1 day	9.07 b (± 1.59)	14.06 a (± 3.09)	5.64 a (± 1.20)	187.48 a (± 35.44)	3.63 c (± 0.29)	11.14 bc (± 1.18)	3.06 ab (± 0.59)	4.37 a (± 0.53)	57.61 a (± 9.25)	23.62 a (± 6.13)	319.65 a (± 55.86)	14.57 a (± 1.33)
	10 °C/ 2 days	9.70 ab (± 1.03)	13.67 a (± 1.84)	4.89 a (± 0.88)	180.96 a (± 6.42)	3.69 bc (± 0.27)	11.28 bc (± 1.99)	3.16 ab (± 0.31)	4.39 a (± 0.38)	57.93 a (± 2.50)	24.87 a (± 1.51)	314.54 a (± 12.66)	14.21 a (± 4.76)
	10 °C/ 4 days	10.44 ab (± 1.88)	15.00 a (± 2.80)	5.49 a (± 0.91)	195.93 a (± 29.04)	3.78 abc (± 0.53)	9.52 cd (± 0.19)	2.69 b (± 1.08)	4.37 a (± 0.69)	63.55 a (± 9.62)	29.57 a (± 5.64)	340.33 a (± 51.23)	11.47 a (± 3.18)
	15 °C/ 1 day	11.02 ab (± 2.00)	15.30 a (± 1.99)	5.39 a (± 0.63)	188.68 a (± 28.65)	4.25 ab (± 0.21)	13.06 ab (± 1.07)	3.39 ab (± 0.43)	4.70 a (± 0.31)	60.50 a (± 9.55)	29.54 a (± 7.73)	335.85 a (± 50.55)	14.28 a (± 2.41)
	15 °C/ 2 days	12.09 a (± 0.88)	16.60 a (± 1.03)	4.96 a (± 0.54)	200.93 a (± 5.89)	4.34 a (± 0.32)	11.72 bc (± 1.07)	3.86 a (± 0.14)	4.59 a (± 0.04)	66.11 a (± 1.86)	31.91 a (± 2.57)	357.11 a (± 11.47)	14.96 a (± 4.72)
	15 °C/ 4 days	10.51 ab (± 1.01)	15.03 a (± 1.75)	4.83 a (± 0.29)	181.39 a (± 25.51)	4.15 abc (± 0.23)	15.00 a (± 0.64)	3.35 ab (± 0.27)	4.34 a (± 0.18)	61.00 a (± 10.47)	31.78 a (± 9.07)	331.40 a (± 49.29)	16.30 a (± 3.42)
2002	Control	10.24 a (± 0.93)	14.36 ab (± 0.67)	5.71 a (± 0.57)	186.08 ab (± 4.56)	3.62 ab (± 0.14)	7.06 bc (± 1.29)	3.18 abc (± 0.06)	4.69 a (± 0.14)	56.23 ab (± 1.04)	28.68 ab (± 1.86)	319.85 ab (± 4.70)	8.92 a (± 2.63)
	10 °C/ 1 day	10.57 a (± 1.96)	15.27 ab (± 2.29)	5.85 a (± 0.74)	197.64 a (± 15.21)	3.88 ab (± 0.58)	5.96 c (± 1.67)	3.44 a (± 0.24)	5.14 a (± 0.56)	60.21 a (± 3.07)	30.93 a (± 2.56)	338.90 a (± 26.27)	11.26 a (± 2.84)
	10 °C/ 2 days	10.65 a (± 1.31)	15.48 a (± 1.27)	5.71 a (± 0.88)	193.50 ab (± 13.48)	4.06 a (± 0.05)	9.49 ab (± 1.52)	3.48 a (± 0.24)	5.07 a (± 0.39)	57.25 ab (± 3.54)	28.47 ab (± 3.06)	333.16 ab (± 22.32)	10.20 a (± 1.65)
	10 °C/ 4 days	10.02 a (± 1.51)	14.65 ab (± 1.51)	5.54 a (± 0.89)	188.47 ab (± 14.65)	3.72 ab (± 0.16)	10.33 a (± 2.61)	3.05 bc (± 0.25)	4.85 a (± 0.44)	55.14 ab (± 4.00)	28.24 ab (± 3.64)	324.03 ab (± 26.24)	12.26 a (± 2.39)
	15 °C/ 1 day	10.76 a (± 1.32)	15.73 a (± 1.52)	6.18 a (± 1.11)	195.74 a (± 18.76)	3.69 ab (± 0.27)	7.45 abc (± 1.05)	3.36 ab (± 0.28)	4.80 a (± 0.43)	57.47 ab (± 7.22)	27.61 ab (± 4.07)	332.78 ab (± 32.50)	10.66 a (± 1.23)
	15 °C/ 2 days	9.91 a (± 0.23)	14.36 ab (± 0.05)	5.34 a (± 0.53)	176.94 ab (± 10.08)	3.88 ab (± 0.25)	10.57 a (± 1.83)	3.15 abc (± 0.14)	4.73 a (± 0.24)	52.21 b (± 5.21)	26.02 ab (± 3.72)	307.11 ab (± 17.63)	10.92 a (± 2.05)
	15 °C/ 4 days	8.82 a (± 0.23)	13.00 b (± 0.25)	5.13 a (± 0.46)	171.77 b (± 5.73)	3.50 b (± 0.27)	8.69 abc (± 1.40)	2.95 c (± 0.06)	4.69 a (± 0.07)	50.64 b (± 2.69)	23.70 b (± 3.85)	292.88 b (± 11.00)	10.19 a (± 1.07)

[a] mg/L unless otherwise noted; [b] mg corresponding anthocyanin-3-glucoside equivalents/L; [c] sum of phenolic group content; [d] mg malvidin-3-glucoside equivalents/L; [e] different letters within a group in a column denote significant differences ($P < 0.05$); [f] SD; Dp = delphinidin; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Pn = peonidin; Pt = petunidin; Mv = malvidin.

Table 3. Flavonol content[a] of the 2001 and 2002 Pinotage wines produced according to different pre-fermentation maceration protocols.

	Treatment	Unknown flavonol[b]	Quercetin-3-glucoside	Quercetin-3-rhamnoside	Quercetin	Total[c]
2001	Control	20.74 bc[d] (± 4.42)[e]	16.34 a (± 3.51)	10.95 a (± 1.92)	2.58 a (± 0.86)	30.80 a (± 3.45)
	10 °C/1 day	17.90 c (± 1.35)	17.91 a (± 1.23)	12.49 a (± 1.18)	2.03 a (± 0.81)	33.34 a (± 1.61)
	10 °C/2 days	19.08 bc (± 3.11)	16.07 a (± 2.69)	11.23 a (± 1.27)	2.38 a (± 0.67)	31.27 a (± 1.66)
	10 °C/4 days	19.34 bc (± 2.89)	17.06 a (± 4.46)	12.01 a (± 1.88)	3.23 a (± 1.46)	30.01 a (± 1.08)
	15 °C/1 day	23.24 ab (± 1.42)	16.59 a (± 2.30)	11.11 a (± 1.34)	2.18 a (± 0.13)	29.67 a (± 1.44)
	15 °C/2 days	22.06 abc (± 1.55)	17.76 a (± 1.49)	11.45 a (± 0.53)	2.70 a (± 0.74)	34.38 a (± 1.73)
	15 °C/4 days	25.41 a (± 1.72)	20.22 a (± 3.20)	12.38 a (± 2.42)	2.88 a (± 1.39)	32.16 a (± 1.58)
2002	Control	10.26 a (± 0.52)	7.47 a (± 0.43)	8.92 a (± 0.66)	2.80 a (± 0.53)	19.32 a (± 1.60)
	10 °C/1 day	9.61 a (± 3.31)	7.46 a (± 0.21)	8.97 a (± 0.97)	3.10 a (± 1.07)	19.83 a (± 2.27)
	10 °C/2 days	10.43 a (± 1.90)	7.32 a (± 0.89)	9.05 a (± 0.21)	2.78 a (± 1.13)	19.30 a (± 2.28)
	10 °C/4 days	10.20 a (± 2.10)	7.06 a (± 2.04)	9.77 a (± 1.50)	3.11 a (± 1.03)	20.14 a (± 4.92)
	15 °C/1 day	10.45 a (± 2.77)	7.59 a (± 0.69)	9.63 a (± 1.03)	3.26 a (± 0.47)	20.60 a (± 2.29)
	15 °C/2 days	12.00 a (± 0.93)	8.17 a (± 0.20)	9.73 a (± 0.52)	3.82 a (± 0.21)	22.23 a (± 0.93)
	15 °C/4 days	8.86 a (± 0.23)	7.27 a (± 0.47)	8.86 a (± 0.03)	2.71 a (± 0.45)	19.02 a (± 0.30)

[a] mg/L unless otherwise noted; [b] mg rutin equivalents/L; [c] sum of phenolic group content; [d] different letters within a group in a column denote significant differences ($P < 0.05$); [e] SD.

Table 4. Phenolic acid, flavan-3-ol, non-coloured polymer and total monomer content[a] of the 2001 and 2002 Pinotage wines produced according to different pre-fermentation maceration protocols.

Year	Treat-	Phenolic acids						Flavan-3-ols			Non-coloured	Total
	ment	Gallic acid	Caftaric acid	Caffeic acid	Coutaric acid[b]	*p*-Coumaric acid	Total[c]	(+)-Catechin	Procyanidin B1	Total[b]	polymers[d]	monomers[c]
2001	Control	13.47 bcd[e] (± 0.36)[f]	157.92 d (± 18.60)	0.96 b (± 0.24)	18.46 d (± 1.03)	0.48 e (± 0.26)	172.35 c (± 18.31)	11.32 c (± 1.12)	19.48 ab (± 2.37)	30.80 bc (± 3.45)	214.88 a (± 9.47)	595.58 d (± 69.70)
	10 °C/ 1 day	16.17 a (± 0.72)	170.79 cd (± 4.10)	1.28 a (± 0.07)	18.52 d (± 0.97)	1.00 d (± 0.02)	188.25 bc (± 3.71)	12.70 ab (± 0.31)	20.65 a (± 1.44)	33.34 ab (± 1.62)	210.40 a (± 46.75)	611.66 cd (± 52.74)
	10 °C/ 2 days	15.19 ab (± 1.58)	195.20 c (± 11.29)	0.88 bc (± 0.11)	21.27 cd (± 1.23)	1.47 bc (± 0.10)	211.26 b (± 12.79)	11.70 bc (± 0.60)	19.57 ab (± 1.20)	31.27 abc (± 1.66)	190.06 a (± 45.72)	629.18 bcd (± 21.96)
	10 °C/ 4 days	12.09 d (± 0.76)	195.32 c (± 26.61)	0.93 b (± 0.15)	22.09 c (± 2.05)	1.19 cd (± 0.30)	208.33 b (± 27.14)	11.26 c (± 0.59)	18.75 ab (± 1.23)	30.01 bc (± 1.08)	205.30 a (± 25.41)	654.54 abcd (± 89.32)
	15 °C/ 1 day	12.76 cd (± 0.15)	251.75 ab (± 15.59)	0.64 c (± 0.05)	28.41 ab (± 2.39)	2.03 a (± 0.29)	265.14 a (± 15.51)	11.94 bc (± 0.31)	17.73 b (± 1.18)	29.67 c (± 1.44)	218.47 a (± 70.74)	714.84 abc (± 69.62)
	15 °C/ 2 days	14.59 abc (± 2.92)	243.03 b (± 27.60)	0.82 bc (± 0.08)	27.63 b (± 3.31)	1.45 bc (± 0.15)	258.44 a (± 30.45)	13.46 a (± 0.62)	20.92 a (± 1.21)	34.38 a (± 1.73)	234.85 a (± 49.59)	733.71 ab (± 46.88)
	15 °C/ 4 days	14.01 abcd (± 0.48)	276.16 a (± 12.24)	0.80 bc (± 0.23)	31.66 a (± 1.03)	1.69 ab (± 0.27)	290.96 a (± 12.63)	13.09 a (± 0.52)	19.07 ab (± 1.06)	32.16 abc (± 1.58)	217.13 a (± 66.20)	749.52 a (± 69.62)
2002	Control	11.73 c (± 1.11)	204.89 a (± 11.98)	0.69 a (± 0.12)	16.47 a (± 1.45)	1.40 a (± 0.25)	217.32 a (± 12.81)	9.69 a (± 0.53)	18.16 a (± 1.13)	27.86 a (± 1.62)	164.59 b (± 18.13)	615.62 a (± 18.26)
	10 °C/ 1 day	11.75 c (± 1.58)	168.55 bc (± 21.28)	0.75 a (± 0.24)	17.03 bc (± 1.34)	1.43 a (± 0.03)	181.05 bcd (± 22.63)	8.88 ab (± 0.51)	16.08 ab (± 1.09)	24.95 ab (± 1.41)	168.40 ab (± 24.42)	592.60 a (± 53.19)
	10 °C/ 2 days	12.58 bc (± 0.98)	175.24 bc (± 11.18)	0.62 a (± 0.16)	15.59 bc (± 1.63)	1.27 ab (± 0.24)	188.44 bc (± 10.38)	9.39 a (± 0.30)	16.12 ab (± 1.56)	25.51 ab (± 1.85)	178.97 ab (± 25.43)	595.49 a (± 38.91)
	10 °C/ 4 days	13.60 ab (± 0.73)	164.37 cd (± 20.11)	0.67 a (± 0.11)	15.31 c (± 0.91)	1.05 b (± 0.23)	178.65 bcd (± 19.52)	9.28 ab (± 0.34)	15.87 ab (± 1.51)	25.14 ab (± 1.76)	204.22 a (± 16.21)	575.35 ab (± 53.71)
	15 °C/ 1 day	12.65 bc (± 0.92)	162.37 cd (± 11.88)	0.61 a (± 0.12)	18.40 cd (± 0.88)	1.34 ab (± 0.15)	175.63 cd (± 12.27)	8.52 b (± 0.54)	14.94 b (± 1.64)	23.46 b (± 2.18)	162.34 b (± 27.12)	580.10 ab (± 51.29)
	15 °C/ 2 days	13.41 abc (± 0.65)	190.69 ab (± 7.23)	0.75 a (± 0.13)	13.26 ab (± 0.74)	1.05 b (± 0.06)	204.85 ab (± 7.01)	9.35 ab (± 0.12)	16.07 ab (± 1.60)	25.43 ab (± 1.72)	198.10 ab (± 16.54)	591.65 a (± 13.39)
	15 °C/ 4 days	14.60 a (± 0.37)	139.99 d (± 5.19)	0.59 a (± 0.05)	13.26 d (± 0.74)	0.53 c (± 0.04)	155.18 d (± 4.88)	9.19 ab (± 0.81)	16.12 ab (± 1.78)	25.31 ab (± 2.59)	165.44 b (± 4.71)	515.52 b (± 18.72)

[a] mg/L unless otherwise noted; [b] mg *p*-coumaric acid equivalents/L; [c] sum of phenolic group content; [d] different letters within a group in a column denote significant differences ($P < 0.05$); [e] SD.

Table 5. Antioxidant capacity of the 2000, 2001 and 2002 Pinotage wines produced according to different pre-fermentation maceration protocols.

Year	Treatment	TAC_M [a]	TAC_{CAL} [b]	TAC_R [c]
2000	Control	12.69 b[d] (± 0.81)[e]	na	na
	10 °C/1 day	13.59 ab (± 1.20)	na	na
	10 °C/2 days	15.38 a (± 1.80)	na	na
	10 °C/4 days	13.64 ab (± 0.95)	na	na
	15 °C/1 day	13.09 b (± 1.20)	na	na
	15 °C/2 days	12.93 b (± 0.62)	na	na
	15 °C/4 days	13.96 ab (± 0.51)	na	na
2001	Control	15.88 abc (± 1.99)	1.81 b (± 0.19)	14.08 ab (± 1.91)
	10 °C/1 day	15.25 bcd (± 0.14)	1.89 ab (± 0.17)	13.36 abc (± 0.04)
	10 °C/2 days	15.72 abc (± 0.62)	1.92 ab (± 0.07)	13.80 ab (± 0.55)
	10 °C/4 days	13.73 d (± 0.39)	1.95 ab (± 0.25)	11.78 c (± 0.30)
	15 °C/1 day	14.46 cd (± 0.66)	2.10 ab (± 0.19)	12.36 bc (± 0.84)
	15 °C/2 days	16.79 ab (± 1.41)	2.19 a (± 0.17)	14.60 a (± 1.24)
	15 °C/4 days	17.27 a (± 1.42)	2.20 a (± 0.20)	15.07 a (± 1.61)
2002	Control	15.05 a (± 0.73)	1.87 a (± 0.07)	13.18 a (± 0.67)
	10 °C/1 day	14.74 a (± 1.68)	1.81 ab (± 0.16)	12.92 a (± 1.52)
	10 °C/2 days	14.89 a (± 0.74)	1.83 ab (± 0.09)	13.06 a (± 0.70)
	10 °C/4 days	16.07 a (± 0.90)	1.79 ab (± 0.14)	14.29 a (± 0.77)
	15 °C/1 day	15.25 a (± 1.04)	1.79 ab (± 0.14)	13.46 a (± 0.93)
	15 °C/2 days	15.80 a (± 0.46)	1.82 ab (± 0.03)	13.98 a (± 0.49)
	15 °C/4 days	15.05 a (± 0.88)	1.64 b (± 0.05)	12.91 a (± 0.23)

[a] total antioxidant capacity in mM Trolox equivalents; [b] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [c] $TAC_R = TAC_M - TAC_{CAL}$; [d] different letters within a group in a column denote significant differences ($P < 0.05$); [e] SD; na = not available.

Table 6. Objective colour parameters of the 2000, 2001 and 2002 Pinotage wines produced according to different pre-fermentation maceration protocols.

Year	Treatment	*C**[a]	*h**[b]	*L**[c]	*a**[d]	*b**[e]
2000	Control	58.63 ab[f] (± 1.63)[g]	16.38 a (± 0.89)	41.44 ab (± 1.57)	56.24 ab (± 1.33)	16.54 a (± 1.32)
	10 °C/1 day	59.68 ab (± 1.28)	17.53 a (± 0.17)	39.92 ab (± 1.19)	56.90 ab (± 1.26)	17.98 a (± 0.26)
	10 °C/2 days	60.40 a (± 2.13)	16.98 a (± 2.22)	37.06 b (± 5.88)	57.74 a (± 1.58)	17.67 a (± 2.58)
	10 °C/4 days	56.06 ab (± 3.82)	15.77 a (± 2.24)	43.65 ab (± 6.92)	53.90 b (± 3.03)	15.33 a (± 3.22)
	15 °C/1 day	57.82 ab (± 1.85)	16.18 a (± 1.75)	40.92 ab (± 3.50)	55.50 ab (± 1.43)	16.14 a (± 2.14)
	15 °C/2 days	55.59 b (± 2.95)	14.78 a (± 0.28)	44.75 a (± 2.99)	53.75 b (± 2.81)	14.19 a (± 0.94)
	15 °C/4 days	58.19 ab (± 3.20)	16.02 a (± 2.73)	41.18 ab (± 5.16)	55.87 ab (± 2.31)	16.14 a (± 3.56)
2001	Control	62.04 ab (± 2.05)	14.43 a (± 3.49)	37.32 ab (± 4.61)	60.28 ab (± 1.38)	13.40 a (± 2.93)
	10 °C/1 day	61.10 ab (± 1.92)	15.67 a (± 2.15)	36.66 ab (± 4.89)	59.04 bc (± 1.42)	14.83 a (± 1.60)
	10 °C/2 days	61.12 ab (± 1.26)	15.80 a (± 1.46)	34.81 b (± 4.14)	60.06 abc (± 1.05)	14.73 a (± 1.19)
	10 °C/4 days	59.53 b (± 1.48)	12.70 a (± 1.15)	41.16 a (± 1.81)	58.15 c (± 1.27)	12.30 a (± 0.83)
	15 °C/1 day	60.86 ab (± 1.83)	14.33 a (± 1.89)	38.08 ab (± 4.19)	59.14 bc (± 1.44)	13.60 a (± 1.45)
	15 °C/2 days	63.24 a (± 0.17)	16.15 a (± 0.76)	34.68 b (± 0.78)	61.14 a (± 0.31)	14.80 a (± 0.72)
	15 °C/4 days	62.27 a (± 0.64)	15.78 a (± 1.70)	34.19 b (± 2.28)	60.22 ab (± 0.33)	14.67 a (± 1.49)
2002	Control	78.19 ab (± 1.19)	37.06 a (± 0.94)	57.35 a (± 2.84)	68.86 b (± 0.85)	28.28 a (± 0.32)
	10 °C/1 day	76.39 b (± 6.65)	37.61 a (± 1.15)	53.49 ab (± 5.76)	70.65 ab (± 2.01)	29.74 a (± 3.67)
	10 °C/2 days	80.34 ab (± 1.65)	38.10 a (± 0.88)	52.67 ab (± 3.38)	70.73 ab (± 1.90)	28.32 a (± 0.92)
	10 °C/4 days	82.02 a (± 1.95)	38.16 a (± 0.19)	50.27 ab (± 3.41)	72.61 a (± 2.22)	27.74 a (± 0.75)
	15 °C/1 day	78.83 ab (± 2.00)	37.10 a (± 0.51)	52.27 ab (± 2.50)	69.55 ab (± 2.30)	28.09 a (± 0.91)
	15 °C/2 days	81.71 a (± 2.54)	37.51 a (± 0.86)	48.59 b (± 5.70)	72.59 a (± 2.44)	27.33 a (± 0.35)
	15 °C/4 days	79.40 ab (± 0.78)	37.08 a (± 0.64)	52.61 ab (± 3.65)	70.21 ab (± 0.93)	27.84 a (± 0.58)

[a] chroma; [b] hue angle (°); [c] lightness; [d] red/green chromaticity; [e] yellow/blue chromaticity; [f] different letters within a group in a column denote significant differences ($P < 0.05$); [g] SD.

Table 7. Anthocyanin content[a] of the 2001 and 2002 Pinotage wines produced according to different juice/skin mixing protocols and frequencies.

Year	Technique/ Frequency	Delphinidin-3-glc	Petunidin-3-glc	Peonidin-3-glc	Delphinidin-3-glc-ac[b]	Petunidin-3-glc-ac[b]
2001	Punching-down	7.23 a (± 1.39)	11.00 a (± 1.83)	3.55 a (± 0.81)	2.40 a (± 0.45)	2.07 a (± 0.29)
	Pumping-over	6.51 a (± 0.64)	10.26 a (± 1.02)	3.19 a (± 0.32)	2.17 a (± 0.20)	1.92 a (± 0.28)
	Rotor	6.03 a (± 1.03)	9.73 a (± 1.28)	3.02 a (± 0.67)	2.24 a (± 0.35)	1.78 a (± 0.25)
2002	Punching-down	7.90 a (± 1.35)	12.57 a (± 1.73)	5.06 a (± 0.83)	2.21 a (± 0.31)	2.01 a (± 0.26)
	Pumping-over	8.04 a (± 1.61)	11.85 a (± 1.77)	4.33 a (± 1.03)	2.22 a (± 0.30)	1.91 a (± 0.25)
	Rotor	8.98 a (± 0.34)	13.45 a (± 0.40)	4.10 a (± 0.32)	2.54 a (± 0.08)	2.15 a (± 0.12)
2001	Every hour	6.68 a (± 0.81)	10.14 a (± 1.08)	3.36 a (± 0.52)	2.31 a (± 0.34)	1.85 a (± 0.22)
	Every 3 hours	6.50 a (± 1.41)	10.51 a (± 1.77)	3.14 a (± 0.76)	2.24 a (± 0.35)	1.99 a (± 0.34)
2002	Every hour	8.26 a (± 1.12)	12.70 a (± 1.08)	4.69 a (± 0.76)	2.27 a (± 0.24)	2.01 a (± 0.20)
	Every 3 hours	8.35 a (± 1.45)	12.55 a (± 1.93)	4.30 a (± 0.93)	2.37 a (± 0.33)	2.04 a (± 0.27)

[a] mg/L unless otherwise noted; [b] mg corresponding anthocyanin-3-glucoside equivalents/L; [c] different letters within a group in a column denote significant differences ($P < 0.05$); [g] SD; glc = glucoside; glc-ac = acetylglucoside.

Addendum E: Chapter 6 Data

Aim

The aim is to tabulate all the actual values for phenolic composition and objective quality (total antioxidant capacity and colour) parameters, including data not shown, for Pinotage wines matured using oak and oxygenation treatments as described in **Chapter 6**.

Table 1. Effect of oak maturation on the anthocyanin content[a] of Pinotage wines.

	Dp-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Dp-3-glc-ac[b]	VitA[b]	Pt-3-glc-ac[b]
Control (0 weeks)	16.31 a[e]	19.57 abc	9.33 abc	191.09 a	5.66 abcd	3.80 ab	5.00 ab
New barrels							
6 weeks	15.95 ab	19.70 abc	9.20 abc	175.22 bcde	4.94 abcde	4.07 ab	4.93 ab
15 weeks	13.73 bcdef	17.20 defg	7.49 bcdef	151.74 g	4.33 abcde	3.45 ab	3.61 abc
28 weeks	10.65 g	13.80 h	5.66 f	119.87 i	2.72 e	2.78 ab	2.46 c
Second fill barrels							
6 weeks	15.65 ab	19.15 abcd	9.34 abc	181.34 ab	4.66 abcde	3.43 ab	4.29 abc
15 weeks	14.83 abcd	17.67 cdef	7.98 abcde	163.94 f	4.72 abcde	3.29 ab	3.65 abc
28 weeks	12.52 efg	14.95 h	7.5 bcdef	138.30 h	3.81 cde	3.26 ab	2.99 bc
Third fill barrels							
6 weeks	16.55 a	19.57 abc	9.72 a	182.84 ab	5.61 abcd	4.57 a	5.23 a
15 weeks	15.64 ab	18.35 abcd	8.33 abcde	170.42 cdef	4.95 abcde	3.61 ab	3.93 abc
28 weeks	13.28 cdef	15.87 efgh	7.09 def	149.30 g	3.37 cde	2.72 ab	4.08 abc
Chips							
6 weeks	16.66 a	19.86 a	9.44 ab	181.42 ab	6.28 ab	4.39 a	5.21 ab
15 weeks	15.03 abc	17.90 abcdef	8.19 abcde	164.56 ef	4.98 abcde	3.55 ab	4.25 abc
28 weeks	12.65 defg	15.13 gh	6.76 def	141.78 gh	3.62 cde	2.92 ab	3.95 abc
Staves							
6 weeks	16.08 a	19.87 a	9.11 abc	178.28 bcd	4.58 abcde	4.08 ab	4.19 abc
15 weeks	14.99 abc	18.36 abcd	8.02 abcde	167.95 def	4.59 abde	3.00 ab	3.30 abc
28 weeks	15.00 abc	18.66 abcd	8.10 abcde	173.71 bcdef	4.01 bcde	2.88 ab	3.29 abc
Oak extract							
6 weeks	16.77 a	19.81 ab	9.32 abc	183.07 ab	6.44 a	4.41 a	5.46 a
15 weeks	14.87 abcd	17.94 abcde	8.66 abcd	172.79 bcdef	5.51 abcd	3.21 ab	4.29 abc
28 weeks	12.24 fg	14.86 h	6.45 ef	144.02 gh	3.30 de	1.96 b	3.67 abc
Oak dust							
6 weeks	16.60 a	19.67 abc	9.40 ab	180.84 abc	5.70 abc	4.62 a	5.23 ab
15 weeks	14.75 abcde	17.72 bcdef	8.63 abcd	167.68 def	4.03 bcde	2.97 ab	384 abc
28 weeks	13.34 cdef	15.83 fgh	7.39 cdef	146.26 gh	4.06 bcde	3.32 ab	3.55 abc

[a] mg/L unless otherwise noted; [b] mg of corresponding anthocyanin-3-glc equivalents/L; [c] sum of phenolic group content; [d] mg Mv-3-glc equivalents/L; [e] means with different letters within the same column differ significantly ($P < 0.05$); CP (HPLC) = coloured polymers measured using HPLC; Dp = delphinidin; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; PA (pH shift) = polymeric anthocyanins measured using the pH shift assay; Pt = petunidin; Pn = peonidin; MA (HPLC) = monomeric anthocyanins measured using HPLC; MA (ph shift) = monomeric anthocyanins measured using the pH shift assay; Mv = malvidin; VitA = vitisin A.

Table 1. (continued)

	Pn-3-glc-ac[b]	Mv-3-glc-ac[b]	Mv-3-glc-coum[b]	MA (HPLC)[c]	CP (HPLC)[d]	MA (pH shift)[d]	PA (pH shift)[d]
Control (0 weeks)	4.45 abcd[e]	55.11 a	20.31 a	330.63 a	39.04 ab	341.7 ab	69.6 abcde
New barrels							
6 weeks	5.01 a	52.71 abcde	19.72 ab	311.43 abcde	37.88 ab	324.0 ab	73.3 abcd
15 weeks	3.63 abcde	45.24 fg	14.69 efg	265.11 fgh	39.26 ab	283.9 c	71.4 abcde
28 weeks	2.61 e	35.34 i	10.81 h	241.18 i	42.93 a	244.3 d	72.2 abcde
Second fill barrels							
6 weeks	4.73 ab	53.43 abc	19.36 ab	315.38 abcd	30.75 b	337.7 ab	72.7 abcd
15 weeks	3.79 abcde	48.61 ef	16.50 cdef	284.99 efg	37.05 ab	315.8 b	75.7 abc
28 weeks	3.30 bcde	40.27 h	14.23 fg	241.18 h	35.82 ab	263.2 cd	66.3 cde
Third fill barrels							
6 weeks	4.80 ab	53.23 abcd	19.46 ab	321.97 abc	33.76 ab	329.9 ab	77.2 ab
15 weeks	3.88 abcde	50.15 bcde	18.64 abc	297.90 bcde	38.82 ab	334.7 ab	74.3 abcd
28 weeks	3.06 cde	42.96 gh	15.53 defg	257.24 gh	37.06 ab	284.5 c	65.6 de
Chips							
6 weeks	4.83 ab	53.45 abc	19.77 ab	321.32 abc	41.80 ab	320.0 ab	74.8 abcd
15 weeks	3.83 abcde	49.01 ef	17.24 bcde	288.54 def	36.15 ab	321.7 ab	75.5 abc
28 weeks	2.96 de	41.06 h	13.69 g	244.53 h	39.86 ab	265.7 cd	62.9 e
Staves							
6 weeks	4.70 abc	53.52 abc	18.98 abc	313.40 abcd	31.95 ab	343.6 a	78.8 a
15 weeks	3.63 abcde	49.22 def	17.22 bcde	290.28 def	39.94 ab	333.2 ab	72.1 abcde
28 weeks	3.47 abcde	49.81 cde	16.68 cdef	295.61 cde	41.81 ab	279.6 c	68.1 bcde
Oak extract							
6 weeks	4.74 ab	54.29 ab	20.10 a	324.40 ab	39.03 ab	342.7 a	70.8 abcde
15 weeks	3.59 abcde	49.09 def	17.94 abcd	297.89 bcde	41.81 ab	337.4 ab	73.6 abcd
28 weeks	2.51 e	40.96 h	13.66 g	243.65 h	37.95 ab	277.4 c	63.0 e
Oak dust							
6 weeks	5.00 a	53.90 abc	19.05 abc	320.02 abc	35.66 ab	330.8 ab	74.6 abcd
15 weeks	3.56 abcde	48.80 ef	16.58 cdef	288.57 def	40.33 ab	322.1 ab	72.3 abcde
28 weeks	3.52 abcde	42.91 gh	14.80 efg	254.99 h	38.93 ab	281.4 c	66.9 cde

Table 2. Effect of oak maturation on the flavonol content[a] of Pinotage wines.

	Unknown flavonol[b]	Quercetin-3-glc	Quercetin-3-rham	Quercetin	Kaempferol	Isorhamnetin	Total[c]
Control (0 weeks)	14.31 a[d]	10.77 abcd	8.63 a	5.17 abcd	0.37 a	0.53 a	144.03 a
New barrels							
6 weeks	13.05 b	11.38 ab	8.27 abcd	4.84 abcdefgh	0.25 a	0.43 a	139.23 abc
15 weeks	11.69 cde	9.27 abcde	7.66 defghi	3.93 fgh	0.23 a	0.38 a	141.05 abc
28 weeks	9.84 fgh	8.90 cde	7.08 ij	3.73 h	0.24 a	0.45 a	1347.03 bc
Second fill barrels							
6 weeks	12.96 b	10.80 abcd	8.36 ab	5.08 abcde	0.30 a	0.47 a	138.80 abc
15 weeks	11.44 de	9.87 abcde	7.91 bcdef	4.45 abcdefgh	0.24 a	0.44 a	139.18 abc
28 weeks	9.41 h	8.54 de	7.17 ij	3.88 fgh	0.22 a	0.42 a	132.30 c
Third fill barrels							
6 weeks	12.53 bcd	11.07 abc	8.23 abcd	5.31 ab	0.30 a	0.52 a	140.13 abc
15 weeks	10.78 efg	9.36 abcde	7.82 bcdefgh	5.00 abcdef	0.29 a	0.54 a	138.19 abc
28 weeks	9.46 h	8.50 de	7.36 fghij	4.18 bcdefgh	0.26 a	0.47 a	135.01 bc
Chips							
6 weeks	13.69 ab	11.64 a	8.39 ab	5.42 a	0.32 a	0.51 a	141.75 ab
15 weeks	10.93 ef	9.00 bcde	7.33 fghij	4.11 defgh	0.29 a	0.45 a	138.21 abc
28 weeks	9.72 gh	8.59 de	7.21 hij	3.97 efgh	0.30 a	0.49 a	136.35 abc
Staves							
6 weeks	12.88 b	11.00 abc	8.10 abcde	5.28 abc	0.32 a	0.46 a	137.22 abc
15 weeks	11.19 e	9.85 abcde	7.87 bcdefg	4.64 abcdefgh	0.30 a	0.48 a	137.00 abc
28 weeks	9.66 gh	8.80 cde	7.25 ghij	4.14 cdefgh	0.27 a	0.48 a	133.78 bc
Oak extract							
6 weeks	13.30 ab	10.54 abcde	8.33 abc	5.49 a	0.31 a	0.52 a	141.44 ab
15 weeks	11.34 e	8.93 cde	7.70 cdefghi	4.66 abcdefgh	0.34 a	0.57 a	138.26 abc
28 weeks	9.49 h	8.23 e	7.22 hij	4.93 abcdefg	0.29 a	0.51 a	134.29 bc
Oak dust							
6 weeks	12.87 bc	10.52 abcde	8.11 abcde	4.37 abcdefgh	0.26 a	0.41 a	141.15 ab
15 weeks	10.99 ef	10.25 abcde	7.48 efghi	4.24 bcdefgh	0.28 a	0.43 a	141.29 ab
28 weeks	9.17 h	10.17 abcde	6.75 j	3.81 gh	0.23 a	0.40 a	135.58 abc

[a] mg/L unless otherwise noted; [b] mg rutin equivalents/L; [c] sum of phenolic group content; [d] means with different letters within the same column differ significantly ($P < 0.05$).

Table 3. Effect of oak maturation on the phenolic acid content[a] of Pinotage wines.

	Gallic acid	Caftaric acid	Caffeic acid	Coutaric acid[b]	*p*-Coumaric acid	Total[c]
Control (0 weeks)	23.99 hi[d]	88.48 a	21.25 a	6.90 abc	3.41 a	144.03 a
New barrels						
6 weeks	25.05 fg	83.94 abcd	20.26 abc	6.58 abcde	3.41 a	139.23 abc
15 weeks	26.36 cde	84.86 abc	19.73 abcde	6.52 abcdef	3.58 a	141.05 abc
28 weeks	27.72 a	79.03 cd	18.33 e	5.69 g	3.26 a	134.03 bc
Second fill barrels						
6 weeks	24.63 fghi	83.14 abcd	20.63 ab	6.86 abcd	3.53 a	138.80 abc
15 weeks	26.03 de	83.76 abcd	19.36 bcde	6.58 abcde	3.44 a	139.18 abc
28 weeks	27.60 ab	77.21 d	18.46 e	5.94 efg	3.12 a	132.30 c
Third fill barrels						
6 weeks	24.33 ghi	85.14 abc	20.43 abc	6.61 abcde	3.61 a	140.13 abc
15 weeks	25.00 fg	83.48 abcd	19.24 bcde	6.81 abcd	3.66 a	138.19 abc
28 weeks	27.00 abc	79.79 bcd	18.66 de	6.18 cdefg	3.39 a	135.01 bc
Chips						
6 weeks	24.79 fghi	86.90 ab	20.07 abcd	6.64 abcde	3.36 a	141.75 ab
15 weeks	25.48 ef	83.17 abcd	19.16 bcde	6.78 abcd	3.63 a	138.21 abc
28 weeks	27.30 abc	80.81 bcd	18.51 de	6.20 bcdefg	3.55 a	136.35 abc
Staves						
6 weeks	23.90 i	82.68 abcd	20.67 ab	6.69 abcde	3.29 a	137.22 abc
15 weeks	24.94 fgh	82.63 abcd	19.38 bcde	6.73 abcde	3.31 a	137.00 abc
28 weeks	26.57 cd	79.43 cd	18.46 e	6.07 defg	3.24 a	133.78 bc
Oak extract						
6 weeks	24.24 ghi	86.61 ab	20.40 abc	6.77 abcd	3.42 a	141.44 ab
15 weeks	24.83 fghi	83.64 abcd	19.56 bcde	6.62 abcde	3.61 a	138.26 abc
28 weeks	26.71 bcd	80.03 bcd	18.45 e	5.74 fg	3.36 a	134.29 bc
Oak dust						
6 weeks	24.72 fghi	85.46 abc	20.74 ab	7.01 ab	3.21 a	141.15 ab
15 weeks	25.49 ef	85.68 abc	19.77 abcde	7.08 a	3.27 a	141.29 ab
28 weeks	27.03 abc	79.98 bcd	19.02 cde	6.44 abcdefg	3.13 a	135.58 abc

[a] mg/L unless other wise noted; [b] mg *p*-coumaric acid equivalents/L; [c] sum of phenolic group content; [d] means with different letters within the same column differ significantly ($P < 0.05$).

Table 4. Effect of oak maturation on the flavan-3-ol, total monomer and total phenol content[a] of Pinotage wines.

	(+)-Catechin	Procyanidin B1	Non-coloured polymers[b]	TF (DAC)[b]	Total monomers[c]	TP (Folin-Ciocalteau)[d]
Control (0 weeks)	33.73 ab[e]	19.27 a	67.70 a	188.0 a	567.42 a	1984.4 abc
New barrels						
6 weeks	30.72 bcde	16.87 abc	67.81 a	175.4 abc	536.46 abcd	2049.0 ab
15 weeks	28.76 de	15.32 c	67.81 a	162.1 cd	483.36 efghi	1949.0 bcd
28 weeks	26.99 e	15.93 bc	38.44 b	154.5 d	413.84 k	1857.5 d
Second fill barrels						
6 weeks	32.70 abcd	18.13 abc	59.24 a	172.1 abcd	542.98 abc	1962.1 bcd
15 weeks	30.43 bcde	16.25 abc	70.71 a	172.7 abcd	505.20 defgh	1948.8 bcd
28 weeks	28.92 cde	17.31 abc	60.64 a	160.0 cd	449.36 j	1922.7 cd
Third fill barrels						
6 weeks	32.07 abcd	19.21 a	61.59 a	184.1 ab	551.33 ab	2027.4 ab
15 weeks	29.86 bcde	16.42 abc	70.47 a	171.0 abcd	516.13 cde	1962.9 bcd
28 weeks	30.79 bcde	18.28 abc	66.57 a	166.3 bcd	471.53 hij	1941.2 bcd
Chips						
6 weeks	31.65 bcd	18.84 ab	74.18 a	170.2 abcd	553.54 ab	2056.4 ab
15 weeks	29.30 cde	18.46 ab	65.01 a	169.5 abcd	506.63 defg	1942.6 bcd
28 weeks	33.15 abc	17.31 abc	66.21 a	165.8 bcd	475.71 ghij	1850.9 d
Staves						
6 weeks	32.41 abcd	17.47 abc	60.05 a	183.2 ab	538.55 abcd	1990.9 abc
15 weeks	33.95 ab	16.79 abc	65.52 a	169.2 abcd	512.35 cdef	1911.0 cd
28 weeks	30.44 bcde	17.46 abc	64.68 a	164.2 bcd	507.89 defg	1909.0 cd
Oak extract						
6 weeks	32.41 abcd	17.58 abc	69.49 a	175.5 abc	554.31 ab	2096.4 a
15 weeks	36.24 a	18.31 abc	67.80 a	173.3 abcd	524.24 bcd	1956.2 bcd
28 weeks	31.31 bcd	18.31 abc	63.74 a	159.8 cd	458.24 ij	1921.0 cd
Oak dust						
6 weeks	30.95 bcde	16.97 abc	64.12 a	178.9 abc	545.62 abc	2007.4 abc
15 weeks	32.74 abcd	17.13 abc	65.07 a	178.4 abc	513.39 cdef	2005.2 abc
28 weeks	28.88 de	16.34 abc	66.44 a	159.5 cd	481.01 fghij	1950.0 bcd

[a] mg/L unless other wise noted; [b] mg (+)-catechin equivalents/L; [c] sum of all monomeric phenolic compounds; [d] mg gallic acid equivalents/L; [e] means with different letters within the same column differ significantly ($P < 0.05$); TF (DAC) = total flavan-3-ols measured using the DAC assay; TP (Folin-Ciocalteau) = total phenols measured using the Folin Ciocalteau assay.

Table 5. Effect of oak maturation on the antioxidant capacity and objective colour parameters of Pinotage wines.

	TAC_M [a]	TAC_{CAL} [b]	TAC_R [c]	*C** [d]	*h** [e]	*L** [f]	*a** [g]	*b** [h]
Control (0 weeks)	14.33 cd[i]	2.01 a	12.31 de	61.90 defg	17.67 hij	31.52 ab	58.98 cd	18.79 hij
New barrels								
6 weeks	15.34 ab	1.93 abcd	13.41 ab	64.36 a	17.86 ghi	30.64 cd	61.26 ab	19.74 de
15 weeks	nd	1.79 efg	nd	62.49 c	18.16 fgh	28.19 i	59.38 c	19.48 efg
28 weeks	14.36 cd	1.61 h	12.75 bcde	61.12 i	19.53 d	26.23 j	57.61 ef	20.44 c
Second fill barrels								
6 weeks	14.34 cd	1.95 abcd	12.40 cde	63.99 ab	17.30 jkl	31.69 ab	61.10 ab	19.03 ghi
15 weeks	14.58 bcd	1.85 def	12.73 bcde	62.24 cd	18.51 ef	29.81 ef	59.02 cd	19.77 de
28 weeks	14.77 bcd	1.72 gh	13.06 abcde	61.38 hi	20.30 c	28.21 i	57.58 ef	21.29 b
Third fill barrels								
6 weeks	15.02 abc	1.97 abc	13.04 abcde	63.80 b	17.16 jklm	31.99 a	60.96 b	18.82 hij
15 weeks	14.99 abc	1.88 bcdef	13.11 abcde	62.13 cde	18.84 e	30.88 c	58.81 d	20.06 cd
28 weeks	14.95 abc	1.77 fg	13.17 abcd	61.36 hi	21.00 a	29.34 fg	57.29 ef	21.99 a
Chips								
6 weeks	15.29 ab	1.98 ab	13.30 abc	64.03	17.41 ijk	31.42 b	61.10 ab	19.16 fgh
15 weeks	14.79 bcd	1.85 def	12.94 abcde	61.95 defg	18.53 ef	30.18 de	58.74 d	19.69 def
28 weeks	14.02 d	1.79 efg	12.22	61.57 fghi	20.59 abc	28.73 h	57.64 ef	21.65 ab
Staves								
6 weeks	14.91 abcd	1.93 abcd	12.98 abcde	64.17 ab	16.92 klm	31.65 ab	61.39 ab	18.68 hij
15 weeks	14.65 bcd	1.86 def	12.79 bcde	62.01 cdefg	18.58 ef	30.34 d	58.78 d	19.76 de
28 weeks	14.65 bcd	1.87 bcdef	12.77 bcde	61.68 efgh	20.52 abc	28.89 gh	57.77 e	21.62 ab
Oak extract								
6 weeks	15.76 a	1.98 abc	13.79 a	64.14 ab	16.87 lm	31.79 ab	61.39 ab	18.62 ij
15 weeks	14.98 abc	1.90 abcde	13.07 abcde	61.95 defg	18.43 ef	30.78 cd	58.78 d	19.58 def
28 weeks	14.58 bcd	1.73 g	12.85 bcde	61.25 hi	20.84 ab	28.85 gh	57.25 f	21.79 ab
Oak dust								
6 weeks	15.13 abc	1.96 abcd	13.17 abcd	64.28 ab	16.65 m	31.59 ab	61.59 a	18.42 j
15 weeks	15.42 ab	1.87 cdef	13.55 ab	62.03 cdef	18.24 fg	30.21 de	58.91 cd	19.42 efg
28 weeks	14.99 abc	1.80 efg	13.45 ab	61.52 ghi	20.39 bc	28.73 h	57.67 ef	21.44 b

[a] total antioxidant capacity in mM Trolox equivalents; [b] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [c] unexplained TAC = measured TAC – calculated TAC; [d] chroma; [e] hue angle (°); [f] lightness; [g] red/green chromaticity; [h] yellow/blue chromaticity; [i] means with different letters within the same column differ significantly ($P < 0.05$).

Table 6. Effect of oxygenation on the phenolic composition (measured spectrophotometrically) of Pinotage wines.

Treatment	Monomeric anthocyanins[a]	Polymeric anthocyanins[a]	Total anthocyanins[a]	Total flavan-3-ols[b]	Total phenols[c]
Control	324.75 a[d] (± 10.57)[e]	60.92 c (± 4.50)	385.67 ab (± 14.96)	229.36 a (± 1.22)	2569.37 a (± 65.76)
No oxygen					
2 months	339.67 a (± 18.48)	59.44 c (± 8.72)	399.10 ab (± 24.89)	223.81 a (± 4.98)	2443.81 abcd (± 101.72)
4 months	325.61 a (± 11.96)	63.46 c (± 8.08)	389.07 ab (± 4.04)	230.65 a (± 8.78)	2472.28 abc (± 143.83)
6 months	355.52 a (± 17.56)	62.72 c (± 1.39)	418.24 a (± 17.46)	223.37 a (± 8.56)	2523.24 ab (± 113.78)
Low level					
2 months	284.00 b (± 8.48)	72.32 bc (± 9.64)	356.32 bc (± 16.30)	200.33 b (± 14.32)	2258.83 de (± 132.11)
4 months	208.87 cd (± 18.78)	85.91 ab (± 18.78)	294.78 de (± 37.56)	185.02 b (± 5.88)	2195.13 ef (± 102.95)
6 months	219.28 c (± 19.04)	84.05 ab (± 3.03)	303.32 de (± 16.02)	185.50 b (± 6.56)	2336.53 bcde (± 78.02)
High level					
2 months	235.71 c (± 26.75)	84.14 ab (± 14.23)	319.85 cd (± 40.98)	191.34 b (± 3.16)	2313.96 cde (± 145.31)
4 months	179.80 d (± 20.82)	88.11 ab (± 10.43)	267.91 ef (± 21.01)	182.14 b (± 21.73)	2236.89 e (± 116.37)
6 months	142.03 e (± 16.27)	95.91 a (± 18.84)	237.94 f (± 28.77)	158.67 c (± 8.87)	2019.62 f (± 66.72)

[a] mg malvidin-3-glucoside equivalents/L; [b] mg (+)-catechin equivalents/L; [c] total phenol content in mg gallic acid equivalents/L; [d] means with different letters within the same column and group differ significantly ($P < 0.05$); [e] SD.

Table 7. Effect of oxygenation on the anthocyanin content[a] of Pinotage wines.

Treatment	Monomeric anthocyanins											Coloured polymers[d]
	Dp-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Dp-3-glc-ac[b]	Vitisin A[b]	Pt-3-glc-ac[b]	Pn-3-glc-ac[b]	Mv-3-glc-ac[b]	Mv-3-glc-coum[b]	Total[c]	
Control	11.68 a[e] (± 0.82)[f]	14.86 a (± 0.65)	5.83 a (± 0.29)	162.72 (± 3.64)	3.00 ab (± 0.18)	6.82 d (± 0.70)	2.28 a (± 0.03)	3.84 a (± 0.12)	43.33 a (± 0.41)	17.33 a (± 1.52)	271.69 a (± 7.54)	15.58 c (± 5.16)
No oxygen												
2 months	11.71 a (± 0.63)	15.01 a (± 0.77)	5.81 a (± 0.27)	162.51 (± 7.64)	2.98 ab (± 0.12)	7.35 cd (± 1.52)	2.23 a (± 0.10)	3.78 a (± 0.10)	43.16 a (± 2.35)	17.93 a (± 0.96)	272.47 a (± 12.36)	15.16 c (± 3.48)
4 months	11.93 a (± 0.47)	15.08 a (± 0.74)	5.90 a (± 0.29)	163.74 a (± 7.63)	3.06 ab (± 0.25)	7.96 cd (± 2.04)	2.27 a (± 0.13)	3.72 a (± 0.40)	43.42 a (± 2.18)	18.12 a (± 1.22)	275.19 a (± 11.45)	19.20 bc (± 8.97)
6 months	12.91 a (± 0.07)	16.31 a (± 0.24)	6.14 a (± 0.14)	172.67 a (± 2.86)	3.18 a (± 0.00)	7.01 d (± 0.28)	2.38 a (± 0.14)	3.95 a (± 0.32)	45.30 a (± 0.97)	18.97 a (± 1.41)	288.81 a (± 5.87)	16.28 bc (± 1.02)
Low level												
2 months	7.80 b (± 0.75)	10.10 b (± 0.78)	3.98 b (± 0.06)	107.85 b (± 8.27)	2.23 bc (± 0.22)	9.24 abc (± 0.38)	1.49 b (± 0.11)	2.70 b (± 0.18)	29.19 b (± 2.31)	9.62 b (± 0.34)	184.19 b (± 13.10)	14.75 c (± 1.91)
4 months	5.06 cd (± 0.31)	6.41 c (± 0.49)	2.07 c (± 0.47)	66.28 c (± 3.56)	0.91 e (± 1.28)	8.61 bcd (± 1.07)	0.69 c (± 0.97)	1.97 cd (± 0.24)	18.68 c (± 1.51)	4.34 cd (± 0.31)	115.00 c (± 7.80)	19.29 bc (± 1.85)
6 months	5.41 c (± 0.56)	6.97 c (± 0.69)	2.20 c (± 0.18)	72.04 c (± 10.36)	1.65 cde (± 0.07)	8.12 cd (± 1.04)	0.00 d (± 0.00)	2.10 cd (± 0.15)	19.48 c (± 2.35)	4.62 cd (± 0.06)	122.55 c (± 13.03)	21.22 bc (± 1.97)
High level												
2 months	5.96 c (± 0.59)	7.46 c (± 0.93)	2.87 c (± 0.56)	76.79 c (± 8.08)	1.91 cd (± 0.21)	10.52 ab (± 0.74)	0.00 d (± 0.00)	2.51 bc (± 0.04)	20.76 c (± 1.64)	5.80 c (± 0.95)	134.58 c (± 13.35)	22.18 bc (± 4.77)
4 months	3.99 d (± 0.92)	4.67 d (± 1.40)	1.10 d (± 0.98)	47.06 d (± 12.85)	1.05 de (± 0.91)	11.07 a (± 0.69)	1.62 b (± 0.29)	1.59 d (± 0.22)	13.06 d (± 3.55)	2.90 d (± 2.63)	88.11 d (± 23.82)	35.79 a (± 2.50)
6 months	1.68 e (± 1.08)	1.45 e (± 1.34)	0.00 e (± 0.00)	22.90 e (± 6.55)	0.00 f (± 0.00)	7.28 cd (± 1.11)	0.00 d (± 0.00)	0.39 e (± 0.68)	6.70 e (± 1.50)	0.00 e (± 0.00)	40.39 e (± 11.53)	23.75 b (± 4.78)

[a] mg/L unless otherwise noted; [b] mg of corresponding anthocyanin-3-glc equivalents/L; [c] sum of phenolic group content; [d] mg Mv-3-glc equivalents/L[e] means with different letters within the same column differ significantly ($P < 0.05$); [f] SD; Dp = delphinidin; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Pt = petunidin; Pn = peonidin; Mv = malvidin.

Table 8. Effect of oxygenation on the flavonol content[a] of Pinotage wines.

Treatment	Unknown[b]	Q-3-gal	Q-3-glc	Q-3-rham	Quercetin	Kaempferol	Isorhamnetin	Total[c]
Control	18.36 a[f] (± 2.24)[g]	3.60 abc (± 0.52)	15.09 abc (± 2.22)	13.99 a (± 0.95)	6.31 ab (± 2.09)	1.06 abcd (± 0.46)	0.46 a (± 0.07)	58.88 ab (± 8.49)
No oxygen								
2 months	18.40 a (± 1.03)	3.84 ab (± 0.18)	15.86 ab (± 0.94)	14.28 a (± 0.38)	6.87 a (± 0.97)	1.14 ab (± 0.11)	0.43 ab (± 0.06)	60.82 a (± 3.61)
4 months	18.32 a (± 1.05)	3.81 ab (± 0.18)	16.08 a (± 1.30)	14.45 a (± 0.52)	7.43 a (± 1.30)	1.18 a (± 0.17)	0.46 a (± 0.08)	61.73 a (± 4.57)
6 months	17.74 ab (± 0.87)	3.92 a (± 0.08)	15.71 ab (± 0.52)	14.52 a (± 0.31)	6.52 ab (± 1.51)	1.10 abc (± 0.20)	0.44 a (± 0.06)	60.17 ab (± 3.11)
Low level								
2 months	16.22 bc (± 0.25)	3.40 bc (± 0.16)	13.66 cd (± 0.68)	13.15 b (± 0.06)	4.47 bc (± 2.13)	0.57 bcde (± 0.49)	0.24 bc (± 0.21)	51.71 bc (± 3.84)
4 months	14.79 c (± 0.47)	3.36 c (± 0.01)	13.04 d (± 0.59)	12.48 b (± 0.23)	5.17 abc (± 0.52)	0.68 abcde (± 0.07)	0.18 cd (± 0.25)	49.69 c (± 2.14)
6 months	15.01 c (± 0.49)	3.22 cd (± 0.08)	13.01 d (± 0.29)	12.56 b (± 0.17)	5.46 abc (± 0.13)	0.71 abcde (± 0.01)	0.36 abc (± 0.02)	50.32 c (± 0.57)
High level								
2 months	15.90 bc (± 0.91)	3.58 abc (± 0.41)	13.95 bcd (± 0.89)	13.06 b (± 0.40)	5.57 abc (± 0.84)	0.51 cde (± 0.44)	0.39 ab (± 0.03)	52.96 bc (± 3.72)
4 months	14.71 c (± 0.86)	3.30 c (± 0.07)	13.69 cd (± 0.78)	12.71 b (± 0.36)	5.97 ab (± 0.91)	0.49 de (± 0.42)	0.40 ab (± 0.05)	51.26 c (± 3.29)
6 months	11.90 d (± 0.75)	2.84 d (± 0.09)	10.62 e (± 0.73)	11.10 c (± 0.47)	3.52 c (± 0.51)	0.19 e (± 0.33)	0.00 d (± 0.00)	40.17 d (± 2.69)

[a] mg/L unless otherwise noted; [b] mg rutin equivalents/L; [c] sum of phenolic group content; [d] means with different letters within the same column differ significantly ($P < 0.05$); [e] SD; Q = quercetin; gal = galactoside; glc = glucoside; rham = rhamnoside.

Table 9. Effect of oxygenation on the phenolic acid, flavan-3-ol, non-coloured polymer and total monomer content[a] of Pinotage wines.

Treatment	Phenolic acids						Flavan-3-ols			Non-coloured	Total
	Gallic acid	Caftaric acid	Caffeic acid	Coutaric acid[b]	*p*-Coumaric acid	Total[c]	(+)-Catechin	Procyanidin B1	Total[c]	polymers[d]	monomers[e]
Control	14.56 cd[f] (± 0.26)[g]	248.43 abc (± 4.35)	0.70 a (± 0.03)	22.72 b (± 0.48)	0.62 abc (± 0.07)	287.03 abc (± 5.08)	11.58 a (± 0.11)	17.04 a (± 0.14)	28.62 a (± 0.12)	212.07 ab (± 52.40)	600.94 b (± 17.29)
No oxygen											
2 months	14.60 cd (± 0.19)	251.06 ab (± 5.39)	0.67 ab (± 0.11)	22.96 ab (± 0.61)	0.60 abc (± 0.09)	289.88 ab (± 6.23)	11.41 a (± 0.21)	16.35 a (± 0.39)	27.77 a (± 0.59)	207.52 b (± 47.68)	605.14 ab (± 14.69)
4 months	14.62 cd (± 0.29)	254.89 a (± 10.77)	0.70 a (± 0.07)	24.58 a (± 2.18)	0.65 ab (± 0.07)	295.44 a (± 12.05)	11.34 a (± 0.07)	16.14 a (± 0.63)	27.48 a (± 0.68)	242.26 ab (± 74.41)	612.48 ab (± 11.45)
6 months	14.45 d (± 0.38)	252.45 ab (± 1.75)	0.62 ab (± 0.09)	23.09 ab (± 0.29)	0.67 a (± 0.06)	291.29 ab (± 2.50)	11.69 a (± 0.31)	16.89 a (± 0.83)	28.13 a (± 1.14)	200.87 b (± 28.76)	635.88 a (± 23.39)
Low level											
2 months	14.91 c (± 0.22)	240.10 c (± 4.37)	0.60 abc (± 0.03)	21.85 b (± 0.56)	0.52 abc (± 0.02)	277.99 c (± 5.06)	10.78 b (± 0.29)	14.17 b (± 1.00)	24.95 b (± 1.29)	170.44 b (± 29.49)	496.85 c (± 6.45)
4 months	15.39 b (± 0.10)	243.51 bc (± 2.21)	0.70 a (± 0.06)	21.86 b (± 0.27)	0.51 abc (± 0.03)	281.96 bc (± 1.96)	9.96 c (± 0.01)	11.24 d (± 0.16)	21.20 c (± 0.18)	196.67 b (± 21.75)	427.33 de (± 7.38)
6 months	15.59 ab (± 0.09)	248.16 abc (± 8.49)	0.57 bc (± 0.01)	23.93 ab (± 1.18)	0.61 ab (± 0.14)	288.85 abc (± 7.07)	9.97 c (± 0.01)	11.54 cd (± 0.07)	21.51 c (± 0.09)	195.43 b (± 10.30)	440.46 d (± 21.31)
High level											
2 months	15.40 b (± 0.21)	244.45 bc (± 6.11)	0.60 abc (± 0.01)	22.12 b (± 0.60)	0.52 c (± 0.11)	283.11 bc (± 5.81)	10.27 c (± 0.05)	12.48 c (± 0.20)	22.75 c (± 0.17)	216.54 ab (± 44.68)	451.26 d (± 20.97)
4 months	15.68 ab (± 0.03)	250.22 ab (± 1.02)	0.57 bc (± 0.03)	22.53 ab (± 0.60)	0.57 abc (± 0.10)	289.55 ab (± 0.33)	9.39 d (± 0.47)	9.63 e (± 0.05)	19.01 d (± 1.52)	287.23 a (± 15.97)	407.07 e (± 26.43)
6 months	15.84 a (± 0.19)	246.46 abc (± 3.90)	0.50 c (± 0.07)	23.27 b (± 2.37)	0.53 bc (± 0.04)	286.61 abc (± 6.36)	8.69 e (± 0.34)	7.00 f (± 0.79)	15.69 e (± 0.99)	184.15 b (± 33.09)	344.30 f (± 14.48)

[a] mg/L unless other wise noted; [b] mg *p*-coumaric acid equivalents/L; [c] sum of phenolic group content; [d] mg (+)-catechin equivalents/L; [e] sum of all monomeric phenolic compounds; [f] means with different letters within the same column differ significantly ($P < 0.05$); [g] SD.

Table 10. Effect of oxygenation on the antioxidant capacity of Pinotage wines.

Treatment	TAC_M [a]	TAC_{CAL} [b]	TAC_R [c]
Control	14.76 a[d] (± 0.45)[e]	1.99 a (± 0.07)	12.77 a (± 0.41)
No oxygen			
2 months	14.32 a (± 0.40)	2.01 a (± 0.05)	12.32 abcd (± 0.38)
4 months	14.41 a (± 0.44)	2.03 a (± 0.04)	12.38 abc (± 0.46)
6 months	14.61 a (± 0.21)	2.06 a (± 0.05)	12.46 ab (± 0.12)
Low level			
2 months	13.41 b (± 0.67)	1.68 b (± 0.01)	11.73 bcde (± 0.67)
4 months	13.17 b (± 0.12)	1.48 cd (± 0.02)	11.69 bcde (± 0.10)
6 months	12.85 bc (± 0.45)	1.52 cd (± 0.06)	11.33 e (± 0.50)
High level			
2 months	12.96 bc (± 0.23)	1.55 c (± 0.07)	11.40 de (± 0.19)
4 months	12.93 bc (± 0.98)	1.42 d (± 0.09)	11.51 cde (± 0.96)
6 months	12.20 c (± 0.35)	1.22 e (± 0.05)	10.98 e (± 0.40)

[a] total antioxidant capacity in mM Trolox equivalents as measured; [b] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [c] unexplained TAC = measured TAC – calculated TAC; [d] means with different letters within the same column differ significantly ($P < 0.05$); [e] SD.

Table 11. Effect of oxygenation on the objective colour parameters and sensory quality of Pinotage wines.

Treatment	Objective colour parameters					Sensory quality				
	*C**[a]	*h**[b]	*L**[c]	*a**[d]	*b**[e]	Colour	Berry/plum intensity	Astringency	Fullness	Overall quality
Control	64.61 ab[f] (± 0.60)[g]	14.80 e (± 0.15)	33.81 a (± 1.20)	62.47 a (± 0.62)	16.51 e (± 0.05)	54.13 e (± 4.83)	60.17 ab (± 4.34)	44.60 bcd (± 5.90)	46.77 d (± 6.31)	53.53 ab (± 7.66)
No oxygen										
2 months	64.83 ab (± 0.27)	15.83 de (± 0.50)	33.48 a (± 0.98)	62.37 a (± 0.11)	17.69 de (± 0.62)	60.00 de (± 6.06)	64.93 a (± 1.91)	42.73 d (± 2.11)	51.00 cd (± 7.85)	61.80 a (± 5.05)
4 months	64.62 ab (± 0.70)	16.82 cd (± 0.83)	32.99 a (± 1.99)	61.85 ab (± 0.41)	18.71 cd (± 1.09)	58.60 de (± 3.64)	62.07 a (± 3.16)	50.00 abc (± 4.67)	50.43 cd (± 3.79)	56.60 ab (± 2.20)
6 months	63.56 cd (± 0.56)	15.98 d (± 0.24)	35.36 a (± 0.66)	61.11 bc (± 0.58)	17.50 de (± 0.23)	57.13 e (± 4.45)	52.17 bc (± 2.45)	47.33 abcd (± 2.45)	48.23 d (± 6.00)	53.93 ab (± 5.83)
Low level										
2 months	65.46 a (± 0.25)	17.98 b (± 0.90)	29.60 b (± 2.75)	62.26 a (± 0.49)	20.21 ab (± 0.94)	65.47 cd (± 4.30)	51.43 c (± 7.11)	47.17 abcd (± 5.18)	50.17 cd (± 2.60)	51.73 ab (± 11.37)
4 months	62.81 de (± 0.57)	17.93 bc (± 0.50)	23.89 cd (± 0.21)	59.77 d (± 0.71)	19.33 bc (± 0.35)	59.90 de (± 4.38)	53.60 bc (± 6.93)	53.85 a (± 6.86)	52.30 bcd (± 2.12)	49.60 bc (± 0.57)
6 months	62.38 e (± 0.74)	17.55 bc (± 0.10)	23.16 cde (± 0.42)	59.48 de (± 0.67)	18.81 cd (± 0.33)	73.20 ab (± 1.98)	33.75 de (± 1.48)	50.90 ab (± 3.68)	60.85 a (± 0.92)	32.70 d (± 6.65)
High level										
2 months	64.31 bc (± 0.22)	19.21 a (± 0.06)	25.09 c (± 0.28)	60.73 c (± 0.19)	21.17 a (± 0.14)	69.07 bc (± 3.00)	39.83 d (± 2.78)	43.67 cd (± 1.67)	59.03 ab (± 2.25)	40.00 cd (± 6.61)
4 months	62.47 e (± 1.03)	17.95 b (± 0.86)	22.53 de (± 1.12)	59.42 de (± 0.73)	19.27 bc (± 1.18)	75.80 ab (± 3.74)	28.00 ef (± 6.53)	44.97 bcd (± 3.62)	57.17 abc (± 2.76)	9.60 e (± 5.07)
6 months	61.19 f (± 0.25)	16.18 d (± 0.92)	20.79 e (± 0.51)	58.76 e (± 0.51)	17.04 e (± 0.87)	77.67 a (± 0.81)	20.93 f (± 4.74)	44.83 bcd (± 0.76)	59.30 ab (± 1.39)	5.20 e (± 4.16)

[a] chroma; [b] hue angle (°); [c] lightness; [d] red/green chromaticity; [e] yellow/blue chromaticity; [f] means with different letters within the same column differ significantly ($P < 0.05$); [g] SD.

Addendum F: Chapter 7 Data

Aim

The aim is to tabulate all the actual values for phenolic composition, objective quality (total antioxidant capacity and colour) and sensory quality parameters, including data not shown, for Pinotage wines produced according to various enological protocols as described in **Chapter 7**.

Table 1. Phenolic composition (measured spectrophotometrically), antioxidant capacity, objective colour parameters and sensory quality of Pinotage wines produced according to different enological protocols .

	Control	Pumping-over	Oak tannin	Grape tannin	Extended maceration	Oxygenation
Phenolic composition						
Monomeric anthocyanins[a]	422.2 ab[b] (± 43.2)[c]	394.1 b (± 14.4)	465.5 a (± 23.7)	427.3 ab (± 24.2)	402.8 b (± 4.2)	398.8 b (± 37.3)
Polymeric anthocyanins[a]	53.6 abc (± 6.9)	41.0 c (± 4.8)	61.7 a (± 9.2)	56.5 ab (± 6.1)	45.3 bc (± 5.0)	52.5 abc (± 10.7)
Total anthocyanins[a]	475.8 ab (± 50.1)	435.0 b (± 18.6)	527.2 a (± 32.9)	483.9 ab (± 30.1)	448.2 b (± 8.8)	451.4 b (± 47.2)
Total flavan-3-ols[d]	138.6 ab (± 6.8)	120.1 c (± 7.7)	140.8 ab (± 7.8)	152.4 a (± 7.2)	154.1 a (± 1.4)	129.1 bc (± 18.2)
Total phenols[e]	1808.3 ab (± 27.9)	1573.0 c (± 67.5)	1899.9 a (± 153.7)	1906.5 a (± 89.2)	1874.8 a (± 86.1)	1666.4 bc (± 154.5)
Antioxidant capacity						
TAC_M[f]	13.33 ab (± 0.35)	11.66 c (± 0.52)	13.89 a (± 0.69)	13.63 a (± 0.58)	14.11 a (± 0.54)	12.29 bc (± 1.02)
TAC_{CAL}[g]	1.98 a (± 0.17)	2.06 a (± 0.02)	2.08 a (± 0.11)	2.02 a (± 0.09)	1.99 a (± 0.02)	1.94 a (± 0.03)
TAC_R[h]	11.35 ab (± 0.22)	9.60 c (± 0.54)	11.48 ab (± 0.64)	11.61 a (± 0.55)	12.11 a (± 0.51)	10.35 bc (± 1.01)
Objective colour parameters						
*C**[i]	60.18 a (± 1.16)	58.69 a (± 1.50)	61.47 a (± 0.20)	61.68 a (± 0.30)	53.03 b (± 2.06)	58.95 a (± 3.36)
*h**[j]	13.42 b (± 0.30)	10.63 c (± 1.25)	14.32 b (± 0.75)	13.36 b (± 0.99)	12.77 bc (± 1.90)	17.00 a (± 1.45)
*L**[k]	36.06 bc (± 2.54)	43.15 ab (± 2.25)	34.25 c (± 2.80)	35.98 bc (± 2.45)	45.44 a (± 3.28)	39.32 abc (± 8.02)
*a**[l]	58.53 ab (± 1.12)	57.67 ab (± 1.45)	59.56 a (± 0.29)	60.00 a (± 0.38)	51.69 c (± 1.68)	56.35 b (± 2.80)
*b**[m]	13.96 bc (± 0.44)	10.82 d (± 1.35)	15.20 ab (± 0.78)	14.25 bc (± 1.04)	11.75 cd (± 2.14)	17.29 a (± 2.37)
Sensory quality						
Colour	73.8 a (± 7.3)	60.5 c (± 9.5)	74.9 a (± 8.2)	73.0 ab (± 7.2)	60.3 c (± 7.6)	68.4 b (± 9.5)
Berry/plum intensity	64.1 ab (± 9.9)	59.4 bc (± 9.5)	67.8 a (± 7.8)	66.8 a (± 7.5)	51.0 d (± 12.2)	56.8 c (± 14.0)
Astringency	51.0 ab (± 14.1)	49.7 b (± 20.0)	56.9 a (± 10.8)	54.4 ab (± 15.1)	50.6 b (± 18.3)	51.4 ab (± 17.5)
Fullness	61.0 ab (± 11.0)	53.7 cd (± 13.9)	64.8 a (± 10.4)	59.0 bc (± 11.9)	52.2 d (± 10.8)	55.1 cd (± 12.4)
Overall quality	63.8 a (± 11.5)	55.7 b (± 13.2)	66.0 a (± 10.6)	63.7 a (± 11.2)	53.6 b (± 8.8)	55.8 b (± 14.3)

[a] mg malvidin-3-glc equivalents/L; [b] different letters within a row denote significant differences ($P < 0.05$); [c] SD; [d] mg (+)-catechin equivalents/L; [e] mg gallic acid equivalents/L; [f] total antioxidant capacity in mM Trolox equivalents; [g] total antioxidant capacity in mM Trolox equivalents as calculated from the content of monomeric phenolic compounds and their Trolox equivalent antioxidant capacity; [h] unexplained TAC = measured TAC – calculated TAC; [i] chroma; [j] hue angle (°); [k] lightness; [l] red/green chromaticity; [m] yellow/blue chromaticity.

Table 2. Anthocyanin and flavonol content[a] of Pinotage wines produced according to different enological protocols .

	Control	Pumping-over	Oak tannin	Grape tannin	Extended maceration	Oxygenation
Dp-3-glc	7.71 ab[b] (± 1.18)[c]	8.92 ab (± 0.82)	9.49 a (± 1.20)	8.72 ab (± 1.40)	5.29 c (± 0.26)	7.68 b (± 0.80)
Pt-3-glc	15.34 ab (± 2.23)	15.55 ab (± 1.18)	17.50 a (± 1.63)	16.17 a (± 1.82)	13.34 b (± 0.46)	15.05 ab (± 1.58)
Pn-3-glc	2.70 ab (± 0.22)	2.43 bc (± 0.42)	3.24 a (± 0.66)	2.78 ab (± 0.36)	1.78 c (± 0.13)	2.59 ab (± 0.24)
Mv-3-glc	271.92 ab (± 24.44)	254.64 b (± 2.51)	284.75 a (± 13.39)	272.78 ab (± 14.50)	283.75 a (± 5.80)	259.25 b (± 6.68)
Dp-3-glc-ac[d]	2.90 ab (± 0.39)	3.00 a (± 0.35)	3.35 a (± 0.33)	2.92 ab (± 0.43)	2.38 b (± 0.13)	2.75 ab (± 0.38)
Vitisin A[d]	3.86 ab (± 1.13)	2.50 bc (± 0.44)	4.57 a (± 0.87)	3.45 abc (± 0.70)	2.30 c (± 0.62)	4.50 a (± 0.67)
Pt-3-glc-ac[d]	3.10 ab (± 0.29)	3.13 ab (± 0.11)	3.49 a (± 0.28)	2.90 b (± 0.45)	2.94 b (± 0.24)	3.04 ab (± 0.27)
Pn-3-glc-ac[d]	4.79 ab (± 0.78)	4.20 b (± 0.50)	5.00 a (± 0.22)	4.48 ab (± 0.37)	4.03 b (± 0.11)	4.15 b (± 0.34)
Mv-3-glc-ac[d]	94.92 ab (± 7.53)	90.10 b (± 3.21)	100.10 a (± 5.30)	92.12 b (± 3.46)	97.72 ab (± 1.64)	90.55 b (± 2.07)
Mv-3-glc-coum[d]	38.79 a (± 6.28)	44.05 a (± 3.46)	39.26 a (± 5.40)	41.71 a (± 2.14)	39.71 a (± 0.44)	37.74 a (± 3.33)
Monomeric anthocyanins[e]	446.02 ab (± 44.02)	428.52 b (± 6.76)	479.51 a (± 29.69)	448.04 ab (± 25.47)	453.22 ab (± 6.62)	427.29 b (± 6.26)
Coloured polymers[f]	14.61 a (± 4.97)	6.72 b (± 2.16)	14.99 a (± 0.48)	16.74 a (± 5.39)	15.07 a (± 2.91)	13.76 ab (± 5.60)
Unknown flavonol[g]	14.06 a (± 2.95)	14.14 a (± 1.75)	15.29 a (± 2.06)	16.24 a (± 1.73)	9.66 b (± 0.76)	13.00 ab (± 1.35)
Q-3-glc	8.14 ab (± 1.42)	6.98 bc (± 0.90)	8.31 ab (± 0.63)	9.11 a (± 0.75)	6.08 c (± 0.50)	7.60 abc (± 0.60)
Q-3-rham	9.16 a (± 0.82)	6.86 b (± 0.17)	8.54 a (± 0.79)	9.07 a (± 0.70)	8.23 a (± 0.72)	9.06 a (± 0.38)
Quercetin	2.77 a (± 1.12)	1.91 a (± 0.26)	2.04 a (± 0.14)	2.63 a (± 0.78)	1.84 a (± 0.65)	1.70 a (± 1.50)
Total flavonols[f]	35.78 ab (± 6.16)	30.85 bc (± 3.07)	34.63 ab (± 3.66)	38.10 a (± 3.57)	26.47 c (± 3.67)	31.87 abc (± 2.50)

[a] mg/L unless otherwise noted; [b] different letters within a row denote significant differences ($P < 0.05$); [c] SD; [d] mg corresponding anthocyanin-3-glucoside equivalents/L; [e] mg malvidin-3-glucoside equivalents/L; [f] sum of phenolic group content; [g] mg rutin equivalents/L; Dp = delphinidin; gal = galactoside; glc = glucoside; glc-ac = acetylglucoside; glc-coum = *p*-coumaroylglucoside; Pn = peonidin; Pt = petunidin; Mv = malvidin; Q = quercetin; rham = rhamnoside.

Table 3. Phenolic acid, flavan-3-ol and non-coloured polymer content[a] of Pinotage wines produced according to different enological protocols.

	Control	**Pumping-over**	**Oak tannin**	**Grape tannin**	**Extended maceration**	**Oxygenation**
Gallic acid	6.05 c[b] (± 0.29)[c]	5.30 c (± 0.41)	8.06 b (± 0.46)	6.24 c (± 0.70)	12.01 a (± 1.34)	5.65 c (± 0.16)
Caftaric acid	158.93 b (± 12.95)	211.36 a (± 4.16)	156.61 b (± 9.51)	165.28 b (± 3.21)	127.55 c (± 5.28)	169.22 b (± 8.62)
Caffeic acid	0.58 a (± 0.30)	0.66 a (± 0.14)	0.51 a (± 0.27)	0.72 a (± 0.21)	0.48 a (± 0.08)	1.01 a (± 0.57)
Coutaric acid[d]	16.23 b (± 1.97)	23.45 a (± 0.75)	15.96 b (± 1.29)	17.36 b (± 0.70)	12.41 c (± 0.25)	17.82 b (± 1.60)
p-Coumaric acid	1.33 a (± 0.55)	1.47 a (± 0.40)	1.27 a (± 0.39)	0.96 a (± 0.41)	1.25 a (± 0.18)	0.95 a (± 0.45)
Total phenolic acids[e]	183.12 b (± 14.82)	242.24 a (± 4.10)	182.41 b (± 11.08)	190.56 b (± 2.98)	153.70 c (± 5.53)	194.64 b (± 9.89)
(+)-Catechin	7.26 bc (± 0.23)	8.53 a (± 0.26)	7.07 c (± 0.15)	8.59 a (± 0.27)	8.67 a (± 0.35)	7.58 b (± 0.40)
Procyanidin B1	14.78 c (± 0.42)	15.28 bc (± 0.68)	15.19 bc (± 0.91)	16.55 ab (± 0.64)	17.24 a (± 1.30)	15.23 bc (± 0.36)
Total flavan-3-ols[e]	22.04 c (± 0.64)	23.80 bc (± 0.94)	22.26 c (± 1.06)	25.15 ab (± 0.89)	25.91 a (± 1.64)	22.81 c (± 0.76)
Non-coloured polymers[f]	196.77 a (± 50.40)	113.32 b (± 10.21)	217.04 a (± 23.02)	229.01 a (± 52.35)	229.73 a (± 0.94)	186.43 ab (± 63.03)
Total monomers[g]	686.96 a (± 63.37)	725.41 a (± 4.43)	676.69 a (± 68.45)	701.85 a (± 30.06)	659.30 a (± 7.71)	676.61 a (± 11.76)

[a] mg/L unless otherwise noted; [b] different letters within a row denote significant differences ($P < 0.05$); [c] SD; [d] mg *p*-coumaric acid equivalents/L; [e] sum of phenolic group content; [f] mg (+)-catechin equivalents/L; [g] mg rutin equivalents/L; [h] sum of all quantified monomeric phenolic compounds.