Sensory characterisation of several red cultivar (*Vitis vinifera* L.) wines, using berry sugar accumulation as a physiological indicator and sequential harvest

by

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Summary

The global wine industry has shifted to a more adopted 'consumer-preference' production. Modern wine consumers are more knowledgeable and cultivated in their understanding of wine quality, value and style. The quality of red wines mainly depends on grape composition, the wine making process and the ability of tasters to recognise sensory attributes. The harvest date/stage has an influence on the grape composition, thus making the decision on when to harvest an important factor in the production of quality wines or different wine styles.

The traditional indicators used in the wine industry to determine time of harvest are more related to the perception of taste and mouthfeel and give little indication of the style of wine in terms of aromatic profile. A new physiological indicator using berry sugar accumulation for the purpose of sequential harvest is proposed to assist the winemaker in producing wines with possible different sensory profiles. This indicator can be used in association with the classical indicators of ripening to affect the diversity of wine styles from a single vineyard or a group of vineyards. The wines could thus have different potential aroma profiles, depending on when the grapes were harvested.

The main aim of this study was to assess the effect of performing sequential harvest using a physiological indicator on red wine's sensory composition. This was done to study the possible relation between harvest time (e.g. fruit composition evolution) and the wine styles/sensory attributes across the different harvest times, thereby possibly increasing the diversity of wine styles. A theoretical berry sugar loading concept was compiled and displays a phase of rapid sugar loading starting at véraison followed by a plateau phase. Depending on whether grapes were harvested in the beginning, mid or end of the plateau phase of fruit sugar accumulation the wines could have different potential aroma profiles. Three main stages: fresh fruit (FF), neutral (N) or pre ripe and mature fruit (MF) has been previously proposed using the sugar loading concept and in terms of harvesting dates.

Cabernet Sauvignon and Merlot grapes form Eikendal Vineyards, Stellenbosch were used to make wines according to sequential harvest. Four harvest stages were considered, pre fresh fruit (Pre FF), fresh fruit (FF), mature fruit (MF) and over ripe (OR). The wines were tasted and analysed using two different sensory techniques. In both Merlot and Cabernet Sauvignon wines, the PreFF and OR stages could be more easily discriminated than the two harvest stages in-between, FF and MF. The results suggested that the wines made from the FF and MF stages could not be distinguished from each other in general when the attribute citation frequency method or sorting tasks were performed. However, a trend could be observed for both Cabernet Sauvignon and Merlot wines in terms of aroma attributes with attributes changing from green to ripe fruit during ripening using expert tasters. Relevant research should be

engaged to refine sequential harvest in order to obtain more diverse wine styles from a single site or a group of vineyards.

Opsomming

Die wêreldwye wynbedryf het ’n verskuiwing ondergaan na ’n verbruikersvoorkeurbenadering in produksie. Wynverbruikers is deesdae beter ingelig en meer ontwikkeld ten opsigte van hulle kennis van wyngehalte, wynstyl, asook die waarde van wyn. Die gehalte van rooiwyn hang hoofsaaklik af van die druifsamestelling, die wynmaakproses en die vermoë van proewers om sensoriese eienskappe te herken. Aangesien die oesdatum/-fase ’n invloed het op druifsamestelling, is die besluit oor wanneer daar geoes moet word ’n belangrike faktor in die vervaardiging van gehaltewyne of verskillende wynstyle.

Die tradisionele aanwysers wat in die wynbedryf gebruik word om oestyd te bepaal, hou verband met die waarneming van smaak en mondgevoel en gee weinig aanduiding van die wynstyl op grond van die aromatiese profiel. ’n Nuwe fisiologiese aanwyser wat gebruik maak van suikerakkumulasie in die druiwekorrel in opeenvolgende oeste, het ten doel om die wynmaker te help om wyne met verskeie moontlike sensoriese profiele te vervaardig. Hierdie aanwyser kan saam met die klassieke aanwysers van rypwording gebruik word om ’n verskeidenheid wynstyle uit een wingerd of wingerdgroep te vervaardig. Die wyne kan dus potensieel oor verskillende aromatiese profiele beskik, afhangend van wanneer die druiwe geoes is.

Die hoofdoel van die studie was om die invloed van opeenvolgende oes te toets deur ’n fisiologiese aanwyser op rooiwyn se sensoriese samestelling toe te pas. Dit word gedoen deur die moontlike verhouding tussen die oestyd (bv. ontwikkeling van vrugsamestelling) en die wynstyle of wyn se sensoriese kenmerke op verskillende oestye te bestudeer ten einde die verskeidenheid wynstyle potensieel te vermeerder. ’n Teoretiese konsep van druifsuikeropname is saamgestel wat dui op ’n fase van vinnige suikeropname wat by véraison begin, gevolg deur ’n plato-fase. Wyn kan oor verskillende moontlike aromatiese profiele beskik, afhangend daarvan of die druiwe aan die begin, middel of einde van die plato-fase van suikeropname geoes is. Drie hooffases is al voorheen voorgestel deur gebruik te maak van die konsep van suikeropname volgens oesdatum, te wete vars vrugte (VV) (“fresh fruit”, FF), neutraal (N) (“neutral”, N) of voor ryp (“pre ripe”), en ryp vrugte (RF) (“mature fruit”, MF).

Cabernet Sauvignon- en Merlot-druiwe van Eikendal, Stellenbosch, se wingerde is gebruik om wyn volgens opeenvolgende oes te maak. Vier oesfases is oorweeg, te wete voor vars vrugte (VVV) (“pre fresh fruit”, Pre FF), vars vrugte (VV) (“fresh fruit”, FF), ryp vrugte (RV) (“mature fruit”, MF), en oorryp (OR) (“over ripe”, OR). Die wyn is geproe en geanaliseer deur gebruik te maak van twee verskille sensoriese tegnieke. In die geval van beide die Merlot- en Cabernet Sauvignon-wyn kon die VVV- en OR-fases makliker onderskei word as die twee tussenin-fases, VV en RV. Resultate dui daarop dat wyn wat van die VV- en RV-fases gemaak is, oor die algemeen nie van mekaar onderskei kan word wanneer die frekwensie van

kenmerkaanhaling-metode en sorteringstaak uitgevoer word nie. ’n Tendens kon egter waargeneem word vir Cabernet Sauvignon- én Merlot-wyn ten opsigte van aromatiese kenmerke, deurdat kenmerke gedurende rypwording van groen na ryp vrugte verander het indien ekspertproewers gebruik is. Verdere navorsing moet gedoen word om opeenvolgende oes te verfyn ten einde ’n wyer verskeidenheid wynstyle van ’n enkele area of wingerdgroep te verkry.

This thesis is dedicated to

My family and friends for their continuous support, encouragement and motivation

Biographical sketch

Marisa Nell was born on 16 May 1983 in Port Elizabeth and matriculated at Brandwag High School, Uitenhage in 2001. Marisa obtained a Bachelors degree in Agricultural Science (Viticulture and Oenology) in 2005 at Stellenbosch University. After working at the University of Stellenbosch for 5 years as technical officer in the experimental cellar, in 2012 Marisa enrolled for a Masters degree in Oenology at the same University.

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Preface

This thesis is presented as a compilation of 4 chapters.

Chapter 1	**General Introduction and project aims**
Chapter 2	**Literature review** Berry development, parameters to assess grape optimum ripening, volatile and non-volatile compounds responsible for aroma and flavour in red wine and sensory characterisation methods
Chapter 3	**Research results** Sequential harvesting of red cultivars using berry sugar accumulation and the relationship with wine sensory attributes
Chapter 4	**Conclusion**
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Chapter 1

Introduction and project aims

1. INTRODUCTION AND PROJECT AIMS

1.1 Introduction

Salvador Dali once said: "*Quien sabe degustar no bebe jamás el vino, sino que degusta secretos*". This translates to: "*He who knows how to taste never again drinks wine but tastes its secrets*". With a global tendency evolving to prioritise customers' needs, the global wine industry has shifted to a more adapted 'consumer-preference' production, focusing to create these "secrets" that will lead the consumer to enjoy the sensory expectations in each glass of wine (Bisson et al., 2002; Lesschaeve, 2007).

The South African wine industry has changed to such an extent that quality is now defined as sustainable customers and consumer satisfaction (Pretorius & Bauer, 2002). The modern wine consumer has quick and easy access to wine information, making him more knowledgeable and cultivated in their understanding of wine quality, value and style. The power to define wine quality thus no longer lies with only the wine producer but also with the consumer (Bisson et al., 2002).

Quality of red wines mainly depends on the grape composition, wine making process and the ability of the tasters to recognise sensory attributes. Well-balanced sugars and acids, phenolic compounds and aroma precursors are closely related to the production of quality wines. These compounds accumulate in the grape berry during different stages of the ripening process and are influenced by climate and soil (site) as well as viticultural practices such as irrigation (Jackson and Lombard, 1993; Jones and Davis, 2000; Conde et al., 2007). Grape quality will thus be determined by the ripening process and the decision when to harvest will thus be an important factor in the making of quality wine or different wine styles (Du Plessis, 1984; Hamilton and Coombe, 1992; Pérez-Magariño and González-San-José, 2006; Boss et al., 2014).

Traditional indicators used in the wine industry to determine time of harvest include sugar concentration, sugar / acid ratio, glycosyl-glucose index, phenolic compounds and physical properties like the firmness and deformability of the berry, the colour of the stems and the colour, texture and brittleness of the seeds (González-San José et al., 1991; Jackson & Lombard, 1993; Boulton et al., 1996; Francis et al., 1998; Bisson, 2001; Conde et al., 2007). However, these indicators are more related to the perception of mouthfeel and give little indication of the style of wine in terms of aromatic profile (Deloire, 2011). New physiological or morphological indicators are required which can assess the harvest date/time in relationship with the wine aromatic profiles. Therefore, in association with the classical indicators of ripening, Deloire (2011, 2012, 2013_{ab}) suggested a new physiological indicator using berry sugar accumulation for the purpose of sequential harvest. The method is based on the fact that during

the ripening process there is an evolution of the fruit aromatic composition in parallel with the evolution of the other classical compounds such as sugar, organic and amino acids and phenols (Coombe, 1992; Conde et al., 2007; Boss et al., 2014). Berry sugar loading was defined by Deloire (2011, 2013$_b$) as “the evolution of the quantity of sugar per berry, expressed as mg per berry, from véraison onwards”.

A theoretical berry sugar loading concept (Figure 1.1) based on data obtained over five years using at least 20 different red grape varieties in mainly France, Spain, Argentina, Chile and South Africa was compiled and displays a phase of rapid sugar loading starting at véraison followed by a plateau phase. The plateau is reached when the rate of sugar loading is ≤ 3 mg/berry/day. Key point (KP) or Day 0, corresponds with the beginning of the sugar loading plateau or slowdown of accumulation (Deloire, 2011). Sequential harvest (Figure 1.2) allows, using a physiological indicator, to affect the diversity of wine styles from a single vineyard or a group of vineyards (Deloire, 2011; Bindon et al., 2014; Boss et al., 2014).The wines could thus have different potential aroma profiles, depending on whether grapes were harvested in the beginning, mid or end of the plateau phase of fruit sugar accumulation. Wines made from these stages could be characterised by fresh fruit/green plant like/unripe plum flavours (beginning of the curve), a possible neutral-spicy flavours (mid curve) or pre mature stage, and mature fruit/blackcurrant, raspberry, cherry flavours (end of the curve), followed by an over ripe stage characterised by dried fruit/ prune flavours (Deloire, 2012).

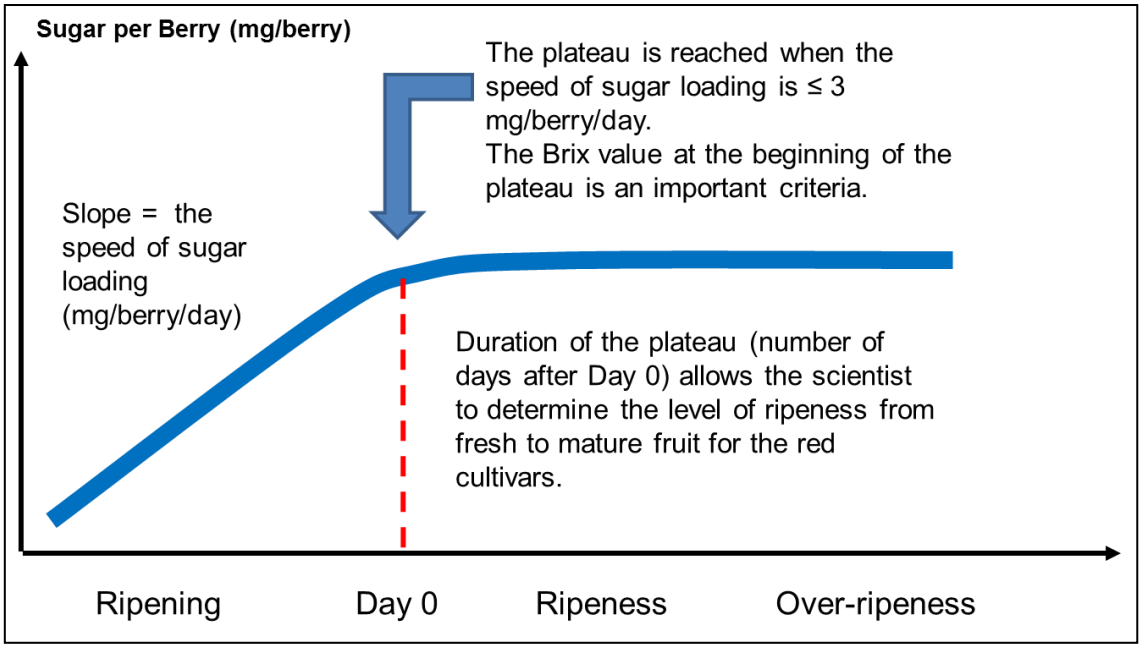

Figure 1.1: Berry sugar loading concept: a few principles (Deloire, 2013$_b$).

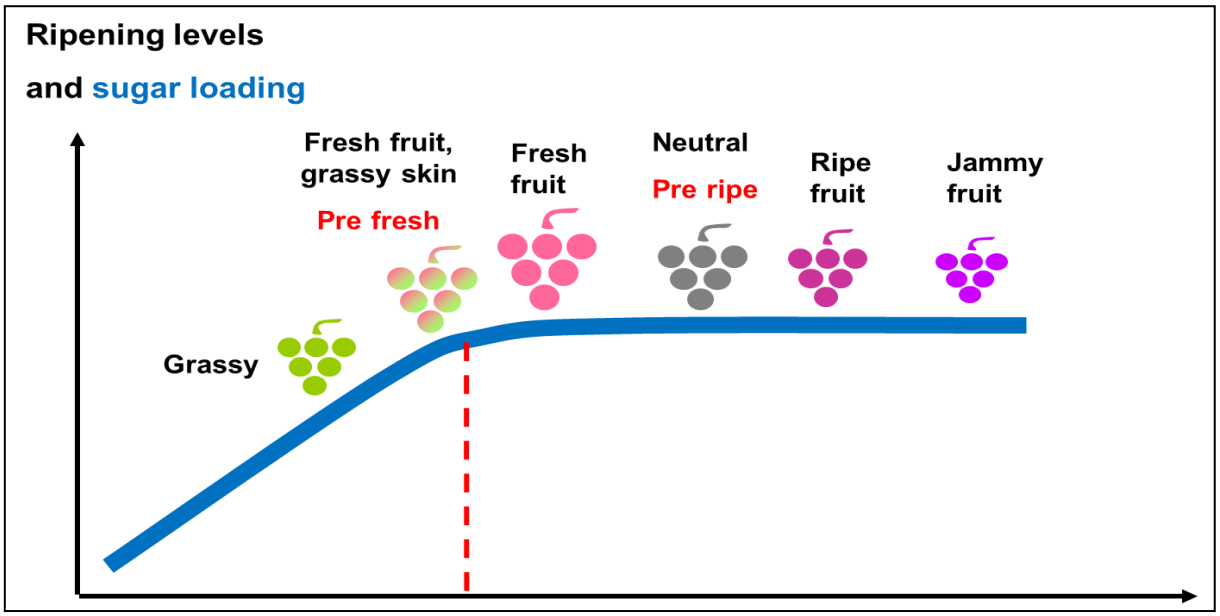

Figure 1.2: The possible evolution of red wine aromatic profiles indirectly correlated to berry sequential harvest using fruit sugar accumulation as a physiological indicator (Deloire, 2013_b).

According to Deloire (2011) three main stages: fresh fruit (FF), neutral (N) or pre ripe and mature fruit (MF) have been identified using the sugar loading concept and in terms of harvesting dates, these stages were determined according to the number of days after the key point. FF stage occurs 10 to 20 days and MF stage 20 to 40 days after sugar per berry has reached a plateau or slowed down, depending on the cultivar. The N stage (which is characterised by a deficiency of fruitiness) is between the FF and MF stages, in some situations this stage is closer to pre mature. Figure 1.3 shows an example of the possible aromatic stages for Cabernet Sauvignon.

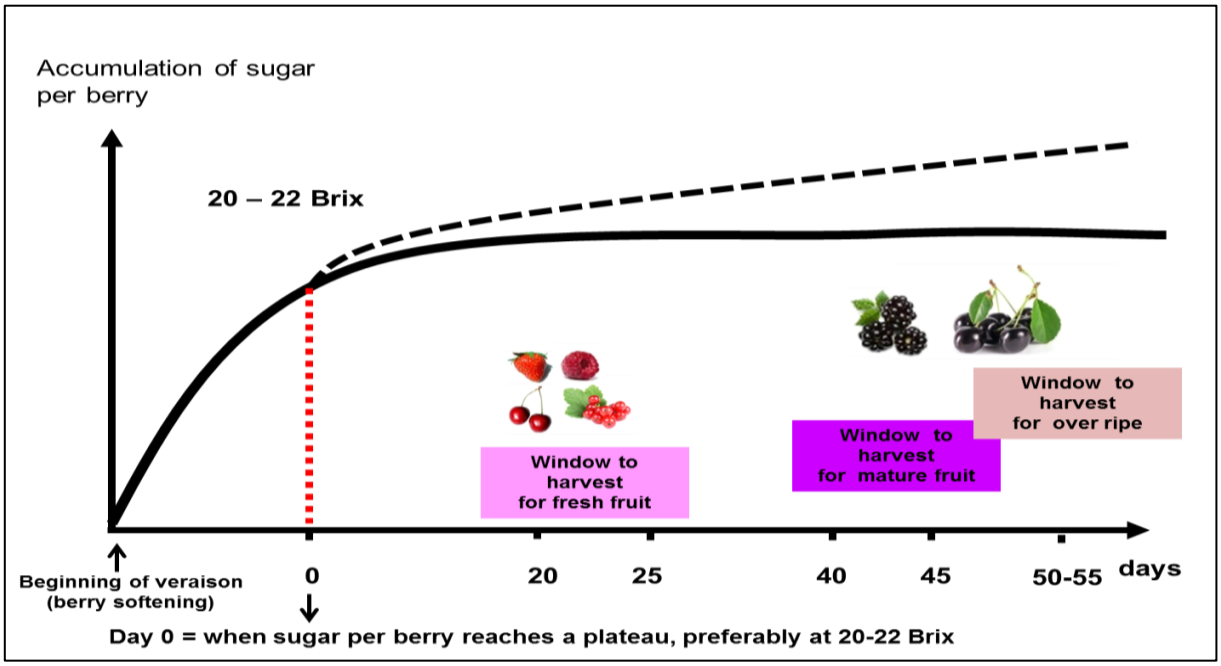

Figure 1.3: Example of Cabernet Sauvignon wine potential aromatic profiles from sequential harvest using berry sugar accumulation as a physiological indicator to assess the harvest time (Deloire, 2013_a).

However, it should be noted that the stages as classified according to sequential harvest have to be studied under the South African conditions and need a calibration phase before it is used by the wine industry. The main aim of this study was thus to test the hypothesis that wine sensory attributes can be influenced by the fruit ripening stages (sequential harvest). For this purpose, a new sensory evaluation technique, frequency of attribute citation, was introduced to the Department Viticulture and Oenology, Stellenbosch University (McCloskey et al., 1996; Piombino et al., 2004).

1.2 Project aims

The specific aims of this project were as follow:

a) to assess, using sensory evaluation, whether there are quantifiable sensory differences between wines made from sequential harvested grapes using a fruit physiological indicator for some red cultivars under South African conditions;
b) to define the core sensory attributes for Merlot and Cabernet Sauvignon wines made from sequential harvesting.

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Chapter 2

Literature review

Berry development, parameters to assess grape optimum ripening, volatile and non-volatile compounds responsible for aroma and flavour in red wine and sensory characterisation methods.

2. LITERATURE REVIEW

2.1 Introduction

The chemical composition and therefore the quality of grape berries for winemaking are influenced by the vine's genotype, the environmental conditions and viticultural practices. The aromas of wine are not dependent on a specific aroma compound but rather on the interaction of different odour-active compounds extracted from grape berries or formed during fermentation and wine ageing. Optimum ripeness of grape berries for winemaking purposes is a critical criterion that will have a direct influence on the style and quality of wine produced. This chapter will summarise the different volatile and non-volatile compounds responsible for aroma and flavour in red wine, their interactions and the viticultural and winemaking practices that can affect their concentrations in wine, as well as the sensory methods used to characterise them.

2.2 Berry development and sugar accumulation

Berry development after flowering can be divided into three stages: green berry growth (herbaceous phase), a lag phase before véraison and the ripening stage from véraison (berry softening and coloration) onwards. Véraison is an important developmental stage because it is the onset of anthocyanins accumulation for the red cultivars. The up and down regulation of other important compounds also occur during ripening (Coombe, 1992; Patrick 1997; Bondada et al., 2005; Zhang et al., 2006).

2.2.1 Berry development

The grape berry consists of three major types of tissue/organ: skin, flesh and seeds. During ripening the berry undergoes modification in size, composition, colour, texture and flavour (Conde et al., 2007). Grape berry development consists of two sigmoid cycles as shown in figure 2.1 (Coombe, 1992). The first cycle is characterised by a rapid growth period during which the ovary starts cell multiplication after fecundation and the growth continues with cell enlargement and seed formation. During this stage, tartaric, malic, and hydroxycinnamic acids as well as tannins accumulate in the berry. The first stage is followed by a lag phase during which no growth takes place. The length of the lag phase is site and cultivar dependent. The end of the lag phase corresponds with the end of the herbaceous phase of the berry (Conde et al., 2007) and coincides with the onset of ripening, the second sigmoid stage. Véraison, a French word that has been used to describe the onset of ripening, is the beginning of fruit

maturation and is characterised by the softening of the grape berry and skin colouring due to the biosynthesis of anthocyanins in red cultivars.

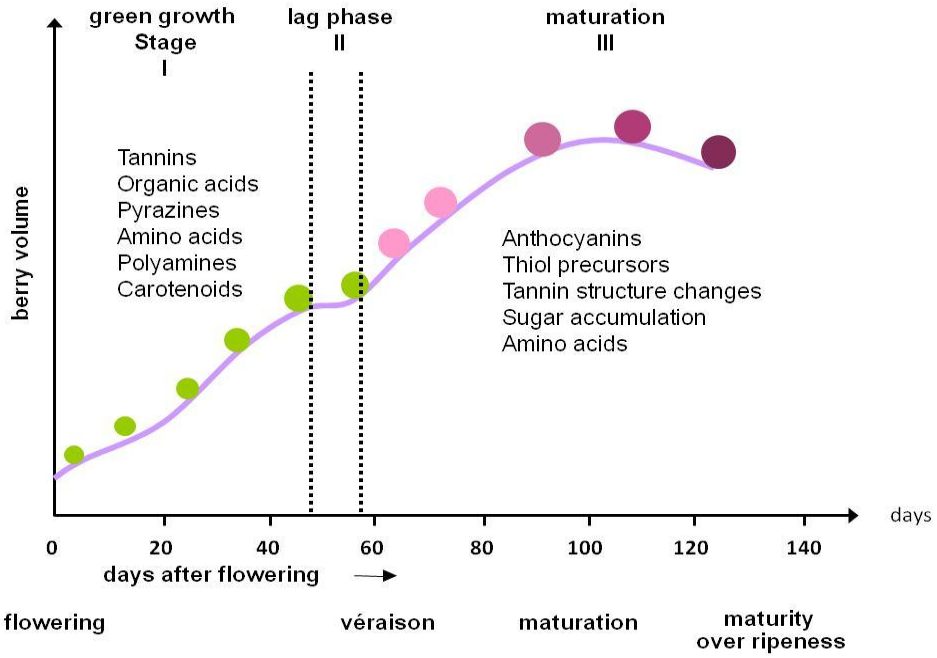

Figure 2.1: Grape berry development.

2.2.2 Sugar accumulation

During grape berry ripening and the onset of the second sigmoid phase, sucrose is transported mainly from the leaves and accumulates in the berry vacuoles as glucose and fructose (Kennedy, 2002). Transport of sugar, water, minerals, hormones and nutrients occur via the vascular system that consists of xylem and phloem. Xylem is functional in the grape berries in the early phases of ripening until véraison where after its function is reduced. Phloem has reduced function early in grape berry ripening, but becomes the main source of transport after véraison (Coombe &McCarthy, 2000).

2.2.3 Parameters to assess grape optimum ripening

Determining grape optimum ripeness is a critical decision in winemaking as it will have a direct influence on the quality of wine produced. What exactly constitutes optimum ripeness depends upon the style and category of wine produced (sparkling, still, fortified, rosé, dessert wine) as well as factors such as cultivar, clone, rootstock, cultural practices and site (climate x soil). Deciding on a harvest date will also be influenced by factors beyond the winemaker's control such as seasonal changes (rain, heat waves) as well as tank space and logistic considerations such as labour availability in the cellar (Bisson, 2001).

2.2.3.1 Sugar concentration

Sugar concentration increases during ripening and thus gives a good indication of berry ripening. Sugar concentration (Brix) is the most common parameter used to determine ripeness and measuring the brix (ºB) of berries is fairly easy using a refractometer or a hydrometer. The sugar concentration level alone however is not a good indication of optimal ripeness seeing that acid levels decrease during ripening and a balanced sugar / acid ratio is needed to make a good quality wine (Jackson & Lombard, 1993). Sugar concentration gives a good indication of the ethanol level in the corresponding wine. A conversion factor of 0.59 has been proposed (Marsh, 1958).

2.2.3.2 Sugar / acid ratio

Acidity, measured as titratable acidity, can also be used to determine the harvest date. An historic index of ripeness proposes that optimal sugar / acid ratio is achieved when the product of the Brix (ºB) value x pH^2 is in the range of 220 to 260. However, the sugar / acid ratio differs across cultivars and sites and might be too general as indicator of wine quality. It is also not clear if the optimal ripeness of grape flavorants corresponds with the optimal sugar (ethanol) / acidity ratio (Bisson, 2001). High malic acid levels in grape berries are also an indication of poor ripening (Boulton et al., 1996).

2.2.3.3 Glycosyl-glucose index

The glycosyl-glucose (G-G) index is an analytical tool that can be used to analyse the composition of grapes, juice and wine. The G-G index measures the concentration of glycosides in the grapes, which have flavour potential in wine and could therefore be used as an indication of grape quality (Francis et al., 1998).

2.2.3.4 Phenolic compounds

Phenolic compounds in the grape berry can be divided into two groups of compounds; the flavonoids and the non-flavonoids. Flavonoids are the most important group and include proanthocyanidins (tannins), anthocyanins and flavan-3-ol monomers. Tannins are polymers of flavan-3-ols (Kennedy et al., 2006).

Phenolic compounds contribute directly to the quality of red wine due to their contribution to sensory characteristics. Anthocyanins are responsible for colour in red grapes and young wines and flavonoid phenols are the main compounds responsible for bitterness and astringency in wine (Somers, 1970; Noble, 1994). The concentration of phenolic compounds in

the grapes will depend on the cultivar and is influenced by the growing environment and viticultural practices.

The concentration of phenolic compounds increases during berry development. Anthocyanin accumulation in the skin starts at the onset of véraison and increase during berry ripening (Pirie and Mullins, 1979). It has been reported that anthocyanins can decline late in berry ripening (Kennedy et al., 2002). Anthocyanin accumulation is closely related to sugar accumulation (Boss and Davies, 2001). A small portion of the grape tannins are located in the skin while the most significant part is located in the grape seeds. Skin tannins are synthesised early in the grape berry development (before véraison) and the quantity per berry does not change significantly during berry ripening, however, their concentration decline during berry growth due to possible increase in berry volume. Seed tannins tend to decline during berry ripening, possibly due to tannin oxidation causing the grape seeds to turn brown (Adams, 2006).

Phenolic compounds are extracted from grapes during winemaking practices and according to Cagnasso et al., (2008) and Du Toit and Visagie, (2002) a correlation exists between the anthocyanins and flavonoid indexes of grapes and colour indexes of wines. Phenolic compounds can thus be used as an indication of ripening as well as of the quality of the grapes and resulting wines (González-San José et al., 1991; Conde et al., 2007).

2.2.3.5 Physical properties

Physical properties like the firmness and deformability of the berry and the colour of the stems can also be assessed as indicators of ripeness. It is common practice in the vineyard to taste the seeds in order to assess berry maturity, however the colour, texture and brittleness of the seed might be better indicators of maturity (Bisson, 2001).

2.3 Flavour development

Wine flavour is very complex due to the chemical composition and the molecular interactions between wine components. Aroma and flavour compounds develop in the grape berry during ripening and consist of both volatile aromatic compounds as well as non-volatile aroma precursors. These compounds can change in concentration and complexity during berry ripening and are extracted during winemaking practices. The non-volatile fraction of wine that mainly contributes to taste and tactile sensations are deemed just as important as the volatile composition due to their influence on wine quality/style (Sáenz-Navajas et al., 2010).

2.3.1 Contribution of volatile composition to red wine flavour

Volatile compounds such as higher alcohols, esters, monoterpenes, C_{13} norisoprenoids, methoxypyrazines and volatile sulphur compounds are responsible for wine aroma. Some compounds such as methoxypyrazines are present in the grape and juice in the volatile, free form, however most aroma compounds in grapes are in a bound form (non-volatile). The bound compounds (glycosides) consist of a volatile aglycone bound to a sugar molecule. These glycosides are non-volatile and therefore odourless and must undergo acid and/or enzymatic hydrolysis to release the volatile aglycons, which will in turn contribute to the aroma of the wine (Marais, 1983).

2.3.1.1 Methoxypyrazines

Methoxypyrazines are found predominantly in the skin of grape berries and contribute to the vegetative/herbaceous aroma of wines made from Sauvignon blanc, Merlot, Cabernet Sauvignon, Cabernet Franc and Carmenere grapes. Three methoxypyrazines, 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 2-*sec*-butyl-3-methoxypyrazine (SBMP), have been identified in grapes and wines (Lacey et al., 1991). IBMP, a very odorous compound with a sensory threshold of 2 ng/L in water (Buttery et al., 1969), is predominant in grapes and depending on variety, clone, maturity and ripening conditions can be present at levels ranging from 4 - 30 ng/L. IBMP contributes to bell pepper, green and gooseberry aromas, IPMP to asparagus and green bean aromas and SBMP to pea and bell pepper aromas (Ebeler & Thorngate, 2009).

Methoxypyrazine levels decrease during maturation of the berry (Suklje et al., 2012) with a maximum concentration two to three weeks before véraison. Viticultural practices such as leaf removal in the fruit zone which changes bunch microclimate (light exposure and temperature), vine water status, and the vigour of the canopy can influence the concentration of methoxypyrazines in the grapes and wine (Sala et al., 2004; Belancic and Agosin, 2007; Ebeler & Thorngate, 2009). Methoxypyrazines are sensitive to light, thus early leaves removal, 10 days after flowering, will decrease the concentration in the grapes and wine (Scheiner et al., 2010). Grapes from cooler climate regions contain higher concentrations of methoxypyrazines than grapes from warmer climate regions. Oenological practices such as extended skin contact will increase methoxypyrazine levels in the must due to the highly soluble nature of the compound whiles clarification of the must can cause a decrease in concentration (Roujou de Boubée et al., 2002; Maggu et al., 2007). It has been reported by Marais (2001) that Sauvignon blanc wines made with yeast strains producing higher levels of esters were found to mask some of the green notes associated with methoxypyrazines.

2.3.1.2 Monoterpenes

Free and bound monoterpenes of which most are in the bound (glycoside) form, are found mainly in the skin of grape berries and contribute to the floral and citrus aromas of wines (Ebeler and Thorngate, 2009). They are considered varietal impact compounds for Gewürztraminer, Riesling and Muscat cultivars. More or less 50 monoterpenes of which the most prominent are geraniol, linalool and nerol have been identified in *Vitis vinifera* L. grapes and wine (Strauss et al., 1986).

Free and bound monoterpenes are formed in the grapes during ripening and their concentration is influenced by climate and viticultural practices (Park et al., 1991). Increased light exposure during ripening enhances the forming of monoterpene glycosides (Bureau et al., 2000). Extended skin contact at low temperatures, different pressing techniques, heat-treatment or pasteurization and the use of enzymes in the winemaking process will increase the concentration of monoterpenes in the wine (Marais, 1983; Marais, 1990; Park et al., 1991).

2.3.1.3 C_{13} norisoprenoids

C_{13} norisoprenoids are a diverse group of aroma compounds generated by carotenoid breakdown. They are present in grapes and wine at trace levels, but because of their very low sensory thresholds, they could have a large sensorial impact on wine aroma (Mendes-Pinto, 2009). The most abundant C_{13} norisoprenoids with sensory properties are β-damascenone (cooked apple/floral/quince), β-ionone (violet/woody/raspberry), vitispirane (camphorous/eucalyptus) and 1,1,6-tri-methyl-1,2-dihydronaphthalene (TDN).

β-damascenone has been identified as an important impact aroma compound in red wines. In wine this compound enhances the fruity aromas of esters and masks the herbaceous aromas of IBMP (Pineau et al., 2007). β-damascenone and β-ionone have sensory thresholds of 200 ng/L and 700 ng/L respectively (Ebeler and Thorngate, 2009).

Most of the C_{13} norisoprenoids are present in grapes as glycosides and needs to undergo hydrolysis during fermentation and storage in order to release the volatile aroma compounds. According to Francis et al. (1998), glycosidic precursors of norisoprenoids can contribute to the honey, tea and lime aromas of Cabernet Sauvignon wines whereas, acid hydrolysis during storage generates TDN, which is responsible for the kerosene aroma of aged Riesling wines (Winterhalter, 1991). A recent study done by Janusz et al. (2003), showed that (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB), with a sensory threshold of 40 ng/L in white wine, has a strong green or cut-grass aroma and studies suggest that this compound might belong to the C_{13} norisoprenoids class.

The concentration of carotenoids in the grapes and thus C_{13} norisoprenoids in wine is influenced by factors such as cultivar, light exposure (climate), stage of maturity, soil

characteristics and viticultural practices (Mendes-Pinto, 2009). Grapes from warm climate regions as well as unripe grapes (before véraison) that have been exposed to sunlight have higher concentrations of carotenoids. However, grapes exposed to sunlight during ripening (after véraison) show a decrease in carotenoid concentration. Carotenoids are synthesised before véraison and decrease during ripening.

2.3.1.4 Higher alcohols and esters

Higher alcohols are produced by yeast during alcoholic fermentation through the conversion of the branched chain amino acids. They may have a significant influence on the sensory characteristics of wine due to their strong and overpowering aromas (Lambrechts & Pretorius, 2000). At high concentrations (above 400 mg/L) generally found in wine they have a harsh and unpleasant aroma, but below 300 mg/L they contribute to the pleasant aroma attributes of wine (Rapp & Mandery, 1986). Table 2.1 lists some important higher alcohols produced by yeast during alcoholic fermentation (Lambrechts & Pretorius, 2000). The quantity of higher alcohols formed during alcoholic fermentation depends on viticultural practices, juice clarity as well as the type of yeast used.

Table 2.1: Concentrations, threshold values and odour linked to different higher alcohols generally found in wine (Lambrechts & Pretorius, 2000).

Compound	Conc. in wine (mg/L)	Threshold value (mg/L)	Odour
Propanol	9 – 68	500 (wine)	Stupefying
Butanol	0.5 – 8.5		Fusel
Isobutyl alcohol	9 – 28	500 (wine)	Alcoholic
Amyl alcohol	15 – 150	65 (beer)	Marzipan
Isoamyl alcohol	45 – 490	300 (wine)	Marizipan
Hexanol	0.3 – 12	4 (beer)	Resin, floral, green
Tyrosol	-	-	Bees wax, honey
Phenethyl alcohol	10 – 180	125 (beer)	Floral, rose

Higher alcohols are important precursors for ester formation during alcoholic fermentation. Esters contribute to the fruity aroma of young red wines and are one of the largest and most important groups of aromatic compounds found in alcoholic beverages. They are usually found above their sensory threshold values in wine. A specific aroma attribute is seldom associated with a single ester, but rather the result of a mixture of esters. The type of yeast, fermentation

temperature, the availability of yeast nutrients during fermentation, skin contact time, grape maturity, sugar content, juice clarity, pH, sulphur dioxide and cultivar have an important impact on the formation of esters (Lambrechts & Pretorius, 2000; Ebeler & Thorngate, 2009).

Saccharomyces cerevisiae produces fatty acid ethyl esters as well as acetate esters of different higher alcohols. Two of these esters are ethyl acetate and isoamyl acetate of which the latter is an important impact ester in young Pinotage wines (Van Wyk et al., 1979). Table 2.2 lists some esters produced by yeast (Lambrechts & Pretorius, 2000; Ebeler & Thorngate, 2009).

Table 2.2: Concentrations, threshold values and odour linked to different esters generally found in wine (Lambrechts & Pretorius, 2000; Ebeler & Thorngate, 2009).

Compound	Concentration in wine (mg/L)	Threshold value (mg/L)	Odour
Ethyl acetate	0 - 150	12.3	Solvent, nail polish, fruity
Isoamyl acetate	0.5 – 10	-	Banana, pear
2-Phenethyl acetate	0.01 – 4.5	-	Rose, honey, fruity,
Ethyl isovalerate	0 – 0.7	-	Apple, fruity
Isobutyl acetate	0.01 – 0.8	-	Banana
Ethyl butanoate	0.01 – 1.8	0.4 (beer)	Floral, fruity
Ethyl 2-methyl-butanoate	0 – 0.9	-	Strawberry, Pineapple
Ethyl hexanoate	Trace – 3.4	0.08	Apple, Banana, Violets
Ethyl octanoate	0.05 – 3.8	0.58	Pineapple, pear
Ethyl decanoate	Trace – 2.1	0.5	Floral

In a study done by Moio and Etievant (1995), four esters, ethyl anthranilate (sweet-fruity, grape-like aroma), ethyl cinnamate (cinnamon, sweet-balsamic, sweet-fruity, plum and cherry aromas), ethyl 2,3-dihydrocinnamate and methyl anthranilate (sweet-fruity, grape-like and floral aroma), were identified that contribute to the characteristic aroma profile of Burgundy Pinot Noir wines. Esters thus also contribute to the varietal characteristics of grape cultivars.

2.3.1.5 Volatile sulphur compounds

Volatile sulphur compounds such as hydrogen sulphide (rotten eggs), dimethyl sulphide (asparagus, corn, molasses), diethyl sulphide (cooked vegetables, onion, garlic) dimethyl disulphide (cooked cabbage, onion-like), diethyl disulphide (garlic, burnt rubber), methyl mercaptan (rotten eggs, cabbage) and ethyl mercaptan (onion, rubber) are responsible for

undesirable off-flavours in wine. These compounds are extremely reactive with very low sensory thresholds and even trace amounts can have a significant impact on the aroma of wine (Lambrechts & Pretorius, 2000). Hydrogen sulphide (H_2S), with a sensory threshold of 10 – 100 *u*g/L, form during alcoholic fermentation of low nitrogen must, fermentation at high temperatures, fermentation at high pH values, fermentation of must with a high solid content and due to the reduction of residual elemental sulphur applied to grapes as a fungicide. Certain yeast strains are also known to overproduce H_2S (Ebeler & Thorngate, 2009). Although low concentrations (20 – 30 *u*g/L) of H_2S in wine contribute to positive 'yeasty' aromas, high levels can lead to the formation of other undesirable volatile sulphur compounds (Lambrechts & Pretorius, 2000). Dimethyl sulfide was reported to significantly enhance the fruity notes of wines made from Syrah and Grenache Noir (Segurel et al., 2004).

However, a number of sulphur containing compounds produced by yeasts during alcoholic fermentation have been found to contribute to positive aromas to wine. These compounds known as volatile thiols are important impact compounds in Sauvignon blanc. Three volatile thiols, 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercapto-hexyl acetate (3MHA) and 3-mercapto-hexan-1-ol (3MH) have been identified as impact compounds in Sauvignon blanc wines (Darriet et al., 1995; Tominaga et al., 1998_a). These volatile thiols are enzymatically released during alcoholic fermentation from cysteinylated and glutathionylated precursors. Known cysteinylated precursors are S-4-(4-methylpentan-2-one)-L-cysteine, S-4-(4-methylpentan-2-ol)-L-cysteine, and S-3-hexan--ol)-L-cysteine (Tominaga et al., 1998c). Volatile thiols have also been isolated from Bordeaux red wine cultivars, Cabernet Sauvignon and Merlot (Bouchilloux et al., 1998). In recent year the so-called coffee/chocolate Pinotage wine has become very popular. The thiol compound responsible for this aroma is 2-furfurylthiol (Tominaga et al., 2000).

Thiols associated with the aroma of Sauvignon blanc have very low sensory thresholds. 4MMP whose aroma reminiscent of black currant, box tree and broom has a threshold value of 0.8 ng/L in a wine model solution. 3MH that have an aroma of passion fruit, grape fruit, guava and cat urine has a sensory threshold of 60 ng/L in wine model solution, 3MHA whose aroma reminiscent of passion fruit, guava, box tree, grape fruit, cat urine and broom has a sensory threshold of 4.2 ng/L in wine model solution (Darriet, et al., 1995; Tominaga et al., 1996; Tominaga et al., 1998_a; Tominaga et al., 1998_b; Dubourdieu et al., 2006).

Viticultural as well as oenological practices influence the concentration of volatile thiols and their precursors in the must and wine. Precursors will increase with grape maturation, moderate water deficit, machine harvest and *Botrytis cinerea* infection, while low nitrogen content will decrease the concentration of precursors in the grapes. Oenological practices that could increase precursors in the grapes and must include moderate SO_2 and ascorbic acid additions, oxygen exposure, skin contact, pressing and higher maceration temperatures. Factors that will increase the concentration of thiols during fermentation are temperature and

yeast strain selection while oxidation and copper additions will decrease the thiol concentration (Coetzee & Du Toit, 2012).

2.3.2 Contribution of non-volatile composition to red wine flavour

In recent years, much research has been conducted on wine flavour and its associated volatile compounds. However, the non-volatile compounds have also been deemed important for wine flavour and consequently for wine quality/style. A recent study that focused on the influence of the non-volatile matrix of wine on its aroma properties, has demonstrated that the non-volatile matrix influences the release of odorants so strongly that it can make a red wine's aroma smell like that of a white wine, and vice versa. It also changes the perceived aroma of red wines; therefore the sensory properties generated by non-volatiles cannot be recognised in the absence of volatile molecules and vice versa (Sáenz-Navajas et al., 2010). However, the non-volatile compounds are primarily responsible for taste and tactile sensation in the mouth.

2.3.2.1 Non- volatile wine molecules influencing taste

Taste is the sensation produced when chemical molecules react with receptor cells in the taste buds on the tongue. There are five basic tastes: sweet, sour, bitter, salty and umami (Jackson, 2009).

2.3.2.1.1 Sweetness

The main contributors to the sweet sensation in wine are glucose, fructose, glycerol and ethanol. Glucose and especially fructose are sweet and contribute to the sweetness of wine if still present after alcoholic fermentation. The amount of glucose and fructose present in the final wine will depend on the desired wine style, the initial sugar content of the juice, the yeast strain used and its ability to utilise fructose effectively and the fermentation parameters such as pH, temperature, SO_2 and nutrients (Boulton et al., 1996).

Glycerol in its pure form is colourless, odourless, viscous and has a slight sweet taste. Depending on the wine style and the concentration of glycerol present in the wine, it can add to its perceived sweetness and viscosity above its threshold taste level of 5.2 g/L in wine. Glycerol levels for different wine styles of commercial South African wines are as follow: dry red, 10.49 g/L; dry white, 6.82 g/L; off-dry white 6.55 g/L; special late harvest, 8.26 g/L and noble late harvest, 15.55 g/L (Nieuwoudt et al., 2002). Ethanol imparts sweetness at low levels, 2% - 4%, however at the high levels found in wine, >10%, ethanol is more likely to be perceived as bitter (Noble, 1994; Sáenz-Navajas et al., 2012).

2.3.2.1.2 Sourness

The main contributors to sour sensation in wine are the non-volatile organic acids synthesised in young leaves and immature green grape berries. Malic acid and tartaric acid generally account for 69 % to 92 % of all organic acids in grape berries and leaves (Kliewer, 1966). The two acids are mostly synthesised in the grape berry during the herbaceous phase (Ruffner, 1982). During véraison and berry ripening the amount of tartaric acid in the berry is relatively stable, however, the concentration may decrease due to an increase in berry size. Malic acid reaches its highest concentration just prior to véraison. The concentration as well as the quantity of malic acid per berry decrease during grape ripening due to respiration. Citric, succinic, lactic and acetic acids are present in ripened grape berries at much lower levels (Conde et al, 2007).

Tartaric and malic acids play an important role in the potential grape quality and the ultimate wine quality. Tartaric acid has the ability to alter the sour sensation in wine, making it fresher and giving the wine a longer aging potential, although too much acid in wine results in a sharp and unpleasant taste. Citric, lactic, oxalic and succinic acids also contribute to wine acidity (Boulton et al., 1996). Hufnagel and Hofmann (2008b) suggested that L-tartaric acid, D-galacturonic acid, acetic acid, succinic acid, L-malic acid and L-lactic acid impart sourness and that it was slightly suppressed by the chlorides of potassium, magnesium and ammonium respectively.

Malolactic fermentation (MLF) plays an important role on the acidic taste of red wines. MLF is a de-acidification process defined as the conversion of L-malic acid to L-lactic acid and the production of carbon dioxide. MLF thus replaces the strong ‘green’ taste of the L-malic acid with the less aggressive taste of the L-lactic acid (Beelman and Gallander, 1979). Table 2.3 summarises the sensory thresholds of described sour compounds found in the literature.

Table 2.3: Sensory thresholds, type of sensory test and medium for described sour compounds present in wine (Sáenz-Navajas et al., 2012).

Compound	Sensory threshold (mg/L)	Sensory test	Medium
Tartaric acid	44	Triangle test[a]	Water
Galacturonic acid	125[b]	Triangle test	Water
Acetic acid	119	Triangle test	Water
Succinic acid	106	Triangle test	Water
Malic acid	494	Triangle test	Water
Lactic acid	1393	Triangle test	Water
Citric acid	499	Triangle test	Water
Oxalic acid	506	Triangle test	Water

a: Detection thresholds for sourness determined in a triangle test using tap water (pH 6.5) as the solvent.

b: Sensory thresholds for both sourness and astringency.

2.3.2.1.3 Bitterness

Bitterness is normally associated with flavonoid phenols. Noble (1994), stated that flavonoid phenols and ethanol are primarily responsible for bitterness in wines with the two wine flavan-3-ol monomers, (-)-epicatechin and (+)-catechin being the main monomeric flavonoid phenols contributing to bitterness. (-)-Epicatechin is more bitter than (+)-catechin and has a longer duration of bitterness. Furthermore, monomeric flavonoid phenols are primarily bitter, but their astringency increases more rapidly than their bitterness with polymerization. Ethanol both imparts bitterness and enhances the bitter sensation and duration of flavonoids in wine. Polyphenolic compounds with low molecular weights such as flavonol aglycones (myricetin and quercetin) also contribute to bitterness (Preys et al., 2006). Bitterness is suppressed by the presence of sugars and glycerol.

In contradiction to Noble (1994), it was found in recent studies that phenolic acid ethyl esters and not flavan-3-ols monomers are the major contributors of bitterness. A series of hydroxybenzoic acid ethyl-esters and hydroxycinnamic acid ethyl-esters were identified as being the main bitter compounds in wine (Hufnagel and Hofmann, 2008a). Table 2.4 summarise the sensory thresholds for compounds described with bitter properties in the literature.

Table 2.4: Sensory thresholds, type of sensory test and medium for described bitter compounds present in wine (Sáenz-Navajas et al., 2012).

Compound	Sensory threshold (mg L-1)	Sensory test	Medium
Flavanols			
(+)-Catechin	290	Triangle test	Water
(−)-Epicatechin	270	Triangle test	Water
Epigallocatechin gallate	87	Triangle test	Water
Procyanidin C1	347	Triangle test	Water
Procyanidin B1	231	Triangle test	Water
Procyanidin B2	280	Triangle test	Water
Procyanidin B3	289	Triangle test	Water
Phenolic acid ethyl esters			
Gallic acid ethyl ester	438	Triangle test	Water
p-Coumaric ethyl ester	137	Triangle test	Water
Syringic acid ethyl ester	130	Triangle test	Water
Vanillic acid ethyl ester	294	Triangle test	Water
Caffeic acid ethyl ester	229	Triangle test	Water
Ferulic acid ethyl ester	158	Triangle test	Water
Protocatechuic acid ethyl ester	182	Triangle test	Water
Flavonols			
Quercetin	10_a	Paired	5% ethanol
Kaempferol	20_a	Paired	5% ethanol
Mirycetin	10_a	Paired	5% ethanol
Quercetin-3-*O*rhamnoside	8.9_a	Triangle test	Water

a: Sensory threshold for both bitterness and astringency.

2.3.2.1.4 Saltiness and Umami

Saltiness and umami sensations have never been detected in sensory research using red wines (Hufnagel and Hofmann, 2008b).

2.3.2.2 Non-volatile wine molecules influencing tactile

Tactile sensation is caused by increased friction perceived by touch via mechanoreceptors in the mouth (Gawel, 1998).

2.3.2.2.1 Astringency

Astringency is an oral sensation produced primarily by the interaction of wine polyphenols with salivary protein commonly described as ‘drying’, ‘roughing’ and ‘puckering’. The interactions appear to consist of both hydrophobic interactions and hydrogen bonding and for flavanols this interaction is influenced by polymerization and percentage of galloylation. High quality red wines have a balanced level of astringency, but at too high or low levels astringency can have a negative effect on red wine perception (Gawel, 1998).

Proanthocyanidins are present in skins, seeds and stems of grape berries. Astringency is directly associated with the composition of proanthocyanidins, also known as condensed tannins. The degrees of polymerization, percentage of galloylation as well as the monomeric composition of the proanthocyanidins have a great influence on the astringency sensation (Del Llaudy et al., 2008; Sun et al., 2013). The degree of maturation of the grape berries at harvest influences the phenolic composition of red wines. Unripe grape berries have a lower extractability of proanthocyanidins from the skins and a higher extractability from the seeds. Proanthocyanidins extracted from the seeds are more galloylated than proanthocyanidins from the skins, thus red wine made from unripe grapes tend to be more astringent (Peyrot des Gachons and Kennedy, 2003; Ryan and Revilla, 2003; Canals et al., 2005; O-Marques et al., 2005). Bindon et al. (2013), found similar results in a study done on Cabernet Sauvignon grapes. The study showed an increase in the concentration of skin tannins as ripening progressed while seed tannin concentration decreased.

Monomeric (catechin and epicatechin) and polymeric flavan-3-ols impart both astringency and bitterness to wine. Monomeric flavan-3-ols contributes more to the bitterness of red wine (see section 2.3.2.1.3), whereas the polymeric flavan-3-ols (proanthocyanidins or condensed tannins) contribute more to the astringency. Epicatechin tends to impart a higher maximum astringency and is more persistent than catechin (Gawel, 1998). Gawel (1998) also suggested that pigmented polymers, as well as the hydroxycinnamate, are responsible for red wine astringency. Hufnagel and Hofmann (2008) identified 26 sensory active non-volatiles among which several hydroxybenzoid acids and hydroxycinnamic acids as well as a structurally

undefined polymeric fraction exhibiting molecular masses above 5kDa, as puckering astringent components, whereas flavon-3-ols glucosides and dihydroflavon-3-ols rhamnosides exhibited a more velvety, silky-type of astringency.

Sweetness, acidity, viscosity and ethanol may also affect the perception of astringency (Preys et al., 2006). Numerous studies have been conducted on the effect of ethanol on astringency, but the results are contradictory (Serafini et al., 1997; Gawel, 1998; Noble, 1998; Scinska et al., 2000; Fontoin et al., 2008; Meillon et al., 2009).

2.3.2.2.2 Other tactile sensations

Wine tasters frequently describe a wine by its body. Although a common definition for this parameter is still needed that would lead to the identification of wine molecules that contribute to this tactile sensation, it is believed that non-volatile compounds such as proline, glycerol, polysaccharides, ethanol and tannins contribute to this attribute (Sáenz-Navajas et al., 2012).

Temperature also has an effect on tactile perception. Cool temperature can decrease sweet perception while enhancing acidity, bitterness and astringency (Jackson, 2009). However, according to Ross and Weller (2008), the serving temperature of red wine significantly impacts the aroma intensity but not the perceived bitterness and astringency.

2.4 Sensory Methods

Sensory evaluation was defined by Stone and Sidel (2004) as a scientific method that uses the senses of sight, smell, taste, touch, and hearing to measure, analyse, evoke and interpret reactions on samples. Depending on the goal of the sensory evaluation there are three types of test: discrimination/similarity, descriptive and hedonic.

2.4.1 Discrimination test

Discrimination tests are used to answer the question of whether there is a difference between two or more samples. The data analysis is very simple and results can be obtained rapidly and easily (Lawless and Heymann, 2010). The test can be performed by either inexperienced or experienced wine drinkers, however a panel should not be a combination of both (Kemp et al. 2009). The most used discrimination tests are the triangle test, paired comparison, duo-trio, and A-not-A. Only the triangle test will be discussed further in this chapter.

Triangle tests

In the triangle tests, three samples each labelled with a three-digit code are presented to the panel members simultaneously. The panel members have to assess the samples in the given order and determine which sample is the odd one out or which two out of the three samples are the most similar (Lawless & Heymann, 2010).

In the experimental layout there are six possible orders in which the samples can be presented: AAB, ABA, BAA, BBA, BAB, ABB. The ideal experimental layout will be a balanced design where each order presentation will be used an equal number of times and depending on the significance level selected, with 24 – 30 panel members (Kemp et al, 2009).

The data analysis is performed by counting the number of correctly identified 'odd one out' responses and comparing it to the Roessler statistical tables. The statistical table gives the minimum number of correct identified responses at different significance levels, required to reject the null hypothesis of 'no difference' (Kemp et al, 2009).

2.4.1 Descriptive test

Descriptive tests are used to describe and quantify the sensory characteristics on which samples differ. It is the most informative sensory evaluation tool and the quantitative data obtained can be linked to consumer preference and instrumentally measured data by means of statistical analyses such as regression and correlation (Greenhof & MacFie, 1994; Lee et al., 1999; Lawless & Heymann, 2010). The most used descriptive test is Quantitative Descriptive Analysis ® (QDA ®) and in recent years frequency of attribute citation has also become popular (Campo et al, 2008; Campo et al, 2010). Both QDA ® and frequency of attribute citation require the panel to be extensively trained before they can be used as an evaluation tool. This can cause a study to take from weeks up to several months to be completed and thus the need for faster and more cost-effective methods ensued. One of these rapid methods that were developed was the free sorting task that is based on similarity measurements between samples (Valentin et al, 2012).

2.4.2.1 QDA ®

QDA® was developed by Stone and Sidel to deal with problems associated with the Flavor Profile method. QDA® is performed by a small number of panellists (10 - 12) that use an unstructured line scale to give the intensity ratings for selected attributes (Stone et al., 1974; Stone and Sidel, 2004).

The QDA® methodology can be divided into three main steps. The first step is for the panellists to familiarise themselves with the samples and to build an attribute vocabulary that

accurately describes the different samples. The panellist then decides on the reference standards that will be used to define each attribute. The second step is to train the panellists to recognise these attributes in the samples and to calibrate on intensity levels. The panel needs to be tested on their performance. Each individual panel member needs to be tested on repeatability and the panel needs to be tested to see if they have reach consensus. The last step is for the panellists to score the samples on an intensity scale for each attribute defining the sample. The intensity scale consists of a 10 or 15 cm line with word anchors at each end. The scale is typically ankered at none on the left-hand side and intense on the right-hand side to indicate an increase in intensity form left to right. Anker words such as 'weak' and 'strong' are typically used. Depending on sample variability, the anticipated degree of difficulty and the expected use of the results; four replications from each panellist on each sample would be optimal (Stone and Sidel, 2004; Lawless & Heymann, 2010).

QDA® is a relative assessment method and the panel leader acts only as a facilitator and does not lead or direct the panel. Data are analysed statistically using analysis of variance (ANOVA) and multivariate statistical techniques such as principal component analysis (PCA). Results are frequently presented graphically in spider plots (Stone and Sidel, 2004).

2.4.2.2 Frequency of attribute citation method

The frequency of attribute citation method is used to evaluate wine odour attributes (McCloskey et al., 1996; Piombino et al., 2004). In this method, the panellists have to select the most relevant odour attributes from a list containing a rather high number of attributes. Although this method does not use intensity scales, it does have some similarities to conventional descriptive analysis. Campo et al. (2010) found that the frequency of attribute citation method can detect more subtle differences between samples than with conventional descriptive analysis.

The frequency of attribute citation method has a similar training schedule to conventional descriptive analysis but it requires the use of as many as possible attributes and their consensus-derived reference standards during training. The number of attributes retained on the aroma list used by the trained panel varies from 10 to 144 (McCloskey et al., 1996; Le Fur et al., 2003; Campo et al., 2008; Campo et al., 2010;). The panel size for frequency of attribute citation is also much larger than the panel for conventional descriptive analysis ranging from 14 panellists (Le Fur et al., 2003) to 38 panellists (Campo et al., 2010). During wine evaluation each panellist are asked to evaluate the wines in duplicate or triplicate by picking from the aroma list the most descriptive attributes for each wine sample. The panel is asked to pick a set number of attributes per wine sample or to use a set maximum of attributes for each wine sample (McCloskey et al., 1996; Le Fur et al., 2003; Campo et al., 2010).

The reproducibility index (R_i) is calculated to assess individual panellist performance across replicate evaluation sessions (Campo et al., 2008).

$Ri = \Sigma [(2 \times des_{com}) / (des_{rep1} + des_{rep2})] / n$

Where

des_{com} = number of common terms used by the specific panellist in the two replicates
des_{rep1} = number of terms used by the specific panellist in Replicate 1
des_{rep2} = number of terms used by the specific panellist in Replicate 2
n = number of replicated wines

The Ri values can range from 0 (no reproducibility across replicates) to 1 (perfect agreement between replicates). Campo et al. (2010) suggested that the data from panellists with Ri values equal or lower than 0.20 should be excluded from further data analyses.
The attributes are ranked by their citation frequency (C_f) to determine the most relevant attributes for data analysis. Only attributes with a $C_f \geq 15$ % are used for data analysis. A chi-square analysis can be performed on the mean C_f of each attribute and wine to determine the discriminating attributes. The data is organised into a contingency table of the mean C_f with rows as the wines and the attributes as the columns in order to perform correspondence analysis to create two- or three-dimensional maps of the wine-attribute spaces (Greenacre, 2007; Murtagh, 2005).

2.4.2.3 Free sorting task

The free sorting task is based on exploring the similarity/dissimilarity between different samples. It is a time and cost effective way of obtaining information about sensory similarities and dissimilarities among a large set of samples (Falahee & MacRae, 1997; Tang & Heymann, 2002; Faye et al., 2004; Saint-Eve et al., 2004; Cartier et al., 2006; Faye et al., 2006; Blancher et al., 2007; Sinesio et al., 2010; Chollet et al., 2011).

All samples are presented simultaneously in a single session with each panel member having a different presentation order. The panellists are asked to look, smell and/ or taste a set of samples and to group them according to similarities/dissimilarities. The panellists can use any criteria to do the sorting and they are free to make as many groups with as many samples in each group as they want. The sorting task needs at least 20 or more panelists, however the sorting task can be performed with trained or/and untrained panelists (Chollet et al., 2011). Depending on the objectives of the study, the panellists can be asked to give descriptors/attributes to characterise each group formed (Lawless et al., 1995; Tang & Heymann, 2002; Saint-Eve et al., 2004; Faye et al., 2004, 2006). A pre-established list with attributes can be provided to the panellists to help them characterise the groups. This will simplify the task of the panellist as well as data analysis (Lelièvre et al., 2008).

Multidimensional scaling (MDS) analysis is done on the sorting data. MDS produces a spatial representation of the sample similarity/dissimilarity which is represented by data points on a map. According to Chollet et al. (2011), this method produces similar sensory spaces to those obtained with conventional profiles. The distances between points reflect the similarity/dissimilarity of the samples. The coordinates of the MDS data points can be subjected to a cluster analysis to reveal sample groupings in the MDS representation. The cluster analysis however only generates group data and thus an additional step is necessary to assess the sensory characteristics of the individual samples. The attributes used to describe the groupings are typically projected onto the MDS space by calculating correlation coefficients between the occurrence of the attributes and MDS factor scores (Valentin et al., 2012).

2.4.2 Hedonic test

Hedonic tests are used to quantify the degree of liking or disliking of a product (Lawless & Heymann, 2010). Hedonic tests fall outside the scope of this thesis.

2.5 Conclusion

Although a lot of research has been done on the biochemical and chemical origins of aroma compounds and the influences of environmental, viticultural and winemaking practices on the concentration of these compounds, there is still room for further research in order to develop strategies for producing wines with specific aroma attributes for specific targeted markets. Optimum ripeness can be measured by numerous methods (see section 2.2.3), however further research is needed in developing methods that can link optimum ripeness with wine aroma profiles. One of the methods suggested make use of the concept of sequential harvest (Bindon et al., 2014; Deloire, 2011, 2012, 2013_a, 2013_b).

The perception of wine aroma can be attributed to chemical molecules and their interactions. The perceived aroma can be enhanced or masked by the presence of other chemical molecules (Marais et al., 1999, 2001; Segurel et al., 2004; Pineau et al., 2007; Hufnagel en Hofmann, 2008). All the volatile and non-volatile chemical molecules responsible for aroma, taste and tactile sensations, and their different interactions and influences on perceived aroma, have not yet been identified. Further research is needed on sensory active molecules, their sensory thresholds and sensory interactions in wine. Methods to detect sensory thresholds for non-volatile chemical molecules in wine also need to be developed.

Sensory evaluation has become a popular science in recent years and depending on the research question and product type, different well-established methods are available in literature to be used as sensory evaluation tools. Descriptive analysis has been used for year to characterise products and a fairly new method, the frequency of attribute citation method that

can detect more subtle differences between samples than the conventional descriptive analysis, has been developed. This method can be used in studies where the differences between products are not that immense. The need for quick, reliable and less restrictive methods has emerged and one of these methods developed is the free sorting task. It has become a very popular sensory evaluation tool because of its simplicity. Chollet et al. (2011) have shown that this method produces similar sensory spaces to those obtained with conventional profiles but that the descriptions of the samples are more robust.

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Chapter 3

Research results

Sequential harvesting of red cultivars using berry sugar accumulation and the relationship with wine sensory attributes.

3. RESEARCH RESULTS

3.1 Introduction

Sugar concentration is a parameter used by winemakers to determine the ripeness of grapes. However, this parameter gives little indication on the possible style of wine in terms of aroma profile. Grape berry development consists of two sigmoid cycles (Coombe, 1992). The first stage is characterised by cell division and cell expansion during which the berry and seeds are formed. During this stage, tartaric acid, malic acid and hydroxycinnamic acids as well as tannins accumulate in the berry. The first stage is followed by a lag phase during which no growth takes place. The end of the lag phase corresponds with the end of the herbaceous phase of the berry growth (Conde et al., 2007) and coincides with the onset of ripening, the second sigmoid stage. Véraison, a French word that has been used to describe the onset of ripening, is the beginning of fruit maturation and is characterised by the softening of the berry and skin colouring due to the biosynthesis of anthocyanins in red cultivars. Berry sugar loading was proposed by Deloire (2011), as “the evolution of the quantity of sugar per berry, expressed as mg per berry, from véraison onwards”. Berry sugar loading and biosynthesis of some flavour and aromatic compounds or precursors take place during the ripening phase (Conde et al., 2007).

In recent years, a number of studies have been conducted with the aim of developing new methods to monitor grape ripening and to determine the optimum harvest date that correlates with specific wine aromas. Although most of these studies have used brix (ºB) to determine the optimal harvest date (Gallander, 1983; Reynolds et al. 1996; Koundouras et al., 2006; Heymann et al. 2013), other methods have also been developed. For example, a mechanical texture test has been developed to monitor ripening in grape berries and a correlation has been found between texture parameters and sensory attributes (Maury et al., 2009).

A theoretical berry sugar loading curve based on data obtained over five years using at least 20 different red grape varieties in mainly France, Spain, Argentina, Chile and South Africa was compiled and displays a phase of rapid sugar loading starting at véraison followed by a plateau phase (Deloire, 2011). According to Deloire (2011), the sugar loading curve could be used to assess possible wine sensory profiles; it is an indirect relationship between harvest time and the potential wine sensory attributes/profiles of red wines made from grapes picked at different stages. The wines will thus have a different aroma profile, depending on whether grapes were harvested in the beginning, mid or end of the plateau phase of the sugar loading curve. Wines made from these stages could be characterised by fresh fruit (FF)/green plant like/unripe plum flavours (beginning of the curve), a possible neutral (N)/pre-ripe (PR) spicy flavours (mid curve) or pre mature stages, and mature fruit (MF)/blackcurrant, raspberry, cherry

flavours (end of the curve), followed by an over ripe stage characterised by dried fruit/ prune flavours (Deloire, 2012).

There is no direct correlation between grape, brix or titratable acidity levels and the sequential harvest stages, meaning that FF, N/PR and MF stages can be reached at the same brix levels depending on the volume of the fruit. This led to the conclusion that harvesting using only brix levels is not a good indicator to determine optimum harvest dates (Deloire, 2013). Using the sequential harvesting stages, a better understanding of the vine morphological and physiological parameters is obtained enabling viticultural practices to be adapted to achieve production objectives and it enables the winemaker to determine the optimum ripening levels according to the desired wine style. To date, no peer-reviewed scientific data has been published on this method which is already used by the wine industry, but some studies have been done on sequential harvest using Brix as indicator (Bindon et al., 2013; Bindon et al., 2014; Boss et al., 2014).

The main aim of this study was to evaluate the sensory evolution of South African red wines made from grapes harvested according to sequential harvest and using sugar accumulation as a physiological indicator. This was undertaken to understand the possible berry aromatic evolution in relation with the potential wine styles/sensory attributes across the different harvest times and to increase the diversity of wine styles from a single or a group of vineyards for the benefit of the wineries and consumers.

3.2 Material and Method

3.2.1 Vineyard and winemaking techniques

3.2.1.1 Origin of grapes

2 Vineyard blocks, Merlot (clone 348) and Cabernet Sauvignon (clone 46), situated on Eikendal Vineyard, Stellenbosch, South Africa were used in this trial (GPS Coordinates: S 34° 0' 46.7" | E 18° 49").

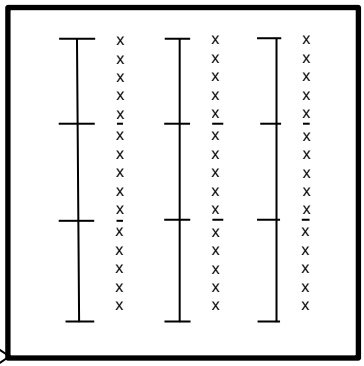
3.2.1.2 Experimental layout of vineyards

From each vineyard block a certain section was allocated for this trial. Visual observation was used at budburst to determine the section of each vineyard deemed the most homogeneous which was used for this study. Each section of vineyard for both Cabernet Sauvignon and Merlot had three repeats (repeats indicated in different colours in table 3.1). In order to consider vineyard variability each repeat was sub divided into 3 randomly chosen sub repeats which was harvested together to form one vineyard repeat (Table 3.1). Each sub repeat consisted of 3

rows with 3 panels and each panel comprised of five vines. Nine vines per sub repeat [one vine in each panel (9 panels)] times three sub repeats (27 vines) were combined to produce one vineyard repeat which was used for determining harvest stages and winemaking. In this way, three wine repeats for each of the five harvesting stage was obtained, yielding 15 wines per cultivar.

Table 3.1: Experimental layout of vineyard for Merlot and Cabernet Sauvignon. Each cultivar had 3 repeats (indicated with different colours) which were subdivided into 3 random sub repeats in order to consider/integrate vineyard variability. Each sub repeat consisted of 3 rows with 3 panels each. Each panel had 5 vines (x).

Repeat 2	Repeat 1	Repeat 3
Repeat 3	Repeat 2	Repeat 1
Repeat 1	Repeat 3	Repeat 2



3.2.1.3 Determination of harvesting stages

Sequential harvest using berry sugar accumulation as a potential physiological indicator allows for assessing the possible relation between harvest time and potential wine styles (Deloire, 2011; Bindon et al., 2014; Boss et al., 2014). According to Deloire (2011) three main stages: fresh fruit (FF), neutral, (N) and mature fruit (MF) are identified linking harvest time according to the berry sugar accumulation curve to potential wine styles along the ripening period. These stages were determined according to the number of days after the key point (KP). KP corresponds to the beginning of the plateau of sugar accumulation or slow down (Deloire, 2011; 2014).

Brix (°B) and berry fresh mass were measured once a week starting at véraison (data not shown) to calculate sugar loading per berry and per berry per day to assess the tempo of fruit ripening. As the rate of sugar loading increased these measurements were done twice a week. The speed of sugar loading per berry per day was calculated and plotted on a graph. The key point or ‘Day 0’ for each cultivar was determined by looking at when the graph

plateaued or rather when sugar loading slowed down, which was around 85% of total sugar per berry and likely corresponds to 20 - 22°B. This key point was used to harvest grapes according to five harvest stages, pre fresh fruit (PreFF), fresh fruit (FF), neutral (N), mature fruit (MF) and an over ripe (OR) stage. The model assessed in various situations (Deloire, 2011; Deloire, 2012; Suklje et al., 2014), showed that for Cabernet Sauvignon, the stages are the following: FF stage is reached 20 – 25 days after key point and a MF stage 40 – 45 days after key point with a N (i.e. possible deficiency of fruitiness in the corresponding wine) or pre mature stage in between. Merlot has a FF stage 10 – 15 days after key point and a MF stage 20 – 25 days after key point with an N (i.e. possible deficiency of fruitiness in the corresponding wine) or pre mature stage in between. Deloire (2011; 2014) named stages 2, 3 and 4 FF, N or pre mature and MF respectively and it was decided to use this terminology in this study as well. A PreFF and an OR stage (stages 1 and 5 respectively) have been added in order to have five date's sequential harvest (see sections 3.3.1.1 and 3.3.2.1).

3.2.1.4 Winemaking protocol

The grapes were harvested early morning on the required harvest stage and brought to the experimental cellar of the Department of Viticulture and Oenology, Stellenbosch University.

The grapes were crushed and destemmed into 50L plastic drums and 30 mg/L SO_2 was added. Juice samples for pH, titratable acidity and °B was taken before the SO_2 addition. The crushed grapes were inoculated with 30 g/hL *Saccharomyces cerevisiae* (ICV-D21, Lallemand) with an addition of 30 g/hL Go Ferm Protect (Lallemand) in the rehydration water. Co-inoculation with 0.01 g/L *Oenococcus oeni* (Enoferm Alpha, Lallemand) was carried out 24 hours after the yeast inoculation in order to start the malolactic fermentation. Fermentation took place at 25 °C and punch downs were done three times a day. The rate of fermentation was measured daily with a hydrometer. After 5 °B sugar was fermented 0.25 g/L Fermaid K (Lallemand) was added. The fermentation took about 5 days after which the skins were pressed at -1 °B (press to 1 bar) and moved to 20 °C in order to finish the malolactic fermentation. Once the malolactic fermentation was completed (malic and lactic acids determined enzymatically by the Central Analytical Facility, Stellenbosch University, South Africa), the wines were racked off the lees and an addition of 50 mg/L SO_2 was made. The wines underwent cold stabilization for 3 weeks at -4 °C before adjusting the free SO_2 to 40 mg/L and bottled under screw cap. The wines were analysed six months after bottling.

3.2.2 Sensory evaluation

All wines were evaluated six months after bottling to induce some bottle aging and to enhance possible sensory differences.

3.2.2.1 Discrimination test: Triangle test

In sensory evaluation, before describing the sensory characteristics of a set of products, discrimination tests are generally done to determine if all the products are perceived different. Only these products with sensory differences are then further characterised by a trained panel. The objective of the discrimination tests was to determine if aromatic differences could be perceived between wines made according to sequential harvest.

Among the different discrimination tests, triangle tests were performed to determine if a detectable sensory difference existed between two harvest stages. Triangle test is a forced-choice procedure. It is a rapid and fairly easy method which does not require a lot of participants (Næs et al., 2010).

Triangle tests were conducted on odour only. The wines made from the two extreme harvest stages i.e. pre fresh fruit and over ripe (PreFF vs. OR) were compared as well as the fresh fruit with mature fruit (FF vs. MF). The neutral stage (N) was also compared with FF and MF respectively.

3.2.2.1.1 Participants

Twenty-seven trained panellists and 33 wine connoisseurs participated in the first triangle tests (PreFF vs. OR and FF vs. MF) and 29 wine connoisseurs participated in the second triangle tests (FF vs. N and MF vs. N). The trained panellists were the same persons which participated in the attribute citation frequency method (See section 3.2.2.2.1 for more detail). Wine connoisseurs were winemakers from the South African wine industry as well as researchers from the Department of Viticulture and Oenology (DVO) and the Institute for Wine Biotechnology (IWBT), Stellenbosch University, South Africa.

3.2.2.1.2 Test Procedure

Three wine samples were simultaneously presented to the participants. Two of the wine samples were the same and one differed. The participants had to identify the odd wine sample by smelling the wines from left to right (odour only). Twenty-five mL samples were presented in black ISO (International Standards Organization) glasses and each wine sample wascoded with a three-digit number. In order to avoid order effect,t the three wine samples were presented in equal amounts of possible combinations (BAA, ABA, AAB, ABB, BAB, BBA) to different participants.

The evaluation sessions took place in the sensory laboratory of the Department of Viticulture and Oenology, University of Stellenbosch (South Africa). Each panellist had their own tasting booth. An optimum tasting environment was provided; controlled temperature (± 20°C),

natural light and limited distractions (Lawless & Heymann, 2010). Each tasting booth was equipped with an instruction sheet, an answer sheet, a pencil and rubber, a spittoon, a glass of water and crackers. Figure 3.1 presents a classical tasting booth during an evaluation session.

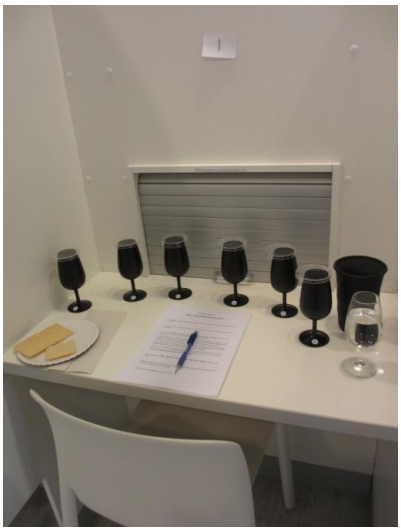


Figure 3.1: A classical tasting booth during an evaluation session

3.2.2.2 Descriptive test: Attributes citation frequency method

After the initial discrimination tests (triangle tests), it was decided to only characterise the sensory properties of wines elaborated from four harvest stages i.e PreFF, FF, MF and OR. The aim of the descriptive analysis was to generate attributes for these four different stages of sequential harvest. The attributes citation frequency method was used to characterise the aroma profiles of the wines (Campo et al., 2008; McCloskey et al., 1996; Piombino et al., 2004). Panellists had to select from a list the most appropriate aroma attributes for each wine. It is an alternative to the conventional descriptive analysis method (DA), more suitable when a detailed description of a complex aroma product such as wine is required (Campo et al., 2010). In combination with attributes citation frequency method a conventional DA was used to quantify taste and mouthfeel.

3.2.2.2.1 Participants

A panel of 37 people was recruited in January 2013 for the entire year on the basis of their interest and availability, no remuneration was given. Four panellists had to quit due to limited availability on their part resulting in a final panel of 33 judges.

The final panel of 33 judges (19 females and 14 males) consisted of students and staff of the University of Stellenbosch. Among them, nineteen were between the ages of 20 - 35 and fourteen were older than 35 years. Ten of the judges had oenology training and 32 of the judges consumed wine more than once a month with 21 of them consuming wine more than once a

week. The one judge that stipulated that he never consumes wine joined the panel because he wanted to learn how to appreciate wine. The 33 panel members were divided into five groups for training and evaluation. Each group had to attend a one-hour session per week.

3.2.2.2.2 Training

The training consisted of two phases: general and specific training. Initially, each panel member had to participate in 11 one-hour general training sessions over a period of four months. Each panel member had to undergo an additional seven sessions of specific training for the two cultivars that had to be assessed.

3.2.2.2.2.1 General Training

Each of the general training sessions was divided into three parts. The first part was an aroma recognition exercise, the second a taste and mouthfeel exercise and lastly a wine description exercise, each which will now be presented in more detail.

3.2.2.2.2.1.1 Part 1: Aroma recognition

In this part of the general training, the panel members were asked to smell aromatic reference standards and to recognise the aroma (20 minutes). The general idea was for the panel member to become familiar with the terms on the aroma list that was provided. The aroma list was taken from a previous study that used the frequency of citation method (Campo et al., 2008).

The terms were divided into eight odour families: Fruity, Vegetative/Green, Floral, Spicy, Animal, Forest Floor, Toasted/Wood and Others. The last family, Others, grouped unclassifiable descriptors such as alcohol, lactic, chalky, rubber, sulphur and solvent/chemical. The Fruity family was further subdivided into: White fruit, Yellow fruit, Citrus, Red fruit, Black fruit, Dried fruit, Nut fruit, Tropical fruit and Other fruits. The Vegetal family was subdivided into Vegetables, Fresh and Dried and the Toasted/Wood family subdivided into Toasted and Woody. The rest of the families were not subdivided.

About 15 aroma standards were presented during a session. Standards were presented in small 60 ml bottles that were wrapped in foil to avoid visual influence. Commercially available standards were taken form Firmenich (Geneva, Switzerland) and Aux Parfums de Grasse (Grasse, France). The standards not commercially available were prepared with natural products. In order to guarantee good aroma quality, all the standards were assessed every morning and newly made if required.

The first general training session focused on the Fruit family aromas with the focus shifting to a different family in consecutive training sessions. During the course of the training the list of terms was adjusted, new descriptors were added (gooseberry, guava, eucalyptus, lemon grass, tomato leaf), synonyms were combined and terms not used were removed. The final list consisted of 109 terms. The final aromatic descriptors list and odour reference standards presented during the training is shown in Addendum A.

3.2.2.2.2.1.2 Part 2: Taste and Mouthfeel

During this part of the session (10 minutes), the panellists had to familiarise themselves with the different tastes (sweet, sour, bitter, saltiness and umami) and mouthfeel (astringency). Panel members first had to identify these taste and mouthfeel compounds in water. During the latter sessions, these compounds were presented in a wine base. Solutions combining taste and mouthfeel were also presented.

Once the panel members could recognise the different tastes and mouthfeel they had to be calibrated. Panel members were asked to rank samples with different concentrations of the various taste and mouthfeel compounds from the lowest concentration to the highest concentration. During the last couple of training sessions, the panel members were asked to score spiked wines on a 6-point scale varying form 0 “Absent” to 5 “Very High”. This part of the session ended with a discussion during which the panel had to reach consensus on the score given to each solution.

3.2.2.2.2.1.3 Part 3: Wine Evaluation

The last part of the session focused on wine evaluation (30 minutes). The panellists had to evaluate three or four different wines and describe their aroma properties with the aid of the list provided. Each panellist was presented with the wines in black (ISO) glasses covered with a Petri dish. The session ended with a discussion led by the panel leader highlighting the terms most frequently cited to describe each wine.

Panel members also had to rate the tastes and mouthfeel for each wine on a 6-point scale varying form 0 “Absent” to 5 “Very High”. The tastes and mouthfeel terms rated were sweetness, sourness, bitterness, saltiness and astringency. Umami was omitted seeing that it is not a well-recognised taste in wine (Jackson, 2002). Scores were revealed, a mean established for each compound and the panel members that scored a wine too high or too low were asked to taste that specific wine again in order to calibrate themselves against the panel. A vast selection of wines was used for this part of the training in order to allow the panel for evaluating several wine styles.

3.2.2.2.2.2 Specific Training

The structure of the specific training was exactly the same as the structure of the general training. The main difference was that the wines used for the wine evaluation part were wines of similar characteristics as those of this study which included some of the wines made for the current study. This was done specifically to familiarise the panellists with the sensory characteristics of the wines produced according to sequential harvest. The initial aroma descriptor list was adapted and shortened to contain only the relevant descriptors for this study.

The specific training consisted of four one-hour sessions for Merlot in which 13 Merlot wines were evaluated (11 experimental and 2 commercial) and three one-hour sessions for Cabernet Sauvignon in which seven Cabernet Sauvignon wines were evaluated (4 experimental and 3 commercial). Throughout the sessions for both Merlot and Cabernet Sauvignon some of the wines were replicated in order to assess the reproducibility of each panel member. Each session started with an aroma recognition exercise (see section 3.2.2.2.2.1.1. for more detail).

In the second taste and mouthfeel part, panel members were asked to rate sweetness, sourness, alcohol, bitterness and astringency on a 6-point scale. Alcohol was included since the study involved wines made from sequential harvest dates, thus yielding wines with increase in alcohol levels. The aroma descriptors used to characterise the Merlot wines and the Cabernet Sauvignon wines were individually compiled. The terms cited by at least 3 panellists for the same wine were used to compile a short descriptors list for each cultivar. The final list for Merlot included a total of 58 terms and that of the Cabernet Sauvignon included a total of 54 terms. The final list for Merlot and Cabernet Sauvignon are shown in table 3.2 and table 3.3 respectively.

Table 3.2: Short descriptor list for Merlot wines

AROMATIC DESCRIPTORS LIST

FRUITY		VEGETATIVE / GREEN	SPICY	TOASTED / WOOD	OTHER
WHITE FRUITS	DRIED FRUITS	VEGETABLES	Bay Leaf / Laurel	TOASTED	Alcohol
YELLOW FRUITS	Date	Cabbage	Clove	Caramel	Rubber
CITRUS	Dried Apricot	Green Beans	Black Pepper		Solvent / Chemical
RED FRUITS	Dried Fig	FRESH	FLORAL	Roasted Coffee	Sulphur
Cherry	Prune	Herbaceous	Honey	Toffee	Wet mop
Raspberry	Raisin	Green / Cut Grass		WOODY	
Redcurrant	NUT FRUITS	DRIED	ANIMAL	Planky	
BLACK FRUITS	TROPICAL FRUITS	Hay / Dried Grass	Horsy / Sweaty	Toasted / Smoked Wood	
Blackberry	OTHER	Tobacco	Meat Stock	Vanilla	
Blackcurrant	Fruit Jam			FOREST FLOOR	
	Ripe Fruit				

OTHER:

Table 3.3: Short descriptor list for Cabernet Sauvignon wines

AROMATIC DESCRIPTORS LIST

- ☐ **FRUITY**
  - ☐ **WHITE FRUITS**
  - ☐ **YELLOW FRUITS**
  - ☐ **CITRUS**
  - ☐ **RED FRUITS**
    - ☐ Cherry
    - ☐ Raspberry
    - ☐ Redcurrant
  - ☐ **BLACK FRUITS**
    - ☐ Blackberry
    - ☐ Blackcurrant
    - ☐ Blueberry
  - ☐ **DRIED FRUITS**
    - ☐ Dried Apricot
    - ☐ Dried Fig
    - ☐ Prune
    - ☐ Raisin
  - ☐ **NUT FRUITS**
  - ☐ **TROPICAL FRUITS**
  - ☐ **OTHER**
    - ☐ Fruit Jam
    - ☐ Glazed / Crystallized Fruit
    - ☐ Oxidized Apple
    - ☐ Ripe Fruit
- ☐ **VEGETATIVE / GREEN**
  - ☐ **VEGETABLES**
  - ☐ **FRESH**
    - ☐ Herbaceous
    - ☐ Green / Cut Grass
    - ☐ Mint
  - ☐ **DRIED**
    - ☐ Hay / Dried Grass
    - ☐ Tobacco
- ☐ **SPICY**
  - ☐ Bay Leaf / Laurel
  - ☐ Cinnamon
  - ☐ Clove
  - ☐ Juniper
  - ☐ Liquorice
  - ☐ Black / White Pepper
- ☐ **FLORAL**
  - ☐ Honey
- ☐ **ANIMAL**
  - ☐ Horsy / Sweaty
  - ☐ Meat Stock
- ☐ **TOASTED / WOOD**
  - ☐ **TOASTED**
    - ☐ Caramel
  - ☐ **WOODY**
    - ☐ Planky
- ☐ **FOREST FLOOR**
- ☐ **OTHER**
  - ☐ Alcohol
  - ☐ Solvent / Chemical

OTHER:

3.2.2.2.3 Wine Evaluation

Wine evaluation for Cabernet Sauvignon and Merlot was conducted separately but the same structure was used. The three repeat wines from the N stage were excluded from further evaluation resulting in 12 wines per cultivar to be evaluated (see section 3.3.1.2.1.). The 24 wines (12 wines x 2 repetitions) were divided into six blocks of four wines each. Within a block the four wines were simultaneously presented according to an incomplete block design in order to reduce bias related to the order, carry-over or expectation effects (Lawless & Heymann, 2010).

Each panel member had to participate in two individual evaluation sessions of 45 minutes each. Both sessions consisted of three blocks of four wines each; the second was a repetition of the first. A 10-min break was compulsory between each block to reduce panellist fatigue.

Panellists were asked to evaluate the wines from left to right. They first had to choose a maximum of 5 aromatic descriptors from the list provided to characterise each wine. An option (“Other:”) was given to allow panellists to add a descriptor that was not on the list. For each wine, panellists were also asked to rate the intensity of taste (sweetness, sourness, bitterness) and mouthfeel (astringency and alcohol) on a 6-point scale (0 = absence, 1 = very low and 5 = very high). A different bottle of wine was used for each session. Twenty-five mL wine sample was poured half an hour before the tasting. Samples were presented at room temperature (20°C) in black (ISO) wine glasses covered with a petri dish and coded with a random 3-digits number. The evaluation sessions took place under the same conditions as those described for

the triangle tests (see section 3.2.2.1.2). The instruction sheet for attributes citation frequency method can be seen in addendum B.

3.2.2.3 Descriptive test: Sorting tasks

In order to further characterise the different stages sequential harvest a sorting task was performed on the Merlot and Cabernet Sauvignon wines using wine professionals. The sorting task was performed on the wines six months after bottling.

Sorting tasks are an efficient alternative method to descriptive analysis. It is a time and cost effective way of obtaining information about sensory similarities and dissimilarities among a large set of samples (Blancher et al., 2007; Cartier et al., 2006; Chollet et al., 2011; Falahee & MacRae, 1997; Faye et al., 2004, 2006; Saint-Eve et al., 2004; Sinesio et al., 2010; Tang & Heymann, 2002). The sorting task method has become popular in recent sensory evaluation studies because of its simplicity. Sorting is based on categorization which is a normal process regularly used in daily life and therefore does not require a quantitative response. It only requires participants to group products according to similarity. According to Chollet et al. (2011), this method produces similar sensory spaces to those obtained with conventional profiles.

In this study, sorting tasks were followed by a descriptive step where participants were asked to describe each group formed. Sorting tasks followed by descriptions of each group were performed in two sessions respectively (40-minute each) separated with a 10 minute break. Each session consisted of two sorting tasks, one on aroma and the second on taste and mouthfeel. Twelve wine samples were used in every session. According to Chollet et al. (2011) twelve samples is the optimum amount to perform a good sorting task.

3.2.2.3.1 Participants

Twenty nine wine professionals from the South African wine industry were recruited for the sorting tasks. A demographic data questionnaire was given to them to complete between the two sessions. A summary of the data is shown in table 3.4.

Table 3.4: Demographic data of the 29 wine professionals whom performed the sorting task.

Characteristics	Modalities	Frequency (%)
Gender	Male	45
	Female	55
Age	Less than 30	41
	Between 30 and 50	59
Activity	Wine Research	26
	Winemaking	62
	Viticulture	6
	Wine sales	6
Oenology training	Yes	90
Previous exposure to Sensory test	Yes	90

3.2.2.3.2 Procedure

Each participant had to participate in two sessions, one session with Merlot wines and the other session with Cabernet Sauvignon wines. A 10-min break was compulsory between each session to reduce fatigue. The 29 participants were divided into two groups. Group 1, consisting of 15 wine professionals, formed the morning panel and their first session was done on Merlot with their second session using Cabernet Sauvignon. The second group, consisting of 14 wine professionals, formed the afternoon panel and their first session was performed on Cabernet Sauvignon and their second session on Merlot. This was done to exclude order effect.

At each session, the participants were first required to only smell the 12 wine samples from left to right. Thereafter, they were allowed to smell the wines as many times as they wanted and in any order. The participants had to sort the wines into groups according to aroma similarities and dissimilarities. The participants were allowed to form as many groups as they wanted with as many wines in a group as they wished as long as it was more than one group and less than 12. Then, participants were asked to describe each group they have formed using the same list compiled by the frequency of citation panel (table 3.2 and table 3.3 respectively). They were thus provided with a list of descriptors and asked to choose a maximum of 5 descriptors to describe each of the groups.

The second step was for participants to sort the 12 wine samples into groups according to taste and mouthfeel using the same sorting protocol as for aroma. Thereafter, the participants were asked to score each group of wines on a six-point scale (0 = absence, 1 = very low and 5 = very high) for sweet, sour, alcohol, bitter and astringent.

For each session, 25 ml samples of wine were poured half an hour before they were presented in black ISO wine glasses closed with a petri dish to assure aroma stability. Each

glass was coded with a random three digit number. Different codes were used for the aroma sorting task and for the taste and mouthfeel sorting task. The wine samples were presented simultaneously in a random order and each participant had a different presentation order. The order was also different for each sorting task.

The sorting tasks were done in the undergraduate laboratory, Paul van der Bijl building, Stellenbosch University (South Africa). An optimum tasting environment was provided; controlled temperature (±20 °C), natural light and limited distractions (Lawless & Heymann, 2010). Participants were provided with an instruction sheet (Addendum B), pencil, rubber, spittoon, a glass of water, crackers and a set of 12 wines. Participants were only informed about the cultivar of the samples.

3.2.3 Data Analysis

3.2.3.1 Discrimination test: Triangle test

In order to determine the minimum number of correct judgments to indicate a significant difference between the samples in the discrimination tests, the Roessler statistical table for triangle tests (Roessler et al., 1978) was used.

3.2.3.2. Descriptive test: Attributes citation frequency method

3.2.3.2.1 Panel performance

The performance of the panel was analysed separately for aroma description (frequency of citation) and taste and mouthfeel (intensity scores).

3.2.3.2.1.1 Aroma description

As previously mentioned, the second session was a repetition of the first. In other words, the same wines were tasted in both sessions. In order to establish whether the panellists described each wine and its repeat with the same aromatic descriptors the repeatability for each panellist was calculated. Repeatability was performed on the families or subfamilies and not on the individual aromatic descriptors. To assess the individual performance, an average reproducibility index (Ri) was calculated for each panellist across the duplicate evaluations (Campo et al., 2008). Firstly the Ri for each replicated wine was calculated as follows:

$(2 \times des_{com}) / (des_{rep1} + des_{rep2})$

Where

des_{com} = number of common terms used by the specific panellist in the two replicates

des_{rep1} = number of terms used by the specific panellist in Replicate 1

des_{rep2} = number of terms used by the specific panellist in Replicate 2

Then the average Ri of each panellists was calculated as follows:

$Ri = \Sigma [(2 \times des_{com}) / (des_{rep1} + des_{rep2})] / n$

Where

n = number of replicated wines

3.2.3.2.1.2 Taste and mouthfeel intensity rating

Three-way analyses of variance (ANOVA) with main effects (wine, panellist and replication) and interaction effects (panellist with wine, panellist with replication and wine with replication) were performed on each attribute to assess discriminability, repeatability and agreement of the panel.

3.2.3.2.2 Wine characterization

3.2.3.2.2.1 Aroma description

Only data from panel members with a Ri > 0.2 were used to characterise the wines (Campo et al., 2008). A contingency table containing the sum of the citation frequencies for each terms used by the most reproducible panellists was constructed. The citation frequency of the terms was averaged across replications and only those terms that were cited by more than 25 % of the panel on at least one wine, was kept. In order to study the relationship between wines and aromatic descriptors, the contingency table was submitted to a correspondence analysis (CA). A hierarchical cluster analysis (HCA) was finally applied to the factorial coordinates of the wines in the spaces defined by CA to identify groups of wines with similar characteristics. This analysis helps to interpret the position of the wines in the CA map. All these statistical analyses were performed with the software XLSTAT.

3.2.3.2.2.2 Taste and mouthfeel intensity rating

The intensity scores were averaged across panellists and replications for each wine and attribute. These mean scores were then submitted to a principal component analysis (PCA) to show the relationships between attributes and wines. A hierarchical cluster analysis (HCA) was finally applied to the factorial coordinates of the wines in the spaces defined by PCA to identify

groups of wines with similar characteristics. This analysis helps to interpret the position of the wines in the PCA map. All these statistical analyses were performed with the software XLSTAT.

3.2.3.2 Descriptive test: Sorting tasks

The data were analysed with two sets of methods: multidimensional scaling analysis (MDS) and clustering analysis. The purpose of multidimensional scaling (MDS) is to provide a visual representation of the perceived dissimilarities and similarities among the samples. For each participant, the data are encoded in an individual dissimilarity matrix, in which 0 stands for two wines set in the same group and 1 for two wines placed in different groups. These individual matrices are summed for all participants resulting in a global dissimilarity matrix in which smaller numbers indicate higher similarity between wines. Dissimilarities between samples were then analysed using MDS. The samples were represented by points on a MDS map which were positioned so that the distances between the pairs of points reflect the dissimilarities between the pair of wines: two wines which have been often sorted together by the participants are close on this representation and two wines which have rarely been sorted together are far apart. Then, assuming that the terms assigned to a group of wines characterise all the wines of this group, citation frequency of each descriptor was computed for each wine. Pearson correlations were calculated between citation frequencies of each term and coordinates of wines on each dimension of the MDS map (Faye et al., 2004; Teillet et al., 2010). These correlations constitute the coordinates of the terms in the MDS configuration and allow interpretation of the underlying dimensions that differentiate the wines. Similar analysis was performed with the intensity scores of taste and mouthfeel.

Finally coordinates of samples in the MDS map were submitted to hierarchical cluster analysis (HCA) to determine clusters of wines with similar characteristics to confirm the interpretation of the clusters of wines in the MDS map (Lawless, 1989).

3.3 Results and discussion

The results and discussion for the Merlot and Cabernet Sauvignon will be deliberated separately, starting with Merlot.

3.3.1 Merlot

3.3.1.1 Sugar loading and harvest dates

According to Deloire (2011), Merlot has a FF stage 10 – 15 days after key point, while the MF stage is reached 20 – 25 days after key point with an N or pre mature stage in between these

two stages. Figure 3.2 shows the average sugar loading curve for the three repeats of 2013 Eikendal Merlot fruit. The first °B and berry fresh mass samples were taken on the 11th of January and the °B was around 9 °B average for the three repeats with an average of 60 mg sugar/berry. The 25th of January was taken as key point or 'Day 0' when sugar loading has slowed down and around 85 % of the total expected sugar loading was loaded at 20.5 °B. Practicing sequential harvest, the dates for Merlot were then fixed for every five days after key point in order to get five different harvest dates. These five harvest dates allowed for a study of possible wine styles obtained from the fruit harvest sequentially from the key point of berry sugar accumulation which was a way to assess the relevance of the physiological indicator. The basic juice and wine analyses of the wines made according to the sequential harvest are summarised in Addendum C Table 5.4.

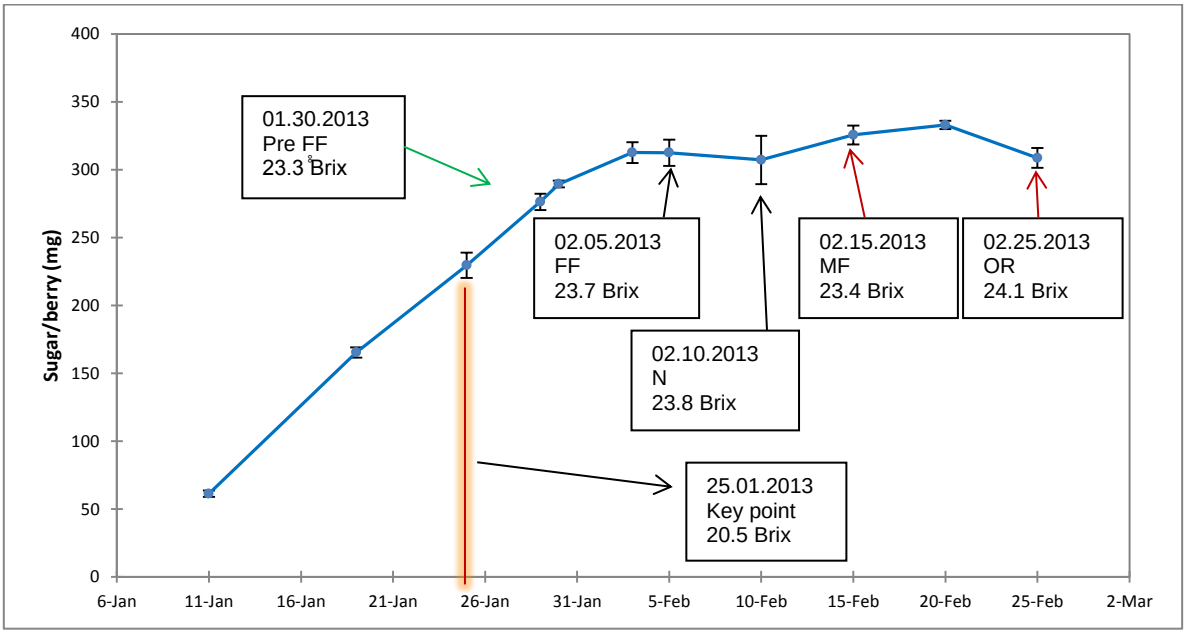


Figure 3.2: Sugar loading curve with harvest dates and corresponding brix levels for Merlot in 2013. Results are presented as averages from 3 replicates ± standard deviation (SD).

3.3.1.2 Sensory results

3.3.1.2.1 Discrimination test: Triangle test

The wines were tasted six months after bottling. The results of the triangle tests for Merlot are summarised in table 3.5.

Table 3.5: Triangle tests result for Merlot. P: trained panelists; C: wine connoisseurs; All: P+C

	PreFF / OR			FF / MF			FF / N	MF /N
	P	C	All	P	C	All	C	C
#correct judgements	15	21	36	13	18	31	18	16
# total judgements	27	33	60	27	33	60	29	29
Significant Difference	p=0.05	p=0.01	p=0.01	NS	p=0.01	p=0.01	p=0.01	p=0.05

A significant difference was observed by all the panellists between the FF and MF stages, but it should be kept in mind that only about half of the panellists could discriminate between the FF and MF stages. There were more correct judgements when PreFF and OR were compared, suggesting that there were bigger differences on aroma profile between these two stages of ripening.

In the triangle tests that included the N stage, a significant difference was found between the three stages (FF, N, and MF) although the number of correct judgements made by the wine connoisseurs was again not high.

Although the variances between the wines made from the different harvest stages were probably not immense, it can be reported that according to the discrimination tests there was a significant aromatic difference between the wines made from the PreFF, FF, N, MF and OR harvest stages.

However, no significant difference was detected between the FF, N and MF harvest stages for Cabernet Sauvignon (Table 3.6). This led to the decision to not include the N stage in the study for both Merlot and Cabernet Sauvignon. Further descriptive analysis performed on Merlot and Cabernet Sauvignon wines therefore only included wines made from the PreFF, FF, MF and OR harvest stages.

3.3.1.2.2 Aroma profiling

3.3.1.2.2.1 Descriptive test: Attributes citation frequency method

3.3.1.2.2.1.1 Analysis of panel performance

The panellists' average Ri ranged from 0.20 to 0.68, with the mean Ri of the panel being 0.40 ± 0.13 (Addendum C Figure 5.1). The Ri values can range from 0 (no reproducibility across replicates) to 1 (perfect agreement between replicates). Campo et al. (2010) suggested that the data from panellists with Ri values equal or lower than 0.20 should be excluded from further data analyses. According to this criterion we kept the data of 31 panellists of the 33 for the attribute citation frequency method.

3.3.1.2.2.1.2 Wine characterisation

The projections of attributes and wines on the first two dimensions of the CA, accounting for 72.27 % of the total variance, are shown in figure 3.3 and figure 3.4 respectively.

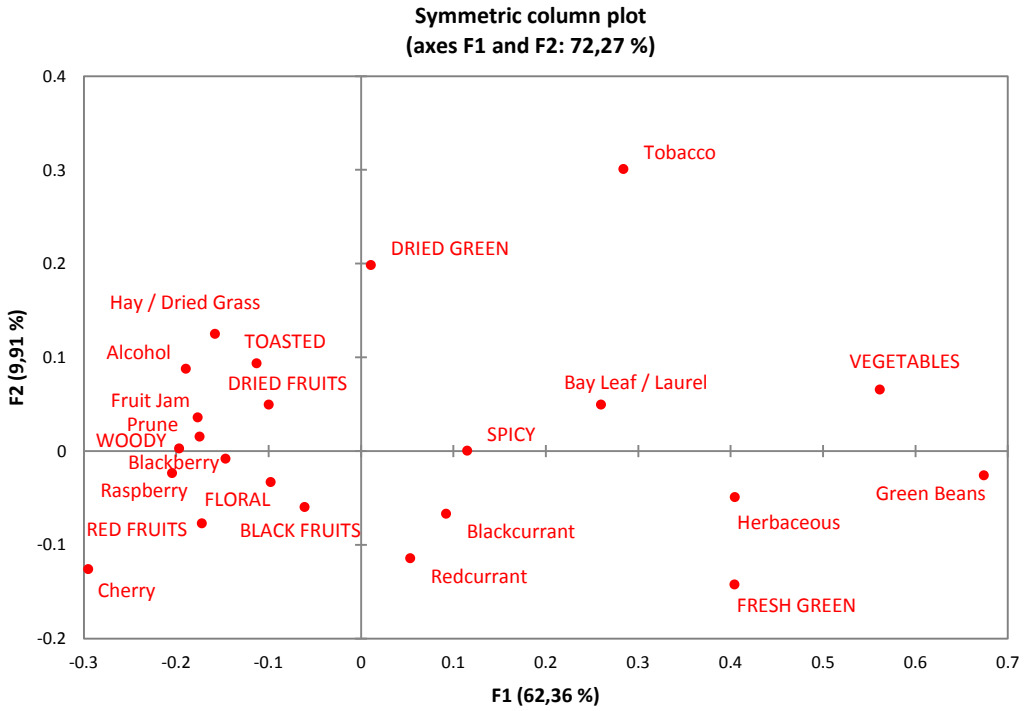


Figure 3.3: Projection of aromatic descriptors on dimensions 1 and 2 of the correspondence analysis for Merlot. Families or sub-families are indicated in capital letters.

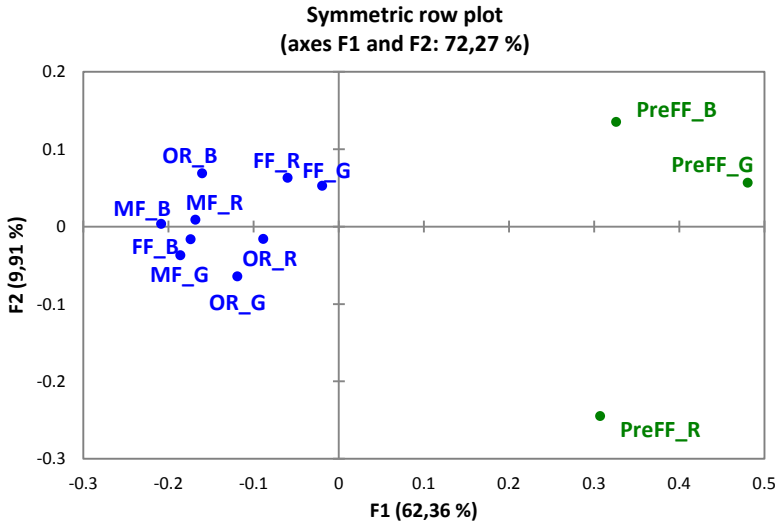


Figure 3.4: Projection of wines on dimensions 1 and 2 of the correspondence analysis for Merlot. Wines with the same colour belong to a same group according to the cluster analysis.

The first dimension, explains 62.36 % of the total variance. Vegetative / green attributes (vegetables, green beans, fresh green and herbaceous) were positively correlated with each other and negatively with the red fruit attributes (red fruits, cherry and raspberry). The second

dimension, explains 9.91 % of the total variance. “Dried’ vegetative / green attributes (dried green, tobacco and hay / dried grass) were positively correlated to each other and negatively to the red fruits attributes (red fruits, cherry and redcurrant) and fresh green attributes. The descriptors which thus contributed the most to the CA’s first two dimensions were from the red fruits family, black fruit family and the green / vegetable family.

The hierarchical cluster analysis yielded two groups of wines clearly different that separated on the first dimension of the CA (wines in different colour in figure 3.4). These were wine repeats from the first harvest date (PreFF) characterised by vegetative green notes (fresh green, vegetable and herbaceous) as well as a group of wines from the other harvest dates with more red fruits notes (red fruits, cherry and raspberry).

The results of the aroma description of the wines elaborated from different harvest stages thus showed a clear difference between the wines from the first harvest stage and the wines from the other harvest stages. However, no clear aromatic difference was observed between the harvest stages FF, MF and OR.

3.3.1.2.2.2 Descriptive test: Sorting task

A three-dimensional MDS configuration resulting from the sorting on aroma gave a stress value of 0.13, which indicates an acceptable representation of the original data. When the MDS configuration perfectly reproduces the input data, the stress is zero. Thus, the lower the stress value, the better is the representation of original dissimilarities. Stress values below 0.1 are considered an excellent fit, values between 0.1 and 0.2 are an adequate fit and values above 0.2 are a poor fit (Kruskal, 1964). Figure 3.5a and Figure 3.5b show the configuration of the wines and the correlations of the descriptors with the MDS dimensions.

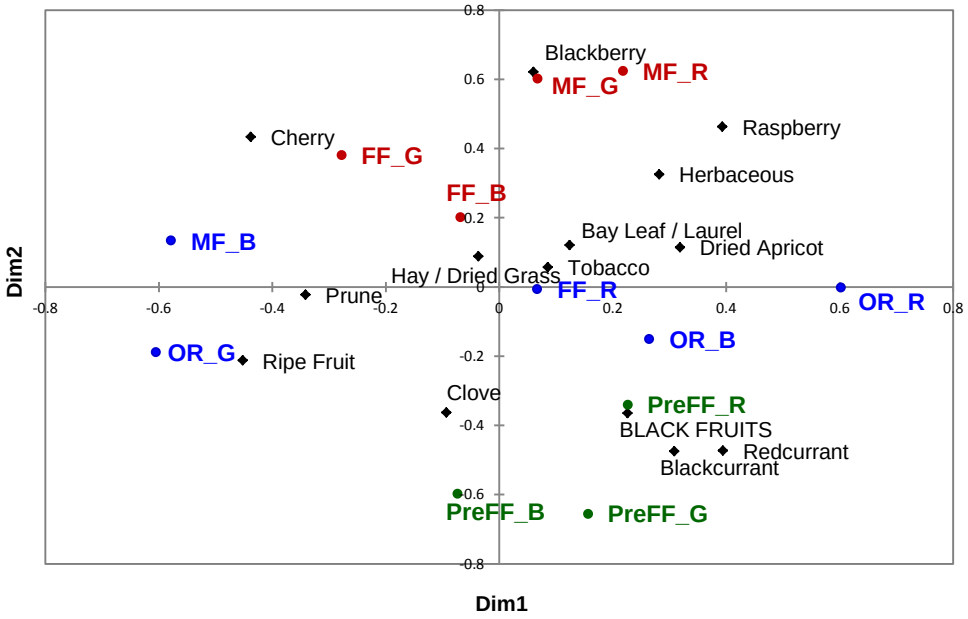


Figure 3.5a: Three dimensional multidimensional scaling plot for aroma sorting on Merlot (stress value = 0.13). Wines with the same colour belong to a same group according to the cluster analysis. Families or sub-families are indicated in capital letters.

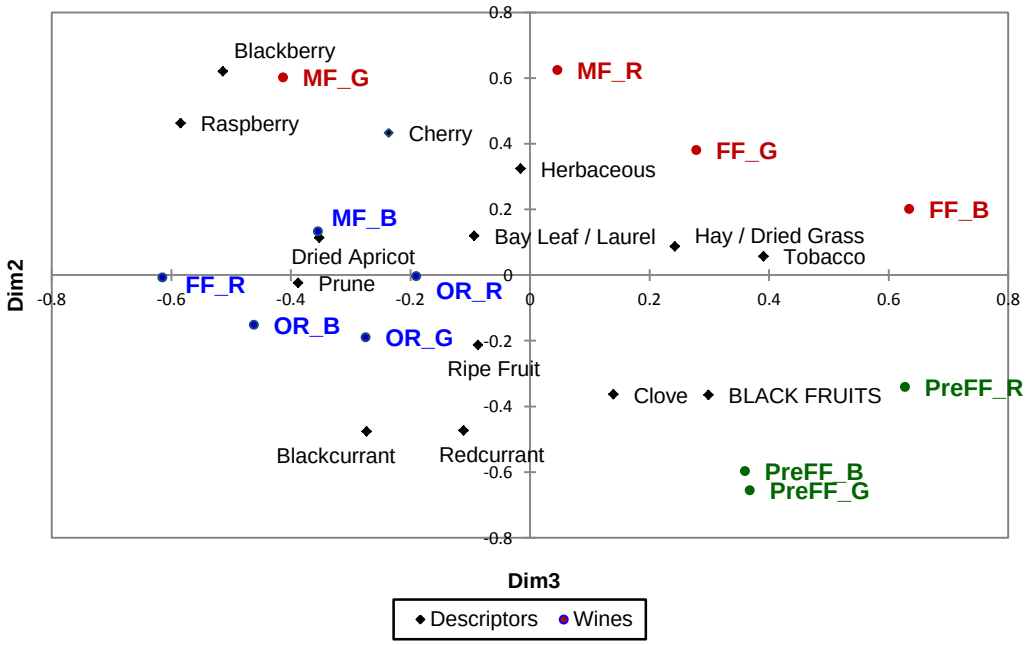


Figure 3.5b: Three dimensional multidimensional scaling plot for aroma sorting on Merlot (stress value = 0.13). Wines with the same colour belong to a same group according to the cluster analysis. Families or sub-families are indicated in capital letters.

The sorting results produced three groups separated on the dimension 1 and 2 and dimension 2 and 3 of the MDS maps (wines in different colour on Figure 3.5a and Figure 3.5b according to the cluster analysis). The wines made from the PreFF stage were grouped together and were characterised by black fruit and clove aroma notes. Wines made from the OR stage were grouped together and characterised by dried apricot, prune and ripe fruit aromas (Figure 3.5b). However, no clear separation was observed between the wines made from the FF and the MF harvest stages.

3.3.1.2.3 Taste and Mouthfeel profiling

3.3.1.2.3.1 Descriptive test: Attributes citation frequency method

The result of the three-way ANOVA for each attribute (Sweet, Sour, Alcohol, Bitter and Astringent) is summarised in addendum C table 5.5. A significant **wine effect** ($p<0.05$) means that panellists could discriminate between sweet, sour, alcohol, bitter and astringent in the wines. The panel could significantly discriminate between all the wines with respect to the above mentioned attributes except for sweet which could be due to the fact that all the wines were fermented dry (< 5 g/L residual sugar) but some panellists mistakenly assessed fruitiness and or ethanol for sweet. As the wine effect was not significant for sweet, this attribute was eliminated for further analysis. Overall, consensuses among panellists were reached and the panel was repeatable.

A correlation circle and PCA (Figures 3.6 and 3.7) were performed on the mean scores for the remaining attributes (sour, alcohol, bitter and astringent) to describe relationships between attributes and wines.

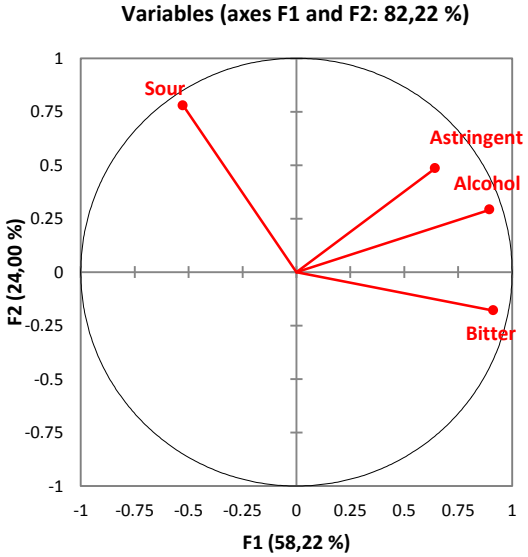


Figure 3.6: Correlation circle of attributes for Merlot (taste and mouthfeel) on principal components 1 and 2.

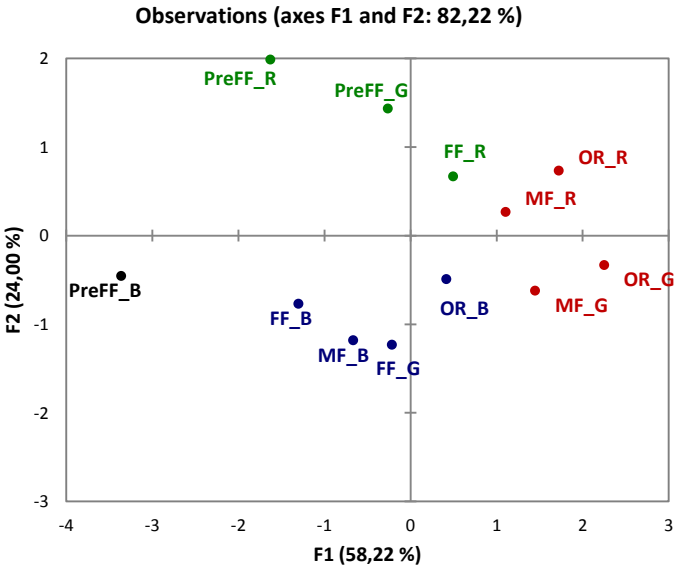


Figure 3.7: Principal component analysis plot of Merlot wines on principal components 1 and 2. Wines in a same colour belong to a same group according to the cluster analysis.

Bitter, alcohol and astringent were located on the positive end of dimension 1 and sour on the positive end of dimension 2 (Figure 3.6). These two dimensions of the PCA explain 82.22 % of the variance in the data. The cluster analysis highlighted four different groups of wines that separated on the two first dimension of the PCA (wines in different colour in figure 3.7). The first dimension seems to separate the wines according to their harvest stages. The wines from the

first harvest stage tended to be on the left of the map and were perceived as being less bitter, alcoholic and astringent than the wines from the last harvest stage on the right of the map. The group with OR_R, OR_G, MF_R, MF_G, (B (Blue) = Repeat 1; G (Green) = Repeat 2; R (red) = Repeat 3), was characterised by astringent, bitter and alcoholic mouthfeel. The second dimension separated the group with PreFF_R, PreFF_G and FF_R from the group with FF_G, FF_B, MF_B and OR_B. PreFF_R, PreFF_G and FF_R were characterised by a more intense sour taste. It is surprising that PreFF_B was not perceived as the other repeats from the first harvest stage.

In general, wines from the first harvest stage were thus perceived as being more sour and less bitter, alcoholic and astringent than those made from later harvests. However, a different result for some of the repeats, such as PreFF_B, MF_B and OR_B was observed which could be due to vineyard differences.

3.3.1.2.3.2 Descriptive test: Sorting task

The three-dimensional MDS plot (Figure 3.8a and Figure 3.8b) gave a stress value of 0.13, which indicated an acceptable representation of the original data.

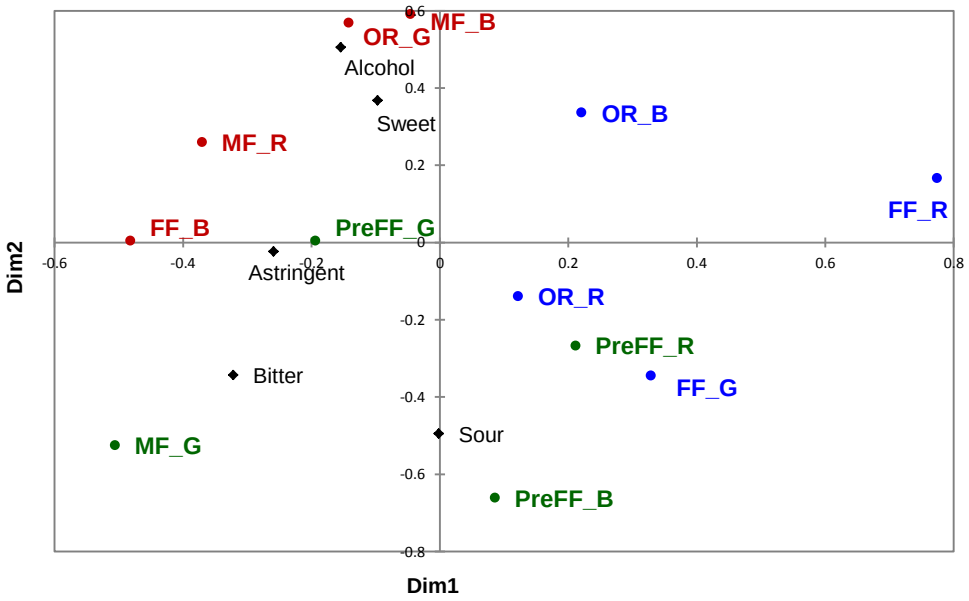


Figure 3.8a: Three dimensional multidimensional scaling plot for taste and mouthfeel of Merlot (stress value = 0.13). Wines with the same colour belong to a same group according to the cluster analysis.

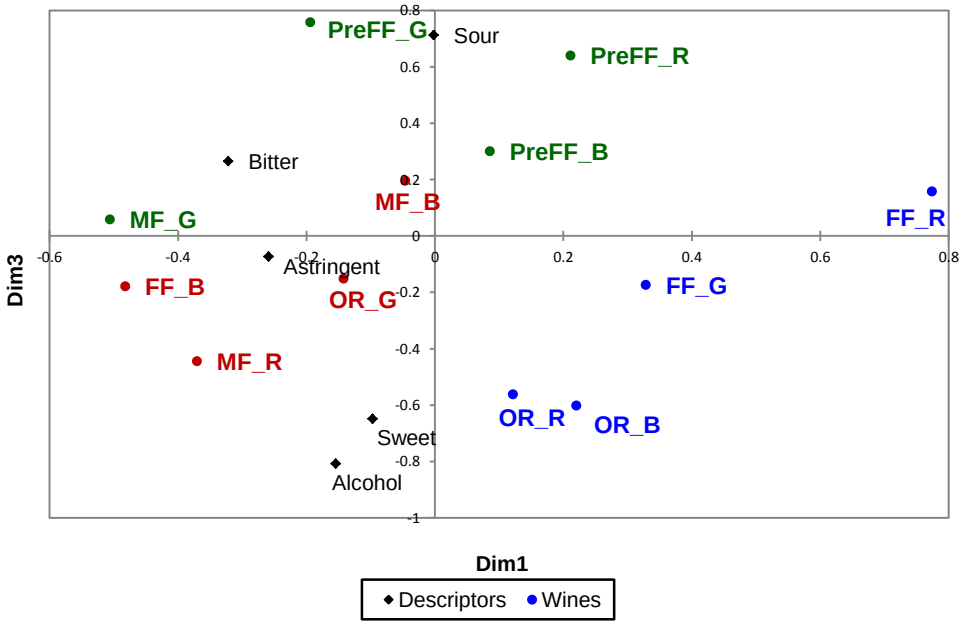


Figure 3.8b: Three dimensional multidimensional scaling plot for taste and mouthfeel of Merlot (stress value = 0.13). Wines with the same colour belong to a same group according to the cluster analysis.

The taste and mouthfeel sorting results produced three groups. Wines made from the PreFF harvest stage were grouped together and described as being more sour and bitter (Figure 3.8a and Figure 3.8b). No clear separation could be made between the wines made from the other three harvest stages. The wines from these three stages were characterised as being more sweet and alcoholic.

3.3.1.3 Discussion

According to the attributes citation frequency method, Merlot wines made from the first harvest stage were characterised by vegetative green notes and were perceived to be more sour. No clear distinction could be made between wines from harvest stages FF, MF and OR. Wines from these harvest stages were characterised as having more red fruit notes and were perceived to be more bitter, alcoholic and astringent.

The aroma results from the sorting tasks done by wine professionals yielded three groups of wines. Wines from the first harvest stages were grouped together and characterised by black fruit and spicy aromas. Wines from the last harvest stage were grouped together and characterised by dried and ripe fruit attributes. No clear separation could be observed between the FF and MF harvest stages. Wines from these harvest stages were characterised by red fruit, black fruit and dried green attributes. The taste and mouthfeel results grouped the wines from the first harvest stage together, but no clear separation could be observed between the other harvest stages. Wines from the first harvest stage were characterised as sour and bitter and wines from the later harvest stages were characterised as being alcoholic, sweet and astringent.

The taste and mouthfeel results obtained by the sorting method reflected the results obtained by the attributes citation frequency method in general. The sorting was done by wine professionals and the attributes citation frequency method was done by a trained panel. The wine professionals characterised the first harvest stage as having black fruit and spicy aromas whereas the trained panel characterised it as being vegetative green. The wine professionals however grouped the wines of the last harvest stage together and characterised them as having more dried and ripe fruit attributes whereas the trained panel could not distinguish between the other three harvest stages. There was thus no consensus about the aroma profiles of the wines between the wine professionals and the trained panel. This could be due to there being very little differences in the aroma profiles of the wines. The attribute citation frequency method has also never been used on experimental wines before.

The different stages of sequential harvest for Merlot could not be characterised by either the attribute citation frequency method or the sorting task. Wines from the first harvest stage were grouped together but no clear separation could be observed for the other consecutive harvest stages. Although the aroma sorting results characterised the first harvest stage wines with black fruit and spicy attributes, no clear trend could be observed for the other harvest dates. Wines from the first harvest stage were more sour and had black fruit and vegetative green notes while the later harvest stages had more red fruit notes and were perceived as more alcoholic and astringent.

Cadot et al. (2012) found similar results from descriptive analysis for Cabernet Franc wines made from different harvest stages. The study has also correlated attributes with biochemical characteristics and in particular red fruit notes with the total phenol index, which in turn can explain the red fruit notes and astringency in the Merlot wines made from the harvest stages FF, MF and OR. The sourness in the first harvest stages was due to higher titratable acid levels in the wine from the earlier harvest stages and the alcoholic perception in the later harvest stages could be due to slightly higher alcohol levels in the wines from these stages. Bindon et al. (2013, 2014) found differences between Cabernet Sauvignon wines using sequential harvest. In their study, the °B levels ranged from 20 °B to 26 °B which led to alcohol levels ranging from 12% to 15.5%. A study done by Heymann et al. (2012) on the effect of extended grape ripening with or without must and wine alcohol manipulation on Cabernet Sauvignon wines sensory characteristics, found that the sensory attributes of wines made from grapes at different stages of ripening, from 20 °B to 30 °B, varied in a systematic fashion. They also found that adding water to higher °B musts to mimic 24 °B musts resulted in wines with similar sensory profiles to wines made from grapes picked at a sugar level close to 24 °B. The study also found that the aroma profiles of wines made from grapes picked at sugar levels under 24 °B differ more than wines made from grapes with higher sugar levels. Alcohol has an immense effect on the matrix of wines as well as the perception of aroma and taste (Fischer and Noble, 1994; Nurgel and Pickering, 2006). The wines made for this study did not have a huge

difference in °B levels (23.3 to 24.1 °B) and therefore alcohol levels. This could explain our results, which showed that the aroma profiles of the wines at the FF, MF and OR stages were perceived rather similar.

3.3.2 Cabernet Sauvignon

3.3.2.1 Sugar loading and harvest dates

According to Deloire (2012) and using berry sugar accumulation as a physiological indicator to practice sequential harvest, Cabernet Sauvignon has a FF stage 20 – 25 days after the key point, with a MF stage 40 – 45 days after key point. In between FF and MF stage, a pre ripe or in some situations a neutral (N = a deficiency of fruitiness in the corresponding wine) stage can be described. Figure 3.9 shows the average sugar loading curve for the three 2013 Eikendal Cabernet Sauvignon repeats.

The first ºB and berry fresh mass samples were taken on the 19th of January, with the average sugar concentration of the three repeats being 13 °B, with an average of 91 mg sugar/berry. The 5th of February was thus taken as key point or 'Day 0' using the same criteria as for Merlot (20.1°B). From the key point it has been decided to do sequential harvest by harvesting every 10 days for up to five dates (Figure 3.9). These harvest dates covered all the different stages described by Deloire (2011) as FF, N or pre ripe and MF. This study has also considered two supplementary stages, pre FF and over ripe (OR). The basic juice and wine analyses are summarised in Addendum C Table 5.6.

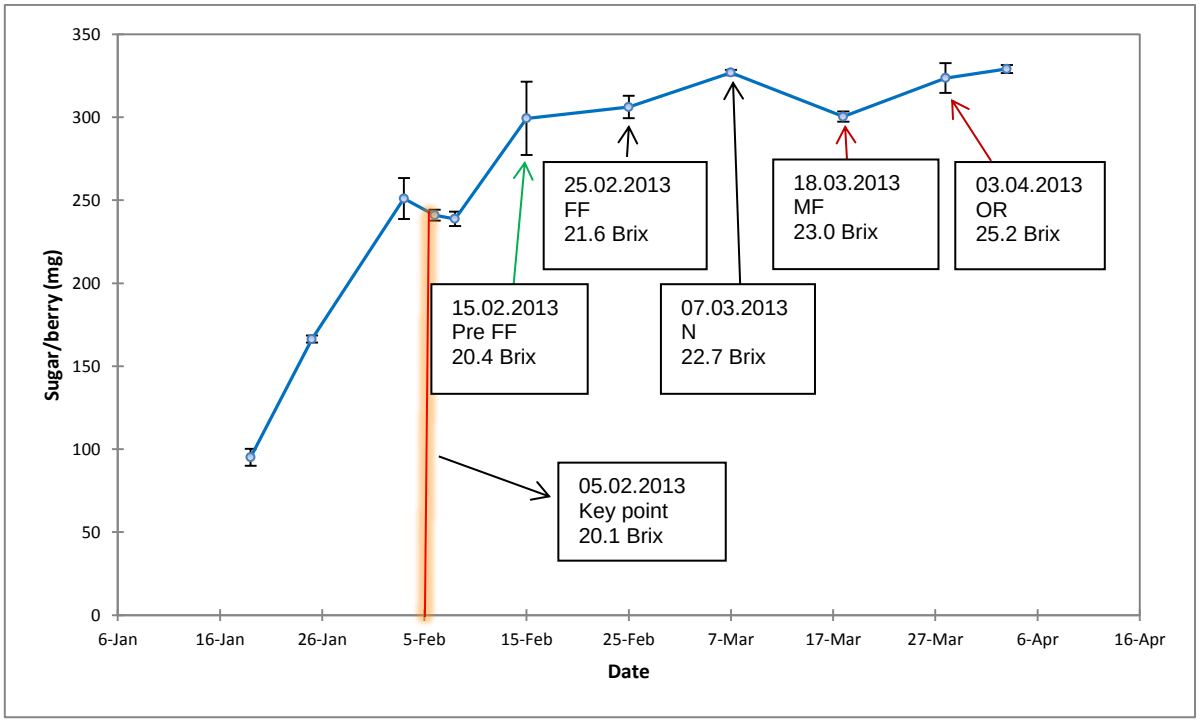


Figure 3.9: Sugar loading curve for Cabernet Sauvignon in 2013. Results are presented as averages from 3 replicates ± standard deviation (SD).

3.3.2.2 Sensory results

3.3.2.2.1 Discrimination test: Triangle test

The results of the triangle tests are summarised in table 3.6.

Table 3.6: Triangle tests result for Cabernet Sauvignon. P: trained panelists; C: wine connoisseurs; All: P+C

	PreFF / OR			FF / MF			FF / N	MF /N
	P	C	All	P	C	All	C	C
# correct judgments	14	29	43	14	15	29	11	8
# total judgments	27	33	60	27	33	60	23	23
Significant Difference	p=0.05	p=0.01	p=0.01	p=0.05	NS	p=0.05	NS	NS

There was a significant difference between the FF and the MF stages for Cabernet Sauvignon although the number of correct judgments was not that high (trained panellists + connoisseurs). There were more correct judgments for the PreFF and OR triangle tests, suggesting that there were more clear differences on perceived aroma between wines made from these two ripening stages when tasted by all (trained panellists + Connoisseurs). In the triangle tests that included the N stage, no significant difference was found between this stage and the FF stage or MF stage. This led to the decision to not include the N stage in the study. Descriptive analysis performed on Merlot and Cabernet Sauvignon wines therefore only included wines made from the PreFF, FF, MF and OR harvest stages.

3.3.2.2.2 Aroma profiling

3.3.2.2.2.1 Descriptive test: Attributes citation frequency method

3.3.2.2.2.1.1 Analysis of panel performance

The panellists' average Ri ranged from 0.19 to 0.63 (Addendum C Figure 5.2a). The mean Ri of the panel was 0.40 ± 0.10 (Addendum C Figure 5.2b). According to the criterion of Campo et al. (2010), 31 panellists out of 32 were kept for the attributes citation frequency method.

3.3.2.2.2.1.2 Wine characterisation

The CA graphs, figure 3.10a and figure 3.10b, show the projections of wines and attributes respectively.

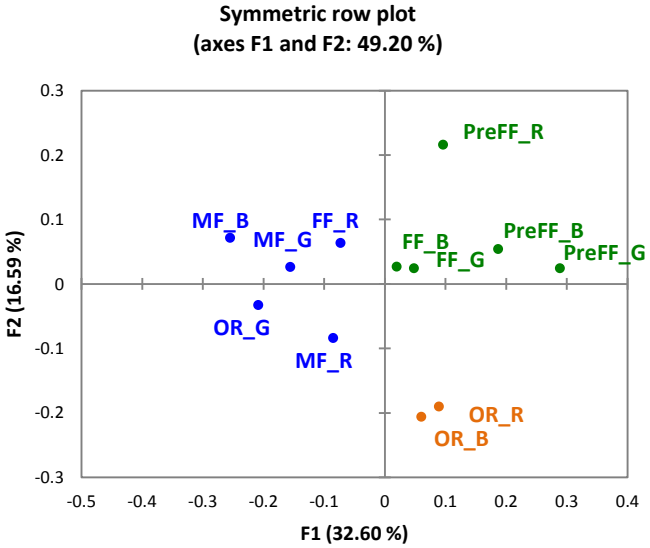


Figure 3.10a: Projection of Cabernet Sauvignon wines on the dimensions 1 and 2 of the correspondence analysis. Wines with the same colour belong to a same group according to the cluster analysis.

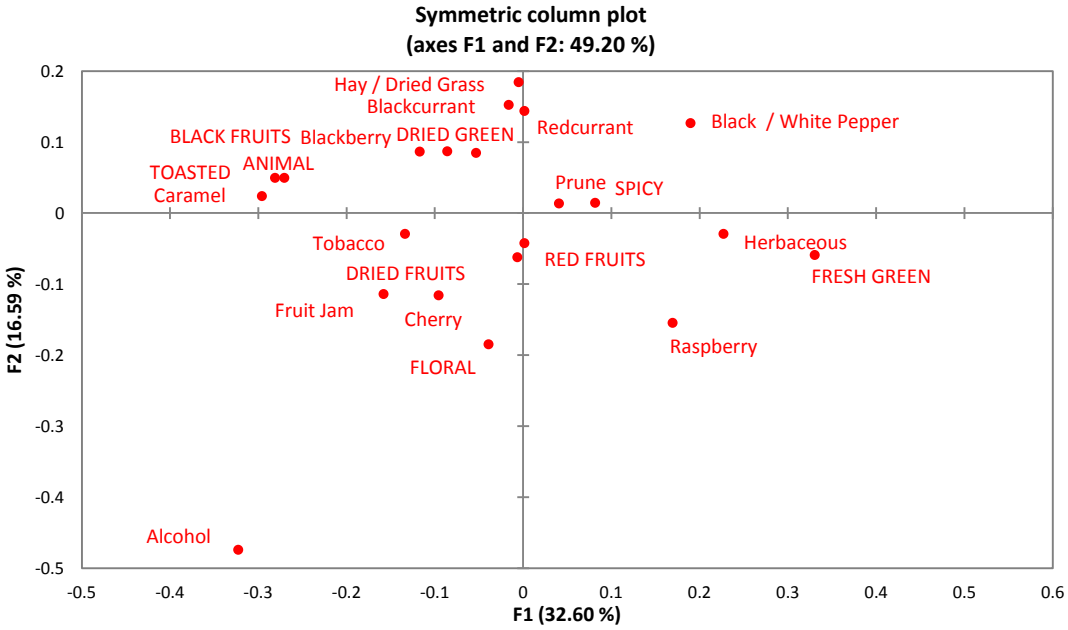


Figure 3.10b: Projection of aromatic descriptors on the dimensions 1 and 2 of the correspondence analysis. Families or sub-families are indicated in capital letters.

The first dimension explained 32.6 % of the total variance. Along the first dimension, the vegetal notes (fresh green and herbaceous) and black/white pepper notes are positively correlated. These attributes were negatively correlated to animal, toasted and caramel notes. The second dimension explained 16.59 % of the total variance. Hay/dried grass; blackcurrant and redcurrant were positively correlated with each other and negatively with alcohol, floral and raspberry.

The cluster analysis yielded three groups of wines clearly different that separated on the two first dimensions of the CA (wines in different colour in figure 3.10a). The three repeat wines from the first harvest stages were characterised by vegetal and black/white pepper notes. Two wines from the last harvest stages (OR_R and OR_B) were characterised mainly by alcohol notes and to a lesser extent by floral and raspberry notes. Again no clear aromatic differences were observed for the FF and MF harvest stages.

3.3.2.2.2.2 Descriptive test: Sorting task

The three-dimensional MDS plots (Figure 3.11a and Figure 3.11b) gave a stress value of 0.08, which indicated an acceptable representation of the original data. The wines were tasted six months after bottling.

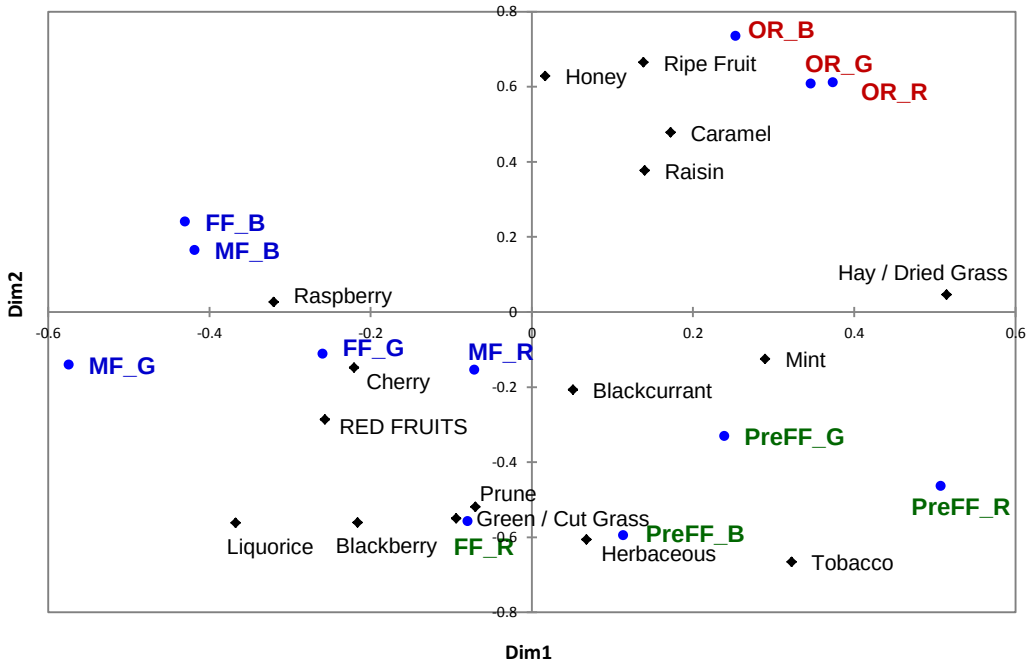


Figure 3.11a: Three dimensional multidimensional scaling plot for aroma sorting (stress value = 0.08). Wines with the same colour belong to a same group according to the cluster analysis. Families or sub-families are indicated in capital letters.

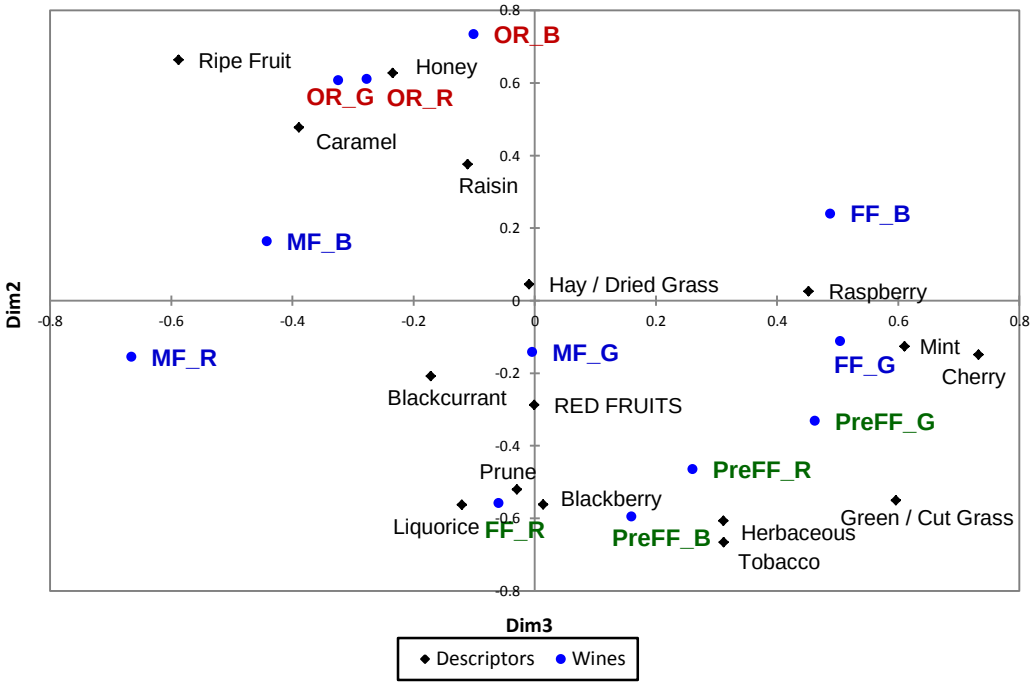


Figure 3.11b: Three dimensional multidimensional scaling plot for aroma sorting (stress value = 0.08). Wines with the same colour belong to a same group according to the cluster analysis. Families or sub-families are indicated in capital letters.

The aroma sorting result showed three clear groups separating on the first three dimensions of the MDS maps, coloured according to the cluster analysis (Figure 3.11a and Figure 3.11b). The wines from the PreFF stage were grouped together as well as the wines from the OR stage. The PreFF stage was characterised by green/cut grass, herbaceous and tobacco aromas. The OR stage was characterised by aromas of ripe fruit, honey, raisin and caramel. No clear separation was observed between the wines of the FF and the MF stages. Wines from these two stages were characterised by raspberry and cherry aromas.

3.3.2.2.3 Taste and Mouthfeel profiling

3.3.2.2.3.1 Descriptive test: Attributes citation frequency method

The result of the three-way ANOVA for each attribute (Sweet, Sour, Alcohol, Bitter and Astringent) is summarised in Addendum C table 5.7. The **wine effect** was significant ($p<0.05$) for all attributes which means that the panel could discriminate between sweet, sour, alcohol, bitter and astringent in all the wines. Overall the panel was repeatable.

Results of PCA on the mean scores of all attributes are shown in figure 3.12 and figure 3.13.

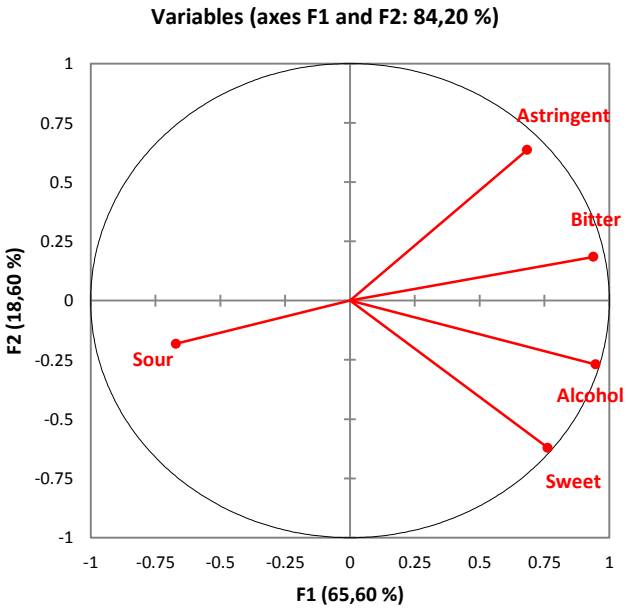


Figure 3.12: Correlation circle of attributes on principal components 1 and 2.

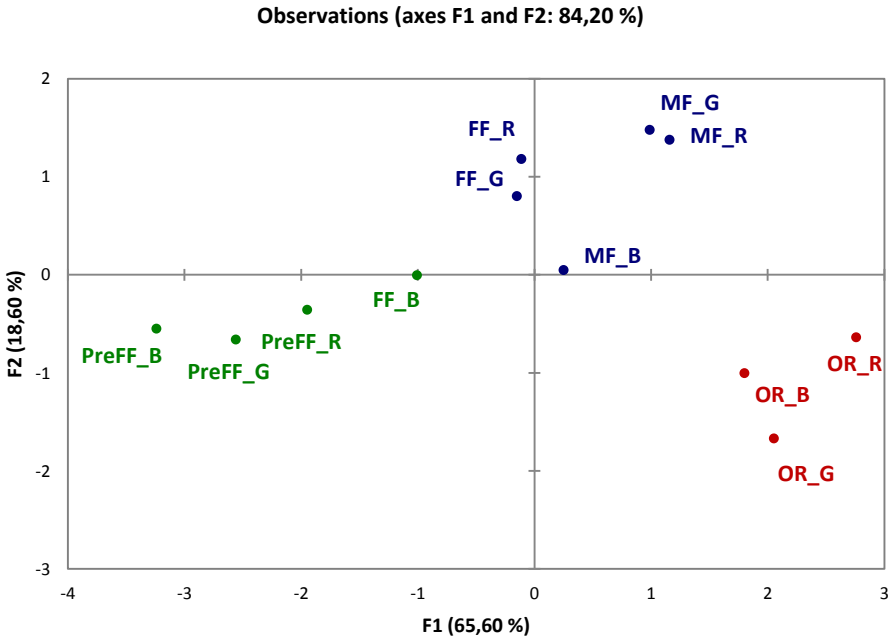


Figure 3.13: Principal component analysis plot of wines on principal components 1 and 2. Wines with the same colour belong to a same group according to the cluster analysis.

The cluster analysis yielded three groups of wines, as indicated with different colours, which mainly separate on the first dimension of the PCA (Figure 3.13). Bitter, alcohol, sweet and astringent were positively correlated with each other but negatively with sour (Figure 3.12). The first dimension separated the wines according to their harvest stages. Wines from the first harvest stage (PreFF), located on the left of the map, were perceived as being less bitter, alcohol, sweet, astringent and more sour than the wines from the last harvest stage (OR) on the right of the map. Astringent and sweet contributed also to dimension 2 which separated the MF repeats from the OR repeats. The OR repeats were perceived sweeter and less astringent than

the MF repeats. The cluster analysis however grouped the FF and MF repeats together, indicating that there was not a big difference between the wines from these two stages.

The harvest stages had an impact on the taste and mouthfeel of Cabernet Sauvignon wines, which is in accordance with Bindon et al. (2014). Wines made from OR grapes tended to be less sour and more sweet, alcoholic and bitter.

3.3.2.2.3.2 Descriptive test: Sorting task

The three-dimensional MDS plots (Figure 3.14a and Figure 3.14b) gave a stress value of 0.10, which indicated an acceptable representation of the original data.

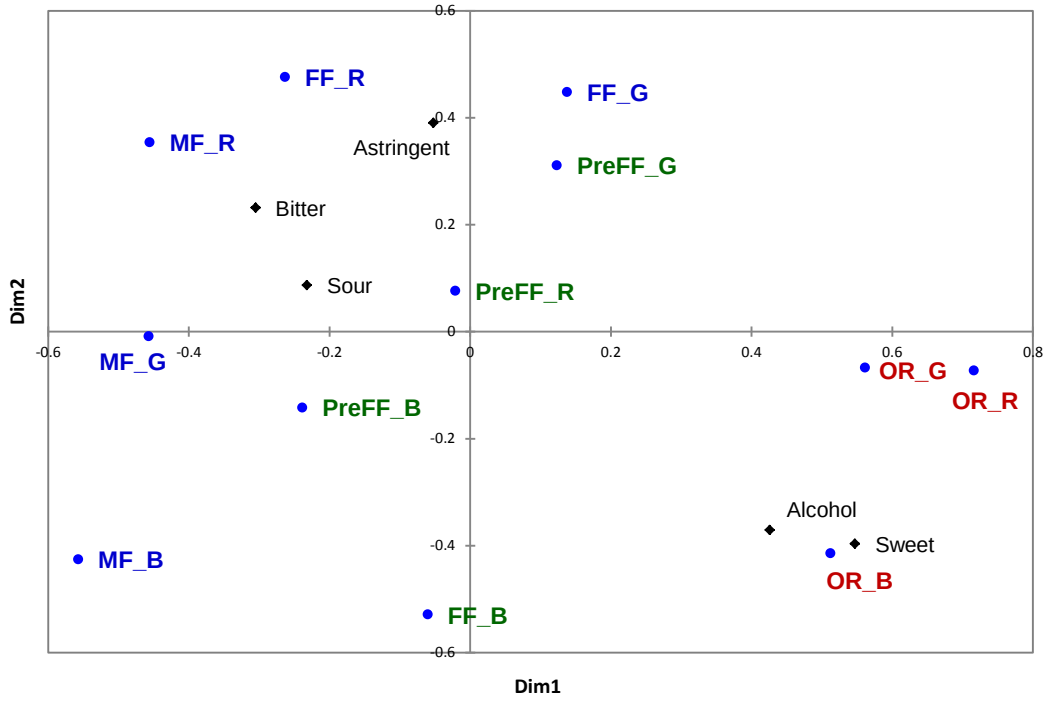


Figure 3.14a: Three dimensional multidimensional scaling plot for taste and mouthfeel (stress value = 0.10). Wines with the same colour belong to a same group according to the cluster analysis.

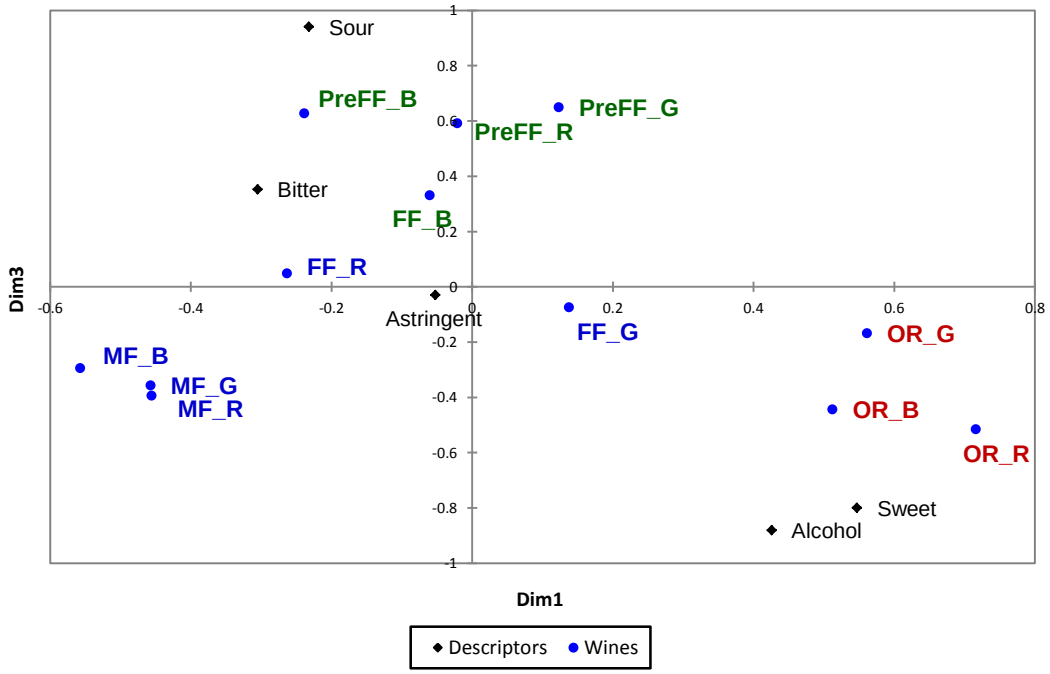


Figure 3.14b: Three dimensional multidimensional scaling plot for taste and mouthfeel (stress value = 0.10). Wines with the same colour belong to a same group according to the cluster analysis.

The taste and mouthfeel sorting results (Figure 3.14a and Figure 3.14b) indicated that the wines were grouped into the same three clusters obtained with the sorting task results on aroma (Figure 3.11a and Figure 3.11b). The wines made from the PreFF stage were grouped together as were the wines made from the OR stage. Wines from the PreFF stage was characterised as sour and bitter and the wines made from the OR stage was characterise as alcoholic and sweet. No clear separation was observed between the wines of the FF and the MF stages. Wines from these two stages were characterised as bitter in general.

3.3.2.3. Discussion

The attributes citation frequency method showed that the Cabernet Sauvignon wines made from the first harvest stage were characterised by vegetal and black/white pepper notes and were perceived to be less bitter, alcoholic, sweet and astringent and more sour that the wines from later harvest stages. Wines made from the last harvest stage were characterised as being more alcoholic with floral and raspberry notes and were perceived to be sweeter and less astringent. No clear aromatic differences were observed for the second (FF) and third harvest (MF) stages although a clear trend could be observed for taste and mouthfeel. In a study done by Bindon et al. (2013, 2014) on Cabernet Sauvignon wines made from sequentially harvest, a decrease in the green notes and red fruit attributes for later harvest dates was observed with an increase in the dark fruit attributes, hotness and viscosity. In their study, a correlation was found between red fruit and fresh green attributes and higher concentration of IBMP and higher levels of C_6 alcohols. Dark fruit aroma attributes correlated with an increase in ester concentration and were

also linked with higher levels of dimethyl sulphide in the wines. The fact that this study did not find the same trend in the fruit progression (red fruit to dark fruit) as in the study of Bindon et al. (2013, 2014), highlights the important role of the interactive effects between wine volatiles as well as the contribution of wine volatiles such as dimethyl sulphide which at high concentration has a negative odour (cabbage) but at low concentrations enhance fruitiness (Segurel et al. 2004, Escudero et al. 2007). It should also be mentioned that Bindon et al. (2014) did not use sugar loading as a physiological tool to predict harvest dates, they harvested sequential according to brix levels. As observed by Bindon et al. (2014), it is important to consider the interaction of IBMP and C_6-alcohols with a change in ester concentration when describing red fruit and dark fruit aroma and flavour. IBMP is commonly associated with the vegetal aroma of Cabernet Sauvignon but a study done by Preston et al. (2008) revealed that other chemical compounds such as sulphur-containing compounds may also contribute to the vegetal aroma.

The wines of the current study underwent malolactic fermentation causing an increase in the pH and a decrease in the titratable acidity of the wines. The titratable acidity in the wines decreased from the earlier harvest stages to the later harvest stages, confirming the observation that the wines from the first harvest stage were more sour than the wines form the last harvest stage. Wine pH influences the perception of astringency (Fontoin et al., 2008). The decrease in titratable acidity and the correlating increase in pH therefore contribute to the astringency perception causing the wines from the last harvest stage to be perceived as less astringent. However, Bindon et al. (2014) found an increase in the astringent attribute in later harvest stages and it was related to the tannin concentration, the proportion of skin tannin in the wines and tannin mDP (mean degree of polymerisation). In their study, astringency was also strongly associated with the acidity attribute, linking our observation that a decrease in acidity with a resulting higher pH correlates with a less astringent perspective. An increase in the alcohol content of the wines from the first to the last harvest stage (11.53% - 14.85%) confirms the observation that the wines from the first harvest stage were perceived as being less alcoholic than the wines form the last harvest stage. Ethanol both imparts bitterness and enhanced the bitter sensation causing the wines from the first harvest stage to be perceived as less bitter (Fontoin et al., 2008).

Doing the sorting task, winemakers and other wine professionals could distinguish between wines made from the first harvest stage and wines made from the last harvest stage but no clear separation could be made between wines for the second and third harvest stages. Wines made from earlier harvest stages were perceived as being more sour and had green notes. Wines made from later harvest stages were characterised by floral and ripe fruit notes and were perceived as sweet and alcoholic. The wines made from the two harvest stages in-between were characterised as having red fruit notes. The results from the sorting tasks done by wine professionals reflected trends obtained by the attributes citation frequency panel. Perrin

et al. (2007), showed the reliability of free profiling by demonstrating that free profiling by wine professionals delivered the same results as conventional profiling by a trained panel.

3.4. Conclusion

It can be concluded that both the Merlot and the Cabernet Sauvignon wines made from earlier harvest stages were characterised as having more green notes and were perceived as being more sour. The wines made from later harvest stages were characterised as having more red fruit notes and were perceived as being more alcoholic. Both the trained panel as well as the wine professionals regularly clustered the PreFF repeat wines together as well as the OR wine repeats. No clear separation could be observed between the FF and the MF repeat wines. More thought should be given to the naming of the stages using sequential harvest seeing that the attributes describing the stages were not reflected by the terms currently describing the stages (Deloire, 2012). Preliminary results showed that harvesting grapes according to sequential harvest let to better classification of the stages for Cabernet Sauvignon than for Merlot.

Further research should be done on investigating the number of days between the different harvesting stages considering the varieties, the sites, fruit zone microclimate and the tempo/profile of ripening using several physiological indicators. A detailed chemical analysis of wine volatiles compounds, non-volatile compounds and esters are needed to explain the interactive effects between chemical compounds and their contribution to aroma profiles of wines produced at different ripening levels (Hjelmeland et al. 2013).

Grapes harvested according to sequential harvest did not yield wines with significantly different aroma profiles. Our study did not show significant difference using sensory analysis between the fresh and mature harvest times, for Merlot and Cabernet Sauvignon.

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Chapter 4

Conclusion

4. CONCLUSION

4.1 Conclusion and future prospects

The perception of wine aroma can be attributed to a large array of different chemical compounds. Wine aroma is complex and not dependent on a specific compound, but on the interactions between them (Robinson et al., 2009; Darriet et al., 2012). The perceived aroma can be enhanced or masked by the presence of other chemical molecules. Marais et al. (1999) reported that the perceived aroma nuances in their study could have been masked or enhanced by the synergistic action of different aromas. Chemical compounds responsible for the aroma of wine develop at different stages during grape ripening making the decision when to harvest a critical one in producing quality wines.

The world is becoming progressively technologically advanced and information has become more freely available to consumers. The modern consumer expects wine to be produced in a healthful and environmentally sustainable manner. The definition of quality has also shifted from that of the producer to that of the consumer (Bisson et al., 2002). These factors have forced the wine industry to evolve into a consumer-focused production industry. The need to produce high quality wines in a specific style or with a specific aroma has thus increased. Sequential harvest in association with a physiological indicator (fruit sugar accumulation) were proposed as a valuable tool to use in conjunction with traditional indicators to help producing high quality wines and increase wine style diversity (Deloire, 2011; Bindon et al., 2014; Boss et al., 2014).

Cabernet Sauvignon and Merlot wines were made according to five stages using sequential harvest and subjected to sensory evaluation to determine if wine experts and a trained panel could distinguish between the different wine aromatic profiles as defined by Deloire (2011, 2012) and Deloire et al. (2014). Attributes were also generated for each stage. In both Merlot and Cabernet Sauvignon, the pre fresh fruit (PreFF) and over ripe (OR) stages could be more easily discriminated than the two harvest stages in-between, fresh fruit (FF) and mature fruit (MF), by both the wine experts and the trained panel. The attributes generated for Merlot at the various stages differed between the expert tasters and the trained panel, however, a better agreement among the expert tasters and trained panel for the attribute of the various stages for Cabernet Sauvignon was observed. This could suggest that the sensory differences between the various stages for Cabernet Sauvignon were greater than for Merlot. The wines made from the FF and MF stages could not be distinguished from each other in general thus further research on fruit and wine composition and sensory during ripening is needed. Aroma attributes characterising the FF and MF wines ranged from red fruit notes to black fruit notes. However, a clear trend could be observed in Cabernet Sauvignon and Merlot wines with attributes changing from green to ripe fruit during ripening in general when using expert tasters.

Practicing sequential harvest using an easy to use fruit physiological indicator is convenient and to certain extends not expensive to perform. Further research is needed to analyse and link fruit and wine composition and wine styles using sequential harvest (Bindon et al., 2014; Boss et al., 2014). This could assist in defining a specific area/site and for a specific cultivar the best harvest times in relation with the desired/potential wine style. Relevant research should be engaged to refine the sequential harvest in order to deliver an increase in the diversity of wine styles from a single site or a group of vineyards for the demanding market/consumer. This will need an integrated approach from the field to the consumer. The study should also include more than one vintage to eliminate vintage influences.

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ADDENDUMS

5. Addendums

Addendum A

Table 5.1: Aromatic Descriptors list used during the training sessions

Aromatic Descriptors List

FRUITY

WHITE FRUIT:
- Quince
- Pear
- Apple

YELLOW FRUIT:
- Apricot
- Peach
- Nectarine
- Melon

CITRUS:
- Bergamot
- Lemon
- Orange
- Grapefruit

RED FRUIT:
- Cherry
- Strawberry
- Raspberry
- Redcurrant

BLACK FRUIT:
- Blackcurrant
- Bilberry / Blueberry
- Blackberry

DRIED FRUIT:
- Dried Apricot
- Date
- Dried Fig
- Prune

HARD FRUIT (Nutty):
- Almond
- Hazelnut
- Walnut

TROPICAL FRUIT:
- Pineapple
- Banana
- Litchi
- Mango
- Coconut
- Kiwi

OTHER FRUIT:
- Fruit Conserve / crystallized
- Bitter Almond
- Muscat
- Cider
- Kirsch

VEGETATIVE

VEGETABLES:
- Artichoke
- Asparagus
- Cabbage
- Celery
- Green Beans
- Green Pepper
- Olive

OTHER VEGETATIVE:
- Hay / Dried grass
- Pine
- Herbaceous
- Tobacco

FLORAL:
- Acacia
- Camomile
- Honeysuckle
- Orange Blossom
- Geranium
- Jasmine
- Lilac
- Rose
- Lemon Blossom
- Violets
- Honey

SPICE:
- Aniseed / Fennel
- Cinnamon
- Clove
- Curry
- Juniper
- Ginger
- Bay leaf / Laurel
- Nutmeg
- Pepper
- Liquorice
- Thyme
- Mint
- Vanilla

ANIMAL
- Leather
- Smoked
- Musk / Civet
- Cat urine
- Wet dog

FOREST FLOOR
- Mushroom
- Humus / Earthy
- Mouldy

TOASTED / WOOD

TOASTED:
- Caramel
- Roasted Coffee
- Toasted bread

WOODY:
- Woody Planky
- Burnt wood
- Smoked Wood

OTHER
- Alcohol
- Butter
- Lactic
- Yeast
- Chocolate
- Rubber
- Tar
- Carton / Dust
- Mineral / Flinty
- Sulphur
- Stuffy / Fusty smell
- Iodine / Salty
- Chemical strawberry
- Wet mop
- Mineral
- Solvent / Chemical
- Chalky

Table 5.2: Odour reference standards presented during the training.

Subfamily/ Family	Descriptor	Odor reference and quantity
		FRUITY
White fruit	Green apple	2 cm3 fresh green apple cut just before the session
	Pear	2 cm3 of canned pear + 10 mL distilled water
	Quince	3 spoons of Quince marmalade "Ann's kitchen"
Yellow fruit	Apricot	20 mL apricot juice "Liquidfruit" + 1/4 of canned fruit "Rhodes"
	Melon	fresh melon cut in pieces (15 min prior to the session)
	Peach	3 cm3 canned peach "Koo" + 3 cm3 fresh peach + 5 mL distilled water
Citrus	Bergamote	Solution standard "Ferminich", 2 drops on a cotton disk
	Grapefruit	3 cm3 of fresh fruit (pulp + flesh)
	Lemon	1 drop essence "Vahine" on a cotton disk
	Orange	1 drop essence "Robertsons" on a cotton disk
Red fruit	Cherry	Solution of 5 mL "Vedrenne" syrup + 15 mL distilled water
	Raspberry	1 big spoon Nappage "Vahine"
	Redcurrant	Solution 5 frozen berries Hillcrest + 10 mL distilled water
	Strawberry	1/2 of a fresh strawberry
Black fruit	Blackberry	Solution 5mL "Vedrenne" syrup + 15 mL distilled water
	Blackcurrant	Solution of 5 frozen berries "Hillcrest" + 10 mL distilled water
	Blueberry	2 spoons Blueberry sauce "St Dalfour"
Dried fruit	Date	1 date "Safari" cut in pieces
	Dried apricot	3 half of dried apricots "Freshers"
	Dried Fig	1 sun dried fig "Freshers"in pieces
	Prune	1 dried prune "Safari" cut in pieces
Hard fruit (nutty)	Almond	Solution 10 drops of almond essence "Vahine" + 10 mL distilled water
	Hazelnut	Solution 2 little spatulas of "Nutella"
	Walnut	An oral comment was provided to panelists
Tropical fruit	Banana	1 cm3 ripe banana +10 mL distilled water
	Coconut	6g dry coconut "Imbo" + 20 mL of hot water
	Goose berry	3 fresh goose berries cut in pieces
	Guava	20 mL guava juice "Darling"
	Litchi	1 dried litchi rehydrated
	Mango	2 cm3 canned mango +1 mL mango juice "Darling" + 5 mL distilled water
	Passion fruit	1/4 of the pulp from a fresh passion fruit
	Pineapple	2 cm3 of a fresh pineapple
Other fruit	Oxidized Apple	2 cm3 fresh apple, leave it for a while to get the oxidation
	Bitter almond	2 drops of bitter almond essence "Vahine"
	Cider	Solution 20 mL dry cider "Hunter's"
	Cristallized fruit	3 cm3 pieces of cristallized fruit "Moir's" + 10 mL boiled water
	Kirschy	An oral comment was provided to panelists
	Muscat	An oral comment was provided to panelists

Subfamily/ Family	Descriptor	Odor reference and quantity
		VEGETATIVE
Vegetables	Artichoke	1/2 piece of a can
	Asparagus	10 mL water from a can "Food Lover's signature"
	Cabbage	Cooked fresh cabbage
	Celery	2 cm3 fresh celery
	Green bean	10 mL water from a can "Koo"
	Green pepper	4 thongs of a fresh green pepper cut in pieces
	Olive	1 olive + 10 mL water from a can
Other vegetative	Eucalyptus	1 drop solution of Eucalyptol
	Hay / dried grass	Finely cut hay - get from pet shop
	Herbaceous Fresh grass	A half bottle of fresh grass
	Lemon grass	1 cm3 cut in little pieces of fresh lemon grass
	Mint	2 fresh smashed mint leaves
	Tobacco	Dried tobacco from 2 cigarettes
	Tomato leaf	Green leaves of cherry tomato + stem
		FLORAL
	Acacia	An oral comment was provided to panelists
	Camomille	1 tea bag into 30 mL of hot water during 10 min
	Geranium	1 drop solution "Aux parfums de Grasse" on a cotton disk
	Honey	1 little spoon of honey + 10 mL of hot water
	Honeysuckle	2 drops solution "Ferminich" on a cotton disk
	Jasmin	1 drop of solution "Aux parfums de Grasse" on a cotton disk
	Lilac	2 drops solution "Ferminich" on a cotton disk
	Linden Tree Flower	2 drops solution "Aux parfums de Grasse" on a cotton disk
	Orange Blossom	2 drops solution "Ferminich" on a cotton disk
	Rose	Solution of 1 mL of rose water +10 mL distilled water
	Violet	Solution 2 mL of "Vedrenne" syrup + 4 mL distilled water
		SPICE
	Aniseed/Fennel	10 drops "Carrefour" aniseed syrup
	Bay leaf / Laurel	1 cut dried bay leaf
	Black pepper	2g whole berries black pepper crushed, "Cheker's Choice"
	Cinnamon	0,05g cinnamon powder, "Chekers's Choice"
	Clove	0,05g "Robertsons" clove powder
	Curry	1 small spatula of curry powder
	Ginger	1g of ginger powder "Robertsons"
	Juniper	2 crushed berries of Juniper
	Liquorice	2 cm of "Mister sweet"
	Nutmeg	0,03g Nutmeg powder "Robertsons"
	Thyme	1 spatula of "Robertsons" dried thyme
	White pepper	2g "Robertsons" white pepper in powder + 5 mL distilled water
		ANIMAL
	Cat urine	An oral comment was provided to panelists
	Horsy / sweaty	An oral comment was provided to panelists
	Leather	Leather pieces were passed among panelists
	Meat Stock	Solution 25 g of beef stock "Ina Paarman's kitchen" + 10 mL of diluated Beefy Borril
	Musk / Civet	An oral comment was provided to panelists
	Smoked	1 little spoon of BBQ sauce Jack Daniels
	Wet dog	wet dog hair

Subfamily/ Family	Descriptor	Odor reference and quantity
		FOREST FLOOR
	Humus / Earthy	wet earth (a half bottle)
	Mouldy	An oral comment was provided to panelists
	Mushroom	Solution 1/2 fresh mushroom cut in pieces + 10 mL distilled water
		TOASTED / WOOD
Toasted	Caramel	Solution 1 big spoon "Vahine" caramel + 5 mL hot water
	Chocolate	1 spoon Nappage "Vahine" (sauce)
	Roasted Coffee	Solution 1 g instant coffee "Jacobs" + 10 mL hot water
	Toasted bread	1x1 cm toasted bread
	Toffee	Solution 1 and a half toffee sweet + 5 mL hot water, to melt in the microwaves
	Vanilla	1/2 teaspoon "Vahine" vanilla essence
Woody	Burnt wood	Pieces of burnt chips wood
	Toasted / Smoked wood	5 g toasted wood
	Woody / Planky	5 g new wood
		OTHER
	Alcohol	5 mL of alcohol at 96%
	Butter	2 cm3 of fresh butter
	Carton / dust	Pieces carton + a few drops of water
	Chalky	Chalky pieces were passed among panelists
	Chemical strawberry	3 drops of strawberry flavour "IFF" on a cotton disk
	Iodine / Salty	1/4 crushed oyster shell
	Lactic	20 mL of fresh pasteurized cream "Darling"
	Mineral / Flinty	An oral comment was provided to panelists
	Rubber	1 cm of a rubber pipe, to warm up in the microwaves
	Solvent / Chemical	Ethyl acetate 200 microL
	Stuffy / Fusty smell	An oral comment was provided to panelists
	Sulphur	Solution 300 microL SO_2 at 15% + 15 mL distilled water
	Tar	1 little spatula of Creosote
	Wet mop	Pieces of wet mop
	Yeast	20 mL rehydrated yeasts from the wine industry

Addendum B

Table 5.3: Instruction sheet for attributes citation frequency method.

Evaluation Session

Odour, Taste and Mouthfeel Description

The session is split in **3 parts** with a break in between. Each part consists of the evaluation of **4 wines**.

Please read carefully the instructions below before you start and ask me if you have any questions.

You need **to evaluate samples in the given order (from left to right)**. Once you have finished evaluating a sample you won't be able to evaluate it again.

For each sample, you need to smell it and choose the most relevant descriptors on the aromatic descriptors list by ticking the corresponding box (**5 descriptors maximum per wine**). You can eventually mention one or more descriptors which are not on the list.

Then, you need to taste the sample to evaluate the intensity of sweetness, sourness, bitterness, astringency and alcohol on the 6-points scales (**from 0: Absent to 5: Very High**).

WE ARE ASKING YOU TO SPIT THE WINE AS YOU HAVE 12 WINES TO EVALUATE.

Once you have finished evaluating a sample, take some crackers and a sip of water to rinse your mouth. You can now follow the same steps (smell and taste) with the next sample. **You are not allowed to go back to the previous samples.**

For each sample, **DON'T FORGET TO INDICATE THE THREE DIGIT CODE** on your answer sheet.

After evaluating 4 samples, leave the room for your break and come back after 5-10 minutes to evaluate the next 4 samples.

Thank you for participating in our experiment.

Table 5.4: Instruction sheet for sorting method.

Instructions - Sorting Task

Thank you for participating in this study. It consists of the evaluation of **Merlot** and **Cabernet Sauvignon** wines and lasts about 1h.

For each wine the experiment consists of 2 parts with a short break in between. Each part consists of 2 steps. There are no correct or wrong answers; it's your personal opinion which interests us. Please read carefully the instructions below before you start and ask the experimenters if you have any questions.

PART I – AROMA

Step 1

12 samples of wines are presented to you in black wineglasses. Each sample is coded with a three-digit number. We ask you:

- **to smell all samples in the given order** (from left to right)
- **to sort samples into groups according to your perceptions of odor similarities and differences among the wines**. You will group together the wines that you perceive odors as similar.

  You are allowed to form as many groups as you want and put as many samples as you want in each group. Groups can be formed by a different number of samples. After smelling each sample in the given order, you can smell them as many times as you need and in any order. You can take all the time you need.
- **to write the wine codes that form each group in the table of the answer sheet "AROMA".**

Please don't change the groups and call the experimenter to move on the next step.

Step 2

Now we ask you to give a **description of each group** that you have formed. **You are not allowed to change the groups anymore**.

You can smell the wines again.

Please **choose the most relevant descriptors from the list on the answer sheet "AROMA"** to describe the odor that define each group **(no more than 5 descriptors per group)**.

Once you have finished this step, call the experimenter.

Part II –TASTE AND MOUTHFEEL

Step 1

For this part, the procedure to follow is the same as the part I, but now **you only base your sorting on taste and mouthfeel and not on aroma of wines**.

- **Taste** all samples in the given order (from left to right)

- **Sort samples into groups according to your perceptions of taste and mouthfeel similarities and differences among the wines**. You will group together the wines that you perceive taste and mouthfeel as similar.

  1. You are allowed to form as many groups as you want and put as many samples as you want in each group. Groups can be formed by a different number of samples. After smelling each sample in the given order, you can smell them as many times as you need and in any order. You can take all the time you need.

- **Write the wine codes that form each group in the table of the answer sheet "TASTE & MOUTHFEEL".**

Water and crackers are available to rinse your mouth between samples. A spittoon is also available to spit out wines.

Please don't change the groups and call the experimenter to move on the next step.

Step 2

Now we ask you to give a **description of each group** that you have formed. **You are not allowed to change the groups anymore**.

You can taste the wines again.

Please **rate the intensity of sweetness, sourness, alcohol, bitterness and astringency** that characterise each group.

Evaluate the intensity of each descriptor by giving a score on the intensity scale, from “0: Absent” to “5: Very High”, on the answer sheet “TASTE & MOUTHFEEL”.

The part II is over, you can take a break of 5-10 min.

The experimenter will give you a short questionnaire to fill in. Then come back for the second experiment, the instructions to follow will be the same.

Addendum C

Table 5.4: The basic juice and wine analysis of the Merlot wines made according to sequential harvest.

			Juice analysis			Wine analysis		
Harvest stage	**Harvest date**	**Repeat**	**ºB**	**pH**	**TA**	**pH**	**TA**	**Alcohol**
PreFF	1/30/2013	Blue	23.2	3.24	7.63	3.39	7.31	12.76
PreFF	1/30/2013	Green	23.6	3.22	7.68	3.35	7.50	13.49
PreFF	1/30/2013	Red	23.2	3.22	7.83	3.37	7.48	13.37
FF	2/5/2013	Blue	23.7	3.29	6.48	3.46	6.75	13.83
FF	2/5/2013	Green	23.7	3.28	6.28	3.42	6.92	13.99
FF	2/5/2013	Red	23.8	3.27	6.56	3.44	7.04	13.88
N	2/10/2013	Blue	23.7	3.39	5.84	3.49	6.90	14.08
N	2/10/2013	Green	23.8	3.37	5.72	3.49	6.84	14.10
N	2/10/2013	Red	23.8	3.35	5.71	3.48	7.01	14.06
MF	2/15/2013	Blue	23.7	3.45	5.55	3.51	6.36	13.87
MF	2/15/2013	Green	23.7	3.43	5.49	3.52	6.38	13.87
MF	2/15/2013	Red	22.8	3.44	5.53	3.49	6.11	13.88
OR	2/25/2013	Blue	23.9	3.44	5.46	3.58	6.92	14.39
OR	2/25/2013	Green	24.3	3.47	5.33	3.52	7.18	14.64
OR	2/25/2013	Red	24.1	3.48	5.21	3.55	7.07	14.42

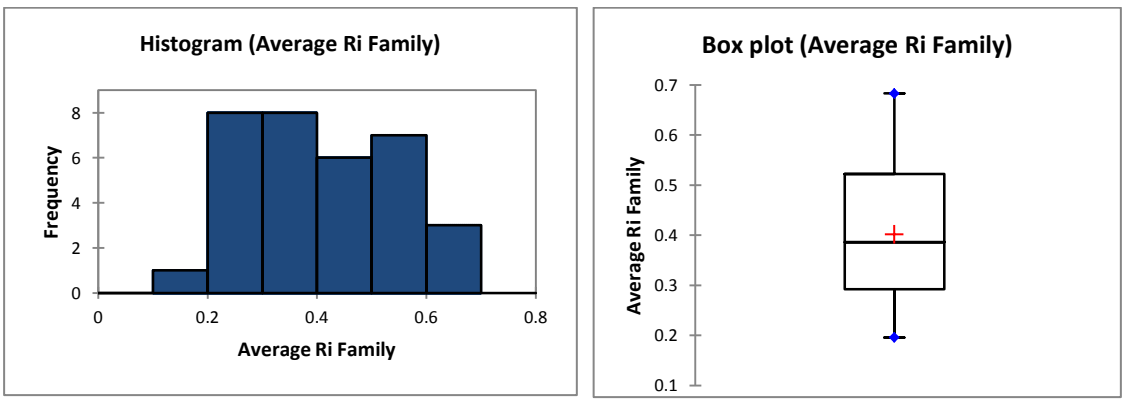

Figure 5.1: Reproducibility index of the trained panel (N=33) for aroma evaluation of Merlot wines

Table 5.5: Results of the 3-way ANOVA for taste and mouthfeel descriptions of Merlot wines.

	Sweet	Sour	Alcohol	Bitter	Astringent
R^2	0.82	0.81	0.86	0.81	0.85
F	3.54	3.52	4.93	3.31	4.53
P > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Judge	34.43	32.89	55.12	31.16	48.28
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Wine	0.87	4.68	3.32	2.17	3.54
	0.57	< 0.0001	0.00	0.02	0.00
Repeat	0.73	1.02	0.00	4.41	5.30
	0.39	0.31	0.95	0.04	0.02
Judge*Wine	1.00	1.06	0.89	1.04	0.94
	0.49	0.29	0.85	0.37	0.72
Judge*Repeat	2.57	1.63	1.39	1.70	2.05
	< 0.0001	0.02	0.09	0.01	0.00
Wine*Repeat	1.01	1.32	0.88	0.97	0.58
	0.43	0.21	0.56	0.47	0.85

Table 5.6: The basic juice and wine analysis of the Cabernet Sauvignon wines made according to sequential harvest.

			Juice analysis			Wine analysis		
Harvest stage	**Harvest date**	**Repeat**	**ºB**	**pH**	**TA**	**pH**	**TA**	**Alcohol**
PreFF	2/15/2013	Blue	20.5	3.21	7.50	3.60	6.75	11.77
PreFF	2/15/2013	Green	20.4	3.23	7.51	3.62	6.85	11.58
PreFF	2/15/2013	Red	20.2	3.22	7.89	3.64	6.19	11.25
FF	2/25/2013	Blue	21.8	3.33	6.62	3.69	6.33	11.76
FF	2/25/2013	Green	21.5	3.32	6.75	3.72	6.00	12.35
FF	2/25/2013	Red	21.5	3.31	7.00	3.73	6.06	12.27
N	3/7/2013	Blue	22.6	3.50	6.35	3.73	5.68	12.44
N	3/7/2013	Green	22.7	3.46	6.05	3.77	5.34	13.00
N	3/7/2013	Red	22.7	3.46	6.30	3.82	5.27	12.93
MF	3/18/2013	Blue	23.2	3.54	5.51	3.68	5.46	13.38
MF	3/18/2013	Green	22.9	3.53	5.40	3.64	5.37	13.27
MF	3/18/2013	Red	23.0	3.50	5.86	3.67	5.16	13.23
OR	4/3/2013	Blue	25.1	3.87	4.81	3.80	5.20	14.81
OR	4/3/2013	Green	25.5	3.83	4.86	3.82	5.18	15.08
OR	4/3/2013	Red	25.1	3.77	4.98	3.78	5.30	14.65

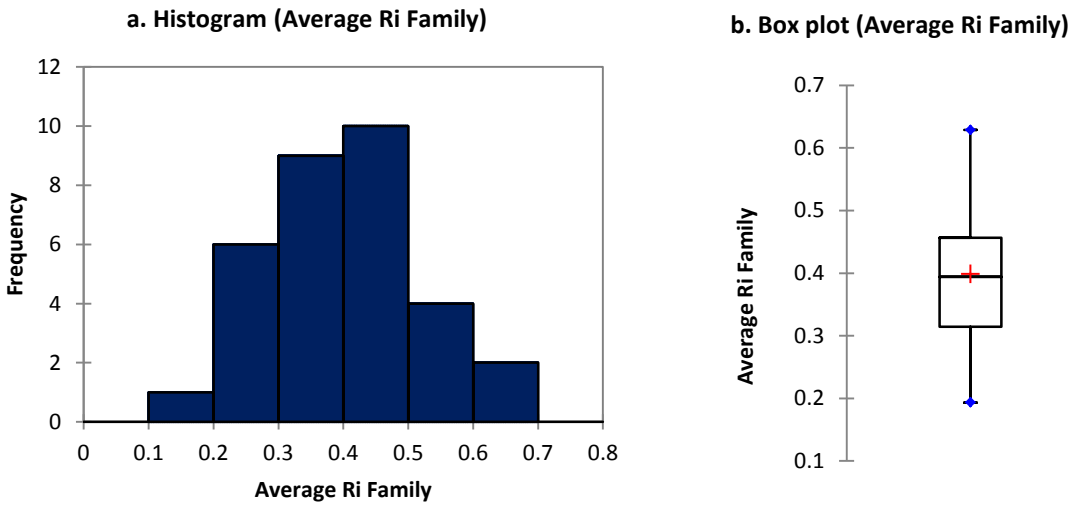

Figure 5.2: Reproducibility index of the trained panel (N=32) for aroma evaluation of Cabernet Sauvignon wines

Table 5.7: Results of the 3-way ANOVA for the taste and mouthfeel descriptions of Cabernet Sauvignon wines

	Sweet	Sour	Alcohol	Bitter	Astringent
R^2	0.84	0.80	0.83	0.77	0.82
F	4.18	3.13	3.96	2.65	3.61
P > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Judge	30.66	24.77	37.59	21.47	31.32
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Wine	18.05	2.07	10.59	2.34	6.00
	< 0.0001	0.02	< 0.0001	0.01	< 0.0001
Repeat	7.72	15.82	12.30	13.00	19.87
	0.01	< 0.0001	0.00	0.00	< 0.0001
Judge*Wine	1.51	1.27	0.96	1.04	1.11
	< 0.0001	0.01	0.65	0.36	0.16
Judge*Repeat	3.21	2.37	2.00	1.80	3.05
	< 0.0001	< 0.0001	0.00	0.01	< 0.0001
Wine*Repeat	0.81	1.91	0.56	1.30	0.92
	0.63	0.04	0.86	0.22	0.52