Microclimate and Grape Ripeness Effects on the Phenolic Composition of Grapes and Wine (*Vitis vinifera* L. cv. Syrah/101-14 Mgt)

By

Phillipus Petrus Minnaar

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Supervisor: PROF JACOBUS JOHANNES HUNTER

Co-supervisor: PROF ANDRÉ DE VILLIERS

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Summary

The study was aimed at the impact of canopy microclimate by means of grapevine row orientation (GVRO) and grape ripeness levels (GRL) on individual phenolics of *Vitis vinifera* L. cv. Syrah/101-14 Mgt grapes and wine. Grapes were harvested over four consecutive vintages at *ca.* 22°Brix, 24°Brix and 26°Brix GRL and representative of NS, EW, NE-SW and NW-SE GVRO. Wines were made from harvested grapes. Phenolics were quantified in lyophilised grape skin and wine samples. Treatment affects were distinguishable by HPLC and sensory analyses and confirmed by ANOVA, PCA and MFA. Grapes from NE-SW GVRO were highest in anthocyanins, flavonols and flavan-3-ols at *ca.* 22°Brix, whereas those from NW-SE GVRO were highest in anthocyanins, flavonols, flavan-3-ols, and phenolic acids at *ca.* 24°Brix. At a GRL of *ca.* 26°Brix, grapes from NW-SE GVRO were highest in flavonols and phenolic acids, whereas anthocyanins were highest from NS GVRO and flavan-3-ols highest from NS and NE-SW GVRO. Lowest anthocyanins and phenolic acids were in grapes from NW-SE GVRO at *ca.* 22°Brix GRL. Grapes at *ca.* 24°Brix and 26°Brix GRL from NS GVRO were lowest in flavonols. Flavan-3-ols seemed lowest in grapes from NS and NW-SE GVRO at *ca.* 24°Brix and 26°Brix GRL, respectively. At GRL of *ca.* 24°Brix and 26°Brix, lowest anthocyanins and phenolic acids were found for NE-SW GVRO. Wines from NW-SE GVRO had highest anthocyanins at *ca.* 22°Brix and 24°Brix GRL, but wines at *ca.* 26°Brix from EW GVRO had highest anthocyanins. Flavonols, flavan-3-ols and phenolic acids were highest in wines from NE-SW GVRO at *ca.* 22°Brix GRL. At a GRL of *ca.* 24°Brix and 26°Brix, wines from NS and NW-SE GVRO, respectively, were highest in flavonols, flavan-3-ols and phenolic acids. Lowest anthocyanins, flavonols, flavan-3-ols and phenolic acids were found in wines from EW GVRO at *ca.* 22°Brix GRL. Phenolic acids were also lowest in wines from EW GVRO at *ca.* 24°Brix GRL. At *ca.* 24°Brix GRL, lowest anthocyanins were found in wines from NS GVRO, lowest flavonols from NE-SW GVRO and lowest flavan-3-ols from NW-SE GVRO. At *ca.* 26°Brix GRL, lowest anthocyanins, flavonols and flavan-3-ols occurred in wines from NS GVRO and lowest phenolic acids from NE-SW GVRO. Phenolics, sensory attributes, GVRO and GRL were associated with each other. Sensory attribute scores of wines differed among GVRO. Wine quality was associated with NE-SW and NW-SE GVRO. Despite the complexity of impacting factors and different phenolics, results showed the likelihood that a chosen GVRO and GRL may affect wine style. Grapevine row orientation enables a “natural” change in canopy microclimate, leading to grape quality improvements. In practice, a desirable GVRO may not necessarily be applicable to all environments. Management of the fruiting zone remains an option for increasing or decreasing grape exposure, irrespective of GVRO. Further research is needed to understand the relationships of vine phenology, light intensity, temperature and GVRO with grape and wine phenolic profiles and wine quality. Phenolic concentration differences in wines in this study and association thereof with GVRO is important in oenology, because phenolics can be affected by vineyard practices, which may further lead to a desired wine style. However, phenolics of grapes and ultimately of wine, are affected by multiple factors, e.g. climate, grape cultivar, viticultural practices, GRL and berry size, all of which must be considered when a specific wine style is intended.

Opsomming

Die studie was gemik op die invloed van lowermikroklimaat d.m.v. wingerdryrigtings (WR) en druifrypheidsgraad (DRG) op fenole van *Vitis vinifera* L. cv. Syrah/101-14 Mgt druiwe en wyn. Die druiwe was oor vier opeenvolgende seisoene by drie DRG, naamlik *ca*. 22°Brix, 24°Brix en. 26°Brix, geoes en wyn was gemaak. Die druiwe het NS, OW, NO-SW en NW-SO, WR verteenwoordig. Wingerryrigting verskille was onderskeibaar deur HPLC en sensoriese analises en bevestig deur ANOVA, PCA en MFA. Druiwe van NO-SW WR was die hoogste in antosianiene, flavonole en flavan-3-ole by *ca.* 22°Brix, terwyl dié van NW-SO WR die hoogste in antosianiene, flavonole, flavan-3-ole en fenoliese sure by *ca.* 24°Brix was. By ’n DRG van *ca.* 26°Brix, was druiwe van NW-SO WR die hoogste in flavonole en fenoliese sure, terwyl antosianiene die hoogste was in NS WR en flavan-3-ole die hoogste in NS en NO-SW. WR. Laagste antosianiene en fenoliese sure was in druiwe van NW-SO WR by *ca*. 22°Brix gevind. Druiwe van NS WR, by *ca*. 24°Brix en 26°Brix, was laagste in flavonole. Flavan-3-ole was oënskynlik laagste in druiwe van NS en NW-SO WR by onderskeidelik *ca*. 24°Brix en 26°Brix. By DRG van *ca.* 24°Brix en 26°Brix was die laagste antosianiene en fenoliese sure in NO-SW WR gevind.

Wyne van NW-SO WR het die hoogste antosianiene by DRG van *ca.* 22°Brix en 24°Brix gehad, maar by *ca.* 26°Brix, het OW WR die hoogste getoon. Flavonole, flavan-3-ole en fenoliese sure was die hoogste in wyne van NO-SW WR by ’n DRG van *ca.* 22°Brix. By DRG van *ca.* 24°Brix en 26°Brix was wyne van onderskeidelik NS en NW-SO WR, hoogste in flavonole, flavan-3-ole en fenoliese sure. Laagste antosianiene, flavonole, flavan-3-ole en fenoliese sure het in wyne van die OW WR by ’n DRG van *ca.* 22°Brix voorgekom. Fenoliese sure was ook laagste in wyne van OW WR by ’n DRG van *ca.* 24°Brix. By laasgenoemde DRG was die laagste antosianiene in wyne van NS WR, terwyl laagste flavonole in dié van NO-SW WR en laagste flavan-3-ole in dié van NW-SO WR gevind. By *ca.* 26°Brix DRG het die laagste antosianiene, flavonole en flavan-3-ole in wyne van NS WR en die laagste fenoliese sure in NO-SW WR voorgekom.

Fenole, sensoriese eienskappe, WR en DRG was met mekaar geassosieerd. Sensoriese eienskap punte van wyne het tussen WR verskil. Wyngehalte was met NO-SW en NW-SO WR geassosieerd. Ten spyte van die kompleksiteit van impakfaktore en verskillende fenole, het die resultate die waarskynlikheid getoon dat ’n gekose WR en DRG wynstyl kan beïnvloed. Wingerdryrigting bring ’n “natuurlike” verandering in lowermikroklimaat teweeg, wat lei tot druifgehalte verbeterings. In praktyk is ’n gewenste WR nie noodwendig vir alle omgewings toepaslik nie. Bestuur van drasone bly ’n opsie vir verhoging of vermindering van druifblootstelling, ongeag die WR. Verdere navorsing is nodig om die verband tussen wingerdfenologie, ligintensiteit, temperatuur en WR en druif- en wyn fenool profiele en wyngehalte te verstaan. Fenoliese verskille in wyne van hierdie studie en assosiasie daarvan met WR is belangrik in wynkunde omdat fenole deur wingerdpraktyke beïnvloed kan word en verder tot ’n gewenste wynstyl kan lei. Die fenoolinhoud van druiwe en uiteindelik die van wyn word egter ook deur faktore soos, klimaat, druifkultivar, wingerdpraktyke, DRG en korrelgrootte beinvloed, wat oorweeg moet word wanneer ’n spesifieke wynstyl beoog word.

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Preface

This thesis is presented as a compilation of six chapters. Each chapter is introduced separately.

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Microclimate and grape ripeness effects on the phenolic composition of grapes and wine (*Vitis vinifera* L. cv. Syrah/101-14 Mgt).

Chapter 2 Literature review

A brief overview of phenolics related to grapes and wine is presented, including a discussion of grapevine growth, grape microclimate, grape phenolic compounds (flavonoids and non-flavonoids) and wine quality.

Chapter 3 Chromatographic methodology

Methodology to Quantify Selected Anthocyanins, Flavonols, Flavan-3-ols and Phenolic Acids in Syrah (*Vitis vinifera* L. cv.) Grapes and Wine Using RP-HPLC-DAD.

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Chapter 1

INTRODUCTION AND STUDY AIMS

1. Introduction and Aims of the study

Microclimate and grape ripeness effects on the phenolic composition of grapes and wine (*Vitis vinifera* L. cv. Syrah/101-14 Mgt)

1.1 Introduction

Wines that are produced from South Africa's red wine grape varieties compete on overseas markets and are ranked among the best in the world (SAWIS, 2013). Climatic diversity, which characterises the South African grape growing regions, particularly in the Western Cape Province, can lead to different wine styles. In addition, soil variation and topographic complexity complicate grape growing, but also increase the diversity of wine styles (Conradie *et al.*, 2002). Long- and short-term vineyard practices need to be adapted to these *terroir*-related factors for successful vine cultivation (Hunter *et al*., 2010). Microclimatic conditions in the grapevine canopy and grape ripening are also affected by topographic complexities (Hunter *et al*., 2007).

Researchers continue to investigate the flavonoid and non-flavonoid phenolic compounds of plants for their medicinal properties and their ability to contribute to the defence against cancer, cardiovascular diseases, certain pathological disorders of gastric- and duodenal ulcers, allergies, vascular fragility, and viral- and bacterial infections (Habauzit & Morand, 2011; Lorrain *et al.*, 2013; Ivanova-Petropulos *et al*., 2015; Lingua *et al.*, 2016). The concentrations of these compounds in a variety of matrices, such as fruit, vegetables and beverages are therefore of significant importance (Andersen & Markham, 2007; Vermerris & Nicholson, 2008). Anthocyanins, flavan-3-ols, flavonols, and phenolic acids form part of the berry phenolic profile of *Vitis* spp (Ribéreau-Gayon *et al.*, 2006). Environmental factors (Andrades & González-San José, 1995; Brossaud *et al.*, 1998; Bergqvist *et al*., 2001; Guidoni *et al.*, 2008; Chorti *et al*., 2010) and certain vineyard treatments (Hunter *et al*., 1991; Price *et al*., 1995; Haselgrove *et al.*, 2000; Downey *et al*., 2004; Mori *et al.*, 2005; Joscelyne *et al*., 2007; Ristic *et al*., 2007; Kocsis *et al*., 2008; Tarara *et al.*, 2008; Rustioni *et al*., 2011) can change the concentrations of these compounds in grape berries (Spayd *et al.*, 2002; Pérez-Magariño & González-San José, 2006; Mattivi *et al*., 2009; Ristic *et al.*, 2010). The importance of phenolics in grapes and ultimately in wine, lends itself to an investigation into the effect of climatic and vineyard practices, such as vineyard row orientation and grape ripeness levels, on the flavonoid and non-flavonoid phenolic concentrations of grapes.

Different groups of flavonoid and non-flavonoid phenolic compounds are present in grapes and wine (Andersen & Markham, 2007). They include the phenolic acids, flavonols, flavan-3-ols, and anthocyanins.

These compounds are collectively known as phenolics or polyphenols (Ribéreau-Gayon *et al.*, 2006). Phenolic/polyphenol compounds are constituents of grapes and wine and impact on sensory attributes, such as colour, mouth feel, body, taste, astringency, and wine stability (Gawel, 1998, Ribéreau-Gayon *et al.*, 2006). Vineyard practices and the timing of harvesting can be useful tools by which grape phenolic concentrations can be changed (Kocsis *et al.*, 2008; Friedel *et al.*, 2012). Knowledge on the variation in individual phenolic concentrations in grapes subjected to different vineyard row orientations, which affect canopy microclimate, and harvested at different ripeness levels, can be applied to optimise decision-making regarding the selection of viticultural and oenological practices to produce wines of desired characteristics. However, the extent to which individual phenolic concentrations in grapes and wines are affected by the combined effect of microclimate and grape ripeness level has not yet been investigated under South African conditions. Therefore, in this study, the focus was to obtain information regarding the manner in which vineyard microclimate, as brought about specifically by vineyard row orientation, and grape ripeness levels affect phenolic concentrations in South African Syrah grapes and wine.

1.2 Hypothesis and aims

The study aimed to investigate individual phenolic compound concentrations of Syrah (*Vitis vinifera* L. cv. Syrah/101-14 Mgt) grapes and wine, in relation to canopy microclimate and grape ripeness levels. It was postulated that grapevine microclimate and grape ripeness levels impact on the individual phenolic compound concentrations. The aims of the study were to provide answers on the following main questions:

a. How do vineyard row orientation (microclimate) and grape ripeness levels affect the individual and total phenolic compound concentrations in grapes and wine?
b. At what stage of grape ripeness are the individual phenolic concentrations highest or optimal?
c. Do the concentrations of phenolic compounds, individually and collectively, relate to grape ripeness levels, *i.e*. total soluble solids (TSS), pH, and total acidity (TA)?

1.3 Description of key concepts

In this section, the key concepts that appear in the title of this thesis will be described briefly. The concepts include microclimate, grape ripeness, and phenolic composition.

1.3.1 Microclimate

Microclimate refers to the immediate environment surrounding the grapes, which is affected by macro- and meso-climate as well as viticulture practices (Hunter, 2000; Hunter *et al*., 2004). Environmental factors such as topography, agro-pedology, and microclimate are parameters affecting grape composition and wine quality (Dokoozlian *et al.*, 1996; Ferrer-Gallego *et al.*, 2012).

The precise dependence of the major grape attributes on the environment is however still unclear (Koundouras *et al.*, 2006). Suitable sites for viticulture are important and can enhance and improve complete grape ripening of appropriately chosen grape varieties by creating favourable microclimatic conditions and adequate vine vigour through the control of soil fertility and water status (Koundouras *et al*., 2006).

1.3.2 Grape ripeness

The ripening of grapes entails a range of physical and physiological/biochemical processes that begin with véraison (change of berry colour) and culminate in berry maturity. These changes are necessary in order for grapes to reach a condition/level of maturity suitable for their transformation into wine (Winkler *et al.*, 1974). Chemical and physical changes during grape ripening do not occur simultaneously. They include skin softening as well as development and evolution of chemical constituents in relation to the impact of plant genetics (Czemmel *et al*., 2009), *terroir* (Castillo-Muñoz *et al.*, 2007) and viticultural practices (Pérez-Magariño & González-San José, 2004). The process of grape ripening is a determining factor in the quality of grapes (Hamilton & Coombe, 1992; Coombe & McCarthy, 2000; Pérez-Magariño & González-San José, 2004; Hunter *et al*., 2007; Li *et al*., 2013).

1.3.3 Phenolic composition

Phenolic or polyphenol composition refers to the anthocyanin, flavan-3-ol, flavonol, and phenolic acid concentrations that occur in grapes and wine. The phenolic compound concentrations of grapes, which include both flavonoids and non-flavonoids, are affected by grape cultivar, viticulture practices, and *terroir* (Rustioni *et al*., 2011).

1.4 Preview of thesis chapters

Chapter 2 comprises a **Literature Review**, which guides and underpins this research. The literature is a brief overview of phenolics related to grapes and wine, including a discussion of grapevine growth, grape microclimate, grape phenolic components (flavonoids and non-flavonoids) and wine quality.

Chapter 3 reports the **Chromatographic Methodology** used for the analysis of phenolics in grape and wine samples by means of liquid chromatography. Chromatographic results for the quantitative analysis of anthocyanins, flavonols, phenolic acids, and flavan-3-ols are presented. This chapter is written in the form of a manuscript, titled: **Methodology to Quantify Selected Anthocyanins, Flavonols, Flavan-3-ols and Phenolic Acids in Syrah (*Vitis vinifera* L. cv.) Grapes and Wines Using RP-HPLC-DAD.**

Chapter 4 presents **Research Results I** for the quantitative analysis of anthocyanins, flavonols, phenolic acids, and flavan-3-ols in grape skin samples. This chapter is written in the form of a manuscript, titled: **Impact of Microclimate (Row Orientation) and Ripeness Levels on Selected Anthocyanins, Flavan-3-ols, Flavonols and Phenolic Acids in *Vitis vinifera* L. cv. Syrah Grapes.**

Chapter 5 presents **Research Results II** for the quantitative analysis of anthocyanins, flavonols, phenolic acids, and flavan-3-ols in wine samples. This chapter is written in the form of a manuscript, titled: **Impact of Microclimate (Row Orientation) and Grape Ripeness Levels on Selected Anthocyanins, Flavan-3-ols, Flavonols, Phenolic Acids and Sensory Attributes of *Vitis vinifera* cv. Syrah Wines.**

Chapter 6 comprises the **General Discussion and Conclusions**.

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Chapter 2

LITERATURE REVIEW

2. Literature Review

A brief overview of phenolics related to grapes and wine is presented, including a discussion of grapevine growth, grape microclimate, grape phenolic compounds (flavonoids and non-flavonoids) and wine quality

2.1 Phenols

A phenol is a simple aromatic compound with chemical formula C_6H_5OH and contains a hydroxyl group (-OH) bonded to an aromatic benzene ring (Margalit, 2004). The hydrogen of the phenolic hydroxyl is labile due to the aromatic ring, which renders it a weak acid. The physical and chemical properties of phenols are also affected by their delocalised electrons, which can be shared between the oxygen atom and the benzene ring. Phenolic compounds are responsible for some of the most important attributes of wine quality (Singleton, 1980). Their chemical behaviour during winemaking is affected by many factors. Wine phenols are continuously altered due to chemical reactions in the wine medium. An overview of current knowledge on phenolics related to grapes and wine will be presented in this chapter.

2.2 Grape and wine phenolics

Phenolics are a group of chemical substances found in plants and are characterised by the presence of more than one phenol unit or building block per molecule (Margalit, 2004). There are three major groups of phenolics related to wine and winemaking (Singleton, 1980). The first group, denoted as $C_6 – C_1$, *i.e.* a phenolic ring plus one carbon, represents the benzoic acids. The second group, $C_6 – C_3$, represents an aromatic ring plus a 3-carbon chain (the cinnamic acids), and the third group, $C_6 – C_3 – C_6$, represents two aromatic rings connected by a 3-carbon chain or ring. The $C_6 – C_1$ and $C_6 – C_3$ groups are known as the *non-flavonoid* phenolics to distinguish them from the $C_6 – C_3 – C_6$ group, which comprises the *flavonoids* (Margalit, 2004; Vermerris & Nicholson, 2008; Andersen & Markham, 2007).

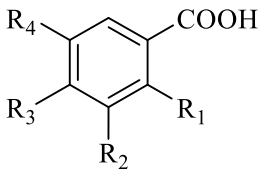
2.3 Non-flavonoids

Non-flavonoid phenols are present in red and white grape juice and wine (Margalit, 2004; Andersen & Markham, 2007; Vermerris & Nicholson, 2008). There are two groups of non-flavonoids related to grapes.

They contain the benzoic- and cinnamic acid systems (Ribéreau-Gayon *et al*., 2006). These phenolic acids are mainly present in the grape berry pulp and occur as glycosides and in combination with other compounds, such as flavonoids (Ribéreau-Gayon *et al*., 2006). Phenolic acid glycosides are released by acid hydrolysis and phenolic acid esters are released by alkaline hydrolysis (Cheynier *et al.*, 2003). The free forms of phenolic acids are more prevalent in red wine, due to the hydrolysis of their derivatives and thermal breakdown reactions involving complex molecules, especially anthocyanins. Phenolic acids are also involved in the formation of anthocyanin-derived pigments during bottle aging of wine (Schwarz *et al*., 2005). These low molecular weight compounds are referred to as pyrano-anthocyanins. Benzoic acids, such as gallic- and *p*-hydroxybenzoic acids, are also responsible for the stabilisation of anthocyanins in young red wines through co-pigmentation (Boulton, 2001; Friedel *et al*., 2012).

2.3.1 Benzoic acid group ($C_6 - C_1$)

Benzoic acids are characterised by the presence of a carboxylic acid group substituted on a phenol (Margalit, 2004; Ribéreau-Gayon *et al.*, 2006). Examples include *p*-hydroxybenzoic acid, protocatechuic acid (*p*-pyrocatechuic acid), gallic acid, vanillic acid, and syringic acid (Fig. 2.1). This group also includes the derivatives of *ortho*-hydroxybenzoic acid, which includes *o*-pyrocatechuic acid, salicylic acid and gentisic acid (Andersen & Markham, 2007). The content of phenolic acids in grape must and wine varies in a range of concentrations (Lee & Jaworsk, 1990). The main sources of gallic acid in wine are grape seeds (during maceration) and oak barrels (during wine maturation) (Jackson, 2000; Margalit, 2004). Gallic acid is present in seeds in its free form as well as esterified to proanthocyanidin polymers. As gallic acid is also present in grape stems, its concentration in wine increases following whole bunch fermentations (Jackson, 2000). The concentration of this compound in wine ranges from 5 mg/L to 100 mg/L, with concentrations in the upper end of this range usually associated with the use of new oak barrels. Syringic acid is a naturally occurring *O*-methylated trihydroxybenzoic acid (Vrhovsek, 1998). It is also present in grapes and is released by the breakdown of the compound malvidin during fermentation.



Benzoic acids	**R_1**	**R_2**	**R_3**	**R_4**
p-Hydroxy benzoic acid	H	H	OH	H
Protocatechuic acid	H	OH	OH	H
Vanillic acid	H	OCH_3	OH	H
Gallic acid	H	OH	OH	OH
Syringic acid	H	OCH_3	OH	OCH_3
Salicylic acid	OH	H	H	H
Gentisic acid	OH	H	H	OH

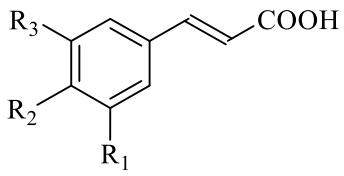
Figure 2.1 Examples of the most common benzoic acids found in grapes and wine (Monagas *et al*., 2005).

2.3.2 Cinnamic acid group ($C_6 – C_3$)

There are four common cinnamic acids in wine, namely *p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid (Fig. 2.2). Cinnamic acids (hydroxycinnamic acids) are an important group of non-flavonoid phenolics of wine for they are responsible for the browning of white grape must (Ribéreau-Gayon et al., 2006). The hydroxycinnamic acids are in the *trans* or *cis* form with regards to the cinnamic acid double bond (Margalit, 2004).

In grape samples, the benzoic acids are commonly present as glycosides or esters (Singleton *et al.*, 1986). Esters are formed with tartaric acid, then called caftaric acid (caffeoyl tartaric acid), fertaric acid (feruloyl tartaric acid) and coutaric acid (coumaroyl tartaric acid).

Cinnamic acids also combine with anthocyanin glucosides to form acylated anthocyanins *via* the esterification of mainly caffeic acid and *p*-coumaric acid on the glycosidic group (Vrhovsek, 1998). Fertaric acid is present in grapes and wine as an ester, formed from ferulic acid bound to tartaric acid (Ribéreau-Gayon et al., 2006). Caftaric acid forms during caffeic acid and tartaric acid esterification. Caftaric acid is responsible for the yellowish-gold colour of some white wines. Winemakers measure caftaric acid concentration as a method to estimate the levels of oxidation in wine (Ribéreau-Gayon et al., 2006). Coutaric acid is a hydroxycinnamoyl tartaric acid present in wine pomace (Maier *et al.*, 2006) and grapes (Singleton *et al.*, 1986). It is an ester formed from coumaric acid and tartaric acid. There are three isomers of coutaric acid, i.e. *o*-coumaric acid, *meta*-coumaric acid and *para*-coumaric acid. The *p*-coumaric acid is the most abundant isomer in nature (Vermerris & Nicholson, 2008).

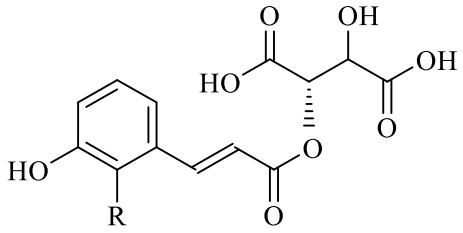


Cinnamic acids	**R_2**	**R_3**	**R_4**	**R_5**
p-Coumaric acid	H	H	OH	H
Caffeic acid	H	OH	OH	H
Ferulic acid	H	OCH_3	OH	H
Sinapic acid	H	OCH_3	OH	OCH_3

Figure 2.2 Examples of the most common hydroxycinnamic acids in grapes and wine (Monagas *et al.*, 2005).

The common hydroxycinnamic acid esters that occur in grapes and wine are listed in Fig. 2.3. Hydroxycinnamic acids in the *trans* form with regard to the cinnamic acid double bond are found in grapes and wine (Cheynier *et al.*, 1989; Rodriguez *et al.*, 2006). The hydroxycinnamic acid derivatives listed in Fig. 2.3 are rarely present in their acidic form (Ribéreau-Gayon *et al.*, 2006). These derivatives combine through an esteric bond to alcohols or sugar molecules. The four most abundant hydroxycinnamic acid esters are *trans*-caftaric acid, *cis*- and *trans*-coutaric acid, and *trans*-fertaric acid (Vrhovsek *et al.*, 1998; Ribéreau-Gayon et al., 2006).

Examples are coutaric acid [*p*-coumaroyl tartaric acid], caftaric acid [caffeoyl tartaric acid] and fertaric acid [feruloyl tartaric acid] (Ong & Nagel, 1978; Margalit, 2004).



Hydroxycinnamic esters	**R**
trans-caffeoyltartaric acid (caftaric acid)	OH
trans-*p*-coumaroyltartaric acid (coutaric acid)	H
trans-feruloyltartaric acid (fertaric acid)	OCH_3

Figure 2.3 Examples of the most common hydroxycinnamic esters found in grapes and wine (Monagas *et al.*, 2005).

2.4 Flavonoids

Flavonoids are synthesized by the phenylpropanoid metabolic pathway (Fig. 2.4) in which the amino acid phenylalanine (and also tyrosine) is used to produce 4-coumaroyl-CoA (Andersen & Markham, 2007). Coumaroyl-CoA is an important enzyme in the flavonoid and stilbenoid biosynthesis pathways of plants (Vermerris & Nicholson, 2008). It combines with malonyl-CoA to yield the backbone of flavonoids, a group of compounds called chalcones. The latter contain two phenyl rings. Conjugated ring-closure of chalcones results in the familiar form of flavonoids, the three-ringed structure of a flavone. The metabolic pathway continues through a series of enzymatic modifications to yield flavanones, dihydroflavonols, and anthocyanins (Fig. 2.4).

The term flavonoid refers to a class of plant phenolics that consist of the $C_6 – C_3 – C_6$ backbone, *i.e.* the phenylbenzopyran functionality (Andersen & Markham, 2007; Vermerris & Nicholson, 2008). The flavonoid structure is based on two aromatic rings, A and B (Fig. 2.4), which are connected by a three-carbon chain. The latter is in most cases closed by oxygen; forming a heterocyclic ring (C-ring), (the exceptions are chalcones, which contain an open three-carbon chain). The A-ring originates from the condensation of three malonyl-CoA molecules and the B-ring from *p*-coumaroyl-CoA (Chemler *et al*., 2005). The A-ring is dihydroxylated at carbons 5 and 7, while the B-ring is mono-hydroxylated, *o*-dihydroxylated or *vic*-trihydroxylated; the B-ring can also be methoxylated.

Flavonoids commonly exist in nature as glycosides and in some cases in polymerised form (Andersen & Markham, 2007). The C-ring is most commonly glycosylated at position 3. Furthermore, glycosylated flavonoids can be esterified with a range of organic and phenolic acids to form acylated derivatives. In addition to these variations, their diversity stems from the different substitution patterns of the flavonoid backbone.

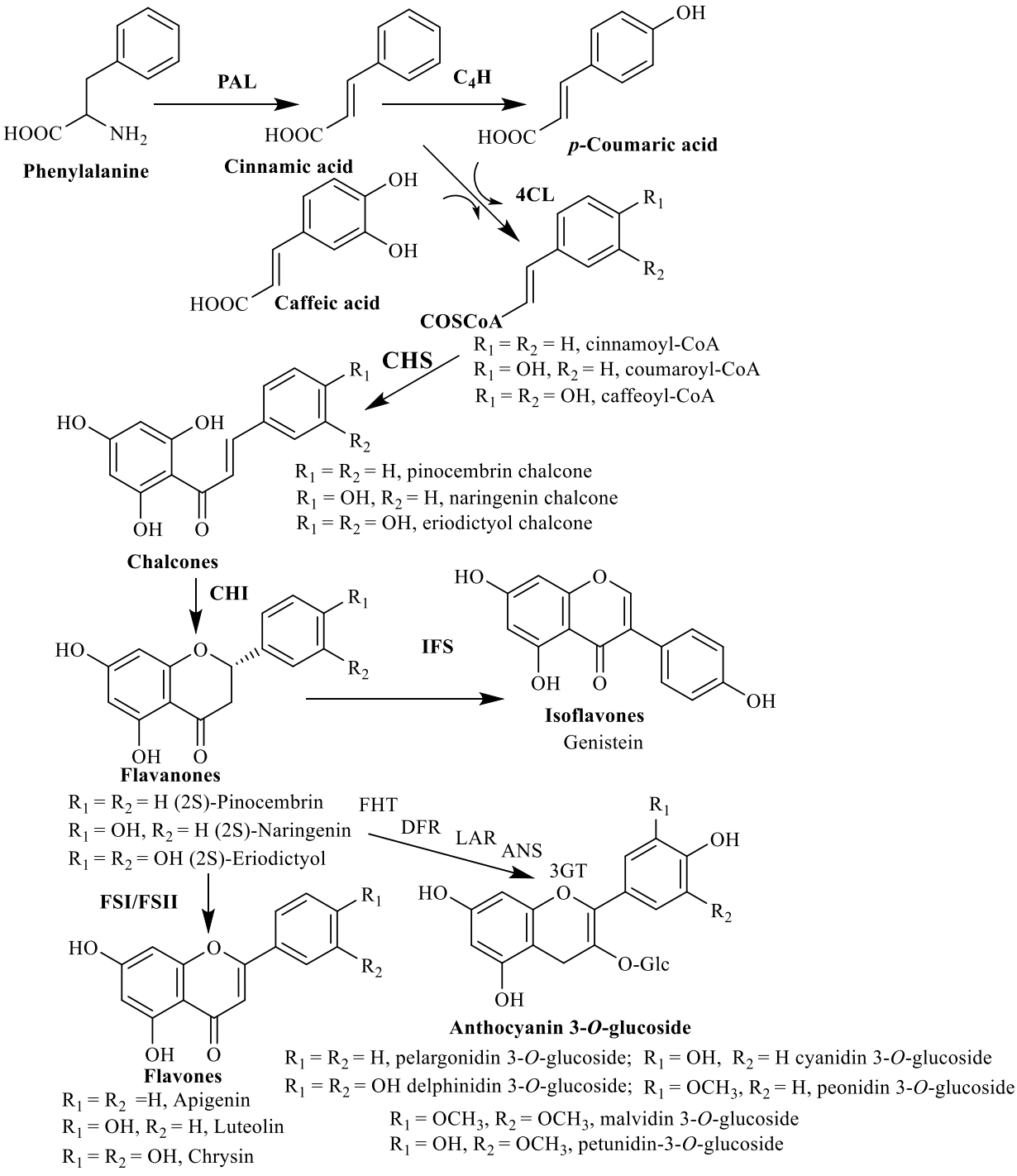


Figure 2.4 The general phenylpropanoid and flavonoid biosynthesis pathways (Chemler *et al.*, 2005).

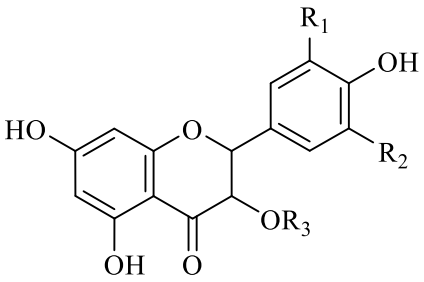
PAL = Phenylalanine ammonia-lyase, C4H = Cytochrome P450 Cinnamate-4-hydroxylase, 4CL = 4-Coumaroyl CoA-ligase, CHS = Chalcone synthase, CHI = Chalcone isomerase, FSI = Cytochrome P450 Flavone synthase, IFSII = Cytochrome P450 Flavone synthase II, IFS = Cytochrome P450 Isoflavone synthase, FHT = Flavanone 3 β-hydroxylase, DFR = Dihydroflavonol 4-reductase, LAR = Leucoanthocyanidin reductase, ANS = Anthocyanidin synthase (also known as leuco-anthocyanidin dioxygenase), 3GT = UDP flavonoid 3-*O*-glucosyltransferase.

The differentiation between flavonoids is derived from the degree of oxidation and saturation present in the central heterocyclic ring (C-ring), which classifies the different flavonoid groups: flavan-3-ols, flavan-3,4-diols, flavanones, flavanonols, flavones, flavonols, dihydroflavonols and anthocyanins (Margalit, 2004; Vermerris & Nicholson, 2008). Three predominant groups of flavonoids are present in grapes, *i.e.* flavanols, flavonols, and anthocyanins. Flavonoids are located in the grape skins, seeds and stems (Ribéreau-Gayon *et al*., 2006). Anthocyanins, which are present in berry skins, are largely responsible for the colour of grapes and ultimately the colour of young wines. Flavan-3-ols, including monomeric catechins and epicatechins as well as proanthocyanidins, are responsible for wine astringency, perceived bitterness and structure (Singleton & Esau, 1969; Gawel, 1998; Cadot *et al*., 2006).

2.4.1 Flavonols

Flavonols are a class of flavonoids that have the 3-hydroxyflavone backbone, *i.e.* IUPAC name 3-hydroxy-2-phenylchromen-4-one (Margalit, 2004). Their diversity originates from different positions of hydroxyl and methoxyl groups and the wide variety of glycosylated forms (Fig. 2.5). Flavonols are one of the most widespread families of phenolics present in grape skin, pulp, leaves of both red and white cultivars, and provide protection against ultra-violet radiation (Downey *et al*., 2006; Ribéreau-Gayon *et al*., 2006). In grapes, these molecules are present as glycosides (Andersen & Markham, 2007), whereas in wine they occur as aglycones (without the sugar moiety). The glycosides are mostly enzymatically hydrolysed during fermentation or hydrolysed through acid or alkaline hydrolysis (Williams *et al*., 1996; Zoecklein *et al*., 1997; Cordero-Ortero *et al*., 2003). The major flavonols in red grapes are kaempferol (4'-hydroxy), quercetin (3',4'-dihydroxy) and myricetin (3',4',5'-trihydroxy) derivatives (Fig. 2.5) (Margalit, 2004). The most abundant flavonol in grapes is 3-glycoside of quercetin that is dihydroxylated on the B-ring in its 3-glucoside and 3-glucuronide form (Downey *et al*., 2003a). Isorhamnetin, the 3'-methoxylated derivative of quercetin, is also present in red grapes and wine, as are the methoxyl-derivatives laricitrin and syringetin (Figure 2.5). Laricitrin (3'-*O*-methylmyricetin) is formed from myricetin by the action of the enzyme myricetin *O*-methyl transferase, and is further methylated by laricitrin 5'-*O*-methyltransferase to form syringetin. Glucose is one of the sugars attached at the 3-carbon position on the C-ring of kaempferol and isorhamnetin (Andersen & Markham, 2007). Quercetin and myricetin have glucuronide, rhamnoside, glucoside, and rutinoside as common sugars attached at position 3 of the C-ring. Quercetin is present in grapes as 3-rhamnosylglucose (rutin), 3-glucosylgalactose, and 3-glucosylxyloside (Andersen & Markham, 2007; Castillo-Muñoz *et al*., 2007; Li *et al*., 2011; Lambert *et al*., 2015). Kaempferol-glycosides include the 3-rhamnoside, 3-glucoside, 3-gluco-arabinoside, 3-glucuronide and 3-galactoside derivatives (Li *et al*., 2011; Zhu *et al*., 2012). Their presence is based on retention times, UV-visible spectra, and ESI-MS/MS *m/z* values (molecular ion [MS]; product ions [MS^2]). Laricitrin glycosides present in red grapes include 3-galactose and 3-glucoside (Li *et al*., 2011).

Flavonols contribute directly to the colour of white wines, but in red wines, their colour is masked by anthocyanins (Price *et al.*, 1995). The flavonols, quercetin, laricitrin, myricetin, kaempferol, isorhamnetin and their glycosides, contribute to the phenomenon of haze formation in white wine and perceived bitterness in red wine (Ribéreau-Gayon *et al*., 2006). They are also involved in the phenomenon of co-pigmentation in red wines (Ribéreau-Gayon *et al*., 2006), where the formation of co-pigmentation complexes between anthocyanins and flavonols causes an enhancement of the extraction of anthocyanins during winemaking. Co-pigmented complexes reflect a more intense red colour, together with a bathochromic spectral band shift to purplish hues of the red colour (Vermerris & Nicholson, 2008).



[1]Gal = Galactoside; [2]Glc = Glucoside; [3]Gluc = Glucuronide,

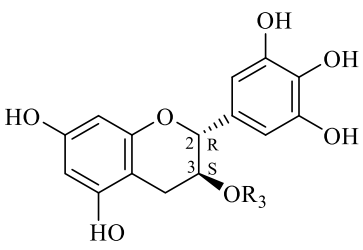
Flavonols	**R_1**	**R_2**	**R_3**
Kaempferol	H	H	H
Kaempferol 3-*O*-glucoside	H	H	Glc[1]
Kaempferol 3-*O*-galactoside	H	H	Gal[2]
Kaempferol 3-*O*-glucuronide	H	H	Gluc[3]
Quercetin	OH	H	H
Quercetin 3-*O*-glucoside	OH	H	Glc
Quercetin 3-*O*-glucuronide	OH	H	Gluc
Myricetin	OH	OH	H
Myricetin 3-*O*-glucoside	OH	OH	Glc
Myricetin 3-*O*- glucuronide	OH	OH	Gluc
Isorhamnetin	OCH_3	H	H
Isorhamnetin 3-*O*-glucoside	OCH_3	H	Glc
Laricitrin	OCH_3	OH	OH
Laricitrin 3-*O*-galactoside	OCH_3	OH	Gal
Syringetin	OCH_3	OCH_3	H
Syringetin 3-*O*-galactoside	OCH_3	OCH_3	Gal
Hyperoside	OH	H	OH
Morin	H	H	OH

Figure 2.5 Examples of grape and wine flavonols (Monagas *et al.*, 2005).

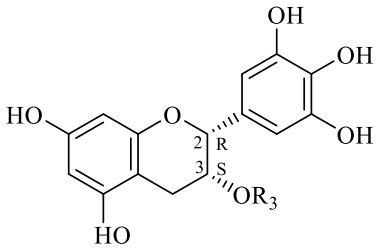
The levels of flavonols in grape berries, as with flavan-3-ols, depend on the developmental stages of the grape berry, plant genetics and environmental factors (Downey *et al.*, 2003b). Flavonol biosynthesis first occurs during flowering and again at véraison. A constant increase in flavonol levels per berry occurs during grape ripening. Castillo-Muñoz *et al*. (2007) suggested that the branch of the flavonoid pathway leading to flavonol biosynthesis is light dependent.

2.4.2 Flavan-3-ols

Flavan-3-ols contain a 2-phenyl-3,4-dihydro-2H-chromen-3-ol skeleton (Fig. 2.6). These compounds include (+)-catechins, (-)-epicatechins, epigallocatechins, epigallocatechin gallates and proanthocyanidins (procyanidins and prodelphinidins). The principal monomers are (+)-catechin, (-)-epicatechin and (-)-epicatechin 3-*O*-gallate (Su & Singleton, 1969). Catechin and epicatechin are epimers with (-)-epicatechin and (+)-catechin being the common optical isomers present in nature (Andersen & Markham, 2007). Catechins have different stereoisomers with respect to asymmetric carbons 2 and 3 in the C-ring (Fig. 2.6), with four stereoisomers for monomeric catechins (Margalit, 2004; Vermerris & Nicholson, 2008): (+)-catechin [2R-3S], (-)-epicatechin [2R-3R], (-)-catechin [2S-3R] and (+)-epicatechin [ES-3S]. The R and S positions refer to carbons 2 and 3 on the C-ring, which refer to the absolute configuration of the B-ring and OH, respectively. In figure 2.6, the S refers to the D configuration of OH (above ring plane) and the R refers to the L configuration, *i.e.* below the ring plane (Margalit, 2004; Vermerris & Nicholson, 2008). The B-ring on carbon 2 and the hydroxyl group on carbon 3 form four conformations with respect to the C-ring plane (Vermerris & Nicholson, 2008). The isomers 2S-3R and 2S-3S are rare in nature, whereas the 2R-3S and 2R-3R are more abundant. The two conformations are written as (*d*+) catechin for 2R-3S and (*l*-) epicatechin for 2R-3R, respectively. The (+) and (-) indicate the orientation of polarised light deflection (left or right), respectively (Vermerris & Nicholson, 2008). These conformations are also valid for flavanones and flavanes, which have a single bond between carbons 2 and 3 of the C-ring. The relative distribution of (*d*+) catechin and (*l*-) epicatechin varies in plants. They are present in roughly equal proportions in grapes. Catechin (+)-and (-)-epicatechin are orthohydroxylated at position 3' and 4' of the B ring, while (+)-gallocatechin and (-)-epigallocatechin possess a third hydroxyl group at position 5'. These flavan-3-ols are present in grapes as free aglycones or polymers of aglycones. In flavan-3-ol classes, further diversity results in modification of their three-ringed skeleton (Vermerris & Nicholson, 2008).



(+)-Catechin



(-)-Epicatechin

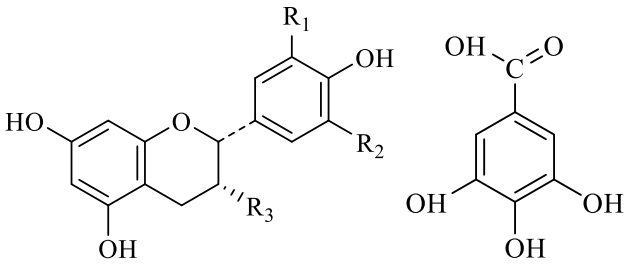
Figure 2.6 The most common catechin isomers (Monagas *et al*., 2005).

Modification includes hydroxylation, methylation of the phenolic hydroxyls, acylation of the alcoholic hydroxyl groups and polymerisation. Flavan-3-ols represent the largest class of naturally occurring monomeric flavonoids in grapes and wine (Andersen & Markham, 2007). They are the precursors and building blocks for tannins, are present in the skin, stems, and seeds of red grape cultivars (*Vitis vinifera* spp), and are extracted during the maceration process of winemaking (Andersen & Markham, 2007). These compounds can also be introduced into wine by the use of oak barrels and oak chips or with the addition of tannin powder (Del Alamo-Sanza 2004; Ribéreau-Gayon *et al.,* 2006; Andersen & Markham, 2007; Mattivi *et al*., 2009).

Flavan-3-ols can be an essential positive contribute to wine quality (Su & Singleton, 1969; Boselli *et al*., 2006; Boido *et al*., 2011; Gil *et al*., 2015) through their effect on wine texture, mouth feel, and body. They are also responsible for the stability of wines because they can form polymers with anthocyanins to produce stable pigments (Noble, 1980; Boulton, 2001; Lorenzo *et al*., 2005; He *et al*., 2012; Gil-Muñoz *et al.,* 2010). Flavan-3-ols can however have a negative effect on white wine quality, since they are responsible for the oxidative browning, haze and precipitation of white wines. The concentrations of catechins in grapes vary among grape cultivars and a grape cultivar such as Pinot noir contains higher concentrations of catechins, compared to grape cultivars such as Merlot and Syrah (Ojeda *et al.,* 2002; Cortell *et al.,* 2007).

Grape skin extracts have high concentrations of monomers and low molecular weight oligomers (mean degree of polymerization < 8). Grape skin extracts contain four monomers, *i.e.* (+)-catechin, (-)-epicatechin, gallocatechin and epigallocatechin as well as procyanidin and prodelphinidin oligomers (Fig. 2.7). Grape seed extracts contain three monomers, *i.e.* (+)-catechin, (-)-epicatechin and epicatechin 3-gallate units, as well as procyanidin oligomers (Rodriguez-Montealegre *et al.,* 2006; Mattivi *et al*., 2009; Boido *et al*., 2011). Dimers are the most abundant compounds among the oligomers (Ferrer-Gallego *et al*., 2010). Oligomers consist of a few monomer units. The C1 trimers epicatechin-(4ß→8)-epicatechin-(4ß→8)-epicatechin and epicatechin-(4ß→8)-epicatechin-(4ß→8)-catechin are well represented in red grapes (Ferrer-Gallego *et al*., 2010). A characteristic of the flavan-3-ol composition of grape seeds is that galloylation occurs on an epicatechin unit. Compounds with catechin-*O*-gallate have not yet been isolated (Ferrer-Gallego *et al*., 2010). Grape pulp extracts contain monomers and low molecular weight oligomers of proanthocyanidins (Kennedy & Hayasaka, 2004; Margalit, 2004; Ribéreau-Gayon *et al*., 2006; Mattivi *et al*., 2009).

Polymerised flavan-3-ols, referred to as condensed tannins or proanthocyanidins because of their ability to release red anthocyanin pigments when they are heated in an acidic solution, are formed naturally by the metabolic processes of the grapevine (Ribéreau-Gayon *et al.,* 2006; Andersen & Markham, 2007; Vermerris & Nicholson, 2008). Concentrations of proanthocyanidins found naturally in grapes vary depending on the grape cultivar. For example, Cabernet Sauvignon, Nebbiolo, Syrah, and Tannat have the highest concentrations of proanthocyanidins (Kennedy & Hayasaka, 2004; Mattivi *et al*., 2009). The reaction of tannins and anthocyanins leads to the creation of pigmented tannins, which affects the colour of red wine (Kennedy *et al*., 2001).



R_3 = Gallate = Gal.

Flavan-3-ols	**R_1**	**R_2**	**R_3**
(+)-Catechin	OH	H	OH
(-)-Epicatechin	OH	H	OH
(-)-Gallocatechin	OH	OH	OH
(-)-Epigallocatechin	OH	OH	OH
(-)-Gallocatechin-3-gallate	OH	OH	Gal
(+)-Catechin-3-gallate	OH	H	Gal
(-)-Epicatechin-3-gallate	H	OH	Gal
(-)-Epigallocatechin-3-gallate	OH	OH	Gal

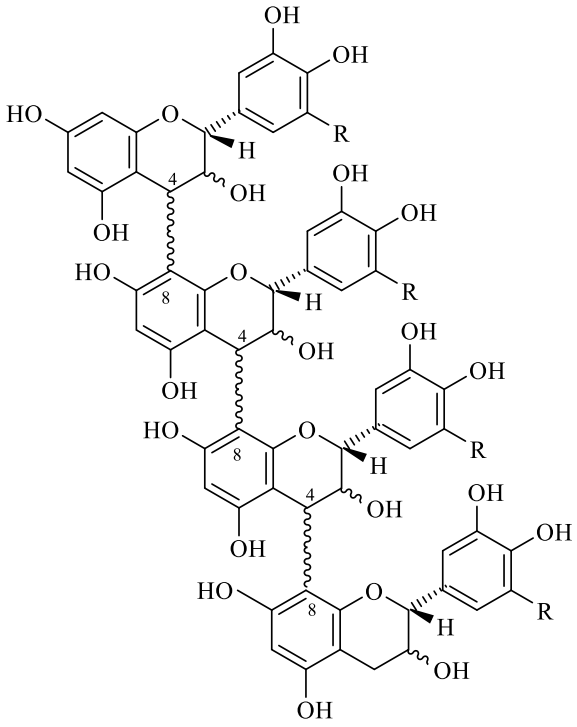
Figure 2.7 Examples of the principal monomeric flavan-3-ols found in grape seed and wine (Monagas *et al*., 2005).

2.4.3 Proanthocyanidins, procyanidins and prodelphinidins

The majority of naturally occurring proanthocyanidins are oligomers and polymers (Czochanska, *et al*., 1980). The procyanidins and prodelphinidins, which hydrolyse to cyanidin and delphinidin, respectively, are the most abundant condensed tannins in grapes and wine. Condensed tannins in grapes and wine are more-or-less complex polymers of flavan-3-ols or catechins (Ribéreau-Gayon *et al.*, 2006). Proanthocyanidins generally occur as polymers of flavan-3-ols (Garrido & Borges, 2013). Proanthocyanidins in *Vitis vinifera* spp. are mainly oligomers and polymers of (+)-catechin and (-)-epicatechin linked through the $C_4 - C_8$ bonds, although monomeric units can also link through the $C_4 - C_6$ bonds (Fig. 2.8).

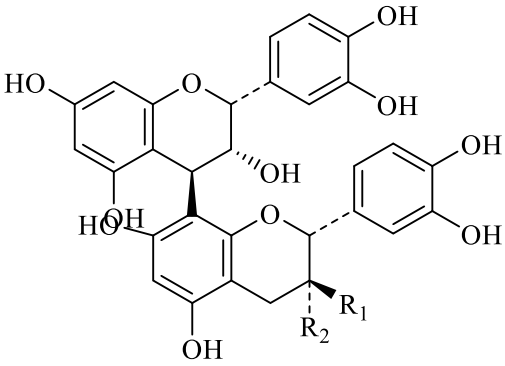
The relationship between total polymer content and flavan-3-ol content has been established in grapes and wine (De Freitas *et al.*, 2000; Kennedy *et al*., 2000; Garrido & Borges, 2013; Rinaldi *et al*., 2014). Oligomers with a maximum degree of polymerisation of 16 have been identified in *Vitis vinifera* spp. Grape seed tannins are primarily polymers of (-)-catechin and gallated catechins, but also of gallocatechins (Yilmazer-Musa *et al*., 2012; Prodanov *et al.*, 2013). Grape skin tannins contain (-)-epigallocatechins and low levels of (+)-gallocatechins and (-)-epigallocatechin 3-*O*-gallates (Zhao *et al.*, 2010).

The elucidation of the structures of dimeric procyanidins and the existence of analogous prodelphinidin dimers (Fig 2.9) have been demonstrated by Fletcher *et al*. (1977) and Engel *et al*. (1978). These compounds are responsible for the sensory characterisation of wine taste, -astringency and -bitterness. Proanthocyanidins also play an important role in the wine ageing process because of their oxidative, condensation and polymerisation properties (Garrido & Borges, 2013).



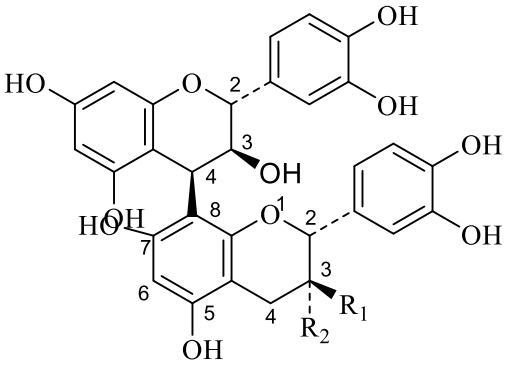
R = H, procyanidin units; R = OH, prodelphinidin units

Figure 2.8 General structure of a B-type condensed proanthocyanidin of degree of polymerisation 4 (Ribéreau-Gayon *et al.*, 2006).



Procyanidin B1: R_1 = OH, R_2 = H

Procyanidin B2: R_1 = H, R_2 = OH

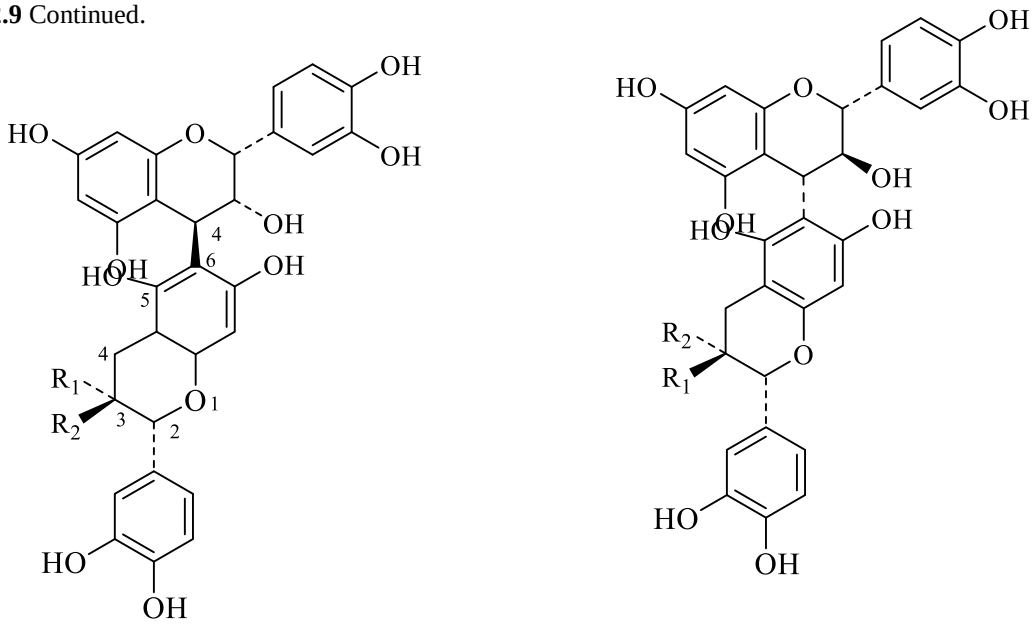


Procyanidin B3: R_1 = OH, R_2 = H

Procyanidin B4: R_1 = H, R_2 = OH

Figure 2.9 Dimeric procyanidins identified in grapes and wine (Garrido & Borges, 2013).

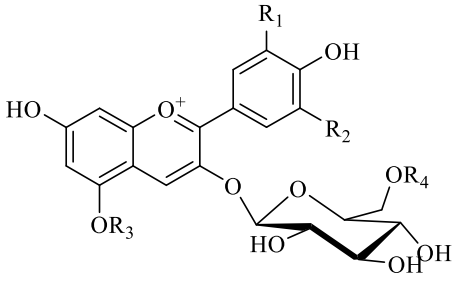
Figure 2.9 Continued.



Procyanidin B5: R_1 = H, R_2 = OH; Procyanidin B7: R_1 = OH, R_2 = H; Procyanidin B6: R_1 = OH, R_2 = H; Procyanidin B8: R_1 = H, R_2 = OH

2.4.4 Anthocyanidins

Anthocyanidins in their predominant flavylium cation form consist of two benzene rings bonded by an unsaturated cationic oxygenated heterocycle (Fig. 2.10), derived from a 2-phenyl-benzopyrylium nucleus (Andersen & Markham, 2007). The flavylium ion and glycolated anthocyanins contain conjugated double bonds responsible for absorption of light at *ca.* 500 nm, causing the flavylium ion to appear red to the human eye. Aglycones (anthocyanidins are per definition aglycones) occur in penta-(3,5,7,3',4') or hexa-(3,5,7,3',4',5') substituted forms, and are unstable (Margalit, 2004). They differ in the number of hydroxyl and methoxyl groups in the B-ring of the flavylium cation (Ribéreau-Gayon *et al*., 2006). Aglycones are only stable, water soluble and chromogenic when linked to a sugar molecule, and are then referred to as anthocyanins.



Anthocyanidin	**R_1**	**R_2**
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Pelargonidin	H	H

R_3 = H, glucose, $_4$ = H, acetyl, *p*-hydroxycinnamoyl, caffeoyl

Figure 2.10 Structures of *Vitis vinifera* spp. anthocyanins (He *et al*., 2010; Flamini *et al.*, 2013).

2.4.5 Anthocyanins

Anthocyanins are anthocyanidin-glycosides, the coloured pigments present in grape skin (Margalit, 2004; Ribéreau-Gayon *et al*., 2006). The coloured appearance of this group of phenolics is mainly due to their ionic character. The red colour of wine originates from the small proportion of anthocyanin that exists in the flavylium state. This proportion depends on the pH and free sulphur dioxide content of the wine. The proportion of anthocyanins in the flavylium form markedly affects both wine hue and colour stability (Ribéreau-Gayon *et al*., 2006). Wine hue and colour are directly affected by the hydroxylation pattern on the B-ring of the anthocyanin. The common factor affecting colour intensity is not pH, but rather the concentration of free sulphur dioxide when in a wine medium (Brouillard *et al.*, 2003). Sulphur dioxide is effective anthocyanin bleach but the process is reversible.

The glycosidic bond is of the form C-O-C between carbons carrying hydroxyls in both the flavonoid system and the sugar molecules. The preferred position in the flavonoid molecule (anthocyanin) is at carbon-3 *via* carbon-1 with the δ-orientation of the sugar. A second glucose can link to the carbon-5 and carbon-7 of the anthocyanidin to form anthocyanidin-3,5-*O*-diglucosides (Margalit, 2004; Andersen & Markham, 2007). Dextrose [(D)-glucose] is the preferred sugar molecule, but glycosidic bonds with (L)-rhamnose, (L)-arabinose and (D)-galactose are also common (Vermerris & Nicholson, 2008).

The glucoside may be acylated at position 6 of the sugar molecule with acetic acid, *p*-coumaric acid or caffeic acid (Ribéreau-Gayon *et al*., 2006). Coumaroylation and acetylation of anthocyanins occur on carbon-6 of the sugar molecule. Acetylated and *p*-coumaroylated glucoside derivatives are present in most grape cultivars (Andersen & Markham, 2007). Acylation with sugar renders the anthocyanin molecule less soluble in water (Mazza & Francis, 1995). The quantity of (acetic-, caffeic- and coumaric acids) acylated monoglucosides is highly variable according to the grape cultivar. Anthocyanins in grapes and wines from *Vitis vinifera* spp. are the 3-*O*-monoglucosides and the 3-*O*-acylated monoglucoside derivatives (Ribéreau-Gayon *et al*., 2006; Andersen & Markham, 2007). Anthocyanins are mainly located in the skin of grape berries. The hydroxylation pattern on the B-ring produces mainly five anthocyanins, which include cyanidin, delphinidin, peonidin, petunidin, and malvidin (Fig. 2.10). The five-anthocyanin compounds have been identified in red grapes and wine (Margalit, 2004). The principal anthocyanin pigment in *Vitis vinifera* spp. is malvidin 3-*O*-glucoside (Margalit, 2004). It is the phenolic compound with the highest concentration in young red wine. Ratios of different anthocyanins vary in different grape cultivars (Timberlake & Bridle, 1967; Singleton, 1980).

2.4.5.1 Reduction/oxidation of anthocyanins

The colours of anthocyanin solutions are directly linked to pH (Ribéreau-Gayon *et al*., 2006). Anthocyanins can lose their colour through reduction and oxidation by ascorbic acid (Sondheimer *et al*., 1953), sulphur dioxide (Jurd, 1964) or hydrogen peroxide (Jurd, 1966).

Anthocyanins are red in an acid medium, losing their colour as the pH increases. Carbons 2 and 4 are partially positively charged. These carbons attract nucleophilic groups such as HSO^{-}_{3} to produce colourless sulphonic acid (Fig. 2.11). The reactions are however reversible. Resistance to oxidation increases in the absence of an *ortho* or adjacent hydroxyl group in the glucose, as in malvidin- and peonidin 3-*O*-glucoside.

Since monomeric anthocyanins are subject to hydrolysis, oxidation and polymerisation (Ribéreau-Gayon *et al*., 2006) their concentrations are usually highest in wine during the early stages of fermentation.

Anthocyanin concentrations decrease during fermentation and continue to decrease during ageing of wine. Indeed, monomeric anthocyanins are the most labile phenolic compounds in wine, typically transforming at a rate of *ca*. 50% per year (Ribéreau-Gayon *et al*., 2006).

The wine standard analysis parameters, pH, SO_2, and acetaldehydes, can be used to estimate the potential rate and extent of anthocyanin interactions with other phenolic compounds (Andersen & Markham, 2007).

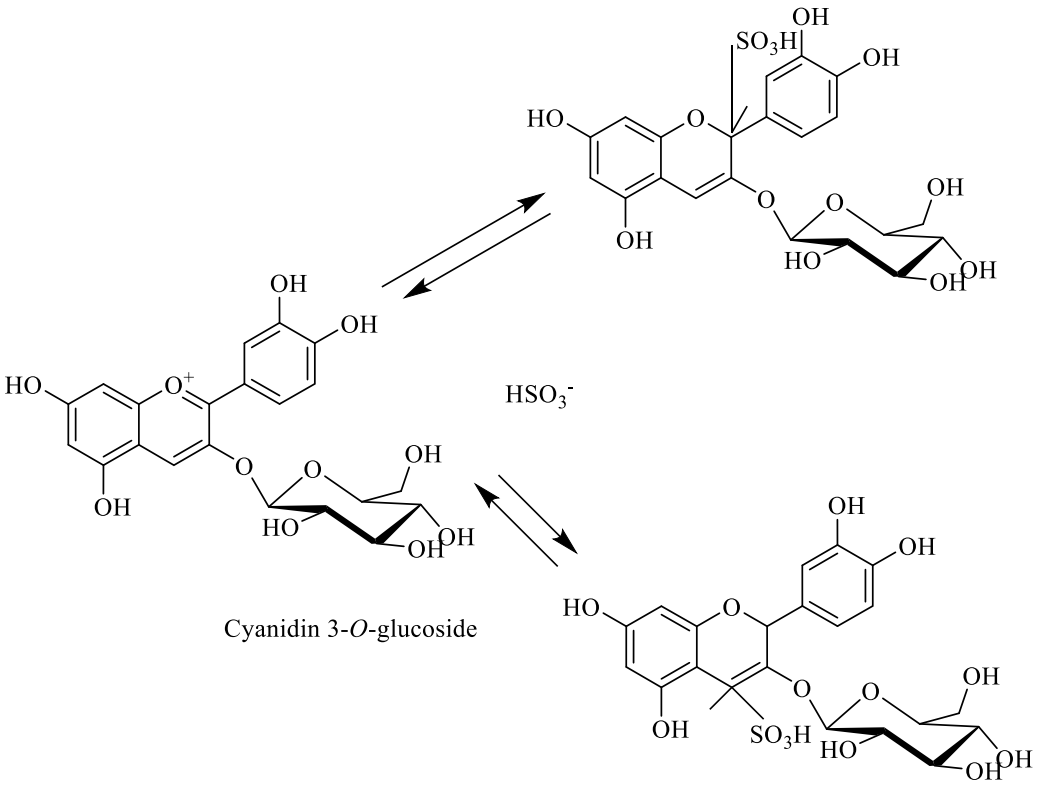


Figure 2.11 Bleaching of anthocyanins (losing their colour) through reduction or oxidation by *e.g.* ascorbic acid (Margalit, 2004).

2.4.5.2 Dependence of anthocyanin colour on pH

Anthocyanins in solution are present in several chemical forms in equilibrium with one another (Margalit, 2004). These equilibria are pH dependent and entail two sets of equilibria involving the flavylium cation: (1) The equilibrium between flavylium cation (A^+) and the *carbinol* base (AOH), which involves a water molecule, followed by a proton transfer, which leads to the formation of the colourless carbinol pseudobase; these species are in equilibrium with the yellow open ring tautomer form, the corresponding *chalcone*, and (2) The conversion of the flavylium cation (A^+) to the *quinonic* base (AO), which occurs due to a proton transfer (Brouillard & Dubois, 1977); the quinonic base has a blue colour. Brouillard and co-workers (2003) investigated the equilibria in detail; the summary presented in Fig 2.12 was derived from this.

Anthocyanins are therefore red in an acidic medium due to the prevalence of the flavylium cationic species, and change colour as the pH increases (Margalit, 2004): from red to blue, colourless, and eventually yellow at a pH higher than 4.

The relatively blue colour of anthocyanins increases with the number of free hydroxyl groups (OH), whereas an increasing degree of methylation (OCH_3) will intensify redness (Brouillard *et al.*, 2003). In young red wines, anthocyanins occur predominantly in a dynamic equilibrium among five major molecular states; one bonded to sulphur dioxide and four free forms. At a pH of *ca.* 3.0, 10 to 15% of the monomeric anthocyanins present in wine are in the flavylium form (Jackson, 2000). Vinification practices can therefore affect the anthocyanin extraction and stability.

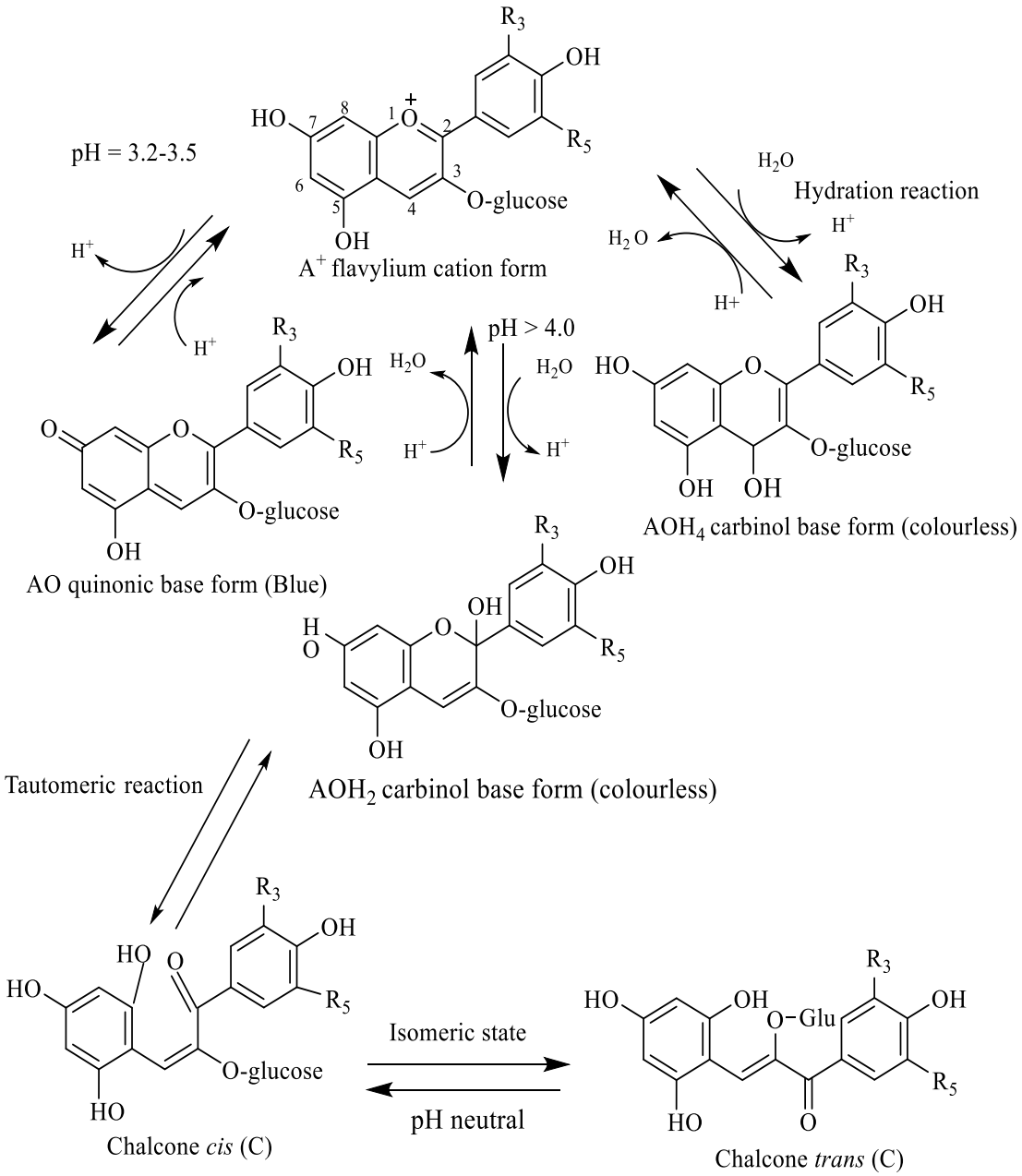


Figure 2.12 Various forms of anthocyanins (Brouillard & Dubois, 1977).

The colour and intensity in anthocyanin solutions are dependent on a particular pigment's equilibria, which are also pH dependent. The following equilibria equations serve to estimate the distribution of colour pigments in red wine:

In equilibrium: $F^+ \rightleftharpoons F + H^+$

The equilibria constant is $K_a = [F]\,[H^+]/[F^+]$

$\log K_a = \log [F]/[F^+] + \log [H^+]$

$-\log K_a = \log [F^+]/[F] - \log [H^+]$

By definition: $-\log K_a = pK_a$; and $-\log [H^+] = pH$

$\longrightarrow pK_a = \log [F^+]/[F] + pH$

$$\boxed{\text{Log } [F^+]/[F] = pK_a - pH}$$

F^+ = Flavylium ion (anthocyanin); $[F^+]$ = Flavylium ion concentration (mol/L).

F = Deprotonated anthocyanins (carbinol or quinoidal); [F] = deprotonated anthocyanin concentration (mol/L).

The above equation serves to calculate the ratio between the concentrations of the flavylium cationic species and the other relevant species at a given pH when the pK_a values are known. Note that the flavylium ion is not isolated in a single equilibrium, therefore the pK_a values are not true constants but rather pH dependent. The pK_a values for the two equilibria of malvidin-glucoside are known to be 2.6 and 4.25.

2.5 Flavonoid and non-flavonoid levels in wine

The average total phenolics of red grape cultivars are 6216 mg/kg fresh weight, whereas the average total for white grape cultivars is 3060 mg/kg fresh weight (Jackson, 2000). These grape cultivars include Pinot noir, Alicante Bouschet, Cabernet Sauvignon, Syrah, Tempranillo, Carignan, Cinsaut, Grenache, Gamay noir and Malbec (red cultivars) and Chardonnay, Sauvignon blanc and Villard blanc (white cultivars). Total average anthocyanins in red grape cultivars are in the order of 2015 mg/kg fresh weight.

Flavonol compounds are present in red wine in the region of 100 mg/L. In white wine, where fermentation takes place usually in the absence of grape solids, typical concentrations are from 1.0 - 3.0 mg/L depending on the grape cultivar (Ribéreau-Gayon *et al*., 2006). Monomeric anthocyanin concentrations in Pinot noir wines are *ca*. 100 mg/L, whereas in Syrah and Cabernet Sauvignon wines it can be as high as 1500 mg/L after fermentation. Condensed tannin (proanthocyanidins) concentrations also vary according to the grape cultivar. Concentrations of 1.0 - 4.0 g/L of condensed tannins have been reported in red wine (Kondo *et al.*, 2000). In dry white wine, the quality of settling determines the tannin concentration of the wine (Ribéreau-Gayon *et al*., 2006). Tannin concentrations can range from 100.0 - 200.0 mg/L if fermentation takes place in the presence of lees.

Benzoic- and cinnamic acids are present in the order of 100.0 - 200.0 mg/L in red wine and 10 - 20 mg/L in white wine (Ribéreau-Gayon *et al*., 2006).

2.6 Analytical techniques used for the identification and quantification of phenolics

2.6.1 Spectroscopic techniques

Typical analysis involving spectroscopic techniques comprises structural elucidation as well as determination of stereochemical characteristics (Santos-Buegela & Williams, 2003). Spectroscopic techniques are also aimed at tracing specific compounds and presenting quantitative characteristics or identifying colour depiction. The application of UV or UV-Vis spectroscopy has been used in the analyses of flavonoids for some time (Andersen & Markham, 2007). These polyphenolic compounds have two characteristic UV absorption bands with maxima in the 240 - 285 nm and 300 - 550 nm ranges. The various flavonoid classes can be recognised by their UV spectra (Markham, 1982) and the UV spectral characteristics of individual flavonoids, including the effect of the number of aglycones, hydroxyl groups, and glycosidic substitution pattern (Harborne, 1967). Today, the use of UV-Vis spectroscopy applied to flavonoids is in quantitative analyses and the value of UV-Vis spectroscopy for structural analyses is diminishing, compared to the level of information that can be gained by other methods such as NMR and MS (Cabretta *et al*., 2000; Andersen & Fossen, 2003). The combination of HPLC equipped with a UV-Vis DAD has been the standard method for the last two decades for the detection of flavonoids in different matrices (George & Maute, 1982; Andersen & Markham, 2007). This type of detection allows for the simultaneous recording of chromatograms at different wavelengths. This increases the power of HPLC analysis because the UV-spectrum information may help to identify the compound subclass or perhaps even the compound itself.

2.6.1.1 UV-Vis absorption spectroscopy for anthocyanins

The UV-Vis spectral data on anthocyanins provide information about the nature of the aglycone and aromatic acyl groups (Gíustí & Wrolstad 2001). The hydrochloric acid method is readily used to estimate the total anthocyanin content in a solution containing other phenolic compounds. This is possible because of the unique absorption band exhibited by anthocyanins, *i.e*. 490-550 nm (Giusti & Wrolstad, 2001). Another tannin assay method for the measurement of anthocyanins in grape extract and wines is the bisulfite bleaching method. This method is based on change sdue to pH and bisulfite bleaching effect (Ribéreau-Gayon & Stonestreet, 1965; Aleixandre-Tudo *et al.*, 2017). The pH differential method proposed by Ribéreau-Gayon & Stonestreet is another method commonly used in tannin assays.

2.6.1.2 UV-Vis absorption spectroscopy involving flavonoids in complexes

The interaction of various flavonoids, *i.e.* compounds having C_6–C_3–C_6 configuration with sodium dodecyl sulphate (an anionic surfactant), was studied through absorption spectroscopy as a function of the concentration of surfactants. A mechanism was proposed for the interaction between the various flavonoids and anionic surfactants.

The approximate number of flavonoid molecules incorporated per aggregate of surfactant molecules (micelle concentration) was estimated at a particular concentration of sodium dodecyl sulphate. Incorporation of additive in micelles resulted in a shift of UV absorption bands towards higher wavelengths (Naseem *et al*., 2004).

2.6.1.3 Tannin assay

Tannins comprise chains of polymerised flavanols (Margalit, 2004). These procyanidins are either homogenous (regular linking) or heterogeneous (different bond linking).

In both cases, certain bonds are broken when these molecules are heated in an acid medium and the resulting carbocations are partially converted into cyanidin (Ribéreau-Gayon & Stonestreet, 1966). The procedure requires two samples diluted 1/50 with H_2O in an acidified medium. One sample is heated to 100°C with the addition of 95% ethanol. Ethanol is added to the second sample without undergoing heat treatment. The difference in the optical density at 520 nm is measured in a 10 mm optical path (Ribéreau-Gayon *et al.*, 2006).

A second method for calculating the tannin concentration is based on examining the visible spectrum of the above reaction (Ribéreau-Gayon *et al*., 2006). Regardless of the degree of polymerisation and concentration of the procyanidins, the following applies: OD 520, OD 470, and OD 570 represent the difference in OD, with or without heating for the three corresponding wavelengths.

Another method for determining tannins is the gravimetric method. This method is based on tannins binding with insoluble polyvinylpyrrolidone (Harinder *et al.*, 1993). The procedure gives the absolute concentration of tannins without the use of standards associated with spectrophotometric methods. Results obtained through this method correlate with spectrophotometric and protein-precipitation capacity methods.

A number of tannin assays based on binding of haemoglobin or bovine serum albumin (BSA) and subsequent determination of unbound protein in a protein-tannin complex (protein precipitation capacity) have also been used, but with limitations. These methods are unable to estimate protein-binding capacity if the quantity of tannin available is low. The protein in the tannin-protein complex is measured spectrophotometrically after staining with Ponceau S (acid red 112) dye. The methyl cellulose precipitable (MCP) tannin assay is another method to determine the tannin concentrations in wine (Mercurio & Smith, 2008). The methyl cellulose precipitable tannin assay is based on the absorbance of phenolics at 280 nm before and after tannin precipitation (subtractive approach) can be obtained by exploiting the polysaccharide polymer methylcellulose to precipitate tannins, thus enabling selective easurement of tannin only (Sarneckis *et al*., 2006). This methylcellulose precipitable tannin assay allows complete precipitation of tannin from red wine and from grape homogenate extracts.

2.6.2 Nuclear magnetic resonance

Identification of proanthocyanidin dimers, including the determination of the linkage position is achievable by using two-dimensional NMR techniques (Andersen & Markham, 2007).

Two-dimensional NMR techniques have been used for the identification of tannin-like derivatives (Mateus *et al*., 2002). Proton-proton and proton-carbon correlation can distinguish between different flavonol moieties and establish their sequence. The two-dimensional NMR approach can also identify tannin-like derivatives (Es-Safi *et al*., 2002).

2.6.3 Fluorescence spectroscopy

Fluorescence spectroscopy is a fast and simple method to determine the concentration of an analyte in solution based on its fluorescent properties (Andersen & Markham, 2007). It can be used for relatively simple analyses, where the compound to be analysed is known in order to quantify and determine the concentration of a specific compound. Fluorescence is mainly used for measuring compounds in solution. The potential of fluorescence spectroscopy for anthocyanins in Pinot noir and Pinot Meunier grapes was investigated by measuring chlorophyll-fluorescence excitation spectra (Agati *et al*., 2007). Less excitation light is transmitted to the deeper chlorophyll layers of the grape berry with increasing anthocyanin concentration in the grape skin, with a proportional decrease of the chlorophyll-fluorescence signal.

2.6.4 Matrix assisted laser desorption/ionisation-time-of-flight mass spectrometry

Matrix assisted laser desorption/ionisation-time-of-flight mass spectrometry (MALDI-TOF) has been successfully applied for the direct analysis of grape and wine extracts (Fulcrand *et al*., 1999). A suitable matrix for phenolics is *trans*-3-indole-acetic acid and 2,5-dihydroxybenzoic acid. This matrix allows identification and confirmation of the presence of the dominant compounds in wine and grape skin samples with minimal fragmentation of the sugar rmoeiety of the anthocyanin (Ivanova *et al*., 2011).

2.6.5 High performance liquid chromatography

Since the early 1990s, high performance liquid chromatography (mainly with UV-detection) became the standard method for the quantification of phenolics, particularly anthocyanins (Cartoni *et al*., 1991; Nogata *et al*., 1994; Fiorini, 1995; Waterhouse *et al*., 1999). Phenolic compounds in grape extracts and wine are usually separated and quantified using normal phase (NP) and especially reversed phase (RP) HPLC (Cheynier *et al*., 1999; Heier *et al*., 2002; Perestrelo *et al*., 2012). A further dimension is added by the introduction of photodiode-array detection (DAD) technology (Nogata *et al*., 1994; Waterhouse *et al*., 1999; Kallithraka *et al*., 2005; Kelebek *et al*., 2007; Abad-García *et al*., 2009; Prodanov *et al*., 2013; Teixeira-Barcia *et al*., 2014; Kumšta *et al*., 2014; Ivanova-Petropulos *et al*., 2015; Moreno *et al*., 2015). HPLC-DAD has been successfully applied to quantify phenolic acids and flavonoids (especially anthocyanins, flavan-3-ols and flavonols) in grapes and wine (De Villiers *et al*., 2004; Jeffery *et al*., 2008; Muñoz *et al*., 2008; Fanzone *et al*., 2010; De Villiers *et al*., 2011; Lorrain *et al*., 2011; Baiano *et al*., 2015; Garaguso & Nardini, 2015).

RP-HPLC-DAD is the most popular chromatographic technique to quantify monomeric flavonoids in grape and wine extracts on a routine basis (Revilla *et al*., 2001; Gómez-Alonso *et al.*, 2007; Abad-Garcia *et al.*, 2009; Liazid *et al*., 2010; Lorrain *et al.*, 2011; Papoušková *et al*., 2011; Perestrelo *et al*., 2012; Fanali *et al*., 2013; Favre *et al.*, 2014; Baiano *et al*., 2015; Garaguso & Nardini, 2015; Nelson *et al*., 2016). This technique enables the distinction of various classes of flavonoid and non-flavonoid compounds based on their characteristic UV-visible spectra and retention times. The technique enables simultaneous recording of chromatograms at different wavelengths (Andersen & Markham, 2007), thereby improving quantification because detection is performed at the wavelength of maximum absorbance for each class of compounds. It also increases the power of HPLC analysis, as it is possible to identify the compound or its sub-class with the information from the UV spectrum (Castillo-Muñoz *et al.*, 2007).

Techniques such as refractive index, light scattering, conductivity, chemiluminescence, and optical rotation detection can also be utilised in chromatography (Skoog *et al*., 2007). Refractive index detection is based on changes in the refractive index of eluting compounds in the mobile phase. The mobile phase itself has a different refractive index to the sample of interest. Temperature control is necessary as it has high temperature sensitivity. Light scattering detectors are useful for the detection of high molecular weight molecules.

After removal of mobile phase by passing through a heated zone, the solute molecules are detected by light scattering depending on molecular sizes (Agilent Technologies, Inc. 2016). Conductivity detectors measure electronic resistance. Solutions containing ionic components will conduct electricity. The measured value is directly proportional to the concentration of ions present in the solution (Thermo Scientific, 2015). Chemiluminescence detection is similar to fluorescence detection but instead of using a light source to excite the atoms, the excitation is initiated by a chemical reaction (Agilent Technologies, Inc., 2016). Optical rotation detection is specific for the optical isomer measurement (Goodall & Lloyd, 1988). The column can separate R- and L- type optical isomers. Infrared detection is based on the reaction to infrared radiation. The two main types of detectors are thermal and photonic.

2.6.6 High performance liquid chromatography - mass spectrometry

The quantification of phenolic compounds in red wine has been performed using a number of UV-detection based chromatographic analytical techniques (Fanzone *et al*., 2010; Liazid *et al.*, 2010; Perestrelo *et al.*, 2012; Sánchez-Ilárduya *et al*., 2012; Ramirez-Lopez *et al.*, 2014; Nelson *et al*., 2016). The diversity of wine phenolics, coupled to their similar UV spectra, does however limit the accuracy and identification power of RP-HPLC-DAD. From this perspective, the hyphenation of HPLC with mass spectrometry (MS) represented a significant improvement in wine phenolic analysis. This hyphenation is most commonly achieved using electrospray ionisation (ESI), a technique used to produce ions by means of an electrospray in which a high voltage is applied to a liquid flow to create a charged aerosol. The ESI is especially useful in ionising macromolecules and polar compounds, because

it overcomes the propensity of these molecules to fragment when ionized (Ho *et al*., 2003). Indeed, ESI is the preferred ionisation mode for flavonoids because of their polarity (Lorrain *et al*., 2013; Teixeira-Barcia *et al*., 2014; Baiano *et al.*, 2015; Ivanova-Petropulos *et al*., 2015).

The first application of HPLC-ESI-MS to grape phenolics reported the identification of anthocyanins in grape extract through their typical fragmentation patterns (Baldi *et al*., 1995; Andersen & Markham, 2007). Detection of anthocyanins in their cationic forms was achieved using ESI in the positive mode in an acidified medium, *i.e.* with 10% formic acid (Revilla *et al.*, 2001). The ESI in the negative ionisation mode is used for uncharged flavonoids, such as flavan-3-ols (Gabetta *et al*., 2000), flavonols (Cantos *et al*. 2002) and dihydroflavanols (Souquet *et al*., 2000), which are detected as the deprotonated $[M-H]^-$ species, and non-flavonoids, such as phenolic acids (Cheynier *et al.*, 2003). The negative ion mode is also more suitable for the detection of anthocyanin-derived pigments bearing carboxylic groups and anthocyanin-flavonol adducts in which the anthocyanin moiety is not in the cationic form (Abad-Garcia *et al.*, 2009). The use of polar eluents under acidic pH conditions is a prerequisite to maintain good chromatographic performance and enhance stability of phenolics.

In recent years a range of commercial mass spectrometers have been used in combination with HPLC for the analysis of flavonoids, including single-stage low resolution [quadrupole (Q), ion trap (IT)] (Kite *et al.,* 2003) and high resolution [magnetic sector, time-of-flight (TOF) and Orbitrap] systems (Fukai *et al*., 2000; Hu *et al.,* 2005).

Ion trap mass spectrometry allows for the rapid identification of most anthocyanins present in grape juice (Andersen & Markham, 2007). Fragmentation patterns on selected individual ions are also obtained. Addition to IT, other multi-stage MS systems, such as IT, Q-TOF, triple quadrupole (QqQ) and IT-TOF have been applied for flavonoid analysis (Waridel *et al*., 2001; Kite *et al.,* 2003).

A recent trend is the increasing use of multi-stage MS systems, such as IT, Q-TOF, triple quadrupole (QqQ) and IT-TOF systems for flavonoid analysis (Waridel *et al*., 2001.

In the last 20 years, numerous phenolics in grapes and wine samples have been identified using HPLC-ESI-MS and -MS/MS (Cameira dos Santos *et al*. 1996; De Villiers *et al*., 2004; Núñez *et al.,* 2004; Guerrero *et al.,* 2009; Fanzone *et al*., 2010; Alberts *et al*., 2012; He *et al*., 2012; Zhu *et al*., 2012; Ramirez-Lopez *et al.,* 2014; Lambert *et al*., 2015; De Villiers *et al*., 2016). In tandem MS (MS/MS) ions produced by ESI and APCI are accelerated inside a mass spectrometer to collide with molecules of the gas to achieve fragmentation (Kite *et al*., 2003). Improved structural elucidation information or improved selectivity can be obtained using MS/MS (Major, 2005).

An alternative ionisation source for hyphenation of HPLC to MS is atmospheric pressure chemical ionisation (APCI). In APCI, a corona discharge is used to ionise the analyte in the atmospheric pressure region. Ions are formed by charge transfer from the solvent as the solution passes through a heated nebuliser into the APCI source (Andersen & Markham, 2007). APCI is therefore based on gaseous-phase ionisation and is most suitable for compounds that are partially volatile and have a medium polarity.

For this reason, the application of APCI for especially condensed tannin and anthocyanin determination is limited (De Pascaual-Teresa & Rivas-Gonzalo, 2003), although APCI has been partially successful in the analysis of various flavonoids (Stewart *et al.*, 2000) in grape and wine samples (Presta *et al.*, 2009).

2.6.7 High performance liquid chromatography - nuclear magnetic resonance

The coupling of HPLC to NMR spectroscopy (LC-NMR) is one of the most powerful methods for the combined separation and structural elucidation of unknown compounds in sample mixtures (Andersen & Markham, 2007), but is to be applied successfully to grape and wine phenolic analysis. The LC-NMR technique has been applied for the in-depth structural investigation of flavonoids and proved to be useful for the structural elucidation of wine secondary metabolites (Andersen & Markham, 2007). However, the advantages of directly coupling NMR and HPLC instrumentation should be compared to compromises in performance made to each technique to achieve a hyphenated system. Although successful advances have been made in LC-NMR technology in wine analysis, HPLC purification of secondary grape metabolites followed by conventional tube NMR is equally useful (Andersen & Markham, 2007).

2.6.8 Capillary electrophoresis

The technique of capillary electrophoresis (CE) has been applied for the quantification of phenolic compounds in wine samples (Carretero *et al*., 2004). Capillary electrophoresis is based on electro kinetic separation methods performed in sub-millimetre diameter capillaries in micro- and nano fluidic channels. Capillary electrophoresis is often referred to as capillary zone electrophoresis. In capillary electrophoresis methods, analytes migrate through electrolyte solutions under the effect of an electric field.

Analytes are separated according to ionic mobility and/or partitioning into an alternate phase via non-covalent interactions. Additionally, analytes may be concentrated by means of gradients in conductivity and pH (Skoog *et al*., 2007). Sáenz-López *et al.* (2003) quantified anthocyanins in wine samples using capillary zone electrophoresis (CZE) in a basic medium. The basic medium allowed for a faster separation than an acid medium. The anionic blue quinonic species were stable enough for quantitative analysis and compared well with HPLC methods in terms of minimal set-up time and costs. Wang & Huang (2004) quantified kaempferol, quercetin, and myricetin in red wine using capillary zone electrophoresis. Detection limits showed improvement when CZE was performed in a borate buffer. Non-coloured phenolic compounds present in Portuguese red wine were analysed qualitatively and quantitatively by CZE with less sensitivity for the detection of flavonols, compared to HPLC (Garcia-Viguera & Bridle, 1995). Capillary electrophoresis coupled to mass spectrometry (MS) may provide valuable structure-selective information about flavonoids in plant extracts. This coupled technique has only been of limited use in flavonoid analysis (Aramendia *et al*., 1995) due to its limited sensitivity, compared to HPLC-MS.

Table 2.1 briefly lists comparative information on phenolic quantification techniques based on the principles of the technique, advantages, disadvantages, and compound suitability.

Table 2.1 Analytical techniques for the quantification of phenolic compounds in grapes and wine.

Technique	Advantage	Disadvantage	Compound Separation
HPLC-NMR Information provided is mainly of ^{1}H NMR spectra or ^{1}H- ^{1} H correlation experiments (Andersen & Markham, 2007). Samples flow in a non-rotating 60 to 180 µL glass tube connected at both ends with HPLC tubing (Andersen & Markham, 2007).	Provides qualitative and (semi-) quantitative information with minor sample preparation and a non-invasive way (Andersen & Markham, 2007). Useful for identification purposes (Bao *et al*., 2002). Verification of wine origin, age and adulteration (Nilsson *et al*., 2004; Andersen & Markham, 2007). Suitable for an in-depth structural investigation of pure compounds (Wilson, 2000).	Identification of compounds in wine is frequently hindered by signal overlap and weak intensities of some resonances, especially in the aromatic regions of the spectra (Wilson, 2000). Extensive compromises in performance of HPLC and NMR to achieve a hyphenated system (Walker & O'Connell, 2008). Low sensitivity and high cost are the main disadvantages.	Phenolic acids, and procyanidin dimers. Structure elucidation of flavonoids (Wilson, 2000). Difficulty in observing analyte resonance in the presence of the larger resonance of the eluent.
MALDI-TOF-MS A laser irradiation technique where molecules are directly desorbed and ionised (Andersen & Markham, 2007).	Direct analysis of grape and wine extracts (Fulcrand *et al*., 1999; Matamoros-Fernández, 2003; Andersen & Markham, 2007). Has a higher mass range compared to other MS techniques and produces fewer multiple charged ions. Rapid analysis of samples. Molecules need not be volatile. Sub picomole sensitivity is easily obtained (Oroian & Escriche, 2015). Wide array of matrices.	Requires incorporation of the sample in a matrix, *i.e. trans*-3-indole-acetic acid and 2,5-dihydroxybenzoic acid (Yang & Chien, 2000). Quantification of non-volatile molecules only. Analyte must have very low vapour pressure (Oroian & Escriche, 2015). Coupling MALDI with chromatography is difficult.	Characterisation of monomeric flavonoids (flavonols, anthocyanins) and proanthocyanidins (Dopke *et al*., 2000; Yang & Chien 2000).
HPLC-DAD The most popular technique for the separation of flavonoids, both on preparative and analytical scale (Andersen & Markham, 2007).	Low temperature operation, simplicity, and low cost (Andersen & Markham, 2007). Quantification of individual compounds in grapes and wine (if separated). UV visible spectra are advantageous since each class of phenolics exhibits a characteristic UV-Vis spectrum (Andersen & Markham, 2007). Multi-wavelength detection (Fontana & Bottini, 2014). Non-degrading. Permits the use of polar eluents under acidic pH conditions to enhance stability (De Villiers *et al.*, 2009).	Restricted to simple low molecular mass compounds and low selectivity.	Flavonoids and non-flavonoids, excluding high MW proanthocyanidins.
LC-APCI-MS It utilises gas-phase ion-molecule reactions at atmospheric pressure and produces primary ions where corona discharges on a solvent spray (Chapman, 1995).	Suitable for MS analysis of compounds with low molecular mass and relatively volatile and less polar compounds. Compatible with MS/MS methods (Heier *et al*., 2002; Zaikin & Halket, 2006; Presta *et al*., 2009). In some cases provides simpler mass spectra than ESI-MS (Gates, 2013).	Harsh vaporisation and ionisation processes, lower sensitivity for most phenolics compared to ESI-MS.	Anthocyanins (limited), flavonols, flavones, flavanones, chalcones. Most suitable for partially volatile compounds with medium polarity (De Pascual-Teresa & Rivas-Conzalo, 2003) and therefore not often used for flavonoids.

Table 2.1 Continued.

Technique	Advantage	Disadvantage	Compound Separation
LC-ESI-MS The most common form of LC-MS for phenolic analysis. Uses a high voltage to create a charged aerosol under atmospheric pressure (Ho *et al*., 2003). Can produce multiple-charged ions.	Useful in producing ions from macromolecules (Andersen & Markham, 2007). Suitable for MS analysis of compounds of high mass and low volatility, such as anthocyanins. MS allows identification of phenolic compounds; useful to distinguish the degree of glycosylation and substitution of phenolic compounds (Heier *et al.*, 2002; Abad-Garcia *et al*., 2009). ESI only produces molecular ions with limited fragmentation information (Andersen & Markham, 2007). LC-ESI-MS/MS is used for structural elucidation. Identification of novel compounds in grapes and wine.	Multiple charges might complicate identification. Prior chromatographic separation required for any MS method used for flavonoids in grapes and wine. Slight fragmentation - this can be advantageous in that the molecular ion (or pseudo molecular ion) is always observed. However, very little structural information is obtained in single-stage MS.	Positive mode for charged molecules (anthocyanins). Negative mode for uncharged molecules (flavanols, flavonols, phenolic acids).
LC-tandem MS (LC-MS/MS) Techniques are based on ions which are accelerated inside the mass spectrometer so as to collide with molecules of the bath gas, which is usually helium (Kite *et al*., 2003).	MS/MS spectra of targeted compounds facilitate compound identification (Andersen & Markham, 2007). Fragmentation patterns on selected individual ions are obtained. Selective detection (QqQ in MRM mode).	Expensive. Different systems more suited to structural elucidation or selective quantification.	Anthocyanin glycosides, flavanols, flavonols, and phenolic acids. Fragmentation patterns can provide insight in flavanol units in proanthocyanidin oligomers (Andersen & Markham, 2007).
CE an electro-kinetic separation method performed in sub-millimetre capillaries and in micro- and nano-fluidic channels (Andersen & Markham, 2007). In CE methods, analytes move through electrolyte solutions as affected by an electric field.	Potentially faster analysis (Carretero *et al*., 2004) and higher separation efficiency (better resolution) than HPLC. Alternative selectivity.	Not a viable alternative to HPLC for routine analysis of wine due to lower sensitivity and reproducibility (Cabooter *et al*., 2007; De Villiers *et al*., 2009).	Wine phenolic compounds such as anthocyanins and flavonols (Carretero *et al*., 2004).

2.7 Grapevine growth, grape microclimate, grape composition (flavonoid and non-flavonoid phenolics) and wine quality

2.7.1 Introduction

Decision-making in terms of harvest date for commercial winemaking requires the consideration of numerous factors, such as adequate knowledge of grape composition and relevant parameters to achieve a targeted wine style (Coombe & McCarthy, 2000). The aim of this overview is to discuss the role of some of these parameters under the following headings: grapevine growth, grape development stages, phenolic compounds in grapes, grape

ripeness and wine quality, effect of light (microclimate) on grape phenolic compounds, wine chemical composition and wine quality.

2.7.2 Grapevine growth

The growth cycle of the grapevine (*Vitis vinifera* spp.) involves many physiological and vegetative growth processes (Creasy & Creasy, 2009). Each step in the process plays a vital role in the development of grapes with ideal characteristics for making wine. The annual growth of grapevines is frequently described using the following stages: 1) dormancy, 2) budburst, 3) flowering, 4) fruit/berry set, 5) berry development, and 6) harvest. Grapevine growth links up with viticultural practices, which can be applied during the annual growth season when phenolic compounds evolve, especially after véraison. Additionally, information regarding growth stages can be useful to estimate harvest time taking in consideration, grape ripeness levels and phenolic concentrations and therefore grape quality.

2.7.2.1 Dormancy

There are three main physiological stages related to dormancy, *i.e.* 1) acclimation, 2) winter dormancy, and 3) de-acclimation (Coombe & McCarthy, 2000; Creasy & Creasy, 2009). Acclimation begins after the vine has ripened its crop and shoot growth has ceased. The second stage is winter dormancy, which occurs during mid-winter months. De-acclimation is the third stage where vines begin to lose their cold hardiness as they start to adjust to warmer temperature conditions. The passing of each event starts the beginning of a new stage in the vineyard growth cycle (Jones & Davis, 2000). The timing and duration of events are subject to variations due to grape cultivar, climate, and seasonal weather. The sequence of events remains constant (Jackson, 2001). From a management point of view, knowledge of a plant's growth stages is advantageous, as viticultural practices can be applied at optimum times during the annual growth cycle. Additionally, information regarding growth stages can be useful to estimate harvest time, yields, and grape quality.

2.7.2.2 Budburst

Budburst is the event when dormant buds begin to grow to produce shoots (Kennedy *et al*., 2002). It depends largely on the climate and number of buds left after winter pruning (Jackson, 2001). Budburst is negatively affected by low carbohydrate storage in vines, drought, or badly timed deficit irrigation in previous seasons, degree of chilling, and frost damage.

2.7.2.3 Flower bunch initiation and flowering

The formation of undifferentiated primordia cells takes place within the buds in the leaf axils as the shoot grows (Winkler *et al*., 1974; Kennedy *et al*., 2002).

The primordia cells are undifferentiated at this point because they can develop into either flower bunches or tendrils, depending on environmental and growing conditions. In other words, bud fruitfulness is determined in the early stages following bud break (Kennedy *et al*., 2002; Hellman, 2003). During this time, the primordia become regulated and differentiated to develop into a flower bunch or a tendril. Flower bunches form opposite a leaf as the new primary shoot develops. Where a flower bunch does not develop, a tendril may grow opposite the leaf. A shoot usually produces one to three flower bunches (inflorescences), primarily depending on the grape cultivar and growing conditions of the previous season under which the dormant bud developed.

2.7.2.4 Fruit set

Flowering is almost immediately followed by fruit-set, when the fertilized flowers develop into berries with seeds (Winkler *et al*., 1974; Kennedy *et al.*, 2002). Ideally, flowering is quick and synchronous, resulting in an even fruit-set with berries developing and ripening uniformly. During fruit-set, *millerandage* (uneven setting and development of berries) is a particularly undesired phenomenon (Hellman, 2003). *Millerandage* is a potential viticultural problem that is the result of metabolic reactions to weather conditions that cause a failure of grapes to develop properly after flowering. *Millerandage* is to a certain extent important because it prevents bunches from becoming too compact. Usually, only 20 to 30% of flowers on a bunch develop into mature berries, but this is adequate to produce a full bunch (Bisson, 2001; Hellman, 2003).

2.7.2.5 Harvest

At harvest, grapes are considered "ripe' when the sugar, remaining acids, and secondary compounds are in balance (Coombe & McCarthy, 2000). A grape grower may choose to harvest grapes before they are ripe, at the point of "ideal" ripeness, or when they are "overripe" or at various points after a certain, desired potential alcohol level is reached. This all depends on the style of wine desired by the winemaker.

2.7.3 Grape development stages

Berry development is characterised by three stages (Coombe, 1960). The first stage of berry development starts soon after fertilization of the flower and is characterized by rapid growth of the seed and berry. During this period, the berries become firm, dark green in colour and rapidly accumulate acid. The next phase is called the lag phase. This is a time of slow growth during which berries remain firm, but begin to lose chlorophyll (Coombe, 1992), reach their highest level of acid content, and begin to accumulate sugar slowly (Somers, 1976; Hamilton & Coombe, 1992). The final stage (véraison) of grape berry growth coincides with the beginning of fruit maturation (ripening). At this stage, berry growth accelerates, berries begin to soften, titratable acidity decreases, and pH and the level of total soluble solids increase (Coombe & McCarthy, 2000; Pérez-Magariño & González-San José, 2002; Hunter *et al.*, 2007).

Grape ripening entails continuous, multiple biochemical processes and physical changes that begin with véraison and culminates in grape berry maturity or ripeness (Coombe, 1960; Winkler *et al*., 1974). The latter indicates readiness for winemaking (Winkler *et al*., 1974). The development of berries consists of two successive sigmoidal cycles (Fig. 2.13) or three successive growing stages or phases (Coombe, 1960; Coombe, 1973; Jackson & Lombard, 1993; Coombe & McCarthy, 2000). During Stage 1 or the first cycle, the pericarp and seed cell numbers increase and the seeds develop to their full size (Coombe, 1973; Bisson, 2001; Kennedy *et al*., 2002). Stage 2 (still within the first sigmoidal cycle) is characterised by changes in grape berry size and the seed embryo develops with a concomitant hardening of the seed coat, which slows down as the first sigmoidal cycle ends. At this stage, the grape berry is hard, green, and slow growing. The second sigmoidal cycle begins with the onset of sugar accumulation, grape berry softening, -berry colouring, and -berry size increase (Coombe, 1973). These events constitute véraison (Fig. 2.13), denoting the beginning of the ripening process. This is also known as Stage 3.

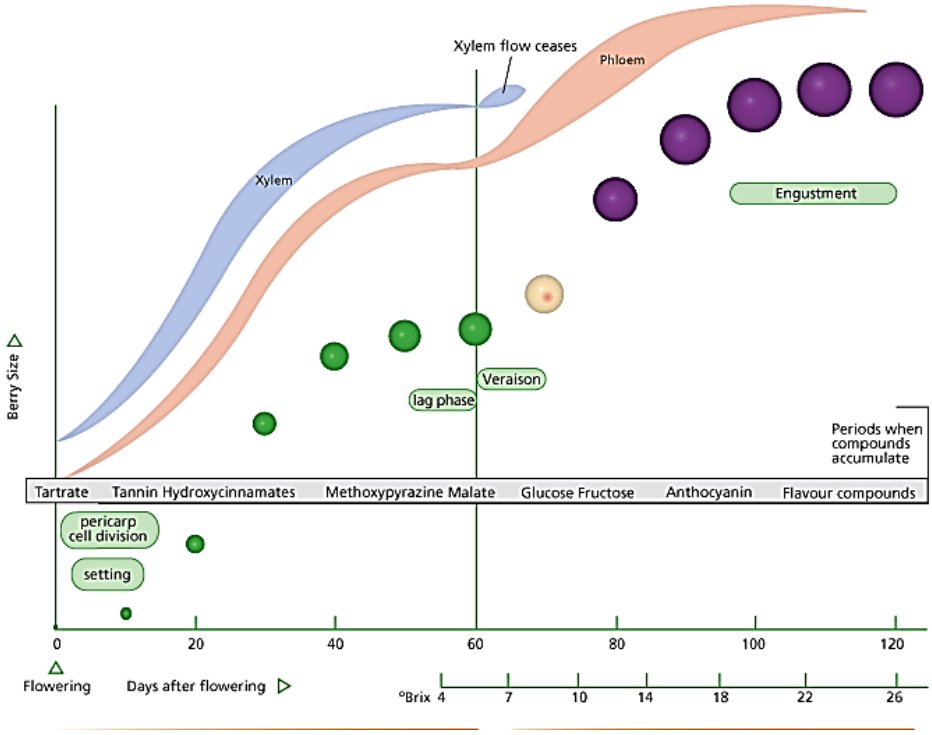


Figure 2.13 Diagram showing relative size and colour of berries at 10-day intervals after flowering and passing through major developmental stages. Also shown are the periods when compounds accumulate, levels of juice °Brix, and an indication of the rate of inflow of xylem and phloem vascular sap into the berry (Kennedy *et al*., 2002).

During the first phases of ripening, berries constantly undergo physiological changes (Coombe, 1987). Berries change from a green colour to a yellow-green colour (in the case of white grape cultivars) or into different shades of red (in the case of red grape cultivars). Véraison is also characterised by berry softening and accumulation of hexose-, glucose-, fructose-sugars, potassium, and phenolics, particularly anthocyanins in the case of red grape

cultivars (Somers, 1976; Coombe & McCarthy, 1997). Changes in grape berry colour occur gradually and not all grape bunches or individual berries within a single grape bunch or vine change colour simultaneously. The most widespread flavonoids in grape skins of both red and white grapes are flavonols, flavan-3-ols (catechins), anthocyanins, and to a lesser extent flavanonols.

2.7.4 Evolution of phenolic compounds in grapes

Grape phenolic compound concentrations are affected by the interaction of the environment (*terroir*) and cultivation practices (Barbagallo *et al*., 2011). Increased phenolic compound concentrations in grapes are associated with increased light exposure of grape bunches, moderate canopy porosity and moderate crop level (Gladstones, 1992; Jackson & Lombard, 1993). Hunter *et al*. (1995), Price *et al*. (1995), and Dokoozlian & Kliewer (1996) showed that canopy microclimate (exposed- or dense canopies) affects the phenolic concentrations of berry skin. Pereira *et al.* (2006), Nadal & Hunter (2007), Hunter *et al.* (2010a), Zorer *et al.* (2013) and Hunter *et al*. (2016) also highlighted row orientation, vine spacing and canopy porosity, including row width and vine height, as factors that may impact on the microclimate in the canopy. Phenolic compound concentrations are also affected by grape cultivar (Ribéreau-Gayon *et al.*, 2006).

Flavonoids effect red wines (red grapes) more than white wines (white grapes). Total flavonoids constitute more than 85% of the phenolic content (≥ 1000 mg/L) in red wines (Jackson, 2000). In white wine, flavonoids typically constitute less than 20% of the total phenolic content (≤ 50 mg/L) (Jackson, 2000). Anthocyanins are present in red grape cultivars, whereas white grape cultivars lack this class of phenolic compounds in the skin.

The degree to which phenolic compounds are extracted from grape must during wine production depends primarily on maceration time, *i.e.* conventional maceration, carbonic maceration or thermovinification (Jackson, 2000). Phenolic compound concentrations are also affected by *inter alia* pH, sulphur dioxide content, fermentation temperature, fermentation time, and ethanol content.

2.7.4.1 Flavonols and anthocyanins

Flavonols and anthocyanins are present in the cellular vacuoles of the grape skin (Coombe & McCarthy, 1997). Flavonols may also be deposited in the grape stem and rachis tissue. The synthesis of both flavonols and anthocyanins is activated by direct exposure of the grape berries to UV and blue radiation. However, anthocyanin synthesis is directly related to the onset of véraison. The anthocyanin contents of grapes increase as berries develop/mature and increase in total pulp soluble solids (TSS, includes sugar content). There are, however, different stages of evolution among the different groups or classes of phenolics (González-San José *et al*., 1990; Andrades & González-San José, 1995; Girard *et al.*, 2001; Mateus *et al.*, 2002; Pérez-Magariño & González-San José, 2006). The evolution of anthocyanins is strongly affected by grape cultivar, climate, soil type and viticulture practices.

Anthocyanins appear in the grape maturation period at véraison as anthocyanin monoglucosides and their respective acylated derivatives, such as acetylated-, coumaroylated- and caffeoylated anthocyanins (Mazza *et al*., 1999; Bisson, 2001; Kennedy *et al*., 2002; Mateus *et al.,* 2002). Accumulation of monomeric anthocyanins occurs in three phases. Anthocyanins show in the first stage of berry development a slow increase in concentration, followed by a rapid, linear increase and ending in a stabilisation stage before the characteristic post- maturation decrease. The acetylated and coumaroylated anthocyanins are most prevalent in the second and third stages. In Syrah grapes, the concentration of flavonols in flowers was high, decreased between flowering and berry set, and then remained relatively constant through berry development (Downey *at al.* 2003a). However, on a per berry basis, the total concentration of flavonols increased during ripening, suggesting a second period of synthesis after véraison. In Syrah grapes, the FLS (flavonol synthase/flavanone 3-hydroxylase) gene was highly expressed around flowering and then decreased to low levels at véraison before increasing again in the last 3-4 weeks of ripening (Downey *et al*., 2003a).

2.7.4.2 Phenolic acids

Grape phenolic acids are initially synthesised from phenylalanine (Packter, 1980). As with anthocyanins and flavonols, phenolic acids are present primarily in the cell vacuoles of grape cells and are therefore easily extracted during grape crushing (Jackson, 2001). The most abundant and variable phenolic acids in the grape tissue are derived from hydroxycinnamic- and hydroxybenzoic acids (Hrazdina *et al*., 1984). They occur esterified to sugars, various alcohols, and organic acids. Examples are caftaric-, coutaric-, and fertaric acids, *i.e.* tartaric acid esters of caffeic-, *p*-coumaric- and ferulic acids. In the presence of the methyl esterase enzyme, these tartaric acid esters are broken down into their monomeric forms. The *o*-diphenol caftaric acid, which is released resulting from methyl esterase activity, plays an important role in oxidative browning and phenol polymerisation in grape must (Jackson, 2000).

2.7.4.3 Flavan-3-ols (proanthocyanidins)

Flavan-3-ol production occurs primarily in grape stems, skins, and seeds (Thorngate, 1992; Jackson, 2000). They are present as both free monomers and polymerised as condensed tannins. Flavan-3-ols can also be further polymerised in wine to condensed tannins (Ribéreau-Gayon *et al.,* 2006). Grape tannins consist mainly of catechin, epicatechin and gallated epicatechin subunits (Jackson, 2000). Grape skin tannins were shown to differ from grape seed tannins primarily by the presence of prodelphinidins, but also by their higher mean degree of polymerisation and lower concentrations of galoylated derivatives (Souquet *et al*., 1996). Kennedy *et al*. (2002) and Downey *et al*. (2003b) showed that most proanthocyanidin biosynthesis occurs prior to véraison.

Condensed tannins are highly reactive and do not occur as glycosylated forms, unlike flavonols and anthocyanins (Margalit, 2004; Andersen & Markham, 2007).

At véraison, most of the grape seeds are fully developed in terms of phenolics (Ribéreau-Gayon *et al.*, 2006). A decrease in flavan-3-ol biosynthesis occurs after véraison (Packter, 1980; Coombe & McCarthy, 1997). It is, however, still unclear to what extent grape seed flavan-3-ols evolve during berry development (González-San José *et al*., 1990a; Mazza *et al*., 1999; Goldner & Zamora, 2010; Rinaldi *et al.*, 2014). According to Czochanska *et al.* (1979), Romeyer *et al.* (1986) and Rinaldi *et al.* (2014), easily extractable seed flavonoids, *i.e.* flavan-3-ol monomers decrease and low molecular weight proanthocyanidins (procyanidins) increase in concentration during berry development. This could be attributed to the fact that catechin polymers, *i.e.* procyanidins, increase in concentration. In the grape berry skin, flavan-3-ols continue to accumulate during berry development. Fournand *et al.* (2006) however reported that grape-berry skin flavan-3-ols remain constant during berry development when expressed on a per berry basis.

2.7.5 Grape ripeness and wine quality

Numerous ripeness indices have been investigated in an attempt to quantify grape berry ripeness (Du Plessis & Van Rooyen, 1982; Du Plessis, 1984; Marais *et al*., 1992; Bisson, 2001; Hellman, 2004; Hunter *et al*., 2004).

These indices include analysis of flavour constituents, phenolic compound concentrations, polysaccharides, potassium, titratable acidity, nitrogenous compounds, turbidity, TSS, and pH. The ratio between degrees Brix (sugar content of an aqueous solution) and titratable acidity (concentration of acid present in a solution - TA) (°B:TA) is another measurable parameter for the determination of grape ripeness (Du Plessis & Van Rooyen, 1982; Hunter *et al*., 2004).

Grape juice component indices such as °B/H^+ (*i.e.* hydrogen ion concentration which relates to wine quality) and °B/pH have been considered as parameters for grape quality (Du Plessis & Van Rooyen, 1982). An average stage of maturity can also be deduced from the attainment of a concentration plateau of summed amino acids (Du Plessis, 1984). Du Plessis (1984) showed that pectin levels in Sauvignon blanc and Sémillon grapes increased to a maximum before decreasing to a minimum during maturation. Suspended material in grape must at levels of 20-30 g/L before fermentation was shown to be an important parameter with respect to wine quality (Marais *et al*., 1999).

Ripeness indices, such as berry skin colour, colour of seeds, assimilable nitrogen, general condition of fruit, and condition of the vine, are also parameters to consider as wine quality indicators (Bisson, 2001). The proportion of anthocyanins, *i.e.* monomers, acetylated and coumaroylated anthocyanins can affect both the hue and colour stability of red wine and is therefore positively associated with overall wine quality (Du Plessis, 1984; Andersen & Markham, 2007; Gawel & Godden, 2008; Baiano, 2015). The proportion of anthocyanins present in flavylium cationic form is primarily affected by pH, which is in turn positively associated with potassium concentrations. Time of harvest is an important aspect to consider for wine quality.

Terpene and pyrazine compounds are also considered as parameters of wine quality (Marias & Swart, 1999). The carbohydrate accumulation in grape bunches, as manipulated by canopy management for the formation of secondary metabolites, is essential for grape ripeness indices (Marais & Swart, 1999). The monitoring of morphological and physiological parameters in the canopy and grapes is a critical aspect in identifying indicators that can be associated with a particular grape cultivar and wine style (Hunter *et al*., 2004). Ripeness indices to consider would therefore be a combination of physiological-, biochemical- and physical changes in the vine and the grapes. There is, however, not a single set of indices that would define berry ripeness for a particular grape cultivar under all circumstances and for all purposes. Sensory techniques have mainly been used to support research protocols in viticulture and oenology (Francis *et al.*, 2004). Wine quality is difficult to qualify, however wine tasting ensures that tasting data are collected in the least biased way that can be analysed and interpreted statistically (Petit & Sieffermann, 2007). This is accomplished by using protocols minimising physiological and psychological factors known to affect human sensory responses and utilizing assessors, which are highly sensitive to sensory stimuli and able to evaluate their perception analytically and objectively (Lesschaeve, 2007).

There are a number of sensory techniques, i.e. good sensory evaluation practices; samples are always presented uniformly, in identical containers coded with random numbers to prevent bias from extraneous clues such as brand or treatment (Lesschaeve &, 2010); Tasting panel, *i.e.* an analytical sensory panel is generally formed of eight to 20 individuals (Meilgaard *et al*., 2007). Sensory methodologies, *i.e.* these methods are categorised into two main types, firstly, the analytical test, which answers one of the following questions (King, 2007): Is there a difference? What is the difference? How large is the difference? Secondly, the hedonic test assessing consumer acceptance and overall preference.

Statistical methods are usually applied to the sensory data; *i.e.* discrimination tests, threshold tests, intensity ranking tests, descriptive analysis which includes conventional profiling methods and free-choice profiling (Perin *et al*., 2008).

2.7.6 Effect of light (microclimate/row orientation) on grape phenolic compounds

Grape growers seek to minimize the heterogeneity of grape material within a single vineyard in order to improve wine quality (Downey *et al.*, 2004; Hunter *et al*., 2010b). Environmental factors, such as topography, soil type, and soil variation are parameters that affect the growth variation amongst vines (Jackson & Lombard, 1993; Downey *et al.*, 2004; Hunter *et al.*, 2010b), whereas other long term practices, such as row orientation, and short term practices, such as canopy management, would primarily affect the canopy microclimate and exposure of grape bunches (Zorer *et al*., 2013; Hunter *et al*., 2016; Zorer *et al*., 2017) (Fig. 2.14). Ambient photosynthetic active radiation measured on top of grapevine canopies was highest during the months of November to January (Southern hemisphere); it decreased during the season as canopies developed and generally peaked just after mid-day (Hunter *et al.*, 2016).

Seasonal patterns of photosynthetic active radiation (PAR) received in bunch zones at microclimate level after being filtered by the canopy showed that, *e.g.*, EW row orientated grapevines maintained lower interior canopy interception than other row orientations (Fig. 2.14).

Grapevines planted to NS orientation displayed highest values in the form of two clear peaks in the morning and in the afternoon, respectively, whereas NE-SW and NW-SE orientations showed peaks primarily in the afternoon and morning, respectively.

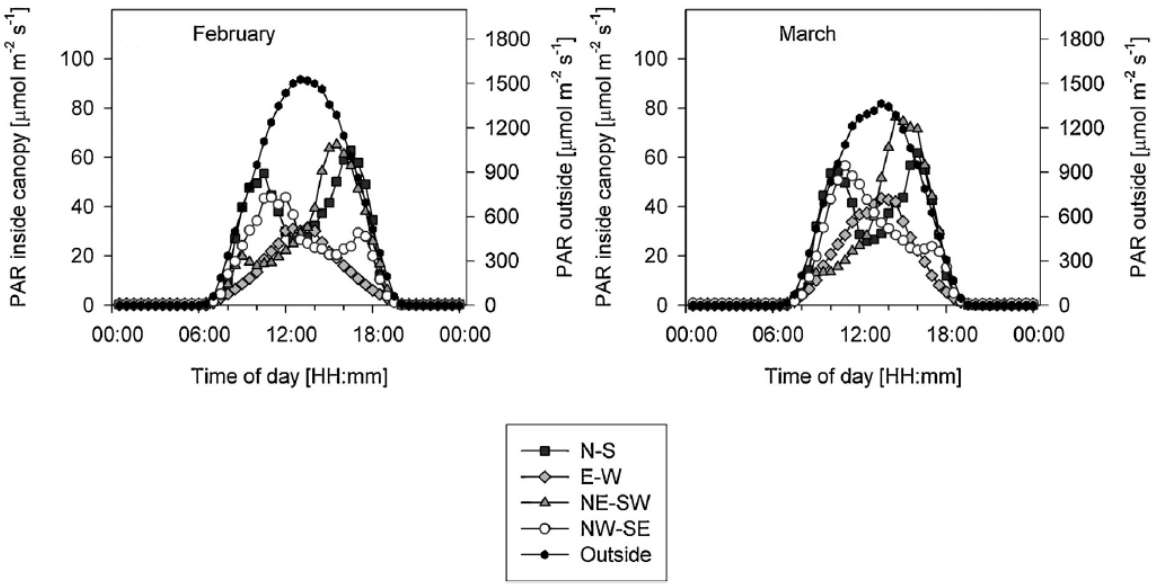


Figure 2.14 Micro hourly mean photosynthetic active radiation for February and March of the Syrah/101-14 Mgt vineyard planted to four different row orientations at Robertson experimental farm of the ARC Infruitec-Nietvoorbij (Hunter *et al.*, 2016).

Factors affecting the grape chemical composition and variability thereof in the vineyard are *terroir*, grape microclimate and viticulture practices (Dokoozlian & Kliewer, 1996).

Shaded or dense canopies with limited interior light exposure are further sources of grape composition variability (Gladstones, 1992). Grape bunches that develop in more open canopy conditions, as opposed to those that develop in shaded or dense canopies, have higher grape juice sugar concentrations, lower juice pH and often increased concentrations of berry skin anthocyanins (Dokoozlian & Kliewer, 1996; Downey *et al*., 2004). Increased concentrations of glycosylated aroma precursors in grapes are indicative of grape bunches exposed to excessive light and elevated daytime temperatures (Baumes *et al*., 2002; Ristic *et al*., 2007).

Jocelyne *et al.* (2007) showed that Syrah grapes originating from dense or shaded canopies are generally lower in TSS content at a given time, compared to grapes originating from light-exposed canopies or low vigour vines. Contrary to Jocelyne *et al.* (2007), Kocsis *et al*. (2008) found that "Furmint" vines planted to an EW row orientation yielded grapes with higher TSS content (sugar) and lower titratable acidity (acid content), compared

to grapes originating from the same vines planted to a NS orientation. Czemmel *et al*. (2009) also showed that microclimate in general affects the TSS content of grape berries.

Ambient and grape-bunch temperature variations during berry development can affect the phenolic content of grapes (Andrades & González-San José, 1995; Mateus *et al*., 2002; Sadras & Moran, 2012; De Oliveira & Nieddu, 2013; Bonada *et al*., 2015). In red grape cultivars such as Syrah, Merlot, Cabernet Sauvignon and Pinot noir, the changes in grape phenolic compound concentrations that occur during berry development have been emphasised as an important aspect in the ripening process (Jackson & Lombard, 1993; Coombe & McCarthy, 1997; Pérez-Magariño & Gonzalez-San José, 2006). This ultimately affects wine quality.

2.7.6.1 Phenolic acids

Benzoic- and cinnamic acids are the third most abundant group of phenolic compounds in grapes (Singleton *et al*., 1986). Price *et al.* (1995) reported that caftaric acid concentrations in Pinot noir wines were inversely related to grape light exposure. Decreased concentrations of caftaric acid in wines from light-exposed grapes appear to be related to the hydrolysis of the tartaric acid esters. Pinot noir wines made with highly light-exposed grape bunches proved 50% higher in caffeic acid concentrations, compared to wines made from grapes that were moderately exposed. In the Northern hemisphere, Friedel *et al.* (2012, 2015) showed that early leaf removal of Riesling grapevines planted to EW row orientations resulted in an increase in the concentrations of hydroxycinnamic acids.

2.7.6.2 Flavonols

Flavonols are one of the most widespread families of phenolics present in grape skin and pulp as well as the leaves of both red and white cultivars, and provide protection against ultra-violet radiation (Downey *et al*., 2006; Ribéreau-Gayon *et al.*, 2006). These compounds are readily measured in grapes, because they are indicators of grapes that have been exposed to excessive light or light in the canopy during anthocyanin biosynthesis and after the induction of véraison (Price *et al*., 1995; Bergqvist *et al.*, 2001; Spayd *et al*., 2002). Increased concentrations of flavonols in Cabernet Sauvignon and Grenache grapes originating from California are also associated with the north-facing canopy side of grapevine rows planted to EW row orientations (Bergqvist *et al*., 2001). Flavonols are thought to act as UV light protectants and free radical scavengers and may contribute to grape (and wine) quality through copigmentation with anthocyanins (Downey *et al*., 2004); this copigmentation results in the increase of vitisin A (pyranoanthocyanins) during aging of bottled wine (Schwarz *et al.*, 2005). Wines with high concentrations of pyruvic acid usually undergo a constant formation of vitisin A during aging (Fulcrand *et al.*, 1998; Asenstorfer *et al*., 2003). Increased flavonol concentrations in Shiraz grapes also translate into increased concentrations in the wine (Ristic *et al*., 2007). According to Downey *et al.* (2003a) and Bonada *et al*. (2015), the principal determining factor for variation in flavonol concentrations of different red

grape cultivars is the transcript levels of flavonol biosynthetic genes. Light and temperature in the canopy are secondary parameters affecting the flavonoid biosynthesis pathway.

Indeed, Cortell & Kennedy (2006), Baiano *et al*. (2015), and Moreno *et al*. (2015) found a positive correlation between light-exposed grapes (partially defoliated vines) and increased flavonol concentrations in Pinot noir, Tempranillo, and Nero di Troia grapes. This positive correlation indicates that biosynthesis of flavonols in grapes of these cultivars is dependent on changes in light exposure of grape bunches with limited effect from water deficit (water status) (Cortell & Kennedy, 2006; Feng *et al*., 2015). This observation may likely be applicable to other red grape cultivars. Castillo-Muñoz *et al*. (2007), Ristic *et al*. (2007) and Czemmel *et al.* (2009) confirmed that Syrah grapes exposed to intense light are conducive to an increase in the concentrations of flavonols during the growth cycle of the grapevine. For example, quercetin 3-*O*-glucoside concentrations per berry were higher in grapes from light-exposed canopies (moderate to high exposure), compared to grapes originating from shaded or dense canopies.

2.7.6.3 Flavan-3-ols

In contrast to the flavonols, direct light or light exposure reportedly has little effect on the grape seed flavan-3-ol content (Haselgrove *et al.,* 2000). On the other hand, grape-skin proanthocyanidin concentrations of Cabernet Sauvignon increased in reaction to direct light exposure of grape bunches (Downey *et al.,* 2004; Cortell & Kennedy, 2006; Ferrandino & Guidoni, 2010; King *et al*., 2014). Ristic *et al*. (2007) reported that Syrah grapes originating from shaded or dense canopy conditions, show increased concentrations of seed proanthocyanidins and decreased concentrations of skin proanthocyanidins. According to Ojeda *et al*. (2002) and Castellarin *et al.* (2006), concentrations of proanthocyanidins remained low in Shiraz grape skin originating from shaded or dense canopies, compared to grapes originating from exposed canopies. Concentrations of skin proanthocyanidin per berry mass were higher in Pinot noir grape berries originating from low vigour vines or partially defoliated canopies (Cortell *et al*., 2007). Such canopies are inevitably more exposed to light. There is however not clear consensus in the literature regarding the concentrations of proanthocyanidins in grapes as affected by viticultural practices. This may also point to the fact that different compounds were investigated in the different studies.

Flavan-3-ols, proanthocyanidins, (-)-epigallocatechin and polymerised flavan-3-ols are also affected by viticultural/environmental factors and water deficits resulted in the decrease of concentrations of flavan-3-ol monomers (Kennedy *et al.,* 2000).

2.7.6.4 Anthocyanins

The biosynthesis of anthocyanins is dependent on the expression of a UDP glucose-flavonoid-3-*O*-glucosyl transferase gene, involved in glycosidation (Jackson, 2000; Downey *et al*., 2003b).

Proportions and concentrations of anthocyanins as well as variation in their accumulation depend on grape cultivar (through genetic factors) (Wenzel *et al.*, 1987; Smart *et al*., 1988). Viticulture practices, degree of grape berry ripeness, temperature around the fruit zone, microclimate and *terroir* are also factors that affect anthocyanin formation/concentration (González-San José *et al.*, 1990; Hunter et al., 1991; Mazza & Francis, 1995; Mazza *et al.*, 1999; Kennedy *et al*., 2001; Pérez-Magariño *et al.*, 2006; Bonada *et al.*, 2015; Gil *et al.*, 2015; Song *et al*., 2015; Hunter *et al*., 2016).

Exposure of grapevines and grape bunches to light (defoliation) during the growth cycle of the vine is led to an increase in concentrations of anthocyanins and flavan-3-ols in Shiraz and Tannat grapes (Hunter *et al*., 2007; Joscelyne *et al*., 2007; Czemmel *et al.*, 2009; Boido *et al*., 2011). Studies conducted by Price *et al.* (1995), Baiano *et al*. (2015) and Song *et al*. (2015) showed that grape bunches originating from moderately light-exposed canopies or more open canopies are higher in glycosylated anthocyanins (monomers), compared to grapes originating from shaded or dense canopies. Cabernet Sauvignon grapes originating from light-exposed or open canopies were higher in total anthocyanin concentrations during the initial stages of berry ripening (22°Brix), compared to grapes originating from dense canopies (Dokoozlian & Kliewer, 1996). Cortell *et al*. (2007) also reported a decrease in concentrations of anthocyanins in grapes originating from high vigour zones.

Contrary to the above, in some studies increased light exposure of the vine and grape bunches decreased anthocyanin accumulation in the grape berry skin, compared to grapes originating from dense canopy conditions (Macheix *et al*., 1990; Gladstones, 1992; Haselgrove *et al*., 2000; Downey *et al*., 2006).

Syrah grapes harvested at *ca*. 22°Brix ripeness level, originating from light-exposed canopies, proved higher in total anthocyanin content, compared to grapes growing in closed or dense canopies (Haselgrove *et al*., 2000). Grapes harvested from the same vineyard at a ripeness level above 22°Brix, showed a decrease in total anthocyanin levels, whereas anthocyanin concentrations in grapes harvested from both exposed and dense canopies at *ca*. 26°Brix showed no significant differences in total anthocyanin concentrations. According to Haselgrove *et al.* (2000), the concentrations of malvidin 3-*O* glucoside were higher in light-exposed grapes, compared to grapes from shaded or dense canopy grapevines during berry ripening (*i.e.* 0 to 35 days after véraison). On the other hand, the authors reported that malvidin 3-*O*-(6-*p*-coumaroyl) glucoside concentrations were higher in grapes originating from dense or shaded canopies, compared to grapes from light-exposed canopy vines. Acetylated derivatives of anthocyanins were also higher in grapes from dense or shaded canopy treatments 35 days after véraison, compared to grapes from open canopies. Mori *et al*. (2007) and Jogaiah *et al*. (2013) showed that Cabernet Sauvignon grapes exposed to light contain higher concentrations of glycosylated anthocyanins in particular malvidin 3-*O*-glucoside.However, elevated temperatures caused a decrease in anthocyanin concentrations, particularly acetylated and coumaroylated anthocyanins. Studies conducted by Bergqvist *et al*. (2001), Spayd *et al.* (2002), Jeong *et al*. (2004) and Tamborra *et al*. (2014) showed that light exposed grapes at *ca*. 24°Brix grape ripeness had

higher total anthocyanin concentrations, compared to grapes from dense canopies. However, acetylated anthocyanin concentrations in particular proved low.

Downey *et al*. (2004) found that total anthocyanin concentrations were lower in Pinot noir and Cabernet Sauvignon grapes originating from exposed canopies or low vigour vines, compared to Merlot and Syrah grapes subjected to the same treatment. Anthocyanin concentrations in Pinot noir grapes were however unaffected when grape bunches were exposed to day time temperatures between 30°C and 35°C, while further anthocyanin formation was inhibited in Cabernet Sauvignon, Merlot and Syrah grapes exposed to temperatures above 35°C (Mori *et al*., 2005; Downey *et al.*, 2006).

Ristic *et al*. (2007) and Tarara *et al.* (2008) reported that Syrah and Merlot grapes originating from dense canopies and harvested from the east side of the canopy, *i.e.* vines planted to NS row orientations (southern hemisphere), had higher anthocyanin accumulation at a maximum daytime temperature of *ca.* 35°C, compared to grapes originating from the west side of the canopy. Accumulation of total anthocyanins in shaded *versus* exposed grapes was similar during berry development (Gil-Muñoz *et al*., 2010). However, shading did alter the individual anthocyanin pigments, *i.e.* cyanidin-, peonidin- and malvidin 3-*O*-glucosides positively in grape berries, whereas sunlight exposure of grape bunches decreased delphinidin- and petunidin 3-*O*-glucosides. Grape bunch shading significantly increased both the concentrations of acetylated- and coumaroylated anthocyanins in Shiraz grapes, confirming the effect of light and temperature on anthocyanin biosynthesis (Bonada *et al*., 2015). There appears to be a point at which the daytime temperature detrimentally affects anthocyanin biosynthesis in grape cultivars, that being above 35°C (Mori *et al*., 2005; Tarara *et al.*, 2008).

Anthocyanin profiles of different grape cultivars are relatively stable, but the absolute concentrations can vary between vintages within the same grape cultivar due to environmental and agronomical factors (Gil-Muñoz *et al*., 2010). Variation in anthocyanin concentration due to temperature and light fluctuation is evident among different grape cultivars, although the precise dependence of the major grape attributes on the environment is still unclear (Koundouras *et al.*, 2006; Chorti *et al*., 2010; Bonada & Sadras, 2014; Bonada *et al*., 2015).

An increase in the accumulation of anthocyanins occurs in grapes of Cabernet Sauvignon vineyards (Mori *et al*., 2005) and autochthonous grape cultivars, *i.e.* Jaén tinto, Palomino Negro and Tintilla de Rota (Guerrero *et al*., 2009) with lower day-night temperatures, compared to grapevines subjected to higher day-night temperatures. Yamana *et al.* (2006) showed that increased anthocyanin biosynthesis occurs in "Aki Queen" grape cultivars (*Vitis vinifera* x *Vitis labrusca*) under low temperature conditions, independent of light intensities. The concentrations of anthocyanins increase relative to an increase in light intensity, whereas an increase in berry temperature, results in a decrease in anthocyanin accumulation and even degradation of anthocyanins (Downey *et al*., 2006; Guerrero *et al*., 2009).

Increases in Cabernet Sauvignon (Mori *et al.*, 2005) and Merlot (Tarara *et al*., 2008) grape skin temperatures of grapes originating from low vigour vines (exposed canopies), resulted in a decrease in anthocyanin

concentrations, compared to grapes originating from shaded or dense canopies. Tarara *et al.* (2008) reported that anthocyanin accumulation and the anthocyanin profile of Merlot grapes appeared to be determined by the synergistic combination of solar radiation and grape berry temperature.

Grapes originating from shaded or dense canopies (northern hemisphere) showed lower levels of 3'-hydroxylated anthocyanins, *i.e.* cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside, compared to grapes originating from light-exposed or open canopies (Chorti *et al.*, 2010). Nebbiolo grapes originating from vines exposed to western light (orientated to NS direction), proved high in total anthocyanin concentrations (Chorti *et al*., 2010). Fruit-zone leaf removal increased light exposure but did not alter anthocyanin accumulation. In contrast to Chorti *et al.* (2010), Joscelyne *et al.* (2007) and Cohen *et al.* (2008) reported that low daytime temperature conditions (southern hemisphere) and high vigour vines (dense canopies) can result in grapes with high concentrations of 3'-hydroxylated anthocyanins. Grape cultivars sensitive to direct light are those with a high proportion of 3'-hydroxylated anthocyanins. In many of these studies, lack of clear definition regarding low/high temperature or open/dense canopies may lead to discrepancies in interpretation of the data.

Pinot noir grapes originating from light-exposed canopies or open canopies planted in NS row directions showed an increase in anthocyanin accumulation (Song *et al*., 2015; Baiano *et al*., 2015), whereas anthocyanins measured in Syrah grapes originating from vines planted in NS and EW directions, showed no significant differences in concentrations (Giacosa *et al.*, 2015).

Anthocyanin concentrations in Nebbiolo grapes originating from NE-SW row orientations with south exposed vineyards in Italy varied between vintages (Guidoni *et al.*, 2008). Cyanidin 3-*O*-glucoside concentrations (3'-hydroxylated) varied in grapes between vintages, whereas 3',5'-dimethoxylated substituted anthocyanins, *i.e.* malvidin 3-*O*-glucoside and the acylated derivatives, were unaffected by seasonal variations in south exposed canopies. Guidoni *et al.* (2008) also reported that cyanidin 3-*O*-glucoside (*i.e.* 3'-hydroxylated) and peonidin 3-*O*-glucoside (*i.e.* 3'- methoxylated) anthocyanin concentrations were higher in Nebbiolo grapes in south exposed canopies, compared to delphinidin (*i.e.* 3',5'-dihydroxylated), petunidin (3'-hydroxy-,5'-methoxylated) and malvidin (3',5'-dimethoxylated) anthocyanins. Hunter & Volschenk (2008) demonstrated that grapes originating from EW and NW-SE row orientations differed in grape berry skin anthocyanins.

Irrigation affects vine physiology, which may directly or indirectly affect yield and grape composition (Cacho *et al.*, 1992; Esteban *et al*., 2001; Bonada *et al.*, 2015; Nelson *et al.*, 2016). Climatic conditions, such as precipitation and prevailing wind, which have a direct effect on the evaporative demand/index and the microclimate of the vine, can also modulate the anthocyanin profile of a grape cultivar and affect vine vegetative behaviour (Cacho *et al.*, 1992; Hunter *et al.*, 2016).

The literature reviewed provides evidence that the concentrations of anthocyanins are determined by a complex interplay of the effects of solar radiation (direct light) and diurnal temperature. Generally, the judicious exposure of grape bunches to light is receptive to increased anthocyanin levels, whereas elevated daytime temperatures

negatively affect the anthocyanins of the grape berry skin. Anthocyanin concentrations in grapes are also affected by factors such as climatic conditions, grape cultivar, degree of ripeness and vineyard practices.

2.7.6.5 Proanthocyanidins

Cortell *et al.* (2007) demonstrated that differences in skin proanthocyanidin concentrations in berries are evident in grapes originating from low vigour vines, compared to grapes originating from high vigour vines. However, wines made from grapes originating from low *versus* high vigour vines proved similar in total grape proanthocyanidin concentrations (Kennedy *et al*., 2001; Cadot *et al.,* 2006). Accumulation of proanthocyanidins in grapes originating from both low and high vigour vines occurs at the same time during berry development (Bergqvist *et al*., 2001; Cortell & Kennedy, 2006; Hunter *et al.,* 2007; Joscelyne *et al.,* 2007; Ristic *et al.,* 2007; Chorti *et al*., 2010; Rustioni *et al.,* 2011). The accumulation of proanthocyanidins is also affected by day-night temperature differences and low night temperatures result in high levels of proanthocyanidins (Mori *et al.,* 2005). Mori *et al*. (2005) and Guerrero *et al.* (2009) reported that high concentrations of anthocyanins were also present in grapes exposed to low day-night temperature conditions, compared to grapes exposed to high day-night temperatures. A reduction in the concentrations of grape proanthocyanidins is evident during warm seasons with temperatures in excess of 35°C (Spayd *et al*., 2002; Mori *et al*., 2005; Downey *et al.,* 2006; Guerrero *et al.,* 2009). Contrary to the above, Cohen *et al*. (2012) showed that artificially heated Merlot grape berries originating from NS row directions were higher in proanthocyanidin concentrations, compared to artificially cooled-down grape berries originating from the same row direction.

Syrah and Pinot noir grapes harvested from low vigour vines proved higher in skin proanthocyanidins and higher in polymeric pigment (*i.e.* anthocyanins bound to tannins) concentration, but lower in colour density (total anthocyanins), compared to high vigour vines (Downey *et al*., 2004; Cortell *et al*., 2007). Grapes originating from low vigour vines may therefore result in improved overall wine quality, considering the contribution of polymeric pigments to wine quality (Guerrero *et al*., 2009).

2.7.7 Wine chemical composition

The chemical composition of red wine includes the primary metabolites, *i.e.* sugars, organic acids, amino acids as well as secondary metabolites, such as flavonoids and non-flavonoids (Gawel, 1998). However, the vast majority of chemical compounds present in wine are the metabolic by-products of yeast activity during fermentation (Jackson, 2000). The most common aromatic compounds present in wine are fusel alcohols, volatile esters and fatty acids (Etiévant, 1991). Carbonyls, phenolics, lactones, terpenes, acetals, hydrocarbons, sulphur and nitrogen compounds are also present in wine, but in low concentrations (Singleton & Noble, 1976). The levels of these compounds would differ according to viticultural practices and grape cultivar and would as such be transferred into the wine during the vinification process.

Grape-derived secondary metabolites (anthocyanins, proanthocyanidins, and monomeric flavan-3-ols) are the principal sources of wine colour and wine stability (Koundouras *et al.*, 2006) and affect the bitterness, astringency, taste, and mouth feel attributes (Robichaud & Noble, 1990; Guinard & Mazzucchelli, 1996). These compounds ultimately affect the quality of wine (Gawel & Godden, 2008).

The anthocyanins are directly associated with wine colour and well related to wine quality (Ribéreau-Gayon, 1964). Flavonols contribute to the yellow colour of white wines.

The addition of SO_2 during vinification, adjustment of pH, duration of skin contact (maceration time) and heat treatment (thermovinification) all have an effect on the phenolic compound concentrations of wine (Somers & Evans, 1977; Amerine *et al*., 1980; Somers & Wescombe, 1982).

Clearly, the phenolic compounds are responsible for some of the most important quality attributes of wine (Margalit, 2004). The proportion of different classes of phenolic compounds in wine varies according to the type of vinification process (Mané *et al.*, 2007). Vinification conditions, such as maceration time and maceration temperature affect the phenolic content of red wine (Jackson, 2000). Vinification techniques *i.e.* pump over instead of punch-down, rotating tanks, thermovinification and carbonic maceration can have an effect on the phenolic concentrations of wine.

Phenolic acids, *i.e.* cinnamic- and benzoic acid derivatives, are present in the pulp or juice of the grape (Kennedy *et al*., 2002; Cheynier *et al*., 2006). The contribution of grape skin to the total flavan-3-ol composition of wine is dependent on the vinification process (Mané *et al*., 2007). During the vinification, the process of maceration or "skin contact" contributes to the concentrations of phenolics in red wine. The skins contribute to the presence of flavan-3-ols (monomers) and proanthocyanidins (condensed tannins) in wine during maceration (Soleas *et al*., 1998; Fulcrand *et al.*, 2006). Ristic *et al.* (2007) showed that a positive relationship exist between grape-skin proanthocyanidin concentration and wine proanthocyanidin concentration.

2.7.8 Wine quality

The quality parameters of grapes and red wine remain deep colour, full body, mouth feel, soft tannins, and fruity aromas (González-San José *et al.*, 1990; Joscelyne *et al.*, 2007; Gil-Muñoz *et al*., 2010; Ristic *et al*., 2010; Boido *et al*., 2011; Sadras *et al*., 2012; Kumšta *et al.*, 2014; Bonada *et al.*, 2015; Song *et al*., 2015). Additionally, soluble phenolic compounds can play a role in certain sensory attributes in determining wine quality (Coombe & McCarthy, 1997; Zoecklein *et al*., 1998; Margalit, 2004; Cheynier *et al*., 2006; Kumšta *et al.*, 2014; Moreno *et al.*, 2015). Phenolic compounds can also be used in the prediction of the sensory properties and oxidative stability of wine (Boselli *et al*., 2006).

Phenolic compounds occur in grapes in free and glycosylated forms, but their proportion is not directly related to wine organoleptic properties (Ribéreau-Gayon *et al*., 2006).

Wine colour is directly related to the type of vinification process applied. The colour of red wine is one of the principal quality variables measured during sensory evaluation. (Somers, 1976; Cliff *et al*., 2007; Ristic *et al*., 2007; Jensen *et al*., 2008; Gil-Muñoz *et al*., 2010; Boido *et al*., 2011; Kumšta *et al.*, 2014). Grape skin anthocyanins are therefore one of the principal measurable variables for the prediction of wine quality (Francis *et al*., 2004; Ristic *et al*., 2010). The potential extractability of anthocyanins (colour pigments) and flavan-3-ols during the vinification process from the grape skin into the must is directly related to the maceration or "skin contact" time and the pH of the must (Somers, 1976; Jackson, 2000; Gil-Muñoz *et al.*, 2010). The presence of copigmentation cofactors, mainly monomeric flavan-3-ols, flavonols and hydroxycinnamic acids (*p*-coumaric- and caffeic acids) or the conjugation of anthocyanins and hydroxycinnamic acids, prevent hydration reactions involving anthocyanins, thereby enhancing the colour intensity (Boulton, 2001; Gil-Muñoz *et al*., 2010; He *et al*., 2012). Accordingly, colourless flavonoids, such as proanthocyanidins, can also contribute indirectly to wine colour and eventually to wine quality (Pérez-Magariño & González-San José, 2002; Rodríguez-Montealegre *et al.* 2006). Anthocyanin extractability is also directly related to fermentation conditions (other than skin contact and pH) and external conditions such as water deficit and climate that in turn affects berry colour density (Somers, 1976; Johnstone *et al*., 1995; Rustioni *et al.*, 2011). Red wine "quality" or different red wine styles are generally associated with different grape ripeness levels (Nadal & Hunter, 2007). In high alcohol wines (over-ripe grapes), anthocyanin concentration and organoleptic properties were not associated with wine quality.

Holt *et al*. (2008) reported that Cabernet Sauvignon grapes subjected to different pruning treatments did not necessarily affect wine anthocyanin concentrations and wine sensory attributes. Fermentation conditions and winemaking procedures can however have a positive effect on anthocyanin extraction from grape skin, consequently resulting in intense red wine colour and improved wine overall quality (Boulton, 2001).

Hunter *et al.* (1991, 1995) examined relationships between grape berry composition and wine quality and sensory attributes. Sensory attributes, which are indicative of wine quality, are however subjective. Nevertheless, there is consensus among researchers regarding the correlation between sensory attributes and wine quality (Francis *et al*., 2004; Preys *et al.*, 2006; Joscelyne *et al*., 2007; Gil-Muñoz *et al*., 2010; Kumšta *et al.*, 2014).

Grape berry size at maturity can affect the overall quality of red wine (Roby & Matthews, 2004; Hunter *et al*., 2010b; Gil *et al*., 2015) and is a factor in determining wine quality (Kennedy *et al.*, 2002; Bravo *et al.*, 2006). Vine water deficit generally leads to smaller berries (Ojeda *et al.*, 2001). Although berry size affects grape quality, little is known about berry size variability in the vineyard and the impact of environmental factors and viticultural practices on the berry variability (Barbagallo *et al.*, 2011). Variability in berry size can lead to changes in grape and wine anthocyanin and flavan-3-ol composition (Mateus *et al.*, 2002; Pérez-Magariño & González-San José, 2004; Pérez-Magariño & González-San José, 2006; Gil *et al*., 2015; Melo *et al.*, 2015) and can affect red wine quality through changes in the skin/flesh ratio and the modification of the levels of flavonoids and non-flavanoids extracted from the skins during skin contact (Roby *et al*., 2004; Roby & Matthews, 2004).

Light manipulation (leaf removal) and altered light quality (UV radiation-reducing sheets) in the fruit zone can positively affect wine sensory properties and therefore improve wine quality (Šuklje *et al.*, 2014).

Koundouras *et al*. (2006) examined the effects of water deficit on wine phenolics, wine sensory attributes (aroma components), and wine quality of Agiorgitiko grapes. Water deficit during the growth period resulted in increased concentrations of total phenolics and anthocyanins in berry skins. Limited water availability also increased the aroma profiles of the wines, resulting in improved wine quality as evaluated by a sensory panel. Vine water status can be highlighted as an essential factor in predicting and determining wine quality.

Bonada *et al*. (2015) showed that water deficit leads to intensely coloured red Syrah wines with improved flavour profiles under moderate temperature conditions. Water status imposed by soil and climate parameters correlated with a high quality potential for Agiorgitiko grape cultivar in Greece (Koundouras *et al.*, 2006). Early water deficit during the growth period showed beneficial effects on anthocyanin- and total phenolic concentrations. The effect of water status on grape quality is linked to the limiting effect of low water uptake on vine vigour rather than the reduction in berry mass. Low vine water status induces early shoot growth cessation, improved microclimate, and accelerates the grape ripening process (Koundouras *et al*., 2006; Hunter *et al*., 2016). This leads to increased berry phenolic concentrations.

Hunter *et al*. (1991) and Johnstone *et al.* (1995) indicated that there is no linear or simple relationship between grape composition and wine quality. Guidoni *et al*. (2008) showed that this non-linear relationship is *inter alia* due to the complexity and behaviour of anthocyanins and their derivatives in grape cultivars and during vinification.

The literature reviewed provides evidence that wine quality is dependent on berry size, water deficit of the vine and fermentation conditions, among many other parameters. The contradicting statements made by different authors can be attributed to many factors, including differences in experimental designs, analytical measurements used and geographical location of experimental sites. It is clear that obtaining knowledge of the relationship between the quality of a particular wine and its phenolic composition is at present one of the major challenges in oenology research (Garrido & Borges, 2013).

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Chapter 3

CHROMATOGRAPHIC METHODOLOGY

3. Chromatographic Methodology

Methodology to quantify selected anthocyanins, flavonols, flavan-3-ols and phenolic acids in Syrah (*Vitis vinifera* L. cv.) grapes and wines using RP-HPLC-DAD

3.1 Introduction

Grape phenolic compounds can be divided into two groups, namely non-flavonoids (hydroxybenzoic- and hydroxycinnamic acids and stilbenes) and flavonoids (anthocyanins, flavanols, flavones, flavanonols and flavonols) (Singleton & Esau, 1969; Ribéreau-Gayon *et al*., 2006). These phenolics contribute to the sensory properties and quality of red and white wine (Ribéreau-Gayon *et al*., 2006).

Anthocyanins are a class of phenolics that are directly responsible for the colour of red grapes and young red wines (Ribéreau-Gayon *et al.*, 2006). During wine production and ageing, grape-derived anthocyanins are altered by a range of reactions involving proanthocyanidins (Ribéreau-Gayon *et al*., 2006), anthocyanin dimers and higher oligomers (Vidal *et al*., 2004; Boido *et al*., 2006) as well as acetaldehyde, pyruvic acid and cinnamic acids (*i.e*. coumaric-, ferulic-, caffeic- and synaptic acids) (Fulcrand *et al*., 1996; Fulcrand *et al*., 1999; Remy *et al*., 2000; Pissarra *et al*., 2003; Schwartz *et al*., 2005), amongst others.

Flavan-3-ols, including monomeric catechins and oligomeric proanthocyanidins, contribute to the perceived astringency, bitterness, complexity, mouth feel, body, and structure of especially red wine (Gawel, 1998; Jackson, 2000; Del Alamo-Sanza *et al*., 2004; Ribéreau-Gayon *et al.*, 2006; Gómez-Alonso *et al*., 2007). Flavan-3-ols also affect the oxidative state and therefore the clarity of wine and are involved in reactions with anthocyanins that lead to the stabilisation of the colour of red wine (Macheix *et al*., 1991; Ribéreau-Gayon *et al*., 2006).

Wine flavonols include quercetin, myricetin, kaempferol, isorhamnetin, and their glycosides (Plumb *et al*., 1999; Boulton *et al*., 2001; Schwarz *et al*., 2005; Gómez-Alonso *et al*., 2007). These compounds contribute to bitterness, affect white wine colour and display antioxidant activity.

The non-flavonoid group of phenolics, *i.e*. hydroxycinnamic- and hydroxybenzoic acids are mainly present in grapes in glycosidic forms (Ribéreau-Gayon *et al.*, 2006). Free forms of benzoic acids, such as gallic-, *p*-hydroxybenzoic- and protocatechuic acids are prevalent in red wine (Cheynier *et al*., 1989). Cinnamic acids mainly occur in esterified form, for example with tartaric acid (caffeoyl-tartaric- and *p*-coumaroyl-tartaric acids) or

anthocyanins (acylated anthocyanins). Cinnamic acids are highly oxidisable, and contribute to the browning of white grape must (Ribéreau-Gayon *et al*., 2006).

The concentrations of anthocyanins, flavonols, flavanols, and phenolic acids in wine are mainly determined by grape cultivar and environmental factors (Ribéreau-Gayon *et al.*, 2006), which affect grape berry development (Broussaud *et al*., 1999; Ojeda *et al*., 2002; Rodriguez-Montealegre *et al*., 2006). Other factors, which affect grape phenolic compounds, include viticultural practices, and grape ripeness levels (Monagas *et al.*, 2003; Barbagallo *et al*., 2011).

Increased phenolic compound concentrations in grapes are associated with moderate canopy light microclimate and moderate crop level (Jackson & Lombard, 1993; Downey *et al.*, 2004; Hunter *et al*., 2010b; Bonada *et al*., 2015). Price *et al*. (1995), Hunter *et al*. (1995) and Dokoozlian & Kliewer (1996) showed that canopy microclimate could affect the phenolic compound concentrations of grape skins. Furthermore, vinification processes play an important role in the extraction of phenolic compounds from grapes and their further stability in wine (Ribéreau-Gayon *et al.*, 2006). Maceration time, fermentation temperature, fining, and bottle ageing are all factors that affect the phenolic compound concentration of wine (Gawel, 1998).

Initially, the quantification of phenolic compounds in grape extracts and wine has been performed using a number of spectroscopic (Markham, 1982; Gíustí & Wrolstad 2001; Andersen & Markham, 2007) or chromatographic analytical techniques (Liazid *et al.*, 2010; Fanzone *et al*., 2011; Perestrelo *et al.*, 2012; Sánchez-Ilárduya *et al*., 2012; Ramirez-Lopez *et al.*, 2014; Moreno *et al*., 2015; Nelson *et al*., 2016). However, with time HPLC with UV-visible detection became the standard method for the quantification of individual wine phenolics (Cartoni *et al*., 1991; Fiorini, 1995; Nogata *et al*., 1994; Waterhouse *et al*., 1999).

This mode of detection provides relatively selective and sensitive quantification of anthocyanins and to some extent flavonols (Downey & Rochfort, 2008; Von Baer *et al*., 2008; Baiano *et al*., 2015; Garaguso & Nardini, 2015; Nelson *et al*., 2016).

Grape and wine phenolics are commonly separated and quantified using either normal phase (NP) or reversed-phase (RP) HPLC, with the latter mode being mostly used (Nogata *et al*., 1994; Cheynier *et al*., 1999; Waterhouse *et al*., 1999; Heier *et al*., 2002; De Villiers *et al*., 2005; Kallithraka *et al.*, 2005; Kelebek *et al*., 2007; Versari *et al*., 2008; Abad-García *et al*., 2009; Perestrelo *et al.*, 2012; Favre *et al*., 2014; Fontana *et al*., 2014; Ferreira *et al*., 2016; Nelson *et al*., 2016; Sen & Tokatli, 2016).

The introduction of photodiode-array detection (DAD) technology has significantly improved the analysis of phenolics, since this enables the distinction of various classes of phenolic compounds on the basis of their characteristic UV-visible spectra (Santos-Buelga & Williamson, 2003; Nelson *et al*., 2016) and enables simultaneous recording of chromatograms at multiple wavelengths for selective detection (Andersen & Markham, 2007; Garaguso & Nardini, 2015). Indeed, RP-HPLC-DAD is nowadays commonly used to quantify phenolics in grape extracts and wine samples on a routine basis (Revilla *et al*., 1999; De Villiers *et al.*, 2005; Castillo-Muñoz

et al., 2007; Gómez-Alonso *et al.*, 2007; Jeffery *et al*., 2008; Muñoz *et al*., 2008; Abad-Garcia *et al.*, 2009; De Villiers *et al.*, 2009; Liazid *et al*., 2010; Fanzone *et al*., 2011; Lorrain *et al.*, 2011; Papoušková *et al*., 2011; Perestrelo *et al*., 2012; Salvatore *et al*., 2013; Favre *et al.*, 2014; Garaguso & Nardini, 2015; Ferreira *et al*., 2016).

The HPLC-electrospray ionisation mass spectrometry (ESI-MS) method was successfully used in the identification of phenolics in grape extracts (Baldi *et al*., 1995). Since then the technique has become an established tool for wine and grape phenolic analysis (Cameira-dos- Santos *et al*., 1996; Liazid *et al.*, 2010; Fanzone *et al*., 2011; Perestrelo *et al.*, 2012; Sánchez-Ilárduya *et al*., 2012; Ramirez-Lopez *et al.*, 2014; Nelson *et al*., 2016), especially due to the inherent power of MS for structural elucidation purposes (Núñez *et al.*, 2004; De Villiers *et al*., 2005; He *et al*., 2006; Andersen & Markham, 2007; Guerrero *et al.*, 2009; Alberts *et al*., 2012; He *et al*., 2012; Zhu *et al*., 2012).

Electro spray ionisation in negative ionisation mode is usually used for neutral phenolics, such as flavan-3-ols, flavan-3,4-diols, flavones and flavonols (which are detected as the deprotonated $[M-H]^-$ species), and non-flavonoids, such as phenolic acids and stilbenes (Cheynier *et al.*, 2003; Castillo-Muñoz *et al.*, 2007). These compound classes have been quantified in grapes (Perestrelo *et al.*, 2012; Ramirez-Lopez *et al.*, 2014). The negative mode is also more suitable for the detection of anthocyanin-flavanol adducts in which the anthocyanin moiety is not in the cationic form (Abad-Garcia *et al.*, 2009). Detection of anthocyanins in their cationic forms is performed using ESI in the positive ionisation mode with highly acidic mobile phases (Revilla *et al.*, 1999; Ivanova *et al*., 2011; Perestrelo *et al*., 2012).

The aim of this study was to conduct an in-house validation of a published RP-HPLC-DAD method (Waterhouse *et al*., 1999) for the quantification of selected anthocyanin, flavan-3-ol, flavonol and phenolic acid compounds in lyophilised Syrah grape skin extract and young non-commercial Syrah wines with direct injection with a multi-wavelength detection function. Chapters 4 and 5 report quantitative data for these compounds in the grape and wine samples. A generic HPLC-ESI-MS method with direct injection was used to confirm the identities of selected phenolics.

3.2 Materials and methods

3.2.1 Grape and wine samples

Syrah grape (harvested at *ca.* 24.0°Brix) extract and wine samples were randomly selected from a batch of lyophilised grape skin extracts and young non-commercial Syrah wine samples (ARC, Infruitec-Nietvoorbij) for the purpose of identification of the phenolic compounds present in the samples, retention time confirmation, and validation of the HPLC technique. Grape extract and wine samples were filtered through 0.22 µm Nylon membrane syringe filters (Separations, Johannesburg, South Africa) prior to reversed-phase HPLC analysis. The grape skin extraction and winemaking procedures are described in Chapters 4 and 5, respectively.

3.2.2 Reagents and standards

Acetonitrile, *ortho*-phosphoric acid, and methanol (HPLC grade) were purchased from Merck, Johannesburg, South Africa. De-ionised water was supplied through a Modulab water purification system, supplied by Separations, Johannesburg, South Africa. Phenolic standards were purchased from Sigma-Aldrich (Darmstadt, Germany) and Sigma-Fluka (Johannesburg, South Africa) as well as Extrasynthese (Genay, France). Table 3.1 lists the phenolic standards with their catalogue numbers, percentage purity, and supplier name.

Table 3.1 Phenolic standards used for the validation of the RP-HPLC DAD method.

Compound compounds	Catalogue no.	Purity	Supplier
Flavan-3-ols			
(+)-Catechin	43412	> 99%	Sigma-Fluka, Johannesburg, South Africa
(-)-Epicatechin	E1753	> 95%	Sigma-Fluka, Johannesburg, South Africa
Epigallocatechin 3-*O*-gallate	E4143	> 95%	Sigma-Aldrich, Darmstadt, Germany
Phenolic acids			
Gallic acid	14291-5	97%	Sigma-Aldrich, Darmstadt, Germany
Caffeic acid	60018	99%	Sigma-Fluka, Johannesburg, South Africa
p-Coumaric acid	C9008	> 95%	Sigma-Aldrich, Darmstadt, Germany
Ferulic acid	46278	99%	Sigma-Fluka, Johannesburg, South Africa
Flavonols			
Quercetin 3-*O*-rutinoside	R5143	95%	Sigma-Aldrich, Darmstadt, Germany
Kaempferol	K-0133	90%	Sigma-Aldrich, Darmstadt, Germany
Quercetin 3-*O*-glucoside	9006	≥ 95%	Extrasynthese, Genay, France
Quercetin 3-*O*-rhamnoside	Q3001	85%	Sigma-Aldrich, Darmstadt, Germany
Quercetin	Q4951	≥ 95%	Sigma-Aldrich, Darmstadt, Germany
Anthocyanins			
Delphinidin 3-*O*-glucoside	73705	97%	Sigma-Fluka, Johannesburg, South Africa
Cyanidin 3-*O*-glucoside	44689	95%	Sigma-Fluka, Johannesburg, South Africa
Petunidin 3-*O*-glucoside	30638	95%	Sigma-Fluka, Johannesburg, South Africa
Peonidin 3-*O*-glucoside	0929	95%	Extrasynthese, Genay, France
Malvidin 3-*O*-glucoside	04288	95%	Sigma-Fluka, Johannesburg, South Africa

3.2.3 Chromatographic conditions and instrumentation

The HPLC-DAD separations were performed on a SpectraSYSTEM HPLC instrument (Thermo Separations Products, Inc., New Jersey, USA) equipped with an autosampler (injection volume 20 µL) using a published method of Waterhouse *et al.* (1999). Ultra-violet visible spectra were recorded for anthocyanins, flavan-3-ols, flavonols, and phenolic acids. Anthocyanins were detected at 520 nm, flavan-3-ols at 280 nm, flavonols at 360 nm and phenolic acids at 316 nm. Detection range was between 190 and 950 nm (DAD). ChromQuest software was utilised for data acquisition and construction of calibration curves. Separation was performed at *ca*. 22°C, using a

polystyrene divinylbenzene RP analytical column (PLRP-S 100 Å, 5 µm, 250 × 4.6 mm), supplied by Agilent Technologies, Santa Clara, USA. Gradient elution was performed using mobile phases comprising water/phosphoric acid [985:15 v/v (pH *ca*. 1.35) - eluent A] and water/phosphoric acid/acetonitrile [185:15:800 v/v/v (pH *ca.* 1.25) - eluent B]. The gradient programme is listed in Table 3.2. The column was equilibrated for 20 minutes after each injection and the flow rate was 1 mL/min.

Table 3.2 Gradient programme used for the HPLC-DAD separation of grape and wine phenolics.

Time (min)	Eluent A composition (%)	Eluent B composition (%)
0	94.00	6.00
73	69.00	31.00
78	38.00	62.00
86	38.00	62.00
90	94.00	6.00

Anthocyanins, flavan-3-ols, flavonols and phenolic acid compounds in the tested grape and wine samples were confirmed by using the area response of individual compounds extrapolated from the corresponding calibration curves based on their spectral data and retention times and expressed in mg/L.

3.2.4 Method validation

The HPLC-DAD method was validated in terms of linearity, precision, sensitivity, detection, and quantification limits.

3.2.4.1 Calibration and detection limits

Stock solutions of individual flavan-3-ols, flavonols and phenolic acid standards were prepared by dissolving the standards in eluent A (water/phosphoric acid 985:15 v/v) and methanol (MEOH concentration <5%). Anthocyanin standards were prepared in eluent A only (1.5% aqueous phosphoric acid). Concentrations of the standards (4 mL total vol.) are listed in Table 3.3. The working standard solutions were prepared by sequential dilutions of the stock solutions with eluent A. Each working solution was placed in an ultrasonic bath for approximately 1 min before analysis. Calibration curves for each compound were constructed with the respective correlation coefficients (R^2) calculated by least-squares linear regression analysis. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the residual standard deviation of a regression line for each compound (equation 3.1 to 3.6) (Singh, 2013). Limit of quantitation is the lowest concentration of a particular compound that can be detected and quantified (Saadati *et al*., 2013). This translates to a signal to noise ratio with the minimum concentration, which when injected gives a minimum detectable peak area.

$$Sa = \frac{Serror}{intercept} \; X \; 100 \qquad 3.\,1$$

$$\boldsymbol{YLOD = a + 3Sa} \qquad 3.\,2$$

$$\boldsymbol{YLOQ = a + 10Sa} \qquad 3.\,3$$

$$\boldsymbol{YLOD = b\,XLOD + a} \qquad 3.\,4$$

Therefore: $XLOD = \frac{YLOD - a}{b}$ 3. 5

$$XLOQ = \frac{YLOQ - a}{b} \qquad 3.\,6$$

Where: Sa: Standard deviation of the regression

$S_{error:}$ Standard error of the intercept

LOD: Limit of detection

LOQ: Limit of quantitation

Y: Denotes absorbance (mAu)

X: Denotes concentration (mg/L)

a: The intercept of the calibration curve

b: The slope of the calibration curve

Table 3.3 Individual phenolic compound concentration data (mg/L) in mixed standard stock solution.

Phenolic compounds	Mass weighed (mg)	Concentration (mg/L)
Caffeic acid	1.40	350.00
Ferulic acid	2.00	500.00
p-Coumaric acid	1.40	350.00
Gallic acid	1.40	350.00
(+)-Catechin	1.80	450.00
(-)-Epicatechin	1.70	425.00
Epigallocatechin 3-*O*-gallate	1.30	325.00
Quercetin 3-*O*-rutinoside	1.40	350.00
Quercetin 3-*O*-rhamnoside	1.30	325.00
Quercetin 3-*O*-glucoside	1.40	350.00
Quercetin	1.70	425.00
Kaempferol	1.40	350.00
Delphinidin 3-*O*-glucoside	1.00	250.00
Cyanidin 3-*O*-glucoside	1.00	250.00
Petunidin 3-*O*-glucoside	1.40	350.00
Peonidin 3-*O*-glucoside	1.10	275.00
Malvidin 3-*O*-glucoside	1.90	475.00

3.2.4.2 Repeatability

The stock solutions described in section 3.2.4.1 were used for repeatability studies of flavan-3-ols, flavonols, phenolic acid, and anthocyanins. Six replicate wine samples (6 x 1000 µL) were spiked with 50 µL each of a 1:1 dilution of standard stock solution mixture of caffeic acid, ferulic acid, gallic acid, *p*-coumaric acid, (+)-catechin, (-)-epicatechin, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-glucoside, quercetin and kaempferol. Additionally, wines were also spiked with 100 µL each of undiluted stock solution of quercetin-3-*O*-rutinoside, epigallocatechin-3-*O*-gallate, and the individual anthocyanins. The final concentrations of the phenolic standards in the spiked wine samples are listed in Table 3.4. A 20 µL volume of phenolic concentrations of standard solution mixture (1:1

diluted and undiluted) (Table 3.4) as well as spiked wine samples (Table 3.4) were separately injected on the same day as well as over 3 consecutive days to assess intra- and inter-day repeatability. Precision of the method was evaluated based on intra- and inter-day repeatability and was assessed by replicate (n = 6) measurements of each compound. Variance between repetitions was expressed as percentage relative standard deviation (% RSD).

Table 3.4 Phenolic compound concentration data (mg/L) in spiked young non-commercial Syrah wines.

Phenolic compounds	**Phenolic concentrations (1:1 diluted) of standard solution mixture (mg/L)**	**Spiked Syrah wine (50 µL) concentration (mg/L)**
Caffeic acid	175.00	8.75
Ferulic acid	250.00	12.50
p-Coumaric acid	175.00	8.75
Gallic acid	175.00	8.75
(+)-Catechin	225.00	11.25
(-)-Epicatechin	212.50	10.62
Quercetin 3-*O*-rhamnoside	162.50	8.12
Quercetin 3-*O*-glucoside	175.00	8.75
Quercetin	212.50	10.62
Kaempferol	175.00	8.75
Epigallocatechin 3-*O*-gallate	325.00	32.50
Quercetin 3-*O*-rutinoside	350.00	35.00
Delphinidin 3-*O*-glucoside	250.00	25.00
Cyanidin 3-*O*-glucoside	250.00	25.00
Petunidin 3-*O*-glucoside	350.00	35.00
Peonidin 3-*O*-glucoside	275.00	27.50
Malvidin 3-*O*-glucoside	475.00	47.50

3.2.5 Analysis of Syrah grape skin extract and Syrah wine samples using HPLC

The identification of the phenolic compounds in a Syrah grape extract (n = 1) and a Syrah wine (n = 1) sample was confirmed by comparing retention times and UV-visible spectra with the standards.

3.2.6 Analysis of Syrah wine using HPLC-ESI-MS

The HPLC-ESI-MS analyses were performed on a UPLC instrument equipped with a binary solvent manager, sample manager, column oven and DAD detector, interfaced through an ESI source to a Synapt G2 quadrupole time-of-flight (Q-TOF) mass spectrometer (Waters, Milford, MA, USA). The mass spectrometer operated in the positive ionisation mode, scanning from 50 to 1500 *m/z*. The ionisation parameters were as follows: capillary voltage of 2.5 kV and sampling cone voltage of 35 V.

The source and desolvation temperatures were 120°C and 275°C, respectively. The desolvation gas flow was 650 L/h and the cone gas flow 50 L/hour (both N_2). A young, non-commercial Syrah wine sample was analysed using the same column as used for the HPLC-DAD method. To ensure compatibility with MS detection, the phosphoric acid mobile phases were replaced by formic acid phases (Downey & Rochfort, 2008): eluent A was 7.5% (v/v) formic acid in water and eluent B 7.5% formic acid in acetonitrile. The high concentration of formic acid employed in this methodology was necessary to ensure good peak shape and peak resolution (De Villiers *et al*., 2011). Gradient separation was performed using the following elution conditions at a flow rate of 0.025 mL/min: 4-35% B (0-35 min), 35-100% B (35-36 min), 100% B for 5 min. Analyses were done at *ca*. 50°C. A 10-µL aliquot of a filtered wine sample was injected. The UV-visible detection was performed at 499 nm, 280 nm, 360 nm and 316 nm, and the flow was split 1:2 before the mass spectrometer. Peak identification was based on accurate mass information, relative retention times, and fragmentation patterns, compared to literature (De Villiers *et al.*, 2004; Abad-García *et al*., 2009; Barnes & Schug, 2011; Willemse *et al.*, 2013).

3.3 Results and discussion

3.3.1 Method validation results

The HPLC method was validated in terms of linearity, limits of detection, and limits of quantitation using the phenolic standards for quantitative purposes (Table 3.5). The R^2 values for all phenolic compounds were greater than 0.9980, confirming the linearity of the method.

The LOQ values were below 1 mg/L for all compounds, indicating the suitability of the method for the quantification of phenolic compounds in grapes and wine.

Table 3.5 Summary of the calibration and sensitivity data for phenolic standards obtained using the HPLC-DAD method.

Phenolic compounds	[1]DW/QW nm	Regression equation	R^2	Range (mg/L)	[2]LOD (mg/L)	[3]LOQ (mg/L)
Gallic acid	280/271	y = 20807x - 10313	0.9998	27.355 to 435.525	0.113	0.204
Caffeic acid	316/276	y = 70046x - 56086	0.9997	6.505 to 216.505	0.032	0.063
(+)-Catechin	280/ 295	y = 57409x - 11519	0.9997	9.350 to 300.001	0.124	0.414
(-)-Epicatechin	280/276	y = 70602x - 33063	0.9989	9.350 to 300.000	0.130	0.434
Epigallocatechin 3-*O*-gallate	280/274	y = 14019x - 99455	0.9998	10.155 to 325.015	0.062	0.209
p-Coumaric acid	316/323	y = 39815x + 36881	0.9981	11.705 to 375.005	0.168	0.560
Ferulic acid	316/321	y = 32142x - 48820	0.9996	18.750 to 350.000	0.015	0.051
Quercetin 3-*O*-rutinoside	360/355	y = 18437x + 31082	0.9994	25.005 to 200.001	0.147	0.492

[1]DW/QW = Detection wavelength/quantification wavelength in nm; [2]LOD = Limit of detection; [3]LOQ = Limit of quantitation.

Table 3.5 Continued.

Phenolic compounds	**[1]DW/QW nm**	**Regression equation**	***R*²**	**Range (mg/L)**	**[2]LOD (mg/L)**	**[3]LOQ (mg/L)**
Quercetin 3-*O*-glucoside	360/355	y = 17649x + 19026	0.9986	13.250 to 212.005	0.236	0.787
Quercetin 3-*O*-rhamnoside	360/355	y = 16562x + 25271	0.9999	16.250 to 260.001	0.269	0.899
Kaempferol	360/337	y = 38663x - 67253	0.9999	25.005 to 320.005	0.255	0.521
Quercetin	360/369	y = 11384x - 75348	0.9998	5.005 to 80.001	0.235	0.455
Delphinidin 3-*O*-glucoside	520/516	y = 54849x - 10206	0.9997	15.650 to 250.005	0.125	0.326
Cyanidin 3-*O*-glucoside	520/510	y = 60218x - 10804	0.9998	15.650 to 250.001	0.145	0.344
Petunidin 3-*O*-glucoside	520/500	y = 42672x - 17078	0.9997	21.850 to 350.005	0.147	0.455
Peonidin 3-*O*-glucoside	520/512	y = 51738x - 11557	0.9999	17.150 to 275.001	0.168	0.561
Malvidin 3-*O*-glucoside	520/520	y = 48522x - 27112	0.9998	29.650 to 475.005	0.148	0.452

[1]DW/QW = Detection wavelength/quantification wavelength in nm; [2]LOD = Limit of detection; [3]LOQ = Limit of quantitation.

3.3.1.1 Repeatability results

Precision of the retention times and concentrations of the phenolic compounds were determined for qualitative and quantitative method evaluation. For this purpose, phenolic standards and spiked wine samples were injected six times on one day to assess intra-day reproducibility and inter-day repeatability over three days. The results are listed in Tables 3.6 and 3.7, respectively. The method showed acceptable repeatability of peak areas (concentrations), as evident from relatively low % RSDs for six repeats (Table 3.6), indicating good precision of the method.

Table 3.6 Inter-day repeatability of phenolic standard concentrations (mg/L) and spiked young non-commercial Syrah wine samples (mg/mL) for flavan-3-ols, flavonols, anthocyanins and phenolic acids, listing mean and percentage relative standard deviation data. Retention time repeatability in %RSD is also reported.

Phenolic compounds	[1]RT (%RSD)	Inter-day repeatability of pure standards						Inter-day repeatability of spiked Syrah wine samples					
		Day 1 (n = 6)		Day 2 (n = 6)		Day 3 (n = 6)		Day 1 (n = 6)		Day 2 (n = 6)		Day 3 (n = 6)	
		Mean	% RSD	Mean	% RSD	Mean	% RSD	Mean	% RSD	Mean	% RSD	Mean	% RSD
Gallic acid	8.249 (1.484)	35.854	5.382	35.643	3.882	36.953	4.762	16.773	2.763	16.753	2.004	16.903	1.893
Caffeic acid	30.798 (1.392)	89.412	4.433	92.123	4.393	92.693	3.303	6.353	2.602	6.644	2.625	6.284	2.313
(+)-Catechin	28.061 (1.481)	61.272	2.164	59.842	3.743	59.315	3.722	16.512	3.223	16.785	2.455	16.664	2.014
(-)-Epicatechin	32.228 (1.150)	96.651	3.983	90.782	4.147	95.852	3.256	19.594	3.653	19.775	2.112	19.449	2.115
EGCG[2]	44.489 (1.043)	84.871	3.752	86.741	4.418	86.962	3.216	49.788	3.457	50.013	3.223	49.663	3.116
p-Coumaric acid	47.154 (1.404)	143.963	3.213	154.412	2.163	156.551	1.577	82.223	3.685	86.083	2.347	88.172	1.682
Rutin[3]	46.456 (0.913)	105.374	3.571	107.802	3.473	104.705	3.194	88.163	3.669	88.284	2.339	88.543	3.453
Ferulic acid	52.242 (0.720)	115.803	3.202	116.946	3.511	121.381	3.375	4.716	3.834	4.732	4.287	5.014	3.931
Quercitrin[4]	50.788 (0.806)	95.672	3.541	97.747	4.482	97.838	4.433	6.929	3.902	6.921	4.085	6.991	4.548
Quercetin 3-*O*-rhamnoside	60.519 (0.720)	128.726	3.013	129.438	3.359	129.849	3.102	25.792	2.351	24.713	3.364	26.082	3.709
Quercetin	80.354 (0.644)	321.946	1.973	326.748	1.277	329.443	1.572	45.893	4.082	44.803	4.807	45.901	3.713
Kaempferol	81.655 (0.651)	906.586	0.941	910.573	1.074	920.073	1.281	26.451	5.116	24.446	3.447	26.667	3.456
Delphinidin 3-*O*-glucoside	23.189 (0.585)	276.642	1.771	281.634	1.463	273.672	1.591	333.996	2.982	316.256	2.983	302.471	3.215
Cyanidin 3-*O*-glucoside	27.388 (0.860)	294.292	1.412	292.714	1.664	294.012	1.615	360.524	2.563	347.482	2.692	338.852	2.424
Petunidin 3-*O*-glucoside	30.528 (0.730)	403.661	1.036	407.652	1.057	406.015	1.614	467.629	1.892	464.214	2.113	447.093	1.842
Peonidin 3-*O*-glucoside	34.728 (0.549)	265.664	1.756	266.493	1.596	264.872	1.545	310.563	2.991	304.314	3.274	294.811	2.915
Malvidin 3-*O*-glucoside	36.419 (0.475)	462.014	1.251	463.442	1.952	461.812	1.037	549.313	1.724	513.142	1.873	505.454	1.964

[1]Retention time repeatability in % RSD; [2]Epigallocatechin 3-*O*-gallate; [3]Quercetin 3-*O*-rutinoside; [4]Quercetin 3-*O*-glucoside.

Table 3.7 Intra-day repeatability of phenolic standard concentrations (mg/L) and spiked young non-commercial Syrah wine samples for flavan-3-ols, flavonols, anthocyanins, and phenolic acids showing mean and percentage relative standard deviation. Retention time repeatability in % RSD is also reported.

Phenolic compounds	[1]RT min (% RSD)	Intraday repeatability of pure standards (n = 6)		Intraday repeatability of spiked wine samples (n = 6)	
		Mean	%RSD	Mean	%RSD
Gallic acid	8.349 (0.584)	36.152	1.9423	16.713	3.232
Caffeic acid	30.78 (0.492)	91.413	1.913	6.422	4.192
(+)-Catechin	28.361 (0.581)	60.142	1.871	16.513	3.553
(-)-Epicatechin	32.258 (1.140)	94.425	3.371	19.594	3.454
Epigallocatechin 3-*O*-gallate	44.539 (1.023)	86.194	1.335	49.783	3.782
p-Coumaric acid	47.264 (0.504)	151.641	4.447	85.492	3.551
Quercetin 3-*O*-rutinoside	46.567 (0.713)	105.952	1.536	88.161	3.457
Ferulic acid	52.452 (0.720)	118.041	2.499	4.828	4.176
Quercetin 3-*O*-glucoside	50.779 (0.706)	97.081	1.268	6.777	3.169
Quercetin 3-*O*-rhamnoside	60.519 (0.610)	129.335	0.434	25.539	3.727
Quercetin	80.449 (0.542)	326.045	1.163	45.535	4.638
Kaempferol	81.785 (0.651)	912.417	0.754	25.856	4.004
Delphinidin 3-*O*-glucoside	23.389 (0.485)	277.781	2.032	325.353	3.055
Cyanidin 3-*O*-glucoside	27.588 (0.751)	295.286	1.431	346.014	2.863
Petunidin 3-*O*-glucoside	30.648 (0.622)	406.317	1.082	495.972	2.122
Peonidin 3-*O*-glucoside	34.738 (0.349)	263.237	2.121	301.283	3.273
Malvidin 3-*O*-glucoside	36.449 (0.375)	460.619	1.216	522.852	1.654

[1]Retention time repeatability in % RSD.

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3.3.2 HPLC-DAD analysis of grape and wine samples

A total of twenty-four individual phenolic compounds were separated in Syrah grape and wine samples using the method of Waterhouse *et al.* (1999) (Table 3.8). Of these, 17 compounds were identified by comparison of retention times and UV-visible spectra with standard compounds.

The remaining compounds for which standards were not available were tentatively identified based on UV-visible spectra and MS data (Table 3.9), compared to literature (De Villiers *et al*., 2004; Liazid *et al*., 2010; Lorrain *et al.*, 2011; Fanali *et al*., 2013; Favre *et al.*, 2014; Garaguso & Nardini, 2015; Nelson *et al*., 2016). Table 3.8 lists the identified flavonols, phenolic acids, flavan-3-ols and anthocyanins with peak numbers corresponding to the chromatograms in Figures 3.1 to 3.8.

Delphinidin 3-*O*-(6-*O*-acetyl) glucoside (grape skin samples), cyanidin 3-*O*-(6-*O*-acetyl) glucoside (grape skin- and wine samples), cyanidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside (grape skin- and wine samples) and peonidin 3-*O*-

(6-*O*-*p*-coumaroyl) glucoside (grape skin- and wine samples) were not detected due to their concentrations being below the LOD of the method.

Table 3.8 Peak identification for flavan-3-ols, flavonols, phenolic acids, and anthocyanins, including anthocyanin derivatives in Syrah grape skin extract and young non-commercial Syrah wine samples as determined by HPLC-DAD as it appears in Figures 3.1 to 3.4.

Phenolic compounds	**Peak no.**	**Phenolic compounds**	**Peak no.**
Gallic acid[1]	1	Petunidin 3-*O*-glucoside[1]	15
(+)-Catechin[1]	2	Peonidin 3-*O*-glucoside[1]	16
(-)-Epicatechin[1]	3	Malvidin 3-*O*-glucoside[1]	17
Epigallocatechin 3-*O*-gallate[1]	4	Delphinidin 3-*O*-(6-*O*-acetyl) glucoside[2, 3*]	18
Caffeic acid[1]	5	Cyanidin 3-*O*-(6-*O*-acetyl) glucoside[2, 3]	19
p-Coumaric acid[1]	6	Petunidin 3-*O*-(6-*O*-acetyl) glucoside[3]	20
Ferulic acid[1]	7	Peonidin 3-*O*-(6-*O*-acetyl) glucoside[3]	21
Quercetin 3-*O*-rutinoside[1]	8	Malvidin 3-*O*-(6-*O*-acetyl) glucoside[3]	22
Quercetin 3-*O*-glucoside[1]	9	Delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside[3]	23
Quercetin 3-*O*-rhamnoside[1]	10	Cyanidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside[2, 3]	24
Quercetin[1]	11	Petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside[3]	25
Kaempferol[1]	12	Peonidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside[2, 3]	26
Delphinidin 3-*O*-glucoside[1]	13	Malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside[3]	27
Cyanidin 3-*O*-glucoside[1]	14		

[1]Compounds identified using authentic standards.[2]Delphinidin, cyanidin and peonidin derivatives were not detected in the Syrah grape and wine samples (below the limits of detection). [3]Compounds tentatively identified based on relative retention, UV-visible spectra and MS data based on literature (Revilla *et al*., 1999; De Villiers *et al*., 2004; De Villiers *et al*., 2009; Sánchez-Ilárduya *et al*., 2012). * Detected in Syrah wine samples but not in Syrah grape samples

Figures 3.1 to 3.4 depict representative examples of chromatograms at 280 nm, 316 nm, 360 nm and 520 nm obtained for the target analytes in a lyophilised Syrah grape skin extract (Figs. 3.1 and 3.2) and a young non-commercial Syrah wine sample (Figs. 3.3 and 3.4) using the validated HPLC-DAD method. Peak labels in these Figures correspond to Table 3.8.

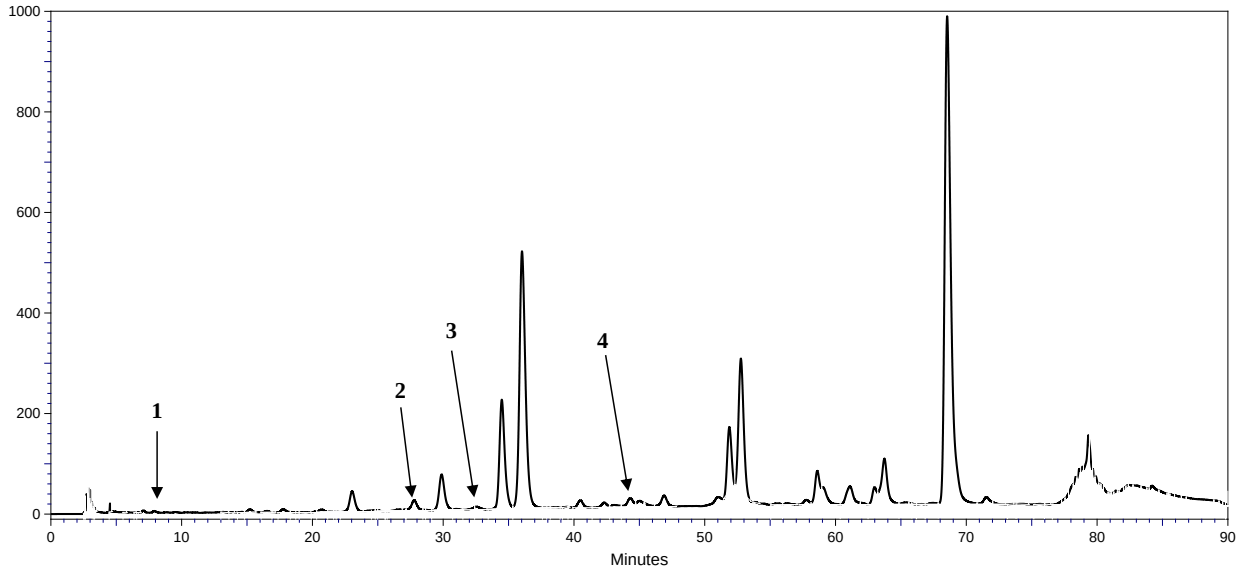

Figure 3.1 HPLC-DAD chromatogram of phenolic acids and flavan-3-ols in lyophilised Syrah grape skin extract measured at 280 nm. Refer to Table 3.8 for compound identification.

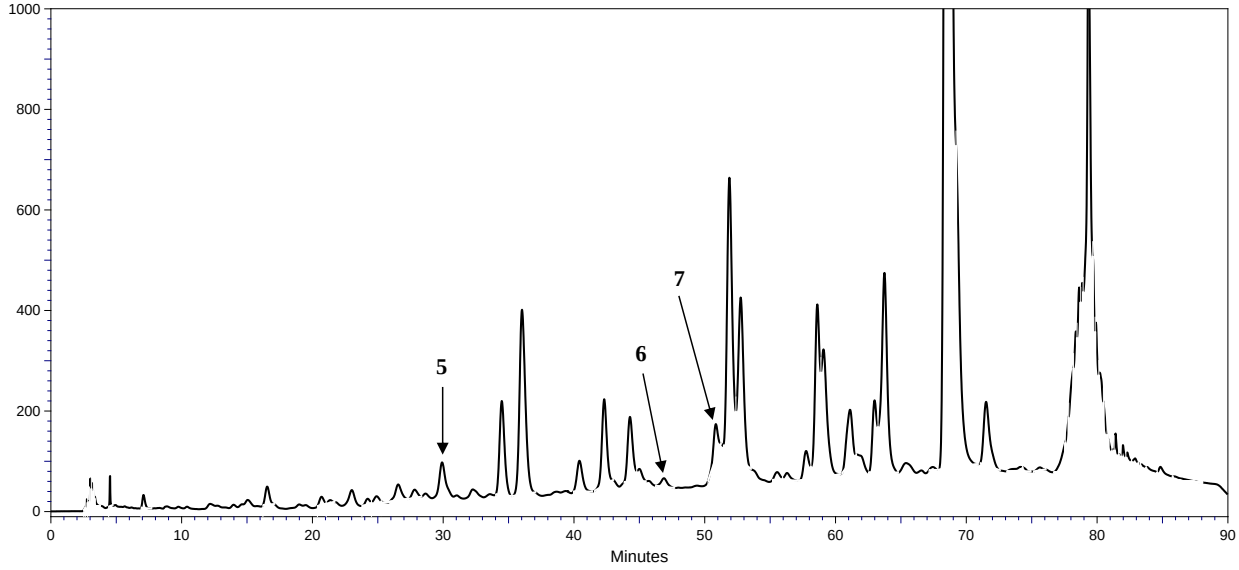

Figure 3.2 HPLC-DAD chromatogram of phenolic acids in lyophilised Syrah grape skin extract measured at 316 nm. Refer to Table 3.8 for compound identification.

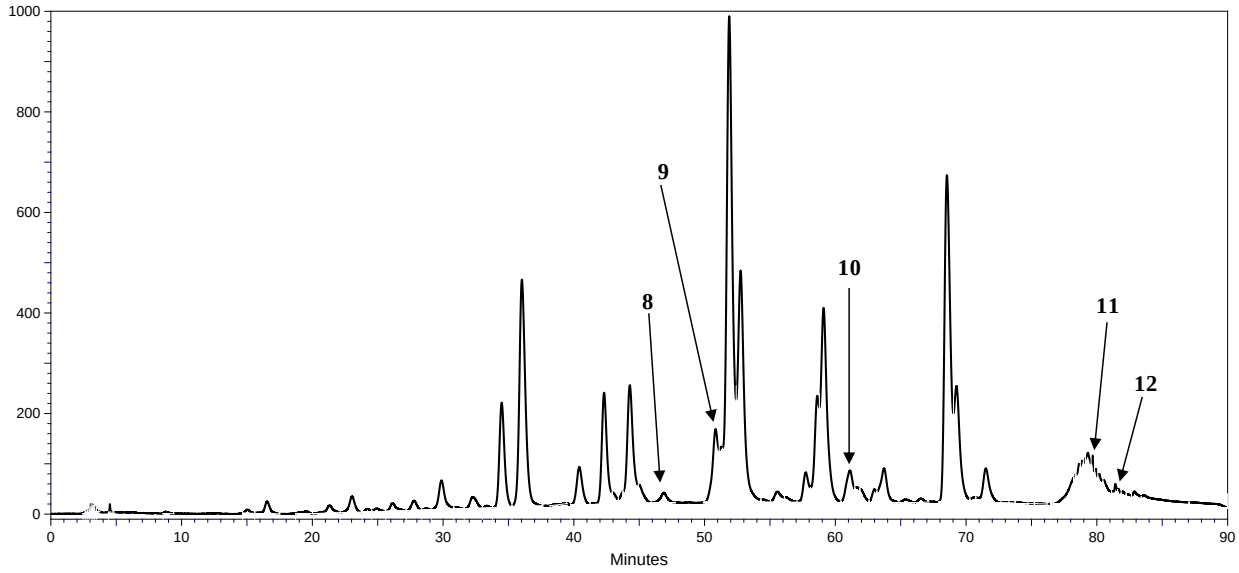

Figure 3.3 HPLC-DAD chromatogram of flavonols in lyophilised Syrah grape skin extract measured at 360 nm. Refer to Table 3.8 for compound identification

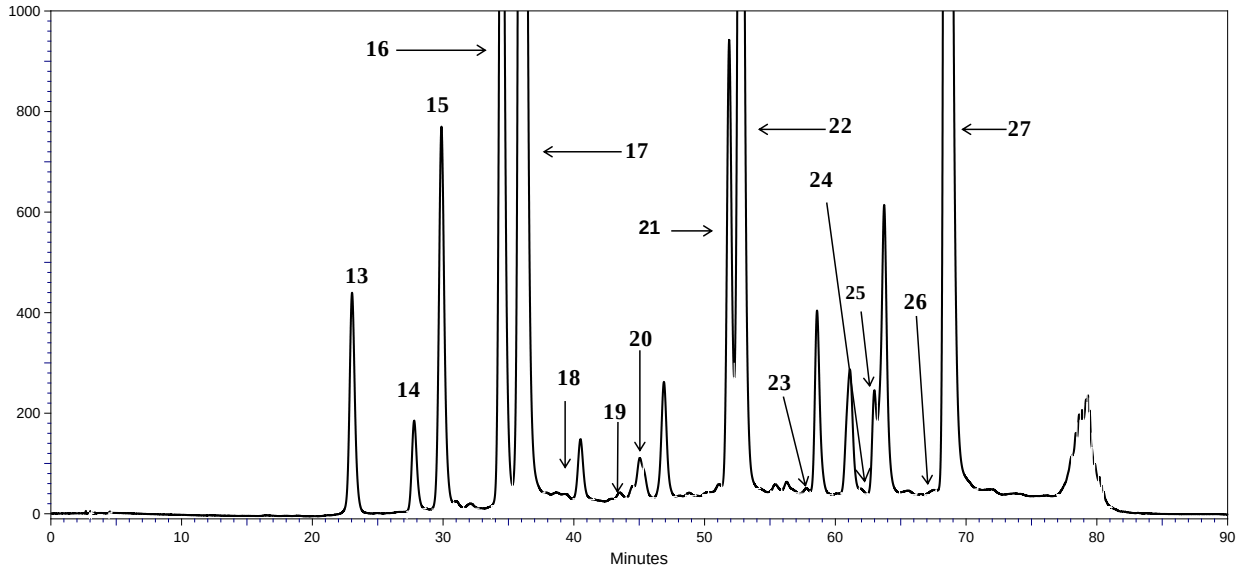

Figure 3.4 HPLC-DAD chromatogram of anthocyanins in lyophilised Syrah grape skin extract measured at 520 nm. Delphinidin 3-*O*-(6-*O*-acetyl) glucoside (18), cyanidin 3-*O*-(6-*O*-acetyl) glucoside (19), cyanidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside (24) and peonidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside (26) were not detected. Refer to Table 3.8 for compound identification.

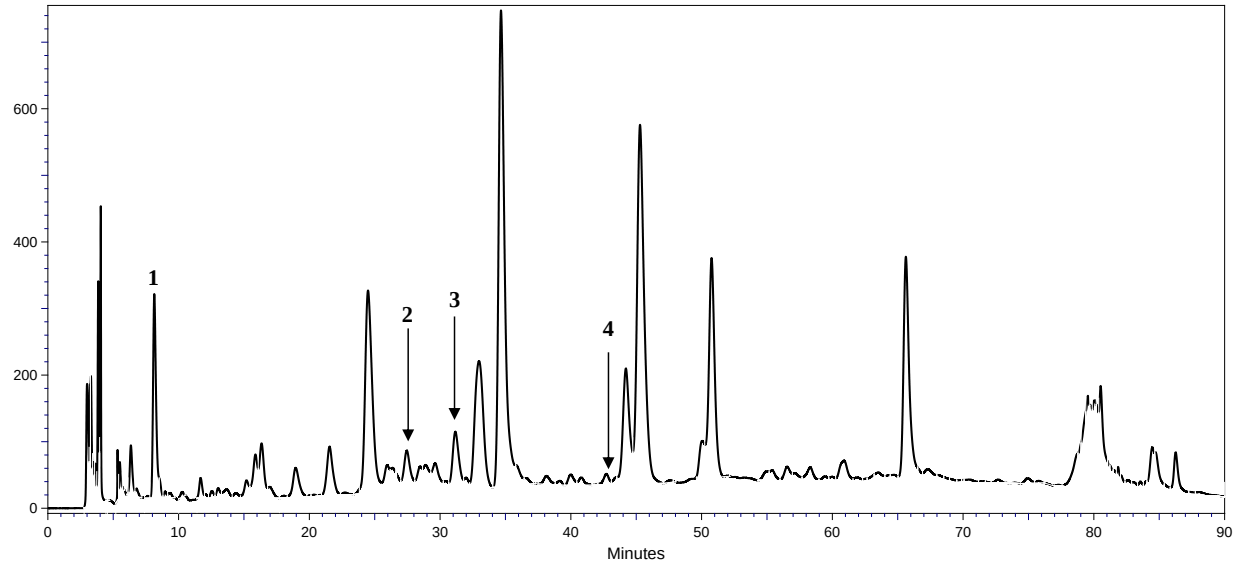

Figure 3.5 HPLC-DAD chromatogram of phenolic acids and flavan-3-ols in young non-commercial Syrah wines measured at 280 nm. Refer to Table 3.8 for compound identification.

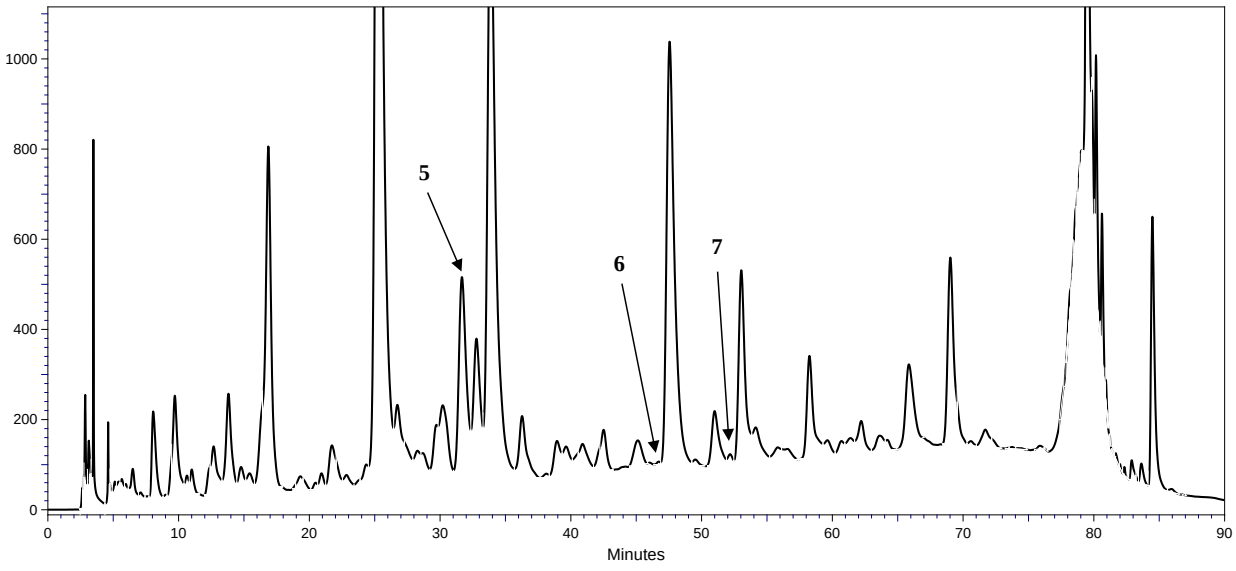

Figure 3.6 HPLC-DAD chromatogram of phenolic acids in young non-commercial Syrah wines measured at 316 nm. Refer to Table 3.8 for compound identification.

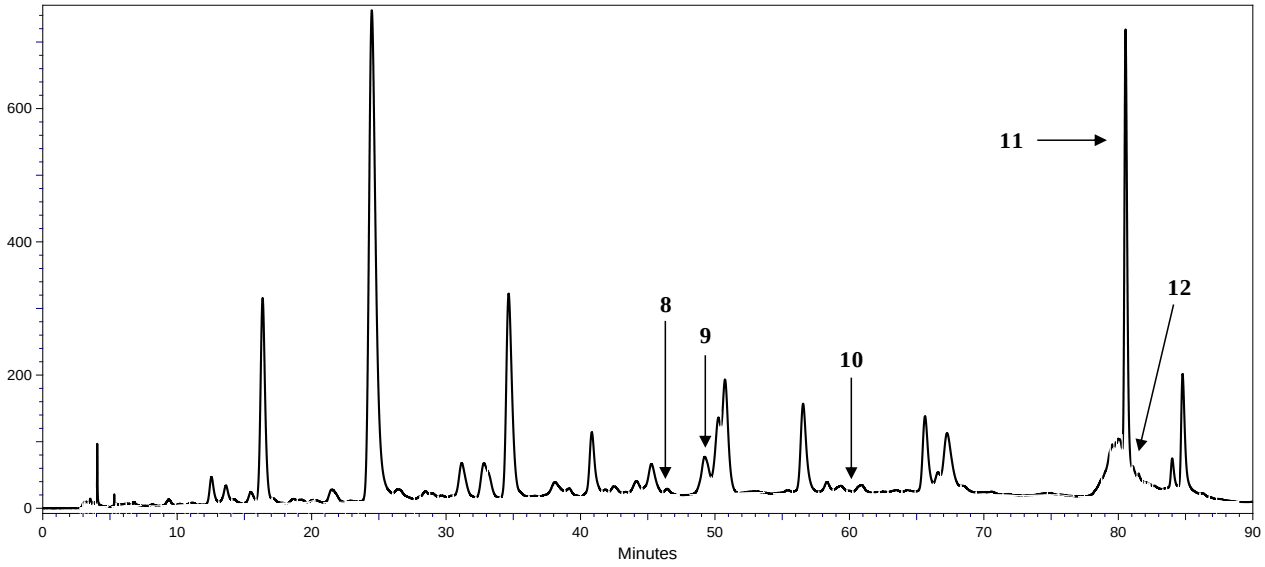

Figure 3.7 HPLC-DAD chromatogram of flavonols in young non-commercial Syrah wine measured at 360 nm. Refer to Table 3.8 for compound identification.

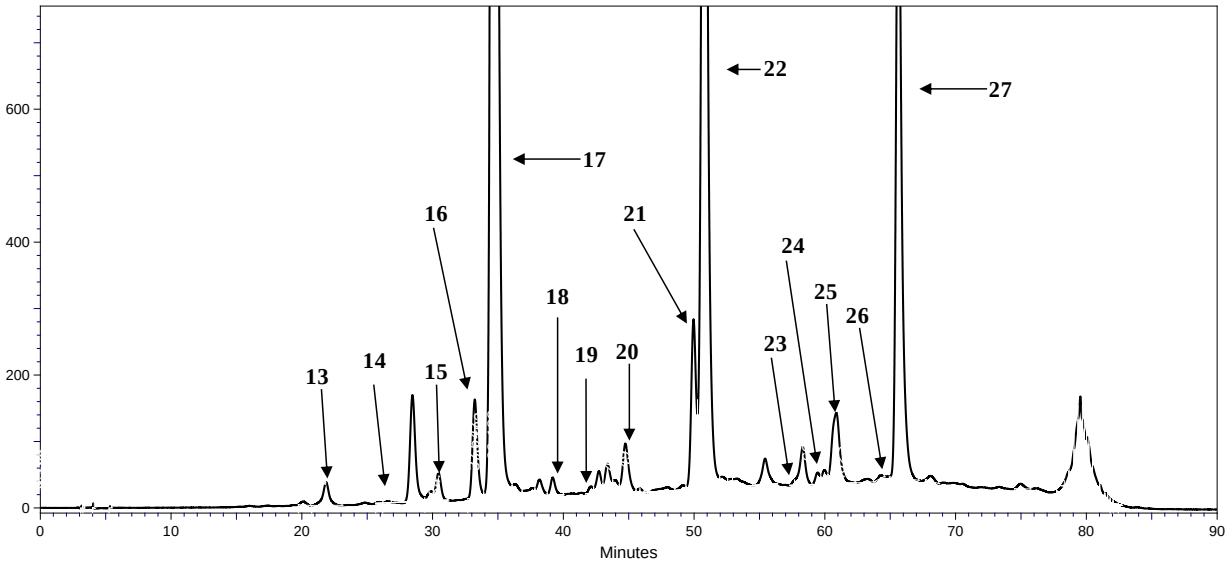

Figure 3.8 HPLC-DAD chromatograms of anthocyanins in young non-commercial Syrah wine measured 520 nm. Cyanidin 3-*O*-(6-*O*-acetyl) glucoside (19), cyanidin (6-*O*-*p*-coumaroyl) glucoside (24) and peonidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside (26) were not detected in the Syrah wine sample. Refer to Table 3.8 for compound identification.

3.3.3 HPLC-ESI-MS analysis of Syrah wine

Anthocyanins are considered important compounds in this study because they are one of the most significant classes of phenolics contributing to wine quality. Because some of the major grape and wine anthocyanins could not be obtained commercially, these compounds could not be identified using authentic standards. For this reason, the HPLC-DAD method was adapted to allow hyphenation to MS to identify additional non-standard anthocyanins. Method adaptation involved replacing the phosphoric acid mobile phase with formic acid. Positive ionisation was used, since this mode is more suitable for anthocyanins (Prodanov *et al*., 2013; Gil *et al*., 2015; Song *et al.*, 2015). In total, 12 anthocyanin-glucosides, -acetyl-glucosides and -coumaroyl-glucosides, comprising the respective delphinidin, petunidin, peonidin and malvidin derivatives, were identified and confirmed on the basis of high resolution mass spectra in young non-commercial Syrah (Table 3.9).

Cyanidin monomers and derivatives were not detected in Syrah wine. Cyanidin comprises only *ca.* 1.0 % of the total anthocyanins of grapes (Margalit, 2004). Non-detection of cyanidin in wine could be because of extraction procedure used and/or type of detection (Kelebek *et al.*, 2007; Ristic *et al*., 2007; Bimpilas *et al*., 2016).

Figure 3.9 shows a typical example of a positive ionisation mode electrospray mass-spectrum acquired by HPLC-ESI-MS of malvidin 3-*O*-glucoside in young non-commercial Syrah wine. The molecular ion was detected with good sensitivity and mass accuracy. Figure 3.10 shows a typical example of a base peak ion chromatogram obtained for a young non-commercial Syrah wine with the corresponding UV chromatograms at 280 nm and 520 nm confirming the presence of anthocyanins as some of the major phenolic compounds.

Table 3.9 Anthocyanins and their derivatives identified in young non-commercial Syrah wines by RP-HPLC-DAD-ESI-MS.

Peak No	Phenolic compounds	t_R min[1] LC-MS*	t_R min LC	Acc Mass[2] [M]$^+$	Acc Mass[3] Cal	Molecular Formula	Error (ppm)[4]	λ_{max}, nm
13	Delph[5] gluc[11]	10.38	23.00	465.1027	465.1033	$C_{21}H_{21}O_{12}$	-1.3	499
15	Petun[6] gluc	15.98	30.00	479.1186	479.1190	$C_{22}H_{23}O_{12}$	-0.8	344/499
16	Peon[7] gluc	21.04	33.90	463.1234	463.1240	$C_{22}H_{23}O_{11}$	-1.3	499
17	Malv[8] gluc	22.57	35.01	493.1348	493.1346	$C_{23}H_{25}O_{12}$	0.4	287/499
18	Delph acet[9] gluc**	25.18	41.00	507.1121	507.1241	$C_{23}H_{23}O_{13}$	0.6	499
20	Petun acet gluc	33.36	45.04	521.1401	521.1447	$C_{24}H_{25}O_{13}$	-0.8	350/499
21	Peon acet gluc	39.82	51.47	505.1351	505.1346	$C_{24}H_{25}O_{12}$	1.0	499
22	Malv acet gluc	41.36	52.36	535.1447	535.1452	$C_{25}H_{27}O_{13}$	-0.9	344/499
23	Delph coum[10] gluc**	45.13	56.31	611.1398	611.1401	$C_{30}H_{27}O_{14}$	-0.5	308/499
24	Petu coum gluc	49.95	61.48	625.1557	625.1549	$C_{31}H_{29}O_{14}$	-0.8	499
25	Peon coum gluc**	58.18	66.15	609.1350	609.1346	$C_{31}H_{29}O_{13}$	0.8	312/499
26	Malv coum gluc	59.25	67.71	639.1702	639.1714	$C_{32}H_{31}O_{14}$	-1.9	318/499

[1]t_R min = Retention time in minutes; [2]Acc. Mass = Experimental accurate mass; [3]Acc. Mass Cal = Theoretical accurate mass; [4]Error = Error between measured mass and theoretical masses in parts per million; [5]Delphinidin; [6] Petunidin; [7]Peonidin; [8]Malvidin; [9]Acetylated; [10] Coumaryolated; [11] Glucoside; *Differences in retention times between LC-ESI-MS method and LC-UV-DAD methods are the result of differences in the mobile phases and delayed volumes between the instruments used; ** Compounds not detected by means of UV-Vis (HPLC).

In addition to the anthocyanins, the presence of the flavan-3-ols (+)-catechin and (-)-epicatechin could also be confirmed based on mass spectral information in the non-commercial Syrah wine sample (Table 3.10). Note that the phenolic acids could not be detected by ESI-MS in positive ionisation mode and the flavonols were not identified due to limited sensitivity because of the flow splitting required before MS detection. Figure 3.11 shows a comparison of the extracted ion chromatograms for the flavanols and anthocyanins identified in young non-commercial Syrah wine.

Table 3.10 Flavan-3-ols confirmed in young non-commercial Syrah wines by RP-HPLC-DAD/ESI-MS.

Peak No	Phenolic compounds	t_R min[1] LC-MS*	t_R min LC	Acc Mass[2] $[M]^+$	Acc Mass[3] Cal	Molecular Formula	Error (ppm)[4]	λ_{max}, nm
2	(+)-Catechin	11.67	28.2	289.0871	290.0869	$C_{15}H_{14}O_6$	0.7	287/279
3	(-)-Epicatechin	15.77	32.2	289.0870	290.0866	$C_{15}H_{14}O_{16}$	0.3	245/330

[1]t_R min = Retention time in minutes; [2]Acc. Mass = Experimental accurate mass; [3]Acc. Mass Cal = Theoretical accurate mass; [4]Error = Error between measured mass, and theoretical masses in parts per million; *Differences in retention times between LC-ESI-MS and LC-UV-DAD methods are the result of differences in the mobile phases and delay volumes between the instruments used.

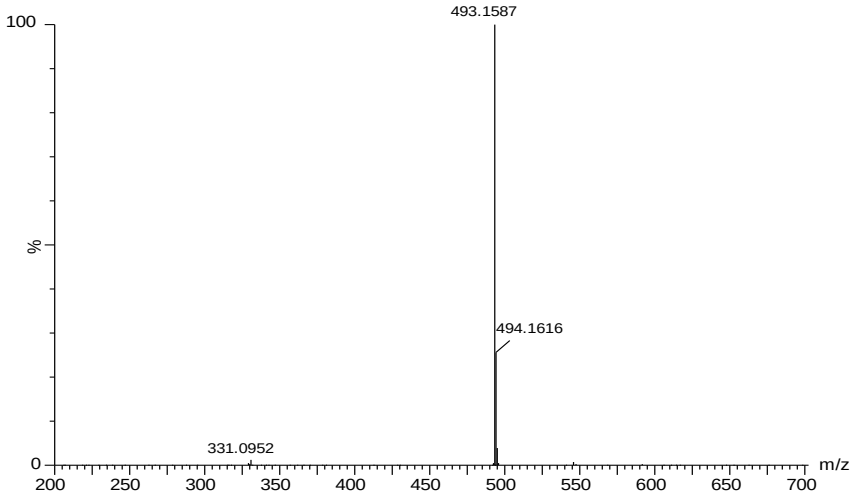

Figure 3.9 Example of the positive ionisation mass spectrum obtained for malvidin 3-*O*-glucoside in a young *non*-commercial Syrah wine by means of HPLC-ESI-MS.

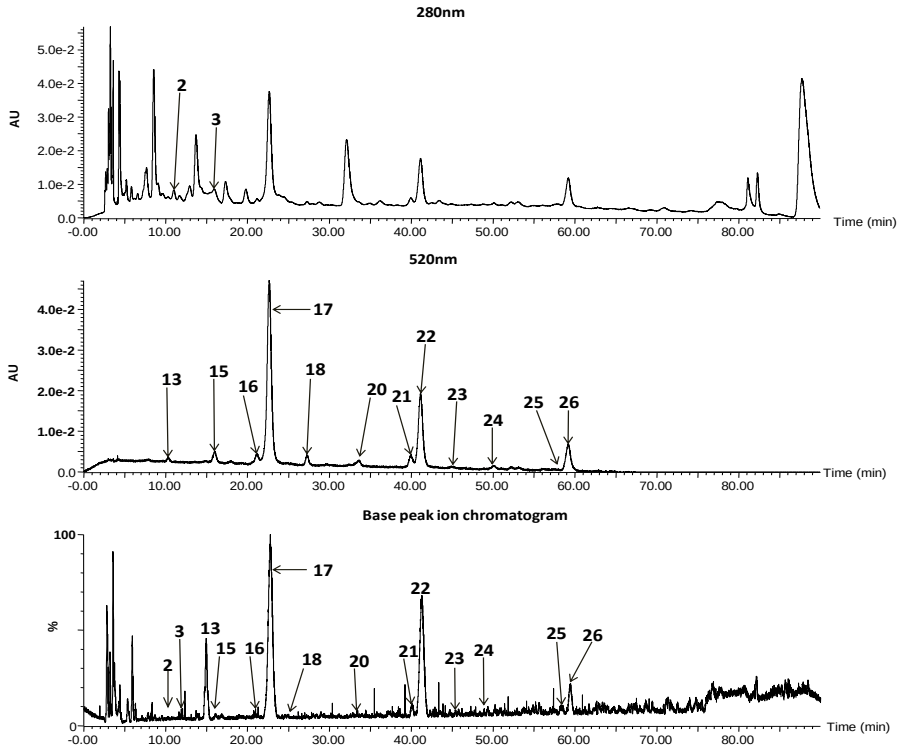

Figure 3.10 UV and visible chromatograms at 280 nm and 520 nm as well as base peak ion chromatogram obtained for the analysis of a young *non*-commercial Syrah wine by HPLC-ESI-MS. Peak numbers correspond to those in Tables 3.9 and 3.10.

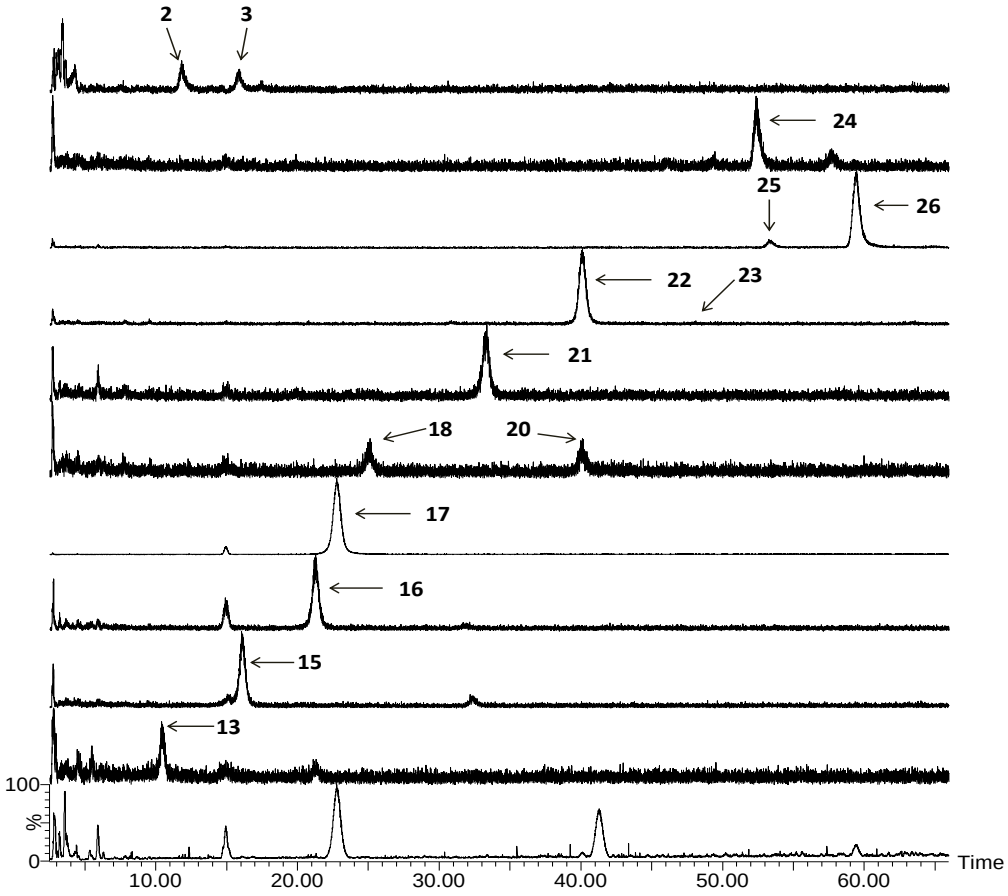

Figure 3.11 Extracted ion chromatograms illustrating the detection of (+)-catechin, (-)-epicatechin, anthocyanin glucosides and anthocyanin derivatives in young *non*-commercial Syrah wine by LC-MS-ESI. See Tables 3.9 and 3.10 for peak identification.

The HPLC-DAD method was validated in terms of linearity, limits of detection (Table 3.5), and limits of quantitation (Table 3.5) using the phenolic standards for quantitative purposes. The R^2 values for all phenolic compounds were higher than 0.9980, confirming the linearity of the method. The LOQ values were below 1 mg/L for all compounds, indicating the suitability of the method for the quantification of phenolic compounds in grapes and wine (Gómez-Alonso *et al*., 2007; Salvatore *et al.,* 2013). The method showed acceptable repeatability of peak areas (concentrations), as evident from relatively low % RSDs for six repeats, indicating good precision of the method.

Photodiode-array detection (DAD) enables the distinction of various classes of phenolic compounds based on their characteristic UV-visible spectra and simultaneous recording of chromatograms at selective multiple wavelengths for detection in grape and wine samples.

Twenty-four (wine) and twenty-three (grape) individual phenolic compounds were separated using the method of Waterhouse *et al.* (1999). Of these, 17 compounds were identified by comparison of retention times and UV-visible spectra with standard compounds. In addition, six (grapes) and seven (wine) acylated anthocyanins, for which standards were not available, were tentatively identified based on their relative retention, UV-visible spectra, and MS data.

Generally good separation performance was achieved and use of various wavelengths improves selectivity, especially for cinnamic acids, flavonols and anthocyanins. Compounds not completely separated were epigallocatechin 3-*O*-gallate (280 nm), ferulic acid (316 nm), quercetin 3-*O*-rhamnoside (360 nm), quercetin 3-*O*-glucoside (360 nm), quercetin (360 nm), and kaempferol (360 nm) in grape and wine samples. These compounds partially co-eluted with unidentified compounds but were sufficiently resolved to allow quantification. Procyanidin B2 could not be quantified because of co-elution with malvidin 3-*O*-glucoside at 280 nm. Baseline separation for peonidin 3-*O*-(6-*O*-acetyl) glucoside (520 nm) and malvidin 3-*O*-(6-*O*-acetyl) glucoside (520 nm) was not achieved, however they were sufficiently resolved to allow quantification. Petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside (520 nm) partially co-eluted with an unknown compound, but was sufficiently resolved to allow quantification. Cyanidin was not detected in the Syrah wine samples. Similarly, cyanidin was also undetected in Pinot noir (Waterhouse *et al.*, 1999), Pinotage (Schwarz *et al.*, 2004) and Merlot wines (Bimpilas *et al.*, 2016). Cyanidin makes up only *ca.* 1.0 % of the total anthocyanins of red wine grapes (Margalit, 2004). Non-detection of cyanidin in wine could be because of the type of extraction procedure applied and detection used (Kelebek *et al.*, 2007; Ristic *et al*., 2007; Bimpilas *et al*., 2016).

The RP-HPLC-DAD method described and used here has several advantages: (1) the wine samples were injected directly without any time consuming sample preparation or previous fractionation, (2) the lyophilised Syrah grape skin extracts were only subjected to a short extraction procedure, where after the samples could be injected directly, (3) the chromatographic separation was acceptable to enable the identification and quantification of the target phenolic compounds, (4) the method is based on DAD detection, which is commonly available and allows multiple wavelength detection, and (5) the method can (therefore) be applied in quality control and industry laboratories interested in acquiring information of the most important grape and wine phenolic compounds.

3.4 Conclusions

A published RP-HPLC-DAD technique was validated and applied for the separation and identification of twenty-three individual phenolic compounds in lyophilised Syrah grape skin extract and twenty-four individual phenolic compounds in young non-commercial Syrah wine samples (three flavan-3-ols, five flavonols, four phenolic acids and twelve anthocyanins (eleven for grape samples), including acetylated and coumaroylated anthocyanins), based on comparison with standards, UV-visible spectra, and relative retention times, including ESI-MS data (twelve anthocyanins). Delphinidin- and cyanidin 3-*O*-(6-*O*-acetyl) glucosides and cyanidin-and peonidin 3-*O*-(6-*O*-*p*-

coumaroyl) glucosides could not be identified (detected) in Syrah grape samples, whereas cyanidin 3-*O*-(6-*O*-acetyl) glucosides and cyanidin-and peonidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides could not be identified (detected) in Syrah wine samples. The method is simple and allows for the quantification of the selected phenolic compounds. The HPLC-DAD technique also allows for the direct injection of samples with simultaneous multi-wavelength detection. The RP-HPLC-DAD method was shown to be robust and reliable for routine analysis of phenolics in the sample matrices under study. Identification of non-standard anthocyanins as well as (+)-catechin and (-)-epicatechin was confirmed by HPLC-ESI-MS in the positive ionisation mode, which made it possible to assign the corresponding peaks in the UV-visible chromatograms.

The HPLC-DAD method therefore provides a simple and reliable technique for the separation and identification of the selected phenolic compounds in lyophilised Syrah grape skin extracts as well as Syrah wine; the method should also be applicable to other red wine grape cultivars as sample preparation for wine only requires membrane filtration. The method can also be adapted for other aqueous/alcoholic solutions for the separation and identification of phenolic compounds.

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Chapter 4

RESEARCH RESULTS I

Impact of Microclimate (Row orientation) and Ripeness Levels on Selected Anthocyanins, Flavan-3-ols, Flavonols and Phenolic Acids in *Vitis vinifera* L. cv. Syrah Grapes

4. Research Results I

Impact of microclimate (row orientation) and ripeness levels on selected anthocyanins, flavan-3-ols, flavonols and phenolic acids in *Vitis vinifera* L. cv. Syrah grapes

4.1 Introduction

Ripening of grapes entails both physical and biochemical processes that begin with véraison and culminate in grape maturity (Coombe, 1960; Coombe & McCarthy, 2000; Creasy & Creasy, 2009). Biochemical processes are necessary in order for grapes to acquire optimum characteristics for their transformation into wine (Coombe, 1973; Winkler *et al.*, 1974; Bisson, 2001; Kennedy *et al.*, 2002).

Compositional changes, which take place during grape ripening, do not occur simultaneously (Coombe & McCarthy, 1997; Pérez-Magariño & Gonzalez-San José, 2006). The development and evolution of chemical constituents, such as phenolic compounds, in grapes are affected by environmental factors (Castillo-Muñoz *et al*., 2007), viticultural practices (Pérez-Magariño & Gonzalez-San José, 2002) and plant genetics (Czemmel *et al*., 2009). Phenolic compound concentrations of grapes can also be affected by grape bunch temperature variations during berry development (Moffat *et al.*, 2013; Santos-Buelga *et al.*, 2014; Bonada *et al*., 2015; Hunter *et al*., 2016). The phenolic compound concentration of grapes increases throughout berry development (Jackson & Lombard, 1993; Coombe & McCarthy, 1997; Pérez-Magariño & Gonzalez-San José, 2006), with different stages of evolution among the different groups of phenolic compounds (González-San José *et al.*, 1990; Andrades & González-San José, 1995; Girard *et al.*, 2001; Mateus *et al.*, 2002; Pérez-Magariño & González-San José, 2006).

Two principal phenolic groups are present in grapes, *i.e.* flavonoids and non-flavonoids (Jackson, 2000). The most common flavonoids in grapes are flavonols, flavan-3-ols and in red grapes, anthocyanins (Margalit, 2004), and the most common non-flavonoids are phenolic acids, which are derivatives of hydroxycinnamic- and hydroxybenzoic acids (Vrhovsek, 1998; Ribéreau-Gayon *et al.*, 2006).

Flavonols, flavan-3-ols and anthocyanins are present in grape skin, whereas phenolic acids are mainly present in the grape pulp (Singleton & Trousdale, 1983; Ribéreau-Gayon *et al.*, 2006). Grape seed phenolic compounds are polymers of (-)-catechin, gallocatechins and gallated catechins (Prodanov *et al.*, 2013). Grape seed phenolic compounds constitute *ca*. 65% of the total content, whereas 30% of the phenolic compound content occurs in grape skin and 4% to 5% in grape pulp (Jackson, 2000).

The percentage extraction of phenolic compounds from grapes into grape must and eventually into wine is 25% to 50% of the total grape phenolic compound content (Singleton & Esau, 1969; Singleton, 1980; Margalit, 2004). The rest of the phenolic compounds after extraction remains in the grape seeds, -skin and -pulp.

Flavonols are the least abundant class of flavonoids in grapes, but are often measured in grape extract and wine samples because they are indicators of grapes that have been exposed to increased light/temperature in the fruiting zone or light in the canopy (Price *et al*., 1995; Spayd *et al*., 2002). Downey *et al*. (2003b) showed that a major determinant for the variation in flavonol concentrations of Syrah and Chardonnay grapes was the intensity of direct sunlight to which the grapes were exposed to during cultivation. Hunter *et al.* (2007) and Czemmel *et al*. (2009) reported that Syrah grape exposure to direct light increased grape flavonol concentrations during the growth period. Cortell & Kennedy (2006) showed that increased flavonol concentration in Pinot noir grapes is associated with direct sunlight exposure in the fruiting zone. This correlation indicates that the biosynthesis of flavonols in grapes is related to sunlight exposure. It was shown that high concentrations of total flavonols in red wines are associated with grapes grown in an exposed canopy (Zou *et al*., 2002; Cortell & Kennedy, 2006). Ristic *et al.* (2007) reported a noticeable decrease in flavonol concentration in Syrah grapes from dense canopies. Martínez-Lüscher *et al*. (2014) reported higher concentrations of kaempferol and quercetin in Tempranillo grapes from vines receiving increased light in the fruiting zone, compared to vines receiving moderate light in the fruiting zone.

Flavan-3-ol monomers present in grapes are (+)-catechin, (-)-epicatechin, and gallic acid esters of (+)-catechin 3-*O*-gallate and (-)-epicatechin 3-*O*-gallate (Su & Singleton, 1969; Andersen & Markham, 2007). Fournand *et al*. (2006) reported that flavan-3-ol concentrations in Syrah grape skin remained constant during grape ripening expressed on a per berry basis. Kennedy *et al*. (2002) and Downey *et al*. (2004) showed a decrease in flavan-3-ol monomer and proanthocyanidin concentrations in Syrah grapes after véraison, but according to Bogs *et al.* (2005) maximum concentrations of flavan-3-ols were reached after véraison in Syrah grapes.

Cortell *et al*. (2005) reported that proanthocyanidin and (-)-epigallocatechin concentrations are positively affected by environmental factors, such as increased sunlight and/or heat exposure in the canopy. Downey *et al*. (2004) and Cortell & Kennedy (2006) showed that Syrah grape-skin proanthocyanidin (polymerised flavan-3-ols) concentrations increased in reaction to grape bunches exposed to light or in grapes from exposed canopies. Increased concentrations of (-)-epigallocatechins were also reported in Pinot noir grape skins from low vigour vines (and therefore most likely more open canopies) (Cortell *et al*., 2005), while low vigour Pinot noir vines also affected the degree of polymerisation of flavan-3-ols (proanthocyanidins). Low concentrations of proanthocyanidins have been found in Cabernet Sauvignon grapes that developed in shaded or dense canopies (Ojeda *et al*., 2002; Castellarin *et al*., 2006). Grapevines grown in cool climates generally produced higher concentrations of (+)-catechins, compared to vines grown in warm climates (Kennedy *et al.*, 2001).

The grape cultivar Pinot noir has higher concentrations of (+)-catechins, compared to Merlot and Syrah grape cultivars (Cortell & Kennedy, 2006). Scrafidi *et al*. (2016) reported increased concentrations of flavan-3-ols in Grillo grapes (white cultivar) harvested at *ca*. 21°Brix ripeness and planted to NS (north-south) row orientations, compared to artificially shaded (boxed) Grillo grape bunches planted to the same orientation. Artificially shaded grape bunches showed a decrease in flavan-3-ol concentrations.

The contribution of grape seeds to the total flavan-3-ol concentration depends on grape cultivar (Jeffery *et al.*, 2008). The concentrations of (+)-catechins can therefore vary among grape cultivars (Mané *et al.*, 2007). Ristic *et al*. (2007) found higher concentrations of seed proanthocyanidins and lower concentrations of skin proanthocyanidins in Syrah grapes from dense canopies, compared to grapes from open canopies (exposed grape bunches).

Phenolic acids in grapes generally respond positively to light exposure. Increased light in the fruiting zone brought about by leaf removal/shoot thinning resulted in increased concentrations of gallic acid in Cabernet Sauvignon grapes from vines planted to NS row orientations (Jogaiah *et al*., 2013). Del-Castillo-Alonso *et al*. (2014) found higher *p*-coumaric acid concentrations in Graciano grapes planted to EW (east-west) row orientations, compared to grapes with artificial UV exclusion treatment from the same row orientation. Work by Tessarin *et al*. (2014) showed that caffeic- and *p*-coumaric acid concentrations were higher in Uva Longanesi grapes after 50% defoliation in vines planted to EW row orientations, compared to no defoliation. Rescic *et al.* (2016) reported increased concentrations of *p*-coumaric acid in Istrian Malvasia grapes planted to NS row orientations with 50% leaf removal in the canopy. The leaf removal most likely also resulted in increased temperature in the fruiting zone (Moffat *et al.*, 2013; Zorer *et al.*, 2013; Hunter *et al*., 2016), compared to control samples with no leaf removal.

Anthocyanin biosynthesis is activated by direct exposure of grapes to UV radiation (Brouilard & Dangles, 1994). The effect of direct light on anthocyanin biosynthesis appears to be grape cultivar dependent (Jeffery *et al*., 2008; Hunter *et al.*, 2016). In a study conducted by Price *et al.* (1995), the anthocyanin concentration in Cabernet Sauvignon grapes from sun-exposed vines was higher than that from shaded grapes or grapes from dense canopies. Direct sunlight was shown to affect anthocyanin concentrations positively in Cabernet Sauvignon, Grenache and Barbera grapes (Smart *et al.*, 1988; Bergqvist *et al.*, 2001; Ferrandino & Guidoni, 2010). Bergqvist *et al*. (2001), Downey *et al*. (2004), Spayd *et al*. (2002) and Jeong *et al*. (2004) also showed an increase in anthocyanin concentrations when Merlot and Cabernet Sauvignon grape bunches were exposed to direct light. However, according to Dokoozlian & Kliewer (1996) exposure of Cabernet Sauvignon and Pinot noir grapes to direct sunlight, negatively affected anthocyanin accumulation during the initial stages of grape ripening. Downey *et al.* (2004) reported a higher response in total anthocyanin concentration in Merlot and Syrah grapes than in Pinot noir and Cabernet Sauvignon grapes with canopy exposure to sunlight.

According to Joscelyne *et al*. (2007) and Ristic *et al.* (2007), shaded Syrah grape bunches of vines planted to EW row orientations (southern hemisphere) have lower concentrations of anthocyanins than that of sun-exposed grape bunches. It was shown by Kocsis *et al*. (2008) that Furmint grapevines planted to EW orientations yielded grapes with higher sugar content and lower acid content, compared to grapes planted to NS row orientations.

Grape cultivars that are sensitive to direct sunlight are those with a high proportion of 3'-hydroxylated anthocyanins (Guidoni *et al.,* 2008). Cyanidin 3-*O*-glucoside concentrations in Nebbiolo grapes from NE-SW (northeast-southwest) row orientations with south exposed vineyards varied between vintages. However, according to Guidoni *et al*. (2008), 3', 5'-dihydroxylated anthocyanins and their acylated derivatives are unaffected by vintage differences in south exposed vineyards. Guidoni *et al.* (2008) also reported that 3'-hydroxylated anthocyanins are higher in Nebbiolo grapes, compared to the 3', 5' dihydroxylated anthocyanins. Guidoni & Hunter (2012) reported an increase in malvidin 3-*O*-glucoside and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside proportions at late ripening stages (*i.e.* 25°Brix to 28°Brix) of Shiraz grapes.

Hunter & Volschenk (2008) demonstrated that Syrah grapes from EW row orientations were lower in grape skin anthocyanins, compared to grapes from NS, NE-SW, and NW-SE row orientations. Nebbiolo grapevines planted to NS row orientations and with artificial shading of grape bunches (netting), resulted in an increase in temperature in the fruiting zone (Chorti *et al*., 2010), causing an unfavourable microclimate for anthocyanin biosynthesis. Rustioni *et al.* (2011) and Song *et al*. (2015) reported a positive relationship between grape anthocyanin concentrations and grape bunch exposure to increased sunlight in Croatina and Pinot noir grapes planted to EW and NS row orientations. Martínez-Lüscher *et al*. (2014) found that artificially reduced light in the grapevine canopy and moderate temperature (*ca.* 30°C) in the fruiting zone of Tempranillo grapevines planted to NS orientations, resulted in an increase in acylated malvidin glucoside concentrations. Feng *et al*. (2015) reported that increased sunlight and elevated temperatures (>35°C) brought about by 50% leaf removal of Pinot noir grapevines planted to NS row orientations, resulted in decreased concentrations of monomeric anthocyanin glucosides. Fernandes De Oliveira & Nieddu (2016) reported lower concentrations of delphinidin- and petunidin 3-*O*-(6-*O*-coumaroyl) glucosides in Bovale Grande and Cannonau grapes from NS orientated vines, when compared to artificially shaded grapes from the same row orientation.

Anthocyanin concentrations in Pinot noir grapes seemed unaffected when grape bunches are exposed to day time temperatures of between 30°C and 35°C, while further anthocyanin formation is inhibited in Cabernet Sauvignon, Merlot and Syrah grapes with exposure to temperatures of >35°C (Mori *et al*., 2005; Downey *et al.,* 2006). Yamane *et al.* (2006) reported increased anthocyanin biosynthesis in "Aki Queen" grape cultivars *(Vitis vinifera* x *Vitis labrusca*) under low temperature conditions (≤ 20°C), independent of light conditions. The concentrations of anthocyanins in Cabernet Sauvignon, Pinot noir and Cardinal grapes increased relative to increases in light intensity, whereas an increase in grape bunch temperature from 30°C to 35°C resulted in a

decrease in anthocyanin accumulation in Cabernet Sauvignon and Cardinal grapes and even degradation of anthocyanins (Downey *et al*., 2006; Guerrero *et al*., 2009).

An increase in grape bunch temperature to above 35°C and low light exposed grapes (dense canopies) of Cabernet Sauvignon and Merlot resulted in a decrease in anthocyanin concentrations, when compared to grape bunches exposed to increased temperatures in combination with high light exposure (*i.e.* 50% defoliation) (Tarara *et al.*, 2008). An increase in the concentrations of anthocyanins occurred in autochthonous (*i.e.* natural crossbreeding or mutations) grape cultivars (Guerrero *et al*., 2009) with lower day-night temperatures, compared to grapes subjected to higher day-night temperatures. Accumulation of anthocyanins and proanthocyanidins in Cabernet Sauvignon, Pinot noir, Syrah and Nebbiolo grapes occurred simultaneously in shaded or dense canopies (Bergqvist *et al*., 2001; Cortell *et al*., 2005; Cortell & Kennedy, 2006; Hunter *et al*., 2007; Joscelyne *et al*., 2007; Ristic *et al*., 2007; Chorti *et al*., 2010; Rustioni *et al.*, 2011). The simultaneous accumulation of anthocyanins and proanthocyanidins is complicated by the effect of day-night temperature differences. Low night temperatures resulted in an increase in anthocyanin monomers and a decrease in proanthocyanidin concentrations.

A simultaneous decrease in anthocyanin and proanthocyanidin concentrations in Pinot noir and Cabernet Sauvignon grapes was observed when cultivated in warm climates with temperatures in excess of 35°C (Spayd *et al*., 2002; Mori *et al.*, 2005; Downey *et al.*, 2006; Guerrero *et al*., 2009).

Nebbiolo grapes from shaded or dense canopies (northern hemisphere), planted to NS row orientations, proved lower in 3'-hydroxylated anthocyanins, compared to those planted to the same row orientation but from sun-exposed or open canopies (Chorti *et al.*, 2010). In contrast to the findings of Chorti *et al.* (2010), Joscelyne *et al.* (2007) and Cohen *et al.* (2008) reported that low day-time temperatures (southern hemisphere) and high vigour vines (dense canopies) can result in Syrah and Merlot grapes with high concentrations of 3'-hydroxylated anthocyanins. The pH of grape juice from exposed grapes is lower than the pH of grape juice from shaded grapes or dense canopies (Cohen *et al*., 2008). This may also affect anthocyanin intensity.

The precedent brief literature overview provides strong evidence that concentrations of phenolic compounds, which contribute to wine and grape colour (anthocyanins) and astringency (flavan-3-ols), are affected by the complex interplay of the combined effects of solar radiation and diurnal temperature as well as grape cultivar specific biosynthesis pathways. Phenolic acid, flavonol, anthocyanin and flavan-3-ol concentrations in grapes also showed differences as a result of different viticulture practices. The literature reviewed mainly provides information on the effect of artificial heating/cooling or boxed-in grape bunches (shading), vine defoliation and shoot thinning, on phenolic compound concentrations. In South Africa, limited work has been published on Syrah grapes reporting the effect of grapevine row orientation on individual phenolic compound concentrations. Guidoni *et al* (2008) reported on the effect of seasonal and agronomical practices on the total skin anthocyanin profile of Syrah grapes. Hunter & Volschenk (2008) reported on the effect of different row orientation on total anthocyanins of Syrah grapes. Hunter *et al*. (2010) also reported on linking grapevine row orientation to a changing climate in

South Africa as well as on the climatic profiles and vine physiology as affected by different row orientation (Hunter *et al.*, 2016). The aim of this study was therefore to investigate the effect of microclimate, induced by different grapevine row orientations, under realistic field conditions and grape ripeness levels on the concentrations of selected individual anthocyanins, flavan-3-ols, flavonols, and phenolic acids in Syrah grapes grown in South Africa.

4.2 Materials and methods

4.2.1 Vineyard layout and management

The experimental vineyard was established in 2003. Syrah, clone SH 9C, was grafted onto 101-14 Mgt rootstock. The vineyard was established on a flat *terroir* with clayey loam soil situated on the experimental farm of ARC Infruitec-Nietvoorbij in the Breede River Valley of Robertson, South Africa. Row and vine spacing were 2.7 m and 1.8 m, respectively. The vines were trained to a vertical shoot positioning trellis. A cover crop of rye grass was sown after harvest (April) and killed before bud burst (October). The grapevine canopies consisted of three to four leaf layers from side to side. Grapevines were supplementary irrigated every seven days. The Robertson region receives on average 325 mm precipitation annually. The basic experimental design was a randomised complete block (Fig. 4.1) with four different row orientations, *i.e.* NS, EW, NE-SW and NW-SE replicated at random in each of five experimental blocks with a total surface area of 1860 m^2. An experimental unit consisted of all the vines within a vineyard block of a specific row orientation within an experimental block.

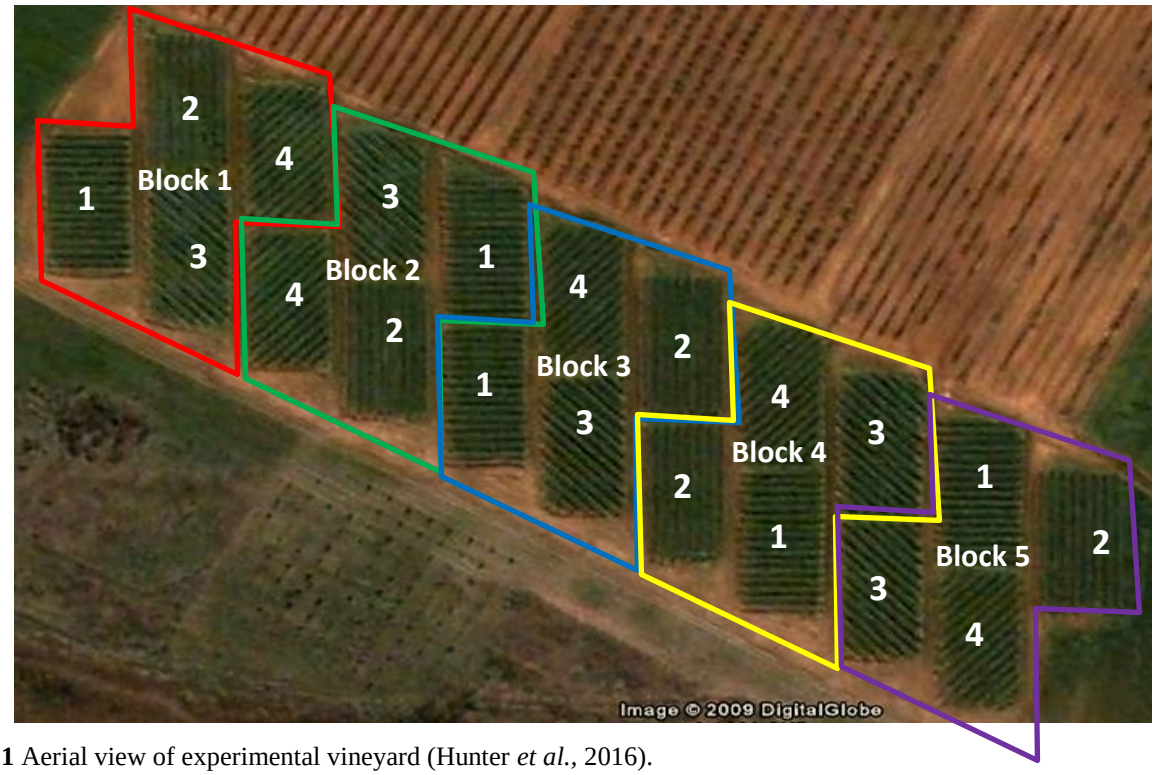

Figure 4.1 Aerial view of experimental vineyard (Hunter *et al.*, 2016).

4.2.2 Grape collection

Grapes were harvested over four consecutive vintages (2008, 2009, 2010 and 2011) from the same experimental units and vintage may be considered as a split plot factor (Little, 1972). Additionally, within each vintage, grapes at three different ripeness levels (*ca.* 22°, *ca*. 24° and *ca*. 26°Brix) [°Brix/°Balling (°Bx/°B) refers to g soluble solids/100 mL] were harvested from each experimental unit, resulting in ripeness levels to be considered as a split-split plot factor (vintage & ripeness factor). Approximately 40 kg of grapes were collected randomly per row orientation replicate/block and per ripeness level. Grapes were harvested from both sides of the canopy and combined. The harvested grapes were representative of the different ripeness levels of each of the NS, EW, NE-SW and NW-SE row orientation treatments, representing different microclimatic conditions according to the movement of the sun over the vertical shoot positioned canopies (Hunter *et al.*, 2016).

4.2.3 Preparation of grape skins

Individual grape berries were removed from grape bunches. Three replicates of 100 berries each per larger replicate (*i.e*. sub-replicates) were collected randomly from the combined grapes (crates) per row orientation treatment, per block replicate and per ripeness level. The grape skins were separated from the flesh by hand. The skins were washed with distilled water, blotted with laboratory absorptive paper, and weighed. The skins were lyophilised and stored in airtight containers until required for analysis.

4.2.4 Reagents

All solvents used were of analytical grade and purchased from Merck®, South Africa. De-ionised water was supplied through a Modulab® water purification system, supplied by Separations.

4.2.5 Extraction of phenolic compounds from lyophilised Syrah grape skin samples

Extraction of phenolic compounds from lyophilised grape skin samples is commonly performed using organic solvents (Waterhouse *et al.*, 1999; Rodríguez-Montealegre *et al*., 2006; Castillo-Muñoz *et al*. 2007; Gómez-Alonso *et al*., 2007). The most commonly used solvents are acidified methanol, -ethanol or -acetone, with or without aqueous dilution. The method described by Castillo-Muñoz *et al*. (2007) was used in this work. The lyophilised grape skins were ground prior to extraction. Separate extractions and analyses were done for each experimental unit (units 1-4 as defined in 4.2.1) from the five block replicates.

A volume of 30 mL methanol/formic acid/water (50:48.5:1.5) was added to 1.0 g of lyophilised ground Syrah grape skins. Extraction was performed in a dark room using a Variomag Poly15 Electronicrührer stirring for 20 minutes at *ca.* 25°C at a rate of 450 rpm.

The samples were centrifuged using a LKB Bromma 2160 centrifuge at 1000 *g* for 15 minutes. The supernatant was collected and filtered through a 0.22 µm nylon membrane syringe filter (Microsep, South Africa) prior to analysis.

4.2.6 High-performance liquid chromatography photodiode-array detection

HPLC determination of anthocyanins, flavonols, flavan-3-ols and phenolic acids were performed on a SpectraSYSTEM HPLC instrument (Thermo Separations Products, Inc., New Jersey, USA) equipped with an auto-sampler (injection volume 20 µL) using a published method of Waterhouse *et al.* (1999). Detection was by means of photodiode array. Detection range was between 190 nm and 950 nm. ChromQuest™ software was utilised for data acquisition. Calibration curves for anthocyanins, flavan-3-ols, flavonols and phenolic acids were constructed by injection of standard solutions (Chapter 3). Calibration parameters and quantification of the target phenolic compounds were performed using ChromQuest™ software. Separation was performed at *ca*. 22°C, using a polymer reversed-phase analytical column (PLRP-S 100 Å, 5 µm, 250 x 4.6 mm) with polystyrene divinylbenzene as stationary phase. Polymer Laboratories, Massachusetts, USA, supplied the column. Gradient elution with two solvents was used (Chapter 3) at 1 mL/min. Ultra violet visible spectra were recorded at 280 nm, 316 nm, 360 nm and 520 nm. An analysis time of ninety minutes was preceded by a twenty-minute equilibration time. Anthocyanins were detected at 520 nm, flavan-3-ols at 280 nm, benzoic acid at 280 nm, flavonols at 360 nm, and cinnamic acids at 316 nm. The identification of the phenolic compounds was confirmed by their relative retention times based on reference standards and UV-visible absorption characteristics. The analytical method is based on that of Waterhouse *et al*. (1999).

4.2.7 Data analysis and strategies

4.2.7.1 Analysis of variance (ANOVA)

Analysis of variance was performed according to the experimental design on all variables accessed using General Linear Models Procedure of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Five replicate samples were analysed per row orientation per vintage, giving a total of 20 (5x4) samples for each ripeness level. This excludes occasional duplicate samples from the same experimental unit, in which case average values were first calculated per experimental unit before analysis, to attain the correct experimental error according to the model for the experimental layout, *i.e*. randomised block design. Data was subjected to XLSTAT 2010 (add-on statistical software for Excel, 2010) to establish averages, variances and standard deviations in the compositional data. Analysis of variance including all three factors (row orientation, vintage and ripeness level), as well as for each ripeness level separately, were performed. Shapiro & Wilk test was performed to test for deviation normality (Shapiro & Wilk, 1965). Fisher's least significant difference test was calculated at the 5% level to compared

treatment means for significant effects (Ott, 1998). A probability level of 5% (≤ 0.05) was considered significant for all significance tests.

4.2.7.2 Principal component analysis (PCA)

The purpose of PCA is to reduce the complexity of data into a principal component (PC) space. The PCA is also a dimension reducing technique. The first *q* principal components ($q < p$) are retained based on the identification of an "elbow" or break in the scree plot of the Eigenvalues associated with each principal component. A *scree plot* displays the Eigenvalues associated with a principal component in descending order *versus* the number of the principal components. Eigenvalues, percentage variability and percentage cumulative variance explained by each principal component are also determined. The principal components are donated as F1 (PC1) to F*q* (PC*q*), depending on the number of variables. The first two principal components, *i.e.* PC1 and PC2, are usually chosen to represent the data, since these explain the highest variance in the data and it is easier to interpret a two-dimensional plot, compared to higher dimensional plots. The PCA results report in vector diagrams (biplots). The vector diagrams describe the relative positions and loadings of the variables in relation to treatments. The first two factor scores (PC1 and PC2) and two factor loadings are used to plot the vector diagrams. The axes (x and y for PC1 and PC2, respectively) represent the principal components and describe the degree of variability in the data. Principal component analysis was applied to the grape phenolic compound data sets using XLSTAT (Version 2015, Addinsoft, New York, USA) to establish correlation, association, and "groupings" between treatments and measured variables of the grape samples, *i.e.* relationships among variables (phenolic compounds) based on treatments.

4.3 Results

4.3.1 Effect of microclimate (row orientation treatment) on measured variables in grape samples

4.3.1.1 Syrah grape skin phenolic compound data

As discussed in the introduction, grape phenolic compound composition changes during different stages of berry development. This evolution of phenolic compounds in grapes is expected to be affected by grape bunch temperature variations (as induced by different grapevine row orientations) during berry development.

Principal component analysis (multivariate analysis), showing association among phenolic compounds, row orientations, vintages and grape ripeness levels

The principal component analysis biplot (Fig. 4.2) illustrates the association of phenolic compounds of Syrah grapes harvested during 2008, 2009, 2010 and 2011 at ripeness levels of *ca*. 22, 24 and 26°Brix with different row orientation treatments, *i.e*. NS, EW, NE-SW and NW-SE row orientations. The PCA biplot shows that row

orientation (treatment) is not consistently associated with the same phenolic compounds at different ripeness levels over the four consecutive vintages. Table 4.1 list abbreviations used in the PCA biplots.

Table 4.1 List of phenolic compound (variables) abbreviations used in Figures 4.2 - 4.5.

Abbreviation = Full name	Abbreviation = Full name	Abbreviation = Full name
EGCG = Epigallocatechin 3- *O*-gallate	PetGluc = Petunidin 3-*O*-glucoside	Gall = Gallic acid
IsoQ = Isoquercitrin (quercetin 3-*O*-glucoside)	*p*-C = *p*-Coumaric acid	Cat = (+)-Catechin
DelGluc = Delphinidin 3-*O*-glucoside	Rut = Rutin (quercetin 3-*O*-rutinoside)	Caff = Caffeic acid
PetGlucAc = Petunidin 3-*O*-(6-*O*-acetyl) glucoside	Qc = Quercetin	Epic = (-)-Epicatechin
MalGlucAc = Malvidin 3-*O*-(6-*O*-acetyl) glucoside	NE-SW = Northeast-Southwest	Fer = Ferulic acid
PetGlucCoum = Petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside	CyGluc = Cyanidin 3-*O*-glucoside	EW = East-West
MalGlucCoum = Malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside	PeoGluc = Peonidin 3-*O*-glucoside	NS = North-South
DelGlucCoum = Delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside	MalGluc = Malvidin 3-*O*-glucoside	Kaem = Kaempferol
PeoGlucAc = Peonidin 3-*O*-(6-*O*-acetyl) glucoside	Qr = Quercitrin (quercetin 3-*O*-rhamnoside)	

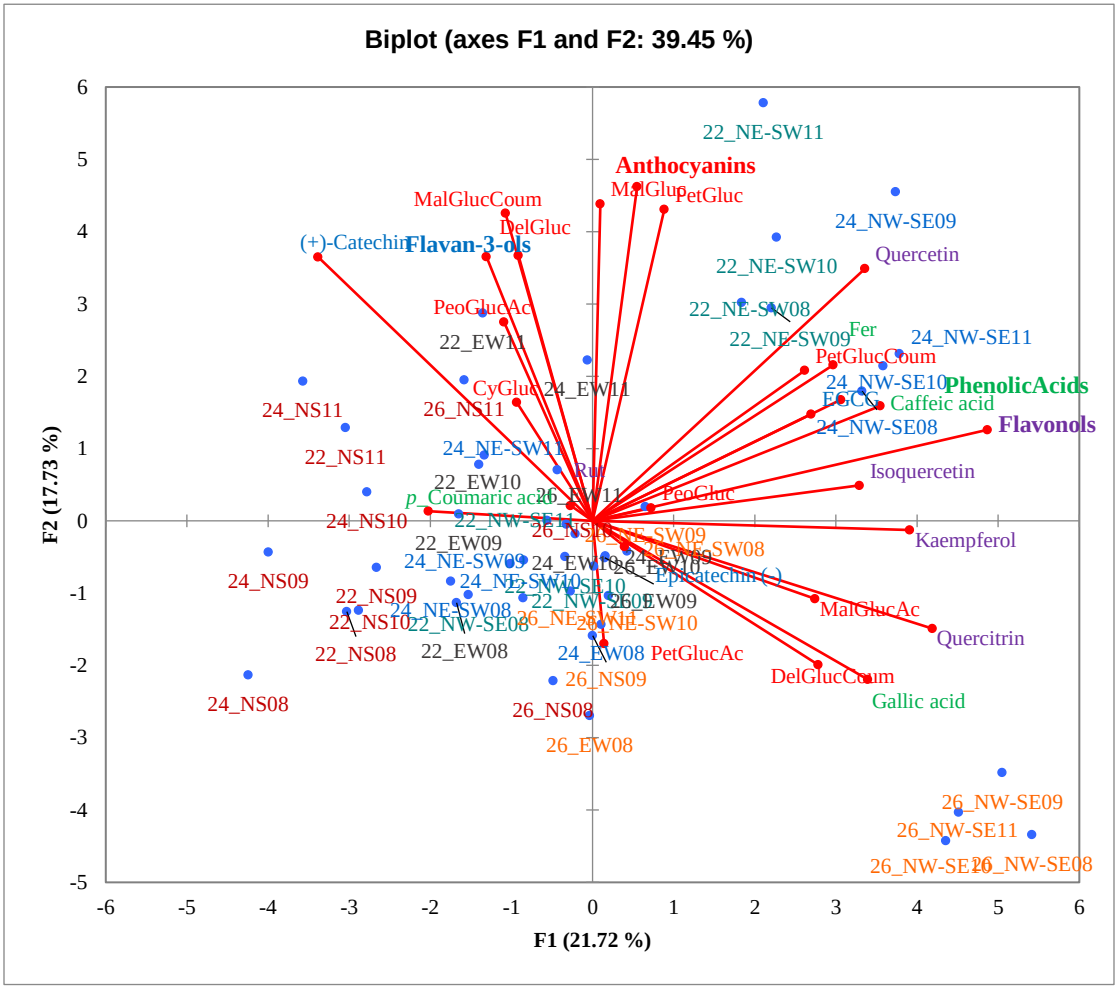

Figure 4.2 PCA biplot illustrating the association of phenolic compound composition of Syrah grapes harvested from NS, EW, NE-SW and NW-SE row orientations during 2008, 2009, 2010 and 2011 at ripeness levels of *ca.* 22, 24 and 26°Brix.

Analysis of variance (3-factor analysis) of grape phenolic data for treatment, vintage and ripeness level

The *p*-values for the 3-factor ANOVA (univariate analysis) including row orientation as main plot factor, vintage as subplot factor and ripeness level as sub-subplot factor are listed in Table 4.2.

Table 4.2 Analysis of variance *p*-values (3-factor ANOVA) for treatment, vintage and ripeness level (°Brix) main effects and interaction for Syrah grapes to determine which effects are statistically significant.

	Main effect			Interaction			
Variables	**Treat[1]**	**Vintage**	**°Brix**	**Treat[1] x Vintage**	**Treat[1] x °Brix**	**Vintage x °Brix**	**Treat[1] x Vintage x °Brix**
Actual °Brix	**<.0001***	0.5532	**<.0001**	0.9385	**0.0293**	0.0518	0.9949
Total flavan-3-ols	**<.0001**	**0.0110**	**<.0001**	0.4443	**<.0001**	0.1469	0.8007
(+)-Catechin	**<.0001**	0.1078	**<.0001**	0.8360	**<.0001**	0.4199	0.7992
(-)-Epicatechin	0.1366	**0.0005**	0.0518	0.1383	**0.0163**	**<.0001**	0.5588
EGCG[2]	**<.0001**	0.0838	**<.0001**	0.3279	**<.0001**	0.1902	0.2808
Total phenolic acids	**<.0001**	0.3603	**<.0001**	0.7623	**<.0001**	0.3497	**0.0359**
Gallic acid	**<.0001**	**0.0064**	**<.0001**	0.1286	**<.0001**	0.0767	0.1089
Caffeic acid	**<.0001**	0.5714	**<.0001**	0.9825	**<.0001**	**0.0431**	**0.0042**
p-Coumaric acid	**<.0001**	0.3072	**<.0001**	0.1120	**<.0001**	0.9782	0.5357
Ferulic acid	**<.0001**	0.7969	**0.0002**	0.7941	**<.0001**	0.9040	0.0883
Total flavonols	**<.0001**	0.9246	**<.0001**	0.2075	**<.0001**	0.0833	0.5695
Rutin	0.1008	0.3645	**0.0012**	0.6091	0.0599	**0.0006**	0.7271
Isoquercetin	**<.0001**	**0.0347**	**0.0001**	0.2232	**<.0001**	0.0776	**0.0124**
Quercetin	**<.0001**	**0.5097**	**<.0001**	0.4065	**<.0001**	**0.0360**	0.5355
Kaempferol	**<.0001**	0.5143	**<.0001**	0.3545	**<.0001**	0.2532	0.2108
Quercitrin	**<.0001**	0.5185	**<.0001**	0.2932	**<.0001**	0.5436	0.4326
Total anthocyanins	0.1204	**<.0001**	**<.0001**	0.1616	**<.0001**	0.5976	**0.0478**
CyGluc[3]	**<.0001**	0.8673	**<.0001**	**<.0001**	**<.0001**	0.2068	**<.0001**
PetGluc[3]	**<.0001**	**<.0001**	**<.0001**	**0.0004**	**<.0001**	**<.0001**	**<.0001**
PeoGluc[3]	**0.0158**	**0.0013**	**<.0001**	0.2048	**<.0001**	**0.0458**	0.3361
MalGluc[3]	0.1476	**<.0001**	**0.0003**	0.3869	**<.0001**	**0.0428**	**0.0188**
DelGluc[3]	0.7556	**<.0001**	**<.0001**	**0.0145**	**0.0454**	0.2869	0.4484
PetGlucAc[4]	**<.0001**	0.4461	**<.0001**	**0.0001**	**<.0001**	0.0619	**<.0001**
PeoGlucAc[4]	**0.0006**	**0.0003**	**<.0001**	**<.0001**	**0.0007**	**0.0027**	**<.0001**
MalGlucAc[4]	0.0847	0.0766	**<.0001**	0.6867	**<.0001**	**0.0104**	**0.0414**
DelGlucCoum[5]	**<.0001**	0.8418	**<.0001**	**0.0220**	**<.0001**	**<.0001**	**<.0001**
PetGlucCoum[5]	**<.0001**	**0.0002**	0.1229	**<.0001**	**<.0001**	0.1434	**<.0001**
MalGlucCoum[5]	0.0628	**0.0005**	**<.0001**	0.3525	**<.0001**	**0.0107**	**0.0274**

**p*-values in bold indicate significant effects. [1]Treatment; [2]Epigallocatechin 3-*O*-gallate; [3]Cyanidin-, petunidin-, peonidin-, malvidin- and delphinidin 3-*O*-glucosides; [4]Petunidin-, peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides; [5]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides.

The purpose of the 3-factor ANOVA is to establish which effects are statistically significant. Row orientation by ripeness level interaction is significant for all compounds, except rutin (quercetin 3-*O*-rutinoside). Vintage by ripeness level and row orientation (treatment) by vintage by ripeness level interactions are significant for certain compounds or in some instances. Interaction refers to the inconsistency of general trends or patterns in the data.

This confirms that phenolic compound concentrations in grapes are affected by different grapevine row orientation treatments at the various grape berry development stages (ripeness levels) and/or vintage conditions, as indicated by exploratory PCA (Figs. 4.3-4.5). It is therefore sensible to interpret results separately for each grape ripeness level.

4.3.1.1.1 Grapes harvested at ca. 22°Brix

Analysis of variance (2-factor analysis) of grape phenolic data for treatment and vintage main effects

The *p*-values for the 2-factor ANOVA including row orientation as main plot factor and vintage as subplot factor for grapes harvested at *ca*. 22°Brix are listed in Table 4.3.

Table 4.3 Analysis of variance *p*-values (2-factor ANOVA) for treatment and vintage main effects and interaction for Syrah grapes harvested at *ca.* 22°Brix to determine which effects are statistically significant.

	Main effect		Interaction
Phenolic compounds	**Treatment**	**Vintage**	**Treatment x vintage**
Total flavan-3-ols	**<.0001***	**0.0009**	0.4099
(+)-Catechin	**0.0015**	0.2171	0.8429
(-)-Epicatechin	**0.0037**	**0.0501**	0.3766
Epigallocatechin 3-*O*-gallate	**<.0001**	**0.0027**	0.1352
Total phenolic acids	**<.0001**	**0.0064**	**0.0015**
Gallic acid	**0.0025**	**0.0039**	0.4090
Caffeic acid	**<.0001**	**<.0001**	**0.0005**
p Coumaric acid	**<.0001**	0.5169	0.1445
Ferulic acid	**<.0001**	0.5144	**0.0042**
Total flavonols	**<.0001**	0.8483	0.0656
Rutin	0.3820	**0.0341**	0.3493
Isoquercetin	**<.0001**	0.4161	0.1723
Quercetin	**<.0001**	0.0605	0.2818
Kaempferol	**<.0001**	0.0895	**0.0393**
Quercitrin	**<.0001**	0.7451	**0.0152**
Total anthocyanins	**0.0012**	**0.0010**	0.6308
CyGluc[1]	**<.0001**	0.6716	**<.0001**
PetGluc[1]	**<.0001**	**<.0001**	**0.0021**
PeoGluc[1]	**0.0017**	0.0822	0.6622
MalGluc[1]	**0.0051**	**<.0001**	0.6973
DelGluc[1]	0.6010	0.0781	0.5539
PetGlucAc[2]	**0.0005**	0.2785	**<.0001**
PeoGlucAc[2]	**0.0487**	0.3406	0.5767
MalGlucAc[2]	**0.0241**	0.0887	0.2734
DelGlucCoum[3]	**0.0017**	**0.0050**	**0.0216**
PetGlucCoum[3]	**<.0001**	0.6020	**<.0001**
MalGlucCoum[3]	0.0623	0.2131	0.4000

**p*-values in bold indicate significant effects. [1]Cyanidin-, petunidin-, peonidin-, malvidin- and delphinidin 3-*O*-glucosides; [2]Petunidin-, peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides; [3]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides.

Row orientation (treatment) by vintage interaction is not significant for most compounds, except total phenolic acids, caffeic acid, ferulic acid, kaempferol, quercitrin, cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, petunidin 3-*O*-(6-*O*-acetyl) glucoside, delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside and petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside. Tables of means to follow are based on these results and are presented for compounds indicated to be significantly affected by a specific interaction or main effect (row orientation).

Principal component analysis showing association among phenolic compounds, row orientation and vintage

In order to highlight the key features of each treatment, PCA was performed using phenolic compounds as variables. The PCA biplot (Fig. 4.3) of the first two principal components (PC1/F1 and PC2/F2) illustrating the association of phenolic compounds of Syrah grapes (harvested during 2008, 2009, 2010 and 2011) at ripeness levels of *ca.* 22°Brix with the treatments, *i.e.* NS, EW, NE-SW and NW-SE row orientations, explained 59.42% of the variation in the data.

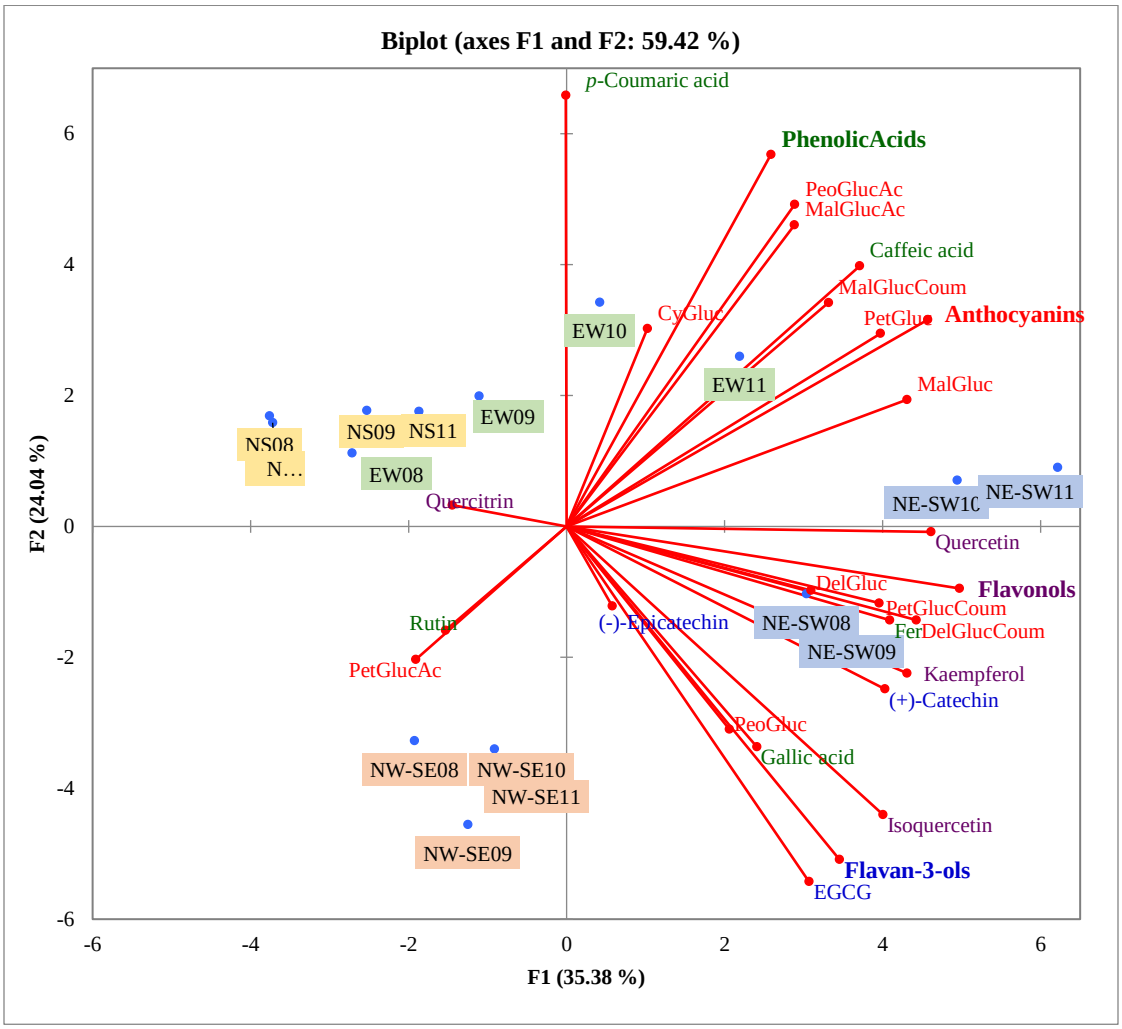

Figure 4.3 PCA biplot illustrating the association of phenolic compound composition of Syrah grapes harvested during 2008, 2009, 2010 and 2011 at ripeness levels of *ca.* 22°Brix with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations.

Principal component 1 shows the main source or greatest variation in the biplot. Variables included in the PCA were limited to those with a significant main effect of squared cosine values ≥ 0.5 (data not shown) or significant effect in the ANOVAs (Table 4.3) Figure 4.3 indicates that the main cause of variation is row orientation, with PC1 mainly separating NE-SW from EW, NS and NW-SE, while PC2 separates NE-SW and NW-SE from EW and NS. The phenolic compounds with the highest squared cosine values on PC1 are (+)-catechin, caffeic acid, ferulic acid, total flavonols, isoquercetin (quercetin 3-*O*-glucoside), quercetin, kaempferol, total anthocyanins, petunidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside and petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside. Flavan-3-ols, epigallocatechin 3-*O*-gallate, total phenolic acids, *p*-coumaric acid, peonidin 3-*O*-(6-*O*-acetyl) glucoside and malvidin 3-*O*-(6-*O*-acetyl) glucoside have the highest squared cosine values on PC2. Principal component 3 explains only an additional 13.85% of variation (data not shown), and does not improve the interpretability/variability, and was therefore not included in the biplot. Only quercitrin (quercetin 3-*O*-rhamnoside), (-)-epicatechin and peonidin 3-*O*-glucosides have the highest squared cosine value on PC3. Fewer close groupings of the same row orientations (treatments) from different vintages may be an indication of variation in phenolic compound composition in grapes harvested during different vintages. In general, the different vintages of the row orientations showed good separation and grouping.

Analysis of variance for grape phenolic compound concentration means for treatment (row orientation) by vintage interaction

Mean concentrations for grape phenolic compounds with a significant treatment (row orientation) by vintage interaction for grapes harvested at *ca*. 22°Brix are listed in Table 4.4. Row orientation treatment by vintage interaction is caused by minor deviations in trends among vintages within row orientation treatments. Therefore, only obvious interactions are discussed.

Grapes from 2010 and 2011 vintages within the EW and NE-SW row orientation treatments had significantly higher concentrations of total phenolic acids (Phen A) than those of the 2008 and 2009 vintages. Caffeic acid (Caff) was significantly higher in grapes from the NE-SW row orientation treatments in 2010 and 2011 vintages.

Vintage interaction was also evident for petunidin 3-*O*-glucoside (PetGluc), because this anthocyanin was significantly higher in the 2010 and 2011 vintages in grapes from the NE-SW row orientation treatments, compared to the 2008 and 2009 vintages. Petunidin 3-*O*-glucoside was also significantly higher in grapes from the 2010 and 2011 vintages from the NS row orientation treatments, compared to the 2008 and 2009 vintages.

Table 4.4 Grape phenolic compound concentration means for treatment (row orientation) by vintage interaction (grapes harvested at *ca.* 22°Brix).*

Treatment	Vintage	Phenolic compounds									
		Total Phen A[2]	**Caff[3]**	**Fer[4]**	**Kaem[5]**	**Qr[6]**	**CyGluc[7]**	**PetGluc[8]**	**PetGlucAc[9]**	**DelGlucCoum[10]**	**PetGlucCoum[11]**
EW[1]	2008	68.919cd** (±7.501)***	33.182gfe (±1.709)	4.455fe (±0.887)	1.417d (±0.311)	8.108ba (±0.730)	8.423dc (±0.558)	10.703dce (±2.716)	3.086cb (±0.088)	7.568e (±0.551)	7.459edf (±0.504)
EW	2009	74.541bc (±5.449)	36.844dc (±5.639)	3.803f (±0.893)	1.443d (±0.244)	8.166a (±1.248)	11.189b (±0.738)	12.565bc (±3.496)	2.473c (±0.561)	7.337ef (±1.389)	7.357edf (±1.230)
EW	2010	**82.018a (±3.997)**	43.636a (±3.961)	3.425f (±0.109)	1.796bac (±0.133)	7.894bac (±1.774)	12.497ba (±1.660)	10.874dc (±1.263)	3.534cb (±0.550)	**9.820bdac (±1.528)**	6.173f (±1.885)
EW	2011	**77.141ab (±2.632)**	36.583dce (±1.336)	5.615dc (±0.652)	1.417d (±0.199)	7.090bdac (±0.582)	13.932a (±0.951)	12.095bc (±1.687)	3.169cb (±0.458)	**10.974a (±0.485)**	8.381edf (±1.051)
NE-SW[1]	2008	66.936de (±4.933)	31.401gf (±4.344)	7.969a (±0.285)	1.674bdc (±0.476)	4.571g (±0.288)	9.272c (±1.397)	10.640dce (±1.127)	3.611b (±0.432)	9.170ebdac (±2.247)	**22.231a (±2.304)**
NE-SW	2009	67.746de (±8.445)	34.150dfe (±3.771)	7.811a (±0.978)	1.894ba (±0.380)	5.653fg (±1.010)	8.874dc (±1.037)	11.341bc (±2.147)	3.502cb (±0.680)	10.364ba (±1.403)	**20.956a (±3.810)**
NE-SW	2010	**77.010ab (±4.244)**	**40.505ba (±3.319)**	7.314ba (±0.248)	1.989a (±0.115)	5.413fg (±1.028)	6.411gfe (±2.405)	**17.023a (±4.472)**	3.593b (±0.368)	10.675a (±0.920)	16.704b (±5.064)
NE-SW	2011	**76.360ab (±3.170)**	**39.825bc (±3.524)**	7.653a (±0.860)	2.071a (±0.065)	5.432fg (±0.844)	4.551ih (±1.642)	**16.484a (±2.382)**	2.679cb (±0.575)	10.809a (±1.960)	13.418c (±0.937)
NS[1]	2008	62.386ef (±6.381)*	26.310ih (±2.278)	4.407fe (±0.442)	0.657e (±0.228	6.301fde (±0.410)	8.025dce (±1.597)	6.518g (±1.458)	**5.711a (±1.176)**	7.504ef (±0.893)	7.013ef (±1.006)
NS	2009	67.775de (±5.843)	29.753gh (±1.492)	6.288bc (±0.569)	0.515e (±0.075)	4.669g (±0.671)	6.140gfh (±1.146)	7.449fg (±1.133)	**5.464a (±1.113)**	5.659f (±1.264)	7.001ef (±2.033)
NS	2010	66.963de (±0.824)	27.626ih (±1.147)	5.359dce (±0.952)	0.490e (±0.044)	5.350fg (±0.885)	5.220gih (±0.910)	**9.746dfce (±1.782)**	3.412cb (±1.199)	7.537e (±0.894)	9.535ed (±1.665)
NS	2011	58.902f (±3.120)	26.802ih (±2.103)	4.481dfe (±0.955)	0.457e (±0.042)	5.478fg (±0.663)	3.730ji (± 0.809)	**14.219ba (±2.807)**	3.025cb (±0.692)	7.990ed (±1.167)	10.215d (±1.466)
NW-SE[1]	2008	50.587g (±3.842)	25.056i (±2.736)	5.215dce (±1.127)	1.449d (±0.237)	5.943fe (±0.793)	2.566j (±0.679)	6.970fg (±0.439)	3.414cb (±0.911)	8.229edc (±0.913)	7.705edf (±1.631)
NW-SE	2009	48.958g (±1.604)	25.372i (±2.808)	5.152dce (±0.384)	1.493dc (±0.216)	7.028bdec (±0.956)	3.621ji (±0.560)	8.076dfge (±1.470)	3.301cb (±0.962)	10.088bac (±1.890)	6.998ef (±1.337)
NW-SE	2010	47.890g (±2.462)	25.421i (±2.195)	5.247dce (±1.489)	1.533dc (±0.165)	6.906dec (±0.610)	7.330dfe (±2.141)	7.821fge (±1.765)	**5.807a (±1.021)**	9.664bdac (±0.803)	8.815edf (±2.193)
NW-SE	2011	49.661g (±0.696)	27.522ih (±1.372)	5.062de (±0.559)	1.775bac (±0.272)	6.929dec (±0.489)	8.243dc (±1.528)	7.811fge (±0.966)	**5.727a (±1.163)**	8.688ebdc (±1.749)	10.343d (±1.709)
p-value		0.0015	0.0005	0.0042	0.0393	0.0152	<.0001	0.0021	<.0001	0.0216	<.0001

[1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast. [2]Total phenolic acids; [3]Caffeic acid; [4]Ferulic acid; [5]Kaempferol; [6]Quercitrin; [7]Cyanidin 3-*O*-glucoside; [8]Petunidin 3-*O*-glucoside; [9]Petunidin 3-*O*-(6-*O*-acetyl) glucoside; [10]Delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside; [11]Petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside. *Means given for compounds with significant treatment (row orientation) and vintage main effects (interaction) as indicated in Table 4.3. **Different letters in the same column indicate significant differences in the content of the compounds measured among the different treatments and vintages according to Fisher's least significant difference test. ***Values in brackets indicate standard deviations.

Petunidin 3-*O*-(6-*O*-acetyl) glucoside (PetGlucAc) concentrations were significantly higher in grapes from the NS row orientation treatments in 2008 and 2009 vintages, whereas grapes from the NW-SE row orientation treatments were significantly higher in the 2010 and 2011 vintages, compared to the 2008 and 2009 vintages for the same compound. Delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides (DelGlucCoum) were significantly higher in grapes from the EW row orientation treatments in 2010 and 2011 vintages, whereas petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides (PetGlucCoum) on the other hand were significantly higher in grapes from the NE-SW row orientation treatments in 2008 and 2009, compared to 2010 and 2011 vintages. Row orientation by vintage interaction of the remaining phenolic compounds caused small differences in trends among vintages within row orientations. This confirms the slight separation of 2008 and 2009 samples from 2010 and 2011 samples for EW and NE-SW row orientations in Figure 4.3.

Analysis of variance for grape phenolic compound concentration means for vintage main effects

Mean concentrations for grape phenolic compounds with significant vintage main effects for grapes harvested at *ca.* 22°Brix are listed in Table 4.5.

Table 4.5 Grape phenolic compound concentration means for vintage main effects (grapes harvested at *ca.* 22°Brix).*

Phenolic compounds	Vintage				*p*-value
	2008	2009	2010	2011	
Total flavan-3-ols	11.820c** (±1.290)***	12.137bc (±1.383)	12.403ba (±1.344)	**12.713a (±1.048)**	0.0009
(-)-Epicatechin	4.958b (±0.185)	5.022ba (±0.330)	5.202a (±0.391)	5.199a (±0.393)	0.0501
EGCG[1]	1.596b (±0.905)	1.666b (±1.167)	1.890a (±0.954)	1.941a (±0.837)	0.0027
Gallic acid	1.510a (±0.296)	1.680a (±0.390)	1.515a (±0.274)	**1.313b (±0.352)**	0.0039
Quercetin 3-*O*-rutinoside (rutin)	0.652a (±0.129)	0.585ba (±0.159)	0.514b (±0.182)	0.657a (±0.138)	0.0341
Total anthocyanins	372.115c (±59.870)	375.960bc (±60.707)	411.303ba (±57.418)	**439.961a (±69.479**	0.0010
Malvidin 3-*O*-glucosides	80.369c (±18.658)	87.711cb (±26.647)	95.139b (±16.026)	**120.428a (±25.486)**	<.0001

*Means given for compounds with significant vintage main effects as indicated in Table 4.3. **Different letters in the same row indicate significant differences in the content of the compounds measured among the different vintages according to Fischer's least significant difference test. ***Values in brackets indicate standard deviations. [1]Epigallocatechin 3-*O*-gallate.

The tendency for certain compounds is that grapes harvested in 2008 and 2009 have significantly lower concentrations than those harvested in 2010 and 2011. The exception is gallic acid for which significantly lower concentrations were observed in 2011. Total flavan-3-ols and total anthocyanins were significantly higher in 2011, but were not significantly different in the 2010 vintage. Malvidin 3-*O*-glucoside concentrations were significantly higher in 2011.

Analysis of variance for grape phenolic compound concentration means for treatment (row orientation) main effects

Mean concentrations for grape phenolic compounds with a significant treatment (row orientation) main effect for grapes harvested at *ca*. 22°Brix are listed in Table 4.6. Among the individual chemical compositional variables quantified, a number of phenolic compound concentration differences were recorded.

Table 4.6 Grape phenolic compound concentration means for treatment (row orientation) main effects (grape harvested at *ca.* 22°Brix).*

Phenolic compounds	Row orientation (treatment)				*p*-value
	EW[1]	NE-SW[1]	NS[1]	NW-SE[1]	
Total flavan-3-ols	11.401b** (±0.846)***	13.199a (±0.521)	**11.008c (±0.796)**	13.404a (±0.720)	**<.0001**
(+)-Catechin	5.386a (±0.452)	5.588a (±0.355)	**5.100b (±0.501**)	5.529a (±0.483)	0.0015
(-)-Epicatechin	5.230a (±0.403)	4.986b (±0.353)	4.931b (±0.140)	5.214a (±0.327)	0.0037
Epigallocatechin 3-*O*-gallate	0.784b (±0.342)	2.625a (±0.355)	0.976b (±0.352)	2.660a (±0.377)	<.0001
Gallic acid	1.504a (±0.240)	1.669a (±0.363)	**1.173b (±0.351)**	1.621a (±0.230)	0.0025
p Coumaric acid	31.723a (±4.535)	25.671b (±3.623)	29.117ba (±5.478)	**16.031c (±2.113)**	<.0001
Total flavonols	16.056b (±1.449)	**24.302a (±2.100)**	**13.032c (±1.388)**	15.534b (±1.288)	**<.0001**
Quercetin 3-*O*-glucoside[2]	**1.711c (±0.291)**	**3.095a (±0.477)**	**1.569c (±0.251)**	2.693b (±0.406)	<.0001
Quercetin	5.041a (±0.799)	4.065b (±1.955)	5.419a (±0.907)	4.600ba (±0.644)	<.0001
Total anthocyanins	407.968b (±67.242)	**452.845a (±70.786)**	387.571cb (±46.590)	352.615c (±41.015)	**0.0012**
PeoGluc[3]	14.802ba (±2.687)	14.159b (±2.079)	**11.221c (±2.189)**	**16.286a (±4.640)**	0.0017
MalGluc[3]	101.381ba (±29.376)	113.582a (±28.181)	86.223bc (±19.717)	84.368c (±19.974)	0.0051
PeoGlucAc[4]	11.203a (±2.467)	10.909a (±2.072)	10.429ba (±1.528)	9.242b (±1.470)	0.0487
MalGlucAc[4]	57.721a (±11.696)	60.936a (±16.439)	56.496a (±13.932)	**43.638b (±12.062)**	0.0240

[1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast. [2]Isoquercetin; [3]Peonidin- and malvidin 3-*O*-glucosides; [4]Peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides. *Means given for compounds with significant treatment (row orientation) main effects as indicated in Table 4.3. **Different letters in the same row indicate significant differences in the content of the compounds measured among the different treatments according to Fisher's least significant difference test. ***Values in brackets indicate standard deviations.

Flavan-3-ols

Total flavan-3-ols were significantly higher in grapes from the NW-SE row orientation treatments, but were not significantly different from the NE-SW row orientation treatments. Significantly lower total flavan-3-ol and (+)-catechin concentrations were recorded in grapes from the NS row orientations treatments.

Phenolic acids

Gallic acid concentrations were significantly lower in grapes from the NS row orientation treatments with significantly lower *p*-coumaric acid concentrations in grapes from the NW-SE row orientation treatments.

Flavonols

Grapes from the NE-SW row orientation treatments were significantly higher in total flavonols, whereas grapes from the NS row orientation treatments were significantly lower in these compounds. Isoquercetin (quercetin 3-*O*-glucoside) concentrations were significantly higher in grapes from NE-SW row orientation treatments. Significantly lowest concentrations of this compound were found in grapes from the NS and EW treatments.

Anthocyanins

Total anthocyanin concentrations were significantly higher in grapes from the NE-SW row orientation treatments with peonidin 3-*O*-glucosides (PeoGluc) significantly lower in grapes from the NS row orientation treatments. Malvidin 3-*O*-(6-*O*-acetyl) glucoside (MalGlucAc) concentrations were significantly lower in grapes from the NW-SE row orientation treatments.

4.3.1.1.2 Grapes harvested at ca. 24°Brix

Analysis of variance (2-factor analysis) of grape phenolic data for treatment and vintage main effects

The *p*-values for the 2-factor ANOVA including row orientation as main plot factor and vintage as subplot factor for grapes harvested at *ca*. 24°Brix are listed in Table 4.7.

Table 4.7 Analysis of variance *p*-values for treatment and vintage, main effects and interaction for Syrah grapes harvested at *ca.* 24°Brix to determine which effects are statistically significant.

Phenolic compounds	Main effects		Interaction
	Treatment	Vintage	Treatment x vintage
Total flavan-3-ols	**0.0005***	**0.0143**	0.5144
(+)-Catechin	**<.0001**	0.1517	0.9102
(-)-Epicatechin	0.1870	**<.0001**	0.1582
Epigallocatechin 3-*O*-gallate	**<.0001**	0.4110	0.5803
Total phenolic acids	**<.0001**	0.7496	0.8200
Gallic acid	**<.0001**	0.0055	**0.0078**
Caffeic acid	**<.0001**	0.9951	0.9502
p Coumaric acid	**<.0001**	0.5285	**0.0526**
Ferulic acid	**<.0001**	0.5399	0.5249
Quercetin 3-*O*-rutinoside (rutin)	**0.0228**	**0.0468**	**0.5053**
Total flavonols	**<.0001**	0.2568	0.1704
Quercetin 3-*O*-glucoside (Isoquercetin)	**<.0001**	**0.0140**	**0.0023**
Quercetin	**<.0001**	0.2752	0.4879
Kaempferol	**<.0001**	0.1663	0.5133
Quercetin 3-*O*-rhamnoside (quercitrin)	**<.0001**	0.3409	0.1366
Total anthocyanins	**0.0023**	**0.0002**	**0.0374**
CyGluc[1]	**0.0020**	0.0530	0.2107
PetGluc[1]	**0.0003**	**0.0001**	**0.0002**
PeoGluc[1]	0.2300	0.1910	0.5479
MalGluc[1]	**0.0232**	**<.0001**	**0.0238**
DelGluc[1]	0.1015	**<.0001**	0.1731
PetGlucAc[2]	0.2054	**0.0423**	0.9217
PeoGlucAc[2]	0.2725	**<.0001**	0.5022
MalGlucAc[2]	**0.0249**	**0.0002**	**0.0175**
DelGlucCoum[3]	0.0672	**0.0333**	0.9756
PetGlucCoum[3]	**<.0001**	**0.0004**	**<.0001**
MalGlucCoum[3]	**0.0280**	0.0828	0.2584

**p*-values in bold indicate significant effects. [1]Cyanidin-, petunidin-, peonidin-, malvidin- and delphinidin 3-*O*-glucosides; [2]Petunidin-, peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides; [3]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides.

Row orientation (treatment) by vintage interaction is not significant for most compounds, except gallic acid, *p*-coumaric acid, isoquercetin, total anthocyanins, petunidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, malvidin 3-*O*-(6-*O*-acetyl) glucoside and petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside.

Principal component analysis showing association among phenolic compounds, row orientations and vintage

The PCA biplot (Fig. 4.4) of the first two principal components (PC1/F1 and PC2/F2) illustrating the association of phenolic compounds of Syrah grapes (harvested during 2008, 2009, 2010 and 2011) at ripeness levels of *ca.* 24°Brix with row orientation treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations, explained 57.61% of the variation in the data. In general, the different vintages of the row orientations separated and grouped reasonably well.

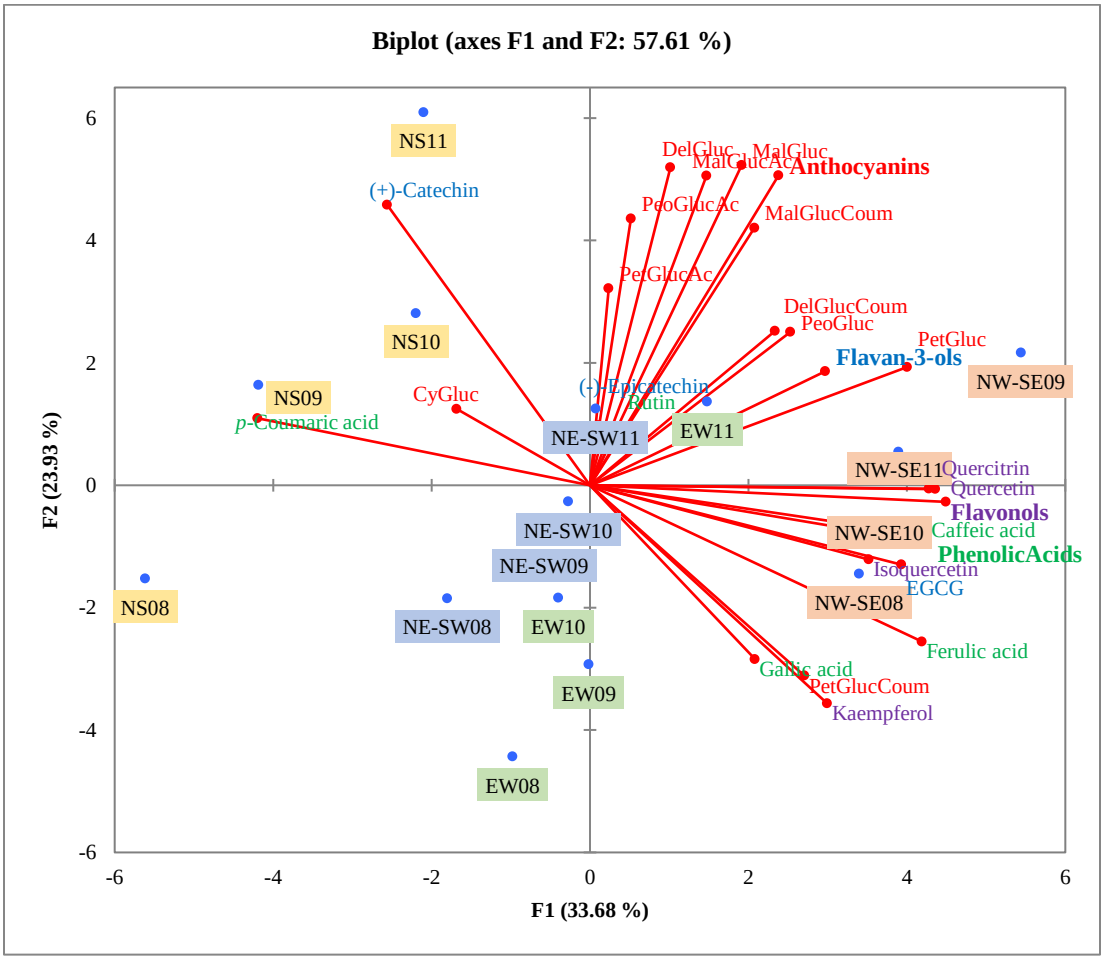

Figure 4.4 PCA biplot illustrating the association of phenolic compound composition of Syrah grapes harvested during 2008, 2009, 2010 and 2011 at ripeness levels of *ca.* 24°Brix with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations.

Principal component 1 shows the main cause or maximum variation in the biplot. Variables included in the PCA were limited to those with a significant main effect of squared cosine values ≥ 0.5 (data not shown) or significant effect in the ANOVAs (Table 4.7). The main cause of variation is row orientation, with PC1 mainly separating NW-SE from EW, NE-SW and NS, while PC2 separates EW, NE-SW and NW-SE from NS. The phenolic compounds with the highest squared cosine values (≥ 0.5) on PC1 are epigallocatechin 3-*O*-gallate (EGCG), phenolic acids, caffeic acid, *p*-coumaric acid, ferulic acid, total favonols, quercetin, quecitrin (quercetin 3-*O*-rhamnoside) and petunidin 3-*O*-glucoside (PetGluc).

Catechin (+), total anthocyanins, malvidin 3-*O*-glucoside (MalGluc), delphinidin 3-*O*-glucoside (DelGluc), peonidin 3-*O*-(6-*O*-acetyl) glucoside (PeoGlucAc) and malvidin 3-*O*-(6-*O*-acetyl) glucoside (MalGlucAc) have the highest squared cosine values on PC2.

Gallic acid, rutin (quercetin 3-*O*-rutinoside) and cyanidin 3-*O*-glucoside have the highest squared cosine values on PC3, but explained only an additional 15.17% of variation in the biplot. It does not improve interpretability and was therefore not included in the biplot (Fig. 4.4).

Analysis of variance for grape phenolic compound concentration means for treatment (row orientation) by vintage interaction

Mean concentrations for grape phenolic compounds with a significant treatment (row orientation) by vintage interaction for grapes harvested at *ca*. 24°Brix are listed in Table 4.8. The data shows that row orientation by vintage interaction is caused by minor deviations in trends among years within row orientations. Therefore, only apparent interaction will be discussed.

Vintage interaction was evident in gallic acid because this phenolic acid was significantly higher in the 2009 and 2010 vintages in grapes from the NS row orientation treatments. Vintage interaction was also distinct in EW row orientation treatments where isoquercetin was significantly higher in grapes from the 2009 vintage.

Total anthocyanins in grapes from 2011 vintage within the EW row orientation treatment were significantly higher than in grapes from the 2008, 2009 and 2010 vintages. Grapes from the NS row orientation treatments within the 2008 and 2011 vintages were significantly lower and significantly higher, respectively, in total anthocyanins.

Petunidin 3-*O*-glucosides (PetGluc) were significantly lower in grapes from 2008 and 2009 vintages for both the EW and NE-SW row orientation treatments.

Table 4.8 Grape phenolic compound concentration means for treatment (row orientation) by vintage interaction (grape harvested at *ca*. 24°Brix).*

Treatment	Vintage	Phenolic compounds						
		Gallic acid	Isoquercetin	Total anthocyanins	PetGluc[2]	MalGluc[3]	MalGlucAc[4]	PetGlucCoum[5]
EW[1]	2008	1.712ba** (±0.337)***	1.906dc (±0.415)	307.471fe (±39.793)	**6.214g (±0.648)**	58.942f (±12.243)	40.462d (±2.687)	13.800ba (±2.297)
EW	2009	1.690bac (±0.291)	**2.609a (±0.283)**	322.701fde (±34.204)	**7.779gf (±1.672)**	72.352fde (±10.832)	41.711d (±1.585)	12.061bc (±1.109)
EW	2010	1.385bdc (±0.305)	1.647def (±0.344)	330.711fde (±56.392)	10.043edc (±1.108)	87.540bdec (±16.512)	44.625cd (±1.069)	11.033dc (±1.346)
EW	2011	1.693bac (±0.249)	1.868dc (±0.586)	**410.701bac (±36.457**)	12.274ba (±1.455)	**112.280a (±16.390)**	**60.418b (±5.636)**	12.504bac (±0.490)
NE-SW[1]	2008	1.204d (±0.116)	2.477ba (±0.180)	341.352fde (±43.486)	**7.767gf (±2.210)**	82.732fbdec (±7.569)	47.910cbd (±1.211)	**12.303bac (±1.289)**
NE-SW	2009	1.802a (±0.286)	2.492ba (±0.242)	322.762fde (±27.878)	**8.455ef (±0.727)**	76.812fdec (±3.988)	46.192cd (±3.778)	**13.585ba (±1.349)**
NE-SW	2010	1.842a (±0.220)	2.600a (±0.268)	334.620fde (±27.484)	11.052bac (±2.200)	87.012bdec (±23.776)	48.060cbd (±3.199)	9.566edf (±3.247)
NE-SW	2011	1.604bac (±0.269)	2.779a (±0.180)	364.671bdec (±50.906)	10.643bdc (±0.979)	98.071bac (±20.851)	44.848cd (±2.628)	8.506ef (±0.451)
NS[1]	2008	0.660e (±0.472)	1.326f (±0.232)	**289.501f (±12.696)**	6.940gf (±1.916)	**66.080fe (±7.778)**	38.677d (±6.494)	5.794g (±1.537)
NS	2009	**1.198d (± 0.301)**	1.336f (±0.244)	357.481dec (±29.377)	7.828gf (±1.008)	93.761bdac (±17.880)	46.376cd (±5.761)	6.204g (±1.203)
NS	2010	**1.096d (±0.167)**	**1**.761de (±0.086)	380.321bdac (±21.574)	8.365ef (±1.087)	104.633ba (±8.801)	57.398cb (±6.833)	7.854gf (±1.028)
NS	2011	0.544e (±0.207)	1.466ef (±0.271)	**441.871a (±63.265)**	8.641edf (±0.378)	112.391a (±31.006)	**75.923a (±9.735)**	7.602gf (±1.379)
NW-SE[1]	2008	1.354dc (±0.162)	2.212bc (±0.213)	362.433dec (±46.303)	11.496bac (±1.311)	87.072bdec (±10.650)	50.324cbd (±9.438)	14.332a (±1.258)
NW-SE	2009	1.423bdc (±0.175)	2.758a (±0.302)	427.750ba (±43.480)	12.977a (±2.846)	118.171a (±23.342)	57.252cb (±6.381)	12.257bac (±2.691)
NW-SE	2010	1.241d (±0.117)	2.460ba (±0.328)	370.501bdec (±50.918)	11.013bac (±0.374)	84.551bdec (±14.923)	50.300cbd (±5.883)	10.502edc (±3.285)
NW-SE	2011	1.384bdc (±0.104)	2.693a (±0.283)	381.891bdac (±62.441)	10.206edc (±0.925)	99.561bac (±19.960)	60.519b (±5.619)	8.443ef (±1.004)
p-value		0.0078	0.0023	0.0374	0.0002	0.0238	0.0175	<.0001

[1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast. *Means given for compounds with significant treatment (row orientation) and vintage (interaction) main effects as indicated in Table 4.7. [2]Petunidin 3-*O*-glucoside; [3]Malvidin 3-*O*-glucoside; [4]Malvidin 3-*O*-(6-*O*-acetyl) glucoside; [5]Petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside. **Different letters in the same column indicate significant differences in the content of the compounds measured among the different treatments and vintages according to Fisher's least significant difference test. ***Values in brackets indicate standard deviations.

Grapes from the EW row orientation treatments in 2011 were significantly higher in malvidin 3-*O*-glucosides (MalGluc), whereas grapes from the NS row orientation treatments in 2008 were significantly lower in malvidin 3-*O*-glucosides. Vintage interaction was also obvious in the EW row orientation treatments for malvidin 3-*O*-(6-*O*-acetyl) glucosides (MalGlucAc), which were significantly higher in grapes from the 2011 vintage than in those of the 2008, 2009 and 2010 vintages. Grapes from the NS row orientation treatments within the 2011 vintage were significantly higher in malvidin 3-*O*-(6-*O*-acetyl) glucosides. Petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides (PetGlucCoum) were significantly higher in grapes from NE-SW row orientation treatments in 2008 and 2009 vintages, compared those in the 2010 and 2011 vintages. Row orientation by vintage interaction of the remaining phenolic compounds was not significant among vintages within row orientations. The results confirm the slight separation of 2009 and 2010 samples from 2008 and 2011 samples for NE-SW row orientation treatments and 2009 samples from 2008, 2010 and 2011 samples for NW-SE row orientations in Figure 4.4.

Analysis of variance for grape phenolic compound concentration means for vintage main effects

Mean concentrations for grape phenolic compounds with a significant vintage main effect for grapes harvested at *ca.* 24°Brix are listed in Table 4.9.

Table 4.9 Grape phenolic compound concentration means for vintage main effect (grape harvested at *ca.* 24°Brix).*

Phenolic compounds	Vintage				*p*-value
	2008	2009	2010	2011	
Total flavan-3-ols	**12.387b** ** **(±0.666)** ***	12.811a (±0.779)	13.157a (±0.656)	12.897a (±0.589)	0.0143
(-)-Epicatechin	5.005b (±0.294)	5.072b (±0.290)	**5.670a (±0.390)**	5.119b (±0.396)	<.0001
Quercetin 3-*O*-rutinoside (rutin)	0.685ba (±0.076)	0.669ba (±0.098)	0.723a (±0.156)	0.635b (±0.078)	0.0468
DelGluc[1]	**1.765c (±0.228)**	2.156ba (±0.381)	1.971bc (±0.318)	2.351a (±0.294)	<.0001
PetGlucAc[2]	2.884b (±0.430)	3.185ba (±0.376)	3.316a (±0.672)	3.368a (±0.340)	0.0423
PeoGlucAc[2]	10.201b (±1.429)	12.493a (±1.854)	11.270b (±1.559)	13.179a (±1.562)	<.0001
DelGlucCoum[3]	6.841b (±1.387)	7.333ba (±1.258)	8.014a (±1.368)	7.685ba (±1.453)	0.0333

*Means given for compounds with significant vintage main effects as indicated in Table 4.7. [1]Delphinidin 3-*O*-glucoside; [2]Petunidin 3-*O*-(6-*O*-acetyl) glucoside; [2]Peonidin 3-*O*-(6-*O*-acetyl) glucoside; [3]Delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside. **Different letters in the same row indicate significant differences in the content of the compounds measured among the different vintages according to Fischer's least significant difference test. ***Values in brackets indicate standard deviations.

Vintage effect on phenolic compounds was not evident except for total flavan-3-ol concentrations in grapes harvested in 2008, which were significantly lower than in grapes harvested in 2009, 2010 and 2011. Grapes harvested in 2010 were significantly higher in (-)-epicatechin concentrations. Delphinidin 3-*O*-glucoside concentrations were significantly lower in grapes harvested in 2008, but were not significantly different from grapes harvested in 2010. Significant differences among vintages for the remaining variables were not evident.

Analysis of variance for grape phenolic compound concentration means for treatment (row orientation) main effects

Mean concentrations for grape phenolic compounds with a significant treatment (row orientation) main effect for grapes harvested at *ca*. 24°Brix are listed in Table 4.10.

Flavan-3-ols

Catechins (+) concentrations were significantly high in grapes from the NS row orientation treatments with epigallocatechin 3-*O*-gallate concentrations significantly lower in grapes from the same row orientation.

Table 4.10 Grape phenolic compound concentration means for treatment (row orientation) main effect (grape harvested at *ca.* 24°Brix).*

Phenolic compounds	Treatment (row orientation)				*p*-value
	EW[1]	NE-SW[1]	NS[1]	NW-SE[1]	
Total flavan-3-ols	12.475b** (±0.577)***	13.209a (±0.749)	12.477b (±0.612)	13.102a (±0.594)	**0.0005**
(+)-Catechin	5.121c (±0.261)	5.452b (±0.338)	**5.774a (±0.491)**	5.214c (±0.286)	<.0001
EGCG[2]	1.993b (±0.427)	2.663a (±0.325)	**1.460c (±0.165)**	2.772a (±0.331)	<.0001
Total phenolic acids	**85.961b (±6.623)**	**57.244d (±3.232)**	**63.865c (±5.108)**	**117.728a (±6.845)**	**<.0001**
Caffeic acid	**48.391b (±6.252)**	**15.036d (±1.205)**	**24.648c (±3.241)**	**83.658a (±6.312)**	<.0001
p Coumaric acid	28.301b (±3.517)	34.413a (±3.039)	34.723a (±4.894)	**25.084c (±1.825)**	<.0001
Ferulic acid	6.996a (±1.124)	5.541b (±1.211)	**2.905c(±0.753)**	6.915a (±0.913)	<.0001
Total flavonols	16.711b (±2.063)	16.440b (±1.333)	**13.836c (±1.942)**	**34.406a (±2.726)**	**<.0001**
Quercetin 3-*O*-rutinoside (rutin)	0.643b (±0.102)	0.632b (±0.116)	0.713a (±0.091)	0.715a (±0.098)	0.0228
Quercetin	5.921a (±1.487)	6.012a (±1.014)	**5.358b (±1.090)**	5.976a (±1.957)	<.0001
Kaempferol	**1.569a (±0.211)**	1.304b (±0.070)	**0.726c (± 0.281**	1.317b (±0.074)	<.0001
Quercetin 3-*O*-rhamnoside (quercitrin)	**7.226a (±1.028)**	6.531b (±0.922)	6.286b (±0.996)	**4.572c (±1.560)**	<.0001
CyGluc[3]	**1.964c (±0.268)**	2.288b (±0.487)	2.654a (±0.579)	2.406ba (±0.549)	0.0020
MalGlucCoum[4]	15.276bc (±2.713)	14.547c (±2.335)	16.403ba (±2.909)	16.871a (±2.699)	0.0280

*Means given for compounds with significant treatment (row orientation) main effects as indicated in Table 4.7. [1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Epigallocatechin 3-*O*-gallate; [3]Cyanidin 3-*O*-glucoside; [4]Malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside. **Different letters in the same row indicate significant differences in the content of the compounds measured among the different treatments according to Fisher's least significant difference test. ***Values in brackets indicate standard deviations.

Phenolic acids

Significant concentration differences were evident for total phenolic acid and caffeic acid concentrations among the four row orientation treatments. Significantly higher total phenolic acid and caffeic acid concentrations were found in grapes from the NW-SE row orientation treatments with significantly lower concentrations in grapes from the NE-SW row orientation treatments. Grapes from NW-SE row orientation treatments were significantly lower in *p*-coumaric acid concentrations, whereas ferulic acid was significantly lower in grapes from the NS row orientation treatments.

Flavonols

Total flavonols were significantly higher in grapes from the NW-SE row orientation treatments with significantly lowest concentrations in grapes from the NS row orientation treatments. Quercetin and kaempferol were significantly lower in grapes from the NS row orientation treatments.

Grapes from the EW row orientation treatments were significantly higher in kaempferol concentrations. Quercitrin concentrations were significantly higher in grapes from the EW row orientation treatments with significantly lowest concentrations in grapes from the NW-SE row orientation treatments.

Anthocyanins

Cyanidin 3-*O*-glucoside concentrations were significantly higher in grapes from the EW row orientation treatments. No significant differences in malvidin 3-*O*-(6-*O*-coumaroyl) glucosides were found among grapes from the EW, NS, NE-SW and NW-SE row orientation treatments.

4.3.1.1.3 Grapes harvested at ca. 26°Brix

Analysis of variance (2-factor analysis) of grape phenolic data for treatment and vintage

The *p*-values for the 2-factor ANOVA including row orientation as main plot factor and vintage as subplot factor for grapes harvested at *ca*. 26°Brix are listed in Table 4.11.

Table 4.11 Anova *p*-values for treatment and vintage main effects and interaction for Syrah grapes harvested at *ca.* 26°Brix to determine which effects are statistically significant.

Phenolic compounds	Main effects		Interaction
	Treatment	Vintage	Treatment x vintage
Total flavan-3-ols	**<.0001***	0.2066	**0.0284**
(+)-Catechin	**<.0001**	**0.0006**	**0.0137**
(-)-Epicatechin	0.3470	0.2872	0.6771
Epigallocatechin 3-*O*-gallate	**0.0008**	0.9208	**0.0469**
Total phenolic acids	**<.0001**	0.0636	0.4158
Gallic acid	**<.0001**	0.3873	0.1293
Caffeic acid	**<.0001**	0.0838	0.4907
p Coumaric acid	**<.0001**	**0.0158**	0.4288
Ferulic acid	**0.0007**	0.4721	0.8558
Quercetin 3-*O*-rhamnoside (rutin)	**0.0180**	**0.0116**	0.3489
Total flavonols	**<.0001**	0.1640	0.4008
Quercetin 3-*O*-glucoside (isoquercetin)	**<.0001**	**0.0091**	0.4195
Quercetin	**0.0079**	**0.0503**	0.0737
Kaempferol	**<.0001**	0.6634	0.4476
Quercetin 3-*O*-rhamnoside (quercitrin)	**<.0001**	0.5543	0.4541

**p*-values in bold indicate significant effects.

Table 4.11 Continued.

Phenolic compounds	Main effects		Interaction
	Treatment	Vintage	Treatment x vintage
Total anthocyanins	0.9236	**0.0006**	0.1170
CyGluc[1]	**0.0085**	0.4473	0.4327
PetGluc[1]	**<.0001**	0.6105	**<.0001**
PeoGluc[1]	**0.0070**	**0.0007**	**0.0351**
MalGluc[1]	**<.0001**	**0.0038**	0.8793
DelGluc[1]	**0.0111**	**0.0001**	**0.0304**
PetGlucAc[2]	**<.0001**	0.2598	**<.0001**
PeoGlucAc[2]	**0.0005**	**0.0167**	**<.0001**
MalGlucAc	**0.0005**	0.5906	0.8032
DelGlucCoum[3]	**<.0001**	**0.0114**	**<.0001**
PetGlucCoum[3]	**<.0001**	**0.0042**	**<.0001**
MalGlucCoum[3]	**0.0008**	**<.0001**	**0.0216**

*p-values in bold indicate significant effects. [1]Cyanidin-, petunidin-, peonidin-, malvidin- and delphinidin 3-*O*-glucosides; [2]Petunidin-, peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides; [3]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides.

Row orientation (treatment) by vintage interaction is not significant for most compounds, except total flavan-3-ols, (+)-catechin, epigallocatechin 3-*O*-gallate, petunidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, petunidin 3-*O*-(6-*O*-acetyl) glucoside, peonidin 3-*O*-(6-*O*-acetyl) glucoside, delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside, petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside.

Principal component analysis showing association among phenolic compounds, row orientations and vintages

The PCA biplot (Fig. 4.5) of the first two principal components (PC1/F1 and PC2/F2) illustrating the association of phenolic compounds of Syrah grapes (harvested during 2008, 2009, 2010 and 2011) at ripeness levels of *ca.* 26°Brix with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations, explained 54.75% of the variation in the data. The different vintages of the row orientation treatments showed excellent separation and grouping.

Principal component 1 is the main source or shows greatest variation in the biplot. Variables included in the PCA were limited to those with a significant main effect of squared cosine values greater or equal to 0.5 (data not shown) or significant effect in the ANOVAs (Table 4.11). The main cause of variation is row orientation, with PC1 mainly separating NE-SW, EW and NS from NW-SE row orientation treatments, while PC2 separates EW, NS and NW-SE from NE-SW row orientation treatments. The phenolic compounds with the highest squared cosine values (≥ 0.5) on PC1 are total flavan-3-ols, (+)-catechin, total phenolic acids, gallic acid, caffeic acid, ferulic acid, total flavonols, kaempferol, quercitrin (quercetin 3-*O*-rhamnoside) and malvidin 3-*O*-(6-*O*-acetyl) glucosides (MalGlucAc).

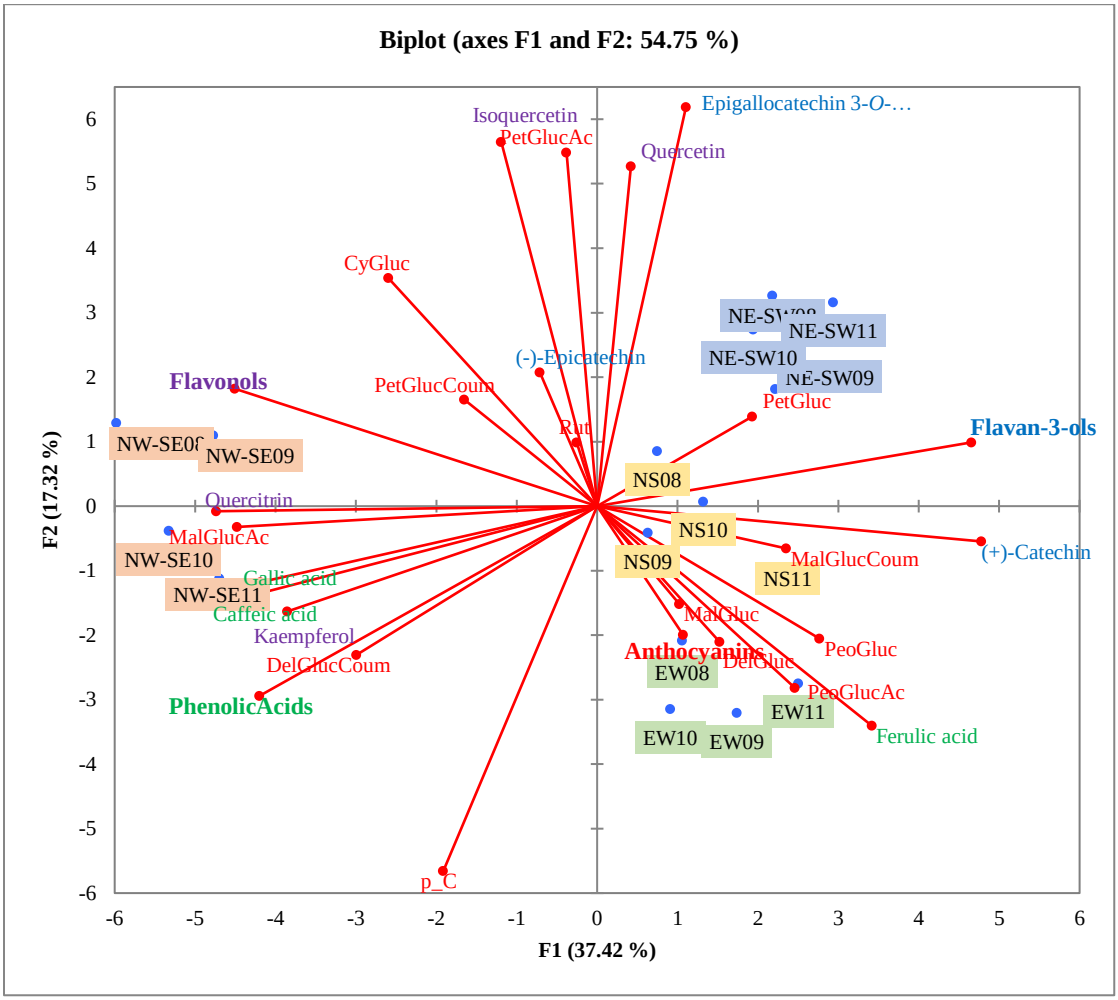

Figure 4.5 PCA biplot illustrating the association of phenolic compound composition of Syrah grapes harvested during 2008, 2009, 2010 and 2011 at ripeness levels of *ca.* 26°Brix with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations.

Epigallocatechin 3-*O*-gallate, *p*-coumaric acid, isoquercetin (quercetin 3-*O*-glucoside), quercetin and petunidin 3-*O*-(6-*O*-acetyl) glucoside (PetGlucAc) have the highest squared cosine values on PC2 (data not shown).

Peonidin 3-*O*-glucoside (PetGluc), malvidin 3-*O*-glucoside (MalGluc) and malvidin 3-*O*-(6-*O*-coumaryol) glucoside (MalGlucCoum) have the highest squared cosine values on PC3 but contributing only an additional 13.76% to the variability in the PCA biplot. It does not improve interpretability and is therefore not included in the biplot.

Analysis of variance for grape phenolic compound concentration means for treatment (row orientation) by vintage interaction

Mean concentrations for grape phenolic compounds with a significant treatment (row orientation) by vintage interaction for grapes harvested at *ca.* 26°Brix are listed in Table 4.12. Row orientation by vintage interaction caused minor deviations in trends among vintages within row orientation treatments.

Therefore, only noticeable interaction will be discussed. Grapes from the 2009 vintage within the NW-SE row orientation treatments had significantly higher concentrations of total flavan-3-ols than those of the 2008, 2010 and 2011 vintages. Vintage interaction was evident in (+)-catechin because this flavan-3-ol was significantly higher in the 2010 and 2011 vintages in grapes from the NS row orientation treatments. Petunidin 3-*O*-glucosides (PetGluc) were significantly higher in grapes in the 2008 and 2009 vintages, compared to those in grapes in the 2010 and 2011 vintages from the NE-SW row orientation treatments.

Significantly higher concentrations were also evident in grapes in the 2008 vintage from the NW-SE row orientation treatments. Peonidin 3-*O*-glucosides (PeoGluc) were significantly higher in grapes in the 2009 vintage from the NW-SE row orientation treatments; whereas delphinidin 3-*O*-glucosides (DelGluc) were significantly lower in grapes in the 2008 vintage from both the EW and NE-SW row orientation treatments.

Vintage interaction was also apparent for petunidin 3-*O*-(6-*O*-acetyl) glucosides (PetGlucAc) since this anthocyanin was significantly higher in the 2010 and 2011 vintages in grapes from the NE-SW row orientation treatments. Petunidin 3-*O*-(6-*O*-acetyl) glucosides also showed interaction in the 2008/2009 and 2010/2011 vintages in grapes from the NS and NW-SE row orientation treatments; it was significantly higher in grapes of the 2010/2011 vintages for both treatments.

Peonidin 3-*O*-(6-*O*-acetyl) glucoside (PeoGlucAc) concentrations were significantly higher in grapes in the 2008 vintage from the EW row orientation treatments. Vintage interaction was also distinct in peonidin 3-*O*-(6-*O*-acetyl) glucosides from the NE-SW row orientation treatments in 2008 and 2009 vintages. This anthocyanin was significantly higher in 2008 and 2009 vintages. Significantly higher concentrations were also evident in grapes in the 2010 and 2011 vintages from the NS row orientation treatments.

Delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides (DelGlucCoum) were significantly higher in grapes in the 2008 and 2009 vintages than in 2010 and 2011 vintages from the NS row orientation treatments.

Petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides (PetGlucCoum) concentrations were significantly higher in both the 2008 and 2009 vintages in grapes from NE-SW and the NW-SE row orientation treatments. This compound was also significantly higher in grapes from the NS row orientation in the 2010 and 2011 vintages, compared to the 2008 and 2009 vintages. Malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside (MalGlucCoum) concentrations in grapes were significantly higher in the 2010 and 2011 vintages from both the NS and NW-SE row orientation treatments.

Row orientation by vintage interaction of the remaining phenolic compounds caused minor differences in trends among vintages within row orientations. These results confirm the slight separation of 2011 samples from the 2008, 2009 and 2011 samples for the NS row orientation treatments and the 2008 and 2009 samples from the 2010 and 2011 samples for NW-SE row orientations in Figure 4.5.

Table 4.12 Grape phenolic compound concentration means for treatment (row orientation) by vintage interaction (grape harvested at *ca*. 26°Brix).*

		Phenolic compounds										
Treat[2]	**Vin[3]**	**Flavan[4]**	**(+)-Catechin**	**EGCG[5]**	**PetGluc[6]**	**PeoGluc[6]**	**DelGluc[6]**	**PetGlucAc[7]**	**PeoGlucAc[7]**	**DelGlucCoum[8]**	**PetGlucCoum[8]**	**MalGlucCou[8]**
EW[1]	2008	11.938ef** (±0.484)***	5.354cb (±0.212)	1.513gh (±0.279)	7.740fegd (±1.418)	20.472a (±1.677)	**1.415gf (±0.081)**	2.683ef (±0.663)	**10.434ed (±0.881)**	12.268ba (±0.956)	6.279cd (±0.918)	13.533bc (±3.281)
EW	2009	11.758f (±0.195)	5.238c (±0.031)	1.467h (±0.308)	10.055bc (±1.021)	19.739a (±3.902)	1.929ebdac (±0.646)	2.443f (±0.405)	13.515ba (±1.836)	11.659b (±1.472)	6.223cd (±1.541)	15.00ba (±1.751)
EW	2010	12.132edf (±0.172)	5.399cb (±0.109)	1.567gh (±0.282)	9.420becd (±1.043)	15.922bdec (±3.927)	2.077bac (±0.346)	2.782def (±0.411)	14.215a (±2.210)	12.983ba (±2.611)	7.909cb (±1.191)	15.010ba (±1.822)
EW	2011	12.577edc (±0.749)	5.658b (±0.254)	1.796gfh (±0.291)	11.083ba (±0.902)	18.888ba (±3.041)	2.271a (±0.399)	3.104def (±0.339)	14.753a (±0.524)	13.096ba (±1.498)	8.199cb (±0.458)	16.390ba (±2.268)
NE-SW[1]	2008	13.376ba (±0.707)	5.243c (±0.094)	2.766a (±0.564)	**12.802a (±0.765)**	15.572bdec (±1.078)	**1.369g (±0.282)**	3.752dc (±0.339)	**12.630bac (±0.973)**	8.116c (±0.790)	**13.105a (±1.654)**	16.121ba (±2.816)
NE-SW	2009	13.107bac (±0.937)	5.444cb (±0.355)	2.328ebdac (±0.574)	**12.619a (±1.908)**	17.276bdac (±3.810)	1.820ebdfc (±0.103)	3.612de (±0.442)	**12.657bac (±2.078)**	7.653dc (±0.630)	**12.965a (±2.560)**	15.295ba (±2.992)
NE-SW	2010	13.284bac (±0.456)	5.639b (±0.168)	2.501bac (±0.249)	9.615bcd (±2.337)	14.072fde (±1.755)	1.900ebdac (±0.276)	**5.467ba (±1.212)**	8.860ef (±2.440)	8.254c (±0.901)	6.526cbd (±3.565)	15.854ba (±3.742)
NE-SW	2011	13.097bac (±0.571)	5.385cb (±0.195)	2.645ba (±0.302)	9.133fbecd (±1.056)	19.384a (±1.501)	2.037bac (±0.240)	**5.861a (±0.645)**	7.609f (±0.601)	8.537c (±0.646)	5.735d (±0.856)	15.824ba (±2.648)
NS[1]	2008	12.726bdc (±0.7333)	5.453cb (±0.283)	2.048edfc (±0.450)	5.200hi (±0.772)	15.354dec (±3.012)	2.022bac (±0.069)	**5.185ba (±0.884)**	11.009edc (±1.324)	**12.581ba (±2.267)**	8.737cb (±1.586)	9.401d (±0.448)
NS	2009	13.567a (±0.385)	5.477cb (±0.287)	2.475bdac (±0.316)	4.127i (±0.620)	17.956bac (±1.538)	2.204ba (±0.275)	**4.770bc (±0.544)**	11.097dc (±1.588)	**12.971ba (±1.329)**	9.366b (±1.833)	9.293d (±0.527)
NS	2010	13.193bac (±0.622)	**5.669a (±0.414)**	2.436bdac (±0.260)	6.747hg (±2.001)	17.197bdac (0.899±)	1.581egdf (±0.205)	3.650de (±0.648)	**13.552ba (±1.470)**	5.058de (±5.779)	**14.301a (±3.455)**	**13.751ba (±3.231)**
NS	2011	13.485a (±0.454)	**6.220a (±0.340)**	2.035edf (±0.378)	7.646feg (±0.728)	17.844bac (±2.678)	2.313a (±0.350)	3.369def (±0.254)	**14.225a (±2.245)**	2.692e (±0.781)	**14.137a (±1.308)**	**16.640a (±1.133)**
NW-SE[1]	2008	8.495h (±0.728)	1.314e (±0.041)	1.961egf (±0.618)	**8.807fecd (±0.810)**	13.293fe (±1.117)	1.541egf (±0.288)	**5.468ba (±0.982)**	10.263ed (±1.853)	14.757a (±0.923)	**15.640a (±1.641)**	10.548dc (±1.526)
NW-SE	2009	**9.485g (±0.635)**	1.830d (±0.410)	2.272ebdc (±0.323)	7.307fg (±1.209)	**18.593bac (±1.935)**	1.973bdac (±0.241)	**4.845ba (±1.280)**	11.764bdc (±2.361)	11.647b (±2.534)	**15.456a (±3.573)**	8.954d (±0.109)
NW-SE	2010	8.623h (±0.234)	1.625ed (±0.209)	1.798gfh (±0.238)	6.673hg (±0.980)	11.668f (±1.430)	1.661egdfc (±0.251)	3.129def (±0.871)	**6.895f (±2.086)**	14.603a (±2.037)	8.990cb (±2.811)	**14.934ba (±3.391)**
NW-SE	2011	8.684h (±0.457)	1.627ed (±0.236)	1.902egfh (±0.238)	6.197hg (±1.099)	14.414fde (±2.887)	1.762 egdfc (±0.202)	2.894def (±)0.296	**6.940f (±1.544)**	14.370ba (±1.545)	8.272cb (±1.058)	**14.546ba (±2.387)**
p-value		0.0284	0.0137	0.0469	<.0001	0.0351	0.0304	<.0001	<.0001	<.0001	<.0001	0.0216

*Means given for compounds with significant treatment (row orientation) and vintage (interaction) main effects as indicated in Table 4.11. [1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Treatment; [3]Vintage; [4]Total flavan-3-ols; [5]Epigallocatechin 3-*O*-gallate; [6]Petunidin-, peonidin- and delphinidin 3-*O*-glucosides; [7]Petunidin- and peonidin 3-*O*-(6-*O*-acetyl) glucosides; [8]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides. **Different letters in the same column indicate significant differences in the content of the compounds measured among the different treatments and vintages according to Fisher's least significant difference test. ***Values in brackets indicate standard deviations.

Analysis of variance for grape phenolic compound concentration means for vintage main effect

Mean concentrations for grape phenolic compounds with a significant vintage main effect for grapes harvested at *ca.* 26°Brix are listed in Table 4.13.

Table 4.13 Grape phenolic compound concentration means for vintage main effect (grape harvested at *ca.* 26°Brix).*

	Vintage				*p*-values
Phenolic compounds	**2008**	**2009**	**2010**	**2011**	
p Coumaric acid	24.071ba** (±3.969)***	24.059ba (±3.890)	25.346a (±4.927)	23.588b (±3.755)	0.0158
Quercetin 3-*O*-rutinoside (rutin)	0.589b (±0.067)	0.594b (±0.069)	0.613b (±0.078)	**0.666a (±0.054)**	0.0116
Quercetin 3-*O*-glucoside (isoquercetin)	2.406bc (±1.011)	2.713a (±1.018)	2.622ba (±1.015)	**2.198c (±0.783)**	0.0091
Quercetin	**7.291a (±1.196)**	6.642ba (±1.297)	6.440b (±1.128)	6.362b (±1.164)	0.0503
Total anthocyanins	**334.007c (±34.541)**	350.910bc (±39.765)	360.768ba (±30.630)	**380.778a (±40.482)**	0.0006
Malvidin 3-*O*-glucosides	**73.751c (±17.240)**	86.864ba (±19.584)	82.221bc (±15.831)	**92.965a (±18.466)**	0.0038

*Means given for compounds with significant vintage main effect as indicated in Table 4.11. **Different letters in the same row indicate significant differences in the content of the compounds measured among the different vintages according to Fischer's least significant difference test. ***Values in brackets indicate standard deviations.

Quercetin concentrations were significantly higher in the 2008 vintage, but were not significantly different from those of the 2009 vintage, whereas rutin concentrations were significantly higher in grapes from the 2011 vintage. Isoquercetin was significantly lower in grapes from the 2011 vintage, but not significantly different in the 2008 vintage.

Total anthocyanins were significantly higher in grapes from the 2011 vintage, but were not significantly different in the 2010 vintage. Significantly lower total anthocyanin concentrations were recorded in the 2008 vintage, but were not significantly different in the 2009 vintage.

Malvidin 3-*O*-glucosides were significantly lower in grapes from the 2008 vintage, but not from the 2010 vintage. Significantly higher malvidin 3-*O*-glucoside concentrations were evident in the 2011 vintage, but were not significantly different in the 2009 vintage.

Analysis of variance for grape phenolic compound concentration means for treatment (row orientation) main effects

Mean concentrations for grape phenolic compounds with a significant treatment (row orientation) main effect for grapes harvested at *ca*. 26°Brix are listed in Table 4.14.

Phenolic acids

Total phenolic acid concentrations were significantly higher in grapes from the NW-SE row orientation treatments with significantly lower concentrations in grapes from the NE-SW row orientation treatments. Gallic acid was significantly higher in grapes from the NW-SE row orientation treatments. No significant differences in gallic acid were found in grapes from the NS, NE-SW and EW row orientation treatments.

Table 4.14 Grape phenolic compound concentration means for treatment (row orientation) main effect (grape harvested at *ca.* 26°Brix).*

Phenolic compounds	Treatment (row orientation)				*p*-values
	EW[1]	NE-SW[1]	NS[1]	NW-SE[1]	
Total phenolic acids	62.689b** (±4.057)***	**45.687c (±5.905)**	65.727b (±4.229)	**83.101a (±5.831)**	<.0001
Gallic acid	1.728b (±0.248)	1.656b (±0.322)	1.630b (±0.244)	**2.207a (±0.346)**	<.0001
Caffeic acid	**29.942c (±2.224)**	**18.491d (±4.046)**	**31.803b (±3.485)**	**48.986a (±5.093)**	<.0001
p Coumaric acid	27.207a (±2.6202)	**18.567b (±2.806)**	25.866a (±1.588)	26.007a (±1.519)	<.0001
Ferulic acid	**7.217a (±1.013)**	6.333b (±1.022)	5.832cb (±0.575)	5.266c (±0.530)	0.0007
Quercetin 3-*O*-rutinoside (rutin)	0.593b (±0.068)	0.637a (±0.063)	0.594b (±0.078)	0.633a (±0.075)	0.0180
Total flavonols	16.921c (±0.950)	19.041b (±1.062)	16.530c (±1.622)	**27.448a (±2.721)**	<.0001
Quercetin 3-*O*-glucoside (isoquercetin)	1.629c (±0.265)	**3.561a (±0.575)**	1.623c (±0.261)	2.962b (±0.562)	<.0001
Quercetin	6.102b (±0.852)	**7.330a (±0.742)**	6.658b (±1.670)	6.558b (±1.188)	0.0079
Kaempferol	**1.682b (±0.177)**	**1.403c (±0.1642)**	**1.219d (±0.069)**	**1.993a (±0.238)**	<.0001
Quercetin 3-*O*-rhamnoside (quercitrin)	7.510b (±0.720)	6.745c (±0.917)	7.029cb (±1.157)	**15.933a (±1.601)**	<.0001
CyGluc[2]	2.000b (±2.000)	2.209ba (±2.209)	2.324a (±2.324)	2.426a (±2.426)	0.0085
MalGluc[2]	78.425b (±13.258)	74.599b (±13.984)	**103.821a (±18.846)**	78.957b (±13.326)	<.0001
MalGlucAc[3]	65.654b (±15.010)	60.996b (±8.429)	63.239b (±14.315)	**86.945a (±12.414)**	0.0005

*Means given for compounds with significant treatment (row orientation) main effects as indicated in Table 4.11. [1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Cyanidin- and malvidin 3-*O*-glucoside; [3]Malvidin 3-*O*-(6-*O*-acetyl) glucoside. **Different letters in the same row indicate significant differences in the content of the compounds measured among the different treatments according to Fisher's least significant difference test. ***Values in brackets indicate standard deviations.

Caffeic- and *p*-coumaric acids were significantly lower in grapes from the NE-SW row orientation treatments. Significant differences in caffeic acid concentrations among all treatments were evident, with highest concentrations occurring in grapes from the NW-SE row orientation treatment. Ferulic acid concentrations were significantly higher in grapes from the EW row orientation treatments.

Flavonols

Total flavonol concentrations were significantly higher in grapes from the NW-SE row orientation treatments with grapes from EW and NS row orientation treatments significantly lower but not significantly different from each other. Isoquercetin (quercetin 3-*O*-glucoside) and quercetin concentrations were significantly higher in grapes from the NE-SW row orientation treatments. Kaempferol and quercitrin (quercetin 3-*O*-rhamnoside) concentrations were significantly higher in grapes from the NW-SE row orientation treatments with grapes from the NS row orientation treatments significantly lower in kaempferol concentrations. Grapes from the NE-SW row orientation treatments were significantly lower in quercitrin, but not significantly different from those of the NS row orientation treatment.

Anthocyanins

Malvidin 3-*O*-glucoside concentrations were significantly higher in grapes from NS row orientation treatments with no significant differences among the EW, NE-SW and NW-SE row orientation treatments.

Malvidin 3-*O*-(6-*O*-acetyl) glucoside concentrations were significantly higher in grapes from the NW-SE row orientation treatments. No significant differences in grapes among the EW, NS and NE-SW row orientation treatments were found.

4.3.2 Comparison between quantitative phenolic compounds of Syrah grape data of this study with results cited in literature

The phenolic compound composition of grapes depends on multiple factors, including climate, degree of grape ripeness, berry size, and grape cultivar (Barbagallo *et al*., 2011).

Other factors which can affect the phenolic compound concentrations are extraction time, composition of extraction medium and temperature when extraction is performed (Pérez- Magariño & Gonzalez-San José, 2006; Gómez-Alonso *et al*., 2007). Table 4.15 lists the phenolic compound concentrations reported by authors in different grape cultivars from different origins.

Grape ripeness levels and sample matrix, *i.e.* lyophilised grape skin, fresh grape skin or whole grape berries, are reported on. Extraction method was based on acidified methanol, -acetone, or -ethanol. Extraction times ranged from 20 minutes to 45 minutes.

Comparisons between results of this study and results published in the cited literature are of grapes harvested at *ca.* 26°Brix (this study) and technological/optimum ripeness, *ca.* 22°Brix and *ca.* 25°Brix.

Table 4.15 Selected quantitative grape phenolic compound concentrations (mg/L) reported in literature.

Grape cultivar	Phenolic compounds	(mg/L)	Grape ripeness	Reference
Syrah (lyophilised grape skin)	(+)-Catechin	8.52	Technological ripeness	Rodríguez-Montealegre *et al.* (2006)
	(-)-Epicatechin	6.90		
Merlot (lyophilised grape skin)	Quercetin 3-*O*-gluc	55.00		
	(+)-Catechin	25.00		
	(-)-Epicatechin	13.00		
	Quercetin 3-*O*-gluc	31.00		
Cencibel (grape skin extracts)	(+)-Catechin	5.49	Optimum ripeness	Gómez-Alonso *et al.* (2007)
	(-)-Epicatechin	2.30		
	Delph 3-*O*-gluc	15.75		
	Cyan 3-*O*-gluc	2.99		
	Peon 3-*O*-gluc	5.77		
	Malv 3-*O*-gluc	37.37		
	Petun 3-*O*-gluc	11.97		
	Delph 3-*O*-(6-*O*-acetyl) gluc	0.61		
	Cyan 3-*O*-(6-*O*-acetyl) gluc	0.23		

Cyan = Cyanidin; Glu = Glucose; Delph = Delpinidin; Peon = Peonidin; Petun = Petunidin; Malv = Malvidin.

Table 4.15 Continued.

Grape cultivar	Phenolic compounds	(mg/L)	Grape ripeness	Reference
Cencibel (grape skin extracts)	(+)-Catechin	5.49	Optimum ripeness	Gómez-Alonso *et al.* (2007)
	Petun 3-*O*-(6-*O*-acetyl) gluc	0.67		
	Malv 3-*O*-(6-*O*-acetyl) gluc	2.56		
	Peon 3-*O*-(6-*O*-acetyl) gluc	0.26		
	Delph 3-*O*-(6-*O*-*p*-coumaroyl) gluc	3.30		
	Petun 3-*O*-(6-*O*-*p*-coumaroyl) gluc	2.80		
	Peon 3-*O*-(6-*O*-*p*-coumaroyl) gluc	1.70		
	Malv 3-*O*-(6-*O*-*p*-coumaroyl) gluc	13.10		
	Cyan 3-*O*-(6-*O*-*p*-coumaroyl) gluc	0.93		
Furmint (grapes)	(+)-Catechin	8.50	Technological ripeness	Kocsis *et al.* (2008)
	(-)-Epicatechin	3.70		
Tannat (grape skin)	Gallic acid	1.70	Technological ripeness	Boido *et al.* (2011)
	(+)-Catechin	4.40		
	(-)-Epicatechin	6.00		
	Epigallocatechin 3-*O*-gallate	1.10		
	Gallic acid	1.70		
Gran negro (grapes)	Quercetin 3-*O*-gluc	7.51	25°Brix	Figueiredo-González *et al.* (2012)
	Quercetin 3-*O*-rutinoside	1.21		
	Malv 3-*O*-gluc	725.00		
	Malv 3-*O*-(6-*O*-acetyl) gluc	17.00		
	Malv 3-*O*-(6-*O*-*p*-coumaroyl) gluc	233.00		
Cabernet Sauvignon (grapes)	(+)-Catechin	2.06	Technological ripeness	Jogaiah *et al* (2013)
	Quercetin	1.85		
	Gallic acid	0.61		
	p-Coumaric acid	0.64		
Pinot gris (lyophilised grape skin)	Gallic acid	16.10	Optimum ripeness	Ferreira *et al.* (2016)
	(+)-Catechin	16.50		
	(-)-Epicatechin	4.70		
	Quercetin 3-*O*-rutinoside	2.50		
	Quercetin 3-*O*-gluc	63.80		
	Delph 3-*O*-gluc	0.60		
	Cyan 3-*O*-gluc	0.10		
	Peon 3-*O*-gluc	7.80		
	Malv 3-*O*-gluc	79.50		
	Malv 3-*O*-(6-*O*-*p*-coumaroyl) gluc	90.80		
Pinot noir (grapes)	Gallic acid	0.03	Technological ripeness	Hendrickson *et al.* (2016)
	(+)-Catechin	0.34		
	(-)-Epicatechin	0.15		
Syrah (lyophilised grapes)	Cyan 3-*O*-gluc	0.70	25°Brix	Lingua *et al.* (2016)
	Delph 3-*O*-gluc	3.30		
	Peon 3-*O*-gluc	48.42		
	Petun 3-*O*-gluc	24.08		

Cyan = Cyanidin; Glu = Glucose; Delph = Delpinidin; Peon = Peonidin; Petun = Petunidin; Malv = Malvidin.

Table 4.15 Continued.

Grape cultivar	Phenolic compounds	(mg/L)	Grape ripeness	Reference
Syrah (lyophilised grapes)	Malv 3-*O*-gluc	380.46	25°Brix	Lingua *et al.* (2016)
	Delph 3-*O*-(6-*O*-acetyl) gluc	1.86		
	Cyan 3-*O*-(6-*O*-acetyl) gluc	0.18		
	Petun 3-*O*-(6-*O*-acetyl) gluc	17.68		
	Malv 3-*O*-(6-*O*-acetyl) gluc	816.78		
	Delph 3-*O*-(6-*O*-*p*-coumaroyl) gluc	8.00		
	Petun 3-*O*-(6-*O*-*p*-coumaroyl) gluc	17.20		
	Peon 3-*O*-(6-*O*-*p*-coumaroyl) gluc	63.86		
	Malv 3-*O*-(6-*O*-*p*-coumaroyl) gluc	251.70		
	Kaempferol	0.05		
	Quercetin	0.38		
	(+)-Catechin	28.70		
	(-)-epicatechin	74.80		
Nebbiolo (grapes)	Cyan 3-*O*-gluc	11.45	25°Brix	Locatelli *et al.* (2016)
	Delph 3-*O*-gluc	75.66		
	Peon 3-*O*-gluc	75.82		
	Petun 3-*O*-gluc	6.64		
	Malv 3-*O*-gluc	53.51		
	Quercetin 3-*O*-gluc	28.16		
	p-Coumaric acid	0.02		
Syrah (grape skin)	Gallic acid	5.85	Technological	Pantelic *et al.* (2016)
	Caffeic acid	0.67	ripeness	
	p-Coumaric acid	4.39		
	Ferulic acid	6.98		
	(+)-Catechin	5.42		
	(-)-Epicatechin	3.02		
	Kaempferol	8.93		
	Quercetin	12.10		
	Quercetin 3-*O*-rutinoside	14.99		
Terrano (grape)	Cyan 3-*O*-gluc	38.00	*ca.* 22°Brix	Bubola *et al.* (2017)
	Delph 3-*O*-gluc	128.00		
	Peon 3-*O*-gluc	169.00		
	Petun 3-*O*-gluc	161.00		
	Malv 3-*O*-gluc	659.00		
Pinot noir (grape)	Delph 3-*O*-gluc	10.00	*ca.* 22°Brix	Feng *et al.* (2018)
	Cyan 3-*O*-gluc	1.75		
	Petun 3-*O*-gluc	18.00		
	Peon 3-*O*-gluc	18.00		
	Malv 3-*O*-gluc	192.00		

Cyan = Cyanidin; Glu = Glucose; Delph = Delpinidin; Peon = Peonidin; Petun = Petunidin; Malv = Malvidin.

Rodríguez-Montealegre *et al.* (2006) reported 8.52 mg/L of (+)-catechin and 6.90 mg/L of (-)-epicatechin concentrations in lyophilised Syrah grape skin samples, whereas Gómez-Alonso *et al*. (2007) reported 5.49 mg/L of (+)-catechin and 2.30 mg/L of (-)-epicatechin concentrations in Cencibel grapes (skin extracts), harvested at optimum ripeness. Furmint grapes harvested at technological ripeness, contained 8.5 mg/L of (+)-catechin and 3.7 mg/L of (-)-epicatechin concentrations (Kocsis *et al.* 2008).

Tannat grape skin samples analysed by Boido *et al.* (2011) contained 4.40 mg/L of (+)-catechin and 6.00 mg/L of (-)-epicatechin concentrations. Ferreira *et al.* (2016) reported (-)-epicatechin and (+)-catechin concentrations in Pinot gris grapes, harvested at optimum ripeness, as 4.70 mg/L and 16.50 mg/L, respectively.

Except for (+)-catechin reported by Ferreira *et al.* (2016) and (-)-epicatechin reported by Gómez-Alonso *et al*. (2007), the above concentrations of (+)-catechin fall within the average concentrations obtained in lyophilised Syrah grape skin samples reported in this study at *ca.* 26°Brix ripeness levels (Table 4.12). However, (+)-catechin concentrations in Syrah grapes from NW-SE row orientation treatments in this study are substantially lower, compared to the literature cited above but fall within (+)-catechin concentrations in Cabernet Sauvignon grapes harvested at technological ripeness reported by Jogaiah *et al*. (2016).

Rodríguez-Montealegre *et al.* (2006) reported 25.0 mg/L of (+)-catechin and 13.0 mg/L of (-)-epicatechin in lyophilised Merlot grape skin samples. This is substantially higher, compared to that quantified in Syrah (Rodríguez-Montealegre *et al.*, 2006), Furmint (Kocsis *et al.* 2008), Tannat (Boido *et al.*, 2011) and Cencibel (Gómez-Alonso *et al*., 2007) grapes. Jogaiah *et al.* (2013) reported notably lower concentrations of (+)-catechin (2.06 mg/L) in Cabernet Sauvignon grapes and Hendrickson *et al.* (2016) reported both lower (+)-catechin and lower (-)-epicatechin concentrations in Pinot noir grapes, *i.e.* 0.34 mg/L and 0.15 mg/L, respectively, compared to those of Syrah, Cencibel, Furmint, and Tannat grapes. Boido *et al*. (2011) reported 1.10 mg/L of epigallocatechin 3-*O*-gallate in Tannat grape skin samples, harvested at technological ripeness. Concentrations found by Boido *et al.* (2011) fall within the range of epigallocatechin 3-*O*-gallate concentrations reported in this study (Table 4.14).

Rodríguez-Montealegre *et al.* (2006) found 55.00 mg/L quercetin 3-*O*-glucosides in lyophilised Syrah grape skin samples and 31.00 mg/L in lyophilised Merlot grape skin samples, whereas Ferreira *et al.* (2016) reported 63.80 mg/L quercetin 3-*O*-glucoside concentrations in Pinot gris grapes (skin extract), harvested at optimum ripeness.

Locatelli *et al.* (2016) found 28.16 mg/L of quercetin 3-*O*-glucosides in fresh Nebbiolo grapes, harvested at 25°Brix ripeness level, whereas Pantelic *et al.* (2016) reported 12.10 mg/L of quercetin and 14.99 mg/L of quercetin 3-*O*-rutinoside in Syrah grape skin samples, harvested at technological ripeness. Contrary to work by Rodríguez-Montealegre *et al.* (2006), Ferreira *et al.* (2016), Locatelli *et al.* (2016) and Pantelic *et al.* (2016); Figueiredo-González *et al.* (2012) reported substantially low quercetin glycoside concentrations in Gran negro grape samples, harvested at 25°Brix, *i.e.* 7.51 mg/L quercetin 3-*O*-glucoside concentrations and 1.21 mg/L quercetin 3-*O*-rutinoside concentrations.

The concentrations of quercetin 3-*O*-glucoside and quercetin 3-*O*-rutinoside reported by Figueiredo-González *et al.* (2012) are higher, compared to the average concentrations obtained in lyophilised Syrah grape skin samples reported in this study, harvested at *ca.* 26°Brix ripeness level (Table 4.14).

Quercetin concentrations reported by Jogaiah *et al.* (2013) in Cabernet Sauvignon grapes are in the order of 1.85 mg/L. Lingua *et al.* (2016) reported 0.38 mg/L quercetin in lyophilised Syrah grapes. This is substantially lower than quercetin concentrations reported in this study in lyophilised Syrah grape skin samples.

Boido *et al.* (2011) reported 1.70 mg/L gallic acid concentrations in Tannat grape skin samples, harvested at technological ripeness. This is in agreement with gallic acid concentrations quantified in Syrah grape skin samples reported in this study (Table 4.14). Contrary to work of this study and work by Boido *et al.* (2011), Jogaiah *et al.* (2013) reported substantially lower concentrations of gallic acid in grapes of Cabernet Sauvignon, *i.e.* 0.61 mg/L.

Kaempferol concentrations (0.05 mg/L) reported by Lingua *et al.* (2016) in lyophilised Syrah grape skin samples are markedly lower than concentrations reported in this study. Concentrations reported by Pantelic *et al.* (2016) in Syrah grape skin samples (8.93 mg/L) are considerably higher compared to kaempferol concentrations reported in this study. Pantelic *et al.* (2016) reported 5.85 mg/L and Ferreira *et al.* (2016) 16.1 mg/L gallic acid concentrations in Syrah grape skin samples and Pinot gris grape skin samples, respectively. Syrah grape samples were harvested at technological ripeness and Pinot gris grapes were harvested at optimum ripeness. Gallic acid concentrations reported by Ferreira *et al.* (2016) and Pantelic *et al.* (2016) are higher, compared to gallic acid concentrations reported in this study.

Locatelli *et al.* (2016) reported 0.02 mg/L of *p*-coumaric acid concentrations in Nebbiolo grapes, which is considerably lower than *p*-coumaric acid concentrations reported in this study. Jogaiah *et al.* (2016) reported 0.64 mg/L *p*-coumaric acid concentrations in Cabernet Sauvignon grapes; whereas Pantelic *et al.* (2016) reported 4.39 mg/L *p*-coumaric acid concentrations in Syrah grape skin samples. Concentrations of *p*-coumaric acid reported by Jogaiah *et al.* (2016) and Pantelic *et al.* (2016) are substantially lower, compared to *p*-coumaric acid concentrations reported in this study. Pantelic *et al* (2016) also reported caffeic acid concentrations of 0.67 mg/L and 6.98 mg/L of ferulic acid in Syrah grape skin samples. Caffeic acid concentrations reported by Pantelic *et al.* (2016) are substantially lower, but ferulic acid concentrations are similar in concentrations reported in this study.

Gómez-Alonso *et al.* (2007) reported 37.37 mg/L malvidin 3-*O*-glucoside concentrations in Cencibel grape skin extract (harvested at optimum ripeness) and Locatelli *et al.* (2016) reported malvidin 3-*O*-glucoside concentrations of 53.51 mg/L in Nebbiolo grapes, harvested at 25°Brix ripeness level. Concentrations reported by Gómez-Alonso *et al.* (2007) and Locatelli *et al.* (2016) are lower than concentrations reported in this study for Syrah grapes harvested at *ca.* 26°Brix ripeness level.

Figueiredo-González *et al.* (2012) reported 725.0 mg/L malvidin 3-*O*-glucosides in Gran Negro grapes and Lingua *et al.* (2016) reported 380.46 mg/L malvidin 3-*O*-glucosides in lyophilised Syrah grape skins. The concentrations reported by these authors are substantially higher than those found in this study.

Ferreira *et al.* (2016) reported 79.50 mg/L of malvidin 3-*O*-glucoside concentrations in lyophilised Pinot gris grape skin extract, grapes harvested at optimum ripeness. Concentrations reported by Ferreira *et al.* (2016) fall within in the range found in lyophilised Syrah grape skin extract of this study, except for grapes from NS row orientation treatments (Table 4.14). Bubola *et al.* (2017) found 659.00 mg/L malvidin 3-*O*-glucoside concentrations in Terrano grapes (*ca.* 22°Brix) and Feng *et al*. (2018) reported 192.00 mg/L in Pinot noir grapes (*ca.* 22°Brix). Concentrations reported by Bubola *et al.* (2017) are substantially higher, compared to values found in this study, whereas concentrations found by and Feng *et al*. (2018) are slightly higher than concentrations reported in this study.

Peonidin 3-*O*-glucoside concentrations reported in this study are not comparable to any of the results listed in Table 4.15 except for concentrations reported by Feng *et al*. (2018) where they found 18.00 mg/L in Pinot noir grapes.

Cyanidin 3-*O*-glucoside concentrations (2.99 mg/L) in Cencibel grape skin extracts reported by Gómez-Alonso *et al*. (2007) are within the range of cyanidin 3-*O*-glucoside concentrations reported in this study. Ferreira *et al*. (2016) and Lingua *et al.* (2016) reported substantially lower cyanidin 3-*O*-glucoside concentrations in Pinot gris (0.10 mg/L) and Syrah (0.70 mg/L) lyophilised grape skin samples, compared to cyanidin 3-*O*-glucoside concentrations reported in Syrah grapes of this study. On the other hand, Locatelli *et al.* (2016) and Bubola *et al.* (2017) reported considerably higher cyanidin 3-*O*-glucoside concentrations in Nebbiolo grapes (11.45 mg/L) harvested at 25°Brix ripeness level and Terrano grapes (38.00 mg/L) harvested at *ca.* 22°Brix than concentrations reported in this study. Feng *et al.* (2018) found 1.75 mg/L cyanidin 3-*O*-glucoside concentrations in Pinot noir grapes (harvested at *ca.* 22°Brix), which is slightly lower than concentrations found in this study.

Gómez-Alonso *et al*. (2007) and Feng *et al.* (2018) reported 11.97 mg/L and 18.00 mg/L petunidin 3-*O*-glucoside concentrations in Cencibel grape skin extract (grapes harvested at "optimum" ripeness) and in Pinot noir grapes (*ca.* 22°Brix), respectively. Lingua *et al*. (2016) found 24.08 mg/L in lyophilised Syrah grape skin samples with grapes harvested at *ca.* 25°Brix and Bubola *et al.* (2017) reported 161.00 mg/L petunidin 3-*O*-glucoside in Pinot noir grapes, harvested at *ca.* 22°Brix. Concentrations reported by Gómez-Alonso *et al*. (2007) fall within the range reported in this study (Table 4.12), except for those of NS and NW-SE row orientation treatments. Lingua *et al*. (2016) and Bubola *et al.* (2017) found notably higher concentrations of petunidin 3-*O*-glucoside concentrations than levels found in this study. Locatelli *et al.* (2016) on the other hand reported 6.64 mg/L of petunidin 3-*O*-glucoside concentrations in Nebbiolo grapes, harvested at 25°Brix. Concentrations reported by Locatelli *et al.* (2016) are similar to concentrations reported in this study (Table 4.12), except for grapes from the EW and NE-SW row orientation treatments.

Delphinidin 3-*O*-glucoside concentrations reported by Gómez-Alonso *et al*. (2007) in Cencibel grapes (15.75 mg/L) and Feng *et al.* (2018) in Pinot noir grapes (10.00 mg/L) are higher than concentrations reported for lyophilised Syrah grape skin samples in this study. Locatelli *et al*. (2016) reported 75.66 mg/L in Nebbiolo grapes and Bubola *et al.* (2017) reported 128.00 mg/L in Terrano grapes. Concentrations reported by these authors are substantially higher, compared to values found in this study. Ferriera *et al.* (2016) reported 0.60 mg/L of delphinidin 3-*O*-glucoside concentrations in Pinot gris lyophilised grape skin samples (at optimum ripeness) and Lingua *et al.* (2016) found 3.30 mg/L in lyophilised Syrah grape skin samples, harvested at *ca.* 25°Brix ripeness. Concentrations reported by Lingua *et al.* (2016) are slightly higher than concentrations reported in this study for Syrah grape skin samples and concentrations reported by Ferriera *et al.* (2016) are lower.

Malvidin 3-*O*-(6-*O*-acetyl) glucoside concentrations reported by Gómez-Alonso *et al*. (2007) in lyophilised Cencibel grape skin (2.56 mg/L) and Figueiredo-González *et al.* (2012) in Gran negro grapes (17.00 g/L) are substantially lower, compared to concentrations reported in lyophilised Syrah grape skin samples in this study. Lingua *et al.* (2016) reported 816.78 mg/L malvidin 3-*O*-(6-*O*-acetyl) glucoside concentrations in lyophilised Syrah grape skin samples. This is considerably higher than concentrations reported in this study.

Gómez-Alonso *et al*. (2007) reported 0.67 mg/L petunidin 3-*O*-(6-*O*-acetyl) glucoside concentrations in Cencibel grape skin extract (harvested at optimum ripeness) and Lingua *et al.* (2016) reported 17.78 mg/L in lyophilised Syrah grape skin extract , harvested at *ca.* 25°Brix. Concentrations reported by Gómez-Alonso *et al.* (2007) and Lingua *et al.* (2016) do not fall within the range reported in this study.

Peonidin 3-*O*-(6-*O*-acetyl) glucoside concentrations (0.26 mg/L) found by Gómez-Alonso *et al*. (2007) in Cencibel grape skin extracts are notably lower than concentrations found in Syrah grape skin extract in this study.

Gómez-Alonso *et al*. (2007) reported 3.30 mg/L delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides in Cencibel grape skin extracts and Lingua *et al*. (2016) 8.00 mg/L in lyophilised Syrah grape skins. Concentrations reported by these authors fall within the concentration ranges reported in Table 4.12 of this study.

Petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside concentrations of 2.80 mg/L (Cencibel grapes) were reported by Gómez-Alonso *et al*. (2007) and 17.20 mg/L (Syrah grapes) reported by Lingua *et al.* (2016). Concentrations reported by Gómez-Alonso *et al*. (2007) do not fall within the range of values found in this study. Concentrations reported by Lingua *et al.* (2016) are slightly higher than those reported in this study.

Malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside concentrations in Cencibel grape skin samples (13.10 mg/L) reported by Gómez-Alonso *et al*. (2007) are in agreement with concentrations reported in this study (Table 4.12). Lingua *et al.* (2016) found 251.70 mg/L of malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside concentrations in lyophilised Syrah grape skins. Concentrations reported by Lingua *et al.* (2016) are markedly higher than malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside concentrations reported in this study.

Differences among results reported in Table 4.15 and those obtained in this study may be ascribed to different viticultural practices, different sampling methods, different application of extraction methods, as well as different extraction solvents and quantification techniques.

4.3.3 Comparison of the effect of grape ripeness levels (°Brix) and microclimate (row orientation treatments) on measured variables

4.3.3.1 Grapes from NS row orientation treatments

Differences in phenolic compound concentrations seemed to occur as a function of grape ripeness level (Table 4.16). Anthocyanins (red pigments) apparently reached highest concentrations already at an average of 22.74°Brix ripeness, whereas flavonols (yellow pigments) and flavan-3-ols, which contribute to body mouthfeel and tannin intensity, seemed highest at an average ripeness level of 24.78°Brix. Anthocyanins concentrations tended to decrease as grape ripeness levels increase. Phenolic acid concentrations were not substantially affected by grape ripeness levels but were highest at an average of 24.78°Brix.

Table 4.16 Total phenolic compound concentrations (mg/L) measured in lyophilised Syrah grape skin extracts at average ripeness levels (°Brix) for Syrah grapes from NS and EW row orientation treatments. Data represents Syrah grape samples collected over four consecutive vintages.

Phenolic compound classes	**Row orientation treatments**					
	NS[1]			**EW[1]**		
	22.74°Brix[2]	**23.89°Brix[2]**	**24.78°Brix[2]**	**22.22°Brix[2]**	**23.67°Brix[2]**	**24.78°Brix[2]**
Flavan-3-ols	11.009	12.479	**13.243**	11.370	**12.482**	12.102
Flavonols	12.882	13.877	**16.542**	16.094	16.765	**16.926**
Phenolic acids	64.007	63.884	**65.977**	75.655	**86.037**	62.690
Anthocyanins	**384.560**	367.293	354.316	**405.774**	342.895	361.836

[1]North-South; [1]East-West; [2]Average °Brix for grape ripeness.

4.3.3.2 Grapes from EW row orientation treatments

Flavan-3-ols and phenolic acids seemed to reach highest concentrations at an average ripeness level of 23.67°Brix (Table 4.16), where after a decrease in concentrations occurs as grapes ripened further. Flavonols appeared to be at highest concentrations at an average ripeness level of 24.78°Brix. This is similar for NS row orientation treatments. Anthocyanins tended to reach highest concentrations already at an average of 22.22°Brix ripeness level, with a slight decrease in concentration as ripeness levels increased. This is similar than that found for NS row orientation treatments. Grapes from both the NS and EW row orientation treatments tended to reach highest anthocyanin and flavonol concentrations at *ca.* 22°Brix and 25°Brix, respectively.

4.3.3.3 Grapes from NE-SW row orientation treatments

Flavan-3-ols showed only slight variation from a low to high ripeness level (Table 4.17). Flavonols and phenolic acids ostensibly reached highest concentration at an average ripeness level of 22.92°Brix, after which at 25.12°Brix and 26.51°Brix ripeness levels they decreased.

Anthocyanins also seemed to reach highest concentrations at an average of 22.92°Brix. This is similar than that found for NS and EW row orientation treatments.

Table 4.17 Total phenolic compound concentrations (mg/L) measured in lyophilised Syrah-grape skin extracts at average ripeness levels (°Brix) for Syrah grapes from NE-SW and NW-SE row orientation treatments. Data represents Syrah grape samples collected over four consecutive vintages.

Phenolic compound classes	**Treatment (row orientation)**					
	NE-SW[1]			**NW-SE[1]**		
	22.92°Brix[2]	**25.12°Brix[2]**	**26.51°Brix[2]**	**23.31°Brix[2]**	**25.41°Brix[2]**	**27.01°Brix[2]**
Flavan-3-ols	13.213	13.202	**13.217**	**13.422**	13.118	8.822
Flavonols	**24.253**	16.437	19.016	25.574	**34.286**	27.395
Phenolic acids	**72.013**	57.249	45.454	49.274	**117.736**	83.072
Anthocyanins	**451.387**	340.851	357.473	352.631	**385.644**	353.010

[1]Northeast-Southwest; [1]Northwest-Southeast; [2]Average °Brix for grape ripeness.

4.3.3.4 Grapes from NW-SE row orientation treatments

Flavan-3-ols appeared to reach highest concentrations at an average of 23.31°Brix ripeness level, after which ripeness level they decreased (Table 4.17). This is similar to what was found for EW row orientation treatments. Flavonols, phenolic acids and anthocyanins tended to reach highest concentrations at an average of 25.41°Brix ripeness.

Grapes from rows planted to NE-SW seemed to reach highest anthocyanin, phenolic acid and flavonol concentrations before those from NW-SE row orientation treatments, whereas grapes from the NW-SE row orientation treatments tended to reach highest concentrations of flavan-3-ols before those from the NE-SW row orientation treatments.

4.4 Discussion

This chapter reports on the effect that NS (high light exposure in the morning and afternoon in the fruiting zone), EW (low light exposure all day in fruiting zone), NE-SW (high light exposure in the afternoon in the fruiting zone) and NW-SE (high light exposure in the morning in the fruiting zone) row orientations (Hunter *et al*., 2016) and grape ripeness levels (*ca*. 22°Brix, *ca*. 24°Brix and *ca*. 26°Brix) have on individual phenolic compound concentrations of experimental Syrah/101-14 Mgt (*Vitis vinifera* L. cv.) grapes.

Grapevines planted to EW row orientations receive a maximum photosynthetic active radiation (PAR) only at mid-day with *ca.* 45 µmol m^{-2} s^{-1} inside the canopy and *ca.* 750 µmol m^{-2} s^{-1} (m^2/s) radiation outside the canopy during March (Hunter *et al*., 2016). The EW rows are the only rows which have a PAR peak only once during the day and maintained lower interior canopy light interception, compared to NW-SE row orientations in the morning. Light quantity and most likely light quality are different between EW and NS row orientations. These orientations may be considered as causing a uniform light distribution in the canopy.

The NS row orientations receive maximum PAR twice a day with *ca.* 60 µmol m^{-2} s^{-1} inside the canopy and *ca.* 1000 µmol m^{-2} s^{-1} radiation outside the canopy during late morning. During late afternoon, *ca.* 60 µmol m^{-2} s^{-1} radiation is measured inside the canopy and *ca.* 1000 µmol m^{-2} s^{-1} radiation measured outside the canopy. Photosynthetic active radiation in NS row orientations is higher, compared to EW row orientations.

The NE-SW row orientations receive maximum PAR twice a day with *ca.* 20 µmol m^{-2} s^{-1} radiation inside the canopy and *ca.* 300 µmol m^{-2} s^{-1} radiation outside the canopy during mid-morning (Hunter *et al*., 2016). During mid-afternoon, these row orientations receive a maximum of *ca.* 80 µmol m^{-2} s^{-1} radiation inside the canopy and a maximum of *ca.* 1300 µmol m^{-2} s^{-1} radiation outside the canopy.

Grapevines planted to a NW-SE direction also receive maximum PAR twice a day with *ca.* 60 µmol m^{-2} s^{-1} measured inside the canopy and *ca.* 1000 µmol m^{-2} s^{-1} measured outside the canopy during late morning. During late afternoon PAR peaks at *ca.* 30 µmol m^{-2} s^{-1} radiation and at *ca.* 450 µmol m^{-2} s^{-1} inside and outside the canopy, respectively?

It is evident that PAR differences occurred among the four differently orientated canopies. Differences in phenolic compound evolution in the grapes may be ascribed to the differences in PAR of the canopies. Photosynthetic active radiation may also affect grape ripeness levels. Among the four different canopies (row orientation treatments), canopy temperatures peaked at *ca.* 30 °C from December onwards during late afternoon (Hunter *et al*., 2016). During January and February, canopy temperatures were highest at just over 30 °C. Generally, temperature differences among the four canopies during January and February were not evident (Hunter *et al*., 2016). Photosynthetic active radiation and temperature are well-known regulating mechanisms/drivers of modification of whole plant and grape berry size and changes in biochemical and physiological processes, which occur at pre- and post vérasion in both canopy and grapes (Tarara *et al*., 2008). Oenological quality, including phenolic compound (anthocyanins, flavonol, and tannin) evolution and synthesis, is largely determined by these factors.

A RP-HPLC-DAD method was used to separate, identify, and quantify 23 target phenolic compounds (Table 4.2) in the lyophilised Syrah grape skin extract samples. The results characterised the phenolic compound concentration variations associated with Syrah grapes subjected to different treatments, *i.e.* row orientations and grape ripeness levels. Flavonoid and non-flavonoid metabolism responded to both treatment (row orientation/microclimate) and grape ripeness levels.

4.4.1 Grapes harvested at *ca*. 22°Brix

Grapes from the NE-SW row orientation treatments were significantly higher in total anthocyanin concentrations at this low ripeness level, followed by those from EW, NS and NW-SE row orientation treatments (anthocyanins were not significantly different between EW and NS) (Table 4.6). This agrees with work by Hunter *et al*. (2010) who reported that EW row orientation treatments are most likely not best for colour development in Syrah grapes, whereas NE-SW row orientations are more suited for grape-skin colour development.

Rustioni *et al*. (2011) reported a positive relationship between total anthocyanin concentrations and grape bunch exposure to increased light in Croatina and Pinot noir grapes planted to EW row orientations (which would normally receive lower light intensities in the fruiting zone) in the northern hemisphere. Caccavello *et al.* (2017) found decreased total anthocyanins concentrations in Aglianico grapes (harvested at *ca.* 22°Brix) planted to NW-SE row orientations. Vines were however defoliated around the fruit zone, exposing grape bunches to excessive light.

Peonidin 3-*O*-glucosides were significantly lower in Syrah grapes from the NS row orientation treatments, whereas Syrah grapes from the NW-SE row orientation treatments showed significantly high concentrations of peonidin 3-*O*-glucosides. Grapes from the NW-SE row orientation treatment were however not significantly different from those of the EW row orientation treatments. Bubola *et al*. (2017) and Feng *et al.* (2017) reported increased peonidin- 3-*O*-glucoside concentrations in Terrano and Pinot noir grapes, respectively, planted to NS orientations. Vines were however subjected to 50% leaf removal around grape bunches, thereby over exposing grape bunches to light.

Hunter *et al*. (2016) reported that NE-SW and NW-SE row orientation treatments are associated with reduced light intensity in the morning and afternoon, respectively. Malvidin 3-*O*-(6-*O*-acetyl) glucosides were significantly lower in grapes from the NW-SE row orientation treatments. Work by Martínez-Lüscher *et al*. (2014) showed that artificial-light reduction in the fruiting zone and in the grapevine canopy of NS row orientated Tempranillo grapevines resulted in increased acetylated malvidin glucoside concentrations in grapes.

Epigallocatechin 3-*O*-gallate concentrations proved significantly lower in Syrah grapes from the NS and EW row orientation treatments, whereas (+)-catechin concentrations were significantly lower in grapes from the NS row orientation treatments. This is in contrast to the findings of Scrafidi *et al*. (2016) who reported increased concentrations of flavan-3-ols in Grillo grapes (white cultivar) from fully exposed grape bunches, harvested at *ca*. 21°Brix and planted to NS row orientations, compared to grape bunches subjected to 50% shading by using net bags to cover the bunches. In this study, decreased concentrations of total flavan-3-ols were evident in Syrah grapes from NS row orientation treatments (Table 4.6).

Quercetin 3-*O*-glucoside concentrations were significantly higher in Syrah grapes from the NE-SW row orientation treatments. Jogaiah *et al*. (2013) found increased concentrations of flavonols (quercetin) in Cabernet Sauvignon grapes harvested at low maturity (*ca.* 22°Brix) from NS row orientation treatments, whereas Martínez-

Lüscher *et al*. (2014) reported highest concentrations of quercetin in Tempranillo grapes from vines receiving high light intensity in the morning and afternoon in the fruiting zone (*i.e.* NS row orientations). This is in agreement with quercetin concentrations reported in this study for NS row orientation treatments, however, significant differences among NS, EW and NW-SE row orientation treatments were not evident.

Total flavonol concentrations were significantly higher in Syrah grapes from the NE-SW and significantly lower in grapes from the NS row orientation treatments. Jogaiah *et al*. (2013) found that kaempferol and quercetin 3-*O*-rutinoside concentrations in Cabernet Sauvignon grapes were not significantly different between treatments, *i.e.* shoot thinning/leaf removal (inducing increased light in the fruiting zone) *versus* control samples (no leaf removal) in NS row orientated vines.

Gallic acid and *p*-coumaric acids were significantly lower in Syrah grapes from the NS and the NW-SE row orientation treatments, respectively. Jogaiah *et al*. (2013) reported increased concentrations of gallic acid in Cabernet Sauvignon grapes from vines planted to NS directions. Vines were however, subjected to 50% leaf removal/shoot thinning, which may have resulted in increased temperature and light in the fruiting zone.

Work by Del-Castillo-Alonso *et al*. (2014) found *p*-coumaric acid concentrations highest in Graciano grapes planted to an EW direction. Vines were subjected to 50% defoliation resulting in increased light exposure in the fruiting zone as well as a change in source:sink relationships in the canopy. This is in agreement with this study where *p*-coumaric acid concentrations were significantly higher in EW row orientation treatments, but not significantly different from NS row orientation treatments.

4.4.2 Grapes harvested at *ca*. 24°Brix

Cyanidin 3-*O*-glucoside (3'-hydroxylated anthocyanin) concentrations were significantly higher in grapes from the NS row orientation treatment, but were not significantly different from the NW-SE row orientation treatments (Table 4.10). This is in agreement with work by Chorti *et al*. (2010). They reported higher concentrations of 3'-hydroxylated anthocyanins in Nebbiolo grapes planted to NS row orientations but with artificial shading treatment, compared to grapes from the same row orientation (NS) but without artificial shading. Grapes represent one vintage and were harvested from the west-facing side of the canopy, which normally received intense sunlight for a short period during the afternoon (Hunter *et al*., 2016). Alteration of light intensity in NS row orientations because of artificial shading can however be linked to row orientations that are associated with slightly lower light intensity in the fruiting zone, *i.e.* NE-SW (lower light penetration in the morning) and NW-SE (lower light penetration in the afternoon) row orientations.

Syrah grapes from the EW row orientation treatments in this study were significantly lower in cyanidin 3-*O*-glucosides. Li *et al.* (2013) reported decreased concentrations of total anthocyanins in Jingxiu grapes from vines planted to an EW direction with increased temperature in the fruiting zone in grapes harvested from north-facing

canopies from the northern hemisphere. Grape bunches were however artificially shaded with netting, therefore further reducing the already lower light conditions.

Syrah grapes from the NS row orientation treatments proved significantly higher in (+)-catechin and significantly lower in epigallocatechin 3-*O*-gallate concentrations. This is in agreement with work by Scrafidi *et al*. (2016) where they reported increased concentrations of (+)-catechins in Grillo grapes (white cultivar) from vines planted to NS directions, compared to artificially shaded grapes from the same row orientation. Peña-Neira *et al*. (2004) reported high concentrations of (-)-epicatechins (flavan-3-ols) in Cabernet Sauvignon grapes from EW row orientations, harvested from north-facing sides of canopies. Likewise, Del-Castillo-Alonso *et al*. (2014) reported increased concentrations of (+)-catechin and (-)-epicatechin in Graciano grapes from EW row orientations. Contrary to works by Peña-Neira *et al*. (2004) and Del-Castillo-Alonso *et al*. (2014), Ristic *et al*. (2007, 2010) showed that Shiraz grapes from EW row orientations had decreased concentrations of flavan-3-ols in artificially shaded grape bunches, whereas higher concentrations of flavan-3-ols were reported in grapes with moderately exposed grape bunches. Although the above results on flavan-3-ols reported by Ristic *et al*. (2007, 2010) and Scrafidi *et al*. (2016) were based primarily on the effect of light manipulation in the fruiting zone, rather than the effect of row orientation, comparisons and deductions are nevertheless relevant in view of this study's results.

Total flavonols were significantly higher in Syrah grapes from the NW-SE row orientation treatments and significantly lower from the NS row orientation treatments. Total flavonols were also significantly lower in grapes harvested at *ca*. 22°Brix from the NS row orientation treatments. Kaempferol and quercetin 3-*O*-rhamnoside (quercitrin) were significantly higher in grapes from the EW row orientation treatments. Significantly lower kaempferol concentrations were found in grapes from the NS row orientation treatments, whereas significantly lower concentrations of quercitrin were evident in grapes from the NW-SE row orientation treatments. Tarara *et al*. (2008) showed that quercetin 3-*O*-glucosides were highest in Merlot grapes planted to NS row orientations and harvested from the east side of the canopy, whereas Azuma *et al.* (2012) reported increased concentrations of kaempferol in Pione grapes with increased light exposure and increased temperature in the fruiting zone. Azuma *et al.* (2012) concluded that increased temperature and light in the fruiting zone have a synergistic effect on flavonol biosynthesis.

Total phenolic acid concentrations were significantly higher in Syrah grapes from the NW-SE row orientation treatments with significantly lower concentrations from the NE-SW row orientation treatments. Significant concentration differences for total phenolic acids were evident among all the treatments. Syrah grapes from the NW-SE row orientation treatments were significantly higher in caffeic acid with significantly lower concentrations from the NE-SW treatments. Significant concentration differences for caffeic acid were evident among all the treatments. Grapes from the NW-SE row orientation treatments were significantly lower in *p*-coumaric acid concentrations, whereas ferulic acid was significantly lower in grapes from the NS row orientation treatments.

Grapes harvested at *ca*. 22°Brix from the NW-SE were also significantly lower in *p*-coumaric acid concentrations. Rescic *et al*. (2016) reported increased concentrations of *p*-coumaric acid in Istrian Malvasia grapes planted to NS row orientations. Vines were however, subjected to leaf removal treatment (50%) which might have resulted in increased temperature and -light in the fruiting zone. This is therefore not the effect of row orientation alone, but also of additional light, temperature and physiological effects.

4.4.3 Grapes harvested at *ca*. 26°Brix

Malvidin 3-*O*-glucosides reached significantly high concentrations in Syrah grapes from the NS row orientation treatments harvested at *ca.* 26°Brix, whereas malvidin 3-*O*-(6-*O*-acetyl) glucoside concentrations were significantly higher in grapes from the NW-SE row orientation treatments (Table 4.14).

The increased malvidin 3-*O*-(6-*O*-acetyl) glucoside concentrations may be due to the effect of more diffused light exposure in the fruiting zone, *i.e.* high light exposure in the morning only for NW-SE row orientation treatments and high light exposure in the afternoon only for NE-SW row orientation treatments, but diffused light for the rest of the diurnal period. Malvidin 3-*O*-(6-*O*-acetyl) glucoside concentrations were significantly lower in grapes from the NE-SW row orientation treatments, but were not significantly different from those of the EW and NW-SE treatments. Cortell *et al*. (2007) reported low concentrations of cyanidin-, peonidin-, petunidin and delphinidin 3-*O*-glucoside concentrations in grapes of high vigour vines of Pinot noir with NE-SW row orientations, harvested at 23.5°Brix ripeness level and high concentrations of malvidin 3-*O*-glucosides.

Cohen *et al.* (2008) showed that total anthocyanin concentrations in Merlot grapes decreased with an increase in grape bunch temperature. Low to moderate temperatures in the fruiting zone resulted in an increase in anthocyanin accumulation. Sadras & Moran (2012) showed the effects of UV radiation and temperature on Syrah and Cabernet Franc grapes, where artificial heating of grape bunches reduced anthocyanin concentrations and artificial cooling increased anthocyanin concentrations. Guidoni & Hunter (2012) reported that grape ripeness levels of 25°Brix and 28°Brix, positively affect skin anthocyanin concentrations with an increase specifically in malvidin 3-*O*-glucoside and its coumaroylated derivative in Syrah grapes planted to NW-SE row orientations. Azuma *et al*. (2012) reported increased accumulation of anthocyanins in Pione grape skins with the induction of moderate temperature/light in the fruiting zone, compared to elevated temperatures in the fruiting zone. They concluded that increased light, but not temperature, in the fruiting zone is conducive to an increase in flavonoid biosynthesis.

Jogaiah *et al.* (2012) reported significant differences in anthocyanin concentrations in Norton grapes planted to NS and EW row orientations. Anthocyanin concentration differences likely occurred because of vines subjected to a 50% shoot thinning treatment that increased light conditions in the canopy. Niu *et al.* (2013) and Li *et al.* (2013) showed that total light exclusion in the fruiting zone inhibited anthocyanin biosynthesis, resulting in decreased concentrations of anthocyanins in Jingxiu grapes at technological ripeness, and planted to NS row

orientations in Japan. Grape bunches were however subjected to artificial shading. Jogaiah *et al.* (2013) reported lower anthocyanin concentrations in Cabernet Sauvignon grapes subjected to shoot thinning/leaf removal, planted to EW row orientations, compared to control samples, *i.e.* no shoot thinning/leaf removal.

Results reported by Niu *et al.* (2013), Li *et al.* (2013) and Jogaiah *et al.* (2013) do not demonstrate the effect of row orientation on phenolic compounds, but rather the effect of increased light in the fruiting zone due to leaf removal/shoot thinning. As shown in this study, these conditions can be related to NS row orientations, which normally receive high light in the fruiting zone in the morning and afternoon.

Rustioni *et al.* (2013) reported acylated-anthocyanin concentration differences between "shaded" and "normal" samples of Sangiovese grapes. Tessarin *et al*. (2014) reported a decrease in dihydroxylated (cyanidin- and petunidin derivatives) and trihydroxylated anthocyanins (delphinidin derivatives) in Uva Longanesi grapes planted to EW row orientations with 50% defoliation treatment.

Total monomeric anthocyanins also showed a significant decrease in concentrations due to increased light (most likely over-exposure) and temperature in the fruiting zone. In agreement with our work, Giacosa *et al.* (2015) found that anthocyanin concentrations in grapes of Syrah grapevines planted to EW row orientations were not significantly different from those in grapes from NS row orientations. In this study, cyanidin 3-*O*-glucoside concentrations in Syrah grape skin extract were not significantly different among the treatments.

Feng *et al*. (2015) reported that increased penetration of light and temperature in Pinot noir grape bunches of vines planted to NS row orientations resulted in decreased concentrations of delphinidin-, cyanidin- and peonidin 3-*O*-glucoside concentrations. Vines were however subjected to 50% leaf removal and the grapes were therefore most likely over-exposed. Degradation of anthocyanins is affected by temperature, light, and the structure of the specific anthocyanin (Downey *et al*., 2006; Guerrero *et al*., 2009). Degradation rate increases as temperature in the fruiting zone rises. It is therefore possible that anthocyanins in grape skins are chemically degraded in response to high temperatures (>35°C). Grapes subjected to temperatures of >35°C would undergo oxidative stress, since genes encoding peroxidase and certain oxido-reduction enzymes are induced (Downey *et al.*, 2003a). This is an indication that the accumulation of anthocyanins is dependent on both moderate temperature and moderate light in the fruiting zone.

Significantly higher concentrations of total flavonols were evident in Syrah grapes from the NW-SE row orientation treatments. Syrah grapes harvested at *ca.* 24°Brix were also significantly higher in total flavonols. This is in contrast to work of Koyama *et al*. (2012) who found that flavonol concentrations in Cabernet Sauvignon grapes (in Japan) increased with increased light exposure in the fruiting zone, *i.e.* NS row orientations, compared to grape bunches artificially shaded with opaque boxes.

Quercetin and quercetin 3-*O*-glucoside concentrations were significantly higher in grapes from the NE-SW row orientation treatments. Quercetin 3-*O*-glucosides were also significantly higher in grapes harvested at *ca.* 22°Brix from the NE-SW row orientation treatments. This is in contrast to work by Spayd *et al.* (2002) where they

reported higher concentrations of quercetin 3-*O*-glucosides in Merlot grapes planted to NS row orientations with increased light exposure brought about by leaf removal, compared to shaded grape bunches (no leaf removal). The results of Spayd *et al.* (2002) are based on the effect of increased fruit exposure to light and not the effect of row orientation. However, comparison and deductions are still relevant in view of the results obtained for grapes harvested at *ca*. 26°Brix in this study.

Feng *et al.* (2015) showed that increased concentrations of quercetin 3-*O*-rhamnoside in Pinot noir grapes planted to NS row orientations, brought about by 50% leaf removal, resulting in increased light and temperature in the fruiting zone. Quercetin 3-*O*-rhamnoside concentrations were significantly higher in grapes form the NW-SE row orientation treatments. Syrah grapes harvested at *ca.* 24°Brix ripeness levels from the EW row orientation treatments were significantly higher in quercetin 3-*O*-rhamnoside concentrations.

Work by Peña-Neira *et al*. (2004) found that quercetin 3-*O*-rutinoside and quercetin 3-*O*-glucoside concentrations were higher in Cabernet Sauvignon grapes from low to moderate exposed bunch zones (EW row orientations), compared to grapes from moderate to high exposed bunch zones (NS row orientations). In our study, significant differences among treatments were not evident.

Kaempferol concentrations of this study were significantly higher in grapes from the NW-SE row orientation treatments, whereas significantly lower concentrations were found in Syrah grapes from the NS row orientation treatments. Jogaiah *et al*. (2013) found that kaempferol concentrations in Cabernet Sauvignon grapes were not significantly different between treatments, *i.e.* shoot thinning/leaf removal (inducing increased light in the fruiting zone) *versus* control samples (no leaf removal) in NS row orientated vines. Martínez-Lüscher *et al*. (2014) reported higher concentrations of kaempferol in Tempranillo grapes from vines receiving increased light in the fruiting zone, compared to vines receiving moderate light in the fruiting zone.

Total phenolic acid concentrations, gallic- and caffeic acids were significantly higher in Syrah grapes from the NW-SE row orientation treatments with significantly lower concentrations of caffeic acid and total phenolic acids in grapes from the NE-SW row orientations treatments. Total phenolic acids and caffeic acid in grapes harvested at *ca.* 24°Brix were also significantly higher from the NW-SE treatments. As with grapes harvested at *ca.* 26°Brix, caffeic acid was significantly lower in grapes harvested at *ca.* 24°Brix from the NE-SW row orientation treatments. Ferulic acid was significantly higher in Syrah grapes from the EW row orientation treatments and *p*-coumaric acid significantly lower in Syrah grapes from the NE-SW row orientation treatments. This is in contrast to work by Gil *et al*. (2013) who found highest concentrations of caffeic-, *p*-coumaric- and ferulic acids in Syrah grapes planted to NS row orientations in Spain. Higher concentrations of the measured phenolic acids were likely because of 50% grape bunch thinning *versus* no thinning, thereby increasing light penetration in the fruiting zone.

Tessarin *et al*. (2014) reported increased concentrations of *p*-coumaric acid in Uva Longanesi grapes in Italy, planted to EW row orientations with 50% defoliation, resulting in increased light and temperature in the fruiting zone and most likely a higher soluble solid level.

Feng *et al.* (2015) reported no significant differences in concentrations of phenolic acids with 50-100% leaf removal of Pinot noir grapevines planted to NS row orientations, compared to control samples with no leaf removal.

Rescic *et al*. (2016) reported an increase in concentrations of hydroxycinnamic acids in Istrian Malvasia grapes from Slovenia, planted to NS row orientations. However, the increased concentrations of hydroxycinnamic acids were likely brought about by leaf removal (50%) treatment, resulting in over-exposed grape bunches to light and increased temperature in the fruiting zone. Results reported by Gil *et al*. (2013) (Syrah grapes), Tessarin *et al*. (2014) (Uva Longanesi grapes), Feng *et al.* (2015) (Pinot noir grapes) and Rescic *et al*. (2016) (Istrian Malvasia grapes) were brought about by grape bunch thinning and defoliation, resulting in increased light penetration in the fruiting zone. Results were therefore primarily due on the effect of light exposure in the fruiting zone because of leaf removal and not because of row orientation. However, these results can be linked to the results and deductions of this study on the effect of different row orientation treatments with respect to the manipulation of sunlight in the fruiting zone. It has already been shown that the microclimate profiles of the canopies are affected by a change in row orientation (Hunter *et al.*, 2016).

4.5 Conclusions

Differences in the concentrations of selected anthocyanins, flavan-3-ols, flavonols, and phenolic acids in Syrah grape skin extracts were found between the row orientation treatments, vintages and grape ripeness levels. Vintage however did not affect the general trend of the data because only single phenolic compound concentrations per ripeness level were affected. Phenolic compound concentrations were strongly affected by row orientation treatment and phenolic compound concentrations were not the same for each grape ripeness level per row orientation treatment. Grape ripeness level therefore exerted a definite effect on the phenolic compound profile obtained for each row orientation treatment. The data was thus separately investigated and analysed.

The characteristic *Vitis vinifera* (Syrah) grape profiles of 3',5'-dihydroxylated anthocyanins (delphinidin derivatives), 3'-hydroxylated anthocyanins (cyanidin- and petunidin derivatives), 5'-hydroxylated anthocyanins (peonidin derivatives) and 3',5'-dimethoxylated anthocyanins (malvidin derivatives) were maintained beyond the variations brought about by the treatments. However, flavonoid and non-flavonoid metabolism responded to both treatment (row orientation/microclimate) and grape ripeness levels. Generally, total anthocyanins were significantly higher in grapes from the NE-SW orientation at *ca.* 22°Brix. No significant differences in total anthocyanins were found amongst grapes harvested at *ca.* 24°Brix and *ca.* 26°Brix from the different row orientation treatments. Total flavan-3-ols were significantly lower in grapes from the NS row orientation treatments, harvested at *ca.* 22°Brix. Total flavan-3-ols for the remaining ripeness levels were not significantly different among the treatments.

Total flavonols were significantly higher in grapes from the NE-SW (*ca.* 22°Brix) and NW-SE (*ca.* 24°Brix and *ca.* 26°Brix) row orientation treatments with total phenolic acids significantly higher in grapes from the NW-SE treatment (*ca.* 24°Brix and *ca.* 26°Brix). Significantly lower flavonol concentrations were found in grapes from the NE-SW (*ca.* 24°Brix and *ca.* 26°Brix) row orientation treatment.

Analysis of variance showed that the effect of vintage was not consistently associated with the same phenolic compounds at the different ripeness levels. The tendency for certain phenolic compounds was that grapes harvested at *ca.* 22°Brix in 2008 and 2009 had significantly lower concentrations than those harvested in 2010 and 2011.

Vintage effect on the phenolic compounds of grapes harvested at *ca.* 24°Brix was not evident except for total flavan-3-ol in grapes harvested in 2008, which were significantly lower than in grapes harvested in 2009, 2010 and 2011. The effect of vintage of certain flavonol concentrations was apparent in the 2008 and 2011 vintages, but was not significantly different from those of the 2009 vintage. The effect of vintage on total anthocyanins was only obvious in the 2008 and 2010 vintage but was not significantly different from the 2009 vintage.

Total anthocyanins were significantly higher in grapes from the 2011 vintage, but were not significantly different in the 2010 vintage. Significantly lower total anthocyanin concentrations were recorded in the 2008 vintage, but were not significantly different in the 2009 vintage.

Results further demonstrated that phenolic compound biosynthesis in grapes is a complex process and involves the interaction of grape ripeness levels as well as viticultural practices, such as grapevine row orientation and canopy management. In the assessment of the impact of row orientation and grape ripeness levels on the phenolic compound accumulation, limited anthocyanin glucosides, acetylated- and coumaroylated anthocyanin compounds with significant differences were observed, whereas more flavonols, phenolic acids and flavan-3-ols were significantly different in concentrations among the treatments and grape ripeness levels. Grape responses of phenolic compounds to light exposure are dependent on the duration and quantity/quality of light exposure. The effect of different light regimes, brought about by the different row orientations, on phenolic compounds was evident.

In a pursuit of improved grape quality, modern viticultural practices such as different row orientations may improve grape quality and reduce sunburn damage. After véraison, several myeloblastosis (MYB) transcription factors controlling the flavonoid biosynthesis are up-regulated by light exposure with a subsequent temperature increase in the fruiting zone, which may lead to an increase in flavonol and flavan-3-ol contents, but a decrease in the anthocyanin content of grapes. Increased temperatures alone may decrease flavonoid content, especially anthocyanin concentrations through a combination of both degradation and synthesis inhibition. Syrah grapes (*ca.* 26°Brix) planted to NE-SW and NW-SE row orientations showed an increase in anthocyanin concentrations and a decrease in flavan-3-ol concentrations.

Light regimes (*e.g.* row orientations) may affect skin anthocyanins negatively or positively. Furthermore, for different grape cultivars, the effects of light on the biosynthesis of anthocyanins can vary. Elevated temperatures may exert a major effect on the accumulation of anthocyanins. High temperatures could reduce the anthocyanin concentrations even in different light conditions. In natural environments, extended duration of elevated temperature, *i.e.* grapevines planted to NS row orientations, may be conducive to increased temperatures in the fruiting zone, compared to EW, NE-SW and NW-SE row orientations, and may have a greater effect than light intensity on the accumulation of anthocyanins, with a negative impact on biosynthesis.

Light intensity differences in the fruiting zone could be an important factor for anthocyanin accumulation, irrespective of row orientation. Accumulation of anthocyanins therefore depends on a combination of exposure of grape bunches to light and moderate temperature. The different treatments, *i.e.* NS, EW, NS-EW, and NW-SE row orientations, in this study involved realistic field conditions that affected the microclimate profiles of the canopies. This is different to many other studies where treatments comprised artificial heating and shading. For this reason, comparison of our results with most published data is not straightforward. The results confirm that different grapevine row orientations (a long-term viticulture practice), investigated under field conditions and harvested at different grape ripeness levels, affect the concentrations of selected phenolic compounds in Syrah grapes. International research on grapevine row orientation only focused on artificially induced temperature and shading effects as well as some seasonal management effects on phenolic compound concentrations. International research lacked a complete statistical comparison of the four different row orientations under similar field conditions. The number of flavonoids and non-flavonoids investigated internationally was also limited.

The results in this chapter showed that Syrah grapes are characterised by a high reactivity to grape microclimate conditions. Light conditions induced in the grape bunch zone by means of row orientation affected grape phenolic compound composition and therefore the potential to make a wider range of Syrah wine styles. A desirable condition for vines growing in a warm climatic environment is where grape bunches are moderately exposed to light. However, in practice a desirable grapevine row orientation may not necessarily be applicable to all environments. Management of the fruiting zone therefore remains an option for increasing/decreasing grape exposure, irrespective of row orientation.

4.6 Literature cited

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Chapter 5

RESEARCH RESULTS II

Impact of Microclimate (Row orientation) and Grape Ripeness Levels on Selected Anthocyanins, Flavan-3-ols, Flavonols, Phenolic Acids and Sensory Attributes of *Vitis vinifera* cv. Syrah Wines

5. Research Results II

Impact of microclimate (row orientation) and grape ripeness levels on selected anthocyanins, flavan-3-ols, flavonols, phenolic acids and sensory attributes of *Vitis vinifera* cv. Syrah wines

5.1 Introduction

The chemical composition of wine reflects the grape cultivar, *terroir* (environment), viticultural practices and the history of the vinification process (Brossaud *et al*., 1998; Gawel, 1998; Jackson, 2000; Mateus *et al.*, 2002; Pérez-Magariño & González-San José, 2006; Ribéreau-Gayon *et al.*, 2006; Gómez-Miguez *et al*., 2007). The chemical composition of red wine includes the primary metabolites, *i.e.* sugars, organic acids, and amino acids, as well as secondary metabolites, *i.e.* flavonoids and non-flavonoids (Andersen & Markham, 2007; Vermerris & Nicholson, 2008). These compounds affect the colour, stability, quality and sensory character of wine (Ribéreau-Gayon, 1964; Su & Singleton, 1969; Coombe & McCarthy, 2000; Gawel & Godden, 2008). Phenolic compounds, which include flavonoids and non-flavonoids, are present in the grape seed, grape flesh and grape skin (Su & Singleton, 1969; Singleton, 1980; Margalit, 2004; Ribéreau-Gayon *et al.*, 2006). Grape phenolic composition is determined by plant genetic factors (Czemmel *et al.*, 2009) and is affected by viticultural practices, environmental factors and the degree of grape ripeness (Mateus *et al.*, 2002; Pérez-Magariño & González-San José, 2006; Ribéreau-Gayon *et al.*, 2006; Hunter & Volschenk, 2008).

Grape ripeness may impact on grape and wine anthocyanin concentration extractability (Guidoni & Hunter, 2012; Giacosa *et al*., 2015). It was shown that grapes with different ripeness levels may result in wines with different colour characteristics, regardless of the anthocyanin concentration in the grape skin (Hunter *et al*., 2004; Del Llaudy *et al*., 2008). Anthocyanin extractability varies throughout grape ripening as a consequence of the compositional changes that occur in the grape skin cell wall during the degradation by pectolytic enzymes (Cagnasso *et al.*, 2011).

Variation in the ratio between anthocyanins and tannins of Pinot noir grape skins and seeds is dependent on grape ripeness levels, which have a direct effect on wine quality (Cortell *et al.*, 2007b). Phenolic compounds and in particular anthocyanin concentrations reached a peak after 72 hours after crushing in Shiraz grapes harvested at *ca.* 23 °Brix, where after a rapid decrease in extraction of phenolics occurred (Guidoni & Hunter, 2012).

Grapes harvested at *ca.* 25 °Brix showed that free forms of phenolics were extracted into the wine until 96 hours after crushing with a modest subsequent decrease in extraction. At *ca.* 28 °Brix, the extraction trend was similar to that of grapes harvested at *ca.* 25 °Brix, but the anthocyanin peak of the former was higher (Guidoni & Hunter, 2012).

Del Llaudy *et al*. (2008) reported a lower extractability of anthocyanins and higher extractability of proanthocyanidins from Cabernet Sauvignon grapes harvested at *ca.* 20 °Brix. This resulted in more astringent wines. Pérez-Magariño & González-San José (2006) found that phenolic acids were higher in Cabernet Sauvignon wines from grapes harvested at *ca.* 24 °Brix and *ca.* 26 °Brix, compared to grapes harvested at *ca.* 22 °Brix. Wines from the lowest ripeness level had higher concentrations of (+)-catechin and (-)-epicatechin, compared to grapes from the second and third ripeness levels. It seems that the decision on a particular grape ripeness level to harvest grapes is critical and has a great effect on the final characteristics of the wine.

Ristic *et al.* (2007) and Tarara *et al*. (2008) reported that environment (*terroir)*, increasing sunlight intensity, and day-night temperature conditions (temperature variation) impact on grape flavonoid concentrations of Syrah and Merlot grapes and corresponding wines.

Grape growers seek to minimize the heterogeneity of grape bunches within a vineyard in order to improve wine quality (Coombe, 1992; Bisson, 2001; Downey *et al*., 2004). A source of variability in a vineyard is vine canopy and grape bunch microclimate (Dokoozlian & Kliewer, 1996; Hunter *et al.*, 2010). Factors affecting the microclimate in the canopy include row orientation, vine spacing and canopy porosity, as well as row spacing and vine height (Pereira *et al.*, 2006; Nadal & Hunter, 2007; Hunter *et al*., 2016). Canopy microclimate can affect the phenolic concentrations of grapes (Hunter *et al*., 1995; Price *et al*., 1995: Dokoozlian & Kliewer, 1996; Haselgrove *et al*., 2000; Fernandes De Oliveira *et al*., 2013; Friedel *et al*., 2015). Canopy microclimate can also affect the concentrations of organic acids (Macheix *et al.*, 1990; Dokoozlian & Kliewer, 1996; Jackson, 2000), amino acids, mineral content (Ribéreau-Gayon, 1964; Du Plessis, 1984; Iland & Coombe, 1988; Price *et al*., 1995) and total soluble solids of grapes (Dokoozlian & Kliewer, 1996; Kennedy *et al*., 2002; Downey *et al*., 2004; Czemmel *et al.*, 2009; Hunter *et al*., 2004).

Syrah grapes from shaded or dense canopies, proved lower in total soluble solids, compared to those from exposed canopies or canopies exposed to increased sunlight by means of *e.g*. leaf removal (Joscelyne *et al*., 2007).

It was reported by Price *et al*. (1995) that anthocyanin concentrations were higher in wines from sun-exposed Pinot noir grapes, compared to wines from grapes from shaded grape bunches or dense canopies. Price *et al.* (1995) also reported that decreased concentrations of caftaric acid in wines from sun-exposed grapes are directly related to hydrolysis of the tartaric acid esters. Quercetin, kaempferol, and isorhamnetin concentrations were higher in wines from Pinot noir grapes with increased sun-exposure, compared to control wines from shaded grape bunches (Price *et al*., 1995).

Studies conducted by Bergqvist *et al*. (2001), Spayd *et al.* (2002), Downey *et al*. (2004), Jeong *et al*. (2004), Cortell & Kennedy (2006), and Tarara *et al.* (2008) confirmed that moderate light exposure of Cabernet Sauvignon, Syrah and Merlot grapes planted to NS row orientations, resulted in increased anthocyanin concentrations, which also corresponded with the respective wines. Downey *et al.* (2004) showed that concentrations of the individual anthocyanins, *i.e*. cyanidin- and peonidin 3-*O*-glucoside, increased in shaded Syrah and Cabernet Sauvignon grapes, compared to exposed grape bunches. They reported that the expression of the gene encoding UDP-glucose flavonoid 3-*O* glucosyl transferase (UFGT), which is a key gene in anthocyanin synthesis, increased after véraison but was similar in both shaded and exposed bunches. Grape bunches were however artificially shaded by means of polypropylene opaque boxes. Downey *et al*. (2006) showed that Cabernet Sauvignon grapes subjected to increased temperatures (>35°C) resulted in a decrease in anthocyanin concentrations and even degradation of anthocyanins. Yamana *et al*. (2006) found an increase in anthocyanin biosynthesis in ”Aki Queen” grapes subjected to low to moderate temperature conditions, independent of light intensities.

Ristic *et al*. (2007) reported that shaded Syrah grape bunches or Syrah grape bunches from dense canopies, planted to EW row orientations, have increased concentrations of seed proanthocyanidins and decreased concentrations of skin proanthocyanidins in the corresponding wines. A decrease in flavonol glycoside concentrations, *i.e.* quercetin-, isorhamnetin-, myricetin- and kaempferol glycosides, was evident in wines from grapes from shaded or dense canopies. Syrah wines produced from grapes of vines planted to EW row orientations with over-exposed canopies, brought about by partial defoliation (removal of leaves in the fruiting zone), proved lower in anthocyanin concentrations, lower in total phenolic compound concentrations, and lower in procyanidin (tannin) concentrations, compared to wines from grapes from denser canopies (Joscelyne *et al.,* 2007; Ristic *et al*., 2007).

An increase in daytime temperature resulted in a decrease in total anthocyanin concentration in Cabernet Sauvignon and Merlot wines from grapes with low light exposure (dense canopies) in a vineyard with NS row orientations (northern hemisphere) (Mori *et al*., 2005; Tarara *et al.,* 2008). Merlot grapes from shaded vines or dense canopies planted to NS row orientations resulted in wines with lower cyanidin- and petunidin 3-*O*-glucosides, but higher delphinidin 3-*O*-glucoside concentrations, compared to wines from sun-exposed grapes or open canopies (Chorti *et al.*, 2010).

Joscelyne *et al.* (2007) and Cohen *et al.* (2008) reported that low daytime temperature conditions (southern hemisphere) and high vigour Cabernet Sauvignon and Merlot grapevines (dense canopies) can result in wines with high concentrations of cyanidin 3-*O*-glucosides.

According to Cohen *et al*. (2008), grape cultivars sensitive to UV-A and UV-B radiation are those grape cultivars with a high proportion of dihydroxylated anthocyanins, *i.e.* cyanidin- and petunidin derivatives, in comparison to trihydroxylated anthocyanins, *i.e.* delphinidin derivatives.

Anthocyanin concentrations monitored over two consecutive vintages in Nebbiolo wines from grapes from the south-east exposed side of the canopy of NE-SW row orientations, varied in cyanidin 3-*O*-glucoside concentrations between vintages (Guidoni *et al*., 2008), whereas concentrations of delphinidin 3-*O*-glucosides and its acylated forms were unaffected by seasonal variation (vintage effect). Guidoni *et al*. (2008) also reported higher cyanidin- and petunidin 3-*O*-glucoside concentrations (dihydroxylated anthocyanins), but lower peonidin 3-*O*-glucoside concentrations in Nebbiolo wines from grapes from south-east exposed vineyards.

Hunter & Volschenk (2008) demonstrated that Syrah wines from grapes from EW row orientations were lower in anthocyanin concentrations, compared to wines from NS, NE-SW, and NW-SE row orientations. Grapevines planted to NS directions in the northern hemisphere seemed favourable for anthocyanin accumulation in Cabernet Sauvignon, Merlot and Nebbiolo wines (Mori *et al*., 2005; Cohen *et al.*, 2008; Chorti *et al.*, 2010).

Wines from Nebbiolo grapes harvested from the west-facing canopy side of NS orientated vines had slightly higher (not significant) anthocyanin concentrations, compared to wines from grapes from EW row orientations (Chorti *et al*., 2010). According to Chorti *et al.* (2010), anthocyanins in Nebbiolo grapes proved insensitive to increased sunlight in the fruiting zone. They concluded that a combination of increased sunlight exposure and high daytime temperature conditions had no effect on anthocyanin biosynthesis. Tardaguila *et al.* (2010) however reported increased colour density in Garignan and Graciano wines from grapes planted to EW row orientations with 50% leaf removal at fruit set, compared to control wines without leaf removal.

Diago *et al.* (2012) showed that wines from Tempranillo grapes planted to EW row orientations proved slightly higher in caffeic acid concentrations after 50% defoliation treatment of vines at fruit-set, compared to control wines, *i.e.* no defoliation. Similarly, Friedel *et al*. (2012) reported increased concentrations of caffeic acid in Riesling wines (white cultivar) from vines with EW row orientations and 50% defoliation. In artificially shaded grapes, *i.e.* no light exposure of grape bunches (boxed-in bunches), a decrease in caffeic acid concentrations was reported.

Cohen *et al.* (2012) showed lower concentrations of kaempferol and quercetin in Merlot wines from grapes planted to NS row orientations and subjected to increased temperatures in the fruiting zone, compared to control wines at ambient temperature. Temperatures were artificially increased by means of heat blowers. They also reported that cyanidin 3-*O*-glucoside concentrations were higher and delphinidin 3-*O*-glucoside concentrations lower in wines from grapes subjected to increased temperatures around the fruiting zone. Acetylated- and coumaroylated anthocyanins were lowest in wines from grapes subjected to increased temperatures.

Lemut *et al*. (2013) showed that Pinot noir wines from grapes planted to EW row orientations, subjected to defoliation (removing the basal four to six leaves from all shoots) after berry set, had increased concentrations of quercetin, kaempferol and isorhamnetin, compared to control wines (no defoliation). Grape skin tannins (procyanidins) were higher in wines from exposed grape bunches or low vigour vines, compared to grapes from shaded bunches or dense canopies. Pinot noir wines from grapes where grapevines were subjected to 50%

defoliation after véraison, proved lower in delphinidin- and peonidin 3-*O*-glucoside concentrations and higher in petunidin- and malvidin 3-*O*-glucoside concentrations, compared to vines with no defoliation treatment (Lemut *et al*., 2013).

Quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside and quercetin 3-*O*-rhamnoside concentrations were higher in Riesling wines from grapes planted to NS row orientations, but subjected to a shading treatment, *i.e.* boxed-in grape bunches, compared to control samples (no artificial shading) in which the concentrations of these compounds were lower (Friedel *et al*., 2015). The wines from shaded Riesling grapes were also lower in (+)-catechin and (-)-epicatechin concentrations, compared to control wines. Feng *et al*. (2015) reported that 50% grape bunch-zone leaf removal treatment at pea-size berry stage, affected Pinot noir grape composition of vines planted to NS orientations; peonidin 3-*O*-glucoside decreased in concentration and quercetin 3-*O*-glucoside and (+)-catechin and (-)-epicatechin increased in concentration, compared to control samples, *i.e.* no leaf removal.

Results emanating from the relevant literature on the research subject are evidence that concentrations of flavonoid and non-flavonoid compounds, which contribute to colour (anthocyanins, flavonols) and astringency (flavan-3-ols) are determined by the complex combined effects of solar radiation, *i.e.* sunlight intensity, light interception and temperature fluctuations, grape ripeness levels, as well as cultivar-related differences. However, limited information on the effect of grape ripeness levels and different light regimes in the canopy, as induced by different grapevine row orientations, on individual phenolic compound concentrations of Syrah wines have been published nationally and internationally. The latter studies mostly dealt with artificially induced changes and seasonal manipulation to the grapevine canopy microclimate. Grapevine row orientation is however a long term viticulture practice that induces ‘’natural’’ canopy microclimate differences that would impact on grape and wine composition. Only limited chemical analyses, particularly phenolic compound composition, were done in this respect. Moreover, phenolic compositional changes related to grape ripeness level were not addressed.

The aim of this study was to evaluate the effect of grapevine row orientation (NS, EW, NE-SW, and NW-SE) as well as grape ripeness level, on the concentrations of selected individual anthocyanins, flavan-3-ols, flavonols and phenolic acids in experimental Syrah wines. The effects of row orientation on the sensory attributes of the wines are also reported. It is a first and novel study in which the combined effect of row orientation treatments and ripeness level are explored under South African conditions in Syrah wines.

5.2 Materials and methods

An experimental vineyard was established in 2003 where Syrah (clone SH 9C/101-14 Mgt) was planted to four different row orientations, *i.e.* NS, EW, NE-SW and NW-SE. The experiment was designed in a randomized way, comprising four row orientations with five replicates per orientation, each confined to a separate vineyard block with a surface area of 1860 m^2.

The vineyard site is a flat *terroir* with clayey loam soil situated on the experiment farm of the Agricultural Research Council (ARC) Infruitec-Nietvoorbij in the Breede River Valley, Robertson, South Africa. Vines were spaced 2.7 m x 1.8 m. The vines were trained to a vertical shoot positioning trellis. A cover crop of rye was sown after harvest and killed before budding. The grapevine canopies consisted of three to four leaf layers from side to side at full canopy development. Shoots were accommodated between four sets of foliage wires and vines were topped approximately three times during the growth season. Grapevines were supplementary irrigated every seven days, since the Robertson region receives an average precipitation of only *ca*. 325 mm annually.

5.2.1 Grape collection

Grapes were harvested over three consecutive vintages (2008, 2009 and 2010) at three different ripeness levels (*ca*. 22°Brix, *ca.* 24°Brix and *ca.* 26°Brix) [°Brix (°B/°Balling) refers to g soluble solids/100 mL]. Grapes were harvested from both sides of the canopy and combined. The harvested grapes were representative of NS, EW, NE-SW and NW-SE vine row orientations, representing different microclimatic conditions according to the movement of the sun over the vertical shoot positioned canopies (Hunter *et al.*, 2016). Five replications of approximately 40 kg of grapes each, representing a specific row orientation (treatment) and ripeness level combination, were collected. The basic experimental design was randomised complete experimental blocks (Chapter 4) with four different row orientations, replicated at random in each of five experimental blocks with a total surface area of 1860 m^2. An experimental unit consisted of all the vines within a vineyard block of a specific row orientation within an experimental block.

5.2.2 Small-scale winemaking

Wines were made according to a standardised small-scale winemaking procedure in the Nietvoorbij Research Cellar. Grape bunches were mechanically de-stemmed and crushed with the addition of 50 mg/kg SO_2. Skin contact occurred for at least an hour before further processing. Di-ammonium hydrogen phosphate (50 g/hL DAP) was added after inoculation with *S. cerevisiae* (VIN 13, Anchor Biotechnologies, South Africa). Fermentation with three cap punch-downs per day was allowed to proceed in a temperature-controlled room (*ca*. 25°C) until the wine reached 0-5°Brix total soluble solids (TSS). After this, the wine and skins were separated by pressing with a small balloon press at 200 kPa (2 bars). The wines were then transferred to stainless steel canisters (20 L) equipped with fermentation air locks. Wines remained in temperature-controlled rooms for approximately one week until dry (glucose levels below 2 g/L as determined by Clinistix, Bayer, South Africa, or digital density meter, DMA 35, Anton Paar, Austria). Malolactic fermentation was not induced for any of the experimental wines. The wines were racked off the yeast lees, SO_2 adjusted to a total of 85 mg/L and cold stabilised for at least two weeks at 0°C.

Wines were first filtered after cold stabilisation by using filter mats (K900 and EK), where after wines were filtered through a 0.45µm membrane and bottled into nitrogen-filled wine bottles at room temperature. Bottled wines were stored at 15°C until required for analysis.

5.2.3 Reagents

Solvents used were of analytical grade and purchased from Merck®, South Africa. De-ionised water was supplied through a Modulab® water purification system, supplied by Separations.

5.2.4 Chemical analysis

High-performance liquid chromatographic (HPLC) determination of selected anthocyanins, flavan-3-ols, flavonols and phenolic acids was performed using a Thermo Separations Products HPLC, supplied by Spectra System Separation Products. The HPLC was equipped with an autosampler, injecting 20 µL.

Photodiode array (DAD) detection was performed for anthocyanins at 520 nm, flavan-3-ols and benzoic acids at 280 nm, and flavonols and hydroxycinnamic acids at 360 nm. ChromQuest™ Software was utilised for data acquisition. Calibration curves for anthocyanins, flavonols, flavan-3-ols and phenolic acids were constructed using available commercial standards (Chapter 3).

The analytical method was based on the method described by Waterhouse *et al*. (1999) for grape and wine phenolic compound separation and quantification. Separation was performed at *ca*. 22°C, using a polystyrene divinylbenzene reversed-phase analytical column (PLRP-S 100 Å, 5 µm, 250 x 4.6 mm, Polymer Laboratories, USA). Gradient elution was performed using mobile phases comprising water/phosphoric acid [985:15 v/v (pH *ca*. 1.35) eluent A] and water/phosphoric acid/acetonitrile [185:15:800 v/v/v (pH *ca.* 1.25) eluent B]. The gradient programme was 90 min. (Chapter 3). The column was equilibrated for 20 minutes after each injection and the flow rate was 1 mL/min. Compound identification was confirmed by comparison of retention times and UV-visible spectra with reference standards. Samples were filtered through a 0.45 µm nylon syringe filter prior to HPLC analysis. Three replicate samples were analysed per row orientation per vintage, giving a total of 9 (3x3) samples for each ripeness level. This excludes occasional duplicate samples from the same experimental unit, in which case average values were first calculated per experimental unit before analysis, to attain the correct experimental error according to the model for the experimental layout, *i.e*. randomised block design. Concentrations were obtained from calibration curves (verified by retention times and spectral data) and expressed as mg/L.

5.2.5 Sensory analysis

Sensory analyses were conducted every year five months after bottling. The tasting panels consisted of between seven to twelve judges comprising winemakers and staff who were experienced in sensory evaluation.

Five repeats of wines representing all four treatments (row orientations) at one ripeness level at a time were presented to the judges in a random order during a tasting session. Sensory analysis was based on the evaluation of pre-determined descriptors, namely colour intensity, overall aroma intensity, fruity intensity, spice aroma intensity, jammy aroma intensity, concentrate intensity, alcohol intensity, tannin intensity, acidity intensity, body mouthfeel, finish persistence, and overall quality. Tasting and evaluation took place in temperature-controlled tasting booths and each taster received approx. 30 mL of each wine in a standard wine-tasting glass. Each taster received his/her wine in a different order from other tasters (randomly). Tasters rinsed their mouths either with water or with carbonated water. Unsalted or sugar-free biscuits were provided for the tasters to clean their palates. The tasters rated the wine attributes on a 10 cm unstructured line-scale from" unacceptable" to "excellent" (colour intensity), "low" to "prominent" (overall aroma-, jammy aroma-, alcohol-, acidity- and tannin intensity), "undetectable" to "prominent" (fruity aroma-, spicy aroma intensity), "thin" to "full" (body mouthfeel), "short" to "long" (finish persistence) and "low" to "excellent" (overall quality.

5.2.6 Statistical analysis

5.2.6.1 Analysis of variance (ANOVA)

Analysis of variance was performed conferring to the experimental design on all variables accessed using General Linear Models Procedure of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Analysis of variance included all three factors (row orientation, vintage and ripeness level) as well as for each ripeness level separately. The Shapiro &Wilk test was performed to test for deviation normality (Shapiro & Wilk, 1965). Fisher's least significant difference test was computed at a 5% level of confidence to compare treatment means [*i.e.* three to four replications (vineyard blocks) per treatment per ripeness level per vintage] (Ott, 1998). ANOVA was used to establish whether significant differences between variables (compound concentrations) were observed as a function of treatments (row orientation and ripeness levels) and to investigate the variation in response to treatment. Data was subjected to XLSTAT 2010 (add-on statistical software for Excel, 2010) to establish averages, variances and standard deviations in the compositional data. An ANOVA was also applied to the sensory data for the same wines.

5.2.6.2 Principal component analysis (PCA)

Principal component analysis was applied to reduce the complexity of data into a principal component space (XLSTAT 2010 add-on statistical software for Excel, 2010). Percentage Eigenvalue variability and percentage cumulative variance for individual principal components in the data are drawn. The principal components are donated as P1 to P15, depending on the number of variables. Although variance in the data can be explained by more than two principal components, the first two, *i.e.* PC1 and PC2, are usually chosen in order to simplify interpretations. Principal component analysis results are reported in biplots illustrating the relative positions and

loadings of the variables in relation to row orientation (treatment). The axes (x and y or PC1 and PC2) represent the principal components and describe the degree of variability in the data. Principal component analysis was applied to the wine phenolic data sets as well as the wine sensory data sets to establish correlation, association and "groupings" between treatments and measured variables of the samples, *i.e.* relationships among row orientation (treatments), grape ripeness levels, sensory attributes, and phenolic compounds.

5.3 Results

5.3.1 Principal component analysis for wines from grapes harvested at *ca.* 22, 24 and 26°Brix

The principal component analysis biplot illustrates the association of phenolic compounds of experimental Syrah wines from grapes harvested during 2008, 2009 and 2010 at ripeness levels of *ca*. 22, 24 and 26°Brix with different row orientation treatments, *i.e*. NS, EW, NE-SW and NW-SE row orientations (Fig. 5.1).

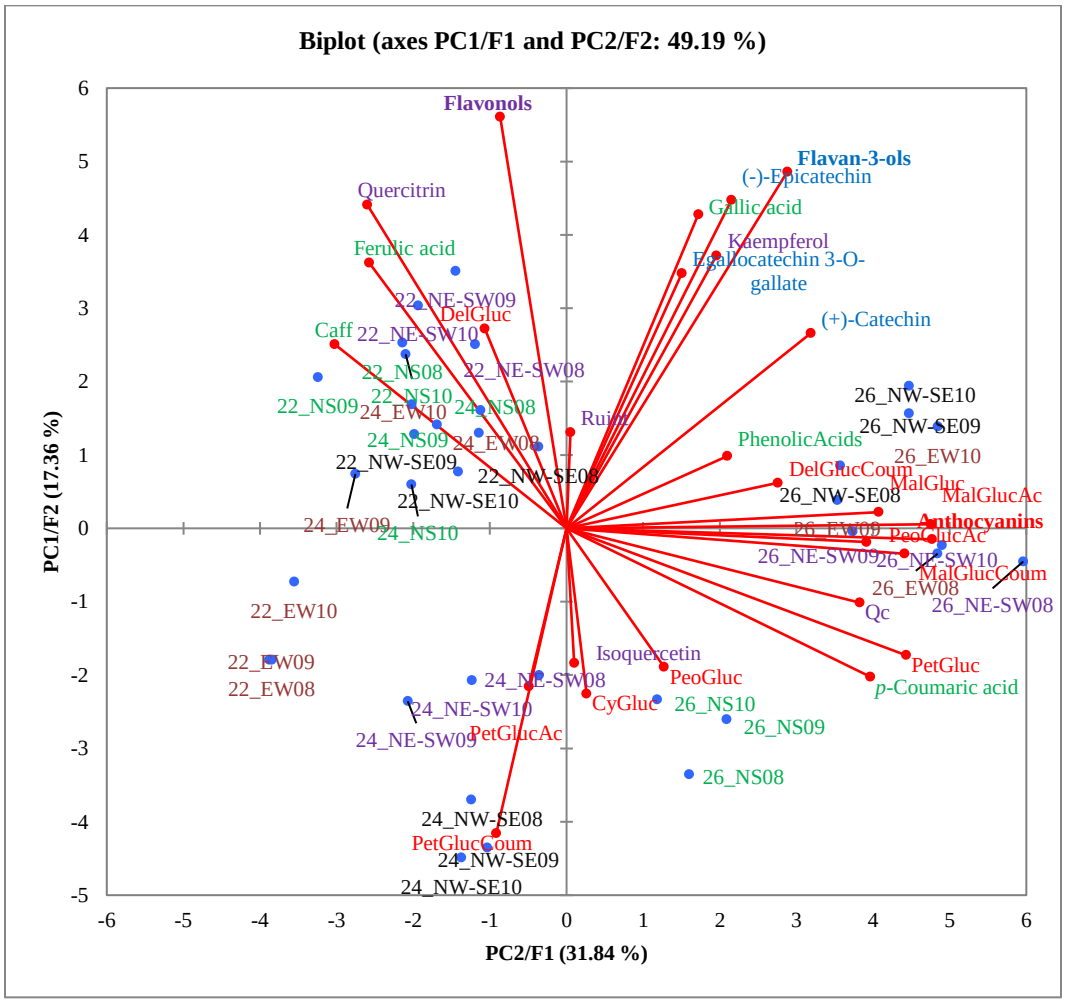

Figure 5.1 PCA biplot illustrating the association of phenolic compound composition of experimental Syrah wines from grapes harvested at ripeness levels of *ca*. 22, 24 and 26°Brix during 2008, 2009 and 2010 from NS, EW, NE-SW and NW-SE row orientation treatments. Abbreviations are defined in Table 5.1.

Table 5.1 list abbreviations used in the PCA biplot. The PCA biplot shows that row orientation treatments are not consistently associated with the same phenolic compounds at different ripeness levels over the three consecutive vintages.

Table 5.1 List of phenolic compound (variables) abbreviations used in Figures 5.1-5.7.

Abbreviation = Full name	Abbreviation = Full name	Abbreviation = Full name
EGCG = Epigallocatechin 3- *O*-gallate	Qr = Quercitrin (quercetin 3-*O*-rhamnoside)	Gall = Gallic acid
IsoQ = Isoquercitrin (quercetin 3-*O*-glucoside)	*p*-C = *p*-Coumaric acid	Cat = (+)-Catechin
DelGluc = Delphinidin 3-*O*-glucoside	Rut = Rutin (quercetin 3-*O*-rutinoside)	Caff = Caffeic acid
PetGlucAc = Petunidin 3-*O*-(6-*O*-acetyl) glucoside	Qc = Quercetin	NS = North-South
MalGlucAc = Malvidin 3-*O*-(6-*O*-acetyl) glucoside	Kaem = Kaempferol	EW = East-West
PetGlucCoum = Petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside	CyGluc = Cyanidin 3-*O*-glucoside	Epic = (-)-Epicatechin
MalGlucCoum = Malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside	PeoGluc = Peonidin 3-*O*-glucoside	NW-SE = Northwest-Southeast
DelGlucCoum = Delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside	MalGluc = Malvidin 3-*O*-glucoside	NE-SW = Northeast-Southwest
PeoGlucAc = Peonidin 3-*O*-(6-*O*-acetyl) glucoside	PetGluc = Petunidin 3-*O*-glucoside	Fer = Ferulic acid

5.3.2 Analysis of variance (3-factor analysis) of wine phenolic data for treatment, vintage and ripeness level

The *p*-values for the 3-factor ANOVA (univariate analysis) including row orientation as main plot factor, vintage as subplot factor and ripeness level as sub-subplot factor are listed in Table 5.2.

Table 5.2 Analysis of variance *p*-values (3-factor ANOVA) for treatment, vintage and °Brix, main effects and interaction for experimental Syrah wines.

Variables	Main effect			Interaction			
	Treatment	Vintage	°Brix[2]	Treat[1] x Vintage	Treat[1] x °Brix[2]	Vintage x °Brix[2]	Treat[1] x Vintage x °Brix[2]
Actual °Brix	0.4783*	**0.0002**	**<.0001**	0.2835	**<.0001**	**<.0001**	**<.0001**
Total flavan-3-ols	**0.0011**	0.2644	**<.0001**	0.5271	**<.0001**	0.1781	0.7751
(+)-Catechin	**0.0003**	0.0673	**<.0001**	0.2149	**0.0136**	0.4846	0.4543
(-)-Epicatechin	0.0591	0.4485	**0.0029**	0.4417	**<.0001**	0.0858	0.1502
EGCG[3]	**<.0001**	0.6521	**<.0001**	0.7001	**<.0001**	0.5997	0.4799
Total phenolic acids	**0.0005**	0.1157	**<.0001**	0.7558	**<.0001**	0.3464	0.1001
Gallic acid	**<.0001**	0.7764	**<.0001**	0.4276	**<.0001**	0.1177	0.2686
Caffeic acid	0.0626	0.0534	**<.0001**	0.9646	**<.0001**	0.8972	0.4380
p-Coumaric acid	**<.0001**	0.7661	**<.0001**	0.5256	**<.0001**	0.5533	0.4272
Ferulic acid	**0.0099**	0.4638	**<.0001**	0.8964	**<.0001**	0.1442	0.2610

*p-values in bold indicate significant effects; [1]Treatment; [2]Degrees Brix; [3]Epigallocatechin 3-*O*-gallate.

Table 5.2 Continued.

Varaibles	Main effect			Interaction			
	Treatment	Vintage	°Brix[2]	Treat[1] x Vintage	Treat[1] x °Brix[2]	Vintage x °Brix[2]	Treat[1] x Vintage x °Brix[2]
Total flavonols	**0.0022**	0.499	**<.0001**	0.6916	**<.0001**	0.3126	0.0596
Rutin	**0.0002**	0.1513	**0.0026**	0.9224	**0.0035**	0.1601	0.6731
Isoquercetin	**<.0001**	0.7346	**<.0001**	0.0858	**<.0001**	0.1054	**0.0109**
Quercetin	**<.0001**	0.8816	**<.0001**	0.4179	**<.0001**	0.2118	0.0707
Kaempferol	0.3813	0.5899	**<.0001**	0.8701	**<.0001**	0.5891	0.9643
Quercitrin	**0.0062**	0.4337	**<.0001**	0.8437	**<.0001**	0.3702	0.0855
Total anthocyanins	**0.0014**	**0.0059**	**<.0001**	0.7869	**0.0004**	0.8772	0.1916
CyGluc[4]	**<.0001**	0.2326	**<.0001**	0.3327	**<.0001**	**0.0057**	**<.0001**
PetGluc[4]	**0.0355**	0.3754	**<.0001**	0.4387	**<.0001**	0.8534	0.4531
PeoGluc[4]	**0.0383**	0.4196	**<.0001**	0.1741	**<.0001**	**<.0001**	**0.0012**
MalGluc[4]	**0.0020**	**0.0073**	**<.0001**	0.5557	**<.0001**	0.2906	**0.0157**
DelGluc[4]	**<.0001**	0.1904	**<.0001**	0.1952	**0.0005**	0.4818	**0.0236**
PetGlucAc[5]	**<.0001**	0.0576	**0.0041**	0.5646	**<.0001**	**0.0415**	**0.0052**
PeoGlucAc[5]	**0.0412**	0.1767	**<.0001**	0.1166	**<.0001**	0.2412	0.1412
MalGlucAc[5]	0.0977	0.0673	**<.0001**	0.7428	**0.0076**	0.8544	0.9729
DelGlucCoum[6]	**<.0001**	0.5108	**<.0001**	0.8642	**<.0001**	0.4352	0.4749
PetGlucCoum[6]	**0.0143**	0.2998	**<.0001**	0.6765	**<.0001**	0.2626	0.0618
MalGlucCoum[6]	**0.0032**	**0.0032**	**<.0001**	**0.0453**	**<.0001**	0.8379	0.4622

[*]*p*-values in bold indicate significant effects; [1]Treatment; [2]Degrees Brix; [4]Cyanidin-, petunidin-, peonidin-, malvidin- and delphinidin 3-*O*-glucosides; [5]Petunidin-, peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides; [6]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside.

Row orientation by ripeness level interaction is significant for all compounds. Vintage by ripeness level interaction are only significant for cyanidin 3-*O*-glucoside, peonidin 3-*O*-glucoside and petunidin 3-*O*-(6-*O*-acetyl) glucoside, whereas row orientation (treatment) by vintage by ripeness level interaction are only significant for isoquercetin, cyanidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside and petunidin 3-*O*-(6-*O*-acetyl) glucoside.

Interaction refers to the inconsistency of general trends or patterns in the data. This confirms that phenolic compound concentrations in experimental Syrah wine are affected by the different grapevine row orientation treatments at the various grape berry development stages (ripeness levels) and/or vintage conditions, as indicated by exploratory PCA (Figs. 5.2-5.4). It was therefore practical to interpret results separately for each grape ripeness level.

5.3.3 Wines from grapes harvested at *ca*. 22°Brix

Titratable acidity (TA) showed significant differences between wines from the NS and EW and those from the NE-SW row orientation treatment (Table 5.3). Significant differences in pH values occurred between wines made from grapes of NS and NW-SE and those from grapes of the NE-SW row orientation treatments.

Table 5.3 Total acidity (TA) and pH of experimental Syrah wines as a function of row orientation from Syrah grapes harvested at *ca.* 22°Brix. Data represents wines from grapes collected over three consecutive vintages.

Measured parameters	Row orientation treatment				*p*-value
	[1]EW [2](20.4-23.7°Brix)	[1]NE-SW [2](21.3-24.6°Brix)	[1]NS [2](21.9-23.1°Brix)	[1]NW-SE [2](21.0-25.1°Brix)	
pH	3.763ba (± 0.1319)	3.634b (± 0.1868)	3.791a (± 0.1984)	3.791a (± 0.1719)	0.1532
Total acidity	5.656a (± 0.6960)	4.664b (± 0.7311)	5.427a (± 0.8626)	5.293ba (± 0.6624)	0.0424

[1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Minimum and maximum total soluble solids (°Brix) for grape must before inoculation.

5.3.3.1 Analysis of variance (2-factor analysis) of wine phenolic data for treatment and vintage main effects

The *p*-values for the 2-factor ANOVA including row orientation as main plot factor and vintage as subplot factor for wines from grapes harvested at *ca*. 22°Brix are listed in Table 5.4. Tables of means to follow are based on these results and are presented for compounds indicated to be significantly affected by a specific interaction or main effect.

Table 5.4 Analysis of variance *p*-values for treatment and vintage, main effects and interaction for experimental Syrah wines (grapes harvested at *ca.* 22°Brix).

Phenolic compounds	Main effect		Interaction
	Treatment	Vintage	Treatment x vintage
Total flavan-3-ols	**0.0001***	0.4925	0.3664
(+)-Catechin	0.2390	0.0630	0.0707
(-)-Epicatechin	**<.0001**	0.5081	0.2809
Epigallocatechin 3-*O*-gallate	**<.0001**	0.8334	0.6069
Total phenolic acids	**0.0001**	0.6098	0.3283
Gallic acid	**0.0409**	0.2943	0.6302
Caffeic acid	0.0705	0.3521	0.3071
p Coumaric acid	**<.0001**	0.5526	0.6450
Ferulic acid	**0.0002**	0.9389	0.7650
Total flavonols	**<.0001***	0.8536	0.4457
Rutin	**0.0115**	0.0755	0.2101
Isoquercetin	**<.0001**	0.6415	0.3023
Quercetin	**0.0028**	**0.0327**	0.0744
Kaempferol	**<.0001**	0.9442	0.9431
Quercitrin	**<.0001**	0.8030	0.5719

*p-values in bold indicate significant effects.

Table 5.4 Continued.

Phenolic compounds	Main effect		Interaction
	Treatment	Vintage	Treatment x vintage
Total anthocyanins	**0.0002**	**0.0090**	0.6930
CyGluc[1]	0.5176	0.2223	0.2374
PetGluc[1]	**0.0090**	0.1009	**0.0025**
PeoGluc[1]	**0.0021**	0.4165	0.0988
MalGluc[1]	**0.0002**	**0.0115**	0.2678
DelGluc[1]	**<.0001**	0.1435	0.1289
PetGlucAc[2]	**0.0209**	**0.0148**	**0.0057**
PeoGlucAc[2]	**<.0001**	0.6530	**0.0104**
MalGlucAc[2]	**0.0155**	0.1049	0.8094
DelGlucCoum[3]	**0.0094**	**0.0011**	0.4890
PetGlucCoum[3]	**0.0182**	**0.0245**	0.1097
MalGlucCoum[3]	**0.0010**	**0.0495**	0.1038

*p-values in bold indicate significant effects. [1]Cyanidin-, petunidin-, peonidin-, malvidin- and delphinidin 3-*O*-glucosides; [2]Petunidin-, peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides; [3]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides.

Row orientation (treatment) by vintage interaction is not significant for most compounds, except petunidin 3-*O*-glucoside, petunidin 3-*O*-(6-*O*-acetyl) glucoside and peonidin 3-*O*-(6-*O*-acetyl) glucoside. Tables of means that follow are based on these results and are listed for compounds that are significantly affected by a specific interaction or main effect (row orientation/vintage/ripeness).

5.3.3.2 Principal component analysis for wines from grapes harvested at *ca.* 22°Brix

In order to highlight the key features of each treatment, PCA was performed using phenolic composition as variables. The PCA biplot (Fig. 5.2) of the first two principal components (PC1/F1 and PC2/F2) illustrating the association of phenolic compounds of experimental Syrah wines from grapes harvested during 2008, 2009 and 2010 at a ripeness level of *ca.* 22°Brix with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations, explained 63.21% of the variation in the data.

Principal component 1 shows the main source or greatest variation in the biplot. Variables included in the PCA were limited to those with a significant main effect (squared cosine values ≥ 0.5) or significant effect in the ANOVAs (data not shown).

Figure 5.2 indicates that the main source of variation is row orientation, with PC1 mainly separating NE-SW and NW-SE from NS and EW row orientation treatments, while PC2 separates NE-SW, NW-SE and EW from NS treatments.

The phenolic compounds with the highest squared cosine values on PC1 are total flavan-3-ols, total phenolic acids, total flavonols, total anthocyanins, (-)-epicatechin, epigallocatechin 3-*O*-gallate, *p*-coumaric acid, isoquercetin (quercetin 3-*O*-glucoside), quercitrin (quercetin 3-*O*-rhamnoside), peonidin 3-*O*-glucoside, peonidin 3-*O*-(6-*O*-acetyl) glucoside and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside.

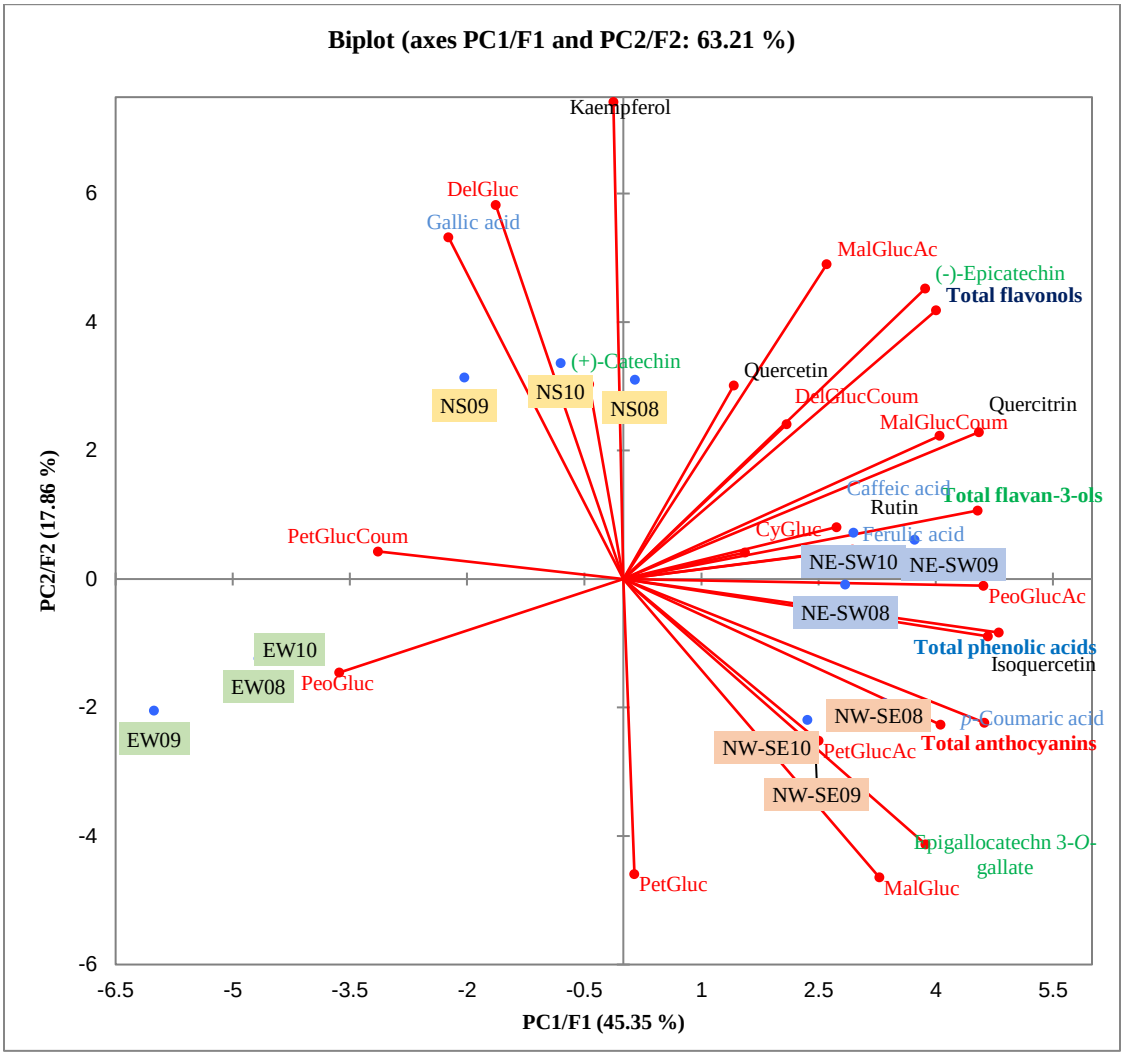

Figure 5.2 PCA biplot illustrating the association of phenolic compound composition of experimental Syrah wines with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations. Grapes were harvested during 2008, 2009 and 2010 at ripeness levels of *ca.* 22°Brix). Abbreviations are defined in Table 5.1.

Kaempferol and delphinidin 3-*O*-glucoside have the highest squared cosine values on PC2. Principal component 3 explains an additional 12.32% of variation (data not shown), and does not improve the interpretability/variability, and was therefore not included in the biplot.

Ferulic acid and quercetin have the highest squared cosine value on PC3. Tables 5.4-5.6 of mean concentrations of phenolic compounds are presented for compounds indicated to be significantly affected by a specific interaction or main effect.

5.3.3.3 Analysis of variance for wine phenolic compound concentration means for treatment (row orientation) by vintage interaction

Mean concentrations for two wine phenolic compounds with a significant treatment (row orientation) by vintage interaction for grapes harvested at *ca*. 22°Brix are listed in Table 5.5. Row orientation treatment by vintage interaction is caused by slight deviations in trends among vintages within row orientation treatments.Therefore, only noticeable interactions are discussed. Only two compounds showed treatment by vintage interaction.

Table 5.5 Wine phenolic compound concentration means for treatment (row orientation) by vintage interaction (grapes harvested at *ca*. 22 °Brix).*

Treatment	Vintage	Phenolic compounds	
		PetGlucAc[2]	PeoGlucAc[3]
EW[1]	2008	2.941bdc[**] (±0.302)[***]	5.112f (±0.304)
EW	2009	2.725dc (±0.334)	4.469f (±0.460)
EW	2010	3.374bac (±0.805)	5.151fe (±0.577)
NE-SW[1]	2008	3.566ba (±0.397)	6.986dc (±0.531)
NE-SW	2009	3.585ba (±0.146)	**9.088a (±0.578)**
NE-SW	2010	3.152bc (±0.477)	**8.185ba (±0.507)**
NS[1]	2008	3.510ba (±0.183)	**7.550bc (±0.670)**
NS	2009	**2.417d (±0.160)**	**6.783dc (±0.250)**
NS	2010	**2.361d(±1.063)**	6.279de (±1.360)
NW-SE[1]	2008	4.066a (±0.261)	8.387ba (±1.178)
NW-SE	2009	**3.295bc (±0.137)**	8.620ba (±0.285)
NW-SE	2010	**2.777dc (±0.377)**	8.737a (±0.743)
p-value		0.0057	0.0104

*Means given for compounds with significant treatment (row orientation) by vintage interaction as indicated in Table 5.3; [1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Petunidin 3-*O*-(6-*O*-acetyl) glucosides; [3]Peonidin 3-*O*-(6-*O*-acetyl) glucosides; **Different letters in the same column indicate significant differences in the content of the compounds measured among the different treatments and vintages according to Fiseher's least significant difference test. ***Values in brackets indicate standard deviations

Wines from the 2009 and 2010 vintages within the NE-SW row orientation treatments had significantly higher concentrations of peonidin 3-*O*-(6-*O*-acetyl) glucosides (PeoGlucAc), compared to wines from grapes in the 2008 vintage.

Peonidin 3-*O*-(6-*O*-acetyl) glucosides in wines from grapes from the NS row orientation in the 2008 and 2009 vintage were significantly higher than wines in the 2010 vintage.

Vintage interaction was also evident in petunidin 3-*O*-(6-*O*-acetyl) glucosides (PetGlucAc) since this acetylated anthocyanin was significantly lower in grapes from the NS row orientation treatments in 2009 and 2010 vintages.

Significantly lower concentrations were also found in wines from grapes from the NW-SE row orientation in the 2009 and 2010 vintages.

5.3.3.4 Analysis of variance for wine phenolic compound concentration means for vintage main effects

Mean concentrations for grape phenolic compounds with significant vintage main effects for grapes harvested at *ca.* 22°Brix are listed in Table 5.6.

Table 5.6 Wine phenolic compound concentration means for vintage main effects (grapes harvested at *ca.* 22 °Brix).*

Phenolic compounds	Vintage			*p*-value
	2008	2009	2010	
Quercetin	1.824ba** (±0.458)***	1.677b (±0.495)	1.900a (±0.389)	0.0327
Total anthocyanins	**202.218a (±27.469)**	175.680b (±25.754)	180.152b (±23.745)	0.0090
MalGluc[1]	**101.320a (±21.559)**	84.759b (±22.882)	86.540b (±15.818)	0.0115
DelGlucCoum[2]	**2.109a (±0.292)**	**1.529c (±0.340)**	**1.848b (±0.473)**	0.0011
PetGlucCoum[2]	**2.330a (±0.818)**	1.760b (±0.357)	1.947b (±0.591)	0.0245
MalGlucCoum[2]	26.785a (±4.520)	23.820b (±5.569)	24.398ba (±6.700)	0.0495

*Means given for compounds with significant vintage main effects as indicated in Table 5.3. **Different letters in the same row indicate significant differences in the content of the compounds measured among the different vintages according to Fischer's least significant difference test; ***Values in brackets indicate standard deviations. [1] Malvidin 3-*O*-glucosides; [2] Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides.

The tendency for certain compounds is that wines from grapes harvested in 2008 had significantly higher concentrations than those harvested in 2009 and 2010. The exception is quercetin for which significant differences between 2008 and 2010 were not evident as well as malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides (MalGlucCoum), which were also not significantly different between the 2008 and the 2010 vintages. Malvidin 3-*O*-glucoside concentrations (MalGluc) were significantly higher in 2008, compare to 2009 and 2010 vintages. Significant differences among the 2008, 2009 and 2010 vintages were evident for delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside (DelGlucCoum) concentrations.

5.3.3.5 Analysis of variance for wine phenolic compound concentration means for treatment (row orientation) main effects

Mean concentrations for wine phenolic compounds with a significant treatment (row orientation) main effect for wines from grapes harvested at *ca*. 22°Brix are listed in Table 5.7. Among the individual chemical compositional variables quantified, a number of phenolic compound concentration differences were recorded.

Flavan-3-ols

Total flavan-3-ols were significantly higher in wines from grapes from the NE-SW row orientation treatments and significantly lower in wines from grapes from the EW row orientation treatments. Epigallocatechin 3-*O*-gallate (EPCG) concentrations were significantly lower in wines from the NS row orientations and (-)-epicatechin concentrations significantly lower in wines from grapes from the EW row orientation treatments.

Phenolic acids

Total phenolic acids were significantly lower in wines from the EW row orientation treatments with significantly higher concentrations in wines from the NW-SE row orientations. Wines from the NW-SE orientation were not significantly different from wines from the NE-SW orientation.

Table 5.7 Wine phenolic compound concentration means for treatment (row orientation) main effects (grapes harvested at *ca.* 22 °Brix).*

Phenolic compounds	Row orientation (treatment)				*p*-value
	EW[1]	NE-SW[1]	NS[1]	NW-SE[1]	
Total flavan-3-ols	**13.885c[**] (±1.507)[***]**	**18.917a (±1.055)**	15.927b (±1.110)	16.956b (±0.838)	0.0001
(-)-Epicatechin	**5.411c (±0.406)**	8.281a (±0.536)	7.821a (±1.117)	7.011b (±0.714)	<.0001
EGCG[2]	2.180b (±0.592)	4.222a (±0.564)	**1.569c (±0.280)**	4.072a (±0.517)	<.0001
Total phenolic acids	**66.406c (±4.093)**	83.426a (±3.741)	75.485b (±5.310)	86.888a (±6.560)	0.0001
Gallic acid	4.198ba (±0.565)	3.818b (±0.407)	4.551a (±0.639)	3.586b (±0.372)	0.0409
p Coumaric acid	**21.249d (±2.235)**	**32.657b (±1.561)**	**25.182c (±2.376)**	**35.594a (±2.160)**	<.0001
Ferulic acid	5.269b (±0.792)	**8.112a (±0.860)**	5.100b (±0.893)	5.436b (±0.790)	0.0002
Total flavonols	**10.610c (±0.934)**	22.728a (±1.030)	22.368a(±1.779)	19.783b (±1.722)	<.0001
Rutin	0.814b (±0.187)	**1.342a (±0.326)**	0.829b (±0.126)	0.869b (±0.268)	0.0115
Isoquercetin	**0.395c (±0.075)**	1.377a (±0.079)	0.784b (±0.081)	1.317a (±0.115)	<.0001
Quercetin	1.694cb (±0.283)	**2.348a (±0.212)**	1.807b (±0.384)	1.393c (±0.212)	0.0028
Kaempferol	1.748c (±0.394)	2.560b (±0.398)	**5.376a (±0.618)**	1.780c (±0.393)	<.0001
Quercitrin	**12.771c (±0.954)**	16.443a (±0.842)	14.400b (±1.914)	15.292ba (±1.483)	<.0001
Total anthocyanins	161.278b (±18.495)	199.487a (±13.095)	172.031b (±21.303)	208.926a (±24.016)	0.0002
PetGluc[3]	4.900ba (±0.619)	3.956c (±1.151)	4.327bc (±0.688)	5.597a (±0.656)	0.0090
PeoGluc[3]	**5.538a (±1.564)**	4.035b (±0.355)	4.280b (±0.735)	3.768b (±0.932)	0.0021
MalGluc[3]	80.983b (±12.551)	100.468a (±9.759)	70.833b (±16.683)	109.474a (±16.763)	0.0002
DelGluc[3]	2.058b (±0.240)	2.042b (±0.330)	**2.344a (±0.428)**	**1.556c (±0.212)**	<.0001
PetGlucAc[4]	3.740b (±6.461)	4.556a (±5.849)	4.833a (±5.270)	4.303ba (±7.301)	0.0155
DelGlucCoum[5]	1.477c (±0.341)	1.714bc (±0.381)	2.093a (±0.312)	2.038ba (±0.474)	0.0094
PetGlucCoum[5]	**2.555a (±0.784)**	1.741b (±0.516)	2.094ba (±0.496)	1.634b (±0.283)	0.0182
MalGlucCoum[5]	**24.107b (±2.418)**	27.171a (±3.573)	26.896a (±3.770)	28.589a (±4.343)	0.0010

*Means given for compounds with significant treatment (row orientation) main effects only as indicated in Table 5.3. [1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [3]Petunidin-, peonidin-, malvidin and delphinidin 3-*O*-glucosides; [4]Petunidn 3-*O*-(6-*O*-acetyl) glucosides; [5]Delphinidin-, petunidin and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides; **Different letters in the same row indicate significant differences in the content of the compounds measured among the different treatments according to Fisher's least significant difference test; ***Values in brackets indicate standard deviations.

Ferulic acid and rutin concentrations were significantly higher in wines from the NE-SW row orientation treatments. Significantly higher *p*-coumaric acid concentrations were found in wines from the NW-SE row orientations and significantly lower concentrations in wines from the EW row orientation treatments. Significant differences for *p*-coumaric acid were evident among all treatments.

Flavonols

Wines from the EW row orientation treatments were significantly lower in total flavonols. Individual flavonols, *i.e*. isoquercetin (quercetin 3-*O*-glucoside) and quercitrin (quercetin 3-*O*-rhamnoside) concentrations were significantly lower in wines from the EW row orientation treatments.

Significantly higher concentrations of quercetin were found in wines from the NE-SW row orientation treatments, whereas kaempferol concentrations were significantly higher in wines from the NS row orientation treatments.

Anthocyanins

Total anthocyanin concentrations were not significantly different among the different row orientation treatments. However, peonidin 3-*O*-glucoside (PeoGluc) and petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside (PetGlucCoum) concentrations were significantly higher in wines from the EW row orientation treatments.

Delphinidin 3-*O*-glucosides (DelpGluc) concentrations were significantly higher in wines from the NS row orientation treatments, but significantly lower in wines from the NW-SE row orientation treatments.

Malvidin 3-*O*-(6-*O*-*p*-coumaryol) glucoside (MalGlucCoum) concentrations were significantly lower in wine from the EW row orientation treatments.

5.3.3.6 Multiple factor analysis of phenolic compounds for Syrah grapes and wine

Multiple factor analysis (MFA) examines observations described by several sets of variables (Abdi, 2003). The MFA identifies the common denominator present in the data sets and is performed in two steps.

Firstly, PCA is performed on each data set, which is then "normalized" by dividing all its elements by the square root of the first eigenvalue obtained from the PCA. Secondly, the normalised data sets are merged to form a unique matrix and a global PCA is performed on this matrix.

The individual data sets are then projected onto the global analysis to examine communalities and discrepancies. The goal of MFA is therefore to integrate different groups of variables describing the same treatment.

Figure 5.3 depicts correlations between phenolic compounds of Syrah grape samples and Syrah wine samples. Four phenolic compounds were identified by MFA as being well correlated between grape and wine samples, *i.e*. epigallocatechin 3-*O*-gallate (R = 0.940), ferulic acid (R = 0.800), isoquercetin (R = 0.871) and quercetin (R= 0.830).

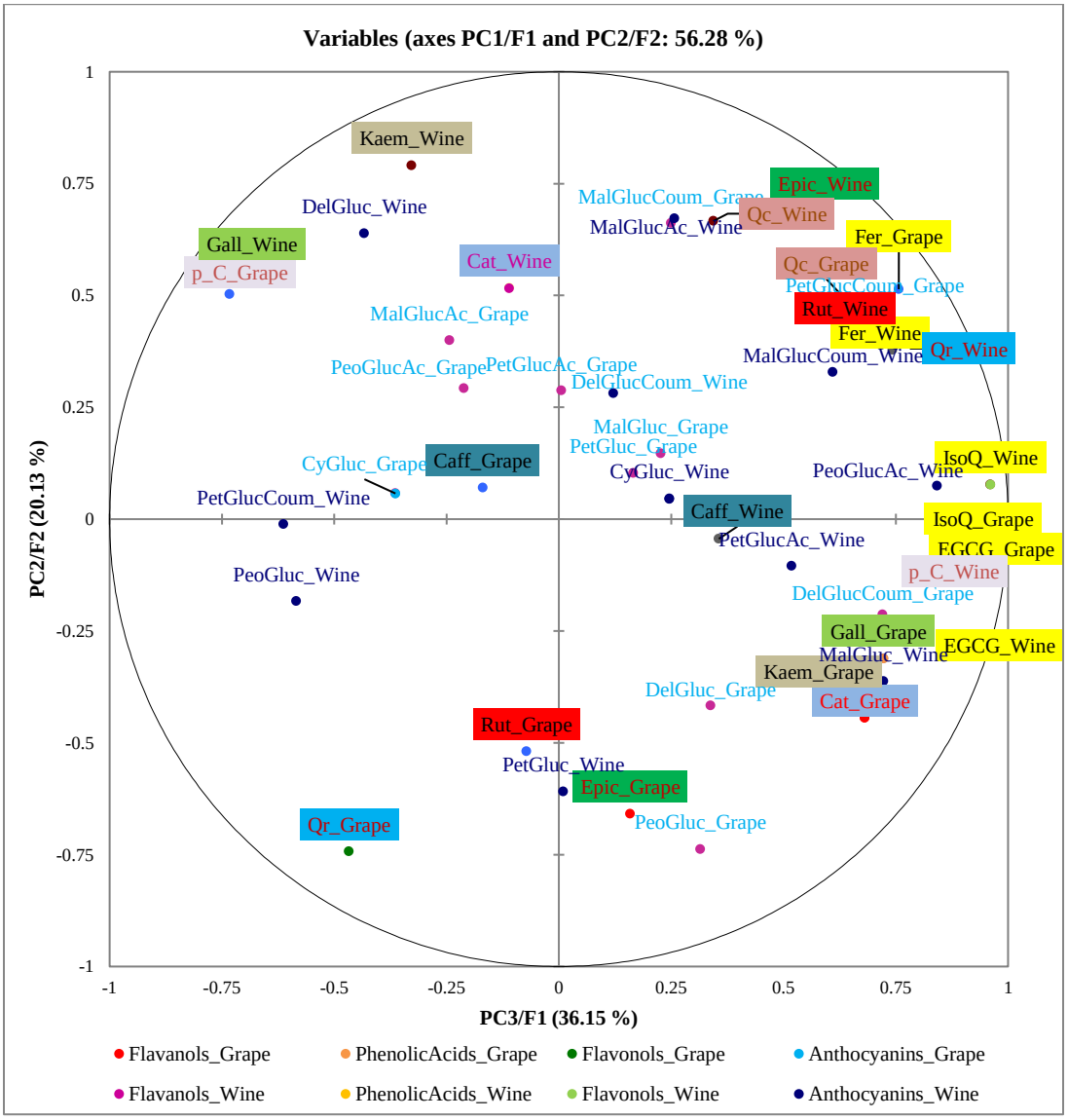

Figure 5.3 MFA variable correlations map illustrating the association of phenolic compound composition of Syrah grapes and wine. Grapes harvested at ripeness levels of *ca.* 22°Brix from NS, EW, NE-SW and NW-SE row orientation treatments.

5.3.3.7 RV (vector correlation) coefficient analysis of phenolic compounds in Syrah grape and wine samples

RV (vector correlation) coefficient is a multivariate generalisation of the squared Pearson correlation coefficient (Abidi *et al.* 2009). It is a correlation between two sets of variables.

The RV coefficient takes values between 0 and 1. It measures the closeness of two set of points that may each be represented in a matrix. Values closest to 1 indicate a good correlation between the measured variables (Table 5.8).

Table 5.8 RV Coefficients of the relationship between the tables of component groups of Syrah grapes and wine. Grapes harvested at ripeness levels of *ca.* 22°Brix from NS, EW, NE-SW and NW-SE row orientation treatments.

Phenolic compound classes	Flavan-3-ols, wine	Phenolic acids, wine	Flavonols, wine	Anthocyanins, wine
Flavan-3-ols, grapes	0.582	0.691	0.587	0.494
Phenolic acids, grapes	0.607	**0.694**	0.675	0.580
Flavonols, grapes	0.612	0.753	**0.768**	0.446
Anthocyanins, grapes	0.436	0.518	0.515	**0.499**

The highest correlation between the grape and wine groups of phenolic compounds was flavonols at R = 0.768, followed by phenolic acids at R = 0.694. The lowest correlation between grape and wine samples was anthocyanins (R = 0.499)

5.3.4 Wines from grapes harvested at *ca.* 24°Brix

The wines from grapes harvested at *ca.* 24ºBrix were significantly higher in titratable acidity for the NS and EW row orientation treatments in comparison to those from the NE-SW and NW-SE row orientation treatments (Table 5.9).

The wine pH values of the NE-SW and NW-SE row orientations appeared slightly higher than those of the NS and EW row orientations.

Table 5.9 Total acidity (TA) and pH of experimental Syrah wines as a function of row orientation from Syrah grapes harvested at *ca.* 24°Brix. Data represents wines from grapes collected over three consecutive vintages.

Measured parameters	Row orientation treatments				*p*-value
	[1]EW [2](21.7-26.0°Brix)	[1]NE-SW [2](22.8-26.3°Brix)	[1]NS [2](22.5-26.3°Brix)	[1]NW-SE [2](23.0-26.9°Brix)	
pH	3.991a (± 0.1479)	4.326a (± 0.8353)	4.073a (± 0.1416)	4.291a (± 0.5071)	0.3441
Total acidity	5.316a (± 6.600)	4.891b (± 0.7803)	5.034a (± 0.6183)	4.824b (± 0.7289)	0.4674

[1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Minimum and maximum total soluble solids (°Brix) for grape must before inoculation.

5.3.4.1 Analysis of variance (2-factor analysis) of wine phenolic data for treatment and vintage main effects

The *p*-values for the 2-factor ANOVA including row orientation as main plot factor and vintage as subplot factor for wines from grapes harvested at *ca*. 24°Brix are listed in Table 5.10.

Row orientation (treatment) by vintage interaction is not significant for most compounds, except for isoquercetin, peonidin 3-*O*-glucoside and delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside.

Tables of means to follow are based on these results and are presented for compounds indicated to be significantly affected by a specific interaction or main effect (row orientation).

Table 5.10 Anova *p*-values for treatment and vintage, main effects and interaction for experimental Syrah wines (grapes harvested at *ca*. 24°Brix).

Phenolic compounds	Main effect		Interaction
	Treatment	Vintage	Treatment x vintage
Total flavan-3-ols	**0.0005**	0.0978	0.5146
(+)-Catechin	**0.0003**	0.7305	0.4619
(-)-Epicatechin	**0.0309**	0.1107	0.1727
Epigallocatechin 3-*O*-gallate	**0.0002**	0.6229	0.9045
Total phenolic acids	**0.0036**	0.5295	0.5692
Gallic acid	**<.0001**	0.7764	0.1260
Caffeic acid	**<.0001**	0.5285	0.6101
p Coumaric acid	**<.0001**	0.8651	0.8932
Ferulic acid	**<.0001**	0.0987	0.3456
Total flavonols	**<.0001**	0.2544	0.3374
Rutin	**0.0006**	0.6946	0.9968
Isoquercetin	**<.0001**	0.3047	**0.0314**
Quercetin	**<.0001**	0.5662	0.5849
Kaempferol	**<.0001**	0.2830	0.1433
Quercitrin	**<.0001**	0.2549	0.2542
Total anthocyanins	**0.0463**	0.1670	0.9502
CyGluc[1]	0.0638	**0.0045**	0.4211
PetGluc[1]	**0.0135**	0.9693	0.7950
PeoGluc[1]	**0.0003**	**0.0114**	**0.0196**
MalGluc[1]	0.5222	0.3337	0.9792
DelGluc[1]	**0.0078**	0.9717	0.0792
PetGlucAc[2]	**0.0005**	**0.0047**	0.2622
PeoGlucAc[2]	0.6655	**0.0357**	0.4964
MalGlucAc[2]	0.2140	0.1688	0.9258
DelGlucCoum[3]	**0.0002**	**0.0131**	**0.0053**
PetGlucCoum[3]	**<.0001**	0.9971	0.3899
MalGlucCoum[3]	**0.0088**	**0.0006**	0.3069

*p-values in bold indicate significant effects. [1]Cyanidin-, petunidin-, peonidin-, malvidin- and delphinidin 3-*O*-glucosides; [2]Petunidin-, peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides; [3]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides.

5.3.4.2 Principal component analysis for wines from grapes harvested at *ca.* 24°Brix

The PCA biplot (Fig. 5.4) of the first and third principal components (PC1/F1 and PC3/F3) illustrating the association of phenolic compounds of experimental Syrah wines from grapes harvested during 2008, 2009 and 2010 at a ripeness level of *ca.* 24°Brix with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations, explained 54.50% of the variation in the data. If PC2 instead of PC3 is included, the percentage variation increases only to 58.68%, but variability does not improve on the biplot. Principal component 1 shows the main source or greatest variation in the biplot. Variables included in the PCA were limited to those with a significant main effect (squared cosine values ≥ 0.5) or significant effect in the ANOVAs (data not shown).

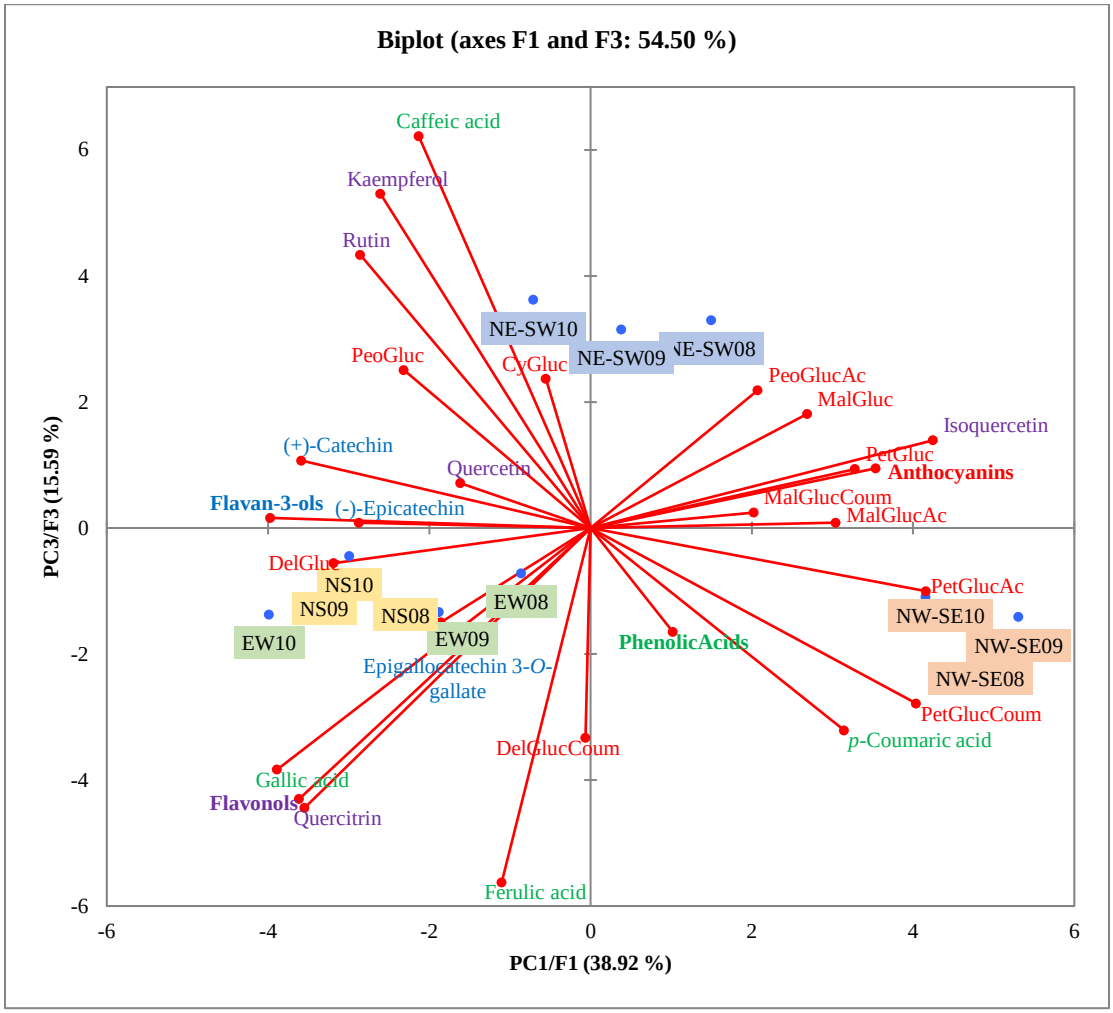

Figure 5.4 PCA biplot illustrating the association of phenolic compound composition of experimental Syrah wines with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations. Grapes were harvested during 2008, 2009 and 2010 at ripeness levels of *ca.* 24°Brix). Abbreviations are defined in Table 5.1.

Figure 5.4 indicates that the main source of variation is row orientation, with PC1 mainly separating NW-SE from NE-SW, NS and EW row orientation treatments, while PC3 separated NW-SE, EW and NS treatments from NE-SW row orientation treatments.

The phenolic compounds with the highest squared cosine values on PC1 are total flavan-3-ols, total flavonols, total anthocyanins, (+)-catechin, gallic acid, isoquercetin (quercetin 3-*O*-glucoside), quercitrin (quercetin 3-*O*-rhamnoside), petunidin 3-*O*-(6-*O*-acetyl) glucoside and petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside. Caffeic acid, ferulic acid and kaempferol have the highest squared cosine values on PC3. Principal component 3 explains an additional 15.58% of variation and does improve the interpretability/variability and was therefore included in the biplot.

Total phenolic acids, epigallocatechin 3-*O*-gallate, quercetin and delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside have the highest squared cosine value on PC2 but do not improve the variability in the biplot. Tables 5.9-5.10 of mean concentrations of phenolic compounds are presented for compounds indicated to be significantly affected by a specific interaction or main effect.

5.3.4.3 Analysis of variance for wine phenolic compound concentration means for treatment (row orientation) by vintage interaction

Mean concentrations for three wine phenolic compounds with a significant treatment (row orientation) by vintage interaction for grapes harvested at *ca*. 24°Brix are listed in Table 5.11. The data shows that row orientation by vintage interaction is caused by slight deviations in trends among years within row orientations. Therefore, only apparent interaction will be discussed.

Vintage interaction was evident in isoquercetin because this flavonol was significantly lower in the 2009 and 2010 vintages in wines from the NW-SE row orientation treatments. Vintage interaction was also evident in EW row orientation treatments where peonidin 3-*O*-glucosides and delphinidin 3-*O*-(6-*O*-*p*-coumaryol) glucosides were significantly lower in wines from the 2008 and 2009 vintages.

Table 5.11 Wine phenolic compound concentration means for treatment (row orientation) by vintage interaction (grapes harvested at *ca*. 24°Brix).[*]

Treatment	Vintage	Phenolic compounds		
		Isoquercetin	**PeoGluc[2]**	**DelGlucCoum[3]**
EW[1]	2008	0.250f[**] (±0.019)[***]	**4.924dc (±0.847)**	**3.931b (±1.652)**
EW	2009	0.288f (±0.025)	**4.462dc (±0.817)**	**3.980b (±1.783)**
EW	2010	0.347ef (±0.017)	6.734ba (±0.953)	5.312a (±1.129)
NE-SW[1]	2008	1.728c (±0.078)	**4.148d (±0.641)**	2.618cd (±0.401)
NE-SW	2009	1.618c (±0.085)	**5.235bdc (±1.273)**	2.092ed (±0.310)
NE-SW	2010	1.598c (±0.225)	7.852a (±1.216)	1.952ed (±0.340)
NS[1]	2008	0.599ed (±0.133)	4.416dc (±0.133)	2.193ed (±0.395)
NS	2009	0.757d (±0.181)	5.505bdc (±0.554)	1.440e (±0.342)
NS	2010	0.631d (±0.064)	6.118bac (±1.308)	2.411d (±0.294)
NW-SE[1]	2008	2.804a (±0.101)	4.119d (±0.296)	2.685cd (±0.136)
NW-SE	2009	**2.311b (±0.434)**	4.472dc (±0.498)	3.508cb (±0.508)
NW-SE	2010	**2.248b (±0.146)**	4.869dc (±1.883)	3.364cb (±0.514)
	***p*-value**	**0.0314**	**0.0196**	**0.0053**

[*]Means given for compounds with significant treatment (row orientation) by vintage interaction as indicated in Table 5.8; [1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Peonidin 3-*O*-glucoside; [3]Delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides. [**]Different letters in the same column indicate significant differences in the content of the compounds measured among the different treatments and vintages according to Fiseher's least significant difference test; [***]Values in brackets indicate standard deviations.

Peonidin 3-*O*-glucoside concentrations were also significantly lower in wines from the NE-SW row orientation treatments in the 2008 and 2009 vintages. Row orientation (treatment) by vintage interaction caused small

differences in trends among vintages within row orientations. This confirms the slight separation of 2008 and 2009 samples from the 2010 samples for EW row orientation treatments and the 2009 and 2010 samples from the 2008 samples for NS row orientations in Figure 5.4.

5.3.4.4 Analysis of variance for wine phenolic compound concentration means for vintage main effects

Mean concentrations for wine phenolic compounds with significant vintage main effects for grapes harvested at *ca.* 24°Brix are listed in Table 5.12.

Table 5.12 Wine phenolic compound concentration means for vintage main effects (grapes harvested at *ca.* 24°Brix).[*]

Phenolic compounds	Vintage			*p* value
	2008	2009	2010	
CyGluc[1]	1.142b[**] (±0.178)[***]	1.180b (±0.151)	**1.296a (±0.120)**	0.0045
PetGlucAc[2]	**3.978a (±0.401)**	3.379b (±0.851)	3.224b (±0.858)	0.0047
PeoGlucAc[2]	8.173a (±0.746)	7.278b (±1.439)	7.352ba (±1.479)	0.0357
MalGlucCoum[3]	35.719a (±4.517)	29.391b (±4.790)	29.983b (±5.737)	0.0006

[*]Means given for compounds with significant vintage main effects as indicated in Table 5.8; [1]Cyanidin 3-*O*-glucosides; [2]Petunidin- and peonidin 3-*O*-(6-*O*-acetyl) glucosides; [3]Malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides; [**]Different letters in the same row indicate significant differences in the content of the compounds measured among the different vintages according to Fischer's least significant difference test; [***]Values in brackets indicate standard deviations.

Cyanidin 3-*O*-glucosides (CyGluc) in wines from the 2010 vintage had significantly higher concentrations than those from the 2008 and 2009. Petunidin 3-*O*-(6-acetyl) glucosides were significantly higher in wines from the 2008 vintage. Peonidin 3-*O*-(6-acetyl) glucoside concentrations were significantly higher in the 2008 vintage but not different from the 2010 vintage. Malvidin 3-*O*-(6-*p*-coumaryol) glucosides in wines were not significantly different in wines from the three vintages.

5.3.4.5 Analysis of variance for wine phenolic compound concentration means for treatment (row orientation) main effects

Mean concentrations for wine phenolic compounds with a significant treatment (row orientation) main effect for grapes harvested at *ca*. 24°Brix are listed in Table 5.13. Among the individual chemical compositional variables quantified, a number of phenolic compound concentration differences were recorded.

Flavan-3-ols

Wines from the NW-SE row orientation treatments were significantly lower in total flavonol and (+)-catechin concentrations. Epigallocatechin 3-*O*-gallate concentrations were significantly higher in wines from the EW row orientation treatments. Epicatechin (-) concentrations did not differ significantly from one another among the treatments.

Phenolic acids

Caffeic acid concentrations were significantly higher in wines from the NE-SW row orientation treatments, followed in a decreasing order by those from the NS and EW with significantly lower concentrations found in wines from the NW-SE row orientation treatments. Ferulic acid concentrations were significantly higher in wines from the EW row orientation treatments, followed in a decreasing order by those from the NW-SE and NS with significantly lower concentrations found in wines from the NE-SW row orientation treatments. Wines from the NW-SE row orientation treatments were significantly higher in *p*-coumaric acid with significantly lower concentrations in wines from the NE-SW and EW row orientation treatments. Wines from the NE-SW and EW row orientation treatments were however not significantly different in *p*-coumaric acid concentrations.

Table 5.13 Wine phenolic compound concentration means for treatment (row orientation) main effects (grapes harvested at *ca.* 24 °Brix).*

Phenolic compounds	Treatment (row orientation)				*p*-value
	EW[1]	NE-SW[1]	NS[1]	NW-SE[1]	
Total flavan-3-ols	15.669a** (±1.362)***	14.504b (±1.168)	15.760a (±1.139)	**12.905c (±0.950)**	0.0005
(+)-Catechin	6.331ba (±0.897)	5.945b (±0.560)	6.566a (±0.644)	**5.140c (±0.463)**	0.0003
(-)-Epicatechin	7.009ba (±0.937)	7.051ba (±0.710)	7.794a(±0.846)	6.363b (±0.795)	0.0309
EGCG[2]	**2.328a (±0.301)**	1.507b (±0.265)	1.399b (±0.353)	1.400b (±0.318)	0.0002
Total phenolic acids	73.031c (±3.636)	77.747bc (±4.235)	83.624a (±4.024)	81.597ba (±6.171)	0.0036
Gallic acid	4.287a (±0.551)	1.512b (±0.214)	4.632a (±0.435)	1.551b (±0.329)	<.0001
Caffeic acid	**31.855c (±3.212)**	**41.338a (±2.774)**	**35.359b (±2.092)**	**26.689d (±1.825)**	<.0001
p Coumaric acid	27.858c (±3.610)	31.326c (±4.287)	37.957b (±3.828)	**47.732a (±5.319)**	<.0001
Ferulic acid	**7.687a (±0.757)**	**2.168d (±0.341)**	**4.501c (±0.318)**	**4.866b (±0.510)**	<.0001
Total flavonols	21.015a (±1.199)	13.051b (±0.761)	22.410a (±2.118)	13.913b (±1.367)	<.0001
Rutin	1.342a (±0.375)	1.401a (±0.240)	1.173a (±0.398)	**0.758b (±0.223)**	0.0006
Quercetin	1.966c (±0.516)	2.873b (±0.188)	**4.088a (±0.531)**	2.491cb (±0.393)	<.0001
Kaempferol	**0.976b (±0.152)**	**1.239a (±0.163)**	**0.854c (±0.078)**	**0.560d (±0.097)**	<.0001
Quercitrin	7.772a (±1.245)	7.315b (±0.940)	6.807a (±1.833)	8.427b (±1.155)	<.0001
Total anthocyanins	186.767ba (±25.984)	191.745ba (±24.870)	176.005b (±28.213)	205.350a (±23.887)	0.0463
PetGluc[3]	5.277ba (±1.151)	5.325a (±0.816)	4.615b (±0.827)	5.806a (±0.682)	0.0135
DelGluc[3]	1.664b (±0.296)	1.673b (±0.367)	**1.974a (±0.278)**	1.381b (±0.128)	0.0078
PetGlucAc[4]	3.176b (±0.855)	3.353b (±0.331)	2.981b (±0.616)	**4.351a (±0.498)**	0.0005
PetGlucCoum[5]	1.803b (±0.274)	2.050b (±0.322)	2.261b (±0.737)	**5.242a (±0.800)**	<.0001
MalGlucCoum[5]	33.170a (±7.112)	30.946ba (±4.608)	28.024a (±4.911)	33.010a (±4.750)	0.0088

*Means given for compounds with significant treatment (row orientation) main effects as indicated in Table 5.8; [1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Epigallocatechin 3-*O*-gallate; [3]Petunidin- and delphinidin 3-*O*-glucosides; [4]Petunidin 3-*O*-(6-*O*-acetyl) glucosides; [5]Petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides; **Different letters in the same row indicate significant differences in the content of the compounds measured among the different treatments according to Fisher's least significant difference test; ***Values in brackets indicate standard deviations.

Flavonols

Quercetin concentrations were significantly higher in wines from the NS row orientation treatments with significantly lower concentrations in wines from the EW row orientation treatments. Wines from the EW and NW-SE row orientation treatments were not significantly different from each other. Wines from the NW-SE row orientation treatments were significantly higher in rutin concentrations.

Kaempferol concentrations were significantly higher in wines from the NE-SW row orientation treatments, followed in a decreasing order by those from the EW and NS with significantly lower concentrations found in wines from the NE-SW row orientation treatments.

Anthocyanins

Wines from the NS row orientation treatments proved significantly higher in delphinidin 3-*O*-glucoside concentrations with significantly lower concentrations in wines from the NW-SE row orientation treatments. Wines from the EW, NE-SW and NW-SE row orientation treatments were not significantly different from one another in delphinidin 3-*O*-glucoside concentrations.

Petunidin 3-*O*-(6-*O*-acetyl) glucosides and petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside concentrations showed significantly higher concentrations in wines of the NW-SE row orientation treatments.

Significantly lower concentrations were found in wines from the NS row orientation treatments for petunidin 3-*O*-(6-*O*-acetyl) glucosides and significantly lower concentrations in wines from the EW row orientation treatments for petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside. However, significant differences among the EW, NS and NE-SW row orientations were not found.

5.3.4.6 Multiple factor analysis of phenolic compounds for Syrah grapes and wine

Figure 5.5 depicts correlations between phenolic compounds of Syrah grape samples and Syrah wine samples.

Only two grape and wine variables that correlated were identified by MFA, *i.e*. isoquercetin (R = 0.579) and petunidin 3-*O*-glucoside (R = 0.510).

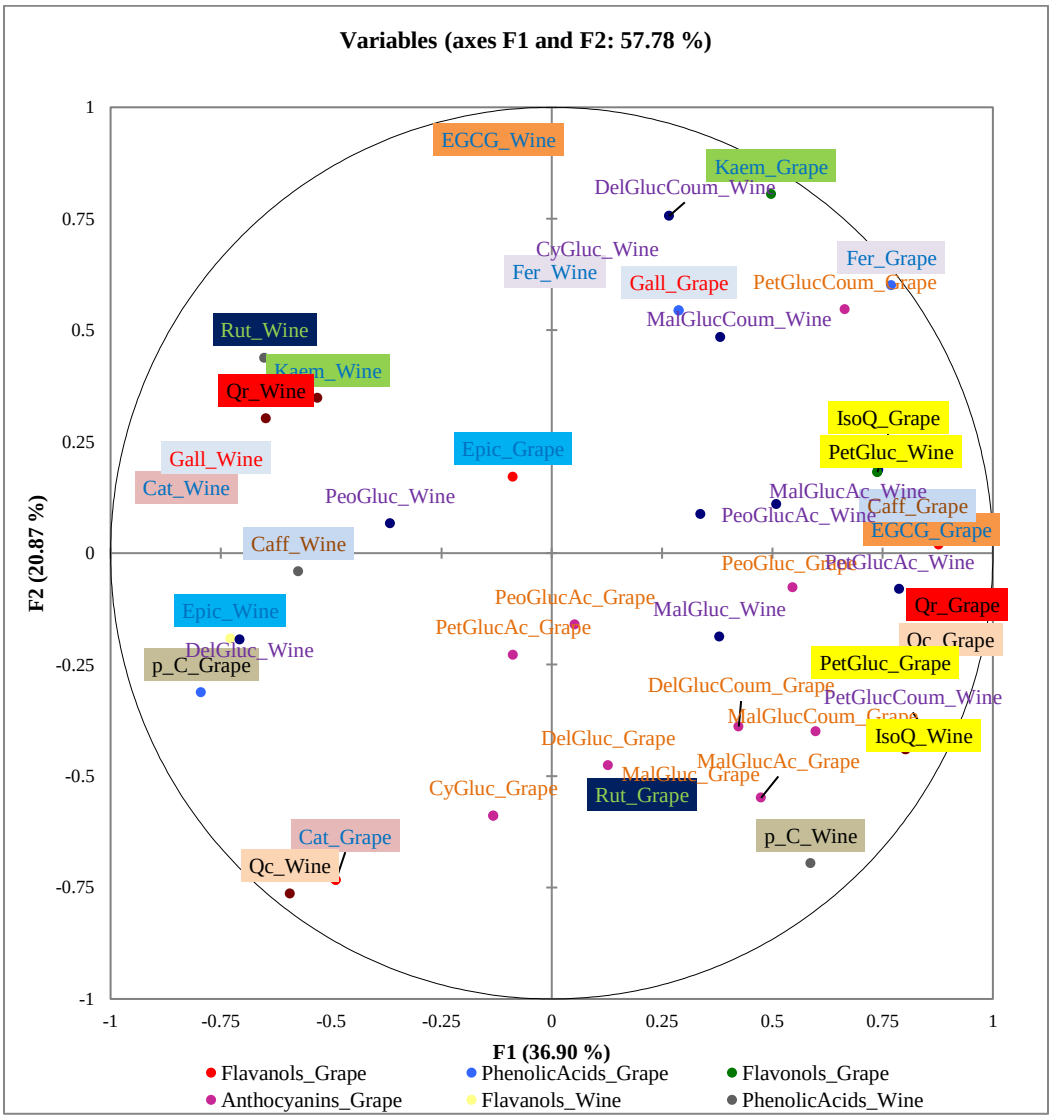

Figure 5.5 MFA variable correlations map illustrating the association of phenolic compound composition of Syrah grapes and wine. Grapes harvested at ripeness levels of *ca.* 24°Brix from NS, EW, NE-SW and NW-SE row orientation treatments.

5.3.4.7 RV (vector correlation) coefficient analysis of phenolic compounds in Syrah grape and wine samples

Values closest to 1 indicate a good correlation between the measured variables (Table 5.14).

Table 5.14 RV Coefficients of the relationship between the tables of component groups of Syrah grapes and wine. Grapes harvested at ripeness levels of *ca.* 24°Brix from NS, EW, NE-SW and NW-SE row orientation treatments.

Phenolic compound classes	Flavan-3-ols, wine	Phenolic acids, wine	Flavonols, wine	Anthocyanins, wine
Flavan-3-ols, grapes	**0.556**	0.318	0.557	0.708
Phenolic acids, grapes	0.510	**0.514**	0.585	0.468
Flavonols, grapes	0.716	0.633	**0.777**	0.593
Anthocyanins, grapes	0.482	0.420	0.472	0.429

The highest correlation between the grape and wine groups of phenolic compounds was flavonols at R = 0.777. The same group of phenolic compounds were also correlated in wines and grapes harvested at *ca.* 22°Brix (R= 0.768). Flavan-3-ols had the second highest correlation (R = 0.556) between grape and wine phenolic compound groups.

The lowest correlation between grape and wine samples was anthocyanins at R = 0.429. The same trend was observed for grape and wine variables where anthocyanins in grapes harvested at *ca.* 22°Brix had the lowest correlation (R = 0.499).

5.3.5 Wines from grapes harvested at *ca.* 26°Brix

Titratable acidity and pH values were not significantly different among wines from the four row orientation treatments at *ca.* 26°Brix ripeness levels (Table 5.15). There were also no significant differences among the four row orientations for pH measured in wines from grapes harvested at *ca.* 24°Brix ripeness levels. However, for grapes harvested at *ca.* 22ºBrix ripeness level, wines from the NS and NW-SE row orientations had significantly higher pH values, compared to wines from the EW and NE-SW row orientation treatments.

Table 5.15 Total acidity (TA) and pH of experimental Syrah wines as a function of row orientation from Syrah grapes harvested at *ca.* 26°Brix. Data represents wine from grapes collected over three consecutive vintages.

Measured parameters	Row orientation treatments				*p*-value
	[1]EW [2](23.3-28.9°B)	[1]NE-SW [2](23.5-29.2°B)	[1]NS [2](25.9-29.9°B)	[1]NW-SE [2](24.5-28.3°B)	
pH	3.982a (± 0.0949)	4.356a (± 0.0738)	4.073a (± 0.0488)	4.255a (± 0.0574)	0.2011
Total acidity	5.417a (± 0.5599)	4.982a (± 0.6096)	5.138a (± 0.4169)	4.946a (± 0.8892)	0.3776

[1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Minimum and maximum total soluble solids (°Brix) for grape must before inoculation.

5.3.5.1 Analysis of variance (2-factor analysis) of wine phenolic data for treatment and vintage

The *p*-values for the 2-factor ANOVA including row orientation (treatment) as main plot factor and vintage as subplot factor for wines from grapes harvested at *ca*. 26°Brix are listed in Table 5.16.

Tables of means to follow are based on these results and are presented for compounds indicated to be significantly affected by a specific interaction or main effect. Row orientation (treatment) by vintage interaction is not significant for any compounds.

Table 5.16 Anova *p*-values for treatment and vintage main effects and interaction for experimental Syrah wines (grapes harvested at *ca.* 26°Brix).

Phenolic compounds	Main effects		Interaction
	Treatment	Vintage	Treatment x vintage
Total flavan-3-ols	**<.0001**	0.0727	0.3211
(+)-Catechin	**0.0131**	**0.0189**	0.1253
(-)-Epicatechin	**0.0256**	0.1868	0.5013
Epigallocatechin 3-*O*-gallate	**<.0001**	0.465	0.7171
Total phenolic acids	**<.0001**	**0.0302**	0.1604
Gallic acid	**<.0001**	0.0548	0.2177
Caffeic acid	**<.0001**	0.1146	0.5958
p Coumaric acid	**0.005**	**0.0183**	0.0632
Ferulic acid	**0.0001**	0.4296	0.0961
Total flavonols	**<.0001**	0.619	0.1754
Rutin	**0.0003**	0.053	0.7234
Isoquercetin	**<.0001**	0.1517	0.4824
Quercetin	**<.0001**	0.0927	0.1547
Kaempferol	**<.0001**	0.2992	0.5678
Quercitrin	**0.0004**	0.4714	0.3427
Total anthocyanins	**0.0007**	0.2735	0.3651
CyGluc[1]	**<.0001**	0.6798	0.0732
PetGluc[1]	**0.0106**	0.3889	0.3085
PeoGluc[1]	0.0583	**0.0052**	0.0757
MalGluc[1]	**0.0003**	0.1221	0.1132
DelGluc[1]	**0.0004**	0.4321	0.7581
PetGlucAc[2]	**<.0001**	0.8478	0.3372
PeoGlucAc[2]	**<.0001**	0.8542	0.7092
MalGlucAc[2]	**0.0201**	0.8131	0.8154
DelGlucCoum[3]	**<.0001**	0.9651	0.9876
PetGlucCoum[3]	**0.0134**	0.9752	0.8617
MalGlucCoum[3]	**0.0003**	0.3291	0.2739

[*] *p*-values in bold indicate significant effects; [1]Cyanidin-, petunidin-, peonidin-, malvidin- and delphinidin 3-*O*-glucosides; [2]Petunidin-, peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides; [3]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides.

5.3.5.2 Principal component analysis for wines from grapes harvested at *ca.* 26°Brix

The PCA biplot (Fig. 5.6) of the first and third principal components (PC1/F1 and PC3/F3) illustrating the association of phenolic compounds of experimental Syrah wines from grapes harvested during 2008, 2009 and 2010 at a ripeness level of *ca.* 26°Brix with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations, explained 53.46% of the variation in the data.

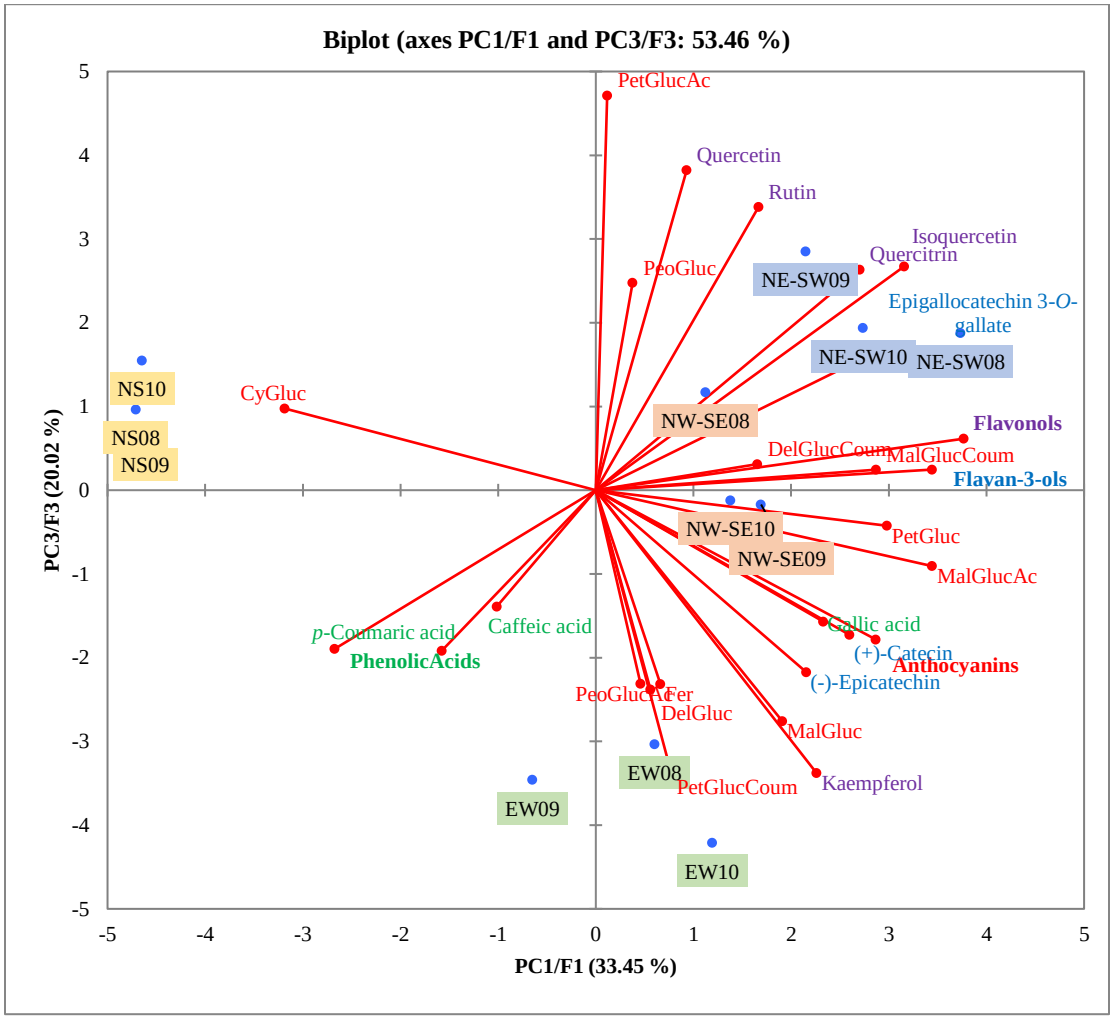

Figure 5.6 PCA biplot illustrating the association of phenolic compound composition of experimental Syrah wines with treatment, *i.e.* NS, EW, NE-SW and NW-SE row orientations. Grapes were harvested during 2008, 2009 and 2010 at ripeness levels of *ca.* 26°Brix). Abbreviations are defined in Table 5.1.

Principal component 1 shows the highest or greatest variation in the biplot. Variables included in the PCA were limited to those with a significant main effect (squared cosine values ≥ 0.5) or significant effect in the ANOVAs (data not shown). Figure 5.6 indicates that the main source of variation is row orientation, with PC1 mainly separating NW-SE, NE-SW and EW row orientation treatments from NS row orientation treatments, while PC3 separated EW row orientation treatments from NW-SE, NS and NE-SW row orientation treatments.

Phenolic compounds with the highest squared cosine values on PC1 are total flavan-3-ols, total flavonols, total anthocyanins, epigallocatechin 3-*O*-gallate, isoquercetin (quercetin 3-*O*-glucoside), cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, malvidin 3-*O*-(6-*O*-acetyl) glucoside and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside.

Quercetin and petunidin 3-*O*-(6-*O*-acetyl) glucoside have the highest squared cosine values on PC3. Principal component 3 explains an additional 20.01% of variation (data not shown) and does improve the interpretability/variability and was therefore included in the biplot.

Total phenolic acids, caffeic acid, ferulic acid, delphinidin 3-*O*-glucoside, peonidin 3-*O*-(6-*O*-acetyl) glucoside and delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside have the highest squared cosine value on PC2. If PC2 instead of PC3 is included, the percentage variation increases to 59.13%, but variability does not improve on the biplot.

5.3.5.3 Analysis of variance for wine phenolic compound concentration means for vintage main effects

Mean concentrations for wine phenolic compounds with significant vintage main effects for grapes harvested at *ca.* 26°Brix are listed in Table 5.17.

Table 5.17 Wine phenolic compound concentration means for vintage main effects (grapes harvested at *ca.* 26 °Brix).*

Phenolic compounds	Vintage			*p*-value
	2008	2009	2010	
(+)-Catechin	6.728b** (±1.086)***	7.214ba (±1.073)	7.491a (±0.773)	0.0189
Total phenolic acids	82.730b (±8.999)	87.236ba (±10.104)	87.504a (±12.021)	0.0302
p-Coumaric acid	48.190b (±3.582)	50.509ba (±5.546)	51.045a (±5.427)	0.0183
Peonidin 3-*O*-glucosides	**6.371a (±0.975)**	4.928b (±0.752)	5.321b (±1.303)	0.0052

*Means given for compounds with significant vintage main effects as indicated in Table 5.13; **Different letters in the same row indicate significant differences in the content of the compounds measured among the different vintages according to Fischer's least significant difference test; ***Values in brackets indicate standard deviations.

The tendency for certain compounds is that wines from grapes harvested in 2010 had significantly higher concentrations than those harvested in 2008. The exception is peonidin 3-*O*-glucosides for which significantly higher concentrations were observed in 2008.

5.3.5.4 Analysis of variance for wine phenolic compound concentration means for treatment (row orientation) main effects

Mean concentrations for wine phenolic compounds with a significant treatment (row orientation) main effect for grapes harvested at *ca*. 26°Brix are listed in Table 5.18. Among the individual chemical compositional variables quantified, a number of phenolic compound concentration differences were recorded.

Flavan-3-ols

Wines from the NW-SE row orientation treatments were significantly higher in total flavan-3-ol concentrations with significantly lower concentrations in wines from the EW row orientation treatments. Significantly lower concentrations of total flavan-3-ols in wines from the EW row orientation treatments were also found in grapes harvested at *ca*. 22°Brix.

Epigallocatechin 3-*O*-gallate concentrations were significantly higher in wines from the NW-SE row orientation treatments, with significantly lower concentrations in wines from the NS row orientation treatments. Wines from the NS row orientation treatments made with grapes harvested at *ca.* 22°Brix were also significantly lower in epigallocatechin 3-*O*-gallate.

Significant differences among wines from the four treatments for both total flavonol and epigallocatechin 3-*O*-gallate concentrations were found.

Table 5.18 Wine phenolic compound concentration means for treatment (row orientation) main effects (grapes harvested at *ca.* 26 °Brix).*

Phenolic compounds	Treatment (row orientation)				*p*-value
	EW[1]	NE-SW[1]	NS[1]	NW-SE[1]	
Total flavan-3-ols	**17.267c[**] (±2.000)[***]**	**19.099b (±1.475)**	**13.945d (±0.892)**	**20.592a (±1.277)**	<.0001
(+)-Catechin	7.748a (±1.410)	7.669ba (±0.565)	6.485c (±0.525)	6.908bc (±0.613)	0.0131
(-)-Epicatechin	8.080a (±1.042)	7.624ba (±1.057)	6.853b (±0.629)	8.064a (±0.838)	0.0256
EGCG[2]	**1.438c (±0.342)**	**3.805b (±0.454)**	**0.605d (±0.093)**	**5.619a (±0.543)**	<.0001
Phenolic acids	86.878b (±5.853)	**71.408c (±4.269)**	88.966b (±7.480)	**95.582a (±6.540)**	<.0001
Gallic acid	5.023a (±0.491)	4.941a (±0.452)	**3.973c (±0.431)**	4.430b (±0.666)	<.0001
Caffeic acid	26.243b (±2.796)	**16.918c (±1.549)**	27.588b (±3.137)	**36.419a (±2.568)**	<.0001
p-Coumaric acid	51.255ba (±3.933)	**45.467c (±3.725)**	53.517a (±4.835)	49.599b (±4.453)	0.005
Ferulic acid	3.636b (±0.548)	2.620c (±0.319)	2.945c (±0.497)	**4.281a (±0.438)**	0.0001
Total flavonols	14.904b (±0.855)	17.141a (±0.816)	**11.736c (±0.681)**	17.080a (±0.943)	<.0001
Rutin	0.719b (±0.140)	**1.460a (±0.232)**	0.941b (±0.295)	0.851b (±0.229)	0.0003
Isoquercetin	0.352b (±0.056)	1.416a (±0.103)	**0.228c (±0.048)**	1.346a (±0.091)	<.0001
Quercetin	**3.491d (±0.454)**	**5.828a (±0.302)**	**4.581b (±0.433)**	**3.976c (±0.627)**	<.0001
Kaempferol	4.792a (±0.556)	2.236b (±0.397)	**0.913c (±0.059)**	4.803a (±0.361)	<.0001
Quercitrin	6.269c (±0.727)	**7.659a (±0.838)**	6.013c (±0.430)	6.954b (±0.770)	0.0004
Total anthocyanins	284.700a (±18.514)	271.323a (±39.889)	**222.256b (±36.406)**	270.178a (±31.371)	0.0007
CyGluc[3]	1.316b (±0.200)	1.346b (±0.049)	**2.055a (±0.305)**	**0.906c (±0.088)**	<.0001
PetGluc[3]	7.807ba (±0.458)	8.091a (±0.577)	6.683c (±0.717)	7.096bc (±1.014)	0.0106
MalGluc[3]	137.027a (±10.485)	111.822b (±27.128)	100.762b (±18.236)	127.281a (±13.796)	0.0003
DelGluc[3]	2.129a (±0.340)	1.879ba (±0.459)	1.599b (±0.194)	**1.236c (±0.142)**	0.0004
PetGlucAc[4]	**1.730b (±0.225)**	3.634a (±0.645)	3.413a (±0.963)	3.477a (±0.768)	<.0001
PeoGlucAc[4]	11.151a (±0.921)	10.390a (±1.042)	9.489b (±0.798)	**8.091c (±1.499)**	<.0001
MalGlucAc[4]	70.028a (±5.564)	71.795a (±14.031)	**54.883b (±9.352)**	66.345a (±12.379)	0.0201
DelGlucCoum[5]	2.999cb (±0.408)	3.228b (±0.690)	2.518c (±0.381)	**6.829a (±1.355)**	<.0001
PetGlucCoum[5]	2.324a (±0.414)	1.980ba (±0.478)	1.727b (±0.276)	1.637b (±0.435)	0.0134
MalGlucCoum[5]	43.475b (±8.550)	**51.254a (±9.190)**	**33.513c (±9.236)**	41.468b (±8.365)	0.0003

*Means given for compounds with significant treatment (row orientation) main effects as indicated in Table 5.13; [1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [3]Cyanidin-, petunidin-, malvidin- and delphinidin 3-*O*-glucosides; [4]Petunidin-, peonidin- and malvidin 3-*O*-(6-*O*-acetyl) glucosides; [5]Delphinidin-, petunidin- and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides; **Different letters in the same row indicate significant differences in the content of the compounds measured among the different treatments according to Fiseher's least significant difference test; ***Values in brackets indicate standard deviations.

Phenolic acids

Wines from the NW-SE row orientations were significantly higher in total phenolic acids and significantly lower in wines from the NE-SW row orientation treatments. Caffeic- and *p*-coumaric acids were also significantly lower in wines from the NE-SW row orientation treatments. Caffeic- and ferulic acids were significantly higher in wines from the NW-SE row orientation treatments.

Flavonols

Total flavonols were significantly lower in wines from the NS row orientation treatments. Wines from the NE-SW row orientation treatments were significantly higher but not different from the NE-SW treatments.

Rutin, quercetin and quercitrin concentrations were significantly higher in wines from the NE-SW row orientation treatments with quercetin concentrations significantly lower in wines from the EW row orientation treatments.

Wines from the NE-SW row orientation treatments made with grapes harvested at *ca.* 22°Brix were also significantly higher in rutin and quercetin concentrations.

Significant differences in wines among all treatments were evident for quercetin. Kaempferol concentrations were significantly lower in wines from the NS row orientations.

Anthocyanins

Total anthocyanin concentrations were significantly lower in wines from the NS row orientation treatments. Delphinidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside and peonidin 3-*O*-(6-*O*-acetyl) glucoside concentrations were significantly lower in wines from the NW-SE treatments. Delphinidin 3-*O*-glucoside concentrations in wines from grapes harvested at *ca.* 22°Brix were also significantly lower from the NW-SE row orientation treatments.

Wines from the NS row orientation treatments were significantly lower in malvidin 3-*O*-(6-*O*-acetyl) glucoside and malvidin 3-*O*-(6-*O*-*p*-coumaryol) glucoside concentrations with delphinidin 3-*O*-(6-*O*-*p*-coumaryol) glucoside concentrations significantly higher in wines from the NW-SE row orientation treatments. Wines from the NE-SW row orientation treatments were significantly higher in malvidin 3-*O*-(6-*O*-*p*-coumaryol) glucoside concentrations.

5.3.5.5 Multiple factor analysis of phenolic compounds for Syrah grapes and wine

Figure 5.6 depicts correlations between phenolic compounds of Syrah grape samples and Syrah wine samples. Multiple factor analysis identified five grape and wine variables which were well correlated. They are caffeic acid (R = 0.941), rutin (R = 0.662), isoquercetin (R = 0.972), kaempferol (R = 0.889) and petunidin 3- *O*-glucoside (R = 0.868).

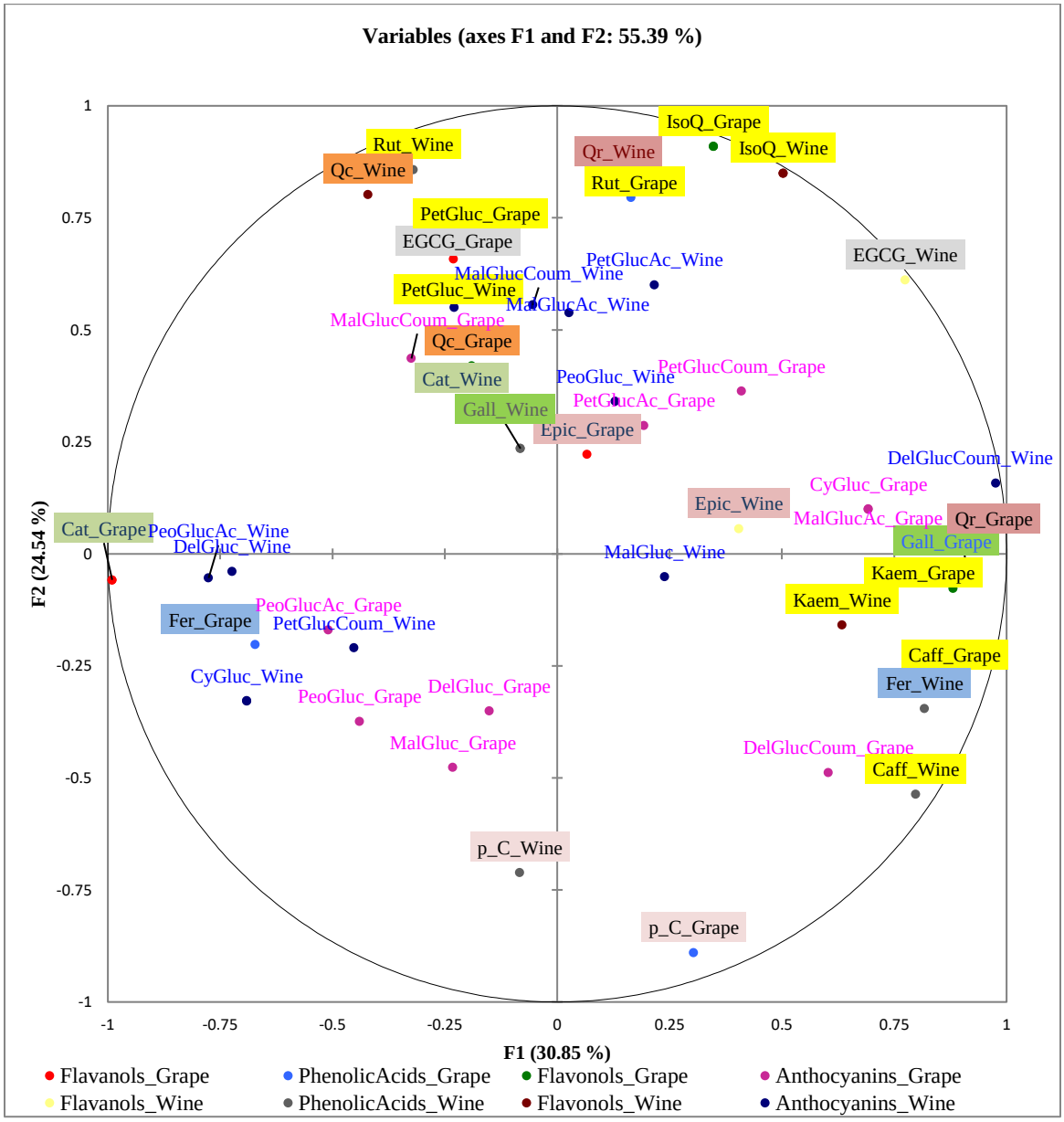

Figure 5.6 MFA variable correlations map illustrating the association of phenolic compound composition of Syrah grapes and wine. Grapes harvested at ripeness levels of *ca.* 26°Brix from NS, EW, NE-SW and NW-SE row orientation treatments.

5.3.5.6 RV coefficient (vector correlation) analysis of phenolic compounds in Syrah grape and wine samples

Coefficients of the relationship between the component groups of Syrah grapes and wine are shown in Table 5.19. Values closest to 1 indicate a good correlation between the measured variables.

The highest correlation between grape and wine groups of phenolic compounds was found for anthocyanins at R = 0.726. Phenolic acids (R = 0.723) had the second highest correlation between grape and wine phenolic compound groups. The same trend was observed for phenolic acids in grape and wine variables at *ca.* 22°Brix.

Table 5.19 RV Coefficients of the relationship between the component groups of Syrah grapes and wine. Grapes harvested at ripeness levels of *ca.* 26°Brix from NS, EW, NE-SW and NW-SE row orientation treatments.

Phenolics compound classes	Flavan-3-ols, wine	Phenolic acids, wine	Flavonols, wine	Anthocyanins, wine
Flavan-3-ols, grapes	0.269	0.494	0.482	0.457
Phenolic acids, grapes	0.459	**0.723**	0.577	0.633
Flavonols, grapes	0.509	0.655	**0.695**	0.497
Anthocyanins, grapes	0.473	0.529	0.434	**0.726**

The flavonol group of phenolics had the highest correlation between grape and wine samples at *ca.* 22°Brix (R = 0.768) and *ca.* 24°Brix (R = 0.777); however, flavonol correlation between grape and wine samples at *ca.* 26°Brix (R = 0.695) was second lowest. The lowest correlation between grape and wine samples at *ca.* 26°Brix was found for flavan-3-ols at R = 0.269.

5.4 Comparison between quantitative phenolic compound data of Syrah wines of this study and results cited in literature

The phenolic compound composition of wine depends principally on grape ripeness and type of vinification process applied (Pérez-Magariño & Gonzalez-San José, 2006; Gómez-Alonso *et al*., 2007). Table 5.20 lists the phenolic compound concentrations reported by different authors in wine from different grape cultivars and from different countries. Grape ripeness levels are listed where possible.

Table 5.20 Selected quantitative wine phenolic concentrations reported in literature.

Grape cultivar	Phenolic compounds	Concentration (mg/L)	Grape ripeness	Reference
Monastrell wines	Gallic acid	22.01	25°Brix	Bautista-Ortín *et al*.
	Caffeic acid	4.45		(2007)
	p-Coumaric acid	1.05		
	Ferulic acid	0.22		
	Quercetin	11.80		
	Kaempferol	1.00		
	(+)-Catechin	15.85		
	(-)-Epicatechin	1.80		
	Delph 3-*O*-gluc	4.35		
	Cyan 3-*O*-gluc	0.90		
	Peon 3-*O*-gluc	2.35		
	Malv 3-*O*-gluc	28.00		
	Petun 3-*O*-gluc	6.25		

Gluc = Glucose; Delph = Delpinidin; Cyan = Cyanidin; Peon = Peonidin; Malv = Malvidin; Petun = Petunidin; Tech. = Technological.

Table 5.20 Continued.

Grape cultivar	Phenolic compounds	Concentration (mg/L)	Grape ripeness	Reference
Cencibel wines	(+)-catechin	31.01	Optimum ripeness	Gómez-Alonso *et al.*
	(-)-epicatechin	12.78		(2007)
	Gallic acid	20.05		
	Kaempferol	0.48		
	Quercetin	56.85		
	Delph 3-*O*-gluc	10.87		
	Cyan 3-*O*-gluc	0.48		
	Peon 3-*O*-gluc	4.07		
	Malv 3-*O*-gluc	55.10		
	Petun 3-*O*-gluc	12.41		
	Delph 3-*O*-(6-*O*-acetyl) gluc	0.76		
	Cyan 3-*O*-(6-*O*-acetyl) gluc	0.33		
	Petun 3-*O*-(6-*O*-acetyl) gluc	0.70		
	Malv 3-*O*-(6-*O*-acetyl) gluc	3.99		
	Peon 3-*O*-(6-*O*-acetyl) gluc	0.51		
	Delph 3-*O*-(6-*O*-*p*-coumaroyl) gluc	1.27		
	Petun 3-*O*-(6-*O*-*p*-coumaroyl) gluc	1.16		
	Peon 3-*O*-(6-*O*-*p*-coumaroyl) gluc	0.96		
	Malv 3-*O*-(6-*O*-*p*-coumaroyl) gluc	7.00		
	Cyan 3-*O*-(6-*O*-*p*-coumaroyl) gluc	0.40		
Red grape juice	Gallic acid	2.01	*Tech. ripeness	Moreno-Montoro *et al.*
	Caffeic acid	0.65		(2015)
	p-Coumaric acid	0.62		
	Ferulic acid	0.25		
	(+)-Catechin	1.64		
	(-)-Epicatechin	1.93		
Red wine	Gallic acid	2.41		
	Caffeic acid	1.52		
	p-Coumaric acid	0.73		
	Ferulic acid	0.22		
	(+)-Catechin	2.30		
Pinot noir wines	Gallic acid	4.46	*Tech. maturity	Hendrickson *et al.*
	(+)-Catechin	18.33		(2016)
	(-)-Epicatechin	6.52		
	Gallic acid	4.46		
	(+)-Catechin	18.33		
	(-)-Epicatechin	6.52		

Gluc = Glucose; Delph = Delpinidin; Cyan = Cyanidin; Peon = Peonidin; Malv = Malvidin; Petun = Petunidin; Tech. = Technological.

Table 5.20 Continued.

Grape cultivar	Phenolic compounds	Concentration (mg/L)	Grape ripeness	Reference
Syrah wines	(+)-Catechin	60.00	Tech. ripeness	Heras-Roger *et al.*
	(-)-Epicatechin	39.00		(2016)
	Caffeic acid	13.50		
	Gallic acid	41.80		
	p-Coumaric acid	9.70		
	Rutin	4.35		
	Quercetin	2.80		
	Quercetin 3-*O*-gluc	6.20		
	Delph 3-*O*-gluc	10.30		
	Cyan 3-*O*-gluc	2.00		
	Peon 3-*O*-gluc	10.00		
	Malv 3-*O*-gluc	93.00		
	Petun 3-*O*-gluc	4.35		
	Cyan 3-*O*-(6-*O*-acetyl) gluc	3.60		
	Petun 3-*O*-(6-*O*-acetyl) gluc	4.30		
	Malv 3-*O*-(6-*O*-acetyl) gluc	14.15		
	Peon 3-*O*-(6-*O*-acetyl) gluc	5.60		
	Peon 3-*O*-(6-*O*-*p*-coumaroyl) gluc	7.60		
	Malv 3-*O*-(6-*O*-*p*-coumaroyl) gluc	15.10		
Syrah wines	(+)-Catechin	41.94	25°Brix	Lingua *et al.* (2016)
	(-)-Epicatechin	40.44		
	Caffeic acid	8.38		
	Gallic acid	62.85		
	Delph 3-*O*-gluc	0.70		
	Peon 3-*O*-gluc	1.58		
	Malv 3-*O*-gluc	87.41		
	Petun 3-*O*-gluc	2.45		
	Delph 3-*O*-(6-*O*-acetyl) gluc	0.15		
	Petun 3-*O*-(6-*O*-acetyl) gluc	0.65		
	Malv 3-*O*-(6-*O*-acetyl) gluc	49.74		
	Peon 3-*O*-(6-*O*-acetyl) gluc	2.05		
	Delph 3-*O*-(6-*O*-*p*-coumaroyl) gluc	1.55		
	Peon 3-*O*-(6-*O*-*p*-coumaroyl) gluc	1.18		
	Malv 3-*O*-(6-*O*-*p*-coumaroyl) gluc	8.88		
Narince wines	*p*-Coumaric acid	0.83	*ca.* 23°Brix	Bekar *et al.* (2017)
	Caffeic acid	2.09		
	Gallic acid	0.63		
	Ferulic acid	0.64		
	(+)-Catechin	13.97		
	(-)-Epicatechin	1.21		

Gluc = Glucose; Delph = Delpinidin; Cyan = Cyanidin; Peon = Peonidin; Malv = Malvidin; Petun = Petunidin; Tech. = Technological.

Table 5.20 Continued.

Grape cultivar	Phenolic compounds	Concentration (mg/L)	Grape ripeness	Reference
Sangiovese wines	Cyan 3-*O*-gluc	7.10	Tech. ripeness	Romboli *et al.* (2017)
	Malv 3-*O*-gluc	49.61		
	Peon 3-*O*-gluc	13.50		
	Petun 3-*O*-gluc	17.81		
	Delph 3-*O*-gluc	10.81		
	(+)-Catechin	39.30		

Gluc = Glucose; Delph = Delpinidin; Cyan = Cyanidin; Peon = Peonidin; Malv = Malvidin; Petun = Petunidin; Tech. = Technological.

Bautista-Ortín *et al*. (2007) reported 15.85 mg/L of (+)-catechin and 1.80 mg/L of (-)-epicatechin concentrations in Monastrell wines (Spain) grapes harvested at *ca.* 25°Brix ripeness level, whereas Gómez-Alonso *et al*. (2007) reported 31.01 mg/L of (+)-catechin and 12.78 mg/L of (-)-epicatechin concentrations in Cencibel wines (Spain) grapes harvested at optimum ripeness.

Moreno-Montoro *et al.* (2015) found 1.65 mg/L of (+)-catechin and 1.93 mg/L of (-)-epicatechin concentrations in red grape juice from grapes (grape cultivar not mentioned) harvested at optimum ripeness in Rioja, Spain. Moreno-Montoro *et al.* (2015) also reported 2.30 mg/L of (+)-catechin in red wine from grapes (grape cultivar not mentioned) harvested at technological ripeness.

Concentrations of (+)-catechin reported by the above-mentioned authors are between 1.65 and 31.01 mg/L. The same authors reported (-)-epicatechin concentrations of between 1.80 and 12.78 mg/L. Although the above results were obtained in Monastrell and Cencibel wines, the concentrations do fall within ranges reported in this study for Syrah wines from grapes harvested at *ca* 26°Brix.

Hendrickson *et al.* (2016) reported 18.33 mg/L of (+)-catechin and 6.52 mg/L of (-)-epicatechin concentrations in Pinot noir wines from grapes harvested at technological maturity from California, USA. Syrah wines from grapes harvested at technological ripeness from Spain, had 60.00 mg/L of (+)-catechin and 39.00 mg/L of (-)-epicatechin content (Heras-Roger *et al.*, 2016). Lingua *et al.* (2016) reported 41.90 mg/L of (+)-catechin and 40.00 mg/L of (-)-epicatechin in Syrah wines from grapes harvested at *ca*. 25°Brix ripeness from Argentina. Concentrations of (-)-epicatechin reported by Hendrickson *et al.* (2016) in Pinot noir wines are similar to concentrations reported in this study for (-)-epicatechin in Syrah wines from grapes at *ca.* 26°Brix (Table 5.18). Concentrations reported by Hendrickson *et al.* (2016) [(+)-catechin only], Heras-Roger *et al.* (2016) and Lingua *et al.* (2016) in Pinot noir and Syrah wines do not fall within the range reported in this study for Syrah wines.

Bekar *et al.* (2017) reported 13.97 mg/L of (+)-catechin and 1.21 mg/L (-)-epicatechin in Narince wines from Turkey whereas Romboli *et al*. (2017) found 39.30 mg/L of (+)-catechin in Sangiovese wines from grapes planted to a NE-SW direction in Italy. Flavan-3-ol concentrations reported by Bekar *et al.* (2017) and Romboli *et al*. (2017) do not fall within the concentration range reported for Syrah wines in this study.

Bautista-Ortín *et al*. (2007) reported 22.01 mg/L of gallic acid, 4.45 mg/L of caffeic acid, 1.05 mg/L of *p*-coumaric acid and 0.22 mg/L of ferulic acid in Monastrell wines.

These concentrations are not comparable to results reported in this study for Syrah wines. Caffeic- (1.52 mg/L), *p*-coumaric- (0.73 mg/L) and ferulic acid (0.22 mg/L) concentrations in red wine reported by Moreno-Montoro *et al*. (2015) are substantially lower, compared to phenolic acid concentrations obtained in this study for Syrah wines. Gallic acid (2.41 mg/L) concentrations reported by Moreno-Montoro *et al.* (2015) fall within the range of gallic acid concentrations reported in this study for Syrah wines (Table 5.13).

Hendrickson *et al.* (2016) reported comparable results to findings in this study for gallic acid concentrations (4.46 mg/L). Lower concentrations of caffeic- and *p*-coumaric acids and higher concentrations of gallic acid in Syrah wines from Spain were reported by Heras-Roger *et al.* (2016) and Lingua *et al.* (2016), compared to results reported in this study. Bekar *et al*. (2017) reported substantially lower concentrations of caffeic- (2.09 mg/L), *p*-coumaric- (0.83 mg/L), gallic- (0.63 mg/L) and ferulic (0.64 mg/L) acids in Narince wines, compared to concentrations reported for Syrah wines in this study. Concentrations reported by Bekar *et al*. (2017) were however in white wine grapes.

Bautista-Ortín *et al*. (2007) reported 1.00 mg/L of kaempferol in Monastrell wines from grapes harvested at *ca.* 26°Brix ripeness from Spain. Gómez-Alonso *et al*. (2007) reported lower concentrations of kaempferol (0.48 mg/L) in Syrah wines from grapes harvested at optimum ripeness in Spain, compared to that of Bautista-Ortín *et al*. (2007). Concentrations reported by Bautista-Ortín *et al*. (2007) and Gómez-Alonso *et al*. (2007) fall within the range of kaempferol concentrations reported in this study for Syrah wines from grapes harvested at *ca.* 24°Brix (Table 5.13).

Heras-Roger *et al.* (2016) found 4.35 mg/L of quercetin 3-*O*-rutinoside, 6.20 mg/L of quercetin 3-*O*-glucoside and 2.80 mg/L of quercetin concentrations in Syrah wines from grapes harvested at technological ripeness in Spain. Quercetin concentrations reported by Heras-Roger *et al.* (2016) are in line with concentrations reported in this study, whereas quercetin 3-*O*-glucoside and quercetin 3-*O*-rutinoside concentrations are higher, compared to those reported in this study for Syrah wines.

Monomeric anthocyanin concentrations found by Bautista-Ortín *et al*. (2007) were 4.35 mg/L of delphinidin-, 0.48 mg/L of cyanidin-, 6.25 mg/L of petunidin-, 2.35 mg/L of peonidin-, and 28.00 mg/L of malvidin 3-*O*-glucosides in Monastrell wines from grapes harvested at *ca*. 25°Brix ripeness levels in Spain. Gómez-Alonso *et al*. (2007), on the other hand, reported higher delphinidin- (10.87 mg/L), petunidin- (12.41 mg/L) and malvidin 3-*O*-glucoside (55.10 mg/L), but lower peonidin- (4.07 mg/L) concentrations in Cencibel wines from grapes harvested at optimum ripeness in Spain, compared to concentrations reported by Bautista-Ortín *et al*. (2007).

Petunidin 3-*O*-glucoside concentrations found by Bautista-Ortín *et al*. (2007) in Monastrell wines from Spain and peonidin 3-*O*-glucoside concentrations reported by Gómez-Alonso *et al*. (2007) in Cencibel wines from Spain are in line with concentrations reported in this study for Syrah wines. Heras-Roger *et al.* (2016) reported 2.0 mg/L of cyanidin-, 93.00 mg/L of malvidin-, and 4.35 mg/L of petunidin 3-*O*-glucoside concentrations in Syrah wines from Spain. Grapes were harvested at technological ripeness.

These reported concentrations are similar to results reported in this study for Syrah wines from grapes harvested at *ca.*22°Brix (petunidin- and malvidin 3-*O*-glucosides) and grapes harvested at *ca.* 26°Brix (cyanidin 3-*O*-glucoosides).

Delphinidin- (10.30 mg/L) and peonidin 3-*O*-glucose (10.00 mg/L) concentrations reported by Heras-Roger *et al.* (2016) are higher, compared to results reported in this study. Contrary to work by Heras-Roger *et al.* (2016) and results reported in this study, Lingua *et al.* (2016) reported lower concentrations of delphinidin- (0.70 mg/L), peonidin- (1.58 mg/L) and petunidin 3-*O*-glucosides (2.45 mg/L) in Syrah wines from Argentina. Malvidin 3-*O*-glucoside concentrations (87.41 mg/L) reported by Lingua *et al.* (2016) are similar to those reported in this study in Syrah wines where grapes were harvested at *ca.* 22°Brix.

Romboli *et al.* (2017) reported 7.10 mg/L of cyanidin 3-*O*-glucosides, 13.50 mg/L peonidin 3-*O*-glucosides, 17.81 mg/L petunidin 3-*O*-glucosides and 10.81 mg/L delphinidin 3-*O*-glucosides in Sangiovese wines (grapes harvested at technological ripeness) from Italy. Values reported by Romboli *et al.* (2017) are substantially higher, compared to concentrations reported in this study for Syrah wines at *ca.* 26°Brix (Table 5.15). Malvidin 3-*O*-glucoside concentrations found by Romboli *et al.* (2017) are lower, compared to concentrations reported in Syrah wines of this study (Table 5.18). Acetylated anthocyanin concentrations reported by Gómez-Alonso *et al*. (2007) in Cencibel wines from grapes harvested at optimum ripeness from Spain were substantially lower, compared to concentrations in Syrah wines from grapes harvested at *ca.* 26°Brix of this study. Heras-Roger *et al.* (2016) reported petunidin-(4.30 mg/L) and peonidin 3-*O*-(6-*O*-acetyl) glucoside (5.60 mg/L) concentrations in Syrah wines from grapes harvested at technological ripeness. This is in line with petunidin 3-*O*-(6-*O*-acetyl) glucoside concentrations reported in this study also for Syrah wines. Peonidin 3-*O*-(6-*O*-acetyl) glucoside concentrations reported in this study (Table 5.18) are slightly higher, compared to results reported by Heras-Roger *et al.* (2016). Malvidin 3-*O*-(6-*O*-acetyl) glucoside concentrations (14.15 mg/L) reported by Heras-Roger *et al.* (2016) are however lower, compared to concentrations reported in this study.

Lingua *et al.* (2016) found lower delphinidin-, peonidin- and petunidin 3-*O*-(6-*O*-acetyl) glucoside concentrations in Syrah wines from grapes harvested at *ca.* 25°Brix ripeness level, compared to results reported in Syrah wines from grapes harvested at similar ripeness level in this study. Malvidin 3-*O*-(6-*O*-acetyl) glucoside concentrations reported by Lingua *et al.* (2016) are slightly higher, compared to concentrations reported in this study for Syrah wines. Delphinidin- (1.27 mg/L) and petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside (1.16 mg/L) concentrations in wines from Cencibel grapes harvested at optimum ripeness found by Gómez-Alonso *et al*. (2007) are similar to those reported in this study for Syrah wines from grapes harvested at *ca.* 22°Brix. Lingua *et al.* (2016) reported 1.55 mg/L for delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides in Syrah wines from grapes harvested at *ca.* 25°Brix ripeness level. Concentrations reported by Lingua *et al.* (2016) fall within the range of concentrations reported in this study in wines from grapes harvested at *ca.* 22°Brix. Malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside concentrations (7.00 mg/L) found by Gómez-Alonso *et al*. (2007) are substantially lower,

compared to malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside concentrations in this study. Heras-Roger *et al.* (2016) reported 7.60 mg/L for peonidin - and 15.10 mg/L for malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside concentrations in Syrah wines from grapes harvested at technological ripeness, whereas Lingua *et al.* (2016) reported 1.18 mg/L for peonidin- and 8.88 mg/L for malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides in Syrah wines from grapes harvested at *ca.* 25°Brix ripeness level. Malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside concentrations reported by Heras-Roger *et al.* (2016) and Lingua *et al.* (2016) were substantially lower, compared to concentrations reported in this study. Peonidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides were not reported in this study due to detection limits of the analytical technique used. Concentration differences of anthocyanins, flavonols, flavan-3-ols and phenolic acids found in the literature cited as well as results of this study could be attributed to differences in vitcultural practices and vinification processes as well as quantification method used.

5.5 Analysis of variance of Syrah wine sensory data

Wine sensory data was analysed using ANOVA to determine differences among wines from grapes subjected to four different treatments (row orientations) and harvested at three different ripeness levels (Hunter & Volschenk, 2017).

5.5.1 Syrah wines from grapes harvested at *ca.* 22°Brix

Wines from grapes harvested at *ca*. 22°Brix, subjected to ANOVA, of four different treatments (row orientations/microclimate) showed significant differences among sensory attributes (Table 5.21).

Table 5.21 Average percentage scores of sensory attributes for Syrah wines as a function of row orientation and grape ripeness levels for grapes harvested at *ca.* 22°Brix. Data represent wine from grapes collected over three consecutive vintages.

Sensory attributes	Row orientation (treatment)				[2]LSD (p = 0.05)
	[1]EW	[1]NE-SW	[1]NS	[1]NW-SE	
Colour Intensity	34.32c±2.63[2]	49.29a±2.32	45.67b±2.56	48.59a±1.89	6.54
Overall Aroma Intensity	44.09c±1.65	49.94a±1.16	48.92a±1.07	49.97a±0.85	3.35
Fruity Intensity	37.80d±1.51	42.68c±1.12	47.41a±0.98	43.11b±1.03	2.78
Spice Aroma Intensity	30.37c±1.73	32.87b±1.16	34.67a±1.11	34.08a±1.33	2.49
Jammy Aroma Intensity	29.35b±1.61	32.47a±1.32	32.41a±1.40	32.63a±1.29	3.11
Concentrate Intensity	28.41c±1.46	33.76a±1.36	32.54b±1.15	32.85b±1.24	3.05
Alcohol Intensity	39.53b±1.24	44.09a±0.93	43.70a±0.96	44.29a±0.94	2.75
Tannin Intensity	35.84d±1.39	41.22b±1.01	40.85c±0.79	42.60a±1.18	2.85
Acidity Intensity	43.78c±0.96	46.19ba±0.66	47.32a±0.72	47.18a±0.68	2.34
Body Mouthfeel	33.13d±1.71	42.07b±1.39	39.83c±1.12	43.39a±1.30	3.64
Finish Persistence	35.45c±1.65	43.17a±1.35	41.68b±1.04	43.77a±1.07	3.51
Overall Quality	34.64c±1.94	44.40a±1.49	41.95b±1.16	44.67a±1.21	4.00

[1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Standard deviation. Different letters in the same row indicate significant differences in the sensory attribute among the different treatments according to the least significance difference test (p = 0.05).

Colour intensity, fruity intensity and tannin intensity were significantly different among wines from grapes from all four row orientations. Significant differences in percentage scores were found between wines from NS and EW row orientation treatments and wines from the EW and NE-SW treatments for all the measured sensory attributes. Wines from the NE-SW and the NW-SE row orientation treatments were only significantly different for colour intensity, fruity intensity, spice aroma intensity, concentrate intensity, and tannin intensity.

Overall quality was significantly different between wines from the NS treatment and those from the EW, NE-SW and NW-SE row orientation treatments. Wines from the NE-SW and NW-SE row orientation treatments were not significantly different in terms of overall quality. Wines from the EW row orientation treatment scored lowest in all attributes, including lowest overall quality.

Wines from the NS row orientation treatment were lower in colour intensity, overall aroma intensity, jammy intensity, concentrate intensity, alcohol intensity, tannin intensity, body mouthfeel, finish persistence and overall quality, compared to wines from the NE-SW and NW-SE row orientation treatments. Wines from the NW-SE and NE-SW row orientation treatments seemed generally higher in all measured sensory attributes, compared to wines from the EW row orientation treatment. Fruity intensity, spice aroma intensity and acidity intensity seemed highest in wines from the NS row orientation treatment.

5.5.2 Radar plot of Syrah wine sensory data

Radar plots (Figs. 5.7-5.9) illustrate the relative percentages of the wine sensory attributes scored by the tasters. Radar plots indicate how the tasters ranked each attribute in the descriptive analysis of the samples. These analyses were performed on the average sensory scores of the wines over four vintages.

Wines from the NE-SW and NW-SE row orientation treatments proved more intense in body mouthfeel, finish persistence, overall quality, colour intensity, overall aroma intensity and fruity intensity, compared to wines from the NS and the EW row orientation treatments (Fig. 5.7).

Wines from the NS row orientation treatment proved higher in body mouthfeel, finish persistence, overall quality, colour intensity and overall aroma intensity, compared to wines from the EW row orientation treatment.

Overall quality rated highest in wines from the NE-SW and the NW-SE treatments, followed by wines from the NS row orientation treatments.

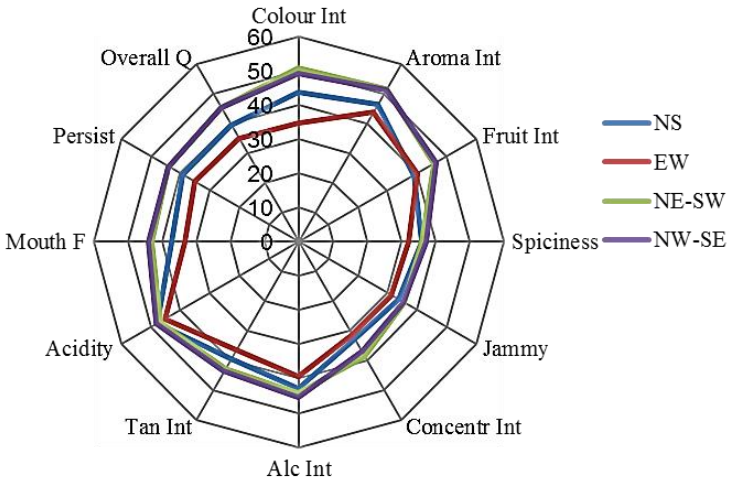

Figure 5.7 Radar plot of descriptive sensory analysis scores (%) of experimental Syrah wines from grapes harvested at *ca*. 22°Brix from four different row orientation treatments. NS = North-South; EW = East-West; NE-SW = Northeast-Southwest; NW-SE = Northwest-Southeast. Abbreviations used in radar plot: Colour Int = Colour intensity; Aroma Int = Overall aroma intensity; Fruit Int = Fruity intensity; Spiciness = Spice aroma intensity; Jammy = Jammy aroma intensity; Concentr Int = Concentrate intensity; Alc Int = Alcohol intensity; Tan Int = Tannin intensity; Acidity = Acidity intensity; Mouth F = Body mouthfeel; Persist = Finish persistence; Overall Q = Overall quality.

5.5.3 Syrah wines from grapes harvested at *ca*. 24°Brix

Analysis of variance showed that wines from grapes harvested at *ca*. 24°Brix ripeness from four different row orientations (treatments/microclimate) were significantly different based on the measured sensory attributes (Table 5.22).

Wines from the EW and the NS row orientation treatments showed significant differences between all measured sensory attributes. Significant differences between all measured sensory attributes were also recorded in wines from grapes harvested at *ca.* 22°Brix ripeness level, from the EW and NS row orientation treatments.

Wines from the NE-SW and NW-SE row orientation treatments were not significantly different in sensory attribute scores, except for spice aroma intensity, jammy aroma intensity, and tannin intensity. Significant differences between wines from the EW and the NE-SW row orientations treatments were also evident for the measured sensory attributes.

Highest scores for colour intensity, concentrate intensity, body mouthfeel, finish persistence and overall quality were found for wines from the NE-SW and the NW-SE row orientation treatments. Lowest scores in measured sensory attributes were found for wines from the EW row orientation treatment.

Table 5.22 Average percentage scores of sensory attributes for experimental Syrah wines as a function of row orientation and grape ripeness levels for grapes harvested at *ca.* 24°Brix. Data represent wine from grapes collected over three consecutive vintages.

Sensory attributes	Row orientation (treatment)				[2]LSD (p = 0.05)
	[1]EW	[1]NE-SW	[1]NS	[1]NW-SE	
Colour Intensity	43.88c±2.84[2]	59.00a±1.81	53.76b±2.26	59.34a±1.87	6.78
Overall Aroma Intensity	47.67b±1.46	52.79a±1.12	52.66a±1.10	52.85a±1.21	3.28
Fruity Intensity	41.80c±1.38	44.86a±1.15	42.80b±1.11	44.42a±1.17	3.44
Spice Aroma Intensity	31.59c±1.41	34.12b±1.03	35.93a±1.29	35.06a±1.19	2.79
Jammy Aroma Intensity	28.90c±1.42	32.79a±1.34	31.98b±1.44	31.66b±1.46	2.80
Concentrate Intensity	31.72c±1.31	37.13a±1.83	34.92b±1.51	36.80a±1.31	3.01
Alcohol Intensity	44.15b±1.03	47.96a±0.92	47.72a±0.91	47.56a±0.87	2.30
Tannin Intensity	37.97c±1.25	42.60b±1.10	42.57b±1.14	43.91a±1.14	2.87
Acidity Intensity	41.53c±0.96	45.88ba±0.66	46.28a±0.72	45.52b±0.68	1.74
Body Mouthfeel	38.61c±1.71	47.14a±1.39	46.41b±1.12	47.95a±130	4.45
Finish Persistence	41.12c±1.65	49.04a±1.35	48.71b±1.04	48.93a±1.07	4.22
Overall Quality	40.18c±1.94	48.48a±1.49	46.43b±1.16	48.64a±1.21	4.33

[1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Standard deviation. Different letters in the same row indicate significant differences in the sensory attribute among the different treatments according to the least significance difference test (p = 0.05).

Lowest scores for all measured attributes were also found in wines from grapes harvested at *ca.* 22°Brix ripeness from the EW row orientation treatment. Wines from the NE-SW and the NW-SE row orientation treatments scored highest in colour intensity, overall aroma intensity, fruity intensity, concentrate intensity, tannin intensity, body mouthfeel, finish persistence, and overall quality.

Wines from grapes harvested at *ca.* 22°Brix ripeness from the NW-SE row orientation treatments also scored highest in overall quality.

5.5.4 Radar plot of Syrah wine sensory data

Wines from the NW-SE and the NE-SW row orientation treatments, showed higher scores in colour intensity and overall quality, compared to wines from the NS and the EW row orientation treatments (Fig. 5.8).

Wines from the EW row orientation treatments proved lower in tannin intensity, acidity intensity, body mouthfeel, finish persistence, overall quality, colour intensity and overall aroma intensity, compared to wines from the NS, NE-SW and NW-SE row orientation treatments.

Fruity aroma intensity, spice aroma intensity, jammy aroma intensity, concentrate intensity, and alcohol intensity scored similar in wines from the four treatments.

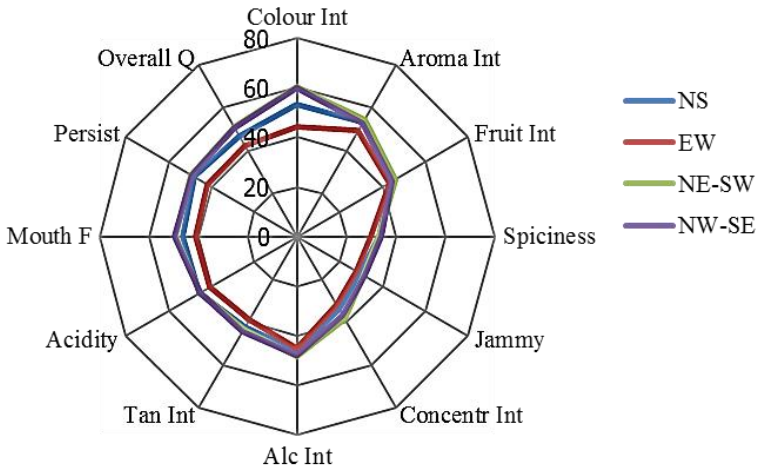

Figure 5.8 Radar plot of descriptive sensory analysis scores (%) of experimental Syrah wines from grapes harvested at *ca*. 24°Brix from four different row orientation treatments. NS = North-South; EW = East-West; NE-SW = Northeast-Southwest; NW-SE = Northwest-Southeast. Abbreviations used in radar plot: Colour Int = Colour intensity; Aroma Int = Overall aroma intensity; Fruit Int = Fruity intensity; Spiciness = Spice aroma intensity; Jammy = Jammy aroma intensity; Concentr Int = Concentrate intensity; Alc Int = Alcohol intensity; Tan Int = Tannin intensity; Acidity = Acidity intensity; Mouth F = Body mouthfeel; Persist = Finish persistence; Overall Q = Overall quality.

5.5.5 Syrah wines from grapes harvested at *ca*. 26°Brix

Wines from grapes harvested at *ca*. 26°Brix from four different row orientations (treatments/microclimates) showed significant differences among sensory attribute scores as per ANOVA (Table 5.23).

Table 5.23 Average percentage scores of sensory attributes for experimental Syrah wines as a function of row orientation and grape ripeness levels for grapes harvested at *ca.* 26°Brix. Data represent wine from grapes collected over three consecutive vintages.

Sensory attributes	Row orientation (treatment)				[2]LSD (p = 0.05)
	[1]EW	[1]NE-SW	[1]NS	[1]NW-SE	
Colour Intensity	53.60c±2.31[2]	69.49a±1.51	63.59b±1.61	69.45a±1.58	5.90
Overall Aroma Intensity	48.75c±1.12	56.94a±0.83	53.39b±0.94	55.66ba±0.99	3.19
Fruity Intensity	40.06d±1.52	46.27a±1.05	42.51c±1.21	44.50b±1.47	3.35
Spice Aroma Intensity	32.29c±1.24	37.76a±1.38	38.07a±1.49	36.64b±1.13	3.04
Jammy Aroma Intensity	34.24c±1.07	38.64a±1.05	36.46b±1.11	36.72b±1.49	3.07
Concentrate Intensity	35.03d±1.46	41.41b±1.25	38.65c±1.27	43.28a±1.76	3.44
Alcohol Intensity	49.70c±1.14	53.94ba±0.81	53.00b±0.81	54.26a±1.04	2.81
Tannin Intensity	41.69c±1.41	46.63a±0.86	45.48b±1.05	46.90a±1.04	3.45
Acidity Intensity	48.15b±0.83	49.66a±0.55	50.77a±0.67	50.05a±0.82	1.90
Body Mouthfeel	43.21d±1.64	53.29a±0.95	50.06c±1.56	51.96b±1.10	4.34
Finish Persistence	44.90c±1.52	52.67a±1.03	49.99b±1.07	53.59a±0.81	3.68
Overall Quality	41.63c±1.58	51.58a±0.85	48.08b±1.07	50.97a±0.97	3.76

[1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast; [2]Standard deviation. Different letters in the same row indicate significant differences in the sensory attribute among the different treatments according to the least significance difference test (p = 0.05).

Wines from the EW and the NS row orientation treatments showed significant differences in scores among the measured sensory attributes. Significant differences in measured sensory attribute scores were also found for wines from grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix ripeness from the EW and the NS row orientation treatments. Lowest scores for all measured sensory attributes were recorded in wines from the EW row orientation treatments. Lowest scores for all measured attributes were also found for wines from grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix ripeness from EW row orientation treatments. Significant differences in sensory attributes were found between wines from the NE-SW and the NW-SE row orientation treatments for fruity intensity, spice aroma intensity, jammy aroma intensity, concentrate intensity, body mouthfeel, finish persistence, and overall quality. Wines from the EW and the NE-SW treatments showed significant differences among all measured sensory attributes, except for acidity intensity. Significant differences were evident between wines from the EW and the NW-SE row orientation treatments for all measured sensory attributes. Wines from the NW-SE row orientation treatments were highest in concentrate intensity, alcohol intensity, tannin intensity, finish persistence, and overall quality, whereas wines from the NE-SW row orientation treatments proved highest in colour intensity (same as for wines from grapes harvested at *ca.* 22°Brix), overall aroma intensity, fruity intensity (same as for wines from grapes harvested at *ca.* 22°Brix), jammy aroma intensity, body mouthfeel, and overall quality.

5.5.6 Radar plot of Syrah wine sensory data

Wines from grapes harvested at *ca.* 26°Brix from the NE-SW and NW-SE row orientation treatments proved higher in all attributes, followed by those of the NS row orientation treatment, whereas the wines from the EW row orientation treatment scored lowest (Fig. 5.9). Overall quality scored highest in wines from the NE-SW row orientation treatments.

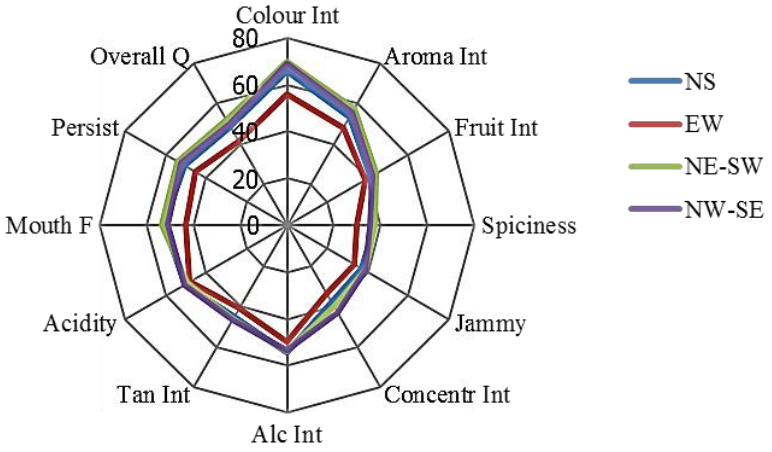

Figure 5.9 Radar plot of descriptive sensory analysis scores (%) of experimental Syrah wines from grapes harvested at *ca.* 26°Brix from four different row orientation treatments. NS = North-South; EW = East-West; NE-SW = Northeast-Southwest; NW-SE = Northwest-Southeast. Abbreviations used in radar plot: Colour Int = Colour intensity; Aroma Int = Overall aroma intensity; Fruit Int = Fruity intensity; Spiciness = Spice aroma intensity; Jammy = Jammy aroma intensity; Concentr Int = Concentrate intensity; Alc Int = Alcohol intensity; Tan Int = Tannin intensity; Acidity = Acidity intensity; Mouth F = Body mouthfeel; Persist = Finish persistence; Overall Q = Overall quality.

5.6 Principal component analysis of Syrah wine sensory data

Vector diagrams (Figs 5.10-5.12) show the loadings of the wine sensory attributes scored by the tasters indicating association and grouping of wines from the NS, EW, NE-SW and NW-SE row orientation treatments. Clustering of vectors indicate that certain variables are associated with one another, whereas vectors at right angles to each other are not. A PCA was performed using each sensory attribute in relation to the total content of the measured sensory attributes.

5.6.1 PCA of Syrah wines from grapes harvested at *ca.* 22°Brix

PCA yielded two principal components with Eigenvalues higher than two, explaining 98.77% of the total variance in the data (Fig. 5.10). The PC1 and PC2 explained 90.38% and 8.39% of the variance, respectively.

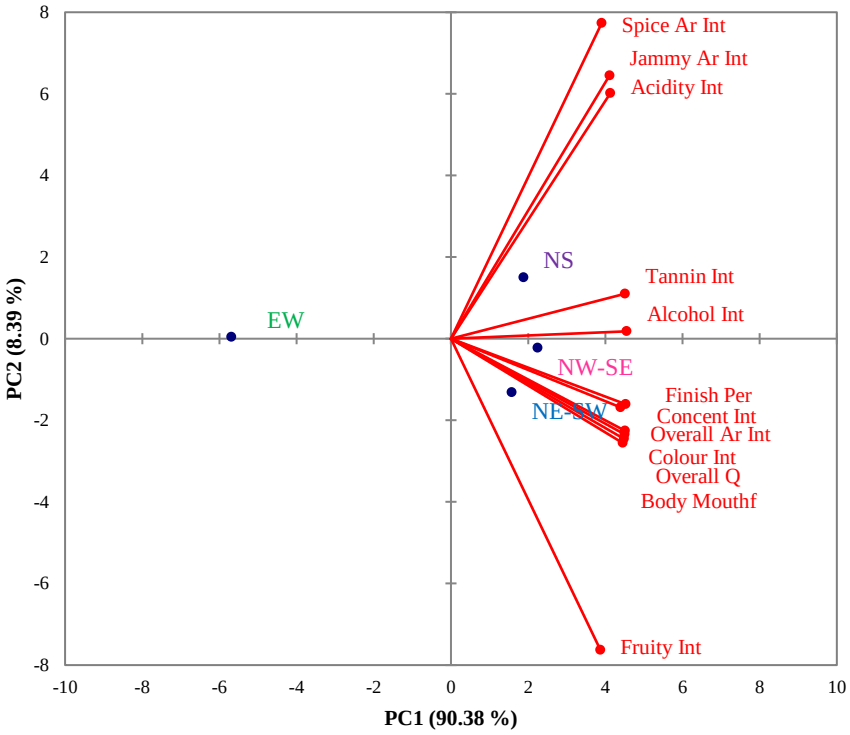

Figure 5.10 Vector diagram (PC biplot) of wine sample average scores and loadings of 12 sensory attributes used in descriptive analysis for experimental Syrah wines from grapes harvested at *ca.* 22°Brix from NS, EW, NE-SW and NW-SE row orientation treatments. Abbreviations used in biplot: Colour Int = Colour intensity; Overall Ar Int = Overall aroma intensity; Fruity Int = Fruity intensity; Spice Ar Int = Spice aroma intensity; Jammy Ar Int = Jammy aroma intensity; Concent Int = Concentrate intensity; Alcohol Int = Alcohol intensity; Tannin Int = Tannin intensity; Acidity Int = Acidity intensity; Body Mouthf = Body mouthfeel; Finish Per = Finish persistence; Overall Q = Overall quality. NS = North-South; EW = East-West; NE-SW = Northeast-Southwest; NW-SE = Northwest-Southeast.

Principal component 1 shows the greatest variation in the biplot. All the sensory attributes were positively associated with PC1. PC1 mainly separated NW-SE, NE-SW and NS row orientation treatments from EW row orientation treatments, while PC2 separated NW-SE, EW and NE-SW row orientation treatments from NS row orientation treatments. The attributes, jammy aroma intensity, acidity intensity, and spice aroma intensity were

associated with wines from the NS orientation treatments. An association between jammy aroma intensity, acidity intensity, and spice aroma intensity exist. These attributes differentiated wines from the NS row orientation treatment and wines from the NE-SW, NW-SE and EW row orientation treatments. Wines from the EW row orientation treatment were not associated with the measured sensory attributes. Wines from the NE-SW and the NW-SE row orientation treatments were associated with finish persistence, concentrate intensity, overall aroma intensity, colour intensity, body mouthfeel and overall quality. Wines from both the NS and the NW-SE row orientation treatments were associated with tannin intensity and alcohol intensity. Fruity intensity seemed poorly associated with all wines.

5.6.2 PCA of Syrah wines from grapes harvested at *ca.* 24°Brix

Principal component analysis yielded two principal components with Eigenvalues higher than two, explaining 98.43% of the total variation in the data of the first two dimensions (PC1 and PC2) with 91.64% and 6.79% explained by PC1 and PC2, respectively (Fig. 5.11).

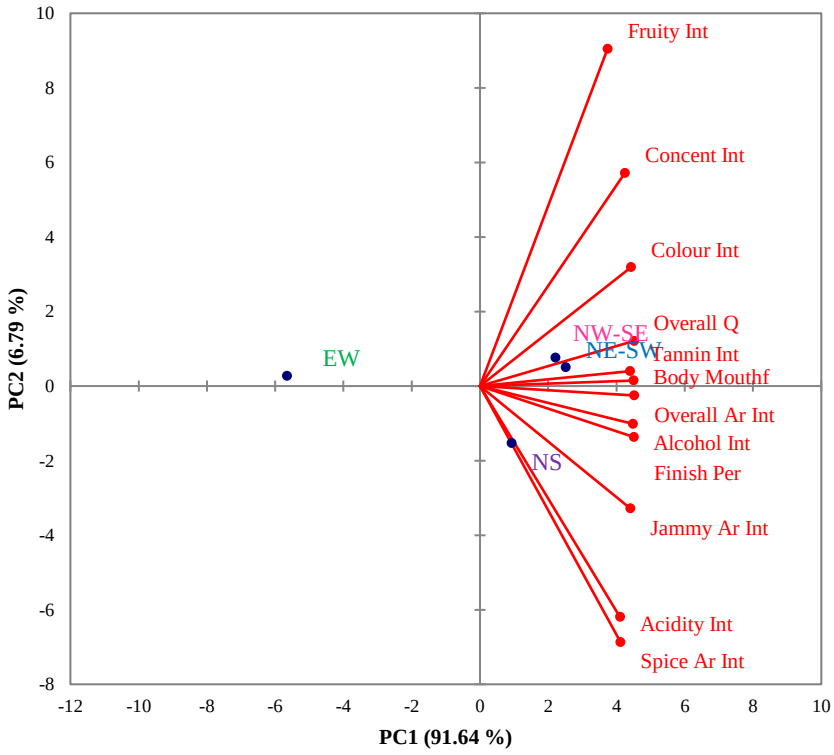

Figure 5.11 Vector diagram (PC biplot) of wine sample average scores and loadings of 12 sensory attributes used in descriptive analysis for experimental Syrah wines from grapes harvested at *ca.* 24°Brix from NS, EW, NE-SW and NW-SE row orientation treatments. Abbreviations used in biplot: Colour Int = Colour intensity; Overall Ar Int = Overall aroma intensity; Fruity Int = Fruity intensity; Spice Ar Int = Spice aroma intensity; Jammy Ar Int = Jammy aroma intensity; Concent Int = Concentrate intensity; Alcohol Int = Alcohol intensity; Tannin Int = Tannin intensity; Acidity Int = Acidity intensity; Body Mouthf = Body mouthfeel; Finish Per = Finish persistence; Overall Q = Overall quality. NS = North-South; EW = East-West; NE-SW = Northeast-Southwest; NW-SE = Northwest-Southeast.

Separation and association occurred only in PC1. All the sensory attributes were positively associated with PC1. PC1 mainly separated NW-SE, NE-SW and NS row orientation treatments from EW row orientation treatments, while PC2 separated NS row orientation treatments from NW-SE, NE-SW and EW row orientation treatments. Wines from the NE-SW and the NW-SE row orientation treatments were associated with most measured sensory attributes, *i.e.* colour intensity, overall quality, body mouthfeel, overall aroma intensity, alcohol intensity, and finish persistence. Similar associations as above were found for wines from grapes harvested at *ca.* 22°Brix from the NE-SW and the NW-SE row orientation treatments. Wines from the NS row orientation treatment were associated with acidity intensity and spice aroma intensity; this also occurred for wines from grapes harvested at *ca.* 22°Brix. Similar to what was found for wines from grapes at *ca.* 22°Brix, wines from the EW row orientation treatment were not associated with any of the measured sensory attributes.Fruity intensity and concentrate intensity were weakly associated with wines from the NW-SE and NE-SW row orientation treatments. Fruity intensity was also weakly associated with wines from the NW-SE and NE-SW row orientation treatments at *ca.* 22°Brix.

5.6.3 PCA of Syrah wines from grapes harvested at *ca.* 26°Brix

Principal component analysis yielded two principal components with Eigenvalues higher than three, explaining 98.26% of the total variation in the data of the first two dimensions (PC1 and PC2) with 94.19% and 4.07% explained by PC1 and PC2, respectively (Fig. 5.12). Separation and association occurred only in PC1. All the sensory attributes were positively associated with PC1. PC1 mainly separated NW-SE, NE-SW and NS row orientation treatments from EW row orientation treatments, while PC2 separated NW-SE, NE-SW and EW row orientation treatments from NS row orientation treatments.

Wines from the NS row orientation treatment were associated with acidity intensity and spice aroma intensity; this also occurred for wines from grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix, respectively. Wines from the NW-SE and the NE-SW row orientation treatments were associated with alcohol intensity, tannin intensity, overall quality, colour intensity, finish persistence, fruity intensity, overall aroma intensity, and jammy aroma intensity. Wines from these treatments from grapes harvested at *ca.* 22°Brix, were also associated finish persistence, colour intensity, overall aroma intensity, and overall quality, whereas the wines from grapes harvested at *ca.* 24°Brix were associated with overall quality, colour intensity, tannin intensity, overall aroma intensity, alcohol intensity, and finish persistence.

Wines from the EW row orientation treatment were not associated with any of the measured sensory attributes, as was the case for wines from grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix ripeness levels.

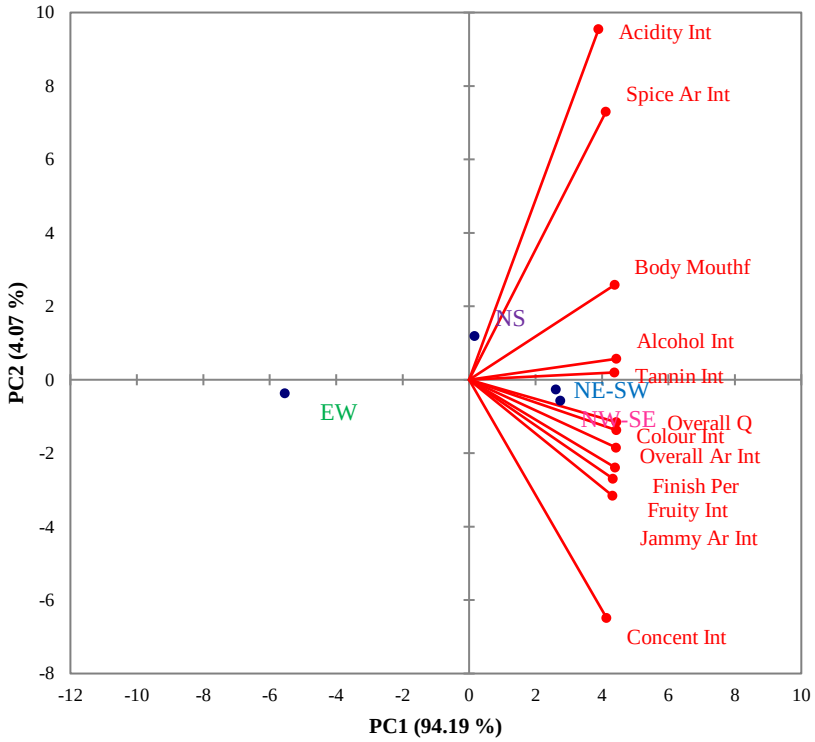

Figure 5.12 Vector diagram (PC biplot) of wine sample average scores and loadings of 12 sensory attributes used in descriptive analysis for experimental Syrah wines from grapes harvested at *ca.* 26°Brix from NS, EW, NE-SW and NW-SE row orientation treatments. Abbreviations used in biplot: Colour Int = Colour intensity; Overall Ar Int = Overall aroma intensity; Fruity Int = Fruity intensity; Spice Ar Int = Spice aroma intensity; Jammy Ar Int = Jammy aroma intensity; Concent Int = Concentrate intensity; Alcohol Int = Alcohol intensity; Tannin Int = Tannin intensity; Acidity Int = Acidity intensity; Body Mouthf = Body mouthfeel; Finish Per = Finish persistence; Overall Q = Overall quality. NS = North-South; EW = East-West; NE-SW = Northeast-Southwest; NW-SE = Northwest-Southeast.

5.7 Discussion

5.7.1 Syrah wines from grapes harvested at *ca.* 22°Brix

Titratable acidity was significantly different between wines from the NE-SW and the EW row orientation treatments. Significantly higher TA was reported in wines from the EW row orientation treatments, but was not significantly different from NW-SE and NS wines. Significantly higher pH values were recorded in wines from the NS and the NW-SE row orientation treatments. Significantly lower pH was reported in wines from the NE-SW row orientation treatment, but was not significantly different from that of EW wines.

Wines from the NW-SE and the NE-SW row orientation treatments were significantly higher in total anthocyanin (monomeric-, acetylated- and coumaryolated anthocyanins) concentrations (Table 5.7). Work conducted by Spayd *et al.* (2002) showed increased concentrations of monomeric anthocyanins in Merlot wines of NS row orientated vines (*i.e.* high light exposure in the morning and afternoon). The fruiting zone was however artificially shaded, resulting in low light exposure of grape bunches.

Wines from EW (low light exposure all day) and NS (high light exposure in the morning and afternoon) row orientation treatments were significantly lower in total anthocyanin concentrations. This agrees with work by

Haselgrove *et al.* (2000), Joscelyne *et al*. (2007) and Hunter & Volschenk (2008). Hunter *et al*. (2010) reported that EW row orientation treatments were not conducive to colour development in Shiraz grapes, whereas NE-SW row orientation treatments were more favourable for grape-skin colour development.

Rustioni *et al.* (2011) reported an increase in anthocyanin concentrations in wines from Croatina and Pinot noir grapes planted to EW row directions. Vines were subjected to leaf removal around grape bunches and therefore higher light penetration. They showed that there is a correlation between increased anthocyanin concentrations and increased light in the fruiting zone of grapes.

In this study, total anthocyanins were significantly higher in Syrah wines from the NW-SE row orientation treatments, *i.e*. high light penetration in the morning but low light penetration in the afternoon (but results were not significantly different from those of NE-SW row orientation treatments). Martínez-Lüscher *et al*. (2014) reported that artificial moderately reduced light penetration (simulating reduced light) and moderate temperature (*ca.* 30°C) of Tempranillo grape bunches showed an increase in total anthocyanin concentrations in the resulting wines. Grapevines were cultivated in glasshouses. The stimulating effect of light intensity / penetration (light interception, increased or reduced light) induced by vine row orientation on total anthocyanins in Shiraz grapes was shown by Hunter *et al*. (2007) and Downey *et al*. (2004). This was also found for flavan-3-ols in Pinot noir and flavonols in Merlot grapes by Cortell & Kennedy (2006) and Spayd *et al.* (2002). Cortell *et al.* (2007a) found that low vigour Pinot noir vines orientated in a NE-SW direction with moderate temperature in the fruiting zone were conducive to increased concentrations of acylated anthocyanins in the grapes, compared to low vigour vines orientated in a NS direction. Manipulating light penetration in the fruiting zone, *i.e.* either increased or decreased light intensities, can also have a negative effect on flavonol and flavan-3-ol concentrations.

Total flavan-3-ol concentrations were significantly higher in wines from the NE-SW row orientation treatments (Table 5.7). Epigallocatechin 3-*O*-gallate showed significantly higher concentrations in wines from the NE-SW row orientation treatments, but was not significantly different from NW-SE wines. Epicatechin (-) concentrations were also significantly higher in wines from the NE-SW row orientation treatment, but were not significantly different from NS wines. This is in agreement with work by Scrafidi *et al.* (2016) where they reported increased concentrations of flavan-3-ols in Grillo (white) wines from grapes harvested at *ca.* 21°Brix from vines planted to NS row orientations. The NS orientated vines were artificially shaded to decrease light penetration / temperature in the fruiting zone. The low light penetration in the fruiting zone at low grape ripeness levels increased total flavan-3-ol concentrations in wines from the NS row orientations (Scarfidi *et al*., 2016).

Significantly higher concentrations of total flavonols were evident in Syrah wines from the NE-SW row orientation treatment, but were not significantly different from NS wines. Quercetin 3-*O*-rhamnoside was significantly higher in wines from the NE-SW row orientation treatment, but not significantly different from NW-SE wines.

Spayd *et al.* (2002) reported high concentrations of quercetin 3-*O*-rhamnoside in Merlot wines from NS row orientated vines. Vines were however artificially shaded resulting in low light exposure and reduced temperature in the fruiting zone.

Quercetin 3-*O*-rutinoside (rutin) and quercetin concentrations were significantly higher in wines from the NE-SW row orientation treatment, followed by those in wines from the NW-SE row orientation treatment for quercetin 3-*O*-rutinoside and NS row orientation treatments for quercetin in this study.

Martínez-Lüscher *et al*. (2014) found higher concentrations of kaempferol and quercetin in Tempranillo wines from grape bunches exposed to increased light, compared to grape bunches subjected to artificially reduced light. In this study it was found that grapevine rows orientated to NE-SW (low light exposure in the morning) may lead to increased concentrations of flavonols in Syrah grapes and ultimately in the wines.

Total phenolic acid concentrations were significantly higher in wines from the NW-SE row orientation treatments, but were not significantly different from NE-SW wines. However, gallic acid concentrations were significantly higher in wines from the NS row orientation treatments, but not significantly different from EW wines. Jogaiah *et al*. (2013) reported increased concentrations of gallic acid in Cabernet Sauvignon wines from grapes of vines planted to NS row orientations. Vines were however subjected to leaf removal / shoot thinning, resulting in increased light in the fruiting zone. The light regime of vines planted to NS row orientations in this study is that of high light exposure in the fruiting zone in the morning and in the afternoon.

Coumaric acid (*para*) on the other hand in this study was significantly higher in wines from the NW-SE row orientation treatments, whereas ferulic acid was significantly higher in wines from the NE-SW row orientations treatment. Diago *et al.* (2012) reported increased concentrations of ferulic acid in wines from Tempranillo grapes. Vines were however planted to an EW direction and subjected to 50% defoliation, thereby exposing grape bunches to increased light as opposed to low light, which is indicative of EW row orientations. Defoliation may change the light / temperature regime (microclimate) of EW row orientated canopies and may lead to increased exposure of grape bunches to light. In this study, no leaf removal was done and the exposure of the vertical canopies and grape bunches resulted (naturally) only from canopy orientation.

Wines from NE-SW and NW-SE orientated vines scored highest in overall quality. Colour intensity was also highest in wines from the NE-SW and the NW-SE row orientation treatments. Increased wine colour relates to increased anthocyanin concentrations in the grapes. Wines from the NE-SW and the NW-SE row orientation treatments also scored highest in jammy aroma intensity, body mouthfeel and tannin intensity. High tannin intensity and body mouthfeel can relate to increased flavan-3-ols or oligomeric tannin concentrations in wine.

5.7.2 Syrah wines from grapes harvested at *ca.* 24°Brix

The NE-SW row orientation treatments were conducive to higher pH values, but wines from grapes harvested at *ca.* 22°Brix, showed highest pH levels from the NW-SE and the NS row orientation treatments.

Titratable acidity was highest in wines from the EW and the NS row orientation treatments. The same trend was found for wines from the EW and NS row orientation treatment at *ca.* 22°Brix.

A trend of high concentrations of total anthocyanin was observed in wines from the NE-SW and the NW-SE row orientation treatments, as were found in wines from grapes harvested at *ca.* 22°Brix. Delphinidin 3-*O*-glucoside concentrations were significantly higher in wines from the NS row orientation treatments (Table 5.13). Lemut *et al.* (2012) reported increased concentrations of malvidin 3-*O*-glucosides in Pinot noir wines from EW row orientations, but the vines were subjected to 50% defoliation, therefore increasing the light intensity in the fruiting zone. Li *et al.* (2013) reported decreased concentrations of total anthocyanins in Jingxiu wines from grapes planted to EW row orientations with increased temperature in the fruiting zone. Grape bunches were however artificially shaded and harvested from north-facing canopies only. Results from this study indicated that both EW and NS row orientation treatments were conducive to decreased wine total anthocyanin concentrations, compared to those of NE-SW and NW-SE row orientation treatments. Grapes were however harvested from both sides of the canopies.

In this study, petunidin 3-*O*-(6-*O*-acetyl) glucosides were significantly higher in wines from the NW-SE row orientation treatments. Work by Chorti *et al*. (2010) reported highest concentrations of acetylated anthocyanins in Nebbiolo wines from grapes planted to NS row orientations; these grapes were however subjected to artificial shading (netting) and harvested from west-facing canopies only.

Total flavan-3-ols were significantly higher in wines from the NS row orientation treatments, therefore stimulated by increased sunlight exposure in the fruiting zone. These wines were however not significantly different from EW wines. This agrees with work by Peña-Neira *et al*. (2004) who found total flavan-3-ol concentrations being highest in Cabernet Sauvignon wines from grapes harvested at 23°Brix from NS row orientated vines. Mori *et al*. (2005), Cohen *et al.* (2008) and Chorti *et al.* (2010), also showed that Cabernet Sauvignon, Merlot and Nebbiolo wines produced from grapes planted to NS row orientations (northern hemisphere) were highest in total flavan-3-ols. Song *et al*. (2015) reported increased total flavan-3-ol concentrations in Pinot noir wines from NS row orientated vines.

Wines from the NS row orientation treatments (high light exposure in the morning and afternoon) were highest in (+)-catechin and (-)-epicatechin concentrations, followed by wines from the EW row orientation treatments. Kemp *et al.* (2011) reported increased concentrations of (+)-catechin in Pinot noir wines from EW row orientations; grapes were harvested from the north-facing side of the canopy. Vines were however subjected to 50% defoliation resulting in increased light penetration and temperature in the fruiting zone.

Epigallocatechin 3-*O*-gallate concentrations were significantly higher in wines from the EW row orientation treatments. Increased concentrations of flavan-3-ol concentrations were reported by Feng *et al.* (2015) (Pinot noir wines) and Scrafidi *et al*. (2016) (Grillo wines) from NS row orientations. The different growth conditions and cultivars may have affected the results.

Kemp *et al*. (2011) reported a decrease in concentrations of epigallocatechin 3-*O*-gallate in Pinot noir wines from EW row orientations. Vines were however subjected to 50% defoliation, which may have increased the light penetration and temperature in the fruiting zone. Bekar *et al.* (2017) reported higher concentrations of (+)-catechin and (-)-epicatechin in Narince wines, compared to wines from vines subjected to leaf removal around the fruit-zone, thereby allowing more light penetration. Row orientation was not revealed. Total flavonol concentrations were highest in wine from the NS row orientation treatments, followed by those from the EW row orientation treatment. North-south row orientations allowed high light exposure into the fruiting zone in the morning and afternoon, whereas east-west row orientations allowed lower light penetration into the fruiting zone all day.

Quercetin and kaempferol were significantly higher in wines from the NS and NE-SW row orientation treatments, respectively. In the case of grapes harvested at *ca.* 22°Brix ripeness levels, quercetin and kaempferol were significantly higher in wines from the NE-SW and NS row orientation treatments, respectively. Peña-Neira *et al*. (2004) found that quercetin 3-*O*-rutinoside and quercetin 3-*O*-glucoside concentrations were higher in Cabernet Sauvignon wines from low light exposed vines (EW), compared to vines with a high light exposure in the morning and afternoon, *i.e.* NS row orientations. This is in agreement to work of this study where quercetin 3-*O*-rutinoside proved higher in wines from the EW row orientation treatments.

Lemut *et al*. (2013) also showed that Pinot noir wines from grapes planted to EW row orientations had increased concentrations of quercetin glycosides. Vines were however subjected to grape-bunch zone leaf removal.

Total phenolic acid concentrations were higher in wines from the NS row orientation treatments, followed by those from the NW-SE row orientation treatments, compared to the NE-SW and the EW row orientation treatments. Caffeic acid concentrations were significantly higher in wines from the NE-SW row orientation treatments. Coumaric acid (*para*) concentrations were significantly higher in wines from the NW-SE row orientation treatments. This compares with *p*-coumaric acid concentrations of wines from grapes harvested at *ca.* 22°Brix ripeness levels. Work by Gil *et al.* (2013) reported highest concentrations of *p*-coumaric acid in Syrah wines from NS row orientations; the concentrations of the measured phenolic acids were likely affected by 50% grape bunch thinning treatment.

Contrary to the above, Tessarin *et al*. (2014) reported increased concentrations of *p*-coumaric acids in Uva Longanesi wines from EW row orientations. Vines were however subjected to 50% defoliation treatment, resulting in increased light penetration in the fruiting zone. Ferulic acid was significantly higher in wines from the EW row orientation treatment. This agrees with work by Gil *et al*. (2013) who reported highest concentrations of ferulic acid in Syrah wines from EW orientated vines. Tessarin *et al*. (2014) reported highest levels of gallic- and ferulic acids in Uva Longanesi wines from grapes planted to EW row orientations. However, these EW row orientated vines underwent 50% defoliation treatment, resulting in increased light in the fruiting zone. Feng *et al.* (2015) found no significant differences in concentrations of phenolic acids in Pinot noir wines from NS row orientations with 50% to 100% leaf removal in the canopies, compared to those of the control treatment (no leaf removal).

Bekar *et al.* (2017) reported higher concentrations of gallic-, *p*-coumaric-, caffeic- and ferulic acids in Narince wines, compared to wines from vines which were subjected to leaf removal around the fruit-zone. Row orientations of vines were not disclosed.

5.7.3 Syrah wines from grapes harvested at *ca.* 26°Brix

Wines from the EW row orientation treatment appeared to have highest titratable acidity, but TA was not significantly different among the treatments. Titratable acidity was also highest in wines from the EW row orientation treatments for grapes harvested at both *ca.* 22°Brix and *ca.* 24°Brix. Although pH values seemed higher in wines from the NE-SW row orientation treatment, pH was not significantly different among the wines from the four row orientation treatments.

Total anthocyanins, *i.e.* monomeric, acetylated and coumaryolated anthocyanin concentrations were highest in wines from the EW row orientation treatments (low light in the morning; low light in the afternoon, respectively), followed by wines from the NE-SW and NW-SE row orientation treatments. This is in agreement with Spayd *et al*. (2002), Downey *et al.* (2004), Tarara *et al.* (2008), and Cortell & Kennedy (2006), who found moderate / low light exposure of Cabernet Sauvignon, Syrah and Merlot grape bunches resulting in wines with increased anthocyanin concentrations. Cyanidin 3-*O*-glucosides of this study was significantly higher in wines from the NS row orientation treatments, whereas significantly lower cyanidin- and delphinidin 3-*O*-glucosides were found in wines from NW-SE row orientation treatments. Cook *et al*. (2015) reported decreased concentrations of delphinidin-, petunidin- and peonidin 3-*O*-glucosides as well as coumaroylated anthocyanins in Merlot wines from NS row orientations (high light exposure in the morning and afternoon) with vines subjected to pre-bloom leaf removal, compared to no leaf removal. Degu *et al*. (2016) reported that Shiraz wines from NS row orientated vines had reduced total anthocyanin concentrations due to prolonged heat and increased light in the fruiting zone. This is in agreement to total anthocyanin concentrations of this study (Table 5.18). Romboli *et al*. (2017) reported higher concentrations of delphinidin-, cyanidin-, petunidin- and peonidin 3-*O*-glucosides in wines from high vigour Sangiovese vines planted to NE-SW directions, thereby reducing light penetration, compared to low vigour vines of the same row orientation. Malvidin 3-*O*-glucoside concentrations were however higher in wines from low vigour vines. Delphinidin 3-*O*-glucoside concentrations reported in this study were significantly higher in wines from EW (low light in the morning; low light in the afternoon) row orientations, followed by wines form NE-SW row orientations.

Work by Giacosa *et al.* (2015) reported no significant differences between individual anthocyanins in Shiraz wines from grapes planted to NS and EW row orientations. In this study, significant differences between wines from the NS and the EW row orientation treatments were found in terms of individual anthocyanins, except for delphinidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides. Lower concentrations of certain individual anthocyanins in wines from the NS row orientation treatments, compared to wines from the NE-SW and the NW-SE row orientation

treatments, could be a result of probable increased light and temperature in the fruiting zone. Degradation or inhibition of the biosynthesis of anthocyanins may occur with excessive light penetration and increased temperatures in the fruiting zone (Cook, 2015).

Wines from the NW-SE row orientation treatments proved significantly higher in total flavan-3-ol concentrations. Wines from the NS row orientation were significantly lower. Peña-Neira *et al*. (2004) found that total flavan-3-ol concentrations in Cabernet Sauvignon wines from grapes harvested at technological ripeness and from NS row orientated vineyards with low vigour vines were higher in (-)-epicatechin and (+)-catechin concentrations, compared to high vigour vines. Total flavan-3-ol concentrations were generally higher in wines from grapes harvested at *ca.* 26°Brix, compared to those in wines from grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix. This agrees with work done by Cadot *et al.* (2012) where Cabernet Franc wines from grapes harvested at *ca.* 25°Brix had higher concentrations of flavan-3-ols, compared to grapes harvested at *ca.* 22°Brix.

Higher flavan-3-ol concentrations in wines from grapes at increased ripeness levels (>24°Brix) can be attributed to delayed biosynthesis of flavonoids (Kennedy *et al*., 2000; Bogs *et al.*, 2006).

Highest concentrations of (+)-catechin and epigallocatechin 3-*O*-gallate were recorded in wines from the EW and the NW-SE row orientation treatments, respectively. Kemp *et al.* (2011) found increased concentrations of (+)-catechin in wines from north-facing Pinot noir grapes planted to EW row orientations, harvested at technological ripeness. Increased temperature and light in the fruiting zone were brought about by 50% leaf removal. Feng *et al.* (2015) reported increased concentrations of (+)-catechin and (-)-epicatechin in Pinot noir wines from NS row orientations. Vines were however also subjected to 50% leaf removal treatment in the fruit zone. Scrafidi *et al*. (2016) reported increased concentrations of (+)-catechin in Grillo (white) wines from NS row orientated vines. Results were likely affected by the 50% leaf removal treatment of the canopy, increasing light penetration into the fruiting zone. Rescic *et al.* (2016) also showed that (+)-catechin concentrations increased in Istrian Malvasia wines from NS orientated vines. The increased levels were again likely brought about by leaf removal (50%) treatment of the canopy; this probably resulted in grape bunches being over-exposed to light. Romboli *et al*. (2017) reported higher (+)-catechin and epigallocatechin-gallate concentrations and lower (-)-epicatechin concentrations in Sangiovese wines from low vigour vines planted to NE-SW, compared to high vigour vines.

Significantly higher concentrations of total flavonols were evident in Syrah wines from the NE-SW and the NW-SE row orientation treatments (Table 5.18). Quercetin 3-*O*-rhamnoside, quercetin and quercetin 3-*O*-rutinoside were significantly higher in wines from the NE-SW row orientation treatment. Peña-Neira *et al*. (2004) found similar results where quercetin 3-*O*-rutinoside and quercetin 3-*O*-glucoside concentrations were higher in Cabernet Sauvignon wines from low to moderate light exposed vines, compared to vines with high light exposure. Koyama *et al*. (2012) reported that flavonol concentrations in Cabernet Sauvignon wines from grapes subjected to artificial shading, *i.e.* light proof boxes, showed a decrease in flavonol concentration.

Wines from row orientations receiving low light penetrations all day, mornings, or afternoons, *i.e.* EW, NE-SW or NW-SE, respectively, are favourable for flavonol formation. For quercetin 3-*O*-rutinoside concentrations, wines from the NE-SW row orientation treatment from grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix compared well with wines from grapes harvested at *ca.* 26°Brix. Quercetin in wines from grapes harvested at *ca.* 22°Brix were also significantly higher from NE-SW row orientation treatments, as in the case of *ca.* 26°Brix. Kaempferol concentrations were significantly lower in wines from the NS row orientation treatments. Significantly higher kaempferol concentrations were found in wines from the NS and NE-SW row orientation treatments for grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix, respectively.

Total phenolic acid concentrations were significantly higher in Syrah wines from the NW-SE row orientation treatments. Wines from grapes harvested at *ca.* 26°Brix compared well with wines from grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix in terms of gallic acid concentrations, except for gallic acid in wines from grapes harvested at *ca.* 24°Brix from the NW-SE row orientation treatments. Ferulic- and caffeic acids were significantly higher in wines from the NW-SE row orientation treatments from grapes harvested at *ca.* 26°Brix ripeness level. Gil *et al.* (2013) reported highest concentrations of ferulic acid in Syrah wines from EW row orientations, where they compared grape bunch thinning *versus* berry thinning. Differences in concentrations were likely because of the treatment, changing the source:sink relationships in the canopy. In this study, ferulic acid concentrations were significantly higher in wines from grapes planted to EW row orientations harvested at *ca.* 24°Brix.

Contrary to results of this study, Tessarin *et al.* (2014) reported increased concentrations of caffeic- and *p*-coumaric acids in Uva Longanesi wines from EW row orientations; vines were however 50% defoliated, which increased light penetration into the fruiting zone. Feng *et al.* (2015) reported no differences in concentrations of phenolic acids in Pinot noir wines from NS row orientations with vines subjected to 50% leaf removal, compared to vines with no leaf removal treatment. Rescic *et al.* (2016) found increased concentrations of caffeic- and ferulic acids in Istrian Malvasia wines from grapes of NS orientated vines. Vines were subjected to leaf removal (50%). Significantly higher concentrations of *p*-coumaric acid were reported in wines of the NS row orientation treatment from grapes harvested at *ca.* 26°Brix, but not significantly different from EW wines.

Wines from grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix from the NE-SW row orientation treatments were significantly higher in *p*-coumaric acid.

Quality wines were best associated with grapes from the NE-SW and the NW-SE row orientation treatments. Principal component analysis showed that it is possible to associate wines from specific row orientations with certain measured sensory attributes.

Wine quality perception is linked to colour intensity, body mouthful, and tannin intensity. The results mainly showed the importance of anthocyanins and flavan-3-ols in the assessment of wine quality, but also the relevance of certain phenolic acids.

Colour-, overall aroma-, alcohol- and tannin intensity as well as body mouthfeel increased as grape ripeness increased over time for all four row orientation treatments, but were highest in wines from the NE-SW and the NW-SE row orientation treatments; this resulted in a higher score also for overall wine quality. Higher alcohol content, as found in wines from the NE-SW and the NW-SE row orientation treatments, may also improve phenolic extraction during maceration, hence improving colour intensity. These findings correspond to work by Cadot *et al*. (2012) where they showed that Cabernet Franc wines had improved colour intensity, body mouthfeel and increased alcohol intensity when grapes were harvested at *ca.* 25°Brix ripeness, compared to *ca.* 22°Brix ripeness. In addition, the de-pectination of cell walls may improve the extraction of phenolics during skin contact (Zietsman *et al.*, 2015).

Sensory results showed that the sensory attribute scores were associated with specific row orientations (treatments) and ripeness levels. The sensory data for colour intensity showed notable differences between row orientation treatments and ripeness levels; in line with similar differences in anthocyanin levels. Wines from the NW-SE and the NE-SW row orientation treatments, harvested at *ca.* 22°Brix and *ca.* 24°Brix ripeness levels, produced wines that had higher colour intensity than wines from the NS and the EW row orientation treatments. At *ca*. 26°Brix, wines from the NE-SW row orientation treatments, again proved highest in colour intensity and body mouthfeel. An association existed between astringency attributes (tannin intensity) and measured flavan-3-ols in wines from grapes harvested at *ca*. 22°Brix and *ca*. 26°Brix ripeness level and from the NW-SE and the NE-SW row orientations, respectively. For wines from grapes harvested at *ca.* 24°Brix no association existed between astringency attributes and measured flavan-3-ols.

Based on the sensorial data, the NW-SE and NE-SW row orientation treatments at all three ripeness levels produced wines with the highest tannin intensity and body mouthfeel scores. This was also confirmed by individual phenolic compound analyses. Wines from grapes harvested at *ca.* 22°Brix and from the NE-SW row orientation treatments were high in (-)-epicatechin and epigallocatechin 3-*O*-gallate, whereas wines from grapes harvested at *ca.* 26°Brix and from the NW-SE row orientation treatments were high in (-)-epicatechin and epigallocatechin 3-*O*-gallate. Thus flavan-3-ol compounds do contribute to the sensory assessment of the wines.

Wines from riper grapes (> 26°Brix) scored higher in body mouthfeel and tannin intensity, compared to grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix, indicating increased levels of monomeric flavan-3-ols and oligomers. This can be attributed to an increased extraction rate of flavan-3-ols because of higher concentrations of ethanol, which is linked to grapes with higher TSS.

Increased colour intensity was also linked to an increase in ripeness level. This is in agreement with work by Cadot *et al.* (2012) where Cabernet Franc wine quality was associated with anthocyanins and flavan-3-ol compounds in grapes harvested at *ca*. 26°Brix. Phenolic compound concentrations therefore contribute to the sensory assessment of the wines or distinctive style.

5.8 Association of phenolics with row orientation, grape ripeness and sensory attributes

Individual phenolic compound concentrations were strongly and consistently affected by row orientation (microclimate / light intensity) and grape ripeness levels. Analysis of variance of wine phenolic data showed that the effect of row orientation and grape ripeness levels on individual phenolic compound concentrations was significant. Differences induced in phenolic compound concentrations by the different treatments (row orientations) were verified mainly by anthocyanin concentrations and flavan-3-ol concentrations. Tables 5.24 and 5.25 list comparative information of phenolic compounds associated with the measured sensory attributes.

Wines from grapes harvested at *ca.* 22°Brix, *ca.* 24°Brix and *ca.* 26°Brix ripeness levels and from the NE-SW row orientation treatment that were high in (-)-epicatechin [(+)-catechin in the case of *ca.* 26°Brix] epigallocatechin 3-*O*-gallate and total anthocyanin concentrations, scored high/highest in tannin intensity, body mouthfeel, colour intensity and overall quality (Table 5.24).

Wines from grapes harvested at *ca.* 22°Brix and *ca.* 26°Brix ripeness level from the NW-SE row orientation treatments that were high in epigallocatechin 3-*O*-gallate concentrations [also (-)-epicatechin in the case of *ca.* 26°Brix], scored high/highest in tannin intensity, body mouthfeel, colour intensity and overall quality. However, most concentrations/scores measured for these variables (phenolics and sensory attributes) were not significantly different between wines from the NE-SW and wines from the NW-SE row orientation treatments. Wines from grapes harvested at the three different ripeness levels and from all the NS and EW row orientation treatments showed no association between measured flavan-3-ols/anthocyanins and sensory attributes.

Wines from grapes harvested at *ca*. 22°Brix ripeness level and from the NE-SW row orientation treatment that were high/highest in ferulic acid and *p*-coumaric acid concentrations, scored high in tannin intensity, body mouthfeel and overall quality (Table 5.25). Wines from grapes harvested at *ca.* 24°Brix ripeness level and from the NE-SW row orientation treatments that were highest caffeic acid concentrations, scored high in tannin intensity, body mouthfeel, and overall quality.

Wines from the NW-SE row orientation treatments from grapes harvested at *ca*. 22°Brix and *ca*. 24°Brix ripeness levels that were high/highest in ferulic acid and *p*-coumaric acid concentrations, scored highest in tannin intensity, body mouthfeel and overall quality. Wines from grapes harvested at *ca*. 26°Brix from the NW-SE treatments that were highest in caffeic acid and ferulic acid, scored high/highest in tannin intensity, body mouthfeel and overall quality. Oberholster (2008) and Romano *et al*. (2011) reported an association between caffeic acid- and *p*-coumaric acid concentrations and wine quality sensory parameters. Although often significant among treatments, the phenolic compound concentration differences were small (often a few mg/L). These mostly monomeric flavan-3-ols comprise a relative small percentage of the total flava-3-ol derived moieties in wine, *i.e.* oligomers and polymers, such as polymeric phenols and tannins. The polymeric phenolic compounds most likely affect the sensory aspects more than monomeric flavan-3-ols. Despite this, monomers constitute a valuable component of the final wine and their role in wine quality can hardly be ignored.

Table 5.24 Association of wine flavan-3-ols and anthocyanins with sensory attributes, including row orientation and grape ripeness level.

Row Orientation	Ripeness levels	Phenolic compounds (mg/L)				Sensory attributes (%)			
		(+)-catechin	(-)-epicatechin	[1]EGCG	[2]Tot. anthocyanins	[3]Tannin Int.	[4]B. mouthfeel	[5]Colour Int.	[6]Overall Q.
[8]EW	*ca.* 22°Brix	6.29a NSD[7]	Lowest (5.41c)[*]	Low (2.18b)	Lowest (161.27b)	Lowest (35.84d)	Lowest (33.13d)	Lowest (34.32c)	Lowest (34.64c)
[8]NE-SW		6.41a NSD	**Highest (8.28a)**	**Highest (4.22a)**	**High (199.48a)**	**High (41.22b)**	**High (42.07b)**	**Highest (49.29a)**	**High (44.40a)**
[8]NS		6.53a NSD	High (7.82a)	Lowest (1.56c)	Low (172.03b)	Low (40.85c)	Low (39.83c)	Low (45.67b)	Low (41.95b)
[8]NW-SE		5.87aNSD	Low (7.01b)	**High (4.07a)**	**Highest (208.92a)**	**Highest (42.60a)**	**Highest (43.39a)**	**High (48.59a)**	**Highest (44.67a)**
[8]EW	*ca.* 24°Brix	High (6.33ba)	Low (7.00ba)	Highest (2.32a)	Low (186.76ba)	Lowest (37.97c)	Lowest (38.61c)	Lowest (43.88c)	Lowest (40.18c)
[8]NE-SW		Low (5.94b)	**High (7.05ba)**	**High (1.50b)**	**High (191.74ba)**	**High (42.60b)**	**High (47.14a)**	**High (59.00a)**	**High (48.48a)**
[8]NS		Highest (6.56a)	Highest (7.79a)	Lowest (1.39b)	Lowest (176.00b)	Low (42.57b)	Low (46.41b)	Low (53.76b)	Low (46.43b)
[8]NW-SE		Lowest (5.14c)	Lowest (6.36b)	Low (1.40b)	**Highest (205.35a)**	**Highest (43.91a)**	**Highest (47.95a)**	**Highest (59.34a)**	**Highest (48.64a)**
[8]EW	*ca.* 26°Brix	Highest (7.74a)	Highest (8.08a)	Low (1.43c)	Highest (284.70a)	Lowest (41.69c)	Lowest (43.21d)	Lowest (53.60c)	Lowest (41.63c)
[8]NE-SW		**High (7.66ba)**	Low (7.62ba)	**High (3.80b)**	**High (271.32a)**	**High (46.63a)**	**Highest (53.29a)**	**Highest (69.49a)**	**Highest (51.58a)**
[8]NS		Lowest (6.48c)	Lowest (6.85b)	Lowest (0.60d)	Lowest (222.25b)	Low (45.48b)	Low (50.06c)	Low (63.59b)	Low (48.08b)
[8]NW-SE		Low (6.90bc)	**High (8.06a)**	**Highest (5.61a)**	Low (270.17a)	**Highest (46.90a)**	**High (51.96b)**	**High (69.45a)**	**High (50.97a)**

[1]Epigallocatechin 3-*O*-gallate; [2]Total anthocyanins; [3]Tannin intensity; [4]Body mouthfeel; [5]Colour intensity; [6]Overall quality; [7]Not significantly different; [8]North-South; [8]East-West; [8]Northeast-Southwest; [8]Northwest-Southeast. It should be noted that only monomeric flavan-3-ols were measured. Body mouthfeel / tannin intensity / overall quality are usually associated with higher oligomers. If there is a relation between monomeric flavan-3-ols and body mouthfeel / tannin intensity, it means the monomers can be measured to approximate these sensory characteristics. [*]Different letters in the same column indicate significant differences in the content of the variables measured among the different treatments according to Fisher's least significant difference test.

Table 5.25 Association of wine phenolic acids with sensory attributes, including row orientation and grape ripeness level.

Row Orientation	Ripeness level	Phenolic compounds (mg/L)				Sensory attributes (%)		
		Caffeic acid	*p*-coumaric acid	Ferulic acid	Gallic acid	[1]Tannin Int.	[2]B. mouthfeel	[3]Overall Q.
[5]EW	*ca.* 22°Brix	34.87a NSD	Lowest (21.24d)[*]	Low (5.26b)	High (4.19ba)	Lowest (35.84d)	Lowest (33.13d)	Lowest (34.64c)
[5]NE-SW		37.49a NSD	**High (32.65b)**	**Highest (8.11a)**	Low (3.81b)	**High (41.22b)**	**High (42.07b)**	**High (44.40a)**
[5]NS		39.82a NSD	Low (25.18c)	Lowest (5.10b)	Highest (4.55a)	Low (40.85c)	Low (39.83c)	Low (41.95b)
[5]NW-SE		41.40a NSD	**Highest (35.59a)**	**High (5.43b)**	Lowest (3.58b)	**Highest (42.60a)**	**Highest (43.39a)**	**Highest (44.67a)**
[5]EW	*ca.* 24°Brix	Low (31.85c)	Lowest (27.85c)	Highest (7.68a)	High (4.28a)	Lowest (37.97c)	Lowest (38.61c)	Lowest (40.18c)
[5]NE-SW		**Highest (41.33a)**	Lowest (31.32c)	Lowest (2.16d)	Lowest (1.51b)	**High (42.60b)**	**High (47.14a)**	**High (48.48a)**
[5]NS		High (35.35b)	High (37.95b)	Low (4.50c)	Highest (4.63a)	Low (42.57b)	Low (46.41b)	Low (46.43b)
[5]NW-SE		Lowest (26.68d)	**Highest (47.73a)**	**High (4.86b)**	Low (1.55b)	**Highest (43.91a)**	**Highest (47.95a)**	**Highest (48.64a)**
[5]EW	*ca.* 26°Brix	Low (26.24b)	High (51.25ba)	High (3.63b)	Highest (5.02a)	Lowest (41.69c)	Lowest (43.21d)	Lowest (41.63c)
[5]NE-SW		Lowest (16.91c)	Lowest (45.46c)	Lowest (2.63c)	Low (4.94a)	High (46.63a)	Highest (53.29a)	Highest (51.58a)
[5]NS		High (27.58b)	Highest (53.51a)	Low (2.94c)	Lowest (3.97c)	Low (45.48b)	Low (50.06c)	Low (48.08b)
[5]NW-SE		**Highest (36.41a)**	Low (49.59b)	**Highest (4.28a)**	Lowest (4.43b)	**Highest (46.90a)**	**High (51.96b)**	**High (50.97a)**

[1]Tannin intensity; [2]Body mouthfeel; [3]Overall quality. [4]Not significantly different; [5]North-South; [5]East-West; [5]Northeast-Southwest; [5]Northwest-Southeast. If there is a relationship between phenolic acids and body mouthfeel / tannin intensity / overall quality, it means phenolic acids can be measured to approximate these sensory characteristics. [*]Different letters in the same column indicate significant differences in the content of the variables measured among the different treatments according to Fisɛher's least significant difference test.

5.9 Conclusions

The results presented in this chapter show that row orientation (treatment), vintage, and grape ripeness level differences are distinguishable in the wines for both phenolic data obtained by HPLC and sensory analysis, as confirmed by ANOVA and PCA analyses. In the assessment of the effect of row orientation treatment, vintage, and grape ripeness levels on the phenolic compound concentrations, slight as well as significant differences in flavan-3-ol-, flavonol-, phenolic acid- and anthocyanin concentrations were found. Sensory attribute differences were also found in wines from the different row orientation treatments, and different grape ripeness levels. Vintage did not affect the general trend of the data because only single phenolic compound concentrations per ripeness level were affected. Differences in phenolic compound concentrations among treatments were not the same at each grape ripeness level. Variables that were associated with treatments were not similar for all three ripeness levels (PCA).

Two important key findings emerged from this investigation. The first was confirmation that wine phenolic concentrations differ significantly as a function of grapevine row orientation and grape ripeness levels. The second key finding was that wine sensory attributes were also affected by row orientation and grape ripeness. Wine quality was related to wine phenolic compound concentration and sensory attributes. Anthocyanin concentrations were associated with colour intensity. Body mouthfeel and tannin intensity were associated with flavan-3-ols. Other phenolic compounds associated with overall quality and body mouthfeel were caffeic- , ferulic- and *p*-coumaric acid concentrations. Row orientation treatments which affect microclimate, specifically light profiles of the canopies, altered the concentrations of selected phenolic compounds in the experimental Syrah wines. The highest chromatographic response in flavan-3-ol concentrations was measured for wines from grapes harvested at *ca.* 26ºBrix and from the NW-SE row orientation treatments, followed by the NE-SW row orientation treatments. Chromatographic responses in flavonol concentrations were highest in wines from grapes harvested at *ca.* 22ºBrix and from the NE-SW row orientation treatment, followed by wines from the NS row orientation treatments. For phenolic acids, highest chromatographic responses were evident in wines from grapes harvested at *ca.* 22°Brix and *ca.* 26ºBrix ripeness level and from the NW-SE row orientation treatments. Average anthocyanin concentrations showed differences amongst all row orientations at each of the ripeness levels. Highest chromatographic responses for anthocyanins were apparent in wines from the NW-SE row orientation treatment from grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix ripeness. Wines from the EW row orientation treatments were highest at a ripeness level of *ca.* 26°Brix. Anthocyanins tended to reach high concentrations in wines from the NE-SW and the NW-SE row orientation treatments, at all ripeness levels. It therefore appears that moderate grape bunch exposure to light favours anthocyanin accumulation. It appeared that flavonols were the compounds with the highest correlation between grape and wine samples at grape ripeness levels of both *ca.* 22°Brix and 24°Brix. Anthocyanins, on the other hand, showed the lowest correlations between grape and wine samples for *ca.* 22 °Brix and *ca.* 24°Brix ripeness.

In contrast to grapes harvested at *ca.* 22°Brix and *ca.* 24°Brix, grapes harvested at *ca.* 26°Brix showed anthocyanins with the highest correlation between grapes and wine samples with flavan-3-ols showing the lowest correlation. Wines from grapes harvested at *ca.* 22°Brix in 2008 showed significantly higher concentrations of compounds than those harvested in 2009 and 2010. The exception was quercetin and malvidin 3-*O*-(6-*O*-*p*-coumaroyl) glucosides, for which significant differences between 2008 and 2010 were absent. Wines made from grapes harvested at *ca.* 22°Brix in 2008 showed a tendency of certain compounds to be significantly higher in concentrations than those harvested in 2009 and 2010 vintages. In wines from grapes harvested at *ca.* 24°Brix, cyanidin 3-*O*-glucosides from the 2010 vintage were significantly higher than those from the 2008 and 2009 vintages. When compared to the 2009 and 2010 vintages, petunidin 3-*O*-(6-acetyl) glucoside was the only acetylated anthocyanin significantly higher in wines from the 2008 vintage. Wines from grapes harvested at *ca.* 26°Brix in 2010, showed a propensity of certain compounds to be significantly higher in concentrations than those harvested during the 2008 vintage. The exception was peonidin 3-*O*-glucosides for which significantly higher concentrations were observed in 2008.

The association between wine phenolic compound concentrations and sensory attributes, including overall wine quality, is neither simple nor direct. The results showed that an association (although not always consistent) existed among phenolic compounds measured, sensory attribute scores, row orientation treatments (microclimate/light intensity) and grape ripeness levels. The results showed that the sensory attributes of Syrah wines differ among the treatments and that overall quality of wine is associated with the NE-SW and the NW-SE row orientation treatments. It was possible to assign the wines to the different treatments to which the vines and grapes were subjected to, based on PCA of the phenolic compound data.

The effect of soil type and *terroir* on wine phenolic compounds is mediated through their effect on vine growth, vine water status and site parameters, such as diurnal temperature, precipitation, heat summation, and soil physical and chemical composition / structure. These parameters exert an independent effect on grape ripening and ultimately on wine quality, and should therefore be considered in phenolic management. This is the first investigation that demonstrates the effect of NS, EW, NE-SW, and NW-SE row orientation treatments (microclimate/light interception) on individual anthocyanin-, flavonol-, phenolic acid- and flavan-3-ol concentrations in Syrah wines. It is also the first study in which the combined effect of row orientation treatments and ripeness level is explored in this regard. Results showed that the concentrations of phenolic compounds in wine depend on complex processes that involve the interaction of grape ripeness and viticulture practices. The findings indicate that viticultural practices, such as row orientation and grape ripeness level, can be applied to regulate the phenolic concentrations in grapes and wines. Results should be viewed as applicable to wines from grapes cultivated on a flat *terroir*, trained to a vertical-shoot-positioned trellis system, and planted in clayey loam soil. Different results may be obtained from a commercial vineyard block that is located in a different *terroir* and with different *hombre influencia* and climatic conditions.

In other words, the observed effects of specific grapevine row orientations are not necessarily applicable to all environments. Further research is required to understand the relationships that exist among vine phenology, row orientation, and wine quality. The knowledge of phenolic compound concentration differences associated with a specific grape cultivar and therefore row orientation is important in oenology, because the phenolic compound concentrations can be controlled through vineyard practices and this can lead to a preferred phenolic composition in the wine and ultimately a desired wine style per cultivar.

5.10 Literature cited

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Chapter 6

GENERAL DISCUSSION AND CONCLUSIONS

6. General Discussion and Conclusions

6.1 Introduction

This study investigated the effects of grapevine row orientation (which directly affect canopy light profiles and intensity as well as general microclimate - Hunter *et al*., 2016), grape ripeness level and vintage on selected individual and combined phenolic compound concentrations of South African experimental Syrah grapes and wines. This has not been investigated before. Literature published on the effect of grapevine row orientation (and per implication light intensity or light interception by the grapevine canopy) on South African Syrah grape and wine quality is limited. Nadal & Hunter (2007) reported the effect of grape ripeness level on different wine styles in terms of total phenolics, anthocyanins and tannins, whereas Hunter & Volschenk (2008, 2017) investigated the implication of row orientation on total anthocyanins and total phenolic compound concentrations in Shiraz grapes. Guidoni & Hunter (2012) studied the effect of grape ripeness level on individual as well as total anthocyanins of Shiraz wines and Giacosa *et al*. (2015) showed the effect of grape ripeness on anthocyanin 3-*O*-glucosides as well as total anthocyanins of Shiraz grapes.

6.2 Overview of research

This study characterised the phenolic compound concentration variations in grapes and wines associated with experimental Syrah grapevines planted to NS, EW, NE-SW and NW-SE row orientations and of which the grapes were harvested for four consecutive years at ripeness levels of *ca*. 22°Brix, *ca.* 24°Brix and *ca.* 26°Brix. Further, in order to establish links, phenolic compounds, individually or combined were related to common wine sensorial attributes.

A reversed-phase liquid chromatography-diode array detection (RP-HPLC-DAD) method was used for phenolic compound quantification. Quantitative grape and wine sample data representing four consecutive vintages was subjected to analysis of variance (ANOVA) to establish significant differences among treatments, grape ripeness levels and vintages. Principal component analysis (PCA) was used to identify associations between the measured variables and differentiation of the grape and wine samples as a function of treatment.

A published RP-HPLC-DAD method was used to quantify a total of 23 phenolic compounds in lyophilised Syrah grape skin samples and 24 phenolic compounds in experimental Syrah wine samples, *i.e*. anthocyanins, flavan-3-ols, flavonols and phenolic acids (Waterhouse *et al*., 1999). The method was validated using a set of 17 phenolic compound reference standards. Identification of acetylated and coumaroylated anthocyanins, for which standards were not available, was performed by RP-HPLC-DAD-electrospray ionisation-mass spectrometry (ESI-

MS) in positive ion mode. These anthocyanins were identified by their molecular ions $[M]^+$ and assigned to their corresponding peaks of the UV-visible chromatogram.

Validation data confirmed that the RP-HPLC-DAD method was suitable for the routine quantification of the target analytes in lyophilised Syrah grape skin samples and experimental Syrah wine samples. The preparation for lyophilised grape skin samples required only a short extraction procedure followed by filtration of the supernatant through a 0.22µm membrane filter, whereas wine samples were directly injected after filtration. Lyophilised grape skin samples were preferred for the analysis, because it would eliminate interfering compounds from grape seeds and flesh. The single, 90 minute RP-HPLC run time allowed the analysis of 27 different phenolic compounds, although only 23 (in grape skin) and 24 (in wine) could be quantified.

6.3 Conclusions from research findings

Row orientation treatment differences were distinguishable by liquid chromatographic and sensory analysis, as confirmed by the application of ANOVA and PCA. Although individual phenolic compound concentrations were strongly and consistently affected by row orientation treatment (per implication light interception/microclimate), differences among treatments were not the same for each grape ripeness level. Variables that were associated with treatments were also not similar at all three ripeness levels. Grape ripeness level therefore exerted a very definite effect on the phenolic compound profile obtained for each row orientation treatment. The effect of vintage on phenolic compound concentrations was limited to only single phenolic compounds and vintage did not affect the general trend of the data. The 2011 vintage seemed to have affected phenolic compounds in grapes harvested at *ca*. 22°Brix and particularly at *ca.* 26°Brix, whereas the 2010 vintage affected phenolic compound concentrations at *ca*. 24°Brix ripeness.

Phenolic compound concentrations of grapes as well as of wines from the four row orientation treatments showed notable differences and trends in concentration as a function of grape ripeness levels. Grapes of the NS row orientation treatments showed a steady increase in flavan-3-ol concentration as the grapes ripened. Flavonol concentrations on the other hand showed a slight increase with ripening in grapes from the EW row orientation. Phenolic acid concentrations decreased for the NE-SW row orientation treatments as grapes ripened further.

Wines from grapes of the EW row orientation treatments showed a steady increase in flavan-3-ol concentration as the grapes ripened. Flavonol concentrations in wines from grapes of the four row orientation treatments did not show any trends. Phenolic acid concentrations increased in wines from grapes of the EW and NS row orientation as grape ripen but phenolic acid concentrations in wines from grapes of the NE-SW row orientation treatments, decreased with grape ripening. Wines from the EW and of the NS row orientation treatments increased in anthocyanins as grapes ripen further. Wines of the EW row orientation treatments showed a steady increase in flavan-3-ol concentration as the grapes ripened. Flavonol concentrations in wines of the four row orientation treatments did not show any trends.

Phenolic acid concentrations increased in wines from grapes of the EW and NS row orientation as grape ripen but phenolic acid concentrations in wines from grapes of the NE-SW row orientation treatments, decreased with grape ripening. Wines from the EW and of the NS row orientation treatments increased in anthocyanins as grapes ripen further. A tendency in anthocyanin concentrations in wines of the NE-SW and NW-SE row orientation treatments over ripeness was not apparent. The level of light exposure of grape bunches from grapevines planted to NE-SW and NW-SE row orientations seemed optimal for berry anthocyanin development and ultimately for improved wine quality.

Differences in sensory attributes as a function of the row orientation associated with individual phenolic compounds were evident in wines made from grapes of the four row orientations treatments. Phenolic compound concentrations of grape and wine samples, in combination with the wine sensory attributes, showed an association between certain variables from the same treatment, notably (+)-catechin, (-)-epicatechin/epigallocatechin 3-*O*-gallate with tannin intensity/body mouthfeel, and anthocyanins with overall quality. An association was also evident between caffeic acid/*p*-coumaric acid/ferulic acid and tannin intensity/body mouthfeel/overall quality. An association between (+)-catechin/epigallocatechin-3-*O*-gallate/total anthocyanins and tannin intensity/body mouthfeel/colour intensity and overall quality was also shown.

Analysis of variance and PCA showed that it was possible to group the different row orientations according to certain measured variables. Therefore, wine phenolic compound concentrations were affected by row orientation treatments and ripeness levels. Wine quality was associated with anthocyanins, and combinations of flavan-3-ols and phenolic acids.

Despite the complexity of various impacting factors and different phenolic compounds, the results showed that it is likely that a chosen grapevine row orientation, in combination with a grape ripeness level, may affect the wine style. The anthocyanins, flavan-3-ols, flavonols and phenolic acids for such wine styles may therefore be identified. Although such compounds are dependent on the environment and cultivation conditions, the study showed that individual phenolic compounds could be of great value in the separation of wine styles.

Subsequent research is needed to increase understanding of the relationships between vine phenology, light regimes (microclimate), diurnal temperature, vine row orientation, and other viticulture practices with grape and wine phenolic profiles and wine quality. The knowledge gained on the phenolic compound concentration differences in the experimental Syrah wine in this study and the association with row orientation is important in oenology. Phenolic concentrations are clearly affected by vineyard practices (e.g. row orientation and harvesting time, as in this study), which can lead to a desired wine style. However, the phenolic content of grapes and ultimately of wine, is affected by multiple factors, including climate (*terroir*), grape cultivar, viticulture practices, degree of grape ripeness, berry size, etc., all of which must be considered when a specific wine style is intended. The relationship among grape- and wine phenolic compounds and wine sensory attributes, *i.e*. overall quality, was therefore neither simple nor direct.

Within the context of global warming, many wine growers are already facing the problem of loss of colour development in grape skin due to higher temperatures and severe sunlight exposure after canopy opening, leading to sunburn on grape-berry skin tissue as well as inhibition of the biosynthesis of certain phenolic compounds, such as anthocyanins and flavan-3-ols. Row orientation has proved to be one of the viticultural practices enabling successful ("natural") canopy microclimate manipulation, leading to important grape quality improvements. There is however still limited information available on its effect on wine quality from different grape cultivars.

Results that emanated from this investigation are applicable to wines from grapes planted to specific row orientations in clayey loam soil and specific *terroirs*, trained to a VSP trellis system. Commercial vineyard blocks, different *terroirs* and the *hombre influencia* may however have a different effect on the eventual grape quality. In other words, a specific grapevine row orientation is not necessarily applicable to all environments. Further research is needed to comprehend the associations that exist among vine phenology, row orientation (microclimate/light penetration/light intensity) and wine quality.

The knowledge of phenolic compound concentration differences of experimental Syrah wine and the phenolic concentration differences associated with a grape cultivar is important in oenology, because the phenolic concentrations can be manipulated by means of vineyard practices and this can lead to improved wine quality or a desired style of wine.

6.4 Limitations and weaknesses of the study

The chemical and sensory results should be viewed as applicable to Syrah grapes grown on a flat *terroir*, trained to a Vertical Shoot Position trellis system at high summer temperatures and in clayey loam soil. Different results may be obtained in vineyard blocks with different *terroirs.* Considering the results, grapevine row orientation should be adapted to the specific environment in which grapes will be grown. Phenolic compounds reported in this study represent only a small portion of the grape and wine phenolic compounds. Importantly, oligomeric flavanols (tannins) were not analysed, and these compounds are responsible for astringency/bitterness perception. Nevertheless, some correlation was observed between levels of quantified flavan-3-ols, *i.e*. (+)-catechin, (-)-epicatechin and epigallocatechin 3-*O*-gallate, and body mouthfeel, indicating that these are good indicators of total tannins. In addition, phenolic acids, *i.e*. only free acids, were quantified, but since their levels are related to those of the esters, the effect of row orientation on phenolic acid content might be obscured. There was however a relationship between phenolic acids measured and sensory attributes, *i.e*. body mouthfeel and overall quality.

6.5 Strengths of the study

This is the first study in South Africa (and to a large extent, in the world) showing that row orientation, which leads to different light interception (microclimate) in the grapevine fruiting zone, in combination with grape ripeness level, affect the individual phenolic compound concentrations of Syrah grapes and wines.

Most phenolic compounds, individually or combined, were related to common wine sensorial attributes, such as body, astringency, colour intensity and overall quality.

The chemical results point the way forward to controlling phenolic concentrations in grapes and wine, and thereby regulating wine style, through vineyard practices. This study confirmed that a simple and reliable RP-HPLC-DAD method can be applied to quantify a range of anthocyanins, flavan-3-ols, flavonols and phenolic acids in lyophilised grape skin and wine samples. This method may not be restricted to Syrah only, but may well be equally applicable to the grapes and wine of other cultivars.

6.6 Recommendations

The results of the study suggest that prolonged exposure of Syrah grape bunches to direct sunlight in warm grape growing regions should be avoided in order to achieve a preferred phenolic compound composition and expression in Syrah grapes and wine. Conversely, excessive shade in the fruit-bearing zone of the vine should also be avoided, In the event of viticultural practices that result in NW-SE row orientations, increased anthocyanin concentrations (colour) may be achievable, with wines having increased body mouthfeel, tannin intensity and overall quality, followed by wines from NE-SW row orientation treatments. Certain phenolic acids, *i.e.* hydroxycinnamic acids, were also highest in wines from NW-SE (caffeic acid and *p*-coumaric acid) and NE-SW (caffeic acid and ferulic acid) row orientations and the wines were judged as having good body mouthfeel and overall quality. Increased flavan-3-ol and hydroxybenzoic/cinnamic acid (phenolic acid) concentrations at the time of harvest may result when grapevines are planted to NS [(+)-catechin, (-)-epicatechin, gallic acid and *p*-coumaric acid] and EW (epigallocatechin 3-*O*-gallate, ferulic acid and gallic acid) row orientations, which may have contributed to body, astringency and perceived bitterness. This may either positively or negatively impact on the sensory attributes of red wine (depending on the winemaking conditions and the wine style required). Syrah wines made from grapes of NW-SE row orientation treatments were found to be of highest quality, followed by wines made from grapes of NE-SW row orientation treatments. Wines made from grapes of the NS orientation showed intermediate quality, whereas an EW row orientation resulted in lowest quality wines.

The specific soil type and other *terroir* conditions, such as climatic factors, also mediate the effect of row orientation and ripeness level on Syrah grape phenolic compound concentrations and wine quality. These parameters exert an independent and mostly indirect effect on Syrah grape berry ripening and ultimately on Syrah wine quality and need to be considered in attempts to manage phenolic composition and levels in the vineyard.

6.8 Literature cited

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