Investigating Peduncle Colour Evolution and Chemistry During Ripening in Vitis vinifera L. cv Shiraz

A thesis presented in fulfilment of the requirements for the degree of

Doctor of Philosophy

Yudan Fang

B.Eng (Viticulture & Oenology)

China Agricultural University

The University of Adelaide

School of Agriculture, Food and Wine

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Abstract

This thesis describes an investigation of how the various sections of the grape stem (peduncle and rachis) in *Vitis vinifera* L. cv Shiraz evolve in terms of their colour and chemical attributes during the ripening period and is compared to the prototypical berry ripeness parameters (^{o}Brix, pH, TA, etc) over three seasons. The grape peduncles turned from green at veraison, to predominantly brown at harvest, whilst the rachises remained green during the maturation period. Certain peduncle and rachis chemical changes were also uncovered during these studies and shed light on the relationships between peduncle colour and chemical composition. Statistical modelling was conducted to examine the relationship between the peduncle colour and chemical composition, and a clear co-development was observed between peduncle evolution (colour and moisture) and the berry ripening continuum. Such observations are yet to be reported and provide for the opportunity to develop a new simple platform to assist in predicting grape berry ripeness and therefore aid in harvest decisions.

Chapter 1 of this thesis comprises a detailed introduction of our current understanding on how both grape berries and their stems evolve over the ripening period and sets the scene by pinpointing key aspects for this exciting research journey.

Chapter 2 details a large study on how the change in morphology (size and colour) of both the grape peduncles and rachises evolve in eight distinct patches of Shiraz from the same vineyard over two seasons. A semi-automatic method was developed to quantify the peduncle morphological details exploiting digital image analysis. The berries associated with the peduncles from the same bunches were also analysed for their prototypical berry ripeness parameters, namely the sugar content (^{o}Brix), acidity (pH and TA), total anthocyanins and total phenolics. The image analysis showed that the overall peduncle colour evolution from green to brown was well reflected in the change of *a** and *b** values in the CIE*L*a*b** colour system coupled with their polar parameters hue and chroma. Importantly, the change in the colour coordinates of the peduncles was found to be in parallel with the change in berry ripeness parameters thus providing a new means to assist in harvest decisions. This work was submitted to the *Australian Journal of Grape and Wine Research* in 2014.

Chapter 3 evaluates a range of key chemical changes in Shiraz peduncles and rachises from veraison to harvest over two seasons. During ripening, peduncles experienced significant decrease in moisture and pigment (chlorophylls and carotenoids) levels while the total phenolics and antioxidant capacity (DPPH) levels did not change significantly. The levels of these chemical traits were always higher in the rachises than the peduncles; an observation yet to be reported. Moreover, it was found that the peduncle moisture content was correlated with the peduncle colour hue value in a strong linear fashion and was negatively associated with the pigments ratio (total chlorophylls / total carotenoids). Finally, the results showed that peduncle moisture content also co-developed with the prototypical berry ripeness parameters during ripening, an observation which excitingly provides for a new hitherto unknown approach to predicting berry ripeness and harvest date via peduncle moisture detection. This work was submitted to the *Journal of Agricultural and Food Chemistry* in 2014.

Chapter 4 evaluates the spatial and temporal variation of peduncle colour in five Shiraz patches from the same vineyard during the 2013/14 season. Peduncles were collected from the same positions based on a regular grid sampling design for each patch of Shiraz; an enormous but fulfilling task! The within-patch variation among individual peduncle colour was found to be mostly driven by the berry maturation stages. It was found that at veraison, the peduncle colour was more homogeneous and displayed a green state, then the peduncle colour became more variable during ripening whilst less heterogeneous at harvest with the majority of peduncles in brownish colour. We also observed that actual fruit harvesting within these vineyard patches began when over 40% of the sampled peduncles appeared visually predominantly brown, providing the sample size was no less than 100 from that particular patch. Thus a simple visual assessment of the proportion of brown peduncles could be used as an easy and quick way to help predict time for harvest. Moreover, the linear relationship between peduncle colour hue value and moisture content was verified and extended to the individual bunch level during the 2014 vintage. Again these observations strengthen the potential use of monitoring peduncle colour evolution to predict harvest date, and pave the way for the development of non-destructive methods of measuring peduncle colour in the field. This work has been prepared in publication style for the *Australian Journal of Grape and Wine Research* in 2014.

Chapter 5 completes these exhilarating research works by summarising the key new findings and their potential benefits to the wine industry and provides suggestions for future research directions.

List of publications

1. Fang, Y.; Kravchuk, O.; Fuentes, S.; Skouroumounis, G. K.; Cotsaris, D.; Taylor, D. K. Assisting Harvest Decisions via the Relationship between Peduncle Colour and Berry Ripeness in *Vitis vinifera* L. cv Shiraz. *Australian Journal of Grape and Wine Research*. **2014**, submitted.

2. Fang, Y.; Kravchuk, O.; Taylor, D. K. Chemical Changes in Grape Stem and Their Relationship to Stem Color Throughout Berry Ripening in *Vitis vinifera* L. cv Shiraz. *Journal of Agricultural and Food Chemistry*. **2014**, submitted.

3. Fang, Y.; Kravchuk, O.; Taylor, D. K. Investigation of Within-Vineyard Variation of Peduncle Colour to Assist in Harvest Decisions in *Vitis vinifera* L. cv Shiraz**.** *Australian Journal of Grape and Wine Research*. **2014**, publication format.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Yudan Fang

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Finally, I would like to thank my parents in China for their care and support along my PhD journey. Although they don't speak English and hardly understand research, their unconditional love has encouraged me continuously during my four years in Adelaide.

Abbreviations

ABA	Abscisic acid
au	Absorbance units
BSA	Berry sensory assessment
C	Chroma
Ca	Chlorophyll *a*
Cb	Chlorophyll *b*
Ca+b	Total chlorophylls
Cx+c	Total carotenoids
cm	Centimetres
DAF	Days after flowering
DPPH	1,1-Diphenyl-2-picrylhydrazyl
g	Grams
GA	Gibberellic acid
GC	Gas chromatography
GPS	Global positioning system
h	Hue
Ha	Hectare
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
Hz	Hertz
JPEG	Joint Photographic Experts Group
kg	Kilogram
L	Litre
LC-MS	Liquid chromatography-mass spectrometry
m	Meter
M	Molar (moles/litre)
mg	Milligrams
MgO	Magnesium oxide
min.	Minutes
mL	Millilitre
mm	Millimeter
mmol	Millimoles

mol	Moles
MS	Mass spectrometry
NIR	Near-infrared spectroscopy
nm	Nanometres
OIV	International Organisation of Vine and Wine
P	Peduncle
pi	π (≈ 3.14)
ppm	Parts per million
R	Rachis
REML	Restricted/residual maximum likelihood
RGB	Red Green Blue
ROI	Regions of interest
ROS	Reactive oxygen species
rpm	Revolutions per minute
SH	Shiraz
SPME-GC-MS	Solid-phase microextration-Gas chromatography-Mass spectrometry
TA	Titratable acidity
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
TSS	Total soluble solids
UV	Ultra-violet
μg	Micro gram
μM	Micro molar

Figures and Tables for Chapter 1.

Learning is the only thing which the mind can never exhaust, never alienate, never be tortured by, never fear or distrust, and never dream of regretting.

— T.H. White

Chapter 1 Introduction

1.1 The Australian Wine Industry and its Evolution.

The birth of the Australian wine industry began in 1788 when Governor Phillip brought grape vine cuttings to Sydney Cove from the Cape of Good Hope, South Africa. Australia's first commercial vineyard and winery was then established in New South Wales in the early 1800s, and by the 1850s, commercial vineyards for wine production were well established in most Australian states. South Australia was one of the very early states to begin the establishment of vineyards, and as a consequence of this, now has some of the oldest vines in the World which are still growing on their original European rootstocks (Wine Australia 2014). During World War II, domestic wine consumption vastly increased, however, up until the 1970s the wine production in Australia consisted largely of sweet and fortified wines, with table wines not starting to thrive until the late 1950s (Allen, M. 2012). Around this time the consumers' wine preferences began to change in Australia, and vineyards and wineries experienced a dramatic technological revolution from a situation of no vineyard irrigation, use of horse-drawn ploughs and mostly manual work at harvest to exploiting drip irrigation, harvesting machines and stainless steel vats for producing wines (Allen, M. 2012). Australia now enjoys the benefits of decades of home grown research, which has allowed it to propel itself from an essentially unknown in the World of wine production and quality, to one who now constantly produces some of the World's best iconic wines.

1.1.1 Wine Regions in Australia.

Australia is the sixth largest country by total area in the World, however this vast country is too hot and dry even for the hardy vine to thrive in most regions. Consequently, most of the established wine regions hug the coast, where it is cooler and there is adequate rainfall. Currently there are over 60 wine regions and 2573 wineries in Australia (Winebiz 2014), most of which are situated near the heavily populated areas along the Southeast Coast, Tasmania and the far Southwest, Figure 1. Wine is produced in every state, and the wine regions in each of these states produce different wine varieties and styles that take

advantage of the local Terroir. During the 2012-13 season, Australia had a gross total wine production of 1,245,601,000 litres, with South Australia producing the most wines among the states with a total production of 568,196,000 litres in that year (Australian Bureau of Statistics 2014).

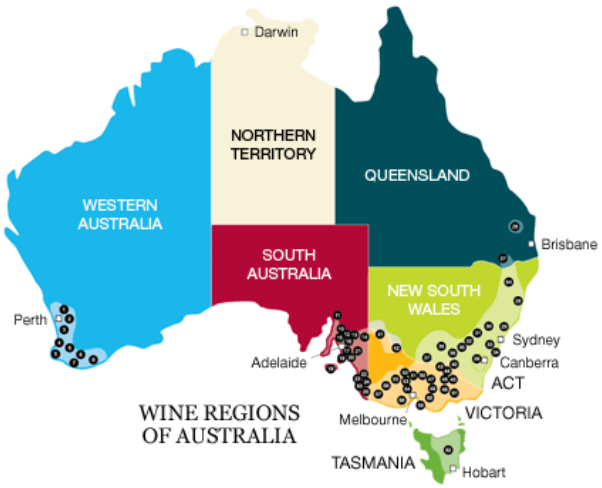

Figure 1. Map of Australian wine regions (adapted from Google image 1).

1.1.2 Australian Wine Grape Varieties.

There are over 100 wine grape varieties grown in Australia, with the top five varieties as of 2012 being Shiraz, Chardonnay, Cabernet Sauvignon, Merlot and Sauvignon Blanc (Winebiz 2014). Over the past 40 years, Shiraz has been Australia's most prominent and iconic wine grape variety, accounting for almost one vine in every four, with a total planting area of 44,000 hectares (Wine Australia 2014).

1.1.3 Australian Wine Production and Export.

Australia, as the World's largest island, is distant from many other wine producing countries. Though Australian wine consumers drink more than five times as much wine per head as they did in the 1960s, they only consume around one third of all the wines they produce (Johnson and Robinson 2013). The Australian wine industry has grown to now be the World's fifth largest exporter of wine by volume with approximately 750 million litres

a year sent into the international export market, just after the leading exporters, Italy, Spain, France and Chile in 2013 (Wine Australia 2014). The top five export markets for Australian wine by volume in 2012 were US, UK, China, Canada and Germany. Of the top five destinations for Australian bottled wine exports, the largest export volume goes to the UK, however China has now become the fastest growing export destination and pays the highest average value of $5.85 per litre in 2010-11 when compared to the US (A$ 3.43 per litre) and the UK (A$3.49 per litre) (Wine Australia 2014).

It is not just the climate and varieties that makes Australian wine unique. As alluded to above, Australia also has world renowned wine research and educational facilities. Their grape and wine research is strongly supported by the Australian Government and the wine industry itself through payment of local levies and export charges (Department of Agriculture 2014). As wine has become more popular in the Australian society, it has become a part of the culture, with every wine region and state having an annual wine festival where local wine, food and culture can be sampled (Australian Government 2014). The wine industry is a significant contributor to the Australian economy overall through its mechanisms of production, employment, export and tourism. South Australia alone currently contributes around $1.7 billion revenue to the state's economy.

1.2 Grape Berry Ripeness.

To use an old adage, '*you can't make a good wine without the growing of good grapes*'. Indeed, producing high quality grapes in Australia has become the norm over the past few decades through a detailed understanding of precision viticulture and pest and disease control, both of which lead to the advanced control of the vines health. The payback for such efforts is that it leads to grapes at harvest that have optimal sugar levels coupled with the most favourable berry aroma attributes in readiness of the vinification process.

1.2.1 Grape Berry Ripening Evolution.

Grape berry ripening is a continuous process that comprises two growth periods separated by a lag phase, Figure 2 (Kennedy 2002). The first growth period starts from bloom and lasts approximately 60 days. During this period, the berry is formed, berry volume expands, and various solutes accumulate, including tartaric acid, tannins, amino acids and some

aroma compounds. In most varieties, the first growth period is followed by a lag phase (veraison) which marks the onset of berry ripening. Veraison is a time when red varieties change berry colour and white grapes transform into berries with translucent skin (Ribereau-Gayon et al. 2000). The second growth period is a time of tremendous accumulation, during which the berries begin to increase rapidly in size, sugar level and the concentrations of secondary metabolites, e.g. anthocyanin and numerous aroma/flavour compounds (Watson 2003). Thus, the evolution of the various compounds in grape berries during ripening does not synchronise with each other; the management and a detailed understanding of which, is critical to producing high quality grapes and underpins the research directions throughout this thesis.

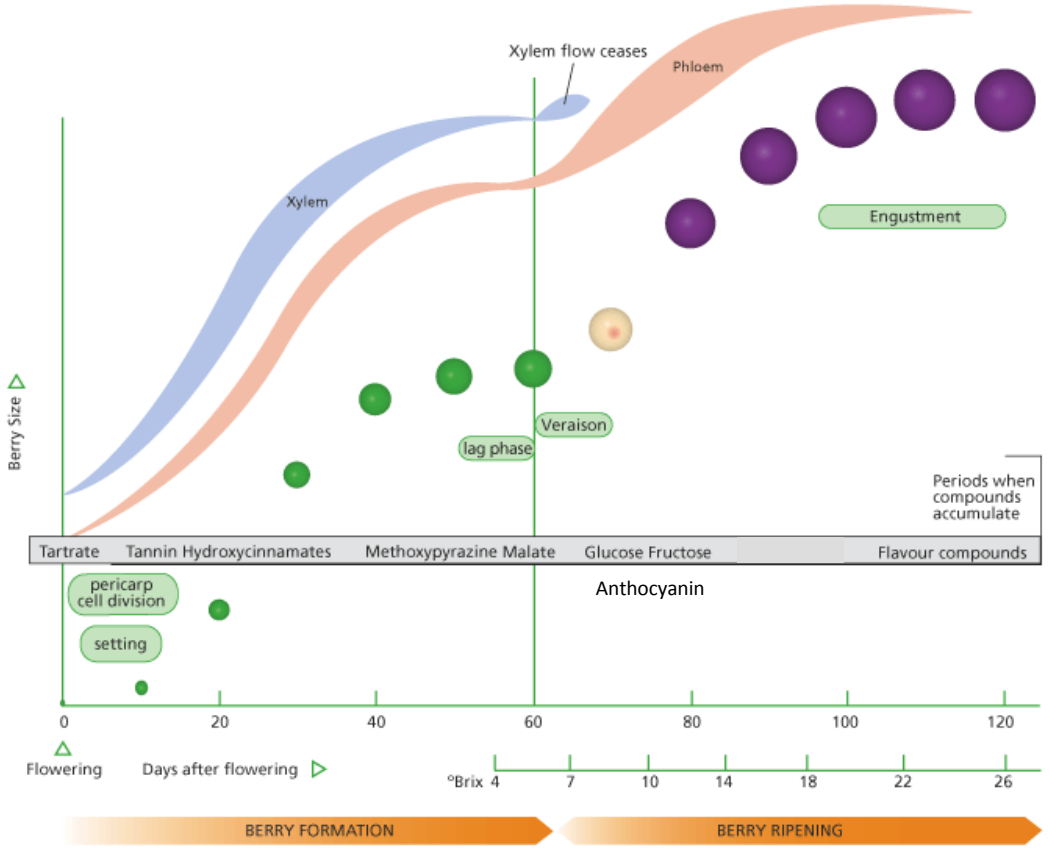

Figure 2: Diagram showing relative size and color of berries at 10-day intervals after flowering, passing through major developmental events (rounded boxes). Also shown are the periods when compounds accumulate, the levels of juice °brix, and an indication of the rate of inflow of xylem and phloem vascular saps into the berry. Illustration by Jordan Koutroumanidis, Winetitles.

Figure 2. Diagram showing the berry development process and when compounds accumulate. (Adapted from Kennedy 2002)

Usually, wine grapes within a vineyard are harvested in one pass, meaning that the decision of harvest date is critical, as the final berry attributes will have a great influence on the potential final wine quality. Unpractical expectations or poor estimations of grape quality and maturity can cause harvest delays which increases the potential of disease, rot and dehydration, and berry spoilage. Consequently, any gains made in the vineyard would have been eroded prior to vinification and should be avoided. In contrast, early harvest may also decrease the final quality of a wine in terms of lacking berry ripeness and quality such as sugar levels and fruit aroma (Hellman 2004). Indeed, Du Plessis and Rossouw (1978) reported that final wine quality of Chenin blanc can decrease by around 10% if the grapes are harvested ±1 week from what was decided to be the harvest date of optimal maturity under South African conditions (Du Plessis 1984). In addition, a recent study conducted by the AWRI focused on the relationship between harvest date and wine composition, and demonstrated that final grape maturity at harvest had a dramatic influence on the chemistry of the final wine which in turn altered the wine's sensory profile (Bindon et al. 2013). It is clear that having a comprehensive understanding of how to define the peak of berry ripeness to allow for harvest is a key driver to producing premium wines.

1.2.2 Prototypical Berry Ripeness Parameters.

As highlighted above, the composition of grape berries is continuously changing during ripening and at a certain point, the accumulation of desirable enological characteristics will cease and the deterioration of these advantageous berry attributes will begin (Bisson 2001). It is therefore extremely important to define the optimal harvest date or peak of ripeness so that winemaking can begin with the most suitable fruit. To make a targeted style of wine, the winemaker would have an expectation of the optimal grape maturity for that particular variety at harvest through knowledge of past vintages, climate, soil type and vineyard management practices (Jackson and Lombard 1993). To aid the winemaker in their decisions, different methods to define optimal grape maturity have been developed. The prototypical berry ripeness parameters used to assess optimal grape berry ripeness have not changed in decades and still focus their attention on assays detailing the chemical composition of the juice from the berries. Both historically and still today, the most commonly used grape maturity indicators employed by viticulturists and winemakers are

sugar content and acidity, with multiple samplings taken between veraison and harvest in order to ascertain optimal harvest date (Watson 2003). Of additional importance are the winemaker's observations on the development of varietal colour, aroma, flavour, and their perceptions of skin, seed, and stem maturity along with observations on the physical condition of the vines and fruit (Watson 2003, Ristic and Iland 2005, Fredes et al. 2010, Ferrer-Gallego et al. 2010, Rodriguez-Pulido et al. 2012, Winter et al. 2004).

Sugar.

Sugar content is the most often used ripening index, the level of which provides the winemaker with an estimate of the expected alcohol level. The juice total soluble solids (TSS) is often used as an estimate of sugar content, which is conveniently measured utilising a refractometer or hydrometer. In normal grape varieties, ripe grapes contain a sugar content ranging between 150 to 240 g/L (Ribereau-Gayon et al. 2000). In Australia winegrapes have high sugar contents displaying TSS levels as high as 26-30 $^{\circ}$Brix. Over the last few decades, Australian wine consumers have developed more acceptance for certain wine styles which contain a higher level of alcohol. Unlike a number of wine regions in the world that need to add sugar to modify the potential alcohol content due to their cooler climates, in Australia the warm and dry climate during vintage leads to a longer ripening season, which in turn produces wines with higher concentrations of alcohol (AWRI, 2013).

Acids.

Similarly, acid levels (titratable acidity, pH, sugar : acidity ratio and malate : tartrate ratio) are also used as ripeness indices (Bisson 2001). Titratable acidity (TA) is commonly expressed as grams of tartaric acid equivalence per litre in Australia, and it can be measured following the procedures reported by Iland et al. (2004a) by mixing 10 mL of juice sample with 50 mL of distilled water and titrating with 0.1 M sodium hydroxide solution until pH 8.2. In simple terms TA provides a snapshot of the amount of the major acids present in a juice or wine and can indicate the sensory attribute of juice/wine acidity. Typically, the titratable acidity in Australian grape musts ranges from 6 to 10 g/L while the pH ranges from 3 to 4 (Hamilton and Coombe 1992). Grapes grown in the warmer regions often have lower TA and higher pH than grapes from cooler regions, thus these low TA fruits may require acid additions to the juice or must prior to fermentation. While malic,

citric and tartaric acid (Figure 3) can all be added to increase acidity, tartaric acid is the most commonly used acidulant (Watson 2003).

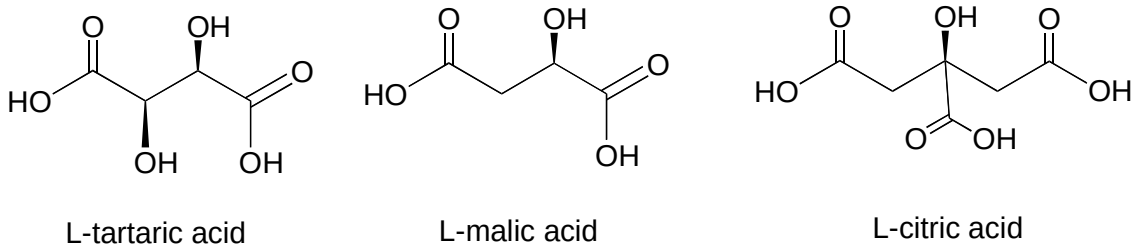

Figure 3. Diagram depicting some of the most important acids in a juice or wine.

Other Chemical Traits in Berries to Assist in Ripeness.

There are some other chemical metabolites within berries examined for their potential use in predicting berry ripeness. For example, Gonzalez-San Jose et al. (1991) applied principle component analysis on different ripening indices and found that levels of the berry phenolic compounds were particularly important in indicating grape maturity. Moreover, Bisson (2001) reported that anthocyanin levels were also correlated with berry maturity. Although other chemical traits in berries such as glutathione and protein content have also been studied to assess berry ripeness, it was found that these chemical metabolite changes during ripening are not necessarily related to the optimal maturity levels of grape flavourants (Girard et al. 2002, Palomo et al. 2007). Consequently, it is generally accepted that optimal grape maturity will also need to be assessed by monitoring levels of grape aroma/flavour compounds themselves and not to utilise predictions based on unrelated metabolites (Bisson 2001).

Thus to determine the evolution profile of the aroma compounds, a number of researchers have identified and quantified the aroma compounds in numerous grape varieties at different ripening stages. For example, during a study of the ripening of Cabernet Sauvignon, terpenes predominated at an early stage, with benzene derivatives increasing towards the later stages of ripening. Moreover, C_6 compounds were firstly exhibited in the form of acetate esters, then aldehydes and finally alcohols (Kalua and Boss 2009). Another study on Muscat Hamburg showed an increasing trend in the levels of the main free terpenols during ripening (Fenoll et al. 2009).

Through the chemical analysis of berry aroma compositions during ripening in other varieties, several compounds have been found to reach a peak at the same time as the desired harvest date, e.g. C_6 aldehydes and alcohols in Monastrell, Cabernet Sauvignon and Tempranillo grapes (Gómez et al. 1995, García et al. 2003), sesquiterpenoids in 'Baga' grapes (Coelho et al. 2006) and terpenoids in 'Fernă-Pires' grapes (Coelho et al. 2007). However, single aroma compounds do not indicate the optimal berry aroma composition desired at harvest (Bisson 2001).

Bisson (2001) has also pointed out that if there is a way that can define optimal berry aroma composition, then solid-phase microextration-gas chromatography-mass spectrometry (SPME-GC-MS) would be a useful tool for routine assessment of optimal aroma ripeness. However, the concentration of volatile compounds varies dramatically during ripening, which means high levels of one desired aroma compound would not necessarily be correlated with high levels of another (Kalua and Boss 2010). Clearly it is a difficult task to attempt to correlate the optimal aroma ripeness for each variety in varying locations according to the grapes aroma compound concentrations (García et al. 2003).

Consequently, it can be concluded that unless detailed aroma profiling studies such as those above are to be performed, and this would certainly not be the case for nearly all vineyards, then it is highly unrealistic that such knowledge could be determined in order to assist in predicting optimal grape berry aroma maturity for a certain variety located within a certain vineyard. Clearly, alternative simple methods to determine the berry aroma/flavour ripeness are needed.

1.2.3 Berry Sensory Assessment to Predict Grape Maturity.

Over the past few decades, grape berry sensory evaluation, particularly berry tasting, is becoming increasingly used in vineyards as it appears to be a good tool to monitor berry maturity (Le Moigne et al. 2008). Tasting berries or juice for a subjective assessment of flavour development from veraison to harvest typically augments the quantitative measure of the prototypical juice chemical traits. Researchers have now developed a range of formalised sensory evaluation methods to assess grape berry ripeness, which are aimed to help viticulturists and winemakers to obtain more consistent information about the timing of harvest. There is now a common language utilised throughout the wine industry.

Winter et al. (2004) in "Winegrape Berry Sensory Assessment in Australia" has introduced a sensory evaluation system to assess berry ripeness. This Berry Sensory Assessment (BSA) system uses 20 standardised descriptors and a 4 point scale scoring system to evaluate the pulp, skin and seeds separately, Table 1. Before evaluating the berries in the field, the personnel must first undergo training on how to use the evaluation system. Table 2 shows part of the BSA criteria chart which focuses on certain aroma/flavour evaluation criteria. The scores are given based on the intensity of each assessed criteria, with rankings of 1 representing unripe characteristics and rankings of 4 relating to desired ripe characteristics. Thus, grapes ready for harvest are anticipated to be ranked with scores of 3 or 4. In the training sessions, approximately 2 kg of the fruit are picked from randomly selected vines and sorted into various maturity levels based on their sugar levels. The berries are then carefully removed from the rachis and the trainees will score each of the 3 batches by chewing them for each of the sensory descriptors, with the expectation that the riper berries are scored at the higher end of the scale. Once training is complete, the trainees are free to assess grape maturity within their own vineyards.

Table 1. The twenty descriptors used for BSA (adapted from Wineter et al. 2004).

BSA	**Pulp**	**Skin**	**Seed**
Visual, tactile and consistency assessment	Softness	Colour	Colour
	Detachment of pulp from the skin and seeds	Stalk removal	Crushability
	Juiciness	Disintegration	
Mouthfeel, aroma and taste assessment	Sweetness	Acidity	Flavours
	Acidity	Herbaceous aromas	Tannic intensity
	Herbaceous aromas	Fruity aromas	Tannic
	Fruity aromas	Tannic intensity	Astringency
		Tannic astringency	

Table 2. Aroma /flavour evaluation criteria in the BSA (adapted from Winter et al. 2004).

Score	**1**	**2**	**3**	**4**
Pulp—herbaceous aromas, in the mouth	Intense	Moderate	Weak	Absent
Pulp—fruity aromas, in the mouth	Absent	Weak	Moderate	Intense
Skin—herbaceous aromas of chewed skins	Intense	Moderate	Weak	Absent
Skin—fruity aromas of chewed skins	Absent	Weak	Moderate	Intense

Although BSA is currently the most important and accurate method to monitor berry flavour maturity, tasting of berries can be very time consuming and fatiguing due to the requirement of professional training. A simplified BSA with less detailed descriptors can also be conducted in the field for people with adequate BSA training (Winter et al. 2004) However, BSA complements, but does not replace the important measurements of the traditional berry ripeness parameters described above (Winter et al. 2004).

1.2.4 Non-Destructive Methods to Evaluate Berry Ripeness.

Since the traditional berry ripeness parameters are usually measured in analytical laboratories and require strict sample preparation procedures along with appropriate analytical tests, there is now a considerable push towards evaluating grape berry ripeness exploiting rapid non-destructive analyses and extensive research has been carried out over the last decade. The non-destructive methods are perceived to be more portable, of lower-cost and faster than the traditional destructive analysis.

A recent non-destructive method of predicting grape maturity relies on using Near-infrared spectroscopy (NIR). By collecting spectral data from the berry surface exploiting an NIR apparatus, grape berry sugar content, acidity (Larrain et al. 2008, Gao 2011), water content (Geraudie et al. 2009) and anthocyanin concentrations (Janik et al. 2007) have been successfully evaluated in some common red *Vitis vinifera* varieties. Furthermore, a fluorescence-based sensor called Multiplex has been developed to monitor the anthocyanin accumulation and chlorophyll degradation in a vineyard of some Italian red grape varieties (Tuccio et al. 2011, Agati et al. 2013), and the flavonol and chlorophyll levels for one

white variety (Agati et al. 2013) over two seasons. These sensor technologies provide a portable tool for analysing a large group of samples, or monitoring the entire vineyard coupled with precision viticulture techniques. However, the instrumentation requires special sensors (Reynolds 2010), which can be expensive and are not widely available. Such sensing techniques are easier to use on single berry samples in the laboratory where calibration is simple, but tend to fail for measurement of berries inside of a bunch on a growing vine within a vineyard, especially for the compact bunch varieties.

Another common non-destructive analytical method is to measure berry mechanical/textural parameters (Rolle et al. 2012a). For example, R ó Segade et al. (2011a) demonstrated that the berry skin break force and berry cohesiveness could be used for varietal differentiation and ripeness differentiation, respectively. R ó Segade et al. (2011b) also related berry skin thickness to anthocyanin extractability and found that thinner skins seemed to release more red pigments. In addition, there are a few observations on the evolution of the mechanical properties of berry seeds during ripening. For example, Rolle et al. (2012b) found that grape seed mechanical and acoustic characteristics could help differentiate berry ripeness. Furthermore, Letaief et al. (2013) investigated the evolution of both the sensory and the instrumental properties of grape seeds during berry development, and found that both properties could be used to describe the ripening process. Although many of these berry mechanical properties can be used to discriminate different grape maturation stages, no clear correlation has thus far been found between the desirable berry sensory attributes and instrumental texture parameters.

During the past decade, researchers have also focused their attention on exploring the application of digital image analysis in viticulture, for yield estimation, quality evaluation, disease detection and determination of grape phenology (Whalleyand and Shanmuganathan 2013). Current digital imaging technology is able to estimate berry size and weight in the field and by exploiting the heterogeneity found in berry size, the heterogeneity in berry maturity may also be assessed (Whalleyand and Shanmuganathan 2013). Furthermore, Rodr guez-Pulido et al. (2012) has also applied digital image analysis to monitor berry colour change, however they found that they could only differentiate the berries at veraison from those at harvest. Moreover, most current methods of digital image analysis were developed for individual grape berries under laboratory conditions and they are unsuitable for cluster or whole bunch analysis (Whalleyand and Shanmuganathan 2013). In the

natural environment of the vineyard, approaches must be able to cope with difficult and variable lighting conditions and with factors such as inclusion of the grapes within the cluster and throughout the canopy. Therefore further research needs to be done to improve these technologies to be able to use in the field (on the vine), so that grape growers are able to monitor grape maturity/quality throughout the vineyard during ripening. Current progress in the arena is still slow as exemplified by the recent works of Reis et al. (2012) who studied the detection and location of grape bunches on vines by processing images captured at night, however, they could not obtain more detailed information at the individual berry level.

Given the clear current excitement by researchers in this research space, it is expected that dramatic leaps forward will be made in the near future. The economic drive behind such studies is driven by the fact that they promise to be simple non-destructive methods that are fast and of low-cost and have the ability to provide berry quality information throughout the grape berry ripening continuum which will assist in harvest decision making.

1.2.5 Sampling Strategies.

It is well known that in order to determine the optimal berry ripeness, grape samples should be taken weekly beginning about three to four weeks before harvest is anticipated, and more frequent sampling should be conducted when close to full ripeness (Watson 2003). However, despite which parameters are chosen for determining berry ripeness, the key to a good estimate of grape maturity is to collect a truly representative grape sample of the entire harvest unit (Hellman 2004). Unfortunately, grape berries do not develop synchronously and it is often problematic to obtain a representative berry sample (Jackson and Lombard 1993, Watson 2003). It is known that the more exposed berries are normally more advanced in sugar and phenol development when compared to those berries on the shaded side of the cluster. Furthermore, grape maturity differences among clusters on a vine can vary. Consequently, there is a need for systematic sampling strategies, which provide for the collection of enough samples in a random fashion, to objectively represent the entire crop that will be harvested.

An important question is, should individual berries or whole bunch samples be collected for measurement? It has been suggested the berry sampling is not only more time consuming, but is also prone to errors from over sampling the outside berries and under sampling the inside berries from tight bunches (Iland et al. 2004b). Therefore it is now considered more advantageous to determine grape maturity at bunch level rather than at the berry level. Moreover, to obtain a representative sample, it is important to select sampling sites randomly. This can be achieved by different methods, such as sampling between different rows in a zig-zag fashion, or using a random number table to select the row numbers and vine numbers to take samples from, or in more recent time designing the sampling grid with the aid of global positional systems (GPS). Whichever sampling method is employed, the samples need to be placed in a cool environment (preferably between 5 to 10 ℃), processed and analysed as soon as possible to avoid degradation of the key chemical attributes being quantified (Iland et al. 2004b).

1.3 Grape Peduncle, Rachis and Stem and their Potential Relationship to Berry Ripening.

With the focus of grape ripeness research squarely placed on the chemical metabolites within the berries themselves, little attention has been paid to grape stem evolution, between veraison and harvest, and how this may be correlated with the berry ripening continuum. According to Iland et al. (2011), the term peduncle sometimes is referred to as the stalk (the entire branched axis apart from the pedicels), but most winemakers regard peduncle as the hypoclade, the portion of the stem attached between the cane and the first branching of the stem. Therefore, within this thesis, we also regard the grape stem as the major axis of a cluster, while the term peduncle is used to refer to the portion of the stem that is attached between the cane and the first lateral branch within the bunch, and the rachis is defined as the remaining portion of the main axis of the stem, Figure 4. It should also be noted that visually there is also a node on the stem that is positioned above the first lateral branching which aids in defining where the peduncle portion ceases and the rachis portion of the stem begins.

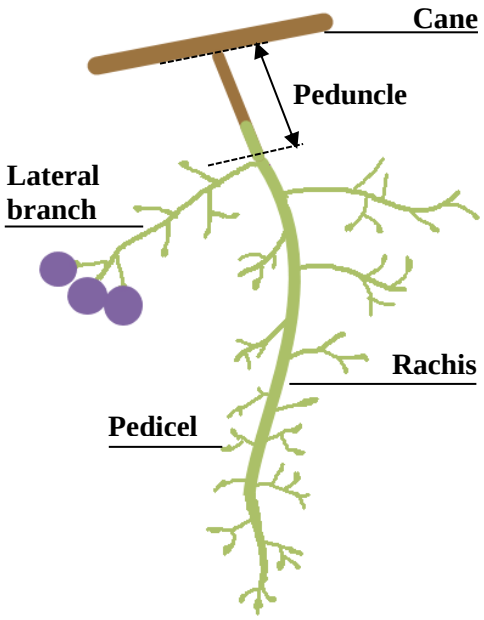

Figure 4. Structure of the grape stem.

Although little attention has been paid to how visual inspection or the monitoring of certain chemical attributes of the various components of a grape stem can be utilised to aid in assessing berry quality attributes, peduncle length and its lignification content has been used as a visual parameter to characterise grape varieties for decades under the code OIV 206 and 207 (OIV 2009, Masi et al. 2001, Boehm and Tulloch 1967, Goussard 2008). Since it has been observed that peduncle length varies from 2 to 10 cm depending on cultivar type (Iland et al. 2011), grapevine varieties may be categorised as belonging to one of a number of groups based on their peduncle length. These are termed very short (up to about 30 mm), short (about 50 mm), medium (about 70 mm), long (about 90 mm) and very long (greater than 110 mm) under the OIV code 206. For example, the varieties Gewürztraminer Rg and Sauvignon Blanc have been characterised as belonging to the short group based on peduncle length under this OIV code.

In addition, under OIV code 207, grape varieties may be divided into three groups depending on their peduncle lignification degree, which essentially means the level of browning colour. The 3 levels of lignification are defined as, at the base only directly attached to the cane, up to about the middle of the peduncle and those showing more than 50% browning. Consequently it has been pointed out by Goussard (2008) that the peduncle

length, thickness and texture may be used as ampelographic characteristics for variety identification. Finally, the textual properties of peduncles have also been used to examine the suitability of grapes for manual versus mechanical harvesting (Goussard 2008).

Most importantly and the key to the research within this thesis is that some anecdotal evidence reported in the literature suggests that grape stem ripening may also parallel berry maturation as indicated by a change in stem colour from green to brown during ripening (Ribereau-Gayon et al. 2000, Bisson 2001, Watson 2003). Perhaps the most interesting of these reports are those of Bisson (2001) and Watson (2003) which highlight that stems of many varieties of grape undergo a change from green unripe to brown or ripe stems to overripe or brittle stems, or that as the fruit reaches maturity, the berry stems (peduncles) turn from green to brown and decrease in green, 'stemmy', herbaceous character as summarised in Table 3 below (Bisson 2001). Furthermore, it was also stated recently that there appears to be a wine industry impression that has assumed there is better tannin maturity (assessed by berry sensory evaluation) with higher peduncle browning (Leal 2007). Naturally, these suggestions in the literature have been well observed for many decades by viticulturists themselves, however, there has been no rigorous study to clearly demonstrate that changes in stem colour do indeed parallel the berry ripening continuum. Consequently, these loosely related observations lay the foundations for the research to be undertaken within this thesis.

Table 3. Characteristics of grape stem during ripening (adapted from Bisson 2001).

Status	**Colour**	**Characteristics**
Unripe	Green	Vegetal, leafy
Ripe	Brown	Resinous wood, spice: cloves, cinnamon, pepper
Over-ripe	Brittle brown	Dried leaf, tea, herbal

1.3.1 Peduncle Physiology.

xylem and phloem.

Similar to vine shoots and roots, water and solutes are transported through the xylem and phloem of grape peduncles. Water is transported into the berries mainly through the xylem

before veraison, whilst the major compound that translocates through the phloem is sucrose before leaf senescence. Almost all plant hormones (auxin, gibberellins, cytokinins, and abscisic acid) and some ions such as potassium are also imported via the phloem (Iland et al. 2011).

To our knowledge, the process of browning of the stem or peduncle has not been investigated deeply. But it is known that the lignification or browning of vine shoots is caused by the death of the green cortex and is accompanied by the deposition of starch in the xylem and phloem parenchyma cells (Iland et al. 2011). The peduncle contains multiple (typically approximately 30) vascular bundles that are separated by rays. The parenchyma cells of the rays and the inside of the vascular bundles accumulate starch, which may serve as transient nutrient storage compartments. The epidermis on the exterior contains numerous stomata and is covered with a cuticle (Iland et al. 2011).

The influence of plant hormones on grape stems.

There have been a few studies in the 1980 investigating the effect of plant growth regulators on the development of grape rachis. For example, Nakamura and Hori (1981) found that after gibberellic acid (GA) treatment, the grape rachis (actually defined in this study as the entire major axis) had an increase in rachis hardness as measured by the deflection angle with force, which caused postharvest berry drop instead of advanced berry maturity. They also investigated the change in rachis structure after GA treatment (Nakamura and Hori 1983) and found that the rachis hardness was accompanied by increases in rachis xylem diameter, the number of cell layers of the secondary xylem without changes in cell size, cell wall thickness and the width of the phloem and primary xylem. Another observation by this group was that early GA treatment would lead to longer rachises at harvest. Later, Theiler and Coombe (1985) and NII (1986) found that GA treatment did not have the same impact on various grape varieties and different plant hormones had different influences on the rachis.

1.3.2 Peduncle Chemical Attributes.

Grape stems contribute 2-8 % of the total bunch weight in grapes, and they are often not utilised during winemaking (Panouille et al. 2007). The production of 100 L of wine gives rise to approximately 4 kg of stalks together with skins and seeds as winery by-products

(Costa 1983). Therefore most current research on grape stems has been focused on their potential economic value as a winery by-product for extraction of nutraceuticals due to their high levels of dietary fibre (Prozil et al. 2012, González-Centeno et al. 2010), phenolics (Doshi et al. 2013, Makris et al. 2007), antioxidants (Llobera and Cañellas 2007, 2008) and functional chemicals such as resveratrol (Makris et al. 2008, Püssa et al. 2006).

The basic chemical composition of grape stems has been reported in a few studies. Grape stems generally contain mostly water, dietary fibre, tannin, ash, protein, but the amount of each component has been found to vary among different grape varieties as summarised within Table 4.

Table 4. Bibliographic values of grape stem chemical composition.

Grape stem composition	Grape variety	Reference
7.29% protein, 1.7% soluble sugars, 1.65% oil, 5.48% ash, 1.04% soluble pectins, 10.3% condensed tannins and 77.2% total dietary fibre, (% dry matter).	Manto Negro (red grape)	Llobera and Cañellas 2007
10.4 g soluble sugars, 11.5 g oil, 51.2 g protein, 14.3 soluble pectins, 69.4 g ash, 79 g condensed tannins, and 790.5 g total dietary fibre, (g/kg dry matter).	Presensal Blanc (white grape)	Llobera and Cañellas 2008
7% of ash, 30% of cellulose, 17% of Klason lignin, 21% of hemicelluloses, 6% of protein and 15.9% of tannins.	local variety from the Dão Region of Portugal (red grape)	Prozil et al. 2012
64.9 g moisture, 26.3 g dietary fibre, 0.9 g lipids, 3 g soluble sugars, 2.4 g protein and 1.7 g ash, (per 100 g fresh stem weight).	Syrah (red grape)	González-Centeno et al. 2010

Stem phenolics studies

Grape stems contain around 5.8% of total polyphenolics content on a dry weight basis (Karvela et al. 2009). Phenolics not only influence the bitterness and astringency of fruit and juices, but they also contribute to the origin of the colour of most fruits and vegetables. When it comes to wines, phenolics are the extremely important compounds that contribute

to a wines flavour and colour (Dai and Mumper 2010). Phenolic compounds include phenolic acids, flavonoids (e.g. flavanols, flavonols, flavanonols), tannins (e.g. proanthocyanidins, anthocyanins), stilbenes (e.g. resveratrol) and lignans (Souquet et al. 2000). Among which, resveratrol is noted as being somewhat famous for associating with the 'French paradox' which is linked to the prevention of coronary artery disease, in spite of the French being known to have a high consumption of fats. In plants, resveratrol is produced as a response to various forms of biotic and abiotic stresses such as fungal disease (Anastasiadi et al. 2012).

Table 5 summarises a range of stem phenolic studies for a range of different grape varieties. Most of the current research has focused on exploring the economic values of the stems in lesser known grape varieties, with fewer reports being conducted on the major grape varieties grown world-wide, such as Syrah, Cabernet Sauvignon and Merlot.

Table 5. Grape stem phenolic studies.

Phenolics	**Grape varieties**	**Reference**
total phenolics, flavoniods, flavan-3-ols, total anthocyanin	red: Pusa Navarang and Merlot	Doshi et al. 2013
total polyphenols, total flavonoids, total flavanols	Roditis (white) and Agiorgitiko (red) cultivars	Makris et al. 2007
gallic acid, *p*-OH-phenethyl alcohol, catechin, epicatechin, et al.	red: Cabernet Sauvignon, Syrah, and Tempranillo	Alonso et al. 2002
total polyphenols, total flavanols	white: Chardonnay, Welschriesling, Pinot gris; blue: Saint Laurent, Andre, Blauer Portugieser	Balik et al. 2008
three stilbenoids and two flavan-3-ols	Hasaine sladki (blue), Zilgam (blue), Yubilei Novgoroda (white)	Makris et al. 2008
total polyphenols, total flavanols	Manto Negro (red) and Presal Blanc (white)	Llobera 2012

While the phenolic composition of grape stems at harvest has been reported more widely, only a limited number of studies have focused their attention on how the phenolic composition within the stem changes during the grape berry ripening continuum. For example, the levels of proanthocyanidin content from bunch stems of two red and one white grape varieties were measured during berry development and it was found that the maximum amount of flavanols was observed in the stem at early developmental stages, and the polymeric fraction was the most abundant during maturation (Jordão et al. 2001). Others (Doshi et al. 2006) have also measured the phenolic composition and antioxidant capacity of grape berries, berry stems (rachis, branches and pedicels), leaves, leaf petiole and shoots of Kishmish Chornyi (seedless variety) during berry ripening, and they not only found that the berry stems had highest amounts of total phenolics as well as antioxidant capacity throughout the entire maturation stage compared to the other plant tissues, but they also observed that the lowest level of flavonoids in the berry stems occurred at veraison. Such studies highlight that there is clearly a lot of chemistry/biochemistry going on within the stems over the ripening period, just like that known to occur within the grape berries themselves, but how these two sections of the plant cross-talk is still to be determined.

Grape stem chlorophyll research.

Comparing to the numerous reports detailing phenolic levels within grape stems, details on other chemical components such as total chlorophylls and carotenoids are less well studied. As photosynthetic pigments, chlorophylls and carotenoids are of great importance in grapevines, and if the components of the stem (peduncle and rachis) undergo significant colour changes over the ripening season then one would naturally wonder what changes are also occurring in the chlorophyll and carotenoid levels. They are not only considered the key biochemical tool for the vine to accumulate sugar through their leaves, but also are important precursors in grape berries for the production of norisoprenoids, which are one of the most important group of compounds for the accumulation of aroma (de Villiers et al. 2012). In addition, Lashbrooke et al. (2010) highlight that the carotenoids also serve as important precursors to the plant hormone abscisic acid (ABA), formed from the cleavage of neoxanthin. Finally, the concentrations of carotenoids and chlorophylls can also provide information about the level of stress the plant is experiencing as well as its ability to endure these stresses (Lashbrooke et al. 2010).

A number of these studies have examined how the total chlorophyll and carotenoid levels of grapes and grapevine leaves change over the grape maturation period. For example, Giovanelli and Brenna (2007) observed a decrease in total chlorophylls of berries from fruit-set to harvest for one white and two red Italian grape varieties. Moreover, Razungles et al. (1988) measured the changes in total carotenoid levels of the berries of three grape varieties and found that the levels decreased progressively from the onset of fruit development to the end of maturation, with a sharp decrease observed during veraison. A number of other studies have reported similar decreasing trends in total chlorophyll and carotenoid levels during the maturation of grape berries (Palliotti and Cartechini 2001, Oliveira et al. 2003). Finally, given that leaves are the primary photosynthetic organs of plants, it is not surprising that researchers have investigated intensively the changes in chlorophyll and carotenoid levels in leaves during vine growth. For example, Lashbrooke et al. (2010) found that the chlorophyll *a* and *b* levels were higher in mature vine leaves when compared to young leaves and their levels dropped post anthesis. However, to the best of our knowledge, there is only one direct report on the changes of chlorophylls within the grape stem in the literature. Palliotti and Cartechini (2001) measured the pigments within young and old rachises and found that the chlorophyll *a*, chlorophyll *b* and the carotenoid levels were higher in young rachises when compared to old rachises. Given this dearth of information of how total chlorophyll and carotenoid levels of grape stems (including peduncles and rachises) change over the berry ripening continuum, coupled with our interest in the observed changes in peduncle colour over the same timeframe we naturally included further studies in this area within the research to be conducted herein.

1.4 Grape Stems and Winemaking.

Compared to grape berries, and the skins and seeds, much less attention has been paid to grape stems during winemaking. This is essentially because grape stems are often considered to be of little value or to have a negative impact on the wine quality since they can impart a woody herbaceous aroma to the wine (Boulton et al. 1995). For example, Hashizume and Samuta (1997) identified seven green-odorant related compounds (five aliphatic carbonyl compounds and two methoxypirazines) in the stems of Cabernet Sauvignon and Chardonnay, and found that the addition of stems during winemaking increased the levels of the two methoxypyrazines in the final wine, which suggested the origin of the stemmy flavour of the wine. The same authors (Hashizume et al. 1998) also

found that by steaming the grape stems, most of the extractable methoxypyrazines from the stems could be eliminated, and the addition of the steamed stems actually increased the level of extractable phenolics into two red wines.

However, some winemakers argue that the stems may sometimes have a positive effect on a wines quality by increasing the concentration of proanthocyanidins which help to stablise the colour and improve the body of the wine (Carmen del Llaudy et al. 2008). The stems also allow more space within the cap of the must / ferment which also facilitates the phenolic extraction of the skins and seeds. In another study, Sun et al. (1999) investigated the transfer rates of catechins, oligomeric and polymeric proanthocyanidins from grape skins, seeds and stems into wine, and found that the stems transferred 100% of their catechins and proanthocyanidin content into the wine during fermentation, while the seeds still contained a small portion of these phenolics within them after fermentation. Furthermore, Carmen del Llaudy et al. (2008) added grape skins, seeds and stems separately from berries of three maturation stages into wine-simulated medias to investigate their different influences on the phenolic composition and astringency of the wine-simulated media. They not only found that the presence of stems increased wine proanthocyanidin levels, but also found that the riper grapes increased in their tannin contribution from stems into the wine whilst additions of stems from unripe grapes caused a higher contribution towards the wines astringency.

It is interesting to note that little research has been done on grape stems, either at harvest time or during berry maturation. It is even more surprising to find that no detailed investigations of grape stem colour evolution have been done. In addition, given the shortage of information on the change of grape stem composition during berry development, particularly the photosynthetic pigments which directly link to the visual colour of the stems, more research on the change in different chemical traits of grape stems is needed to help understand the relationship between stem colour and their associated chemical traits, as well as between stem chemistry and berry chemistry during grape maturation.

1.5 Colour Analysis of Grapes Stems.

Whilst there are numerous well known methods for evaluating the colour attributes of grape berries, juice and the final wine, to investigate the colour change of grape stems over the ripening period which will be required for this research, one needs to consider a few different methods that may wish to be employed. In the early studies, colour charts were often used to grade certain colours with numbers. For example, Ristic and Iland (2005) and Fredes et al. (2010) created a set of colour charts with 12 colours from green to brown for the visual assessment of grape seed colour during berry ripening. Although such colour grading system work for samples such as the grape seeds that go through significant colour change over time, they cannot be used in measuring more subtle colour changes and cannot be reproduced consistently using normal printing technology, and more importantly they are considered to be subjective evaluations.

Another common method used for food colour measurement is to use a spectrophotometer, which may be very expensive and may also have a minimum requirement for the sample size depending on the sensor diameter. Thus, recent food colour studies have focused on the application of digital image analysis for measuring colour. A recent interesting example comes from a study on measuring banana ripeness through the quantification of banana colour (Ji et al. 2013). They measured the CIE$L^*a^*b^*$, chroma, hue colour values and ΔE^*_{ab} values of bananas on a spectrophotometer first, and then collected images of the scanned bananas and measured the colour values utilising Matlab software, Figure 5. By comparing the results obtained from the two methods, they found that digital imaging had better predictions on banana ripeness, and the colour hue value of banana measured by the digital image analysis was highly correlated with the quality grade of the bananas. Thus, this study provided an important starting point for our grape stem colour research to be conducted herein.

There are different colour spaces that can be used in colour quantification. The most commonly used colour spaces include RGB (Red Green Blue), CIE, CMY(K) (Cyan Magenta Yellow (Black)), and HSL (Hue Saturation Lightness). The RGB colour space describes colour by different addition/composition of the three basic colours Red, Green and Blue, while the CMYK works in an opposite way by subtracting the basic colours of Cyan, Magenta and Yellow, Figures 6 and 7. These colour spaces are not linear with visual perception and are device dependent (Ford and Roberts 1998).

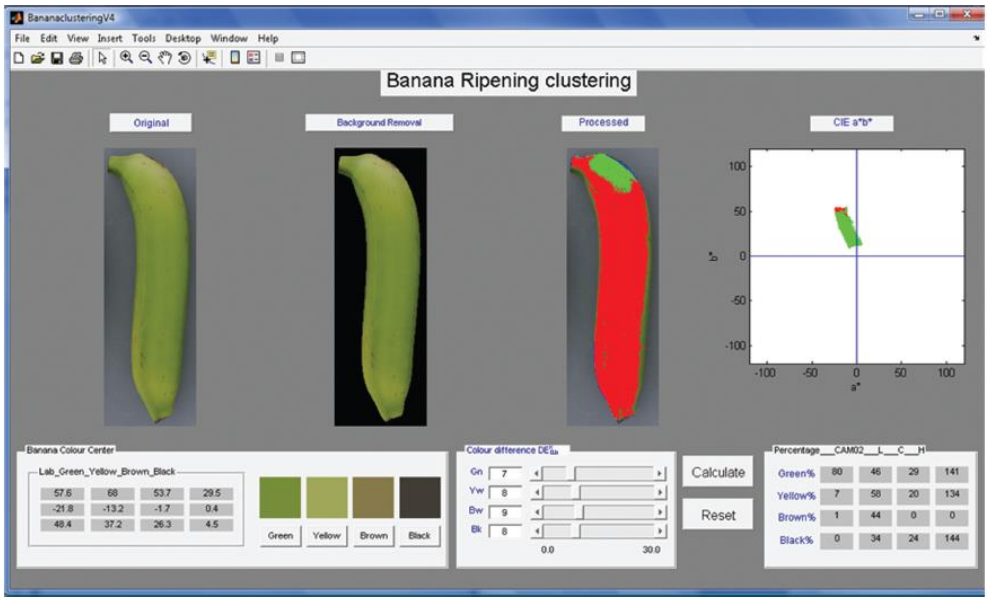

Figure 5. Digital image analysis for banana colour using Matlab software (adapted from Ji et al. 2013).

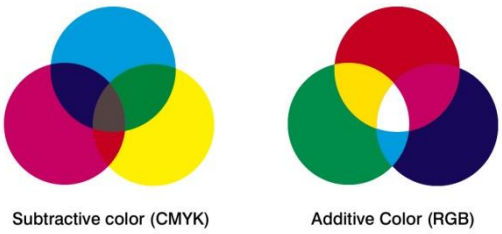

Figure 6. Illustration diagram of CMYK and RGB colour space (adapted from Google image 2).

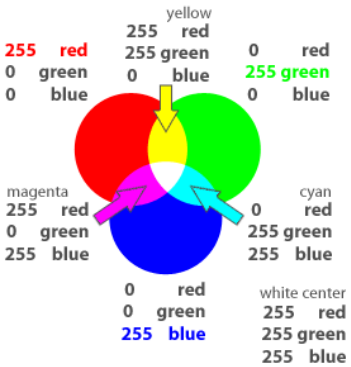

Figure 7. Illustration diagram of RGB space (adapted from Google image 3).

For the CIE colour space, the most commonly used colour system is CIE$L^*a^*b^*$ which measures colour according to characteristics of human vision. As illustrated in Figure 8 and detailed by McLaren (1980), L^* represents the lightness of the colour; $L^* = 0$ means black and $L^* = 100$ indicates diffuse white. The a^* and b^* colour axes are based on the fact that a colour can't be both red and green, or both blue and yellow and each axes will have values running from positive to negative. On the a^* axis, positive values indicate magnitude of redness while negative values indicate magnitude of greenness. On the b^* axis, positive values indicate the magnitude of yellowness whilst blueness is manifested in negative values. For both axes, zero is neutral grey. Thus, if an object was green to begin with and gradually turned brown such as that expected for peduncle colour evolution in our studies, then we would expect to observe a slight decrease in L^* and a change in average a^* from negative to positive.

While the RGB space is non-linear with visual perception, the CIE$L^*a^*b^*$ system can be easily related to human vision (Ford and Roberts 1998). In addition, there are also two polar parameters calculated from the a^* and b^* values which can match the visual experience of colours more closely, as defined by the following equations.

$$C = (a^{*2} + b^{*2})^{0.5}$$

$$h = arctan2\ (b^*, a^*)$$

In Figure 8, hue (h) is an angle in four quadrants and the angle on a colour wheel should be running clockwise from π (green) to 0 (red), whilst chroma (C) is defined as the strength of hue. For the expected change of grape stem colour from green towards brown, the a^* value would be expected to turn from negative to positive towards harvest, and the hue (h) will move from the second quadrant ($> \pi/2$) to the first quadrant ($< \pi/2$), while the chroma (C) will decrease in magnitude. Different colour spaces are suited for different applications and with proper calibration they can be transformed into each other. Thus for the quantification of grape stem colour, different colour space parameters are to be measured in order to determine the most informative ones after data analysis.

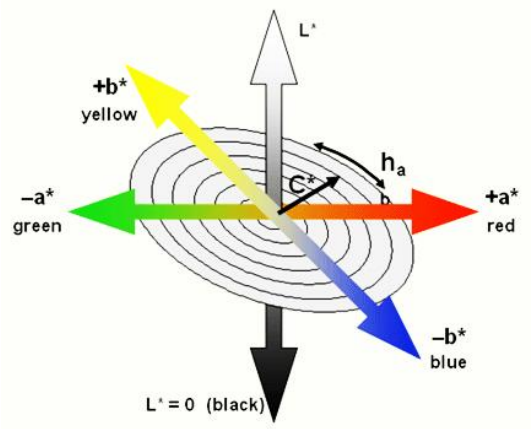

Figure 8. Illustration diagram of CIE*L*a*b**, hue (*h*) and chroma (*C*) colour parameters (From green to red, $0 < h < \pi$, and when $a^* = 0$, $h = \pi/2$) (adapted from Google image 4).

1.6 Key Methods of Analysis: High Throughput Chemical Assays.

Although there are a number of studies on the changes in phenolic levels within grapes as highlighted above, little attention has been paid to the chemical changes within grape stems and how they may be associated with the stem colour change over the ripening period. There were to be a number of key chemical parameters to be measured for all peduncle and rachis samples in these studies, namely the moisture content, total chlorophylls (including chlorophyll *a* and *b* levels), total carotenoids, total phenolics and their antioxidant capacity (DPPH). Considering the large amount of samples required to be analysed, we chose to use the fastest assays to determine the levels of each chemical component.

Water is a major component and important participant in all chemical reactions within grape stem tissues and was targeted as a key parameter to monitor during ripening. Fortunately, such moisture level evaluations are simple to conduct and simply require the measurement of fresh weight and dry weight after desiccation of the samples.

When thinking of the colour change of plant tissues, another important chemical trait related to colour would be the photosynthetic pigments such as the chlorophylls and carotenoids. There are different methods to determine the amounts of chlorophylls and carotenoids in plant tissues. Examples include spectral analysis and High Performance Liquid Chromatography (HPLC), with the former often employed first to estimate the total

amounts of chlorophylls and carotenoids while HPLC is often used to analyse for the levels of individual pigments more accurately (Kamffer et al. 2010). A quick protocol for estimating the total chlorophyll and carotenoid levels in plant tissues was developed by Lichtenthaler et al. (1987, 2001a, 2001b). They have detailed the standard procedures necessary for extracting the pigments and have reported an extensive set of equations needed to calculate the levels of chlorophyll *a*, chlorophyll *b*, total chlorophylls and carotenoids for different extraction solvents based on simple absorbance readings obtained from a UV/VIS spectrophotometer. Consequently this method of analysis was adopted for the studies herein.

The change in stem phenolic levels is highly associated with their antioxidant capacity levels and may possibly be linked to the degradation processes occurring during stem lignification (Tian et al. 2011). Although many research studies have employed High Performance Liquid Chromatography (HPLC), or Liquid Chromatography-Mass Spectrometry (LC-MS) to determine the phenolic composition of grape stems, such techniques are not suitable for large sample analyses and are time-consuming and could not be employed to determine the phenolic contents associated with stem colour that were to be targeted during these studies.

Fortunately, high throughput technology has emerged as an important fast tool that may be applied to determining phenolic levels. The conventional methods for measuring total phenolic content and the DPPH antioxidant capacity are time-consuming and labour-intensive, resulting in low overall throughput, whilst high throughput technology is a highly efficient way to conduct these assays accurately, particularly when coupled with the use of multiple-well microplates and robotic processing. This technology has been employed for decades in the pharmaceutical industry for drug screening (Mishra et al. 2008), and is now being employed for studies in wine research. For example, total phenolics and DPPH antioxidant capacity assays have been determined using 96-well plates for seaweed (Zhang et al. 2006), plant tissues (Ainsworth and Gillespie 2007), berry juice (Horszwald and Andlauer 2011) and grape berries, skins, seeds and wines (Anastasiadi et al. 2010). The high throughput methods for measuring total phenolics have been improved by employing the Folin-Ciocalteau assay of Singleton and Rossi (1965), using gallic acid as a standard. However, there are yet to be any high throughput assays reported in the literature for measuring the phenolics or antioxidant capacity of grape stems.

Considering the high efficiency and cost-saving benefits of using the 96 well plate method, coupled with the fact that we had access to an automatic liquid-handling robot and plate reader, we developed some simple high throughput microplate assays to measure the phenolic and antioxidant levels of the numerous samples of grape stems needed to be analysed.

1.7 Variation within the Vineyard.

Apart from defining the peak of berry ripeness, another important aspect in determining the optimal harvest date is to minimise berry heterogeneity within the vineyard. If this can be controlled precisely then premium quality grapes can be passed from the viticulturist to the winemaker for the opportunity of producing great wines.

1.7.1 Grape Berry Heterogeneity in the Vineyard.

Naturally, grape berries within a bunch or between different grape bunches on the same vine do not develop synchronously. It is known that the grape maturity among berries or bunches on a vine can vary depending on the timeline of flowering and fruitset and their position on the vine (Watson 2003). Specifically those berries or bunches that are more exposed to sunlight are normally more advanced in terms of their ripening stage than those berries or bunches in shaded positions on the vine (Watson, 2003). Even if this aspect is minimised it has been shown by Krstic et al. (2003) that even within relatively uniform vineyards of Southeastern Australia, the vine to vine variability in grape quality can still be significant as exemplified by the findings summarised in Table 6. Notably, the variation in berry colour can vary by some 13-18% and reflects that within any vineyard, there will always be a combination of red and dark purple berries existing at the same time.

Table 6. Typical vine-to-vine variability in berry ripeness parameters (adapted from Krstic et al. 2003).

Quality Parameter	Variability (% coefficient of variation)
°Brix	4-5%
pH	3-4%
TA (g/L)	10-12%
Berry weight	6-20%
Colour (mg/g fresh weight)	13-18%

Generally, many winemakers believe that more homogeneous fruit at harvest will aid in the making of better wines. This notion is supported by a study conducted within two Cabernet Sauvignon vineyards in California by Long (1987) who compared the berry ripeness parameters from a 400-berry sample collection from each of the two vineyards, and found that the vineyard that produced the best quality wine at harvest consistently had less variability in grape maturity. Furthermore, there have been numerous studies on the heterogeneity of grape berry composition within a vineyard and how this affects the final wine quality (Cortell et al. 2005; Kontoudakis et al. 2011). For example, Kontoudakis et al. (2011) separated sampled grapes into different maturity groups according to their density and found that the grapes with higher density produced wines with higher levels of final ethanol content, pH, anthocyanins, colour intensity, and total phenolics and had better balance in flavour and mouthfeel. In addition, Cortell et al. (2005) studied the impact of different vine-vigour zones on the phenolic composition of a final wine and found that wines produced from low-vigour zones had a higher proportion of skin tannins extracted into the wine, higher pigmented polymer levels and higher proanthocyanidin molecular masses which can affect the proanthocyanidin perception of the wine. However, quality is subjective to many and some winemakers may argue that some heterogeneity leads to more complexity and therefore an improvement in quality.

As well stated by Jackson and Lombard (1993), since the ensemble of the grape cluster always has proportions of over-ripe, sufficiently ripe, and under-ripe berries, such heterogeneity not only makes the representative sampling and the accurate assessment of berry ripeness parameters difficult, but also decreases the overall quality of the wine grapes, even if the average ripeness appeared satisfactory. A better understanding of the within-vineyard variation in grape maturity will enable viticulturists to conduct targeted vineyard management in the future and provide winemakers with optimal compositional grapes for their winemaking purposes.

1.7.2 Precision Viticulture.

During the past decade, research into precision viticulture has provided a set of elegant tools aimed at understanding the spatial variability within vineyards, and have included the use of remote sensing imagery (Lamb et al. 2004), yield monitoring systems (Bramley and

Hamilton 2004) and even the employment of high resolution (EM38) soil surveys (McBratney et al. 2005). With the aid of such technologies, the data for understanding vineyard variation can be collected based on a grid sampling design with each sampling position corresponding to a set of coordinates on the map. The geo-referencing for the map is usually conducted by matching each coordinate on the map with their locations in the field using the global positioning system (GPS).

In recent times the vineyard variability has been assessed in terms of soil (Bramley et al. 2011), vine pruning mass (Taylor and Bates 2013), yield (Bramley and Hamilton 2004), juice composition (Brix, pH, TA) (Bramley 2005a, Trought and Bramley 2011), berry anthocyanin concentrations (Baluja et al. 2012) and berry rotundone concentration (Scarlett et al. 2014). While most of the precision viticulture research has been focused on the spatial variation at harvest, only limited research has been done on the change in spatial structures of grape maturity over time. An excellent research study on both spatial and temporal variation in berry ripeness has been conducted by Trought and Bramley (2011). They created a map showing the spatial variation within a Sauvignon Blanc block in New Zealand by collecting data on canopy density using airborne remote sensing and proximal sensing techniques, apparent electrical soil conductivity (EM38) and trunk circumference. In addition, they also produced a set of maps of berry ripeness for this block by measuring the sugar, pH and TA levels of 24 plots of berries at each sampling time point as depicted in Figure 9. Their results demonstrated that grape berry ripeness was not only spatially variable, but there was also temporal variation in the rate of maturation. Thus to make the optimal harvest decisions for a vineyard, both spatial and temporal variability in grape maturity needs to be taken into account. Consequently, various sections of the vineyard may need to be managed differently by the viticulturists.

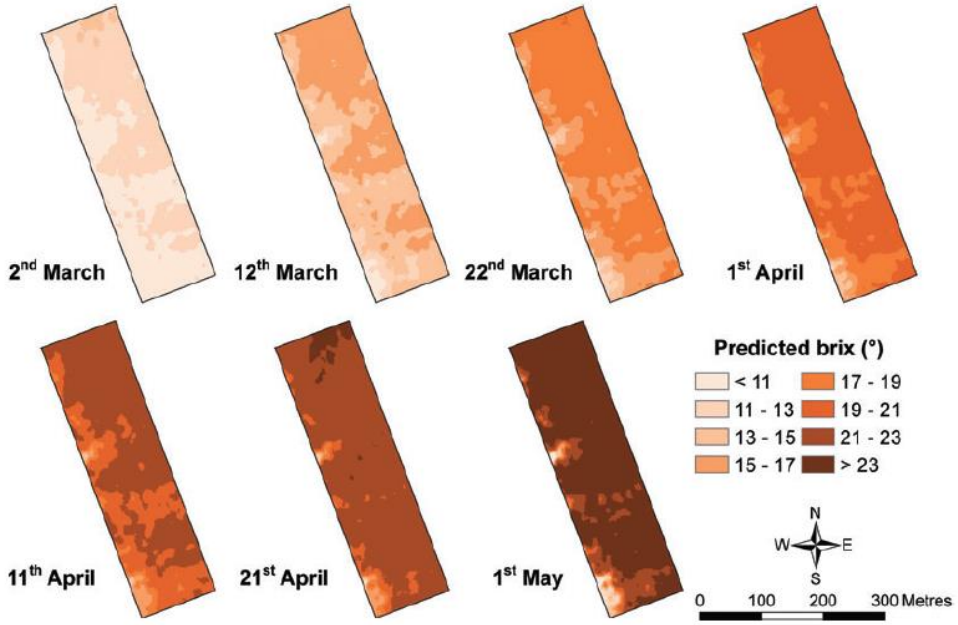

Figure 9. Spatial and temporal variations in level of grape juice total soluble solids (°Brix) (Adapted from Trought and Bramley 2011).

1.7.3 Selective Harvesting.

With more understanding on the within-vineyard variation of berry ripeness through precision viticulture, the wine industry has focused its attention in recent times on applying selective harvesting to promote greater control over final wine quality. As highlighted above, Bramley et al. (2005b) have demonstrated the great economic advantage of applying selective harvesting in Australia with a study conducted in Western Australia showing that the retail value of wine production could increase by over $40,000/ha through the use of selective harvesting of a block of Cabernet Sauvignon. Selective harvesting, according to Bramley et al. (2005b), means the split picking of fruit at harvest based on different yield and quality criteria in order to exploit any observed variation. Selective harvesting is normally conducted in two ways: one may pick the fruits from different zones (determined by investigation of spatial variation) of a block and divide them into different product streams (different fruit bins and fermenters) in a single harvest event or alternatively one may harvest different zones of a block at different times.

However, current investigations on the within-vineyard variation in grape maturity mostly have relied on wet chemistry measurements of the typical berry ripeness parameters, and as

one could imagine these tests are very time-consuming and are usually limited to a relatively small sampling size. Due to the high pressure at vintage time, without a fast way to evaluate the vineyard variation in the fruit quality/ripeness, it is often difficult to answer the question of when to harvest different zones of the vineyard. The benefits of selective harvesting can only be maximised with the aid of fast and portable methods that can measure grape maturity and provide potential harvest date predictions. Consequently, we have focused our attention within this thesis on evaluating the potential use of monitoring peduncle colour evolution during ripening to monitor the within-vineyard variation of grape maturity in a simple fast fashion.

1.8 Aims of this Study.

With the focus of berry ripeness evaluation squarely placed on the chemical and biochemical changes within the grape berries themselves, it is interesting that no comprehensive studies have been conducted on how the grape stem changes its colour and chemical attributes during ripening and how this may relate to the berry maturation process. Consequently, this current study predominantly focused on an investigation of how the grape stem (peduncle and rachis) colour and their chemical attributes evolve during the berry ripening continuum for *Vitis vinifera* L. cv Shiraz. Furthermore, a detailed analysis of whether these attributes may be linked to the prototypical berry ripeness parameters were conducted in order to evaluate whether the colour or chemical changes of grape peduncles or rachises may be utilised as an indicator of grape berry ripeness and quality. If such a scenario turns out to be valid then such findings will provide an opportunity to develop a simple platform to assist viticulturists and winemakers in predicting harvest dates.

Specifically, the focus of the research within this thesis may be divided into three distinct studies:

1) The first study was conducted over two seasons (2012/13) and was designed to examine the change in the grape stem morphology/colour, including the peduncles and rachises from eight different patches of Shiraz from veraison to harvest. At the same time, all prototypical berry ripeness parameters were to be measured (°Brix, pH, TA) along with total anthocyanins and phenolics. With this information in hand, statistical analyses were

conducted in order to evaluate the strength of co-development between peduncle colour evolution and typical berry ripening process seen in the field.

2) The second study was designed to build upon the findings from the first study and was to dig deeper into how certain chemical traits of the peduncles and rachises evolve from veraison to harvest, and how this related to the observed change in colour seen in the first study. Specifically, five key chemical parameters within the peduncle and rachis samples were measured, namely the moisture content, total chlorophylls (including chlorophyll *a* and *b* levels), total carotenoids, total phenolics and their antioxidant capacity (DPPH) for all eight patches of Shiraz over a two year period. Again statistical analyses were utilised in order to evaluate the strength of co-development between peduncle colour evolution and the change in the chemical traits of the peduncles and rachises themselves. To complete this study, an analysis of whether the evolution of these chemical traits is linked to the typical berry ripening parameters was performed with the expectation to uncover new cross-talk links between grapevine stem chemistry and final grape berry quality.

3) The final study of this thesis is centred on evaluating the within-vineyard variation of peduncle colour during ripening of five Shiraz patches. Through a regular grid sampling of grape peduncles from veraison to harvest, the peduncle colour data was presented in a range of maps for each patch, and thus the spatial and temporal variation were investigated. The findings of which were expected to verify our findings from the previous studies and provide viticulturists and winemakers with a simple and fast method of predicting harvest date for each individual patch.

Whilst conducting all the aforementioned research and uncovering numerous new research findings which will aid the Australian wine industry into the future, I often wondered why we don't just ask the vine itself how its fruits are progressing throughout the season. Given that this thesis is to be by publication I dedicate the forthcoming publications and the findings with thanks to my friends, the vines of Longview vineyard.

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Chapter 2

Assisting Harvest Decisions via the Relationship between Peduncle Colour and Berry Ripeness in *Vitis vinifera* L. cv Shiraz

The current focus of grape berry ripening research is generally placed on the measurement of parameters and metabolite concentrations within the berries themselves. Although there has been some anecdotal evidence suggesting that grape stems, particularly the peduncle section, goes through a colour change from veraison to harvest and which possibly parallels berry ripening, no extensive research has been done on how the grape stem (including the peduncle and rachis) changes colour during ripening and how this observed stem colour evolution may assist in harvest decision making.

This paper examines the change in grape stem morphology/appearance at the peduncle and rachis level over eight different patches of Shiraz from veraison to harvest during the 2012 and 2013 vintages. A total of 192 grape peduncle samples (and 192 rachis samples in 2012) from each sampling time point (essentially weekly) were scanned into JPEG pictures and the size and colour parameters of each sample were accurately quantified using digital image analysis. A semi-automatic code was developed in Matlab software for this purpose. The code recognises each individual peduncle/rachis within the sample set and measures their area, perimeter, length, diameter, RGB and CIE*L*a*b** colour values. A number of improvements to the code were also made during this study. For example, at the beginning the code was only able to accurately determine the length of samples with a straight outline, so we then modified it for the precise measurement of length for all samples including any curly peduncles and rachises. Further optimisation of the code allowed each image processing time to be reduced from 10 min to 30 seconds per run. In addition, our initial colour analysis focused on the difference in RGB values, but further data analysis showed that the CIE*L*a*b** colour system is more informative and the polar colour parameters calculated from *a** and *b** would match with our visual colour observations in the field even better.

Apart from collecting this large amount of data from the peduncle and rachis image analyses, all berries associated with these stem samples were also measured for their ripeness parameters, including Brix °, pH, TA, total anthocyanins and phenolics. A total of

32 lots of berry samples from each sampling time point were crushed into juice for °Brix, pH, and TA analysis. Furthermore, a total of 32 to 64 lots of berry samples at each sampling time point were homogenised into paste to measure their total anthocyanin and phenolics levels. Then the berry data set was compared with the peduncle and rachis data.

The image analyses of the peduncle and rachis samples showed that both samples did not change in size, however the peduncles and rachises changed colour asynchronously, i.e. the overall peduncle colour turned from green to predominantly brown while the rachises remained green during berry maturation. The visual colour change within peduncles was well expressed by the change in a^* (CIE$L^*a^*b^*$) and hue values, which allowed us to couple these colour values with the changes in berry ripeness parameters from the same grape bunches. In this way, a clear co-development between peduncle colour evolution and the berry ripening process was presented.

To the authors' knowledge, this is the first in-depth report on grape stem evolution, particularly grape peduncle colour evolution during berry ripening. Our findings on the asynchrony in colour evolution between the peduncles and rachises indicated that stem colour change reported in the previous literature should actually mean that the colour change occurs in peduncles alone, not including the rachises. This study not only provided a simple effective method of monitoring grape stem colour exploiting digital image analysis, but also opens the door of predicting berry ripeness through monitoring grape peduncle colour and therefore assisting in harvest decision making. Presented in the following publication is the first detailed investigation of grape peduncle and rachis colour evolution during berry ripening and has been submitted to the *Australian Journal of Grape and Wine Research*.

Statement of Authorship

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Author contributions

Name of Principal Author (Candidate): Yudan Fang
Contribution to the Paper: Designed experiments, performed experimental work, analysed and interpreted data. Drafted/constructed manuscript.

Signature:

Name of Co-Author: Olena Kravchuk
Contribution to the Paper: Assisted with statistical analysis and manuscript preparation.

Signature:

Name of Co-Author: Sigfredo Fuentes
Contribution to the Paper: Constructed the Matlab code for digital image analysis and aided in early experimental design.

Signature:

Name of Co-Author: George Skouroumounis
Contribution to the Paper: Aided in sampling and experimental design in the early stages of this work.

Signature:

Name of Co-Author: Dino Cotsaris
Contribution to the Paper: Provided vineyard and harvest information details.

Signature

Name of Co-Author: Dennis Taylor
Contribution to the Paper: Supervised the work, aided in designing experiments, assisted with grape sampling and data/results interpretation, drafted/constructed manuscript.

Signature:

Assisting Harvest Decisions via the Relationship between Peduncle Colour and Berry Ripeness in *Vitis vinifera* L. cv Shiraz

Yudan Fang[1], Olena Kravchuk,[1] Sigfredo Fuentes[2], George K. Skouroumounis,[1] Dino Cotsaris,[3] Dennis K. Taylor[1]

Corresponding author. Prof Dennis Taylor, email dennis.taylor@adelaide.edu.au

[1] School of Agriculture, Food & Wine, The University of Adelaide, Waite campus, PMB 1, Glen Osmond, 5064, Australia.

[2] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Melbourne, Victoria, 3010.

[3] Longview Vineyard, Pound road, Macclesfield, Adelaide Hills, SA 5153.

Abstract

Background and Aims: The focus of grape berry maturity or the peak of ripeness is squarely placed on the measurement of parameters and metabolite concentrations of the grape berries themselves, with limited reports on how grape stem evolution during grape ripening may assist in harvest decisions. The objective of this study was to investigate the evolution of grape peduncle colour from veraison to harvest for *Vitis vinifera* L. cv Shiraz and examine if the change in peduncle colour co-develops with berry ripening.

Methods and Results: The change in peduncle colour from veraison to harvest was measured using digital image analysis over eight patches of Shiraz from the same vineyard. Moreover, peduncle morphological details and prototypical berry ripeness parameters (^{o}Brix, pH and TA) were also evaluated. Results showed that overall peduncle colour changes from green to brown in parallel with the berry ripeness parameters thus providing a simple platform to assist in harvest decisions. The a^* (CIE$L^*a^*b^*$) and hue values are the most informative colour coordinates when monitoring peduncle colour change.

Conclusions: Peduncle colour evolution parallels berry ripening and provides an alternative way to assist in the prediction of harvest date.

Significance of the Study: The approach described herein provides a new way to assist in making harvest decisions through monitoring grape peduncle colour evolution. It is simple, fast, non-destructive and has the potential to reduce analytical costs associated with current berry maturity analyses.

Keywords: *grape peduncle, berry ripeness, peduncle colour, Shiraz, image analysis, harvest date*

Introduction

Producing high quality wines requires harvesting grapes at their optimal level of maturity. Timing of harvest is a subjective judgement and is a matter of determining the point along the ripening continuum that best fits the winemaker's objective for the wine, whilst at the same time grape growers must have an appropriate expectation of the targeted fruit ripeness (Hellman 2004). Consequently different methods to define optimal grape maturity have been developed. Both historically and still today, the most commonly used grape maturity indicators employed by viticulturists and winemakers are sugar content (^{o}Brix) and acidity (pH, TA), with multiple samplings taken between veraison and harvest in order to ascertain optimal harvest date (Watson 2003). Of additional importance are the winemaker's observations on the development of varietal colour, aroma, flavour, and their perceptions of skin, seed, and stem maturity along with observations on the physical condition of the vines and fruit. (Watson 2003, Ristic and Iland 2005, Fredes et al. 2010 Ferrer-Gallego et al. 2010, Rodriguez-Pulido et al. 2012, Winter et al. 2004, Olarte et al. 2012). Most of these grape maturity indicators and others (e.g. anthocyanins, aroma compounds etc) are undertaken in analytical laboratories, and require strict sample preparation procedures along with appropriate analytical analysis. Naturally, these procedures are destructive in nature and there is now a considerable push towards evaluating grape berry ripeness, and indeed other fruits maturity levels exploiting non-destructive analyses. (Geraudie et al. 2009, Rolle et al. 2012a, Letaief et al. 2013, Rolle et al. 2012b) Moreover, due to the potential variability in the maturity of berries within a bunch, and the often problematic situation in obtaining a representative berry sample (Watson 2003), it is now considered more advantageous to determine grape maturity at the bunch level rather than at berry level and in a non-destructive manner.

With the focus of grape berry maturity or the peak of ripeness squarely placed on the measurement of parameters and metabolite concentrations of the grape berries themselves, it is not surprising that there have only been limited reports on how grape stem development evolves during grape maturation and how such observations may assist in harvest decisions. Anecdotal evidence reported in the literature suggests that grape stem

ripening also parallels berry maturation as indicated by a change in stem colour from green to brown during ripening (Ribereau-Gayon et al. 2000, Bisson 2001, Watson 2003). Perhaps the most interesting of these reports are those of Bisson (2001) and Watson (2003) which highlight that stems of many varieties of grape undergo a change from green unripe to brown or ripe stems to overripe or brittle stems, or that as the fruit reaches maturity, the berry stems (peduncles) turn from green to brown and decrease in green, 'stemmy', herbaceous character. Furthermore, it was also stated recently that there appears to be a wine industry impression that has assumed that there is better tannin maturity (assessed by berry sensory evaluation) with higher peduncle browning (Leal 2007). The process of browning of the stem or peduncle is known as lignification and is caused by the death of the green cortex and is accompanied by the deposition of starch in the xylem and phloem parenchyma cells (Iland, 2011). Interestingly, peduncle length and lignification have been used as a visual parameter to characterise grape varieties under the code OIV 206 & 207 (Boehm and Tulloch 1967, Masi et al. 2001, Goussand 2008, OIV 2009) for decades. Naturally, these suggestions in the literature have been well observed for many decades by viticulturists themselves, however, there has been no rigorous study to clearly demonstrate that changes in stem colour do indeed parallel the berry ripening continuum.

Currently there is considerable drive to develop non-destructive image analysis tools for a variety of applications in the grape and wine industry. In terms of grape and stem analyses, Lichter et al. (2011) compared photographic image analysis with subjective human evaluation of rachis browning levels during table grape postharvest storage, and determined that image analysis was a more accurate and sensitive approach in monitoring the change in rachis browning degree. Such information is anticipated to lead to new measures to prolong grape quality and shelf life. Furthermore, Rodriguez-Pulido et al. (2012) recently measured the size and colour (CIE*L*a*b**, RGB values) of grape berries and their seeds during maturation through image processing utilising a self-constructed image analysis apparatus, with the colour parameters being able to be employed to discriminate pre-veraison berries from post-veraison berries. In a similar fashion, Fuentes et al. (2010) were the first to utilise colour image analysis tools to quantify berry vitality with morphological information; finding that berry shrivel was correlated with berry vitality across 22 grape varieties. Finally, Cubero et al. (2014) have developed an algorithm that may be employed to locate grape pedicels of individual fruits/berries from digital images. The drive behind this study was to develop a fast and accurate method for

detecting and removing the pedicel in images of berries so that one could accurately determine the size and weight of the berry. Unfortunately, utilisation of such image systems for the grading of an entire grape bunch is yet to be realised. Beside these advances, image analysis systems are also currently being developed for application throughout the entire vineyard. For example, Reis et al. (2012) studied the detection and location of grape bunches on vines by processing images captured at night. The concept was that the information gained may be used to help guide autonomous harvesting robots. Whilst a simple method for detecting the location of the bunches was indeed developed, they also claimed that the location of the grape stem could be detected, however, they were actually estimating the central axis of the bunch from the bunch shape and the system is realistically limited to processing images that contained only one bunch. Further, Diago et al. (2012) utilised a camera to take images of canopies in the vineyard, and developed an algorithm to assess total leaf area and yield by setting up different thresholds to separate leaves, woods, grapes and background from each other. Finally, (Ming and Tien-Fu 2006, Ming 2011) developed and utilised an algorithm of image processing to detect the cutting positions on a vine, which has aided in the development of automatic grapevine pruning systems in the vineyard.

Given the dearth of information on how grapevine stem colour (peduncle and rachis) actually evolves over the ripening season for *Vitis vinifera* and whether this apparent colour change may be coupled to key changes in berry parameters and metabolite concentrations, the aims of this study were threefold. 1) To develop a digital image analysis method to follow the size and colour evolution over time of the peduncles and rachises of the grape variety Shiraz (8 patches) from veraison to harvest over two vintages, 2) to couple the observed colour changes with the traditional grape berry ripeness parameters, namely berry sugar content (^{o}Brix), acidity (pH, TA) and total anthocyanin and phenolics levels, and 3) to statistically evaluate the observed correlations or potential parallel co-development thus providing a predictive model which may be able to be employed to assess grape quality and maturity over time in a non-destructive manner and consequently assist in the making of harvest decisions within a certain vineyard.

Materials and methods

Vineyard, Shiraz patches and sampling schedule

Whole grape bunch samples including the entire peduncle portion of the stem were obtained from eight Shiraz patches at Longview vineyard (GPS Position: 138 °49'54"E, 35 °11'25"S). The vineyard is located on the South Eastern Ridge of the Mt Lofty Ranges, and has a slightly higher average temperature than the rest of the Adelaide Hills although it is still considered a cool-climate vineyard. It has hot dry summers and wet winters. The total rainfall in the 2013 season was 731 mL with only 105.6 mL received between Oct. 2012 and Mar. 2013 (Cotsaris 2013). The 2012 season was slightly cooler and wetter (100 mm in 2012 and 50 mm in 2013 vintage). Figure 1 displays a satellite map of the 8 Shiraz patches at Longview vineyard showing their orientation relative to North. The Shiraz Vine Clone was 1654 for all patches, except for patch SH 6 whose clone was BVCR 12. All patches were planted on their own rootstocks and were under drip irrigation utilising different irrigation regimes. Further details of each patch are collated within Table 1.

Figure 1. Satellite map of 8 patches of Shiraz at Longview vineyard.

Table 1. Details of 8 patches of Shiraz at Longview vineyard.

Patch†	Area (Ha)	# Rows	Row Orientation	Pruning method	Buds per (m)	Vine Space (m)	Row Space (m)	Year of planting
SH 2N	3.33	78	East West	Cane VSP	24	1	2.4	1997
SH 2S	1.41	37	East West	Cane VSP	24	1	2.4	1997
SH 3	2.69	97	North South	Cane VSP	24	1	2.4	1997
SH 6	0.48	55	East West	Cane VSP	24	1	2.4	1997
SH 13E	1.14	31	North South	Cane VSP	16	1.5	3	2000
SH 13B	4.22	63	North South	Spur VSP	20	1.5	3	2000
SH 13T	2.03	63	North South	Cane VSP	16	1.5	3	2000
SH 15	3.19	85	North South	Spur VSP	20	1.5	3	2002

† All 8 patches coded as Shiraz (SH) followed by position within vineyard, see Figure 1; VSP: vertical shoot positioning.

The experiments were conducted over 2 growing seasons: 2011/2012 (season 1) and 2012/2013 (season 2). Data presented herein corresponds to the 2012/2013 season although comparisons with the 2012 data are made wherever relevant. Sampling in 2013 commenced at veraison (Jan. 29th, 67 DAF) and continued weekly to harvest (Mar. 17th or 20th, 114 or 117 DAF). A total of eight samplings were conducted up until the day or night before harvest as determined by the viticulturists and winemakers, Table 2.

For patches considered to have short rows (under 100 vines per row), i.e. SH 2S, SH 6, SH 13E, SH 13T and SH 15, samples were picked from two adjoining rows that were ca. 25% in from the 1st row of the patch and another two adjoining rows that were ca. 25% inward from the last row. For patches SH 2N, SH 3 and SH 13B which were considered to have long rows, two adjoining rows in the middle of the patch were selected for sampling. To ensure that the sun exposed and the shaded parts of the vines selected were equally represented in the sample pool, a zigzag sampling method was adopted. One bunch was picked every five vines and each bunch was carefully cut off the vine as close to the cane as possible utilising secateurs to preserve the entire peduncle, Figure 2. A total of 48 bunches per patch were picked per sampling week in order to ensure an appropriate sampling pool size. The 48 bunches of grapes were separated into four groups of 12 according to their sampling position i.e. one group from each row in patches considered to have short rows or two groups (first half/second half) from each row in patches considered to have long rows. After sampling, the individual bunches were immediately placed into

resealable plastic bags and transported to the laboratory in dry ice cooled containers for analysis.

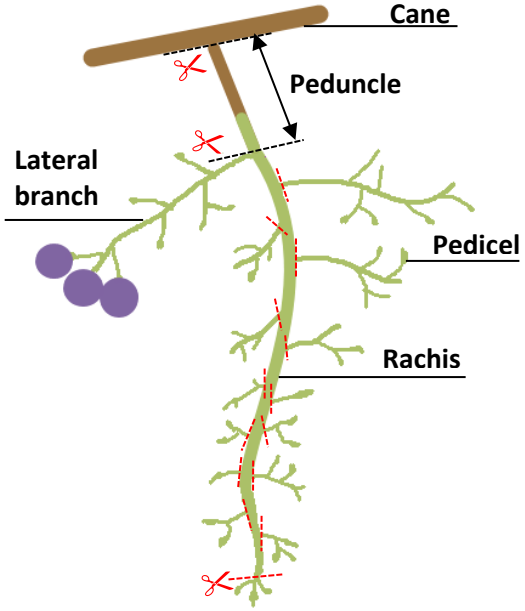

Figure 2. Key structural units of the grape bunch collected for analysis included the peduncle, rachis and berries.

Peduncle, rachis and berry preparation

Peduncle and rachis collection and digital imaging were performed on the day of sampling. Six bunches were randomly selected from each group of 12 bunches. The peduncles were carefully removed by cutting at the peduncle node, which often connects with the first lateral branch, Figure 2. Then the remaining lateral branches with the berries and pedicels were removed in 2012 to obtain rachis. The berries were also removed at this stage. On each sampling day, 24 peduncles per patch (4 groups x 6) were obtained for morphological analysis. For the berries from the 24 bunches, subsamples of 50 berries per group were randomly selected, weighed and stored in 120 mL plastic containers at -20 ℃ for total anthocyanin and phenolics analysis, whilst the remaining berries were crushed into juice and analysed for TSS (^{o}Brix), pH and titratable acidity (TA).

Peduncle image acquisition 2013

Twenty four peduncles from each patch were placed in a 6 x 4 format in the 17'' x 11'' screen of a Canon iR5185 photocopier as illustrated in Figures 3a and 3b for those collected from patch SH 13E on the 1st sampling day (2013, DAF 67), and on the 7th sampling day (2013, DAF 109). The scanning resolution was 300 x 300 dpi and the images were stored as A3 Joint Photographic Expert Group (JPEG) files.

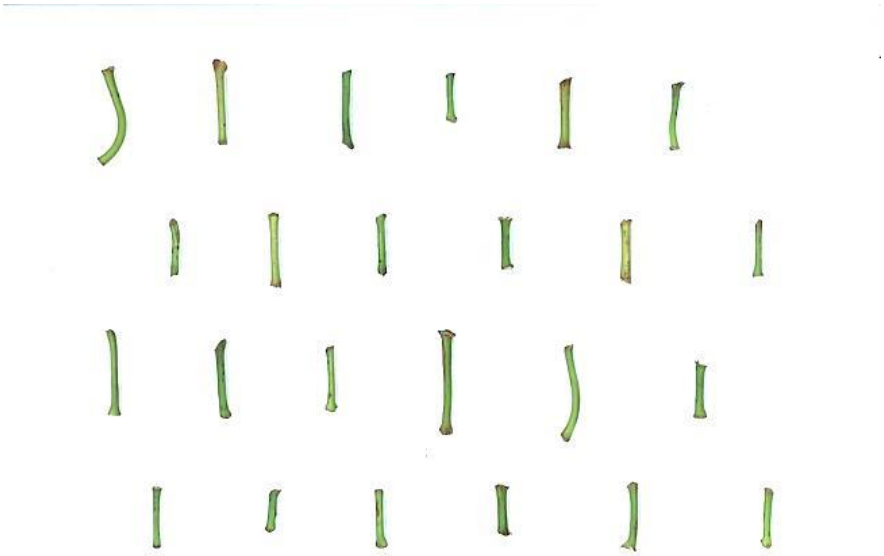

Figure 3a. Scanned Peduncle Image of SH 13E on 1st sampling day (DAF 67), 2013.

Figure 3b. Scanned Peduncle Image of SH 13E on 7th sampling day (DAF 109), 2013.

Peduncle and rachis image acquisition 2012

Twenty-four peduncles and 24 rachises from each patch were scanned in a similar manner to that described above except scanning was done in batches of 6 rachises along with their associated peduncles.

Automatic detection and image analysis of peduncles and rachises

Scanned images of the peduncles in JPEG format were analysed semi-automatically using a customised code developed using Matlab® together with its Image Analysis Toolbox® (Mathworks Inc., Natick, MA, USA). This code was pre-calibrated before each vintage by scanning black square paper cards of specific size and angle to enable the accurate analysis of all the peduncle and rachis images generated by the same scanner. The code recognises all 24 peduncles in each scanned image, and generates regions of interest (ROI) corresponding to the scanned sides of each peduncle. From these ROI's, the code extracts the morphological size parameters: area (cm^2), perimeter (cm), length (cm), and diameter (cm). Furthermore, for each peduncle or rachis in the images, the code obtains the colour value of each pixel and outputs one set of average colour values in both RGB (red, blue and green) and the CIE*L*a*b** colour systems. The code for image analysis is available upon request.

Berry chemical analysis

Berries from each group and patch on each sampling day were crushed into juice and centrifuged at 3500 rpm (2451 x *g*) for 6 minutes. Juice total soluble solids (°Brix) were measured with a refractometer, pH by a pH meter (calibrated daily, pH 510, Eutech Instruments) and TA with a semi-automatic titration instrument (Compact Titrator, Crison Instruments, S.A., Allela, Spain). Berry total anthocyanins and total phenolics were determined according to the method of Iland et al. (2004) with slight modifications. Each sample of 50 berries of known weight was homogenised using a Janke and Kunkel Ultra-turrax IKA T18 homogeniser at 24,000 rpm for approximately 1 minute each. One gram of the homogenised sample was extracted with acidified 50% v/v ethanol (pH 2 adjusted by HCl) and shaken at 100 rpm for one hour. After centrifugation at 3500 rpm for 10 minutes, 1.0 mL of the extract supernatant was mixed with 10 mL 1M HCl and left in the dark overnight (9 hours). Absorbance of the extract was read at 520 nm and 280 nm on a Cintra 40 spectrophotometer using a UV quartz micro rectangular cuvette.

Statistical analysis

Due to substantial spatial variation in the field, the sample transect strategy was modelled as stratified sampling, with each patch representing a stratum. To understand the structure of variation in the vineyard, patches and rows within patches were also modelled as random factors in a simple linear mixed model of repeated measures (a subject being a position within a row within a patch, the samplers were returning to approximately the same position at each sampling time). The corresponding linear mixed models were utilised to estimate the means and medians of peduncle and berry developments in this longitudinal study, with patches treated as fixed for the estimation of the development within patches or as random for the estimation of the development in the vineyard. The analysis was carried out with the REML procedure in GenStat 16.1 software, GenStat for Windows 16th edition. VSN International, Hemel Hempstead, UK.) The diagonal variance structure was allowed for time. All the development parameters except the hue satisfied adequately the normality assumption adequately. For the hue, the medians rather than means were estimated. Graphs were generated with an R computational package (R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.) The code for figures in this publication is available upon request.

Results and discussion

Berry chemical analysis from veraison to harvest for 2012 and 2013 vintages

On each sampling day during the 2012 and 2013 vintages, berries from all eight Shiraz patches were analysed for total soluble solids (°Brix), pH, TA, total anthocyanins, total phenolics and berry weight. Average ripeness parameters for the 2013 season up until 114 or 117 DAF are depicted in Table 2. Overall berry °Brix increased in a facile manner from 11.7 at veraison, tapering off at 88 DAF and finally reaching around 26 °Brix at harvest (DAF 114 or 117). Overall berry weight also increased between 64 DAF to 95 DAF and then tapered off, presumably due to berry shrivel occurring closer to harvest. These two observations are as expected and in accordance with that reported previously for Shiraz (Coombe and McCarthy 2000). As expected, the pH steadily rose from veraison (2.78) to harvest (3.48), whilst TA decreased rapidly in the early stages of ripening and settled

around 5.0 g/L at harvest. Total anthocyanins increased rapidly after veraison and steadily rose until harvest whilst total phenolics also steadily rose during grape maturation. Such observed changes during the ripening of wine grapes have been reported on numerous occasions, Johnson and Nagel (1976), Watson (2003), Fredes et al. (2010), Bindon et al. (2013). The same ripeness parameters for the 2011/2012 season were of similar magnitude over the same time course of ripening (data not shown). The final harvest date was decided by the viticulturists and winemakers based on their independent berry chemical analyses and their tasting records.

Table 2. Sampling schedule and berry ripeness parameters for all patches during the 2012/2013 ripening season.

Sampling date	DAF	°Brix	pH	TA (g/L)	Berry weight (g)	Total anthocyanins (mg/berry)	Total phenolics (au/berry)
29th Jan.	67	11.73 ± 0.12	2.78 ± 0.01	23.27 ± 0.38	0.82 ± 0.02	0.07 ± 0.05	0.96 ± 0.02
5th Feb.	74	13.36 ± 0.16	2.85 ± 0.01	19.15 ± 0.26	0.98 ± 0.02	0.24 ± 0.05	1.05 ± 0.02
12th Feb.	81	17.12 ± 0.26	3.07 ± 0.01	11.74 ± 0.24	1.27 ± 0.03	1.06 ± 0.05	1.38 ± 0.04
19th Feb.	88	21.02 ± 0.28	3.11 ± 0.01	8.74 ± 0.13	1.27 ± 0.02	1.46 ± 0.05	1.49 ± 0.03
26th Feb.	95	21.46 ± 0.30	3.25 ± 0.02	7.06 ± 0.13	1.39 ± 0.02	1.86 ± 0.05	1.61 ± 0.03
5th Mar.	102	23.56 ± 0.25	3.34 ± 0.01	6.08 ± 0.11	1.34 ± 0.02	2.04 ± 0.05	1.67 ± 0.03
12th Mar.	109‡	25.49 ± 0.21	3.49 ± 0.02	5.20 ± 0.10	1.28 ± 0.02	2.19 ± 0.05	1.77 ± 0.03
17th and 20th Mar.	114§ and 117¶	26.43 ± 0.24	3.48 ± 0.01	5.00 ± 0.20	1.34 ± 0.02	2.33 ± 0.05	1.84 ± 0.03

†DAF: days after flowering. ‡ At DAF 109, patches SH 13E, SH 13B and SH 15 were sampled before immediate harvest. § At DAF 114, patches SH 2S and SH 13T were sampled before immediate harvested. ¶ At DAF 117, patches SH 2N, SH 3 and SH 6 were sampled before immediate harvest. Berry ripeness parameters are an average of DAF 114 and 117 data.

Peduncle and rachis colour changes 2012

The colour change of both the peduncles and rachises were monitored from veraison to harvest for all eight Shiraz patches during the 2012 season utilising both standardised RGB and CIE*L*a*b** colour systems. Figures 4a and 4b display typical images obtained for peduncles and rachises collected from patch SH 6 on the 1st sampling day (2012, DAF 64), and on the 7th sampling day (2012, DAF 127), whilst Table 3 summarises the RGB and *L*a*b** values. Simple eyeballing of the images in Figures 4a/4b clearly demonstrates that the peduncles are green at veraison and those at harvest are predominantly brown in texture. Furthermore, whilst the rachises are also green at veraison, they essentially remain that way at time on harvest. These trends were clearly manifested in the observed RGB and *L*a*b** values of the peduncles and rachises, Table 3.

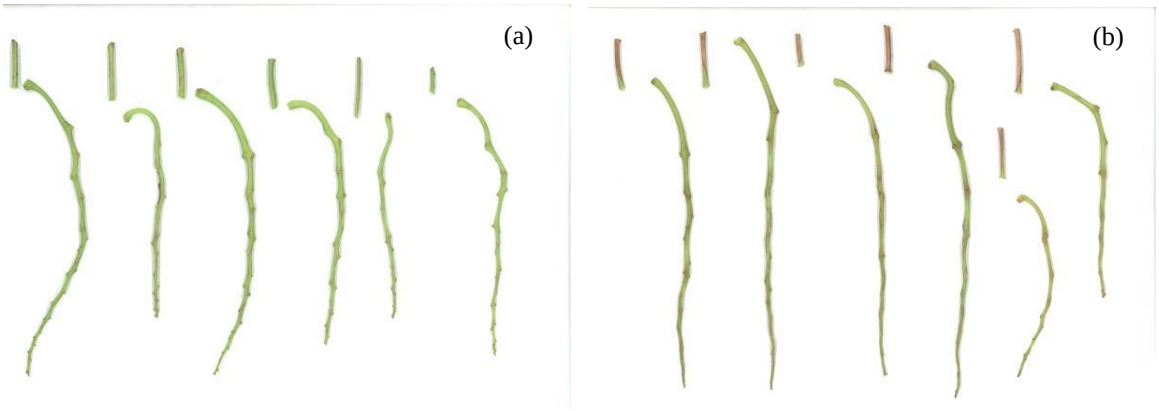

Figure 4. Scanned peduncle and rachis images of Shiraz patch SH 6 in the 2011/2012 season. (a) 1st sampling date (64 DAF) and (b) 7th sampling date (127 DAF).

In terms of the CIE*L*a*b** colour system, as detailed by McLaren (1980), *L** represents the lightness of the colour; *L** = 0 yields black and *L** = 100 indicates diffuse white. The *a** and *b** colour axes are based on the fact that a colour can't be both red and green, or both blue and yellow and each axes will have values running from positive to negative. On the *a** axis, positive values indicate magnitude of redness while negative values indicate magnitude of greenness. On the *b** axis, positive values indicate the magnitude of yellowness whilst blueness is manifested in negative values. For both axes, zero is neutral gray. Thus, if an object was green to begin with and gradually turned brown, then we would expect to observe a slight decrease in *L** and a change in average *a** from negative to positive.

Table 3. RGB and CIE*L*a*b** colour values measured for individual peduncles and rachises depicted in Figure 4a and 4b.

Sample order†	*L**		*a**		*b**		R		G		B	
	Ver	*Har*	*Ver*	*Har*	*Ver*	*Har*	*Ver*	*Har*	*Ver*	*Har*	*Ver*	*Har*
P1	74.36	70.51	-11.05	0.38	21.52	16.97	113.35	124.21	127.74	104.01	70.07	68.77
P2	76.53	73.02	-10.83	-1.01	22.65	18.34	123.70	132.33	136.71	114.44	74.01	73.12
P3	75.49	72.94	-10.67	0.85	24.44	16.25	121.59	135.10	131.95	112.74	67.47	77.21
P4	75.45	69.16	-14.06	4.59	24.09	15.18	111.82	127.98	134.63	96.08	67.90	68.66
P5	75.03	72.65	-10.21	-0.31	22.30	17.08	119.02	131.44	129.81	112.54	70.46	74.61
P6	75.54	74.21	-13.65	2.19	22.25	14.57	111.53	142.82	134.80	116.65	71.82	84.74
R1	75.78	74.07	-13.20	-8.86	23.45	22.85	114.86	119.35	135.40	124.68	70.14	66.78
R2	76.97	74.74	-12.20	-8.70	22.97	22.48	121.89	122.15	139.77	127.33	74.55	69.35
R3	77.15	74.64	-12.85	-7.61	23.60	22.37	121.42	124.68	141.11	126.05	73.76	69.36
R4	78.38	74.23	-13.00	-9.26	24.25	21.93	126.65	118.09	146.68	125.74	75.99	69.00
R5	78.20	76.39	-12.50	-6.68	23.13	22.98	126.29	135.32	145.55	132.53	77.87	73.14
R6	78.74	76.60	-12.22	-7.92	23.21	22.67	129.45	132.32	147.76	134.53	79.34	74.32
P	75.40	72.08	-11.75	1.12	22.88	16.40	116.84	132.31	132.61	109.41	70.29	74.52
R	77.54	75.11	-12.66	-8.17	23.44	22.55	123.43	125.32	142.71	128.48	75.28	70.33

† Sample order P1 to P6 and R1 to R6 correspond to the peduncle and rachis from left to right in Figure 4, respectively. *Ver*: Veraison, DAF 64; *Har*: Harvest, DAF 127. P and R represent the average values of each parameter among 6 samples.

Indeed as can be seen in Table 3, the *L** value slightly decreased almost equally in both peduncles and rachises from veraison to harvest. Importantly, the *a** values of the peduncles began in the range of -10 to -14 at veraison and then transitioned to zero or indeed positive values at harvest. The *a** values of the rachises remained large and negative with an average value of -8.17 at harvest compared to -12.66 at veraison. The *b** values are positive for both the peduncles and rachises, and the average *b** value of the peduncles decreased by around 28% while the rachis *b** value did not change significantly from veraison to harvest. Taken together, these observations indicate that close to veraison, peduncles and rachises display fairly similar *L*a*b** colour values. By contrast, a significant difference between peduncle and rachis colour was found at harvest. The increase of the *a** value from negative to positive and the decrease of the *b** value for peduncles, and the observed small increase of the rachis *a** colour values, reflected what we observed visually in the field; that being the majority of peduncles turned from green to brown while the rachis remained green from veraison to harvest. The same trends and

magnitudes of *L*a*b** colour change were observed for all remaining seven Shiraz patches from veraison to harvest (data not shown).

The RGB colour model was also utilised to analyse the change in colour of the peduncles and rachises during maturation. As an additive colour model RGB represents the three primary colours, red, green, and blue, McLaren (1980). Each colour channel has a value range from 0 to 255. If all three components are at zero the result is black; if all are at maximum, the result is the bright white. The colour is expressed as an RGB triplet; for example, (255, 0, 0) would represent the brightest saturated red in this study. RGB is a device-dependent colour model, and the values are relative to the primary colours instead of absolute.

Inspection of the RGB values within Table 3 indicate that the peduncles had an average increase of 13% in accumulated redness from the veraison and harvest time points while the rachises did not change significantly. The G value for greenness of both the peduncles and rachises went down by 17% and 10%, respectively, indicating that the peduncles lost more greenness than the rachises during ripening and correlates well with the findings found above for the CIE*L*a*b** measurements. The B value for each individual peduncle varies somewhat, whilst the general rachis B value decreased slightly. In fact it appears that the changes in the B values from the RGB colour system are not that meaningful when analysing for colour changes of peduncles and rachises from veraison to harvest. The same trends and magnitudes of RGB colour change were observed for all remaining seven Shiraz patches from veraison to harvest (data not shown).

The findings depicted in Figures 4a/b and Table 3 bode well with the overall aims of this study in that the overall peduncle colour changes from green to brown from veraison to harvest whilst the rachises do not significantly change. Moreover, the peduncles are easier to see visually by the human eye whilst the rachises are obscured by the berries themselves, thus, the development of any downstream automatic image analysis system would have difficulties in pictorially identifying the rachis within a bunch. Consequently, it was decided to only analyse the observed colour change of the peduncles from veraison to harvest in all remaining studies.

Peduncle colour evolution for the 2012/2013 season

The colour change of the peduncles was again monitored from veraison to harvest for all eight Shiraz patches during the 2013 season utilising both the RGB and CIE*L*a*b** colour systems, and is summarised as an average value for all patches in Table 4. In terms of peduncle greenness/redness, the *a** values of the peduncles began with a magnitude of around -9 at veraison and were close to zero at harvest. The *b** values are positive throughout grape berry maturation with the average *b** value of the peduncles decreasing by around 24% from veraison to harvest. These observed changes in peduncle colour coordinates indicated that the peduncles visually appear more brownish in colour at harvest. This finding also has some similarity with that found during recent seed colour maturation studies of 'Graciano' red grapes, Ferrer-Gallego et al. (2010). They observed that during ripening, an increase in *a** value and a decrease in *b** value was observed although whilst their *b** value (from about 15 to 27) is comparable to our peduncle *b** value, their *a** value was always positive.

Table 4. Mean of peduncle colour values for 8 Shiraz patches during the 2012/2013 season.

DAF	*L**	*a**	*b**	R	G	B
67	74.39 ± 0.21	-9.03 ± 0.41	20.32 ± 0.13	118.00 ± 1.59	126.50 ± 0.71	72.74 ± 0.58
74	75.38 ± 0.36	-8.22 ± 0.37	20.18 ± 0.24	124.20 ± 2.37	130.00 ± 1.34	75.96 ± 0.70
81	74.82 ± 0.40	-6.05 ± 0.42	19.76 ± 0.21	127.30 ± 1.44	126.20 ± 1.89	75.31 ± 0.93
88	73.32 ± 0.34	-3.32 ± 0.63	18.11 ± 0.36	127.00 ± 1.38	118.00 ± 1.67	74.39 ± 0.45
95	73.00 ± 0.40	-2.39 ± 0.60	17.2 ± 0.38	127.30 ± 1.22	116.30 ± 1.92	75.40 ± 0.50
102	72.10 ± 0.48	-1.11 ± 0.98	16.18 ± 0.47	126.00 ± 1.50	111.60 ± 2.44	74.80 ± 0.67
109	72.12 ± 0.40	0.26 ± 0.41	15.27 ± 0.25	129.00 ± 1.57	110.70 ± 1.75	76.87 ± 0.90
114 and 117	72.49 ± 0.66	-0.08 ± 0.50	15.53 ± 0.37	130.00 ± 1.76	112.40 ± 2.81	77.45 ± 1.37

Data are the means ± standard errors of 192 peduncles in total on each sampling date (24 peduncles per patch for 8 patches).

Inspection of the average RGB values within Table 4 again indicates that the peduncles clearly change from green to brown during the course of grape berry ripening. The magnitudes of the *L*a*b** and RGB values along with their significant rate and direction of change as an average of all 8 Shiraz patches in 2013 are essentially identical to those found above during the 2011/2012 season at the individual patch level. Consequently, one may utilise observed peduncle colour change either at the individual patch level or at the

vineyard level to probe for correlations with berry ripeness parameters as highlighted below.

Peduncle size development 2013

Besides a few reports on how the grape stem, and in particular the rachis changes diameter and length during maturation under the influence of plant growth hormones such as gibberellic acid, Nakamura and Hori (1981, 1983), Theiler and Coombe (1985), NII (1986), no studies on the evolution of the peduncle size and shape from veraison to harvest have been reported previously. The image analysis and associated code not only allowed us to capture the colour values but also allowed us to determine the associated morphological size parameters; area (cm^2), perimeter (cm), length (cm), and diameter (cm) of the peduncles over time from veraison to harvest. As an example, the morphological size parameters of the peduncles from 67 DAF up until harvest as an average of all 8 Shiraz patches in 2013 are tabulated in Table 5. It was observed that the average peduncle length of the 8 patches of Shiraz was less than 3 cm, and the diameter was around 4 mm whilst the average perimeter of the peduncles was near 7 cm in total. Overall, it appeared that these morphological size parameters do not change significantly from veraison to harvest in 2013. This finding was identical to that found in the 2011/2012 growing season and was apparent even if the analyses were evaluated at the individual patch levels located throughout the vineyard. The finding that peduncle growth has essentially ceased by veraison is similar to that found for rachis evolution by Coombe (1995) who measured the rachis length of several grape varieties for about 19 weeks before full ripeness, and found that there was a rapid increase in rachis length and area before flowering, however, the rachis and pedicel length stopped increasing around fruit set.

Interestingly, the peduncle length is one of the visual parameters employed to characterise grapevine varieties under the code OIV 206 (OIV, 2009). According to code 206, varieties that have a peduncle length up to about 30 mm are categorised as belonging to a very short bunch group. It should be noted that the OIV code 206 describes peduncle length as the length from the attachment to the cane to the second lateral branch along the main axis, however, in our study we have defined it from the same point of attachment to the first lateral branch, and they utilise only a small library of *Vitis* varieties that did not include Shiraz. Irrespective of the definition, our finding that the Shiraz peduncles are less than 3 cm clearly places them in the very short peduncle group according to the OIV code.

Finally, given that the average peduncle size and overall shape did not alter significantly after veraison, it was not surprising that no correlations between peduncle morphological size and berry ripeness parameters (^{o}Brix, pH, TA etc) were observed upon statistical analysis.

Table 5. Mean peduncle size for the 8 Shiraz patches during the 2012/2013 season.

DAF	Area (cm^2)	Perimeter (cm)	Length (cm)	Diameter (cm)
67	1.09 ± 0.06	7.02 ± 0.29	2.93 ± 0.10	0.42 ± 0.01
74	0.98 ± 0.04	6.62 ± 0.18	2.80 ± 0.08	0.39 ± 0.01
81	0.92 ± 0.07	6.41 ± 0.38	2.71 ± 0.15	0.38 ± 0.02
88	1.05 ± 0.04	7.21 ± 0.19	2.98 ± 0.08	0.40 ± 0.01
95	1.01 ± 0.05	6.97 ± 0.22	2.92 ± 0.10	0.41 ± 0.02
102	0.97 ± 0.03	6.97 ± 0.17	2.87 ± 0.07	0.39 ± 0.01
109	0.93 ± 0.03	6.75 ± 0.14	2.79 ± 0.08	0.38 ± 0.01
114 and 117	0.87 ± 0.04	6.69 ± 0.19	2.75 ± 0.08	0.36 ± 0.01

Data are the means ± standard errors of 192 peduncles in total on each sampling date (24 peduncles per patch for 8 patches). † Days after flowering; ‡ At DAF 109, patches SH 13E, SH 13B and SH 15 were sampled before immediate harvest; § At DAF 114, patches SH 2S and SH 13T were sampled before immediate harvested; At DAF 117, patches SH 2N, SH 3 and SH 6 were sampled before immediate harvest. Berry ripeness parameters are an average of DAF 114 and 117 data.

Peduncle colour development 2013, plots of polar colour parameters

Besides simply having tabulated the RGB and CIE*L*a*b** colour values for peduncles from each patch we wished to identify the best way to represent peduncle colour change over the time course of grape berry ripening pictorially. The results of peduncle colour change highlighted above clearly indicate that the most informative coordinates were the R and G values in RGB space, and the *a** and *b** values in the CIE*L*a*b** system, however, an important distinction between the two systems needs to be made. While the RGB space is non-linear with visual perception, the CIE*L*a*b** system can be easily related to human vision, Ford and Roberts (1998). Therefore, our interpretation and use of *L*a*b** values will better correspond to the expected visual changes observed in the field. Moreover, there are several polar parameters that more closely match the visual experience of colours.

$C = (a^{*2} + b^{*2})^{0.5}$

$h = arctan2\ (b^*, a^*)$

(In this study, $0 < h < \pi$, and when $a^* = 0$, $h = \pi/2$.)

Here hue (h) is an angle in four quadrants and in our case, the angle on a colour wheel is running clockwise from π (green) to 0 (red), and chroma (C) is defined as the strength of hue. For the expected change of peduncle colour from green towards brown, the a^* value would be expected to turn from negative to positive as one approaches harvest, and the hue (h) will move from the second quadrant ($> \pi/2$) to the first quadrant ($< \pi/2$), while the chroma (C) will decrease in magnitude. Consequently, it was decided that the best way to pictorially represent the peduncle colour evolution from veraison to harvest for both the overall vineyard and also for each individual Shiraz patch, was to superimpose the evolution of the peduncle chroma and hue values with a^* and b^* values as demonstrated in Figures 5 and 6a-h.

Figure 5 collates the change in colour of all peduncles from all 8 Shiraz patches for the entire vineyard. Overall, the plot displays many small circles with each representing a peduncle, whose positions correspond to their a^* and b^* values. The 7 larger circles derive their positioning from the median of the chroma and hue values of 192 peduncles at each sampling time point. The numbers 1 to 7 on top of each these larger circles represent the sampling time from the 1st sampling (DAF 67) to the 7th sampling (DAF 117). Each circle has different intensities which correspond to the sampling time points, i.e., the circles from the 1st sampling are in very light grey (almost white) colour while the circles with a dark grey colour represents the last samples taken at harvest. The dark green (longer) and brown (shorter) bars at each end of the two quadrants, point to the most green and most brown peduncles at veraison and harvest, respectively. The arrows pointing to each large circle display the changes of chroma and hue. Here hue is represented by the angle of the arrow and chroma is represented by the length of the arrow. As the peduncle a^* value changes from negative to positive, the hue value or the angle of the arrow moves from the second quadrant to the first quadrant, i.e. from veraison (1) to harvest (7), and the arrows also become shorter as chroma decreases during maturation. In this way, we can see a clear trend of overall peduncle colour change from green to brown from veraison to harvest, pictorially.

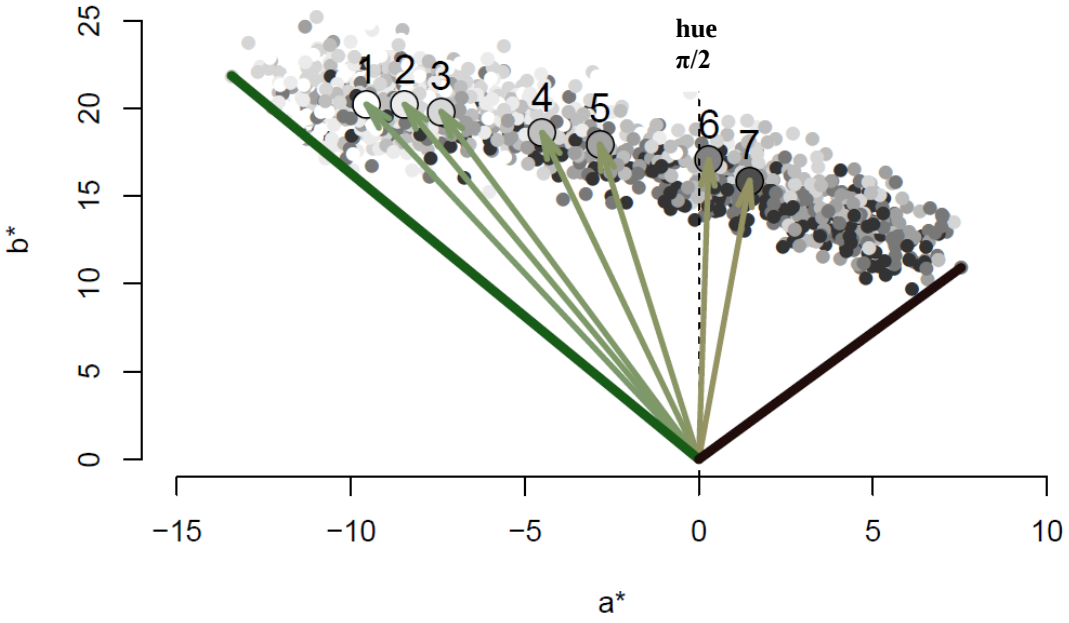

Figure 5. Median plot of overall peduncle chroma and hue for all 8 Shiraz patches in 2013.

Globally the same trends are seen for all individual patches; that is the arrow which represents chroma and hue evolution again begins long and starts from the second quadrant (somewhat yellow-green at veraison) and then becomes shorter and moves towards the first quadrant (earthy brown at harvest), Figures 6a-h. However, and importantly we also observed substantial spatial variation of peduncle colour for each individual patch in the vineyard given that only 24 peduncles were analysed for colour at each sampling time point. Taking Figure 6a (SH 15) as an example, each small circle again represents a peduncle in that patch. One finds that the darker circles (peduncles sampled closer to harvest) are mostly located in the first quadrant but there are still a few located in the second quadrant, which indicates that even at harvest, there were still some green peduncles which gave an average negative a^* value. Similarly, some light grey circles are also present in the first quadrant as well, indicating that at veraison there were a few brownish peduncles with average a^* value > 0. In some patches such as SH 2S and SH 13B, with large spatial heterogeneity, the last few samplings move their representative arrow positions in the figures back and forth to some extent. These changes in the positions of the arrows or in the medians of peduncle chroma and hue values are due to the field variation and highlights that whilst a sample size of only 24 peduncles at each sampling point for each patch was sufficient to verify the overall trend, a larger sample size would remove any such observed variation. Indeed, this was proven to be correct when the peduncles of all patches were combined at the vineyard level as displayed in Figure 5

above in which each arrow representing the medians of peduncle chroma and hue evolution moves in the same direction from the second quadrant to the first quadrant and become shorter in length as one approaches harvest, again indicating the overall change in peduncle colour changes from green to brown.

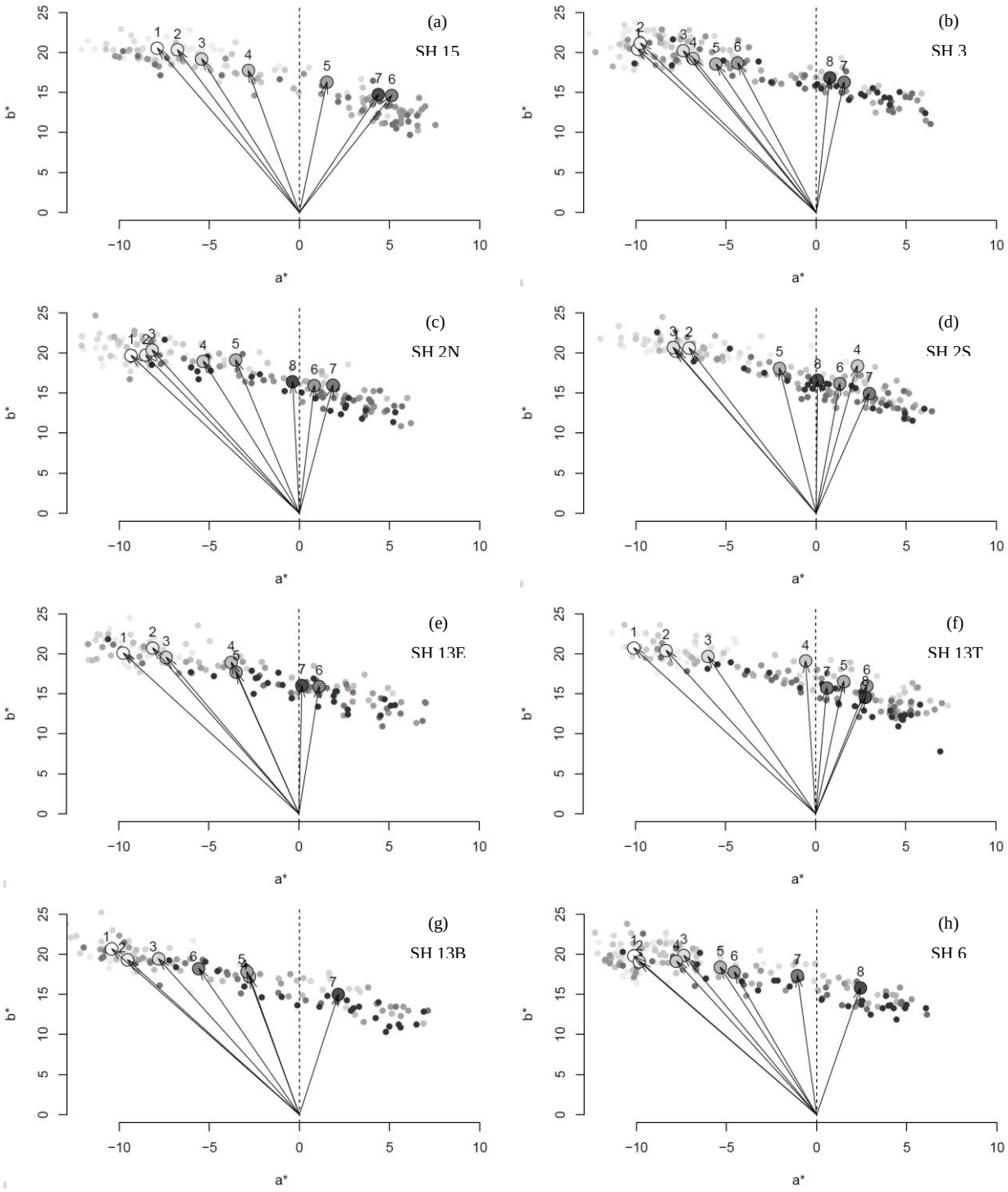

Figure 6. Median plot of peduncle chroma and hue for each individual Shiraz patches in 2013.

As highlighted above, the arrow length and position in Figure 5 and 6a-h represent the median of chroma and hue for each individual peduncle. We also generated the same plots employing the means of chroma and hue, and visually found that they were extremely similar to the median plots (Figures not shown). It was observed that the advantage of using the median plots as opposed to the means plots is that they are not influenced by any outliers, which may change the position of the arrows representing the peduncle chroma and hue evolution significantly. Furthermore, the arrows within the median plots are more representative of the colour proportion or distribution that we would actually observe in the field. Consequently, plots containing the medians of chroma and hue highlight in a simple fashion how the overall peduncle colour was changing from green at veraison to brown at harvest. However, we have identified that for more heterogeneous patches, more than 24 peduncle samples at each sampling date will be required to adequately identify the real trend of the colour evolution for that patch.

Co-development between peduncle colour and berry ripening parameters

As highlighted above, all 8 patches of Shiraz ripened in a normal fashion from veraison to harvest as indicated by their prototypical berry ripeness parameters (^{o}Brix, pH and TA) as well as their total anthocyanin and phenolics levels. At the same time we observed a clear overall peduncle colour change between veraison and harvest from green to brown. Consequently, we analysed the interrelatedness between these two trends to determine the extent to which peduncle colour evolution parallels berry ripening during the 2013 season. As can be observed in Figure 7, the increase in the change of the a^* value of peduncle colour parallels nicely with the accumulation of berry sugar (^{o}Brix) from veraison to harvest, while the decrease of peduncle b^* colour values couples with the observed decrease of TA from the 4th sampling onwards. Moreover, and as indicated in Figure 8, the increase in peduncle a^* colour values and decrease in peduncle b^* colour values were also co-developing with the development of berry pH and total anthocyanin and phenolics levels throughout the ripening season.

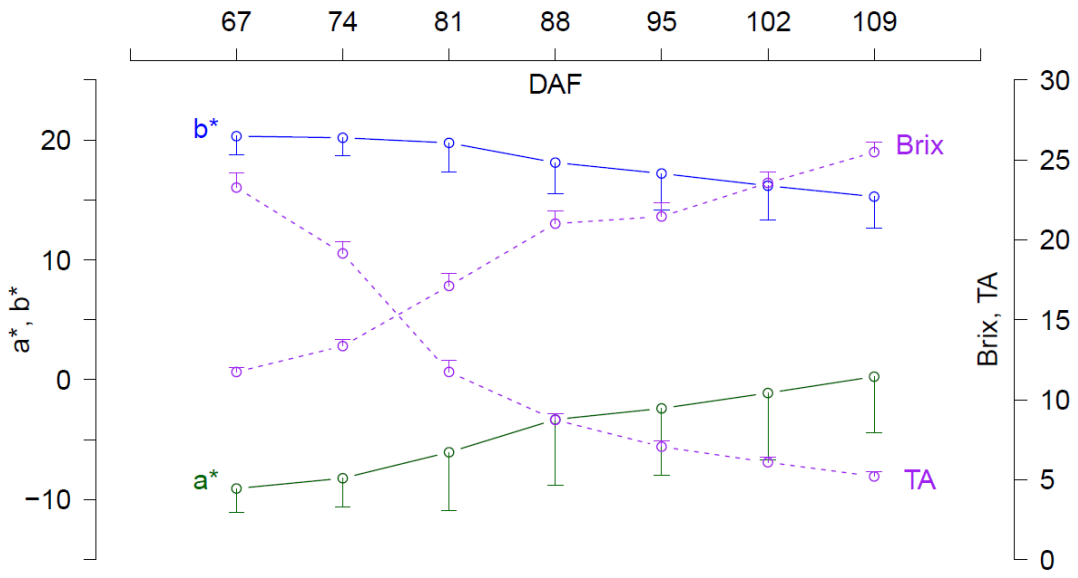

Figure 7. Co-development of peduncle *a**, *b** values and berry °Brix, TA from veraison to harvest. Each point represents the means of the variable, n = 192 for peduncle *a**, *b** values; n = 32 for berry °Brix and TA; error bars correspond to standard deviation. Titratable acidity, g/L.

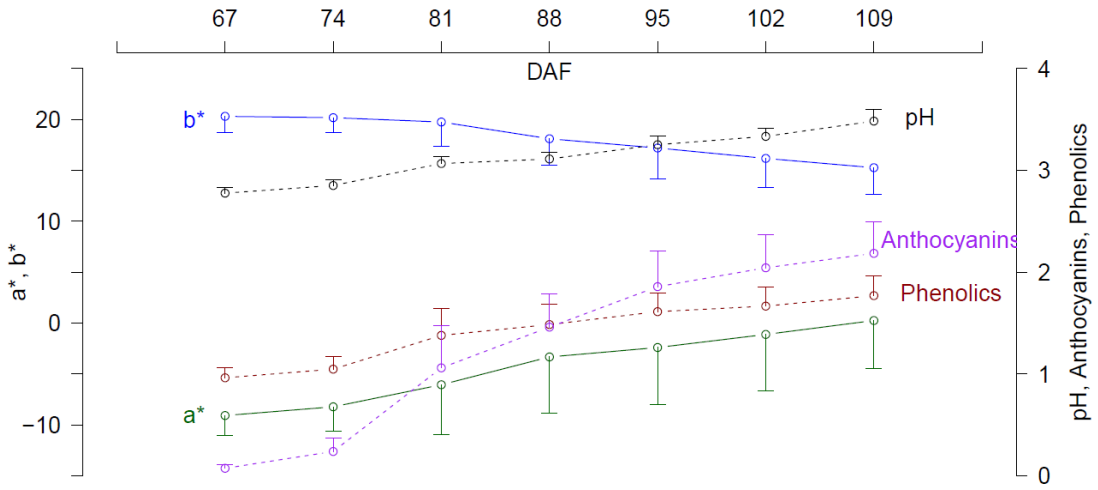

Figure 8. Co-development of peduncle *a**, *b** values and berry pH, total anthocyanins and phenolics from veraison to harvest. Each point represents the means of the variable, n = 192 for peduncle *a**, *b** values; n = 32 for berry total anthocyanins and phenolics; error bars correspond to standard deviation. Note: total anthocyanins, mg/berry; total phenolics, au/berry.

Since *a** or *b** values alone do not ideally reflect what we observe with our own eyes, the polar colour parameters chroma and hue will match better with our visual perception of peduncle colour change in the field. Therefore we also explored the relationship between the changes in peduncle chroma and hue values and the changes in berry ripeness parameters. Thus, when we combine the outcomes from the co-development plots, (Figures 7 and 8), with those found within the chroma and hue evolution plots, (Figures 5 and 6a-h), coupled with the fact that Figure 5 clearly indicates that the change in hue is mostly driven by the *a** value and the change in chroma is mostly influenced by the *b** value, it may be concluded that the berry maturation sequence from veraison to harvest is closely correlated with peduncle visual colour development from green to brown. As an example of potential predictive power, when the average peduncle *a** value increased from veraison to harvest to just surpass zero in magnitude, then the berries would have had an observed 25 °Brix, 5.2 g/L of TA, pH 3.49, 2.19 mg of total anthocyanins and 1.77 au of total phenolics per berry in the 2013 season. In the same way, inspection of the data for the 2012 season also revealed the same co-development trends between the changes in overall peduncle colour and the magnitudes of the berry ripeness parameters between veraison and harvest (Data not shown). Given that the grapes were actually defined by the viticulturists and winemakers as ready for harvest, it appears that we may well be able to utilise the average *a** value or the average hue value as a potential tool to assist in predicting berry maturity levels and even harvest date.

Conclusions

Whilst the focus of grape berry maturity or the peak of ripeness is currently squarely placed on the measurement of parameters and metabolite concentrations of the grape berries themselves, limited reports on how grape stem evolution during grape ripening may assist in harvest decisions have been reported. This study investigated the evolution of grape peduncle colour from veraison to harvest for *Vitis vinifera* L. cv Shiraz and examined if the change in peduncle colour co-develops with the berry ripening process. After developing a digital image analysis method to follow the colour evolution over time of the peduncles and rachises from veraison to harvest, it was found that overall peduncle colour changed significantly from green to brown during grape berry maturation, and the extent of this colour change was well manifested in the observed increase of the *a** value

from negative to zero and the significant decrease of b^* value within the CIE$L^*a^*b^*$ colour system. This change in colour evolution was even more pronounced when the colour polar parameters, chroma (C) and hue (h) which were derived from the a^* and b^* values were considered. This latter aspect allowed us to conveniently pictorially represent the overall colour change of the peduncles during grape berry ripening both at the individual patch and vineyard levels. Whilst the harvest date for each patch of Shiraz was determined by the viticulturists and winemakers based on the berries ^{o}Brix, pH, TA results and their tasting records, we found a remarkably good correlation between the evolution of the traditional grape berry ripeness parameters, namely berry sugar content (^{o}Brix), acidity (pH, TA) and total anthocyanin and phenolics levels and the change in average colour coordinates of the peduncles, namely the a^* and b^* values from the CIE$L^*a^*b^*$ colour system or the change in the median of the polar colour parameters, namely hue and chroma. Such observations are yet to be reported and provide for the opportunity to develop a new simple platform to assist in predicting grape berry ripeness, thus assisting in harvest decisions.

The findings within this study pave the way for the development of automatic detection systems for monitoring grape peduncle colour change to assist harvest timing decisions. These could take the form of an iPhone photo app or automatic robotic imaging system that can capture peduncle images non-destructively in the field and return results of berry ripeness evaluation to the viticulturists/winemakers based on the progress of average peduncle colour change during the ripening period. With current technological advances, such non-destructive devices are envisaged to be relatively simple to design and produce. Furthermore, there is also the opportunity that one could develop a visual grading system (browning index) for viticulturists, by simply observing the proportion of predominantly brown peduncles compared to more green peduncles visually, providing that a reasonably large representative sample of peduncles is utilised. Specifically, peduncles should be mostly green at veraison, but when the majority of the sampled peduncles are predominantly brown (more than 50% brown area for each peduncle), the average a^* value of the peduncles will be above zero, and the hue will reach an angle of $\pi/2$ indicating that this is the point when the grapes are ready for harvest. In this study, 24 peduncles were collected per patch per sampling date and it was found that this sample size is adequate to accurately portray the change in peduncle colour evolution at the vineyard level, however, if one is interested in single individual patches that are very heterogeneous, then a larger

set of peduncles would be needed for colour measurements in order to ensure that the potential field variation does not obscure the real trend in colour evolution. We also estimate by statistical evaluation that approximately 80 to 100 peduncles will need to be measured from an individual patch if we expect to observe over 50% predominantly brown peduncles at harvest time for that patch. Whilst we have focused on Shiraz in this study it would also be of interest to extend the study to alternative varieties and those under different environmental conditions to understand if the observed change in peduncle colour also parallels their berry ripening continuums.

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Chapter 3

Chemical Changes in Grape Stem and Their Relationship to Stem Colour throughout Berry Ripening in *Vitis vinifera* L. cv Shiraz.

Grape stems are often not used during winemaking, thus they are normally considered as a winery waste product. Although many studies have been carried out on the exploration of the potential economic value of extracting nutraceuticals from grape stems at harvest time, very limited research has focused on the changes in grape stem chemical composition during the grape maturation period.

During the 2012 and 2013 vintages, we observed that the grape peduncle and rachis changed colour asynchronously, and we also found the change in peduncle colour parallels berry ripening, thus we have in principle provided a new alternative way to assist in the prediction of berry maturity and potential harvest date. Given the dearth of information on how grape stem chemical composition changes during berry ripening, we continued with this study and further examined the link between grape stem (peduncle and rachis) colour change and their associated chemical traits evolution. In addition we explored the possible relationship between peduncle chemical evolution and the change in berry chemistry during the ripening continuum over two vintages.

After collecting the morphological information of individual grape peduncle and rachis samples through digital image analysis, all samples were freeze dried and ground into a fine powder for chemical analysis. We zeroed in on five key chemical parameters to be measured for all peduncle and rachis samples, namely the moisture content, total chlorophylls (including chlorophyll *a* and *b* levels), total carotenoids, total phenolics and their antioxidant capacity (DPPH). Considering the large amount of samples, we chose to use the fastest assays to determine the levels of each chemical component. The chlorophylls and carotenoids assays were measured by conventional spectral analysis and measured immediately after extraction due to their instability. In addition, two high-throughput assays were developed in this study to measure the total phenolics and antioxidant capacity (DPPH) for the peduncle and rachis samples, exploiting a liquid handling robot and plate reader for spectral analysis on 96 well plates. The methods for

these assays were based on the methods reported in the previous literature but were optimised further for this study.

With all this chemical data on grape peduncles and rachises from veraison to harvest over two vintages in hand, we analysed the data and found that there are significant differences in the moisture content, total chlorophyll (including chlorophyll *a* and *b* levels), total carotenoid, total phenolics and the antioxidant capacity (DPPH) levels between the peduncles and rachises, with the latter case having higher levels of these chemical parameters during ripening. Furthermore, through statistical modelling of the peduncle chemical levels and colour values, we found that peduncle moisture content had an excellent linear correlation with the colour hue value and was negatively correlated with the chlorophyll and carotenoid pigment ratio (*Ca+b/Cx+c*) within the peduncles in a strong fashion. Finally, we demonstrated for the first time that peduncle moisture content co-developed with the berry ripeness parameters (^{o}Brix, pH, TA, total anthocyanins and total phenolics), which improved our understanding of the correspondence between certain peduncle chemical changes and the berry ripening process.

This study represents the first report on the chemical evolution within grape peduncles and rachises separately. Our findings on the significant differences in chemical compositional evolution between peduncles and rachises were in agreement with the colour difference we observed in our previous study (Chapter 2). More importantly, the co-development observed between grape peduncle moisture and berry ripeness levels provide us and the wine industry with an alternative way to evaluate grape maturity through monitoring peduncle moisture levels and therefore could assist in harvest decision making timeframes. Presented in the following publication is the first detailed investigation of how certain chemical traits of grape peduncles and rachises evolve during berry ripening and has been submitted to the *Journal of Agricultural and Food Chemistry*.

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Author Contributions

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Name of Principal Author (Candidate)	Yudan Fang		
Contribution to the Paper	Designed experiments, performed experimental work, analysed and interpreted data, drafted/constructed manuscript.		
Signature		Date	25/7/14

Name of Co-Author	Olena Kravchuk		
Contribution to the Paper	Assisted with statistical analysis, data interpretation and manuscript preparation.		
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Name of Co-Author	Dennis Taylor		
Contribution to the Paper	Supervised the work, aided in designing experiments, assisted with grape sampling and data/results interpretation, drafted/constructed manuscript.		
Signature		Date	25/7/14

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

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Chapter 4

Investigation of within-vineyard Variation of Peduncle Colour to Assist in Harvest Decisions in *Vitis vinifera* L. cv Shiraz.

As highlighted in the previous chapters we found that the change in grape peduncle colour from green to predominantly brown parallels with the berry ripening process, based on 24 peduncles sampled from each of the eight Shiraz patches at each sampling time point over two seasons. Although the overall colour measured by the hue values changed significantly from veraison to harvest, some variation among individual peduncles from the same patches was also noticed. Thus, we decided to investigate both the within-vineyard spatial and temporal variation of peduncle colour and confirm this observed colour change across the entire patch level by utilising tools currently employed in precision viticulture. In addition, by defining the sampling positions of each peduncle, we also had an opportunity to look at the peduncles individually, which allowed us to examine the relationship between peduncle colour and moisture content at the individual bunch level, the results of which would complement those observed in chapter three.

During the 2014 vintage, a total of 545 targeted vines were selected over five Shiraz patches at the same vineyard based on a grid sampling design. One bunch was sampled from each of the targeted vines at each sampling time point from veraison to harvest. Each peduncle was coded by the position of its target vine within the vineyard and the peduncles were scanned in order to collect colour data using the digital image analysis method we developed previously. Consequently, we were able to map the peduncle colour data with their sampling position coordinates to exhibit the variation within each patch over time. In addition, 24 coded peduncles from each patch were weighted individually before and after freeze drying to calculate their individual moisture level at each sampling time.

After collecting this large amount of peduncle colour data from the five patches, a range of maps from veraison to harvest were created for each patch. The results demonstrated that the within-vineyard variation in peduncle colour was dominated by the temporal effect. At veraison, the peduncle colour was more homogeneous with mostly green in

colour, then the colour became more variable and heterogeneous. Finally at harvest the peduncles were predominantly brown. By observing the change in proportions of brown peduncles over time, we found that the percentage of predominantly brown peduncles was around 50-60% for most patches at harvest. Thus, we now propose that when the brown peduncle proportion is over 50% then the grapes of a certain patch are basically ready for harvest. Finally, we have also verified and extended our previous findings of the linear relationship between peduncle colour hue value and moisture content at the individual bunch level. Given their strong and consistent linear relationship over all five Shiraz patches, grape peduncle colour may also be predicted from its individual moisture content at any time point, and vice visa.

This research represents the first study of within-vineyard variability of grape peduncle colour. The findings have allowed us to suggest a new non-destructive method to aid in the prediction of harvest date through the visual assessment of the proportion of brown peduncle. Presented in the following publication is the first detailed investigation of within-vineyard variation of grape peduncle colour during berry ripening and is in publication format ready for submission to the *Australian Journal of Grape and Wine Research*.

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Author Contributions

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Name of Principal Author (Candidate)	Yudan Fang		
Contribution to the Paper	Designed experiments, performed experimental work, analysed and interpreted data. Drafted/constructed manuscript.		
Signature		Date	12/9/2014

Name of Co-Author	Olena Kravchuk		
Contribution to the Paper	Assisted with experimental design, statistical analysis, data interpretation and manuscript preparation.		
Signature		Date	12/9/2014

Name of Co-Author	Dennis Taylor		
Contribution to the Paper	Supervised the work, aided in designing experiments, assisted with grape sampling and results interpretation, draft/constructed manuscript.		
Signature		Date	12/9/2014

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Investigation of within-vineyard variation of peduncle colour to assist in harvest decisions in *Vitis vinifera* L. cv Shiraz.

Yudan Fang, Olena Kravchuk, Dennis K. Taylor*

School of Agriculture, Food & Wine, The University of Adelaide, Waite campus, PMB 1, Glen Osmond, 5064, Australia.

Corresponding author. Prof Dennis Taylor, email dennis.taylor@adelaide.edu.au

Abstract

Background and Aims: Recently we reported that the changes in Shiraz grape peduncle colour and moisture levels occur in parallel with the berry ripening continuum, and thus provides for an alternative method to assist in harvest decisions. The lack of research reported on grape peduncle colour variation within the vineyard, has prompted us to investigate the spatial and temporal variations in grape peduncle colour in *Vitis vinifera* Shiraz for the first time.

Methods and Results: Large numbers of peduncles were collected from five Shiraz patches based on a regular grid sampling design from veraison to harvest, and each peduncle coded based on their sampling position. After scanning and determining the peduncle colour values (particularly CIE*L*a*b** and hue values) through image analysis, the colour data was mapped for each patch over time. The results demonstrated that spatial variation was significant among different patches within the same vineyard, whilst the within-patch variation in peduncle colour was dominated by the temporal effect. Moreover, the sample percentage of predominantly brown peduncles was close to 50-60% for most patches at harvest. Finally, we have verified the linear relationship between peduncle colour hue value and moisture content we observed in a previous study and extended the relationship to the individual bunch level.

Conclusions: Grape peduncle colour change during ripening within each patch is mostly driven by the temporal effect, while the spatial influence is less profound at the individual patch level. We also suggest that once it is assessed that generally when 50% of the peduncles have turned brown by simple visual assessment (more that 40% of peduncles in a random sample of 100) then harvest should be considered. Grape peduncle colour may also be potentially predicted from its moisture level given the strong and consistent linear relationship among all five Shiraz patches.

Significance of the Study: This is the first study of within-vineyard variability of grape peduncle colour. Our findings suggest that a new non-destructive method based on visual assessment of peduncle colour may be utilised to assess grape berry ripeness.

Keywords: peduncle colour, peduncle moisture, berry ripeness, variation/heterogeneity, Shiraz

Introduction

Apart from defining the peak of berry ripeness, another important aspect in making premium wines is to minimise berry heterogeneity within the vineyard so that premium quality grapes can be passed from the viticulturist to the winemaker. Naturally, grape berries between different bunches on the same vine do not develop synchronously. It is known that the grape maturity among bunches on a vine can vary depending on the timeline of flowering and fruitset and their position on the vine (Watson 2003). Specifically bunches more exposed to sunlight are normally more advanced in sugar and phenol development than those in shaded positions on the vine (Jackson and Lombard 1993). It has also been shown by Krstic et al. (2003) that even within relatively uniform vineyards of south eastern Australia, the vine to vine variability in grape quality can still be significant and in the order of 4-5% in °Brix to 13-18% in colour.

Generally, those within the wine industry believe that more homogeneous fruit at harvest aids in the making of better wines. This notion is supported by a study conducted within two Cabernet Sauvignon vineyards in California by Long (1987) who compared the berry ripeness parameters from a 400-berry sample collection from each of the two vineyards, and found that the vineyard that produced the best quality wine at harvest consistently had

less variability in grape maturity. Furthermore, there have been several studies on the heterogeneity of grape berry composition within a vineyard at harvest and how this affects final wine quality. For example, Kontoudakis et al. (2011) separated sampled grapes into different maturity groups according to their density and found that the grapes with higher density produced wines with higher levels of final ethanol content, pH, anthocyanins, colour intensity, total phenolics and had better balance in flavour and mouthfeel. In addition, Cortell et al. (2005) studied the impact of different vine-vigour zones on the phenolic composition of a final wine and found that wines produced from low-vigour zones had a higher proportion of skin tannins extracted into the wine, higher pigmented polymer levels and higher proanthocyanidin molecular masses which can affect the proanthocyanidin perception of the wine.

As well stated by Jackson and Lombard (1993), since the ensemble of the grape cluster always has proportions of over-ripe, sufficiently ripe, and under-ripe berries, such heterogeneity not only makes the representative sampling and the accurate assessment of berry ripeness parameters difficult, but may also decreases the overall quality of the wine grapes, even if the average ripeness appeared satisfactory. A better understanding of the within-vineyard variation in grape maturity will enable viticulturists to conduct targeted vineyard management strategies in the future and provide winemakers with optimal compositional grapes for their winemaking purposes.

During the past decade, research into precision viticulture has provided a set of elegant tools aimed at understanding the spatial variability within vineyards, and has included the use of remote sensing imagery (Lamb et al. 2004), yield monitoring systems (Bramley and Hamilton 2004) and even the employment of high resolution (EM38) soil surveys (McBratney 2005). With the aid of such technologies, vineyard variability has been assessed in terms of soil (Bramley et al. 2011), vine pruning mass (Taylor and Bates 2013), yield (Bramley and Hamilton 2004), juice composition (°Brix, pH, TA) (Bramley 2005, Trought and Bramley 2011), berry anthocyanin concentrations (Baluja et al. 2012), and berry rotundone concentration (Scarlett et al. 2014). While most of the precision viticulture research has been focused on the spatial variation at harvest, only limited research has been conducted on the change in spatial structures of grape maturity over time. An excellent research study on both spatial and temporal variation in berry ripeness has been conducted by Trought and Bramley (2011). They created a map showing the

spatial variation within a Sauvignon Blanc block in New Zealand by collecting data on canopy plant cell density using airborne remote sensing and proximal sensing techniques, apparent electrical soil conductivity (EM38) and trunk circumference. In addition, they also produced a set of maps of berry ripeness for this block by measuring the sugar, pH and TA levels of 24 plots of berries at each sampling time point. Their results demonstrated that grape berry ripeness was not only spatially variable at each fixed sampling point, but there was also spatial variation in the rate of maturation. Thus to make the optimal harvest decisions for a vineyard, both spatial and temporal variability in grape maturity need to be taken into account. Consequently, various sections of the vineyard may need to be managed differently by the viticulturists.

However, current investigations on the within-vineyard variation in grape maturity have relied mostly on measurements of the typical berry ripeness parameters, and as one could imagine these tests are very time-consuming and are usually limited to a relatively small sample size. Due to the pressure at vintage time, without a fast way to evaluate the vineyard variation in the fruit quality/ripeness, it is often difficult to answer the question of when to harvest and whether or not to harvest different zones of the vineyard together or separately. The benefits of selective harvesting can only be maximised with the aid of fast, robust and portable methods that can estimate grape maturity so as to suggest potential harvest date.

We have recently reported that the progression of berry ripeness from veraison to harvest for Shiraz grapes parallels with the changes in colour of the peduncles (Fang. et al. 2014a). This co-development was evident when the change in berry ripeness parameters (Brix, pH, TA) was plotted against the change in peduncle colour hue value (turning from green to brown) over the maturation period. Further investigations focusing on how certain chemical traits within the peduncles themselves changed over time and how these changes may be associated with its colour change uncovered that the change in peduncle colour hue value has a strong linear correlation with the average peduncle moisture levels which were also observed to be in parallel with the berry ripening continuum (Fang. et al. 2014b). The findings of these studies suggest that in principle, one could develop a new non-destructive tool for predicting harvest date through the simple monitoring of the peduncle colour or moisture content levels over time. Furthermore, such measurements

may be used to monitor the expected within-vineyard variation of grape maturity in a simple and facile manner to allow for the adoption of differential management strategies such as selective harvesting within the vineyard.

In our previous studies of peduncle colour evolution, 24 peduncles were sampled from adjoining rows within each patch at each sampling time point during the 2012 and 2013 vintages (Fang, et al. 2014a, 2014b). Though the overall peduncle colour change was found to be significant over time between veraison and harvest, we also noticed a spatial variation existing within the vineyard, especially for the very heterogeneous patches. In order to confirm the potential application of utilising peduncle colour to predict harvest date, one needs certainty that the overall peduncle colour does turn from green to brown gradually during the berry ripening continuum within each patch. Given that there is yet to be any research studies on grape peduncle colour variation within a vineyard, we have now focused our attention on evaluating both the spatial and temporal variation of peduncle colour during ripening at both the entire vineyard level and individual bunch/vine level.

Hence, the objectives of this study were three-fold: 1) examine the spatial variation in peduncle colour within and between five Shiraz patches from the same vineyard; 2) determine the temporal trend of peduncle colour evolution at the vineyard level based on a regular grid sampling design following on from the preliminary work of Fang et al. (2014a, 2014b) and to verify the previous findings at the entire patch level; 3) investigate the relationship between peduncle colour and moisture content at the individual bunch level. It is expected that the results of this study should provide viticulturists with a better understanding of their fruit quality variation within a vineyard through monitoring peduncle colour change during ripening, and as such may aid in precision vineyard management and selective harvesting practices.

Materials and methods

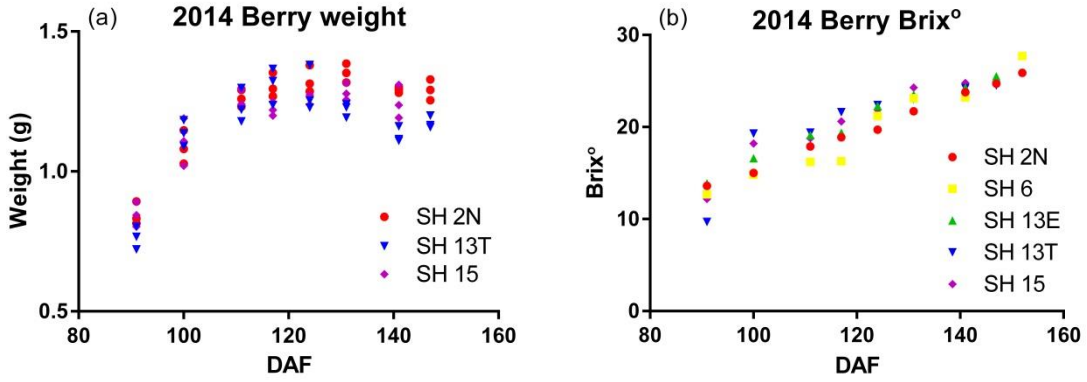
Sampling 2014

Five Shiraz patches designated as SH 2N, SH 6, SH 13E, SH 13T and SH 15 from Longview vineyard (GPS position: 138°49'54"E, 35°11'25"S) were utilised for sampling in this study. A detailed description of the site can be found elsewhere (Fang et al. 2014a). The total rainfall between Feb to Apr 2014 was about 157 mm, which was much wetter

than the previous two vintages (100 mm in 2012 and 50 mm in 2013 vintage). During the 2014 season, a minimum of 2% of the total vine numbers per patch were identified as target vines for analysis, specifically, 235, 34, 43, 88 and 145 vines were selected for SH 2N, SH 6, SH 13E, SH 13T and SH 15, respectively. A regular sampling grid was imposed over the five Shiraz patches and the sampling points were located at the nodes of the grid. The positions of each target vine were referenced onto a map on the *x* and *y* planes. The target vines were sampled at 7 or 8 time points between February 2nd (veraison) to April 4th (last harvest) for each patch and a single bunch including the entire stem and peduncle was randomly picked from each vine at each sampling date. The peduncles were cut from the bunch immediately and placed into 96 deep well plates with each well corresponding to its sampling position. At this stage the peduncle and remaining bunch samples were transported to the laboratory for further analysis under a blanket of dry ice.

Berry chemical analysis

Berries were randomly stripped off the bunches from each of the five patches for juice analysis. In addition, for patches SH 2N, SH 13T and SH 15, three lots of 60 berries were randomly selected from the berry sampling pool and weighted for determination of the mean berry weight. The remaining berry samples for each patch on each sampling day were crushed into juice and centrifuged at 3500 rpm (2451 x *g*), ambient temperature for 6 minutes. Juice total soluble solids (°Brix) were measured with a refractometer.



Figures 1(a-b). Progressive monitoring of berry weight and Brix ° change for the Shiraz patches during 2014 vintage. DAF: days after flowering; At DAF 141, SH 15 was sampled before harvest the next day; At DAF 147, SH 2N (first half), SH 13E, SH 13T were sampled before harvest the next day; At DAF 152, SH 6 and SH 2N (second half) were sampled before harvest the next day.

Peduncle image acquisition

Each coded peduncle was wiped and placed on the 17'' x 11'' screen of a Canon C7270 photocopier in sets of 24 to 32 samples. The scanning resolution was 600 x 600 dpi and the images were stored as A3 Joint Photographic Experts Group (JPEG) files. These scanned images were then analysed semi-automatically using a customised code developed using Matlab® together with its Image Analysis Toolbox® (Mathworks Inc., Natick, MA, USA). The code recognises all peduncles in each scanned image, and generates regions of interest (ROI) corresponding to the scanned sides of each peduncle. After calibration, the code extracts the morphological size parameters: area (cm^2), perimeter (cm), length (cm), and diameter (cm) as well as the colour values in both RGB (red, blue and green) and the CIE*L*a*b** colour systems as previously described (Fang et al. 2014a, 2014b). The code for image analysis is available upon request.

Peduncle moisture analysis

After scanning all the peduncles, 24 peduncles from 24 target vines located in the middle of each patch were then measured for their individual fresh weight on each sampling day, and then dried completely using a freeze drier (FreeZone 6, Labconco, USA) after storing the samples at -20 ℃ overnight. The 24 freeze dried peduncles for each patch were weighed again to calculate their individual moisture levels.

Statistical analysis

Statistical analysis was conducted with GenStat 16.1 software (GenStat for Windows 16th edition, VSN International, Hemel Hempstead, UK). Predicted means and standard errors of the colour (a^* and hue) of peduncles from veraison to harvest were derived using the repeated measures analysis over the target vines for each patch separately. The spatial (x,y) coordinates were also incorporated into the model. Since the degree of variation between individual vines was found to be comparable to the variation among different rows according to our previous study (Fang et al., 2014a), it can be assumed that the systematic grid sampling in this study is equivalent to a simple random sampling when estimating the variation within-vineyard from the variation in the samples taken. The proportion of predominantly brown peduncles was estimated for each patch at each sampling point and was plotted using Prism (GraphPad Software v6, USA). The relationship between moisture and hue of individual peduncles was modelled with a linear regression in

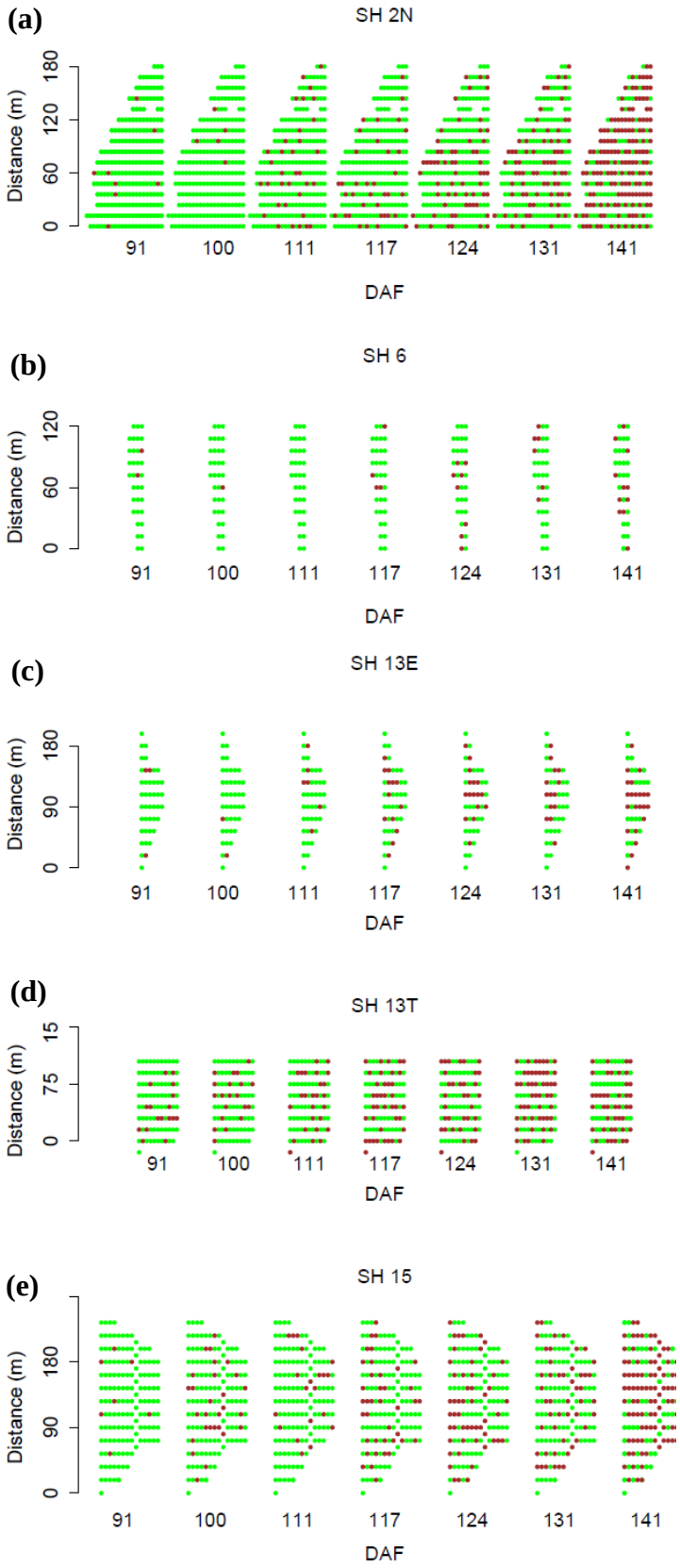
GenStat and plotted using R computational package (R Core Team 2013, R Foundation for Statistical Computing, Vienna, Austria).

Results and discussion

Within-vineyard variation of grape peduncle colour

We previously found that overall grape peduncle colour turned from green to predominantly brown over the ripening period, and this visual colour change was reflected in the change of colour a^* and hue values (Fang et al. 2014a). Moreover, for the vineyard under investigation, when the peduncle colour a^* value turned from negative to zero (half of peduncles becomes brown) or slightly positive, and the hue value turned from over $\pi/2$ (1.57) to nearly or less than $\pi/2$, the grapes were ready to be harvested as determined by the viticulturists and winemakers. Therefore in this study, we chose to use $a^* = 0$ and hue $= \pi/2$ as the border to separate the predominantly green peduncles from the predominantly brown peduncles. By collecting individual peduncle colour data at each sampling position at each sampling time point, a range of schematic maps showing the distribution of the predominantly green peduncles and those that are predominantly brown in colour for each patch were created and analysed for their temporal changes from veraison to harvest.

The variation in peduncle colour within each Shiraz patch was represented by the small circles in two colours to separate the predominantly brown peduncles from the remaining samples, Figure 2. Each peduncle sample that has a colour value of a^* over zero is represented as a red circle, and all those not considered brown as a green circle. The position of each circle represents the location of each target vine, thus the distribution of green and red circles within the maps represents the distribution of the predominantly green peduncles and the predominantly brown peduncles in the field. This allows one to visualise the spatial and temporal variation in peduncle colour from veraison to harvest.



Figures 2(a-e). Within-vineyard variation of peduncle colour for the five Shiraz patches during ripening.

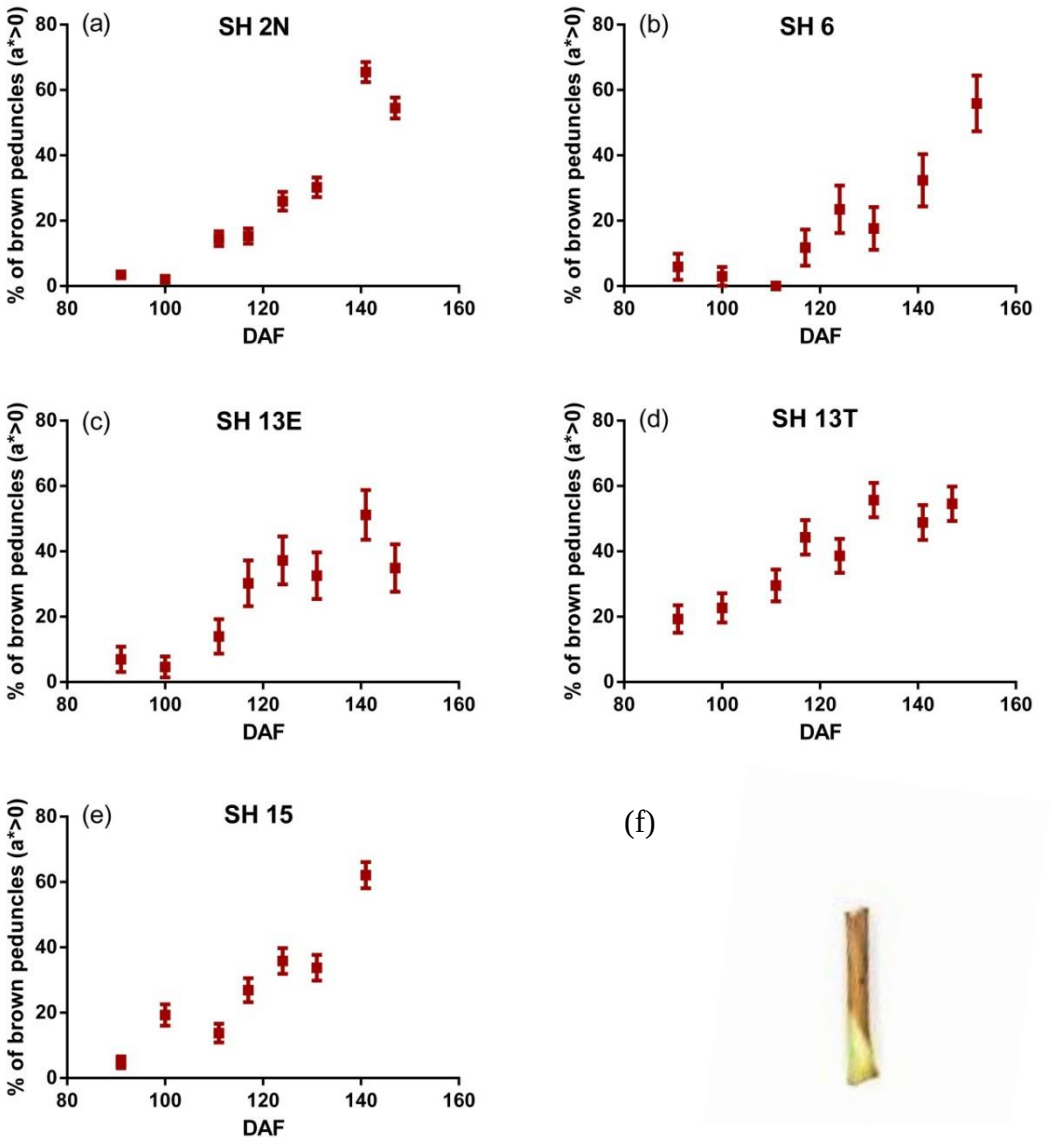
Inspection of Figures 2a-e indicates that it is the temporal effect that dominated the peduncle colour change within each Shiraz patch over the course of ripening. At the individual patch level, through Repeated Measures analysis, the temporal effect was not only found to count for about 50% of variation in peduncle colour and was highly significant ($p<0.001$), but also showed significant differences between patches. On the other hand, the spatial effect within each individual patch explained less than 10% of variation in peduncle colour and was only marginally significant (p value ca. 0.05). Hence not surprisingly, no obvious spatial clustering of peduncle colour within each patch was identified. At the individual vine level, due to the natural heterogeneity of the vineyard and our sampling strategy, peduncle colour from the same position within the vineyard at different sampling times can also vary between green and brown and even reverse from brown to green. Moreover, since our sampling for each patch was proportional to the patch size (2% of vines from each patch), the heterogeneity in peduncle colour was more noticeable in the small patches (SH 6 & SH 13E), while the larger patches (SH 2N, SH 13T & SH 15) presented a more stable progression of brown peduncle distribution.

Change in proportions of brown peduncle within each patch

To better demonstrate the temporal changes in the overall peduncle colour within each patch, the percentage of predominantly brown peduncles ($a^* > 0$) or the ratio of red circles in the maps above was also plotted for each patch from veraison to harvest, Figures 3a-e. The percentage of brown peduncles among most patches began at levels of less than 10% except for SH 13T which began at a level approximately 20%. These levels continued to climb during ripening until 50-60% of the sampled peduncles were predominantly brown. During the 2014 vintage, the actual harvest date for each patch started when around 50% of the sampled peduncles became predominantly brown in that patch, Figures 3a-e. Furthermore, during the previous two vintages, when the harvest decisions were made, the estimated proportion of predominantly brown peduncles was also found to be around 50% for each Shiraz patch, although there could be some variation in the proportion of a random sample ($50\% \pm 2*50\%/\sqrt{n}$, data not shown). Thus, it may be suggested at least for this vineyard that, when it is estimated that 50% of all peduncles in a Shiraz patch are predominantly brown, then this patch may be considered effectively ready for harvest. A simple sample size analysis concluded that in order to estimate the expected 50% of brown peduncles across an entire patch with a sampling error of less than 5%, a sample of at least 100 peduncles would be needed. When the

sampling size is less than 100 from a patch, the standard error of the brown peduncle proportion is much larger than the proportion for 100 samples, making the prediction less reliable. However, when more than 100 representative peduncles are sampled from a patch, the sampling standard error is not much less than the proportion in 100 samples. Hence we can suggest that in a Shiraz patch, when over 40% of peduncles are observed as brown from a random sample of 100 peduncles, one can accept the prediction that the true proportion of brown peduncles is 50% and start preparing for harvest. For example, if we advise to prepare for harvest when the proportion of brown peduncles is larger than 40% in the SH 15 patch (145 samples), then DAF 141 should be close to the predicted harvest day with 62% brown peduncles being sampled. This observation was in agreement with the actual harvest dates of SH 15 at DAF 142 and 143. Similarly, for the other patches of large sampling size, when the observed percentage of brown peduncles was found to be over 40%, the predicted actual harvest dates coincided with those determined by the viticulturists and winemakers.

In addition, when Figures 3a-e were coupled with Figures 1a-b, we could easily find that the increasing trend of the brown peduncle proportion among the Shiraz patches was very similar to the general change happened in berry weight and Brix °. This observation was in accordance with the co-development relationship we found in Fang et al. (2014a) between Shiraz peduncle colour and berry ripeness parameters, and once again proved that grape peduncle colour evolution parallels with berry ripening. Such findings provided an opportunity that with a sampling size of over 100, one could simply count the numbers of predominantly brown peduncles through visual assessment, and calculate the proportion of the brown peduncles among the observed samples to predict if this patch of fruit is ready for harvest, thus providing a simple method to predict harvest date in a non-destructive manner.

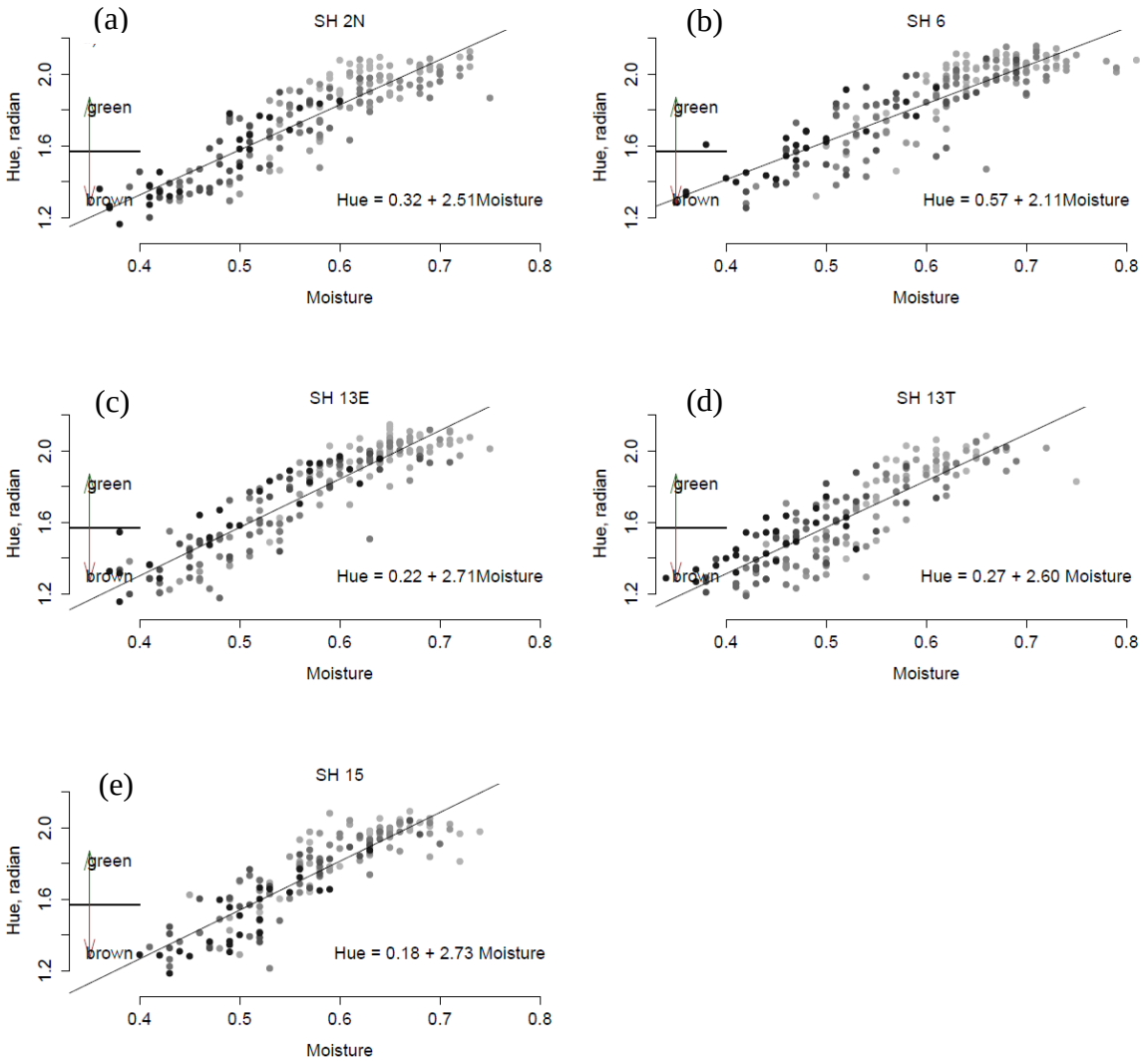


Figures 3(a-e). The change in the percentage of predominantly brown peduncles ($a^* > 0$) during ripening; **3(f)**, Example of a predominantly brown peduncle from SH 15, DAF 147 (harvest date), sampled from target vine No. 50, $a^* = 0.0629$; hue = 1.567.

Correlation between peduncle moisture and colour

Apart from monitoring the change in peduncle colour for all five patches, we also examined 24 peduncle samples individually from each patch for their moisture content. Figures 4a-e display the determined hue values versus the moisture levels within each of the 24 peduncle samples for each patch. Each circle represents an individual peduncle and the colour intensity of the circles progressively represents sampling time point, i.e. the circles in light grey indicate peduncles sampled at veraison, while the dark black circles represent peduncles sampled at harvest. By the nature of the data, the green peduncles of

higher moisture level were located at the upper end while the ash brown and dryer peduncles were at the lower end, and the overall data distribution exhibited an S-shape behaviour with the upper end (bright green (hue = 2.0 – 2.2) and high moisture peduncles) more visible. For practical purposes, the trend can be simply fitted into a straight-line regression model (R^2 > 75% or 85% if we eliminate the veraison measures). There was no significant difference in the regression models of each individual sampling and hence the overall model for all samplings was accepted as the best model.



Figures 4(a-e). Correlation between individual peduncle moisture and hue colour.

Figures 4a-e show a strong regression between the colour hue value and moisture level of individual peduncles among the five different Shiraz patches during the 2014 vintage. Though the individual peduncle colour can vary among different sampling time points, the relationship between peduncle colour and moisture remained strong regardless of

sampling time. For example, a few brown peduncles (hue value less than π/2) were observed at veraison, however these peduncle samples also contained low moisture content at the same time. This indicates that the observed colour change and the observed decrease in moisture content occur at the same time, and is presumably a result of the death of the green cortex which causes the lignification of grape stems (Iland et al. 2011, Fang, 2014b).

Furthermore, from analysis of the equations shown in Figures 4a-e, we found that the decrease in the overall peduncle moisture content from around 80% to 30% is accompanied by a consistent change in peduncle colour hue value from veraison to harvest, and that the rate of change in hue is around 0.025 radian per % moisture across all the five Shiraz patches. In addition, the peduncle colour hue values consistently change from over π/2 (1.57) to less than π/2 at the moisture level of 50% for all the five Shiraz patches. i.e. peduncles tend to turn predominantly brown at 50% moisture. Whereas we reported previously that there is a linear correlation between peduncle colour average hue value and the average moisture content (Fang et al. 2014b), which was calculated from bulk samples of peduncles at the vineyard level over two seasons, the results here have verified and extended this important relationship at the individual bunch level.

Conclusions

Grape peduncles were collected from five Shiraz patches in a orthogonal regular grid sampling design from veraison to harvest during the 2014 vintage, and each peduncle was coded based on their sampling position at each sampling time point. The spatial colour information collected for the peduncles demonstrates that the differences in peduncle colour were significant between patches within the same vineyard, but at the individual patch level, the peduncle colour variation was dominated by the temporal effect. At veraison, the peduncle colour was more homogeneous with most displaying green colour. As time progressed throughout the ripening continuum, the colour distribution became more variable, and displayed less heterogeneity with a predominance of brown peduncles closer to harvest. By observing the change in proportions of brown peduncles over time, we found that the sample proportion of predominantly brown peduncles was around 50-60% for most patches at harvest (when over 100 peduncle were sampled). Thus, we have proposed that a level of around 50% brown peduncle sample proportion may be used as a

visual signal indicating that the grapes are ready for harvest. Finally, we have also re-confirmed and extended the linear relationship between peduncle colour hue value and moisture content at the individual bunch level. Given the strong and consistent relationship among all five Shiraz patches between these attributes, it may be suggested that grape peduncle colour is potentially predictable from its moisture content at any time point. This is the first study of within-vineyard variability of grape peduncle colour and provides an opportunity for the development of new non-destructive methods to predict harvest date through the visual assessment of peduncle colour. In addition, more in-depth spatial analysis of the peduncle colour data using Vesper & ArcGIS software indicated no obvious spatial clustering within each patch. However, we suggest that some adjustment in the sampling strategy to include spatial covariates such as slope, elevation, soil type of the vineyard should aid in the understanding of the spatial variation of peduncle colour within the vineyard whilst a study on the spatial variation of peduncle moisture content can also be recommended.

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Chapter 5
Thesis Conclusions and Future Directions.

This thesis describes an investigation of how the various sections of the grape stem (peduncle and rachis) in *Vitis vinifera* L. cv Shiraz evolve in terms of their colour and chemical attributes during the ripening period and explores possible links to the prototypical berry ripeness parameters (^{o}Brix, pH, TA, etc) over three seasons. As pointed out herein, the focus of grape berry ripening research over the decades has been squarely placed on the measurement of parameters and metabolite concentrations within the berries themselves. Though there has been some anecdotal evidence suggesting that grape stems, particularly the peduncle section, goes through a colour change from veraison to harvest and which possibly also parallels with the berry ripening continuum, no extensive research has been done on how the grape stem (including the peduncle and rachis) changes colour during ripening and how this observed stem colour evolution may assist in harvest decision making in terms of grape berry quality. To this end, chapter 1 of this thesis comprised a detailed summary of our current understanding on how both grape berries and their associated stems evolve over the ripening period and set the scene for this exciting research journey.

The first major study conducted herein reports on how the change in morphology (size and colour) of both the grape peduncles and rachises evolve in eight distinct patches of Shiraz from the same vineyard over two seasons. In particular we were interested in quantifying the colour change of the peduncles and rachises from veraison to harvest and how this information could be used to aid in the prediction of berry quality or harvest date. A semi-automatic method was therefore developed to quantify the peduncle morphological details exploiting digital image analysis. The berries associated with the peduncles from the same bunches were also analysed for their prototypical berry ripeness parameters, namely the sugar content (^{o}Brix), acidity (pH and TA), total anthocyanins and total phenolics. Pleasingly, the image analysis showed that the overall peduncle colour evolution from green to brown was well reflected in the change of a^* and b^* values in the CIE$L^*a^*b^*$ colour system coupled with their polar parameters hue and chroma. Importantly, the change in the colour coordinates of the peduncles was found to be in

parallel with the change in berry ripeness parameters thus providing a new means to assist in harvest decisions.

Given the dearth of information on how grape stem chemical composition changes during berry ripening, we continued with this exciting study and further examined the link between grape stem (peduncle and rachis) colour change and their associated chemical traits evolution. In particular we were interested in finding out why the peduncles changed colour and why such colour changes were found to be linked to berry ripeness. Consequently, we zeroed in on five key chemical parameters to be measured for all peduncle and rachis samples; namely the moisture content, total chlorophylls (including chlorophyll *a* and *b* levels), total carotenoids, total phenolics and their antioxidant capacity (DPPH) from veraison to harvest over two seasons. During ripening, it was found that the peduncles experienced a significant decrease in moisture and pigment (chlorophylls and carotenoids) levels while the total phenolics and antioxidant capacity (DPPH) levels did not change significantly. The levels of these chemical traits were always higher in the rachises than the peduncles; an observation yet to be reported. Moreover, it was found that the average peduncle moisture content was correlated with the peduncle colour hue value in a strong linear fashion and was negatively associated with the pigments ratio (total chlorophylls / total carotenoids). Our results also showed that peduncle moisture content also co-developed with the prototypical berry ripeness parameters during ripening, an observation which excitingly provides for a new hitherto unknown approach for predicting berry ripeness and harvest date via peduncle moisture detection.

The final study within this body of research work evaluated the spatial and temporal variation of peduncle colour in five Shiraz patches from the same vineyard during the 2013/14 season. Peduncles were collected from the same positions based on a grid sampling design for each patch of Shiraz. The within-patch variation among individual peduncle colour was found to be mostly driven by the berry maturation stages. It was found that at veraison, the peduncle colour was more homogeneous and displayed a green state; then the peduncle colour became more variable during ripening with less heterogeneous, whilst at harvest the peduncles were predominantly brown. We also observed that actual fruit harvesting within these vineyard patches began when over 40% of the sampled peduncles appeared visually predominantly brown, providing the sample

size was no less than 100 from that particular patch. Thus, a simple visual assessment of the proportion of brown peduncles could be used as an easy and quick way to predict time for harvest. Moreover, the linear relationship between peduncle colour hue value and moisture content was verified and extended to the individual bunch level during the 2014 vintage. Again these observations strengthen the potential use of monitoring peduncle colour evolution to predict harvest date, and pave the way for the development of non-destructive methods of measuring peduncle colour in the field.

As a result of our research findings on the evolution of peduncle colour over time and how this pertains to berry maturity a number of future research studies can be suggested:

1) Extend the studies to other grape varieties to ascertain if the same strong correlation between peduncle colour evolution and berry ripeness remains true. Furthermore, one should also determine if the moisture content of the peduncles of other varieties is also in parallel with peduncle colour evolution as found here for Shiraz. In addition to these studies, one could also expand the research to include variation studies in vastly different field locations such as cooler and warmer climates.

2) A selective harvesting study should also be carried out at harvest to evaluate that, if grape bunches with brown peduncles were separated from those which contain a green peduncle, and wine was made from these two batches, would the wines be of higher quality from the brown peduncle batch given there more advanced stage of grape maturity and quality? Such studies would improve the understanding of peduncle colour influence on wine quality, especially when coupled with sensory evaluations on the two groups of grape berries and wines produced.

3) Apart from displaying a stable strong linear relationship with moisture content, peduncle colour was also found to be correlated, with the pigments ratio (total chlorophylls/total carotenoids) during ripening, but in a looser manner. This indicates that there are other chemical traits within the peduncles that drive the observed colour change of the peduncles together with a contribution from the chlorophylls and caretenoids. Given that our current studies have only measured some of the chemistry attributes that may be behind the observed peduncle colour change, it would be pertinent to continue

these studies and evaluate additional chemical attributes such as certain plant hormones within the peduncles to further flesh out exactly what causes this colour change and what is the trigger for it to occur.

4) Within-vineyard variation of peduncle colour was observed during all the three vintages, even though the temporal trend dominates the overall colour change for each patch, the colour among individual peduncles still varies considerably. For example, it is always the case that there are a few brown peduncles at veraison and a few green peduncles at harvest. In addition, significant spatial differences were found between different patches in the same vineyard rather than between different rows or columns within each patch. Thus, further investigations into the influence of environmental conditions on peduncle colour are also needed, such as the influence of vineyard canopy and soil moisture level. Such research can be conducted in a simple fashion utilising the known tools of precision viticulture.

5) Our initial findings have suggested that to make a reliable estimate of harvest date based on peduncle colour, approximately 100 peduncles would need to be analysed from an individual patch. Even though we proposed to use the method of observing brown peduncle proportions through simple visual assessment, more objective evaluations of the peduncle colour evolution would be favoured by viticulturists. Hence, the development of simple non-destructive tools that can quantify peduncle colour change accurately would be favoured for application in the field. One such possibility for which we have conducted some initial pilot studies, involves the development of a smart phone app coupled with its camera. The idea being that a small cabin can be attached to the camera on the phone to exclude the influence of natural sunlight and allow consistent use of the light source within the phone to take photo images of the peduncles in real time, followed by image analysis and data interpretation. Thus, the viticulturists would only need to simply take some pictures of peduncles across the vineyard with the app, and would be provided with feedback immediately on the projected harvest date and the prototypical berry ripeness parameters. Such a simple process would avoid the need for destructive picking of the grapes and subsequent wet chemistry analyses in the laboratory that are clearly the norm of today's grape berry ripeness measurements.

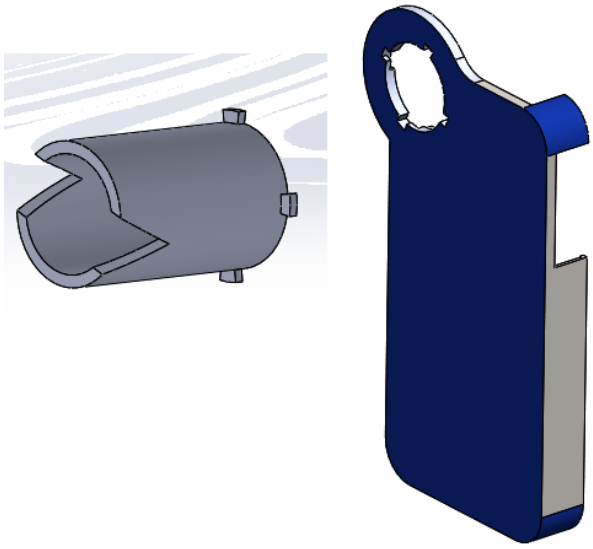

Figure 5.1. An example of the 3D prototype design of a small camera cabin for the iPhone to measure peduncle colour.