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OPTIMISING GRAPE QUALITY AND VALUE TO IMPROVE VINEYARD PROFITABILITY AND SUSTAINABILITY



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Project NWG 1301:

Optimising grape quality and value to improve vineyard profitability and sustainability

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Table of Contents

Abbreviations 7

1 ABSTRACT 10

2 EXECUTIVE SUMMARY 11

Acknowledgements 13

3 BACKGROUND 14

4 PROJECT AIMS AND PERFORMANCE TARGETS 16

5 METHODS 22

5.1 Vineyard Selection and Characterisation—Red Cultivars 22

5.1.1 Vineyard selection 22

5.1.2 Vineyard Characterisation—Red Cultivars 26

5.1.3 Sugar Accumulation Method to Assess Potential Wine Style 29

5.1.4 Sampling, Harvest and Winemaking 31

6 RESULTS AND DISCUSSION 39

6.1 Vineyard Selection Characterisation 39

6.1.1 Climatic data. 41

6.1.1.1 Griffith 42

6.1.1.2 Orange 44

6.1.1.3 South Australia 46

6.1.1.4 Mesoclimatic temperatures 48

6.1.2 Vine water availability and vine water status 51

6.2 Sugar accumulation method to predict harvest dates for red cultivars 55

6.2.1 Outputs: Sugar Accumulation as a Predictor of Harvest Date 60

6.3 Validation of a model of grape aromatic maturity by sensory analysis 60

6.3.1 Shiraz 60

6.4 Wine Sensory Results 2016 66

6.4.1 Shiraz 2016 66

6.4.2 Cabernet Sauvignon 68
6.4.2.1 Cabernet Sauvignon 2016 71
6.4.3 Outputs: Effect of Harvest Date and Yield on Wine Sensory Profile 73
6.5 Grape and wine compositional analysis 74
6.5.1 Multiblock Chemometric Data Analysis 74
6.5.1.1 Chemometric background 75
6.5.1.2 Discussion of Shiraz Models 83
6.5.1.3 Cabernet Sauvignon Models 84
6.5.1.4 Discussion of Cabernet Sauvignon Models 85
6.5.1.5 Global comparisons 89
6.5.1.6 Hierarchical Cluster Analysis (HCA) and Global Summary of Variable Loadings for *Harvest Stage* 90
6.5.1.7 Summary of Variable Loadings for *Region* and *Vineyard Locations* 91
6.6 Grape Composition 100
6.6.1 Basic Grape and Juice Parameters 100
6.6.1.1 Amino acids 102
6.6.1.2 102
6.6.2 Grape volatiles and precursors 105
6.6.2.1 Grape berry terpenes 105
6.6.2.2 Carotenoids and norisoprenoids 108
6.6.2.3 Lipids and green leaf volatiles 108
6.6.2.4 Grape berry phenolics 113
6.7 Effect of yield on Shiraz grape chemical composition in Griffith in the 2015 vintage 114
6.8 Outputs: Effect of Harvest Date and Yield on Shiraz Grape and Juice Composition .. 116
6.9 Wine composition 118
6.9.1 Organic acids and alcohols 118
6.9.2 Wine colour parameters 121
6.9.3 Wine aromatic compounds: grape/wine/sensory nexus 123

6.9.4 Untargeted analysis 124
6.9.5 Targeted analysis 128
6.9.6 Modification of wine volatile composition specific to variety and vintage 130
6.9.6.1 Varietal aroma 131
6.9.6.2 C6-compounds 132
6.9.6.3 Esters 133
6.9.6.4 Volatile Sulfur Compounds (VSCs) 136
6.9.6.5 Lactone 136
6.9.7 Comparison of Shiraz wine volatile composition between Fresh fruit, Mature fruit and Intermediate stages (IF) 139
6.9.7.1 Influence of yield on Shiraz wine volatile composition 142
6.9.8 Outputs: Effect of Harvest Date and Yield on Wine Composition 144
6.10 2016 Vintage 146
6.10.1 Sugar loading models 147
6.10.2 Grape Composition 149
6.10.2.1 Amino acids 150
6.10.2.2 Grape berry volatiles and its precursors 150
6.10.2.3 Grape berry phenolics. 152
6.10.2.4 Wine volatiles. 153
6.11 Influence of berry shrivelling on Shiraz wine chemical and sensory composition 157
6.11.1 Influence of late season berry dehydration on Shiraz wine chemical composition 158
6.12 South Australia—Can Additional Irrigation Delay Berry Water Loss and Influence Lower Alcohol Wines? 161
6.12.1 Outputs 174
6.13 Harvest Dates for White Cultivars 175
6.13.1 Outcomes 186
7 OUTCOMES AND CONCLUSIONS 187

8 RECOMMENDATIONS 189

9 Appendix 1: Communications 191

Publications in international peer review journals: 191

Book Chapters (Conference Proceedings): 191

Publications in journals dedicated to the wine industry: 192

Oral communications at conferences: 192

Reports for the industry 192

Oral communications for the industry 192

Posters: 193

Selected Background Readings 195

10 Appendix 2: Intellectual property 203

11 Appendix 3: References 204

12 Appendix 4: Staff 211

13 Appendix 5: Raw Data 212

Abbreviations

AA	Amino acids
AMOPLS	ANOVA Multiblock Orthogonal Partial Least Squares
ANOVA	Analysis of Variance
ANOVA-PCA	Analysis of Variance Principal Component Analysis
_B	Barossa Valley
BAS	Berry Aromatic Sequence
_CS	Cabernet Sauvignon
CI	Cold Night Index
DA	Descriptive Analysis
DAD	Diode Array Detector
DAP	Diammonium phosphate
Deb	De Bortoli Wines
DMS	Dimethyl sulfide
EMS	Ethyl methyl sulfide
EVI	Enhanced Vegetation Index
FAN	Free Amino Nitrogen
FF	Fresh Fruit
G1-G4	Griffith 1 to 4
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GDD	Growing Degree Days
HCA	Hierarchical Cluster Analysis
HI	Huglin Index
HS-SPME	Headspace Solid Phase Microextraction
HSL	Hue, Saturation, Luminescence
IBMP	Isobutyl methoxypyrazine

IF	Intermediate Fruit
LSD	Least Significant Difference
_McV	McLaren Vale
McW	McWilliam's Wines
MeOH	Methanol
MF	Mature Fruit
MRM	Multiple Reaction Monitoring
NDVI	Normalised Difference Vegetation Index
NOPA	Nitrogen by o-Phthaldialdehyde Assay
NSW	New South Wales
NWGIC	National Wine and Grape Industry Centre
O1/2	Orange 1 or Orange 2
PCA	Principal Component Analysis
PDMS	Potential dimethyl sulfide
PDMS-CAR-DVB	Polydimethylsiloxane Carboxen Divinylbenzene
PLS	Partial Least Squares (aka Projection to Latent Structures)
RIPPLE	Research Institute for Professional Practice, Learning and Education
RSR	Residual Structure Ratio
RSS	Relative Sums of Squares
_SH	Shiraz
SA	South Australia
SB	Sauvignon Blanc
SCD	Sulfur chemiluminescence detector
SD	Standard Deviation
SO2	Sulfur dioxide
SPE-GCMS	Solid Phase Extraction Gas Chromatography Mass Spectrometry
TA	Titratable Acidity

TSS	Total soluble solids
TVI	Transformed Vegetation Index
TWE	Treasury Wine Estates
UPLC	Ultrahigh Pressure Liquid Chromatography
YAN	Yeast assimilable nitrogen

1 ABSTRACT

The objectives of this investigation were to employ predictive models of wine style based upon grape berry sugar accumulation profiles, or berry skin colour, to create a harvest decision framework. Specific wine styles (Fresh, Intermediate and Mature) were predicted for Shiraz and Cabernet Sauvignon cultivars based upon a specified number of days following cessation of sugar accumulation in the grape berry. Compositional measures of grapes, wine and wine sensory scores could be used to consistently predict the specified wine styles from the plateau of sugar accumulation irrespective of vineyard location, altitude or vine yield. Industry partners in the Barossa Valley undertook field based trials incorporating irrigation scheduling with the sugar accumulation models for harvest decision making. For the white cultivars Chardonnay and Sauvignon Blanc, grape berry skin colour measures of hue and tint angle determined using a Dyostem™ instrument were used to optimise harvest decisions for specific wine styles.

2 EXECUTIVE SUMMARY

The ability to determine an optimum harvest time for specific wine styles is one of the main challenges of vineyard and winemaking decisions. Optimising grape quality and value through alignment of harvest decisions with specific wine styles were the practical goals of this project. Traditional measures of grape maturity based upon objective measures of gross composition (Brix, pH, titratable acidity) and berry colour, along with subjective assessment of berry sensory profiles are among the commonly considered indicators for deciding when to harvest grapes. Defining potential wine styles using objective measures of grape berry maturation and having the potential to predict specific wine styles two to four weeks in advance of harvest will provide improved decision making capability for vineyard and winery managers, improve winery logistical operations and provide a means to ensure a consistent wine style profile within the limits of viticultural and regional specific terroir.

This project was undertaken using a model of experimental sites within commercially operated vineyards located in the Riverina, Orange, Tumbarumba, McLaren Vale and Barossa Valley. The main series of experiments focused upon the cultivars Shiraz and Cabernet Sauvignon and was undertaken across three consecutive years. Grape berry maturation was monitored regularly from veraison, by determining a sugar accumulation profile per berry (mg/berry), and harvest decisions for winemaking determined from the cessation of sugar accumulation. Three wine styles were targeted (Fresh Fruit, Intermediate Fruit and Mature Fruit) with the timing for grape harvests determined by the number of days following a plateau of sugar accumulation:

	Grape berry maturation post sugar accumulation plateau (days)	
Target Wine Style	Shiraz	Cabernet Sauvignon
Fresh Fruit	12-14	20-22
Intermediate Fruit	18-20	32-34
Mature Fruit	24-26	40-44

Comprehensive metabolite profiling of grapes, wines combined with winemaking inputs and wine sensory assessments were used to model the flux of compounds of interest that may determine wine style. A consistent trend in wine sensory profiles was evident when grapes were harvested from vineyards according to the indicated maturation times, and when those

vineyards were managed to accommodate excessive heat and avoid water stress, irrespective of vineyard location or climate.

The tempo of change in the sensory profiles of wines was consistent between vintages and sites. There was no apparent link between sugar content (Baumé) and wine styles. The three wine styles could be made from fruit harvested from the same vineyard and Baumé with varying maturity times. These observations indicate that the proposed models for grape maturity are a robust and reliable means to align specific wine styles with a harvest decision based upon berry sugar accumulation.

Clear markers of berry maturation and wine styles in red wines were identified. Dimethyl sulfide is a marker of late maturity for both Shiraz and Cabernet Sauvignon and has an important contribution to the dark stewed fruit profile of wines. Cis-3-hexenol has been recognised as consistent and potent marker of the Fresh Fruit maturity stage in Shiraz. The contribution of higher alcohol acetate esters to Shiraz wine sensory profiles was consistently observed with mature grapes.

γ-nonalactone has been identified as a reliable indicator of berry shrivelling in Shiraz. Berry shrivelling, a phenomenon commonly observed in Shiraz grapes has an important and significant impact on wine sensory profiles. Grape berry shrivelling is also a significant source of harvest losses with grape yield losses of up to 25%, and is also an important contributor to rising ethanol levels in wines. Identification of reliable markers enables targeted and objective measures to assist in determining optimum harvest times.

Grape berry maturation accumulation curves were challenging to measure and to correctly identify a plateau of berry sugar accumulation in some vineyards. This was especially the case for vineyards subject to extreme heat events and in which insufficient water was available, exerting severe abiotic stress to the vines. Industry partners located in South Australia investigated the combined impact of modified irrigation treatments in association with berry monitoring, to determine an optimum window for commercial harvest decisions consistent with the preferred commercial wine style. Additional irrigation did not generally impact the berry sugar accumulation profiles or impact the berry weights, however plant responses were varied. Inconsistent berry sugar accumulation profiles were noted and severe heat and water stress in some vineyards, followed by irrigation, are thought to be responsible for the observed inconsistent berry metabolome profiles.

Berry colour evolution for the white cultivars Chardonnay and Sauvignon Blanc was determined using a Dyostem™ instrument expressing berry skin colour in hue degree angle. Regular

measurements and monitoring of grape berry skin colour evolution from green to yellow hues enabled correlation between the hue angle and commercial wine sensory characters to be explored. The potential to use berry skin colour as an additional measure to traditional grape parameters for harvest decisions in support of specific wine styles is worthy of further investigation. Severe rain and hail events in the 2014/15 growing season prevented an in-depth investigation of this promising technology for predicting wine style and wine sensory characters. The Dyostem™ instrument provided important measures of berry colour and size but feedback from vineyard managers and staff indicated the process was time consuming and onerous. Further work in this area should focus on easier to deploy image capture platforms and real time data analytics.

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The National Wine and Grape Industry Centre is a research centre at Charles Sturt University, created as an alliance with the New South Wales Department of Primary Industry and the New South Wales Wine Industry Association. Input and support from the external partners of the NWGIC is gratefully acknowledged.

3 BACKGROUND

One of the most important and difficult aspects of a viticulturist and winemaker's job is to predict harvest dates, and to produce consumer preferred wine styles. Therefore, considerable interest in the progressive development of grape derived flavour precursors during berry ripening has garnered the attention of researchers over the past several years.

The timing of grape harvest is often decided by viticulturists and winemakers using one of the following criteria:

a) determination of total soluble solids expressed as Brix as the sole criteria or in combination with basic analytical parameters related to acidity (pH, TA) and colour. This approach requires simple, routine analyses and is one of the most commonly used in the wine industry. Knowledge in interpreting analytical results to make the appropriate decision is subsequently required. The cost per analysis and per hectare must be considered;

b) assessment of berry taste. This approach is relevant but highly subjective as the decision is influenced by the taster's personal experience and training;

c) harvest using new decision making tools and taking into consideration new scientific results. This implies the ability to access, understand and assimilate the information and then implement it successfully (extension and adoption process). In addition, the cost of this new technology, which may be expensive, has to be considered.

Overall these indices do not give objective information about the grape aromatic potential or the resulting wine flavour profiles (Calderon-Orellana et al. 2014; Deloire, 2013). Even though wine aromatic feature is one of the most important components of wine quality, the wine industry does not currently use any objective method to assess grape aromatic maturity in order to help to make decisions on harvest date. It would therefore be useful for growers and winemakers to be able to objectively predict specific wine style in terms of aroma by measuring simple physiological grape parameters linked to vineyard growing conditions.

Berry development follows a double sigmoid growth pattern consisting of two distinct growth phases separated by a lag phase (Coombe, 1995). Important compounds such as phenolics, amino acids, as well as some compounds responsible for aroma, for example methoxypyrazines in cultivars including Sauvignon Blanc, Cabernet Sauvignon and Merlot (Diakou-Verdin et al. 2002; Šuklje et al. 2012), accumulate during the first growth period. Berry growth and organic acid accumulation cease during the lag phase. Grape berry ripening is thereafter characterised

by rapid and significant metabolic changes and gene regulation modulations at the onset of veraison, with softening and import of hexoses as the first events. According to Terrier et al. (2005) and Pilati et al. (2007), the most significant changes in gene expression occur during the 24 hour transition between the lag phase and veraison (i.e. individual berry softening and beginning of ripening). Pilati et al. (2007) reported concurrent modulation of the anti-oxidative enzymatic network at veraison whereas the post-veraison phase was characterised by the onset of a ripening-specialised metabolism responsible for the phenotypic traits of the ripe berry (Pilati et al. 2007a). Dal Santo et al. (2013) reported a similar effect on Corvina via a study on the plasticity of berry transcriptome using a network of commercial vineyards to assess the effect of the site (soil x climate) and vintage. The data from this study suggested that "*veraison is a critical period during which the seasonal climate has its greatest effect whereas the microenvironment and agronomic practices had only marginal impact*".

Wine style can be considerably influenced by grape composition, which is largely determined by several vineyard parameters. These include water provided by irrigation and soil moisture, abiotic factors such as light and temperature, mainly at the meso- and micro-climatic levels. A specific wine style or category demands specific ripening conditions (Bindon et al. 2013; Deloire, 2013; Suklje et al. 2014a). The concept of grape quality at harvest should be considered in terms of the required wine composition and winemaking process, resulting in a product with particular sensorial properties. Numerous important works have recently studied the relationship between grape, wine composition and aromatic profile (Capone et al. 2011; Sweetman et al. 2009). Other studies (Bindon et al. 2013; Pons et al. 2008) have emphasised marker compounds that are potentially linked to wine aromatic maturity. Despite these new and important insights, the topic is complex and the relationship between grape, wine composition and the sensory profile of a wine in relation to different stages of fruit maturity remains poorly understood.

Linkage of fruit to wine composition and wine aromatic profile is complicated, with a few exceptions such as methoxypyrazines and thiols present in high concentration in cultivars such as Sauvignon Blanc, or terpenes in Viognier or Muscat. The question therefore is how to comprehend this complexity and to more accurately predict the appropriate harvest date in relation to desired wine style for other grape cultivars.

This project based the principle of sequential harvest on a theoretical concept, developed by Prof. Alain Deloire. The concept suggested that the speed of berry sugar accumulation could be used as a physiological indicator of grape vine functioning and could be used to predict harvest dates 12-40 days in advance, based on the possible wine aromatic profile.

4 PROJECT AIMS AND PERFORMANCE TARGETS

The main practical goals of this project were to optimise grape quality, value and alignment of harvest time with wine style. The investigations were performed by establishing well characterised platform vineyards to measure grape composition and the corresponding wines across several vineyards and vintages, through a sequential harvest. The harvest dates were determined by new physiological indicators and decision making tools allowing precise prediction harvest dates according to the desired wine styles 10-40 days in advance. The project also investigated key grape and wine markers that determined the particular wine style. Calibrating new physiological indicators and decision making tools to predict harvest dates according to the berry aromatic sequence is aimed at helping to stabilise and/or increase vineyard profitability and sustainability.

Therefore the objectives were to:

- establish and characterise experimental vineyards (using existing commercial vineyards) that were used for experimentation, extension and adoption
- determine the optimal harvest time for specific wine styles based upon the sequence of specific berry compounds
- provide growers and wineries with an objective management method and/or tool for harvest decisions and improve winery intake logistics
- characterise key marker compounds specific to different grape maturity levels and corresponding wine styles
- determine relationships between grape and wine composition and link to sensory perception
- improve grape quality with precision irrigation including a reduction of plant and fruit damage due to heat waves
- assess the effect of sequential harvesting using typical winery volumes and also compare the industry produced wines with the small scale vinifications.

The practical objectives were supported by scientific investigation to:

- determine if a common pattern in grape/wine flavour plasticity related to grape ripening exists irrespective of a grape growing region
- determine if commonalities in flavour evolution exist across the vintages
- identify and highlight compounds present in grapes and wines with plasticity related to grape ripening.

The activities and the corresponding outputs that have been completed during this project are listed below in Table 1.

Table 1: Project activities and outputs.

	Output	Target Date	Activities
Year 1			
1	Characterised vineyards for NSW and recommendations for vineyard strategy. Real time climatic data to manage heat waves with irrigation. Recommendations on how to avoid berry shrivelling. Recommendations on how to improve fruit colour. Model to predict harvest dates targeting the 'fresh' and 'mature fruit' stages of the ripening period. Model to predict harvest dates targeting wine diversity for Chardonnay using the Dyostem tool.	30/06/2014	Establish and characterise Shiraz, Cabernet Sauvignon (NSW region, Griffith), Chardonnay (Orange and Tumbarumba) and Sauvignon Blanc (Orange) vineyards. Measurements: meso temperature, relative humidity, rainfall, vigour via NDVI, yield, vine water status, phenological stages, soil moisture. Collect representative grape samples for the calibration of the sugar loading method targeting 'fresh' and 'mature' fruit stages (weekly, from 8 Brix onwards). Collect samples (weekly) from berry lag phase to follow berry colour evolution (Sauvignon Blanc and Chardonnay) using the Dyostem tool. Sequentially harvest according to berry sugar and anthocyanin accumulation for Shiraz and Cabernet Sauvignon at the 'fresh' and 'mature fruit' stages. Sequentially harvest according to berry colour evolution for Chardonnay: three stages. Small scale vinifications (NWGIC) and industry scale winemaking (mainly on

			Shiraz - McWilliams) to assess wine composition and sensory profiles. Analyses: organic acids, sugars (glucose to fructose ratio), pH, yeast assimilable nitrogen (YAN), amino acids (AA).
2	Completed analysis of volatile and non-volatile matrix. Key fruit marker compounds identified (anthocyanins, carotenoids, lipids and amino acids). Sensory characterisation of Shiraz and Cabernet Sauvignon wines.	31/12/2014	Quantify in fruit: anthocyanins, polyphenols, carotenoids, terpenes, norisoprenoids, lipids and some C6-compounds. Proceed with untargeted and targeted analyses on wine (anthocyanins, polyphenols, esters, terpenes, norisoprenoids and C6-compounds). Wine sensory evaluation (difference testing and descriptive analysis).
	At least four industry workshops delivered to demonstrate models of grape berry maturity prediction and wine sensory.	31/12/2014	Hold workshops to demonstrate the developed models to industry and present climatic data from past 50 years.
	Year 2		
3	Characterised vineyards for NSW (low and high yield vineyards in Griffith) and SA. Characterised vineyards for NSW (high and low altitude in Orange). Recommendations for improved vineyard management. Preliminary models to predict harvest dates targeting the 'fresh' and 'mature fruit' stages for Shiraz and Cabernet Sauvignon in high/low yield and/or high/low altitude vineyards in Griffith and Orange. Models to predict commercial harvest dates for Chardonnay and Sauvignon Blanc from Orange vineyards.	30/6/2015	Characterise Shiraz, Cabernet Sauvignon in targeted vineyards (NSW region, Griffith) and Chardonnay (Orange) vineyards including climatic indices (Huglin, Winkler and Fresh Night Index). Characterise new vineyards in SA (Shiraz and Cabernet Sauvignon). Measurements: meso temperature, relative humidity, rainfall, vigour via NDVI, shoot number, yield, vine water status, phenological stages, soil moisture. Collect representative grape samples for the calibration of the sugar loading method targeting 'fresh' and 'mature' fruit stages (weekly, from 8 Brix onwards). Collect samples (weekly) from early veraison to follow berry colour evolution and basic measures of berry composition (Chardonnay and Sauvignon Blanc in Orange) using the Dyostem tool. Sequentially harvest according to berry volume and sugar accumulation for Shiraz

			and Cabernet Sauvignon at the 'fresh' and 'mature fruit' stages. Small scale vinifications (NWGIC) and industry scale winemaking (mainly on Shiraz - McWilliams) to assess wine composition and sensory profiles. Analyses: organic acids, sugars (glucose to fructose ratio), pH, yeast assimilable nitrogen (YAN), amino acids (AA). Predict harvest dates and compare commercial harvest dates for Chardonnay and Sauvignon Blanc from a cool/temperate climate.
4	Completed analysis of volatile and non-volatile matrix. Key fruit marker compounds identified (anthocyanins, carotenoids, lipids and amino acids). Sensory characterisation of wine composition for Shiraz and Cabernet Sauvignon wines from Griffith and Orange vineyards. Objective measures of fruit and wine composition for Shiraz and Cabernet Sauvignon from Griffith and Orange vineyards.	31/12/2015	Quantify in fruit: anthocyanins, polyphenols, carotenoids, terpenes, norisoprenoids, lipids and some C6-compounds. Proceed with untargeted and targeted analysis on wine (anthocyanins, polyphenols, esters, terpenes, norisoprenoids and C6-compounds). Wine sensory evaluation (difference testing and descriptive analysis). Perform multivariate statistical methods to determine the link between fruit and wine composition.
	Industry workshops held. Scientific seminars delivered.	31/12/2015	Hold workshops to demonstrate the developed models to industry. Communicate project progress, through the delivery of seminars.
Year 3			
5	Vineyard characterisation for NSW and SA. Climatic data base (meso climate) for vineyards located in warm/hot and temperate/cool climates covering the period of investigation.	30/6/2016	Characterisation of Shiraz, Cabernet Sauvignon (NSW and SA) and Chardonnay (Orange) vineyards. Measurements: meso temperature, relative humidity, rainfall, yield, vine water status, phenological stages, soil moisture. Collect representative grape samples for the calibration of the sugar loading

	Recommendations for improved vineyard strategy (irrigation, to control fruit volume). Completed objective measures of grape composition for Shiraz grown in targeted vineyards. Improved models to predict harvest dates targeting the 'fresh' and 'mature fruit' stages of the ripening period for Shiraz and Cabernet Sauvignon grown in targeted vineyards.		method targeting 'fresh' and 'mature' fruit stages (weekly, from 8 Brix onwards). Collect samples (weekly) from early veraison to berry colour evolution (Chardonnay and SB in Orange) using the Dyostem tool. Sequential harvest according to berry volume and sugar evolution for Shiraz and Cabernet Sauvignon at the 'fresh' and 'mature fruit' stages for high and low altitude vineyards in selected vineyards. Small scale vinification on Shiraz and Cabernet and selected industry scale winemaking to assess wine composition and sensory profiles. Analyses: organic acids, sugars (glucose to fructose ratio), pH, yeast assimilable nitrogen, amino acids (AA).
Year 4			
6	Completed analysis of volatile and non-volatile matrix. Identification of key fruit marker compounds (anthocyanins, carotenoids, and amino acids). Sensory characteristics of Shiraz and Cabernet Sauvignon wine made from 'fresh', and 'mature' stages.	30/12/2016	Quantify in fruit: anthocyanins, carotenoids, terpenes, norisoprenoids, and some C6-compounds. Targeted analysis on wine (anthocyanins, esters, terpenes, norisoprenoids and C6-compounds). Wine sensory evaluation (difference testing and descriptive analysis). Perform multivariate statistical methods to determine the link between fruit and wine composition.
7	Industry Workshops held. Scientific seminars delivered. Adoption evaluated.	30/09/2016	Hold workshops to demonstrate the models developed to industry. Communicate project outcomes through the delivery of seminars. Linked to ARC Linkage (RIPPLE-NWGIC).
8	AWGA Final Report.	31/12/2016	Submit Final Report to AGWA.

Some activities not initially planned in the FPA but fitting into the scope of the project were performed in order to bring value to the project. These extra activities with their corresponding outputs are listed below.

Output	Target Date	Activities
Extra outputs and Activities		
Completed extra analysis Linkage with project NWG 1401 led by Dr Andrew Clark (metal speciation). Establishment of collaboration between NWGIC and FEMS. New insights on the relationship wine composition*grape maturity*yield. Trial and analysis completed, new insights on the effect of grape maturity on yeast nutrients extractability from grapes to juice. Trial and analysis completed, new insights on the influence of berry shrivelling on grape and wine composition and wine style. Recommendations for how to manage berry shrivelling in the vineyard (harvest date) and winery (sorting) regarding wine style. Trial and analysis completed, new insights on the influence of ripening on the accumulation of grape metabolites impacting wine aromas. Trial and analysis completed, new insights on Australian red wines typicality/regionality and varietal differences between Shiraz and Cabernet Sauvignon wines. Oral and poster presentations in national and international congress. Scientific and industry papers published.	31/12/2016	Extra measurement for targeted and untargeted analysis: IBMP in Cabernet Sauvignon (2014, 2015); Volatile Sulfur Compounds (2014, 2015). Investigation on the impact of grape maturity on yeast nutrients (YAN, lipids) extractability from grapes to juice. Investigation on the influence of berry shrivelling on grape and wine composition and wine style. Investigation on how ripening and environmental factors influence the accumulation of grape metabolites involved in the synthesis of wine volatiles from veraison to harvest (amino acids, lipids, grape volatiles). Investigation on Australian Cabernet Sauvignon and Shiraz typicality/regionality. Focus on 1,4-cineole: new marker of Australian Cabernet Sauvignon uniqueness. Communicate extra outputs of the project.

Moreover, the wines made in 2016 will be used for a PhD project partly funded by Wine Australia, investigating the influence of grape maturity on wine ageing potential. This three year study will focus on the influence of grape maturity on the synthesis of oxidative and reductive aromas during wine ageing.

5 METHODS

5.1 Vineyard Selection and Characterisation—Red Cultivars

5.1.1 Vineyard selection

Experimentation was carried out across three consecutive years, 2014-2016 vintage, in Griffith, Orange and Tumbarumba (NSW) and in the Barossa and McLaren Vale (SA) on red varieties (Shiraz and Cabernet Sauvignon) and white varieties (Sauvignon Blanc and Chardonnay) in collaboration with McWilliam's, De Bortoli wines, Justin Jarrett, Phillip Shaw, Cumulus, Treasury Wine Estate and Courabyra Wines.

All the vineyards were own rooted and grown under drip irrigation. Vines were trellised to an open sprawling canopy in Griffith and SA, whereas in Orange and Tumbarumba vines were trellised to a VSP and lazy VSP.

Slightly different set-ups were utilised across different vintages therefore experimental design for each season will be described individually. A summary of each experimental design for each vintage is presented in Table 2.

Table 2: Summary table for red cultivars across three vintages and three regions that were monitored for berry sugar accumulation and executed harvest dates.

				Harvest stage				
Location	**Vineyard**	**Variable**	**Vintage**	**Pre-H1**	**H1 targeting Fresh Fruit (FF)**	**H2 Targeting Intermediate**	**H3 targeting Mature Fruit (MF)**	**Post H3 (Jammy Fruit)**
Griffith	G1_SH (Deb)	Low yield	2014	•	X	•	X	•
			2015	•	X	X	X	•
			2016	•	•	•	•	•
	G2_SH (McW)	Low yield	2014	•	X	•	X	X
			2015	•	X	X	X	•
			2016	•	•	•	•	•
	G3_SH (Deb)	High yield	2014	•	•	•	•	•
			2015	•	X	X	X	•
			2016	•	•	•	•	•
	G4_SH (McW)	High yield	2014	•	•	•	•	•
			2015	•	X	X	X	•
			2016	•	•	•	•	•
	G_CS		2014	X	X	•	X	X
			2015	•	X	X	X	•
			2016	•	•	•	•	•
Orange	O1_SH	650 m	2014	•	•	•	X	X
			2015	•	X	X	X	•
			2016	•	X	•	X	•
	O1_CS	650 m	2014	X	•	X	X	•
			2015	•	X	X	X	•
			2016	•	X	•	X	•
	O2_SH	950 m	2014	•	•	•	•	•
			2015	•	X	X	X	•
			2016	•	X	•	X	•
	O2_CS	950 m	2014	•	•	•	•	•
			2015	•	X	X	X	•
			2016	•	X	•	X	•
SA	SH_B	Irrigated	2014	•	•	•	•	•
			2015	•	X	X	X	•
			2016	•	•	•	•	•
		Control	2014	•	•	•	•	•
			2015	•	X	X	X	•
			2016	•	•	•	•	•
	CS_B	Irrigated	2014	•	•	•	•	•
			2015	•	X	X	•	•
			2016	•	•	•	X	•
		Control	2014	•	•	•	•	•
			2015	•	X	X	•	•
			2016	•	•	•	X	•
	CS_McV	Irrigated	2014	•	•	•		•
			2015	•	•	X	•	•

				Harvest stage				
Location	**Vineyard**	**Variable**	**Vintage**	**Pre-H1**	**H1 targeting Fresh Fruit (FF)**	**H2 Targeting Intermediate**	**H3 targeting Mature Fruit (MF)**	**Post H3 (Jammy Fruit)**
			2016	•	•	•	•	•
		Control	2014	•	X	X	•	•
			2015	•	•	X	•	•
			2016	•	X	X	X	•
	SH_McV	Irrigated	2014	•	X	•	X	•
			2015	•	X	X	X	•
			2016	•	•	•	•	•
		Control	2014	•	X	•	X	•
			2015	•	X	X	X	•
			2016	•	•	•	•	•

X indicates executed harvest dates whereas • indicates dates which were not harvested. Pre-H1: Pre fresh fruit harvest stage, H1: Fresh fruit (FF) harvest stage, H2: Intermediate (IF) harvest stage, H3: Mature fruit (MF) harvest stage, and Post H3: Jammy fruit harvest stage.

2014 season: Three Shiraz and two Cabernet Sauvignon commercial vineyards were chosen in NSW to monitor sugar accumulation per berry and possibly determine optimal harvest date for selected wine styles. Two Shiraz (G1_SH and G2_SH) and one Cabernet Sauvignon (G1_CS) commercial vineyards were located in Griffith (see Figure 1). G2_SH and G_CS were located on the same farm, with the size 12.7 and 34.4 ha, respectively. The G1_SH block was set approximately 12 km away from G2 and belonged to another producer. To contrast the hot climate of Griffith, a Shiraz (O1_SH) and Cabernet Sauvignon (O1_CS) vineyards were chosen in the cooler Orange region at an altitude of around 650 m above sea level. Evolution of sugar accumulation per berry was also monitored in SA in collaboration with Treasury Wine Estates (TWE) for Barossa Shiraz (SH_B) and Cabernet Sauvignon from McLaren Vale (CS_McV). Due to the distance, SA vineyards were not chosen by members of our project group, but rather by the industry partner and all consecutive measurements were carried out by TWE staff members.

For research purposes a small block consisting of 50 consecutive vines across five rows, (250 vines in total), was chosen inside the existing commercial block described above.

G1_SH, G_CS and G2_SH were mechanically pruned, with adjustment of the number of buds performed by hand in the latter vineyard. Vineyards in Orange, O1_SH and O1_CS, were all hand pruned.

2015 season: In 2015 two additional high yielding Shiraz vineyards, G3 and G4 respectively, were selected in Griffith based on discussions with industry partners. As described above vineyards were own rooted and drip irrigated and trellised to an open sprawling canopy.

At a workshop held in Orange in 2014, growers and winemakers communicated a desire for investigations at higher altitudes to also be completed. Therefore, additional Shiraz (O2_SH) and Cabernet Sauvignon (O2_CS) vineyards were selected at 950 m altitude to be contrasted with the vineyards selected already in 2014 vintage at 650 m (see Figure 2). Newly selected vineyards (O2_SH and O2_CS) were own rooted, manually pruned and trellised to the VSP.

For research purposes a small experimental block consisting of 250 vines, as selected in 2014, was expanded to 400 vines, meaning that three additional adjacent rows of 50 consecutive vines were utilised.

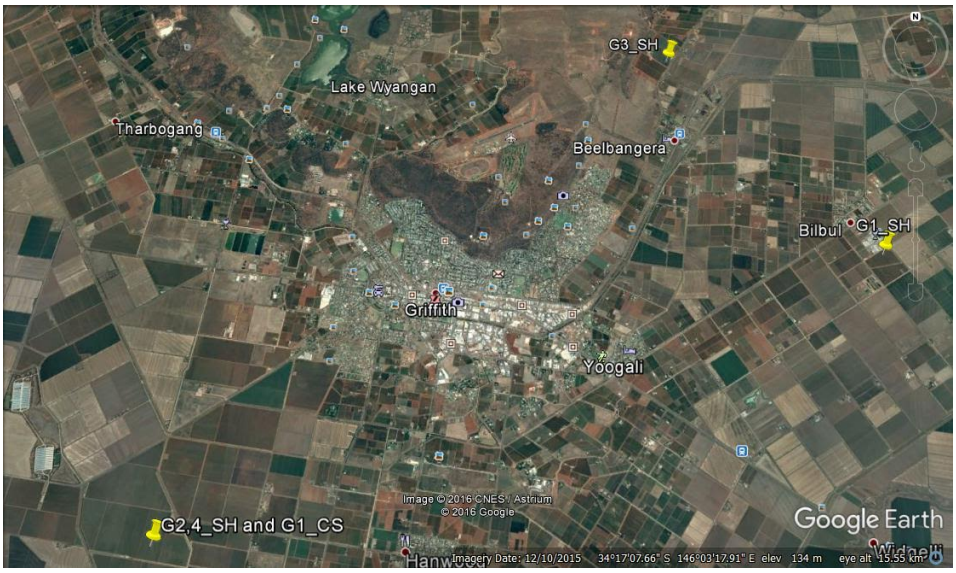


Figure 1: Location of selected Shiraz and Cabernet Sauvignon vineyards in Griffith, NSW.

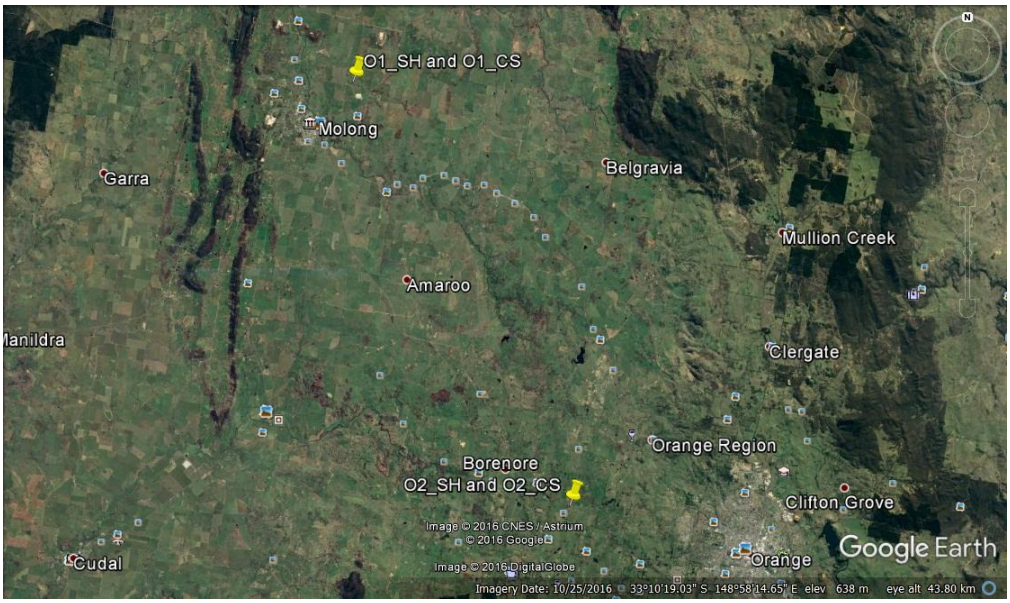


Figure 2: Location of selected Shiraz and Cabernet Sauvignon vineyards in Orange, NSW.

2016 season: In the final season, experimentation was carried out solely using the vineyards in Orange at two altitudes (O1 and O2) on both cultivars (Shiraz and Cabernet Sauvignon) as described above.

5.1.2 Vineyard Characterisation—Red Cultivars

The commercial vineyards used for investigation were characterised by measuring the parameters listed in Table 3.

Mesoclimatic temperature, humidity, rainfall and solar radiation data were collected in G1 and G2 by CR200X loggers (Campbell Scientific Australia, Australia) at 30 min intervals. Mesoclimatic temperatures and relative humidity were collected by Tinytag TGP-4500 (Gemini data loggers, UK) in 30 min intervals in G3_SH, O1_SH and CS, and O2_SH and CS, vineyards. Due to the proximity to G2 no temperature and humidity loggers were installed in G_CS and G3_SH. Similarly, only one weather station was located on site O1 due to the proximity of both Shiraz and Cabernet Sauvignon vineyards and one weather station was required on the O2 site. Loggers were kept in the vineyards throughout the season, excluding several days around harvest, when loggers were removed in order to avoid potential damage by commercial harvesters. Loggers were placed in a white shield and installed at a height of around 2 m. Temperature loggers were not installed in vineyards located in SA. For those vineyards, climatic data for the Barossa Valley were obtained from the weather station located in Nuriootpa, whereas data for McLaren Vale vineyards was collected from the Seaview weather station.

Table 3: Vineyard characterisation parameters carried out in selected vintages and vineyards.

Location	Vineyard	Variable	Vintage	Pruning mass	Primary shoots	Yield /vine	Climate	SM	SWP	NDVI	SILO
Griffith	G1_SH	Low yield	2014	X	X	•	X	X	X	X	X
			2015	X	X	X	X	X	X	•	X
			2016	•	•	•	•	•	•	•	•
	G2_SH	Low yield	2014	X	X	•	X	X	X	X	X
			2015	X	X	X	X	X	X	•	X
			2016	•	•	•	•	•	•	•	•
	G3_SH	High Yield	2014	•	•	•	•	•	•	•	•
			2015	X	X	X	X	X	X	•	X
			2016	•	•	•	•	•	•	•	•
	G4_SH	High Yield	2014	•	•	•	•	•	•	•	•
			2015	X	X	X	X	X	X	•	X
			2016	•	•	•	•	•	•	•	•
	G_CS		2014	X	X	•	X	•	X	X	X
			2015	X	X	X	X	X	X	•	X
			2016	•	•	•	•	•	•	•	•
Orange	O1_SH	650 m	2014	X	•	•	X	•	•	•	X
			2015	X	X	X	X	X	X	•	X
			2016			X	X	X	X	•	X
	O1_CS	650 m	2014	X	•	•	X	•	•	•	X
			2015	X	X	X	X	X	X	•	X
			2016			X	X	X	X	•	X
	O2_SH	950 m	2014	•	•	•	•	•	•	•	•
			2015	X	X	X	X	X	X	•	X
			2016			X	X	X	X	•	X
	O2_CS	950 m	2014	•	•	•	•	•	•	•	•
			2015	X	X	X	X	X	X	•	X
			2016	•	•	X	X	X	X	•	X
SA	SH & CS_B	All treatments	2014	•	•	•	X	•	•	•	X
			2015	•	•	•	X	X	•	•	X
			2016	•	•	•	•	•	•	•	•
	SH & CS_McV	All treatments	2014	•	•	•	X	•	•	•	X
			2015	•	•	•	X	•	X	•	X
			2016	•	•	•	•	•	•	•	•

Long term drilled climatic data used for the calculation of climatic indices were obtained from the SILO database. SILO is an enhanced climate database hosted by the Science Delivery Division of the Department of Science, Information Technology and Innovation (DSITI) and contains Australian climate data from 1889 onwards. Drilled climatic data can be accurately obtained for the selected location. Common viticultural climatic indices such as Huglin index (HI), Cold night index (CI) and the Winkler index were calculated for the period from 1949 onwards for the selected experimental vineyards as proposed by Hall and Jones (2010) and Tonietto and Carbonneau (2004).

Gypsum block soil moisture tension sensors (Measurement Engineering Australia, Australia) were installed to measure soil moisture. Soil moisture tension sensors were installed under a dripper with two probes, one at a depth of 10 cm (GB lite), the other at depth of between 30 and 50 cm (GB heavy). In the Barossa Valley, sensors were positioned on the depth of 10 cm GB lite type and two sensors of GB heavy type at the depth of 30 and 50 cm. Sensors were installed in the Barossa Valley by TWE staff.

During the growing season, stem water potential was measured using a Schollander pressure chamber as described previously by Deloire and Heyns (2011). In selected vintages, pruning mass, number of primary shoots, and yield/vine were measured. Multispectral images of three commercial blocks in Griffith were taken in season 2013/14. Four banded multispectral images, with resolution of 50 cm were captured by Terrabyte Services (Wagga Wagga, Australia) on 5 February 2014 (Figure 3, Figure 4, and Figure 5). Multispectral imaging was performed on the whole commercial plots. Vegetation indices such as NDVI, EVI and TVI were calculated for those plots.

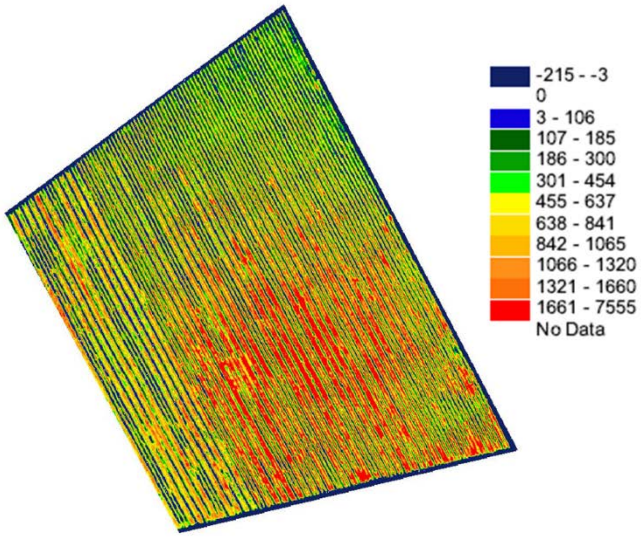


Figure 3: G1_SH EVI image 2014

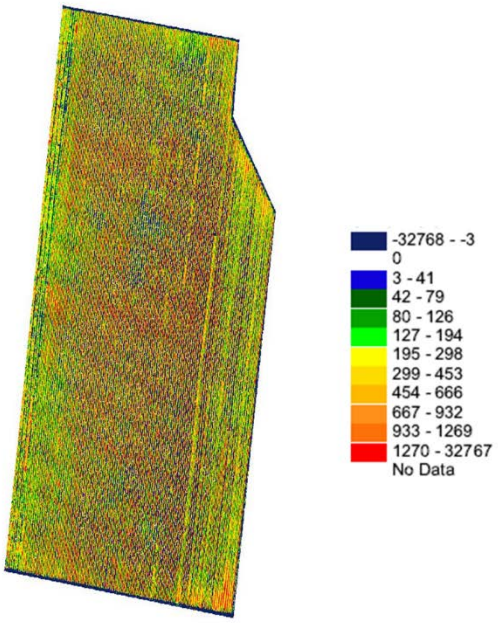


Figure 4: G2_SH EVI image 2014

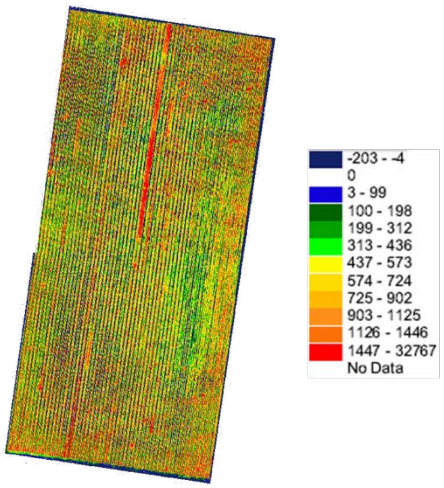


Figure 5: G_CS EVI image, 2014

5.1.3 Sugar Accumulation Method to Assess Potential Wine Style

Harvest dates for red cultivars were predicted based on the protocol: Sugar Loading and Berry Aromatic Sequence (BAS) in relation to the target style of the final wines as outlined in Figure 6. A representative number of 10 bunches in triplicate per block were sampled from veraison onwards. Samples were collected on a weekly basis, except around 18-22 Brix when samples

were collected every three days, to accurately determine the date of the slowdown/plateau of sugar accumulation in the berry. In the laboratory, 100 berries per replicate were carefully excised with the pedicel to avoid juice loss. Berries were weighed and thereafter crushed for measurements of juice total soluble solids (TSS) content expressed as Brix, and sugar accumulation per berry (mg/berry) was calculated (Appendix 1). Harvest dates linked to potential final wine styles were determined by the proposed sugar loading models as seen in Figure 6 A and B, for Shiraz and Cabernet Sauvignon, respectively.

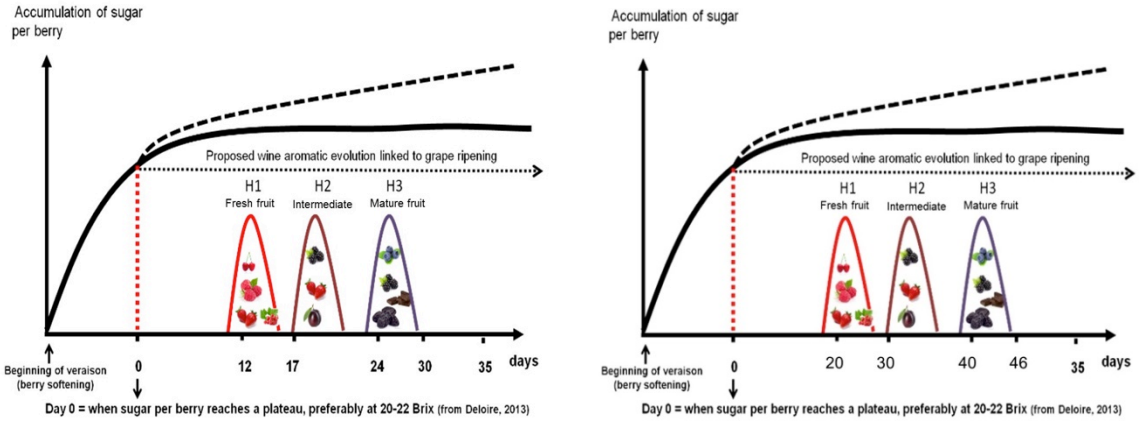


Figure 6: A on left) Proposed model to predict harvest dates in relation to the potential wine style based on the sugar accumulation per berry for Shiraz. B on right) Proposed model to predict harvest dates in relation to the potential wine style based on the sugar accumulation per berry for Cabernet Sauvignon.

For Shiraz it was suggested from previous empirical observations that the first, fresh fruit (FF) harvest date linked to the red fruit notes in wines is reached around 12 days after the slowdown/plateau of sugar accumulation into the berry. The third harvest (H3), targeting mature fruit (MF), reminiscent of dark and stewed fruit characters, is reached around 24 days after the slowdown of sugar accumulation in the berry on a per berry basis as illustrated in Figure 6A. The second harvest date was termed an intermediate stage (Intermediate) as it could not be clearly distinguished from FF or MF and it was suggested to occur around six days after FF and six days before MF for Shiraz.

Cabernet Sauvignon is characterised with longer ripening period and therefore FF was suggested to be reached around 20 days after the slowdown of sugar accumulation into the berry. MF, associated with dark fruit characters in wines, is reached 40 days after the slowdown of sugar accumulation in the berry. Similarly to Shiraz, the Intermediate stage could not be clearly separated from FF and MF based on the wine sensory features and this stage was

reached approximately 30 days after the plateau of sugar accumulation in the berry as shown in Figure 6B.

5.1.4 Sampling, Harvest and Winemaking

Harvest dates for red cultivars were determined based on the sugar accumulation model as described above. Harvest dates were determined 12-24 days in advance for Shiraz and 20-40 days in advance for Cabernet Sauvignon. Actual harvest date did not deviate from the predicted experimental harvest date by more than three days for all the vineyards and vintages. Commercial harvest date often coincided with the predicted harvest date or it was positioned between predicted harvest dates.

Sampling for grape berry analyses. In the 2014 season, grape berry samples were collected weekly from veraison until harvest. One hundred berries were randomly collected across the experimental block located inside the existing vineyards in three biological replicates. Berries were collected evenly from both sides of the canopy and immediately fresh frozen in liquid nitrogen and stored at -80 °C until further processed. The same sampling protocol was followed in 2015 and 2016 season; however, the samples were collected only on the predicted harvest dates.

For grape berry analyses a subsample of 50 frozen berries was deseeded with the mortar and pestle and ground to a fine powder with IKA A11 basic analytical mill (IKA, Malaysia). Grape powder was stored on -80 °C until further analyses.

Harvest for small lot winemaking. Three replicates of 60 kg of grapes were harvested across the experimental plot established inside the commercial block as described above at each harvest date. To minimise the effect of the variability between vines, grapes were hand harvested randomly across the experimental plot picking only few bunches per vine. The harvest strategy was selected as optimal due to the high yielding vines in Griffith and low grape volume needed for experimental winemaking. In Orange vineyards at 900 m randomly selected vines were harvested in whole due to the low yield per vine (2-3 kg/vine). Grapes were handpicked and dosed with 40 mg/kg of sulfur dioxide (SO_2) in the form of dissolved potassium metabisulfite and transported in crates to the NWGIC experimental winery. Grapes were cooled at 4 °C overnight before further processing.

Small-lot winemaking. All biological replicates were kept separate during grape processing. Grapes were destemmed, crushed and transferred to 100 L stainless steel tanks for fermentation. Acidity was adjusted to approximately pH 3.6 with tartaric acid. The must was inoculated with *Saccharomyces cerevisiae* yeast EC118 (Lalvin) and fermentations were carried out at 25-26 °C. For ferments with an initial TSS of less than 23.4 Brix, once alcoholic fermentation had caused a drop of 2 to 3 Baumé, the original yeast assimilable nitrogen (YAN) was adjusted to 200 mg/L with Fermaid K and diammonium phosphate (DAP). For ferments in excess of 23.4 Brix, the YAN was adjusted to 220 mg/L. Malolactic fermentation was carried out by co-inoculation of Enoferm alpha LAB (*Oenococcus oeni*) two days after the start of alcoholic fermentation. Wines were pressed off the skins with a small basket press up to a pressure of 1 bar when residual sugar level dropped below 0.5 g/L. Pressed wines were placed at 22°C until the competition of malolactic fermentation and thereafter dosed with 80 mg/L SO_2, pH adjusted to pH 3.6 and racked of lees. Wines were cold stabilised for 21 days at 4°C, adjusted to 30 mg/L of free SO_2 and bottled in May.

General analyses of grape maturity, yeast available nitrogen, and basic wine parameters. Grape juice samples were collected after crushing for basic parameters of maturity. TSS expressed as Brix was analysed with a portable density meter (Anton Paar DMA 35N, Graz, Austria). Titratable acidity (TA) and pH were determined by sodium hydroxide titration to a pH 8.2 end point with an automatic titrator (Metrohm Fully Automated 59 Place Titrando System, Metrohm AG, Herisau, Switzerland). Ethanol was measured with an Anton Paar Alcolyser DMA 4500 density meter (Graz, Austria) whereas α-amino acids, ammonia and acetic acid in juice were measured by available enzymatic tests purposely designed and deployed on an Arena discrete analyzer (ThermoFisher). Yeast assimilable nitrogen (YAN) was calculated from ammonium and free amino nitrogen (FAN) measurements (Illand et al. 2004).

Organic acids, carbohydrates and ethanol. Grape berry powder was analysed for organic acids and sugars content whereas wines were analysed for residual sugar content by HPLC according to the method previously published (Frayne, 1986). Grape berry powder was prepared according to Eyéghé-Bickong et al. (2012) and filtered through 0.45µm filter (Merck, Frenchs Forest, Australia) prior to injection; wine samples were filtered (0.45µm) and injected directly (Frayne, 1986). For separation of organic acids and sugars, a micro guard cation H^+ cartridge guard column (Bio-Rad Laboratories, Berkeley, USA) and two 300 mm × 7.8 mm Aminex HPX-87H Ion exclusion columns (Bio-Rad Laboratories, Berkeley, USA) at 65˚C were used. Quantification was performed by HPLC using a Waters 600 controller (Milford, USA) connected

to Waters 717 plus autosampler (Waters, USA), coupled to PDA (Waters, USA) and RI (Waters, USA) detectors (Frayne, 1986).

Amino acids. Amino acids were extracted from homogenised frozen grape berry powder as described by Gika et al. (2012) with some modifications. Precisely 100 ± 0.05mg of frozen grape berry powder was weighed into a 1.5mL Eppendorf tube and extracted with 0.1 mL of 80:20 MeOH:MilliQ (v:v) in an ultrasonic bath at room temperature for 15 min. Thereafter samples were centrifuged (Beckman Coulter, Microfuge 20 Series, Brea, USA) at 13000 rpm for 10.5 minutes. Supernatant was collected and used for amino acids determination according to Hynes et al. (1991). Briefly, samples were diluted 1:24 (v/v) with 0.25M borate buffer (pH 8.5) and L-hydroxyproline as internal standard at 13.1 mg/L was added. The amino group was derivatised with 9-fluorenylmethyl chloroformate and samples analysed by HPLC using a Waters 600 controller (Milford, USA) connected to an autosampler (Waters 717 plus, USA), and quantified by a florescence detector (Waters 2475, USA). Amino acid separation was performed on a reverse phase column (Zorbax Eclipse Plus, Agilent Technologies, Mulgrave, Australia) at 45˚C. Quantification was performed as previously reported Hynes et al. (1991).

Grape anthocyanins. Samples were extracted as described in Downey and Rochfort (2008). Precisely 100 mg of homogenised grape powder was weighed into a round bottom 2 mL Eppendorf tube and extracted for 20 minutes in an ultrasonic bath in 1 mL 50:50 Methanol:MilliQ water. Extracts were analysed by Waters Acquity ultrahigh pressure liquid chromatograph (UPLC) equipped with photodiode array detector (DAD). Mobile phases consisted of aqueous A 10% formic acid in MilliQ water and B 10% formic acid in methanol. Linear gradient at flow 0.4 mL/min was employed as follows: 0-1.5 min from 10-12% B; 1.5-6.0 min 22% B; 6.0-8.0 min 25% B; 8.0-9.5 min 35% B; 9.5-10.0 100% B, 10.5-13.0 min 100% B, 13.5-14.0 min 10% B and column equilibration with initial conditions to 15.5 min. Quantification of anthocyanins was carried out at 500 nm. Anthocyanins were quantified as equivalents of Malvidin-3-*O*-glucoside.

Grape polyphenols. Grape polyphenols were measure by spectrophotometer UV-1700 Shimadzu spectrophotometer (Kyoto, Japan) according to Illand et al. (2004).

Grape carotenoids. Carotenoids were extracted from the 1 g frozen homogenised grape berry powder samples according to Wehrens et al. (2013) and analysed according to Young et al. (2015). Dried carotenoid extract was re-dissolved in 100 µL of ethyl acetate. A 50 µL aliquot of re-dissolved carotenoids was mixed with 200 µL methanol (Lashbrooke et al. 2010) and directly injected on Waters Acquity UPLC equipped with UPLC BEH Shield RP18 (2.1 mm x 100 mm, 1.7

µm) protected with a Waters UPLC BEH guard cartridge (2.1 mm x100 mm, 1.7um) and DAD detector. Carotenoids were quantified at 450 nm, whereas 660 nm was used for chlorophyll and pheophytin quantification.

Grape volatiles. Sample preparation for free grape volatiles (C6-compounds, norisoprenoids and terpenes) was carried out according to the protocol published by Matarese et al. (2014)**.** Briefly, 2 g of frozen grape berry powder was weighed into cooled SPME vial. To the frozen sample, 2 mL of phosphate-citrate buffer (0.1 M Na2HPO4 and 50 mM citric acid, pH 5) was added followed by 10 µL of ascorbic acid at concentration 200 g/L. Samples were thereafter spiked with 15 µL of Internal standard mix containing 2-octanol at 5.08 mg/L, [^{2}H3]-linalool at 1.89 mg/L and [$^2H_5$]-ethyl cinnamate at 5.9 mg/L in methanol. Sodium chloride (1 g) was added to the vial which was immediately capped, vortexed and analysed. Analytical sample sequences did not exceed 12 samples and were randomised. Samples were analysed by headspace solid-phase micro extraction (HS-SPME) with a PDMS-CAR-DVB 50/30 µm fibre (Supelco, Bellefonte, USA). Volatiles absorbed by the fibre were released into an Agilent 7890 gas chromatography equipped with a DB-WAXetr capillary column (60 m, 0.25 mm, 0.25 µm film thickness, J&W Scientific, Folsom, CA) and coupled with a Gerstel MPX autosampler with a Peltier tray cooler set at 4˚C. The GC was connected to a 5975C mass spectrometer (Agilent Technologies) as described in Šuklje et al. (2016). Semi quantitative data were normalised on a sample weight and internal standard response.

Total grape volatiles, terpenes and norisoprenoids were analysed with the method used for detection of free grape volatiles as described in Šuklje et al. (2016). Sample preparation was carried out according to the methods of Loscos et al. (2009) and Young et al. (2015). Briefly, 0.5 g of grape berry sample was weighed into the HS-SPME vial followed immediately by 2 mL of tartrate buffer in concentration 5 g/L of tartaric acid and 3 g/L ascorbic acid, pH 3. Samples were spiked with 15 µL of internal standard mix containing 2-octanol at 5.08 mg/L, [^{2}H3]-linalool at 1.89 mg/L and [$^2H_5$]-ethyl cinnamate at 5.9 mg/L in methanol. Sodium chloride (1 g) was added into the vial, which was sealed and heated at 100°C for an hour. Thereafter vials were cooled and injected into the GCMS with conditions as described in Šuklje et al. (2016). Semi quantitative data were normalised on a sample weight and internal standard response.

Grape lipids. Lipids were extracted from 0.5 g frozen homogenised grape berry powder samples according to the method of Della Corte et al. (2015). Samples were spiked with 10 µL of internal standard [d_{33}]-heptadecanoic acid at concentration 50 g/L prepared in chloroform: methanol 2:1. The chloroform lipids rich layer was dried under a stream of nitrogen and derivatised with 1 mL of 6% H_2SO_4 in methanol for 3 hours at 80°C (System biology workshop, 2015). After the

derivatisation the HS-SPME vial was cooled down and 670 µL of hexane and 340 µL of MilliQ water were added to the sample. The upper hexane layer containing methylated esters of fatty acids was collected and immediately injected in 7000C GC-MSMS (Agilent Technologies) (System biology workshop, 2015). 1 µL of sample was injected into the injector set in split 10:1 mode and held at 250°C. Separation was achieved on BPX70 50 m x 0.22 mm, film thickness 0.25 µm (SGE Analytical Science) column and helium flow at 1.2 mL/min. Oven temperature was held at 80°C for 2 min and thereafter oven temperature was ramped at the rate 60°C/min to 130°C. From 130°C temperature increased at the rate 7°C/min to 245°C and held for 3 min. Total run time was 18.2 min. The transfer line was held at 260°C and electron ionisation at 70 eV. Spectra were collected in a mass range *m/z* 35-350, scan time was set at 107 ms and acquisition delay was 4.4 min. Methylated esters of fatty acids were quantified in multi reaction mode (MRM) as presented in Table 4.

The method was also validated, repeatability and recovery were assessed, and limits of detection and quantification were established (not presented). Peak areas were normalised on the internal standard response and sample weight.

Table 4: MRM parameters for lipids quantification.

Compound	(min)	Precursor Ion	Product ion	Collision Energy
Time segment 1	0.00			
caprylic acid		127	57.1	5
Time segment 2	5.50			
lauric acid		171	**101,** 69.2	**5**/10
capric acid		143.2	**101**, 83.1	**5**/5
Time segment 3	8.45			
pentadecanoic A.		256	143, **101**	5/**15**
myristic A.		242.1	143, **101**	5/**15**
myristoleic A.		208.2	**111.1**, 97.2	**10**/10
Time segment 4	9.70			
heptadecanoic Deut (IS)		317	**107.1**, 79.2	**15**/15
heptadecanoic A.		284	**101**, 143	**15**/5
palmitic A		270	**101**, 143	**15**/5
palmitoleic A		236.2	111, **97**	10/**10**
Time segment 5	11.20			
stearic A.		298.3	199, **101**	5/**15**
linoleic A.		294.3	149.9, **81**	0/**20**
oleic A.		264.3	**111**, 97	**10**/10
Time segment 6	13.20			
arachidic A.		326.4	199,**101**	5/**20**
cis-11-eicosenoic A.		292.3	111.1, **98**	10/**5**
linolenic A.		292.3	**92.8**, 78.8	**30**/40
Time segment 7	14.50			
lignoceric A.		382	142, **108**	5/20
behenic A.		354.3	199.3, **101**	5/**20**
erucic A.		320.5	123, **98**	**15**/5

Wine total anthocyanins, colour, polyphenols and tannin analyses. Wine total anthocyanins, colour parameters and polyphenols were analysed as outlined by Illand et al. (2004). Prior to wine colour and polyphenols measurements, wine was adjusted to pH 3.5 and measurements were conducted using a UV-1700 Shimadzu spectrophotometer (Kyoto, Japan). Wine total tannins were analysed by the methyl cellulose precipitable tannin assay as described by Illand et al. (2004) and expressed as equivalents of epicatechin.

General wine volatiles analysis. Esters, higher alcohols, C6-compounds and lactones, were analysed by gas chromatography-mass spectrometry (GC-MS) according to previously outlined methods (Antalick et al. 2010; Antalick et al. 2015a; Šuklje et al. 2016). A mixture of isotopically labelled esters from CDN isotopes (Pointe-Chaire, Canada) was used to quantify esters and higher alcohols (semi-quantification) whereas octan-2-ol (Fluka, Castle Hill, Australia) was used as an internal standard for C6- and lactone compounds. Samples were spiked with the internal standard solution mixed containing 20 mg/L [$^2H_5$]-ethyl butyrate, 20 mg/L [$^2H_5$]-ethyl hexanoate, [$^2H_{15}$]-ethyl octanoate, 4 mg/L [$^2H_{23}$]-ethyl decanoate, 5 mg/L [$^2H_5$]-ethyl cinnamate and 5 mg/L 2-octanol. In this study, 5 mL wine sample was added to a 20 mL SPME vial with 3 g NaCl and 5 mL deionised water, and spiked with 10 µL of internal standard solution. Vials were immediately capped, vortexed, and analysed by headspace solid-phase micro extraction (HS-SPME) with a PDMS-CAR-DVB fibre (Supelco, Bellefonte, USA). Wine volatiles were analysed on the same equipment as detailed for grape volatiles.

Wine Sulfur compounds. Volatile wine sulfur compounds analyses were carried out with an Agilent 7890B gas chromatography system (VIC, Australia or Santa Clara, CA, USA) equipped with a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) and coupled to an Agilent 355 SCD Sulfur Chemiluminescence Detector. The column was an Agilent J&W DB-sulfur SCD 60 m x 0.32 mm x 4.2 µm. GC conditions were adapted from the work of Siebert et al. (2006). Helium (BOC, NSW, Australia-ultra high purity) was used as a carrier gas at a linear velocity of 34.8 cm/s and a constant flow of 2.8 mL/min. The initial oven temperature was 35^{o}C and held for 5 min, followed by an increase to 150^{o}C at a rate of 5^{o}C/min and held at this temperature for 10 minutes. SCD parameters were adjusted based on recommendations by Agilent. SCD burner temperature was 800^{o}C, burner base temperature was 150^{o}C, hydrogen (BOC-ultra high purity) flow rate was 44.5 sccm and oxidiser flow low rate was 63.0 sccm (instrumental grade air-BOC). The ozone regulator pressure was 6 psig, the SCD pressure was 6 Torr while the dual plasma controller pressure was 378 Torr.

Headspace analysis. 10 mL of wine sample and 50 µL of ethyl methyl sulfide (EMS) internal standard were transferred to a vial containing 3 g of NaCl. For potential DMS analysis (PDMS), three pellets of NaOH were also added and the vial was heated at 80˚C for 1h before cooling to room temperature. For VSCS and PDMS analysis the vial was then incubated at 45^{o}C for 30 min with agitation at 400 rpm. Then 800 µL of the headspace was taken, with a 1 mL headspace syringe heater (MSH 01-00B, Shimadzu, Kyoto, Japan) thermostated at 60^{o}C, and further injected into the injector (at 150^{o}C under a 1:8 split ratio).

Method Validation. Linearity was validated by a series of seven standard additions of H_2S, MeSH, EtSH, CS_2, DES, DMS, DMDS, DEDS, MeSAc, and EtSAc in model, red and white wines. For the accuracy study (recovery), two different red and white wines were spiked with the above compounds at the same concentrations as used for repeatability. Instrument control and data analysis were performed with Agilent GC Mass Hunter software, rev. B.07.00.

Untargeted wine volatiles analyses. Untargeted wine volatile analyses were performed as previously described by Schmidtke et al. (2013) and wine sample extraction was performed according to López et al. (2002). Briefly, 50 mL of wine was spiked with 50 µL of 3-tert-butyl-4-hydroxyanisole internal standard at final concentration 4158 µg/L. A sample was extracted with LiChrolute-EN cartridges (Merck) as described by (López et al. 2002). Compounds retained on cartridge were eluted with 1.5 mL of dichloromethane and the sample was spiked with mix of octan-2-ol and 4-hydroxy-4-methyl-2-pentanone to give final concentrations of 4800 and 4310 µg/L respectively, and placed in a vial for injection. GC parameters were kept identical to those described by Schmidtke et al. (2013).

Wine sensory analyses. Descriptive sensory analyses (DA) were conducted six months post bottling according to the method outlined by Blackman and Saliba (2009). Twelve panellists, six females and six males aged 23-45, were selected on the basis of interest and availability from staff and students of the National Wine and Grape Industry Centre. The panellists had previous experience with the DA using the Compusense® five software (Version 5.0.49, Guelph, Canada).

The panellists underwent 10 training sessions, in which attributes were generated and refined to achieve the final list of descriptors (Table 5) through consensus. They also practiced their ability to detect, identify and rank the selected aroma and mouthfeel attributes in a consistent manner. The formal DA of the wines was conducted over three sessions, in individual temperature controlled (22 °C) tasting booths under red lighting to prevent colour bias. At each session, each wine was labelled with a three-digit random number generated by Compusense® and presented using a randomised William Latin Square block design. Samples of 25 mL of wine

were served at 18-19°C in 215 mL covered ISO/INAO clear wineglasses. The panellists familiarised themselves with the reference standards before they entered the booths. Water and unsalted crackers were provided. Attributes were ranked by panellists using a nine-point line scale anchored from 'absent' to 'high intensity'. During the formal DA, panellists also scored the wines for overall liking on a nine-point line scale from 'dislike extremely' to 'like extremely'. The panel performance was assessed using the PanelCheck software (Version 1.4.2, Nofima Mat), to determine the reliability of individual assessors. All assessors were found to be performing to an acceptable standard since no significant panellist by sample interactions were found.

Table 5: List of attributes and composition of standards

Term		Composition of reference standard
Dark fruit	S1	2/3 tsp of blackberry/wild blueberry jam (St Dalfour SAS, Chemin de Cazeaux, Marmande, France) + 1/3 black currant jam (Woolworths, Bella Vista, New South Wales, Australia)
Red fruit	S2	2 slightly mashed frozen strawberries + 2 slightly mashed frozen raspberries
Black cherry	S3	4 slightly mashed canned cherries (SPC Ardmona, Shepparton, Victoria, Australia)
Plum	S4	1 slightly mashed canned plum + 10 mL of plum juice (SPC Ardmona)
Floral	S5	5 µL rosewater (Queen Fine Foods Pty Ltd.)
Black pepper	S6	1 tsp of black peppercorns (MasterFoods, Wyong, New South Wales, Australia)
Green olive	S7	3 'Sicilian' green olives (Sandhurst Fine Foods)
Menthol	S8	Half a crushed lozenge (Vicks, Greensboro, North Carolina, US)
Green pepper	S9	0.15 g green pepper slice
Tomato leaf	S10	4 tomato leaves
Taste/Mouthfeel		
Acidity	S11	1.5 g/ L tartaric acid (Univar) dissolved in standard solution
Alcohol	S12	20 mL 68% v/v grape spirit (Tarac Technologies) in standard solution
Astringency	S13	100 mg/ L quertannin (Laffort, Bordeaux, France) dissolved in standard solution

All standards were freshly prepared in standard tasting glasses (ISO 3591) with glass lids.
*In 50 mL 50:50 filtered water:base wine (Berri Estates, Glossop, South Australia).

Statistical analyses. One-way and two-way analyses of variance (ANOVA) were performed on the chemical data using Statistica, Version 12 (StatSoft, Tulsa, OK, USA) and the means were separated using Stats-Fisher's LSD test (different letters account for significant differences at $p \leq 0.05$). All quoted uncertainty is the standard deviation or standard error of three replicates of one treatment. Principal component analyses were conducted on mean centred concentration profiles of compounds in Statistica, Version 12 (StatSoft, Tulsa, OK, USA).

6 RESULTS AND DISCUSSION

6.1 Vineyard Selection Characterisation

The basic vineyard characteristics for all sites are presented in Table 6.

In addition to the vineyard classifications outlined in Methods, the following summarises additional information that is available for specific vineyards sites;

Both O1_SH and CS_O1 blocks were pruned to approximately 36 buds/vine. This information was not available for G1 and G2 vineyards located in Griffith, but we can assume that number of buds was considerably higher as the number primary shoots counted was 89 and 73, respectively.

In the 2013 season, flowering occurred between 15-30 October in Griffith for both Shiraz and Cabernet Sauvignon vineyards. As expected, the Shiraz flowered later in Orange due to the 650 m altitude, taking place between 12-21 November. Flowering (5%) for Cabernet Sauvignon was observed on 8 November and 80% flowering occurred on 15 November 2013.

In the same season, 50% veraison in the G1_SH and G2_SH vineyards was estimated to occur around 3 January 2014. For the G_CS vineyard, 50% veraison occurred between 11-17 January 2014, and 50% veraison was recorded around 18 January 2014 for both, O1_CS and O1_SH.

In the 2014/15 season, the beginning of flowering in the G1_SH vineyard was noted after 23 October, whereas on 6 November cap fall and berry set was observed. Similar observations were recorded for Shiraz, i.e. G2_SH, G3_SH and G4_SH vineyards in Griffith, and full flowering in G_CS was observed on 6 November 2014. Fruit set had commenced by 18 November 2014 in O1_SH vineyard; whereas O2_SH was observed at 80% flowering, O2_CS at 50% flowering, and O1_CS was at approximately 80% flowering on this date.

In the 2014/15 season, 30% coloured berries in G1_SH vineyard were noted on 30 December 2014, corresponding to the phenophase in vineyard G3_SH. 30% coloured berries were observed in vineyard G2_SH and G4_SH on 6 January 2015, whereas in G_CS first colouration and berry softening was observed only on 16 January 2015. On 6 January 2015 10% coloured berries were observed in O1_SH and O1_CS, whereas around 80% veraison in O2_SH was estimated around 28 January 2015 and on the same date around 30% veraison in CS_O2.

Table 6: Basic vineyard parameters.

	G1_SH	G2_SH	G3_SH	G4_SH	G_CS	O1_SH	O2_SH	O1_CS	O2_CS
Plantation	1995	1997	2008	1997	1997	1995	1989	1995	1989
Clone	Minato	SA1654	BVRC12	SA1654	125	PT23	EVOVS12	G9V3	
Spacing (m)	2.5x3.7	2.5x3.7	2.5x3.7	2.5x3.7	2.5x3.7	2x3		2x3	
Trellis System	Sprawling	Sprawling	Sprawling	Sprawling	Sprawling	Lazy VSP	VSP	Lazy VSP	VSP
Average Yield/vine (kg)	10.3	14.0	18.6	17.7	8.7	5.1	4.2	4.0	1.9
Average No of primary shoots/vine	86	95	92	92	-	42	21	44	20
No of bunches/vine	116	123	149	155	130	84	19	89	20
Average bunch weight (g)	89	109	125	118	68	62	225	45	95
Pruning mass (kg)	1.47	1.00	-	-	0.93	0.53	-	0.45	-

In the 2015/16 season 30-40% coloured berries in O1_SH were observed on 7 January 2016, whereas on 13 January already 80% of berries were coloured. Similarly, on 7 January 2016, 10-20% of berries were already pink in CS_O1. In O2_SH vineyard on 18 January 2016 only 5-10% of berries were coloured whereas in O2_ CS berry softening was noticed on the same date. On 1 February 2016, 80% of berries in O2_SH vineyard were coloured, whereas the proportion of coloured berries is CS_O2 on the same date was only 20-30%.

Data for average yield/vine, average number of primary shoots/vine, average number of bunches/vine, average bunch weight and prunning mass presented in Table 6 were obtained in 2014/15 season and serve as an indication of differences between vineyards. The key parmeters, including average yields/vine, berry weight and number of primary shoots, did not differ significantly across the two vintages for the same vineyard.

6.1.1 Climatic data.

Drilled climatic data obtained from SILO (Queensland, Australia) were used to calculate frequently employed viticultural indices to better describe the climate of chosen regions/vineyards. Indices were calculated from the1949/50 season until 2016.

The Huglin index is a six month (1 October to 31 March for the Southern hemisphere) heat summation for the mean hemeroperiod when most grapevine metabolism is active and provides information regarding the level of heliothermal potential. Combined with the Cold night index, it allows a good discrimination of a region's climate in regards to global heliothermal conditions during the vegetative cycle of the grape and cool night conditions during the ripening (Tonietto and Carbonneau, 2004). In the southern hemisphere the Huglin index divides regions according to heliothermic potential (Table 7).

Table 7: Classification of the regions according to the Huglin index

Huglin index	Viticultural climate description
≤1500	Very cool
>1500≤1800	Cool
>1800≤2100	Temperate
>2100≤2400	Temperate warm
>2400≤3000	Warm
>3000	Very warm

Growing degree days is a seven month active temperatures heat summation that can also provide a good description of heliothermal potential (Hall and Jones, 2010). It is usually calculated from 1 October to 30 April, as in this report, or it can be adapted to the phenology from the bud burst to harvest. Similarly, as for the Huglin index, it divides climates into several classes according to the ability to ripen grapes, Table 8.

Table 8: Classification of the regions according to the Growing degree days index.

Growing degree days	Region	Viticultural climate description
≤850	Too cool	
850-1389	I	Cool
1389-1667	II	Cool or Mild
1667-1944	III	Warm
1944-2222	IV	Hot
2222-2700	V	Very hot
>2700	Too hot	

In addition to the Huglin and Growing degree days index, the Cold night index for the period from 1950 - 2016 was also calculated. The Cold night index is a night coolness variable which takes into the account the mean minimum temperature during the month when ripening usually occurs in the several months before harvest (Tonietto and Carbonneau, 2004). The purpose of this index is to improve qualitative assessment of wine-grape growing region. The Cold night index divides climates according to the number of cold nights, Table 9.

Table 9: Classification of the regions according to the Cold night index.

Cold night index	Viticultural climate description
≤12	Very cool nights
>12≤14	Cool nights
>14≤18	Temperate nights
>18	Warm nights

6.1.1.1 Griffith

For the Griffith region, the Huglin index was only required to be calculated for one location due to the flat terrain of the region. The altitude difference between vineyards was less than 26 m and the furthest distance between vineyards was only 15 km. Minimal temperature differences between vineyards located in Griffith were confirmed by comparing mesoclimatic data obtained from the weather stations positioned inside the vineyards.

According to the Huglin index, Griffith is classified as warm (HI ≥ 2400<3000) to very warm climate (HI ≥ 3000). Figure 7 shows that from 2005 onwards the frequency of very warm vintages increased. The number of vintages with a cold night index exceeding 18°C (Figure 8) and the number of days where Tmax ≥ 35 °C (Figure 9) has also increased in this period. According to the average Growing Degree Days (Winkler scale) of 2338 from 1949/50-2015/16 Griffith is classified in Region V, described as very hot climate (Hall and Jones, 2010). In accordance with Huglin index an increase in warmer vintages was noticed also when looking at GDD in the last decade, Figure 7.

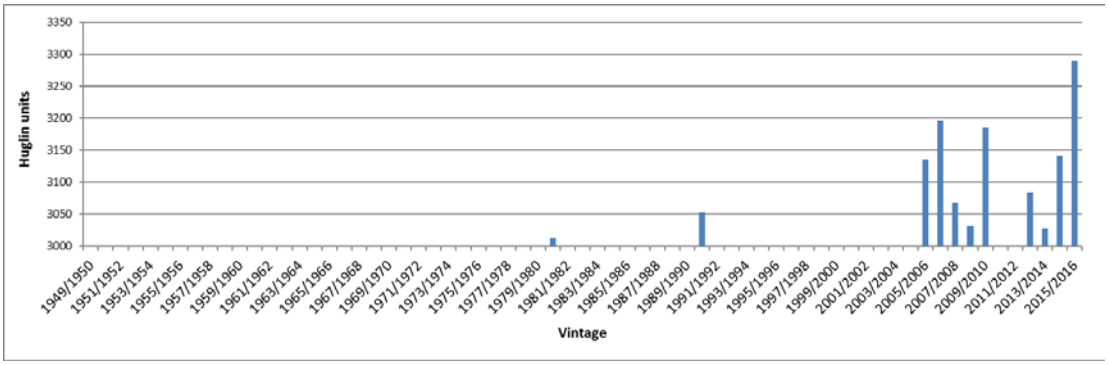


Figure 7: Vintages with Huglin index above 3000 units which are classified as very warm.

According to the cold night index, Griffith is classified as a region with temperate warm to warm nights. The average cold night index for January and February together from 1949/50 to 2015/16 vintage was 17.3°C.

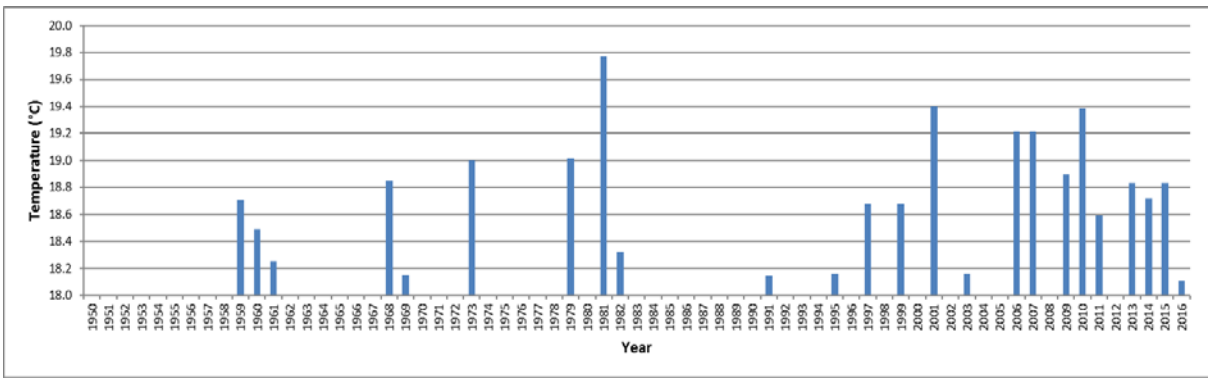


Figure 8: Vintages with calculated Cold night index for January and February above 18°C in Griffith.

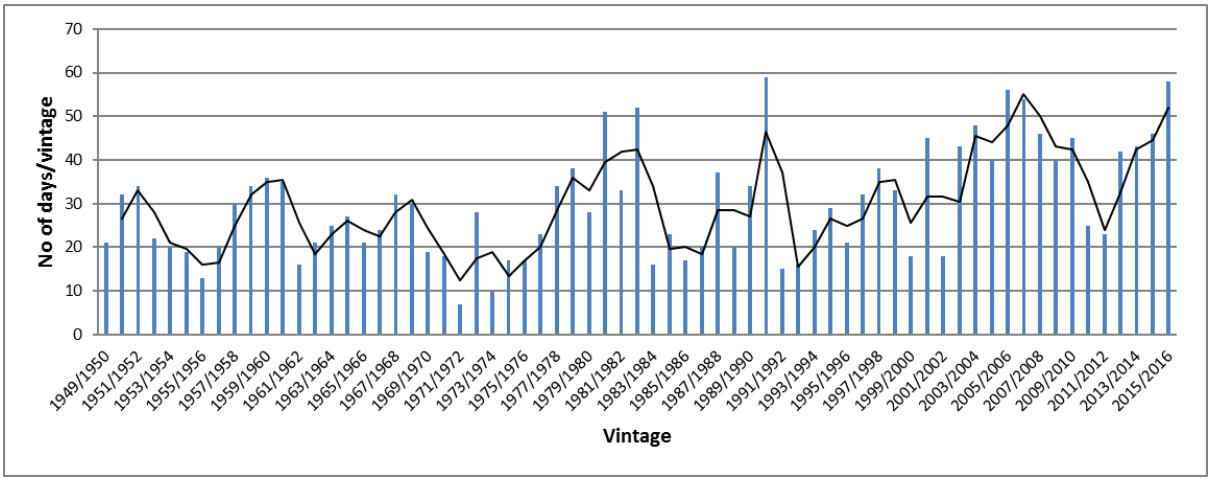


Figure 9: Vintages with days when Tmax ≥ 35 °C in Griffith. Black line represents the moving average of two consecutive vintages.

6.1.1.2 Orange

Orange 650 m: Due to altitude differences and a 35 km air distance between vineyards selected in Orange, viticultural indices were calculated individually for both O1 and O2. O1, situated at 650 m altitude, can be classified as a Temperate-warm grape growing region with an average Huglin index from 1949/50 vintage to 2015/16 of 2370 and a median value of 2337 Huglin units. Figure 10 shows that some vintages over this period were cooler (HI< 2100) and were classified as Temperate vintages. Warmer vintages (HI≥2400) are also evident. An observation of an increase in the number of warmer vintages is less apparent for Orange at altitudes of 650 m compared to the Griffith region. However, the moving average of Huglin index over consecutive years appears to indicate a trend towards warmer vintages since 2002/03.

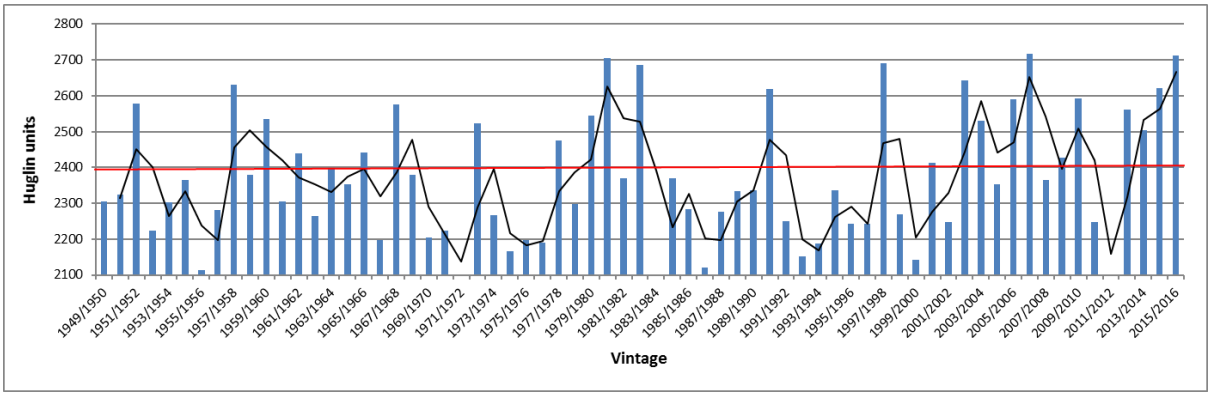


Figure 10: Vintages classified according to the Huglin index as Temperate warm. Vintages with bars above 2400 Huglin Units (red line) can be classified as warm vintages according to the Huglin index. Black line represents Huglin index moving average of 2 years.

According to the Cold night index, vineyards at 650 m altitude in Orange for January-March (months when ripening occurs) are classified as cool areas (CI>12≤14) with temperate nights (CI>14≤18). Figure 11 presents this data for 1950 until 2016. In recent years, there has been a slight increase in nights considered as temperate.

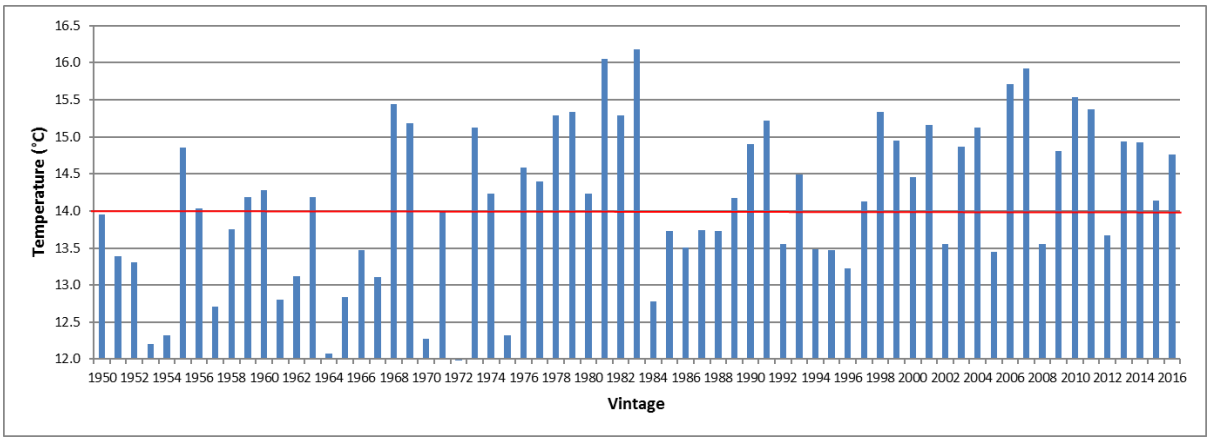


Figure 11: Vintages with calculated Cold night index for January to March above 12°C in Orange 650 m altitude. Red line represents the limitation between cool and temperate nights according to the Cold night index.

Orange 950 m: The average Huglin index (1949/50 - 2015/16) for vineyards located at 950m altitude in Orange (O2) was 1999, classifying this area as a Temperate climate (HI >1800≤2100). Large inter-seasonal variations were observed (Figure 12) with some cooler vintages being classified as Cool (HI >1500≤1800) according to the Huglin index, whereas the majority of vintages fall into the Temperate class (HI >1800≤2100). There were also some vintages with HI>2100 which were classified as temperate warm (HI >2100≤2400), Figure 12.

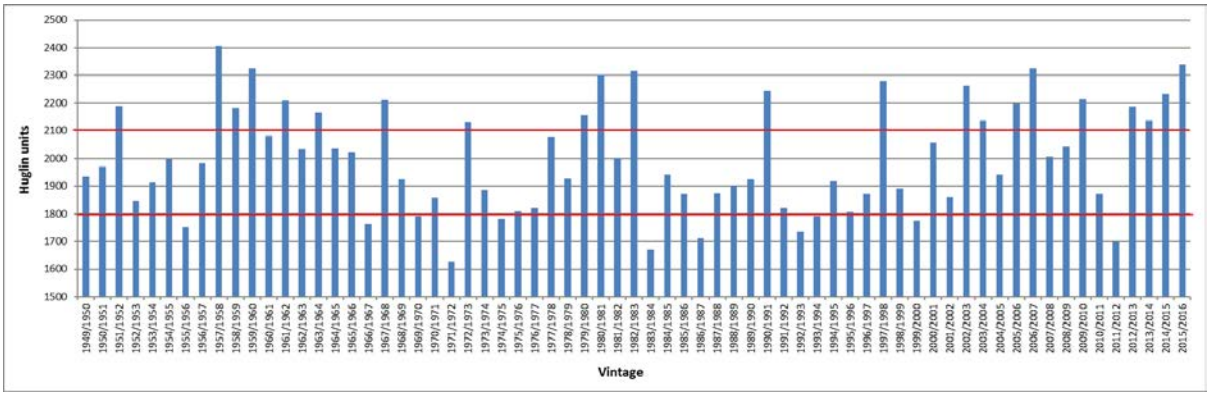


Figure 12: Huglin index for vineyards located at 950 m altitude in Orange. Red line represents the delimiter between cool and temperate climate according to the Huglin classification and temperate and temperate warm climate.

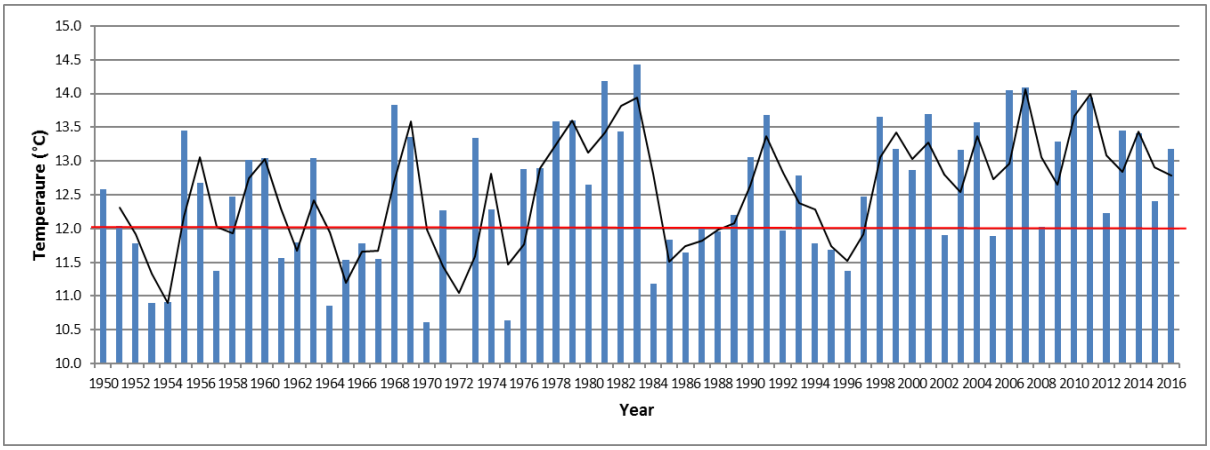
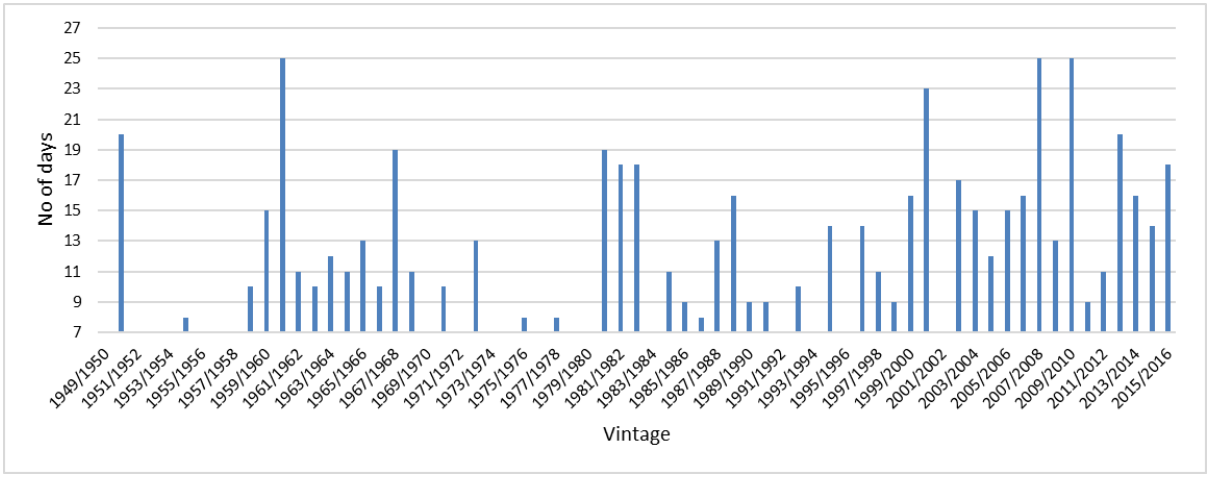


Figure 13: Vintages classified according to the Cold night index having Very cool or Cool nights. Vintages with bars above 12 °C (red line) can be classified as cool night vintages and below the red line as very cool night vintages. Black line represents Cold night index moving average of two years.

6.1.1.3 South Australia

McLaren Vale can be classified as a region with a temperate to temperate warm climate according to the Huglin index. However, an increase in warmer vintages since 2000 onwards is noted (Figure 14), corresponding with the increased temperatures for the Griffith region over the corresponding period. According to the GDD days McLaren Vale can be classified as a warm region (GDD >1667≤1994). Unsurprisingly an increase in warmer vintages according to the GDD index was also observed from 2000 onwards.

McLaren Vale can be classified as a region with temperate warm nights for January-March using the Cold night index (Figure 15, CI >14≤18). Slightly warmer nights are noticed for the last decade,



however, some vintages with warmer nights are evident in earlier years. Interestingly, an increase in the frequency of days with Tmax≥35°C was also noted from 2000 onwards (Figure

16). Climatic data for regions of interest in this investigation and for which interesting comparisons may be made are presented in Table 10.

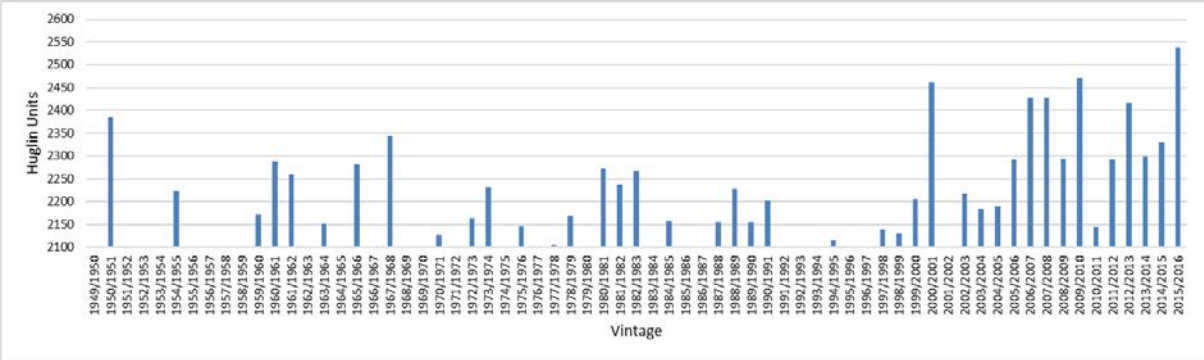


Figure 14: Vintages with calculated Huglin index above 2100 units in McLaren Vale.

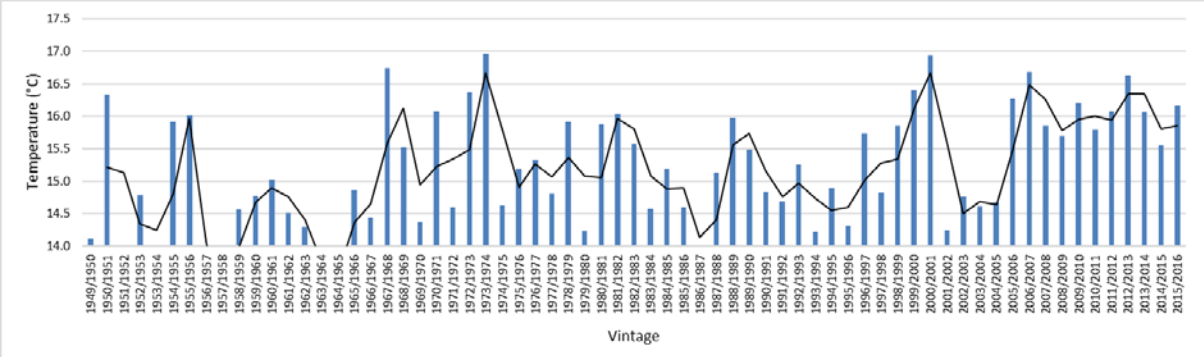


Figure 15: Vintages that can be according to the Cold night index classified as vintages with temperate nights for period January-March in McLaren Vale. Black line represents moving average for two consecutive vintages.

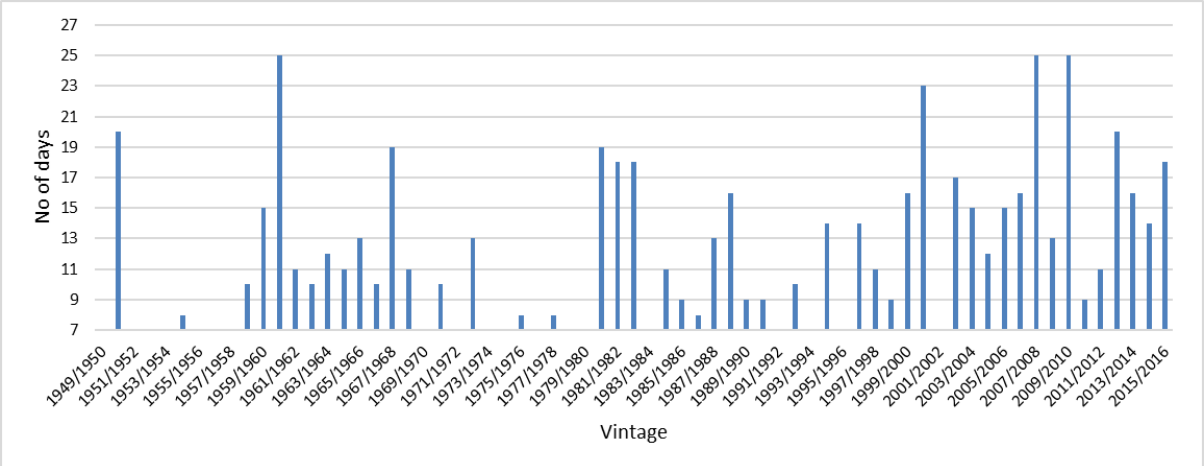


Figure 16: Vintages with number of days with Tmax ≥35°C in McLaren Vale.

Table 10: Summaries of the calculated viticultural indices for interesting locations across Australia.

	Huglin index			Cold night index			GDD		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**
Griffith S 34.35, E 146.00	2452	2809	3289	13.95	16.29	18.18	1983	2312	2859
Orange 650 m S 32.95, E 149.00	2053	2370	2337	11.58	14.2	16.2	1485	1842	2248
Orange 950 m S 33.25, E 149.05	1628	1971	2406	9.8	12.6	14.4	1041	1454	1841
McLaren Vale S 35.20, E 138.55	1828	2152	2538	12.1	15.1	17	1457	1870	2242
Barossa Valley S34.45, E 139.00	2194	2541	2976	12.6	14.7	16.8	1688	2087	2473
Tumbarumba S35.70, E 148.00	1342	1645	2160	8.3	10.8	13.0	748	1086	1624
Yarra Valley S37.60 E 145.40	1460	1762	2073	9.8	12.1	13.7	-	-	-
Yarra Valley S37.95 E 145.55	1298	1604	1887	9.5	11.7	13.4	-	-	-
Yarra Valley S37.65 E 145.45	1690	2008	2263	10.6	12.7	14.6	-	-	-
Yarra Valley S37.70 E 145.40	1668	1988	2238	10.7	12.7	14.7	-	-	-
Coonawarra S36.95 E 139.75	1411	1768	1996	11.3	13.0	14.7	-	-	-
Coonawarra S37.30 E 140.85	1565	1944	2241	9.8	11.4	14.1	-	-	-
Tasmania S41.35, E 147.00	1087	1419	1722	8.9	11.0	12.5	-	-	-
Tasmania S42.80, E 147.45	1223	1484	1830	9.6	11.4	13.3	-	-	-

Yarra Valley locations have been calculated for the period 1949/50 - 2013/14.
Coonawarra locations have been calculated for the period 1949/50 - 2014/15 whereas indices for other locations have been calculated for the period 1949/50 - 2015/16.

6.1.1.4 Mesoclimatic temperatures

No significant differences in mesoclimatic hourly data were noted between vineyards located in Griffith (Figure 17). In all the vineyards the lowest mean temperature was observed between 5 and 6 am, whereas the daily temperature peak was noticed between 3 and 4 pm.

Comparison of the mean hourly temperature evolution for the period from 11 September 2014 to 18 April 2015 for the vineyards selected in two different regions, i.e. Griffith and Orange at 650 m and 950 m altitude, revealed important differences. As expected the highest average mean temperatures were recorded in Griffith. Interestingly, important differences in mean hourly temperatures were noticed also for both locations in Orange, with the vineyards at 950 m characterised particularly with lower night temperatures compared to the vineyards at location O1 (650 m altitude). The latter vineyards also had higher maximum mean temperatures

compared to vineyards at 950 m whereas there were no differences in mean hourly temperatures between both vineyards in the morning, 8-11 am (Figure 17).

Comparison of mesoclimatic data showed differences between the 2013/14 and 2014/15 vintages in Griffith. In the 2014/15 growing season, October, November and December were 2.2˚C, 2.1˚C and 0.2˚C, respectively, warmer compared to 2013/14. A warm start of the 2015 season was followed by milder January and February compared to 2013/14 vintage, which were 2.2˚C and 0.2˚C cooler.

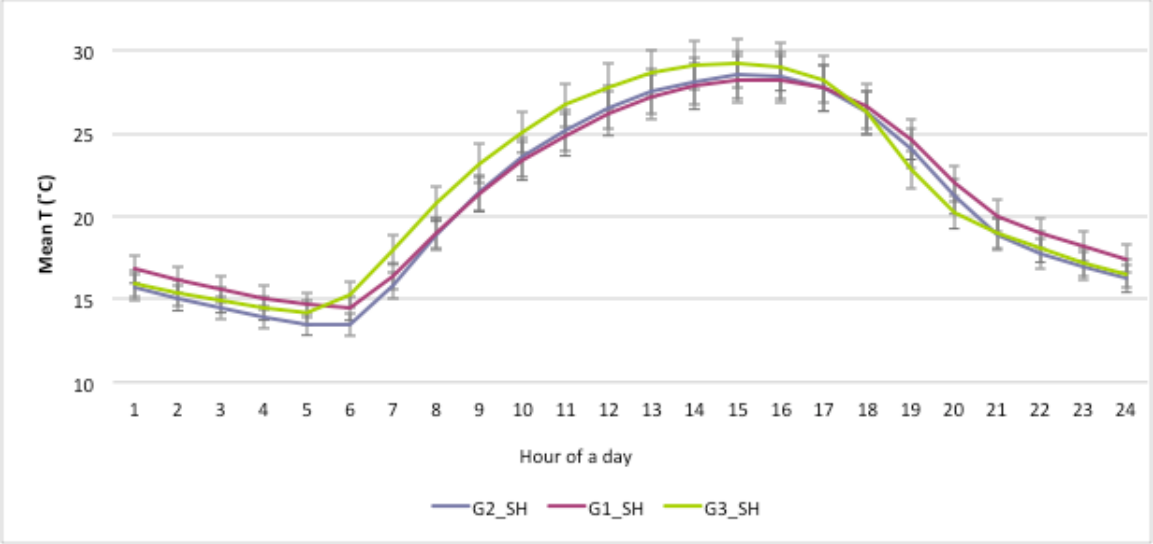


Figure 17: Mean hourly temperature calculated for the period 11 September 2014 to 18 April 2015 for three locations in Griffith.

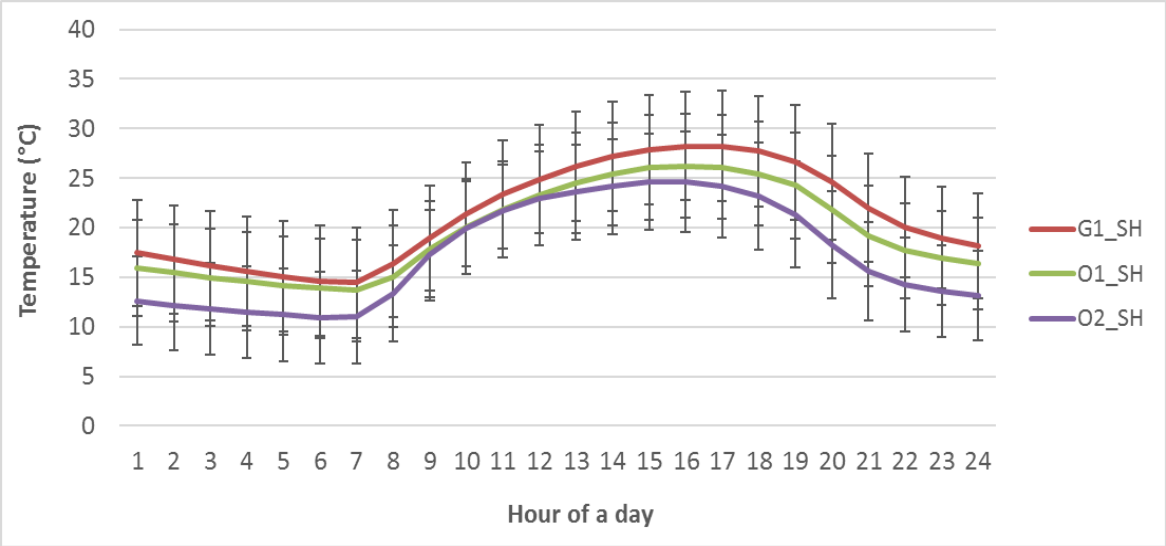


Figure 18: Comparison of a mean hourly temperature evolution calculated for the period 11 September 2014 to 18 April 2015 for the vineyard G1_SH in Griffith and vineyards in Orange at 650 m (O1_SH) altitude and 900 m altitude (O2_SH).

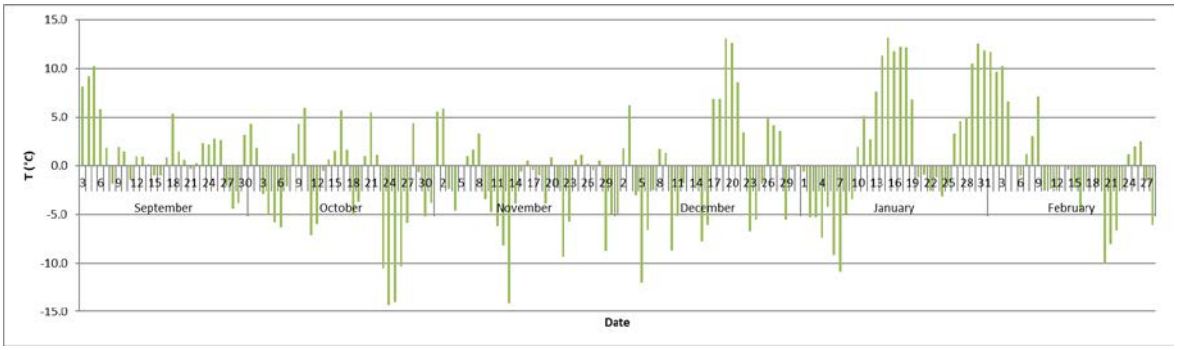


Figure 19: 2013/14 season average daily temperatures normalised against average daily temperatures in 2014/15 season.

Furthermore, the 2013/14 season was also characterised with more heat waves in the period from December to February, compared to the 2014/15 season (Figure 20). Heat waves (Tmax ≥ 35°C) in January and February 2014 were long and the Tmax reached 40°C. In the 2014/15 vintage, only several isolated days with Tmax ≥ 35°C were observed (Figure 21).

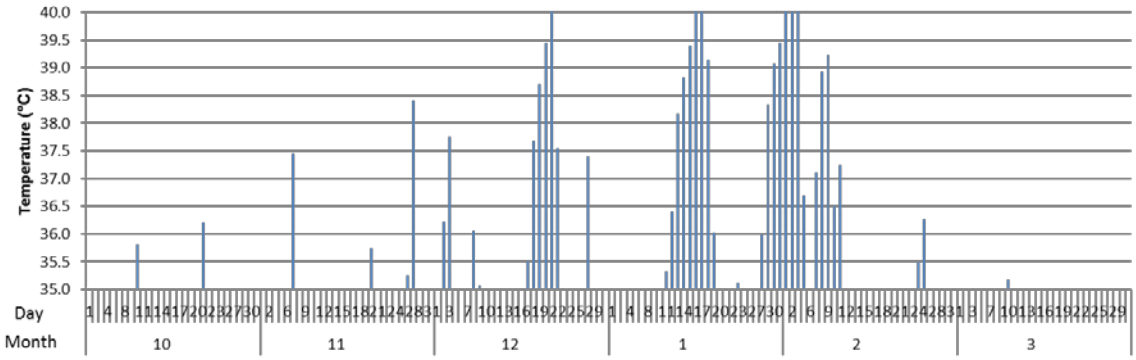


Figure 20: Number of days and average Tmax in G1_SH vineyard when Tmax was equal or higher than 35 ˚C in 2013/14 season.

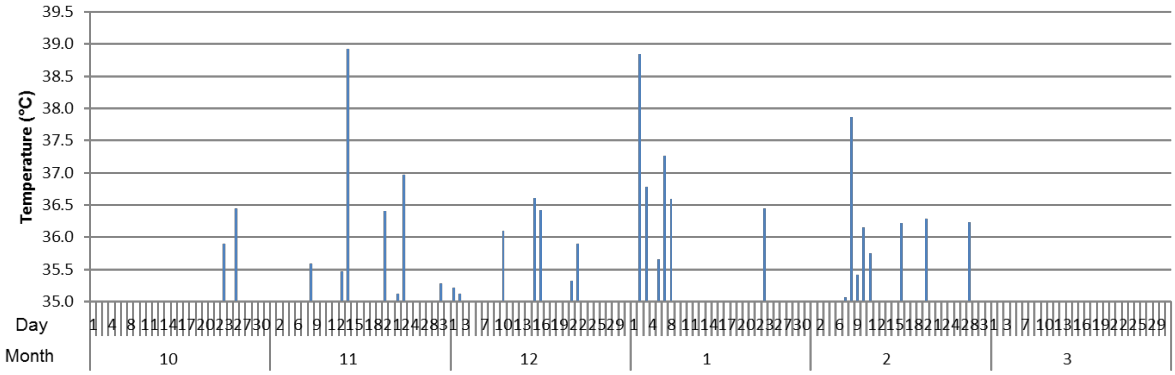


Figure 21: Number of days and average Tmax in G1_SH vineyard when Tmax was equal or higher than 35 ˚C in 2014/15 season.

Mesoclimatic data for Orange revealed the trend for 2014 and 2015 vintages were similar to observations for Griffith. The 2015 vintage was slightly warmer in the first growing period. The average monthly temperature in October, November and December was 18.8, 21.7 and 23.9°C, respectively, which is 2.2, 2.1 and 0.2°C higher than measured in October, November and December 2014, respectively. January 2015 was 2.2°C cooler than January 2014 and February 2015 was 0.2°C cooler. January, February and March were warmer in 2015/16 season compared to the previous vintage. A warmer end of the season could partly explain significant berry shrivelling observed in Shiraz and Cabernet Sauvignon vineyards in Orange for this vintage.

6.1.2 Vine water availability and vine water status

Vine water status was measured using a Schollander pressure chamber six times in the 2013/14 season, in both G1_SH and G2_SH before veraison during application of a restricted irrigation regime. Stem water potential in G1_SH vineyard varied between -1.5 MPa to -1.0 MPa corresponding to a high to moderate water deficit (Deloire et al. 2004). Stem water potential measurements in the G2_SH vineyard varied from -1.5 MPa to -1.1 MPa during the same season. In both vineyards (G1_SH and G2_SH) the least severe water deficit, -1.0 and -1.1 MPa, respectively, was measured around veraison. Restricted irrigation pre-veraison is a common strategy to attempt to control final berry weight and higher irrigation around veraison period is sometimes used to encourage fast and synchronous berry ripening. The pre-veraison period in berry development is crucial as it determines the number of cells in the berry and berry cell enlargement capacity (Ojeda et al. 2001). Therefore, unsurprisingly relatively low average berry weights of 1g/berry were observed in both vineyards. Smaller berries lose less water during the late ripening compared to larger berries.

A similar irrigation strategy was employed in the vineyards where watering was determined by measuring soil moisture with sensors installed in both vineyards. The irrigation strategy comprised of cycles of re-filling (-20 to -50 kPa measured with soil moisture sensors) and draining soil water profiles until dryness (-500 kPa according to the soil moisture sensors).

The average stem water potential in G_CS vineyard was between -1.45 and -1.30 MPa throughout the season 2013/14, which contributed to the small berry weight, i.e. 0.7 g at the last harvest date. In the 2014/15 season, a similar irrigation strategy was applied in the G1_SH vineyard. Stem water potential measurements were between -1.45 and -1.21 MPa. The similar irrigation strategy in 2015 vintage resulted in similar yield/vine and berry size (1g/berry in both vintages) for G1_SH. Contrary, G2_SH and G_SH vineyards received more water in 2014/15 compared to 2013/14 vintage. Soil moisture profiles of G2 and G_CS vineyards for season

2014/15 are shown in Figure 22 and Figure 23, indicating well refilled soil profiles, with little restriction in available water to the plants.

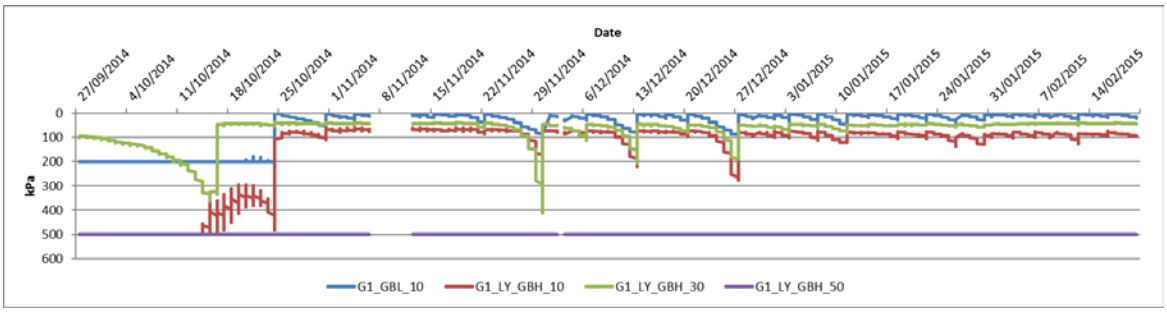


Figure 22: Soil moisture in the G2 vineyard for in vintage 2014/15.

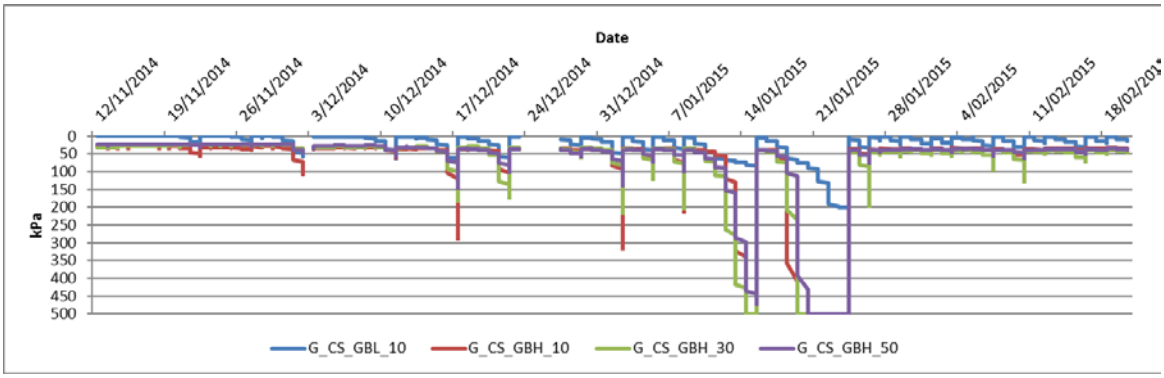


Figure 23: Soil moisture in G_CS vineyard for in vintage 2014/15.

Stem water potential measurements were for the G2_SH vineyard were around -1.00 MPa and for G_CS -0.82 MPa. Higher irrigation rates in the 2015 vintage resulted in higher yields and an increase in average berry weight of 20% in both G2_SH and G_CS vineyards, compared to 2014 vintage. Both high yielding vineyards G3_SH and G4_SH were well watered during the season and no visual water stress could be noted on vines. The stem water potential measurements were between -1.3 and -1.0 MPa for both vineyards.

In theO1_SH and O1_CS vineyards, restricted irrigation was applied due to the natural water limitations (Figure 24 and Figure 25) over the three years of investigation. O1_SH and O1_CS vineyards experienced occasional severe water constraints as evident from soil moisture sensors and visual inspection (Figure 26). Stem water potential measurements in the O1_SH vineyard confirmed severe water stress, with values reaching -1.9 MPa, a level which may cause vine and plant cell damage, in the 2015/16 vintage. As evident from Figure 24 and Figure 25, in both 2015 and 2016 vintages irrigation was reduced around the point of berry sugar accumulation plateau of in O1_SH. Some limited irrigation in O1_SH in the last phase of ripening

was applied and this resulted in berry fresh weight decreasing by around 11% from the maximum in the 2015 vintage. Berry weight loss in 2016 at the MF stage was around 70% of the maximum berry weight. The differences in berry weight loss during these vintages is attributed to difference in climatic conditions (a mild autumn 2015 and a warm autumn 2016) and very limited irrigation schedules following the sugar accumulation plateau in 2016.

High altitude vineyards O2_SH and O2_CS were not subjected to water constraints due to frequent precipitation and lower max temperatures. Therefore O2_SH and O2_CS were not irrigated during the 2014/15 and 2015/16 seasons. While stem water potential was not measured there were no apparent water restrictions or vine growth retardation in the O2 vineyards (Figure 27).

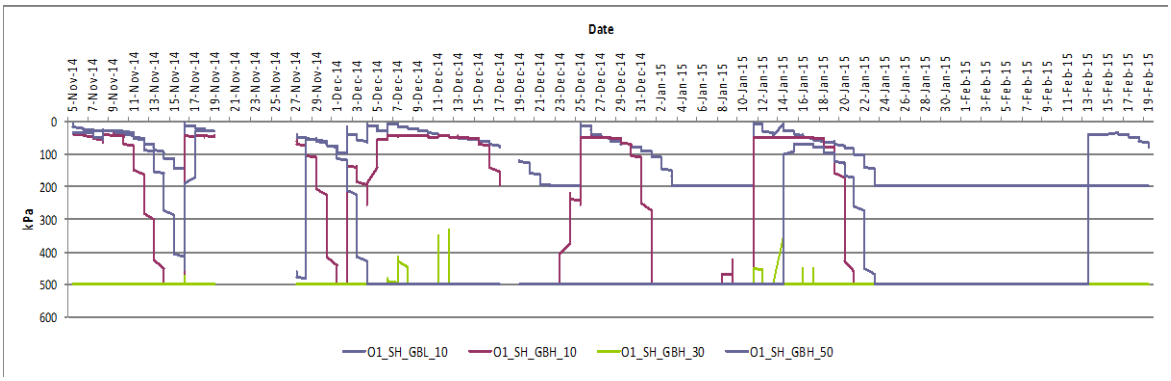


Figure 24: Soil moisture profile of O1_SH vineyard in 2014/15 season.

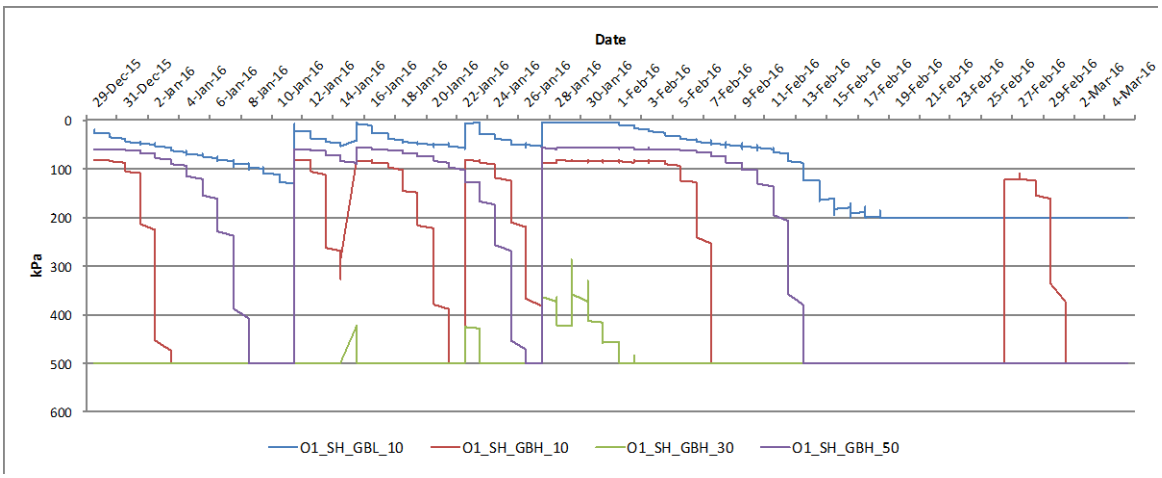


Figure 25: Soil moisture profile of O1_SH vineyard in 2015/16 season.



Figure 26: Left O1_SH and right O1_CS vineyard with severe water constraint.



Figure 27: O2_SH vineyard showing well trellised vines with sufficient water for unrestricted growth.

Additionally to the above described measurements performed in the vineyards, multispectral images of three commercial blocks in Griffith were taken.

6.2 Sugar accumulation method to predict harvest dates for red cultivars

This chapter is addressing following objective: provide growers and wineries with an objective management method and/or tool for harvest decisions and improve winery intake logistics.

Based on previous empirical observations, it was suggested that fresh, red fruit characters in Shiraz wines were reached approximately 12 days after a slowdown of sugar accumulation into the berry, whereas dark/jammy fruit sensorial descriptors appeared after approximately 24 days. This hypothesis was confirmed by the sensory analyses of all investigated vintages of Shiraz, with the FF wines described as having higher perception of red fruit characters. Wines made from grapes harvested at the MF stage were perceived by panellists, irrespective of the site or vintage, as higher in dark fruit and jammy/stewed fruit characters.

In the 2015 season, a third Intermediate harvest date was conducted from both Shiraz and Cabernet Sauvignon vineyards. Wines from this grape maturity stage could not be consistently distinguished from FF or from MF wines by sensory analysis, but were sufficiently different to indicate that some berry characters were influencing wine style. These results suggest that for Shiraz, around additional days of maturation are required to produce wines with different sensory properties compared to the FF stage.

For Cabernet Sauvignon, the ripening period is longer, therefore the berry sugar accumulation model predicted that 20 days maturation post plateau of sugar accumulation were required to achieve the fresh, red fruit characters in wines. Mature fruit stages were expected after approximately 40 days, producing to dark and jammy fruit characters. For Cabernet Sauvignon, an Intermediate harvest stage was performed in the 2015 vintage, which was also difficult to distinguish from either the FF or MF stages with sensory evaluation.

Sugar loading curves and berry fresh mass were determined for all the experimental plots and are shown in Figure 28 and Figure 29. Plateaus of sugar accumulation and berry fresh mass were reached (Figure 28 and Figure 29) for all the vineyards in Griffith in both vintages. Therefore it appears that sugar accumulation per berry was not affected by the heat waves and by deficit irrigation applied prior to veraison in the vineyards. Deloire 2014a suggested that sugar accumulation per berry and berry fresh mass evolution could also be used as indicators of balanced vineyard management. Adequate vine water avaliability directly affects berry cell hyperplasia and hypertrophy in early developmental stages, whereas severe water stress can hasten berry cell death and the onset of berry water loss in Shiraz in the latter stages of maturation (Bonada 2015). The tempo of berry sugar accumulation is linked to plant

photosynthetic activity and can provide information about vine functioning. After the plateau of berry sugar accumulation, any futher change in berry TSS concentration is solely a concentration or dilution effect due to or decreasing or increasing berry fresh weight.

In the 2015 vintage the berry sugar accumulation model was also tested on two high yielding Shiraz vineyards (G3 and G4) at the request of the participating wineries. Plateaus of sugar accumulation were reached in the same manner as seen in the lower yielding vineyards and the G4 vineyard was harvested commercially two days before the predicted MF stage. The G3 vineyard was harvested nine days after the predicted MF.

In the 2014 vintage, both the Shiraz and Cabernet Sauvignon from the O1 vineyards showed no clear plateau of sugar accumulation (Figure 28). Instead, a slow and constant sugar accumulation in the berry throughout the ripening was observed, which was attributed to severe water restrictions (Figure 24). The O1 2014 vintage was characterised by drought in the first ripening period, followed by the several rain events around harvest time. Water supply in the late maturation stage could explain a reactivation of the photosynthetic mechanism, resulting in ongoing sugar accumulation into the berry. To further understand the vine physiology in the O1_SH and O1_CS vineyards, stem water potential was measured and soil moisture sensors installed for the 2014/15 season.

In the 2015 season, berry sugar accumulation curves for both cultivars and both vineyards in the Orange region closely followed the predicted model curve (Figure 29). The observed plateau of sugar accumulation in the 2015 O1 vintage, in contrast to the 2014 vintage, could be explained by higher water availability and milder climatic conditions during the late ripening period. These conditions provided a more balanced grape ripening period. The dates of the berry sugar accumulation plateau, both predicted and actual, in addition to the commercial winery harvest dates, are presented in Table 11.

In Griffith, commercial harvest dates in both vintages coincided with the predicted MF maturity window for both Shiraz and Cabernet Sauvignon (Table 11). In the 2014/15 season some commercial harvest dates in Griffith (G1_SH vineyards) were delayed by approximately 10 days due to rain events at the MF stage. Contrary to observations in Griffith, commercial harvest dates in Orange coincided with the predicted FF or IF harvest dates in both the 2015 and 2016 seasons at both locations for both Shiraz and Cabernet Sauvignon.

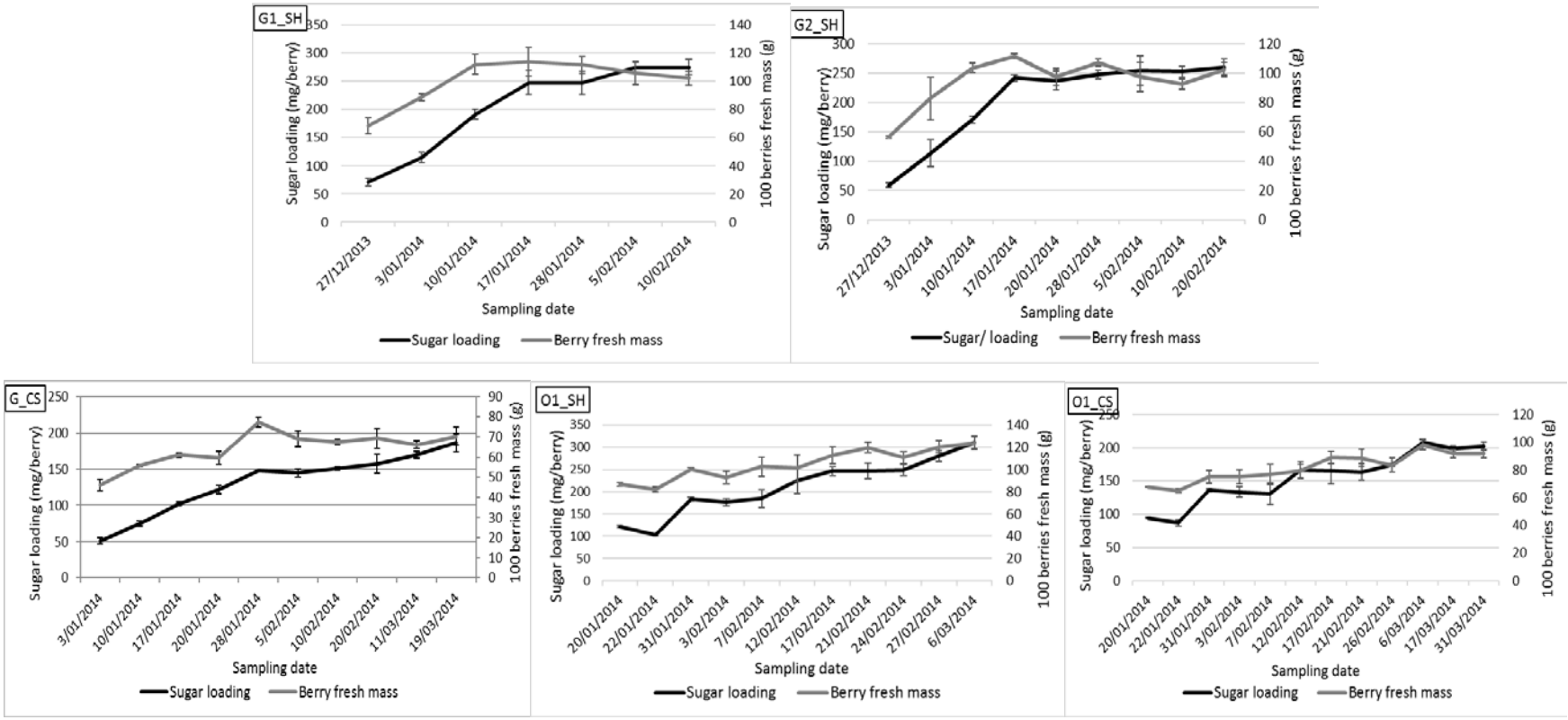


Figure 28: Sugar loading (mg/berry) and berry fresh mass evolution (g) for selected vineyards in Griffith and Orange in 2013/14 vintage.

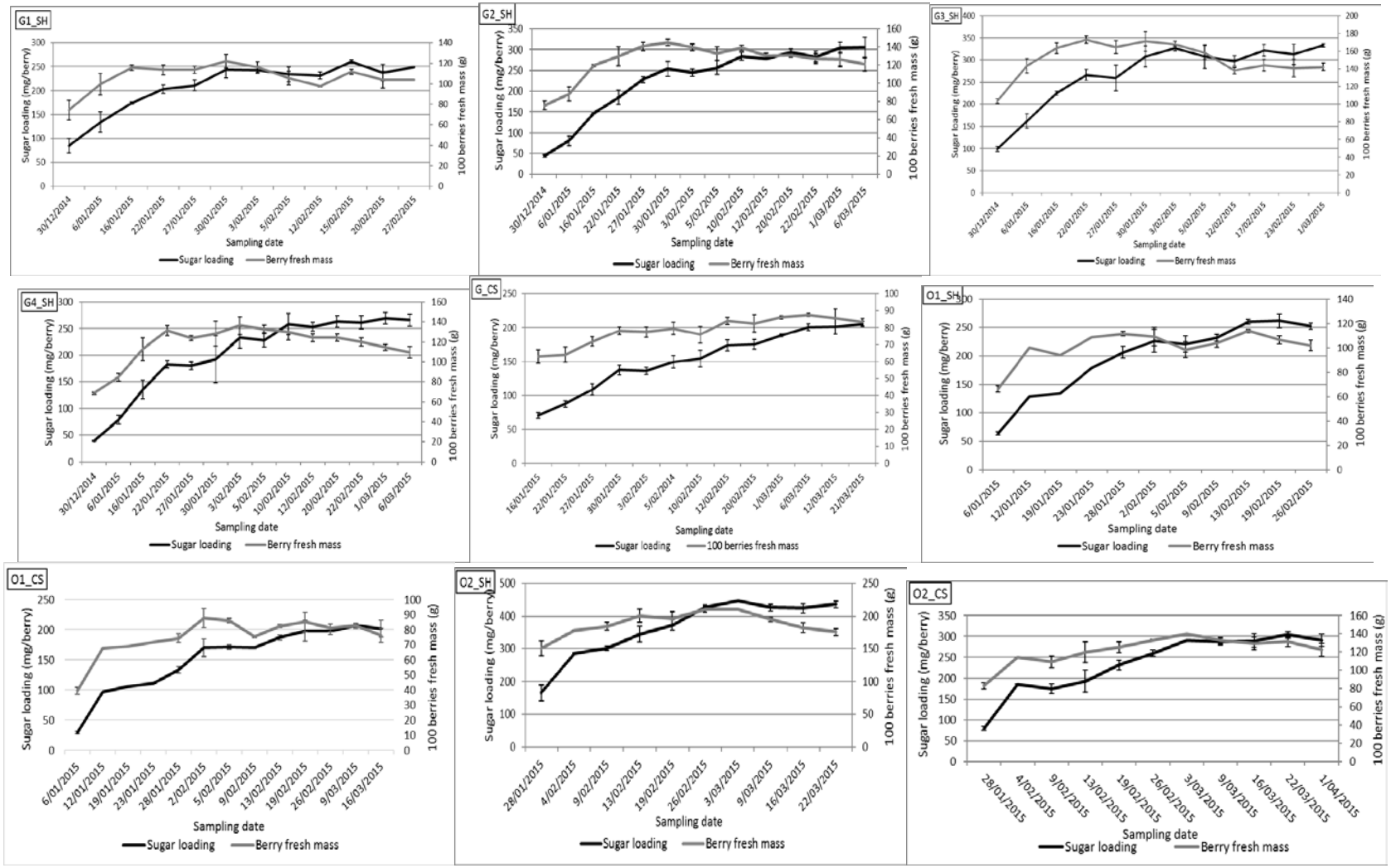


Figure 29: Sugar loading (mg/berry) and berry fresh mass evolution (g) for selected vineyards in Griffith and Orange in 2014/15 vintage.

Table 11: Dates of sugar accumulation plateau, predicted, actual and commercial harvest dates for Shiraz and Cabernet Sauvignon vineyards.

Vintage	Vineyard		Plateau	Pre-FF	Fresh Fruit (FF)	Intermediate Stage (IF)	Mature Fruit (MF)	Post MF	Commercial Harvest
2014	G1_SH	Predicted	17/1/2014	X	29/1/2014•	X	10/2/2014	X	13/2/2014
		Harvested		X	28/1/2014	X	10/2/2014	X	
	G2_SH	Predicted	17/1/2014	X	29/1/2014•	X	10/2/2014	20/2/2014	29/1, 10/2 and 20/2/2014
		Harvested		X	28/1/2014	X	10/2/2014	20/2/2014	
	G_CS	Predicted	28/1/2014	10/2/2014	17/2/1014	X	9/3/2014	17/3/2014	20/2, 9/3 (main harvest)
		Harvested		10/2/2014	20/2/2014	X	11/3/2014	19/3/2014	
	O1_SH	Predicted	31/1/2014	X	15/2/2014	X	27/2/2014	9/3/2014	26/2/2014
		Harvested		X	Rain harvested	X	24/2/2014	6/3/2014	
	O1_CS	Predicted	17/2/2014	X	X	X	29/3/2014	X	15/3/2014
		Harvested		24/2/2014	X	17/3/2014	31/3/2014	X	
2015	G1_SH	Predicted	3/2/2015	X	15/2/2015	21/2/2015	27/2/2015	X	6/3/2015
		Harvested		X	15/2/2015	20/2/2015	27/2/2015	X	
	G2_SH	Predicted	10/2/2015	X	22/2/2015	28/2/2015	6/3/2015	X	24/2 and 4/3/2015
		Harvested		X	22/2/2015	1/3/2015	6/3/2015	X	
	G3_SH	Predicted	5/2/2015	X	17/2/2015	23/2/2015	1/3/2015	X	9/3/2015
		Harvested		X	17/2/2015	23/2/2015	1/3/2015	X	
	G4_SH	Predicted	10/2/2015	X	22/2/2015	28/2/2015	6/3/2015	X	4/3/2015
		Harvested		X	22/2/2015	1/3/2015	6/3/2015	X	
	G_CS	Predicted	10/2/2016	X	2/3/2015	12/3/2015	22/3/2015	X	
		Harvested		X	1/3/2015	12/3/2015	22/3/2015	X	
	O1_SH	Predicted	2/2/2015	X	14/2/2015	20/2/2015	26/2/2015	X	17/2/2015
		Harvested		X	13/2/2015	19/2/2015	26/2/2015	X	
	O1_CS	Predicted	5/2/2015	X	25/2/2015	7/3/2015	17/3/2015	X	4/3 and 11/3/2015
		Harvested		X	26/2/2015	9/3/2015	16/3/2015	X	
	O2_SH	Predicted	26/2/2015	X	10/3/2015	16/3/2015	22/3/2015	X	10/3/2015
		Harvested		X	9/3/2015	16/3/2015	23/3/2015	X	
	O2_CS		3/3/2015	X	23/3/2015	2/4/2015	12/4/2015	X	17/3/2015
				X	23/3/2015	1/4/2015	X	X	

6.2.1 Outputs: Sugar Accumulation as a Predictor of Harvest Date

- Estimations of potential harvest date, using sugar accumulation per berry as a potential physiological indicator, proved to be reliable in balanced vineyards.
- Commercial harvest dates coincided with the predicted FF or MF harvest dates for both vintages. Interestingly, in Griffith commercial harvest dates coincided with the predicted MF stage, whereas in Orange the commercial harvests were aligned with the predicted FF or IF stages.
- A plateau of sugar accumulation was observed for all the vineyards as predicted by the model curve. The only exceptions were O1_SH and O1_CS in 2014, when vines were subjected to severe water constraints which appear to have inhibited vine function.
- Sugar accumulation per berry is a reliable indicator of grapevine function vine performance within a specific a vineyard. Based on the desired wine sensory profiles, it can be used to predict appropriate harvest dates.
- Sugar accumulation per berry could also be used as a tool to help schedule harvesting and grape intake 12-40 days in advance.

6.3 Validation of a model of grape aromatic maturity by sensory analysis

This project has demonstrated sugar accumulation per berry reached a plateau at around 20 Brix. This was observed in Griffith for the both the 2014 and 2015 vintages and in Orange in 2015. It is important to note that vineyards are required to be balanced and have adequate water. The date when sugar accumulation per berry subsequently slowed became the pivotal point of the presented maturity model. The harvest dates were predicted from this date in order to obtain an aromatic sequence in the produced wines. A distinct separation, from fresh/red fruit to mature/dark followed by stewed fruit, was apparent in the corresponding Shiraz and Cabernet Sauvignon wines. These differences were assessed by a trained sensory panel using sensory descriptive analysis (DA).

6.3.1 Shiraz

In 2014, wines from two and three harvest dates from Griffith 1 and 2 Shiraz vineyards respectively, were described using DA. Shiraz wines from the Orange 1 vineyard were analysed by DA as only the mature and jammy fruit stages were able to be harvested. In 2015, all Shiraz wines, from three harvest dates (fresh, intermediate and mature fruit), were assessed. Trials for four vineyards were undertaken in Griffith as both participating wineries expressed a desire to investigate the suitability of the model for both low and high yielding vineyard sites in the second year. Two vineyard sites were trialled in the Orange region in order to investigate the difference between significantly different vineyard altitudes.

Figure 30 shows that in 2014 for both G1 and G2 vineyards, fresh fruit wines were associated with red fruit, acidity and the herbaceous attributes of tomato leaf, green olive and capsicum. Mature and post mature wines were characterised by higher astringency, dark fruit and plum characters. The astringency is probably related to increased tannin extraction with higher alcohol levels. Pepper aroma, often reported in Shiraz wines did not seem to be influenced by grape maturity. However, as Griffith is a very warm to hot viticultural region, the pepper attribute may not be especially evident in wines from this region.

In vintage 2015 wines from Griffith and Orange, fresh fruit wines were correlated with red fruit and the perception of acidity, while mature fruit wines were associated with dark fruit, plum, black cherry, astringency and alcohol perception. Intermediate stages were grouped more closely with the corresponding mature fruit wines from the Orange region but this grouping was not as evident for the IF wines from Griffith.

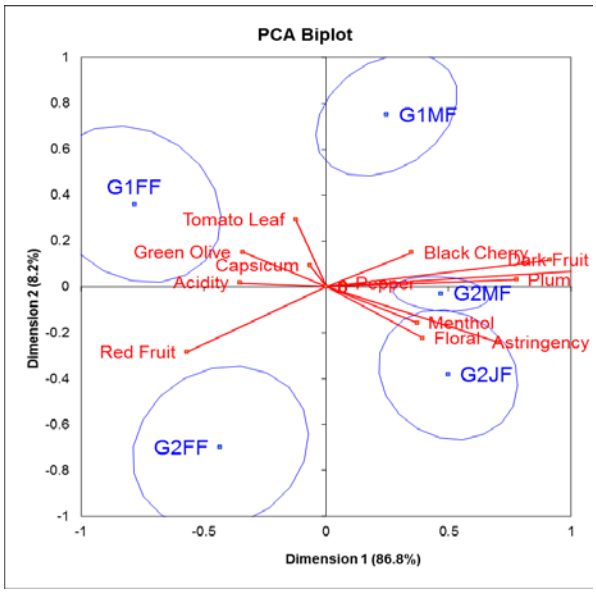


Figure 30: Principal Component Analysis biplot for sensory data of 2014 Shiraz Griffith wines. G1: Griffith 1; G2: Griffith 2; FF: fresh fruit; MF: mature fruit; JF: jammy fruit (post MF).

To further illustrate the existence of the two distinguishable maturity stages, fresh fruit and mature fruit, the mean ratings of red fruit, dark fruit and plum attributes were calculated for all Griffith and Orange wines in 2015 (Figure 31 and Figure 32). The sample groupings and loadings observed on PCAs were tested using Analysis of Variance (ANOVA; Figure 33). Red fruit aroma was perceived with a significantly higher rating in all Shiraz fresh fruit wines compared to those made from the corresponding mature fruit. In contrast, dark fruit and plum characters were rated at significantly higher levels in mature fruit compared to fresh fruit wines. Intermediate stage could not be distinguished from either fresh fruit or mature stages with consistency. Wines from this stage were generally rated in a similar manner as the mature fruit wines for red and dark fruit characters, and more closely to their fresh fruit counterpart for the plum attribute.

These results have demonstrated the occurrence of two harvest windows associated with specific wine styles for Australian Shiraz, from different geographical regions and vintages. These harvest opportunities are able to be predicted 12 and 24 days in advance using berry fresh mass and sugar accumulation as physiological indicators. In general, these two picking periods can be described with fresh/red fruit and mature/dark fruit characters respectively. The existence of an intermediate stage between fresh fruit and mature fruit windows was not readily observed and fruit characteristics were not able to be classified in a distinct manner.

Harvesting during the intermediate phase did not compromise Shiraz wine quality, but instead produced a less discernible wine style.

Once berry sugar accumulation reached a plateau, the aromatic profile evolution was not directly related to Baumé. The Shiraz wines from the G2 vineyard illustrate this finding. While all three harvest dates had very similar sugar levels (and similar alcohol levels in the resultant wines), the corresponding wines showed distinctly different sensory profiles (Figure 34). This example also shows that aromatic profile evolution can change relatively quickly, with observed differences in the wines made from grapes harvested within six days of each other.

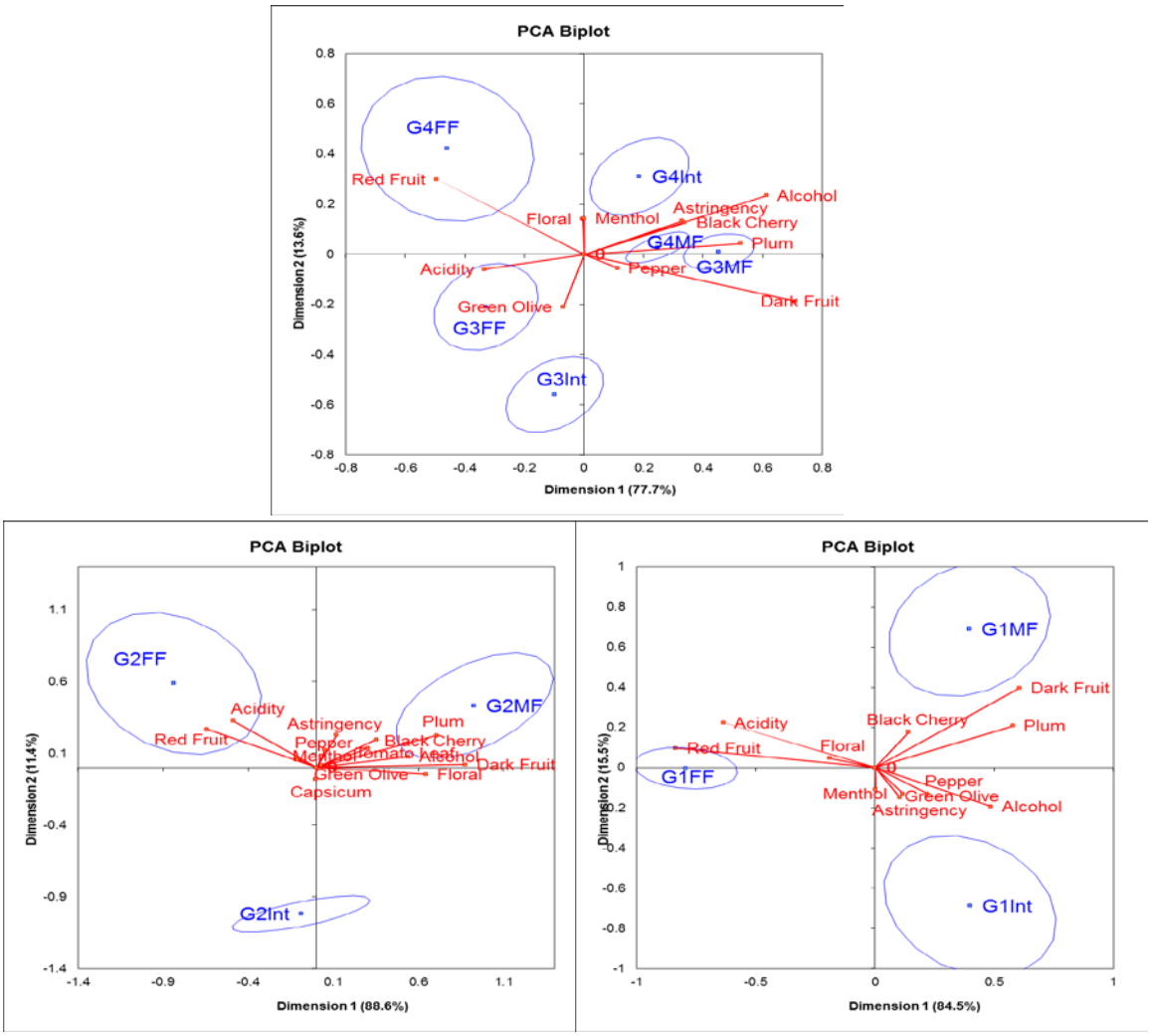


Figure 31: Principal Component Analysis biplot for sensory data of 2015 Shiraz Griffith wines. G1, 2, 3, 4: Griffith 1, 2, 3 and 4 vineyards; FF: fresh fruit; MF: mature fruit; Int, intermediate fruit maturity.

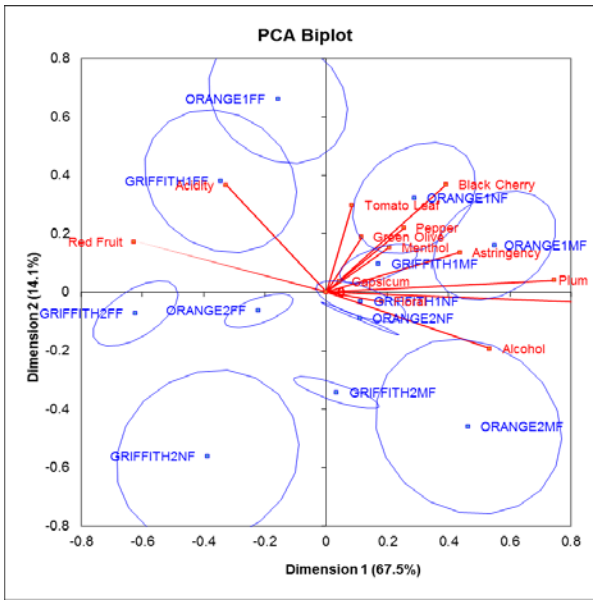


Figure 32: Principal Component Analysis biplot for sensory data of 2015 Shiraz Orange wines. O1, 2, Orange 1 and 2 vineyards; FF, fresh fruit; MF, mature fruit; Int, intermediate fruit maturity.

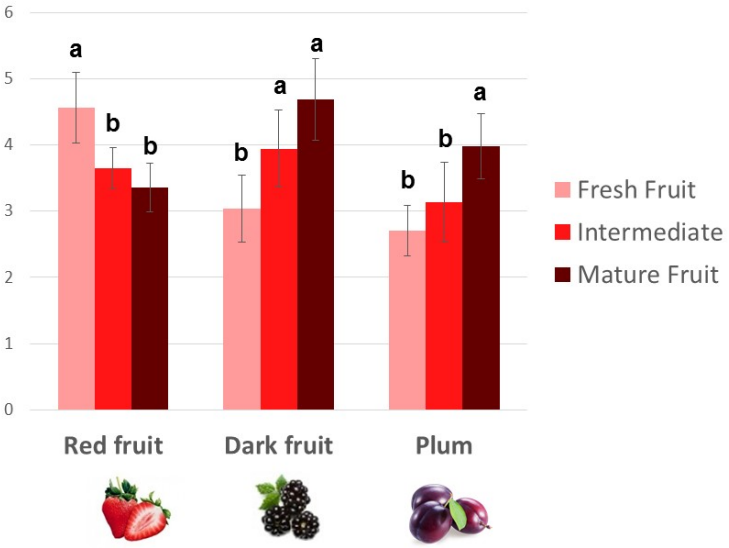


Figure 33: Mean rating ±SD of red fruit, dark fruit and plum attributes assessed in 2015 Shiraz wines (n=18 per harvest date). One way ANOVA was used to compare data. Different letters above bars indicate significance at p ≤ 0.05 (Fischer's LSD). All quoted uncertainty is the standard deviation of 18 wines.

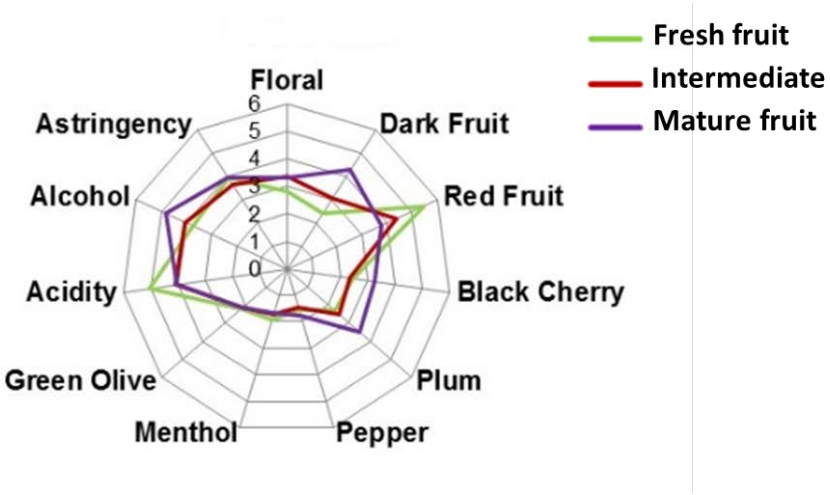


Figure 34: Mean sensory ratings for the 2015 Shiraz wines made from grapes harvested at different predicted harvest maturity dates.

The aromatic sequence previously described was also observed irrespective of harvest yield. The yields investigated in this study ranged from 8 to 20 tonnes per ha. A biplot of the sensory data for 2015 Griffith High yield vineyards (Figure 35) shows separation between Fresh fruit and Mature fruit stages according to the same attributes observed for low yield vineyards (red/dark berry fruit, plum, acidity, alcohol, astringency). The intermediate stage from the G3 vineyard had a sensory profile closer to Fresh fruit style whereas the wines from intermediate stage of the G4 vineyard were perceived closer to mature fruit profile (Figure 35).

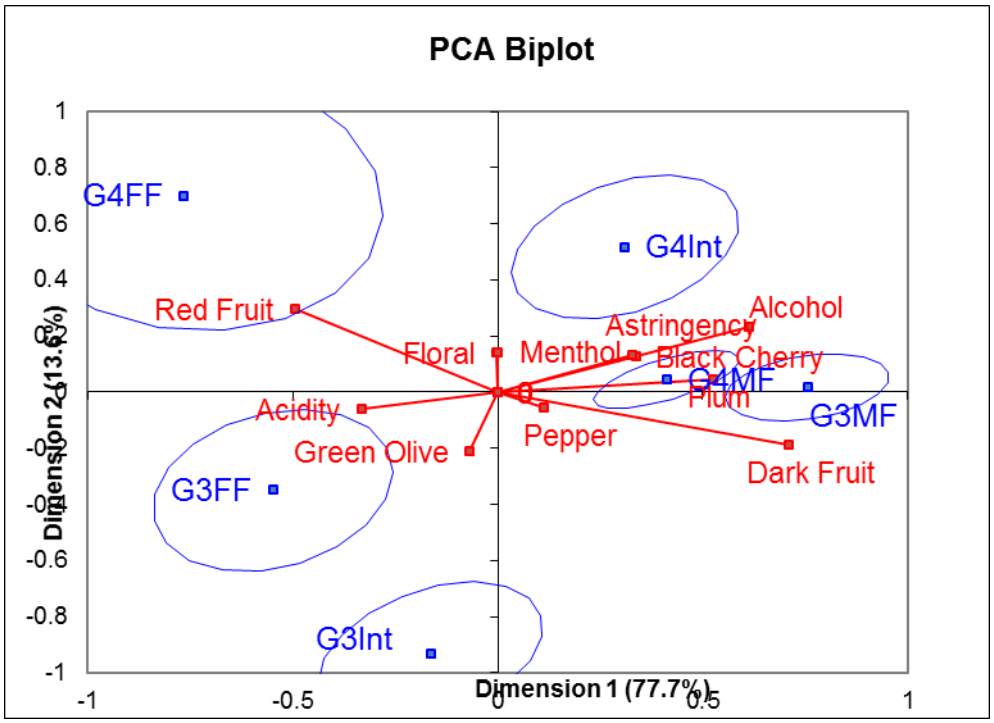


Figure 35: Principal Component Analysis biplot for sensory data of 2015 Griffith HY Shiraz wines. G3, and G4: Griffith 3 and 4 vineyards; FF: fresh fruit; MF: mature fruit; Int: intermediate fruit maturity.

6.4 Wine Sensory Results 2016

6.4.1 Shiraz 2016

The individual differences of the sensory attributes for the two Orange Region vineyards are shown in Table 12. Whilst there are similar trends to both the 2016 Cabernet Sauvignon wines and previous vintages, the differences in sensory results are not particularly well defined. While a consistent red to dark fruit trend is evident between the FF and MF wines from the O2 vineyard, with the FF more associated with red fruit, green olive and perceived acidity attributes, only the dark fruit character was found to be significantly higher (p<0.05) in the O2MF wine in comparison to the O2FF wine.

The PCA (Figure 36) illustrates that the O1 MF wines are more associated with plum, dark fruit and the perception of alcohol than the O1 FF Shiraz wines. Sample positioning indicates that riper fruit characters are the most important distinguishing attributes of these wines. The mean results in Table 12 reinforce this finding, with only the perception of alcohol being significantly higher in O1MF than in O1FF wines. The relative likeness of the Fresh and Mature stage wines from the O1 vineyard seem to the result of a combination of high temperatures, lack of water

and grape shrivel. The small difference in the perception of key sensory attributes in these wines corresponds to the similarity in the aromatic compound profile that was found (Table 12).

Table 12: Shiraz wine sensory attributes for Orange vineyards O2 Fresh and Mature Fruit (Sample Means and Fisher's LSD for Multiple Comparisons).

	O1FF		O1MF		O2FF		O2MF	
Red fruits	4.3	a	3.4	ab	3.8	ab	3.2	b
Dark fruits	4.7	a	5.1	a	3.3	b	4.7	a
Plum	4.4	ab	4.5	a	3.7	b	4.3	ab
Pepper	3.4	ab	3.8	a	2.5	c	3.0	bc
Menthol	3.1	a	3.2	a	2.7	a	3.3	a
Green Olive	3.0	b	3.1	b	4.1	a	3.5	ab
Floral	3.4	a	3.1	a	2.9	a	3.1	a
Acidity	4.7	ab	4.0	b	5.2	a	5.2	a
Alcohol	5.3	b	6.8	a	4.5	b	4.7	b
Astringency	4.7	ab	5.7	a	4.2	b	3.9	b

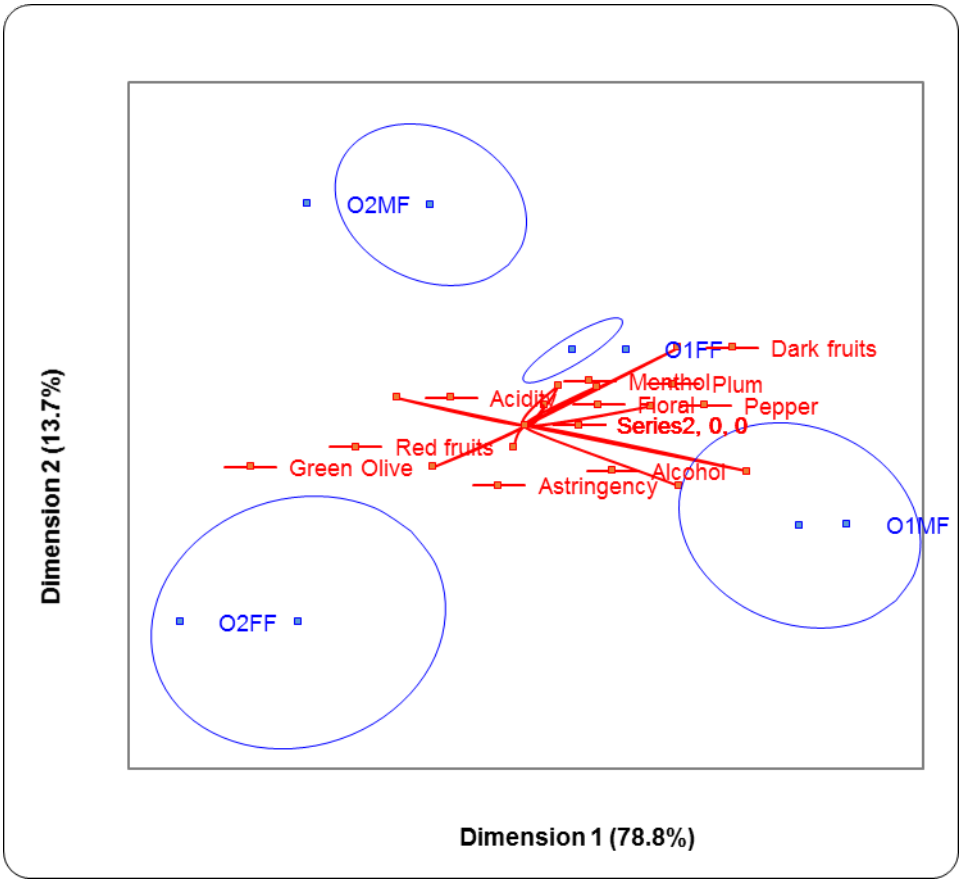


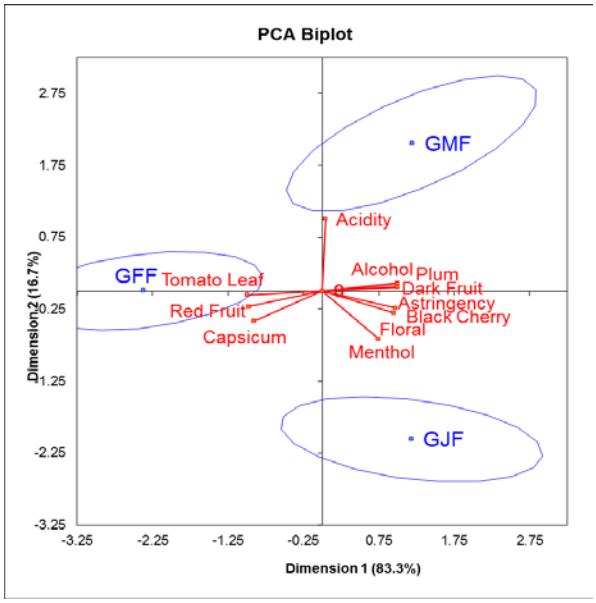
Figure 36: PCA biplot of 2016 Shiraz wines from O1 and O2 vineyards. FF: Fresh Fruit; MF: Mature Fruit maturity harvest stages.

6.4.2 Cabernet Sauvignon

In 2014, wines from four harvest dates (corresponding from pre-fresh to post mature), from the Griffith Cabernet Sauvignon vineyard were evaluated by DA. Wines from three harvest stages (pre-fresh fruit, intermediate and mature fruit) from Orange 1 vineyard were also analysed despite the lack of precision in determining the sugar plateau date due to unbalanced vineyard growth. In 2015, Griffith and Orange 1 wines from three harvest dates (fresh fruit, intermediate and mature fruit) and Orange 2 wines from two harvests (fresh fruit and intermediate) were evaluated.

Similar to the sensory results observed with the Shiraz wines, a consistent trend of aromatic evolution was observed for Cabernet Sauvignon wines irrespective of region and vintage. Wines made from early harvest dates (pre-fresh and fresh fruit) were consistently associated with red fruit and herbaceous attributes (capsicum and tomato leaf). Wines from mature and post mature stages were more correlated to dark fruit, plum and alcohol descriptors (Figure 37 and Figure 38).

A



B

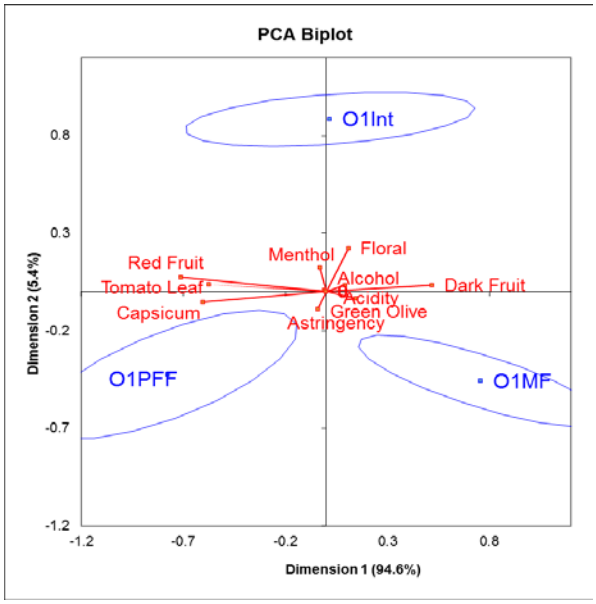
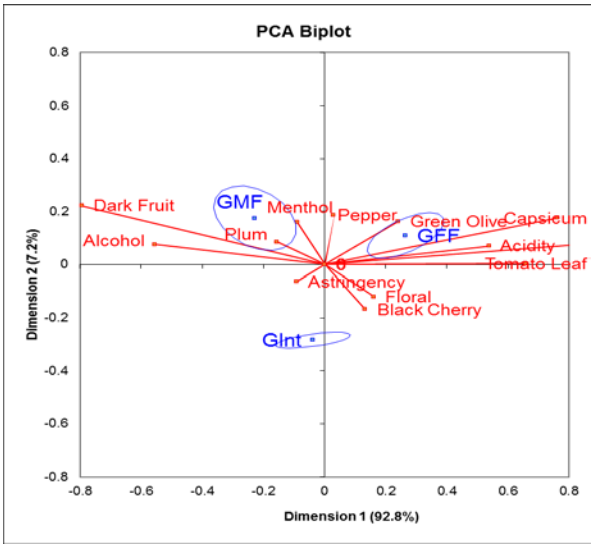


Figure 37: Principal Component Analysis biplot for sensory data of 2014 Cabernet Sauvignon in Griffith (A) and Orange (B) wines. G: Griffith; O1: Orange 1; FF: fresh fruit; Int: Intermediate Fruit: MF: mature fruit; JF: jammy fruit.

A



B

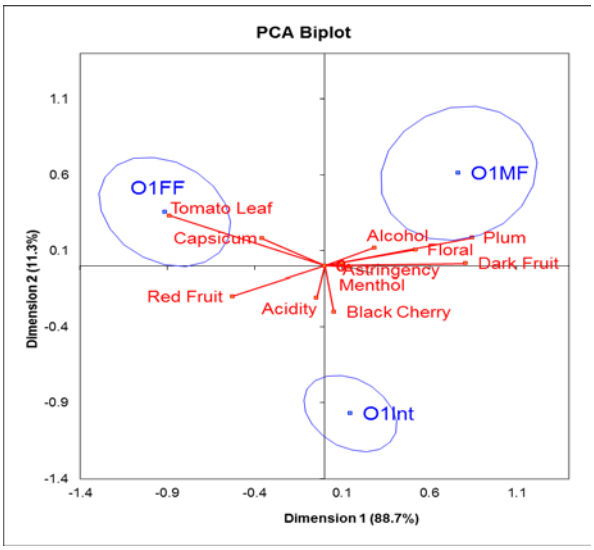


Figure 38: Principal Component Analysis biplot for sensory data of 2015 Cabernet Sauvignon in Griffith (A) and Orange (B) wines. G: Griffith; O1: Orange 1; FF: fresh fruit; Int: Intermediate Fruit; MF: mature fruit.

As for Shiraz, the mean sensory scores for 2015 wines from Griffith and Orange 1 Cabernet Sauvignon vineyards (Figure 39) showed the existence of two distinguishable harvest windows associated to specific wine styles, primarily associated with red fruit and dark fruit aromas respectively. The intermediate stage was not found to be significantly different from either from the fresh fruit and mature fruit stages. The late ripening period of Cabernet Sauvignon is slower than in Shiraz, explaining the delayed fresh fruit and mature fruit windows of 20 and 40 days after sugar accumulation plateau respectively. The existence of a 'neutral' intermediate stage

between fresh fruit and mature fruit windows was also not observed in Cabernet Sauvignon. Again the intermediate stage did not exhibit the same distinct fruit characters of the fresh and mature fruit stages.

The aromatic evolution from red to dark fruit spectrum has been previously reported for Australian Cabernet Sauvignon (Bindon et al. 2014). Nevertheless, the sequential harvest performed in that particular study was based on grape sugar concentration, which is an important indicator for harvest decision but not necessarily directly related to aromatic maturity. In contrast, using sugar accumulation per berry and berry fresh mass as physiological indicators, a synchronised evolution of Australian Shiraz and Cabernet Sauvignon wine styles was observed during grape ripening, irrespective of environment or traditional total soluble solids (TSS) measurements.

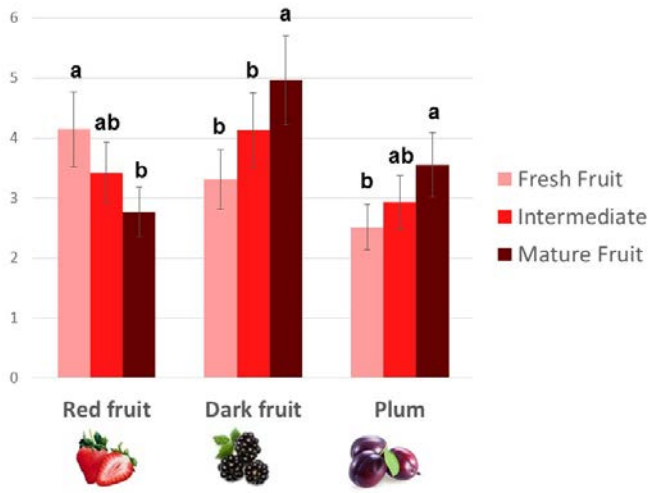


Figure 39: Mean rating ±SD of red fruit, dark fruit and plum attributes assessed in 2015 Cabernet Sauvignon wines from Griffith and Orange (n=6 per harvest date). One way ANOVA was used to compare data. Different letters above bars indicate significance at p ≤ 0.05 (Fischer's LSD). All quoted uncertainty is the standard deviation of six wines.

6.4.2.1 Cabernet Sauvignon 2016

The individual differences of the sensory attributes for the 2016 Cabernet sauvignon wines from both Orange vineyards are shown in Table 13. The differences in characters associated previously between FF and MF Cabernet Sauvignon wines in vintages 2014 and 2015 are again apparent. Tomato leaf, menthol and capsicum are all significantly ($p < 0.05$) lower in the FF replicates in comparison to their MF counterparts. The red fruit characters are higher in the FF samples, but the differences are not significant for both vineyards. The dark fruit, plum and perception of alcohol is significantly higher in the MF wines at both sites.

Table 13: 2016 Cabernet Sauvignon wine sensory attributes for Orange vineyards O2 Fresh and Mature Fruit (Sample Means and Fisher's LSD for Multiple Comparisons).

	O1FF		O1MF		O2FF		O2MF	
Red fruits	4.6	a	3.5	a	4.3	a	3.2	a
Dark fruits	4.4	b	6.3	a	3.1	c	5.5	ab
Plum	3.5	c	6.2	a	3.4	c	4.9	b
Tom Leaf	4.1	a	2.1	b	4.6	a	2.8	b
Menthol	3.5	a	2.4	b	3.7	a	2.4	b
Capsicum	4.0	ab	2.3	c	4.9	a	3.0	bc
Floral	2.6	b	3.4	a	2.8	ab	2.8	ab
Acidity	4.9	b	4.3	b	6.1	a	4.6	b
Alcohol	5.5	b	7.2	a	4.1	c	6.0	b
Astringency	5.0	a	5.8	a	3.7	b	5.1	a

A PCA (Figure 40) was performed on the mean sensory scores for each wine. A two-factor solution was accepted as it accounted for 98% of the total variation and passed the conditions of the scree test and interpretability. The first component accounted for 93.4% of the variance and separated the samples according to relative intensity of acidity, capsicum, tomato leaf and red fruit as opposed to the more mature attributes of dark fruit, plum and a higher perception of alcohol on the right hand side.

The Cabernet Sauvignon wines from warmer vineyard (O1) site at the lower altitude were noticeably different to the wines from the higher altitude vineyard (O2). The MF wines were more characterised by alcohol, dark fruit, astringency and plum attributes whereas the FF wines had less obvious tomato leaf and capsicum characters in particular.

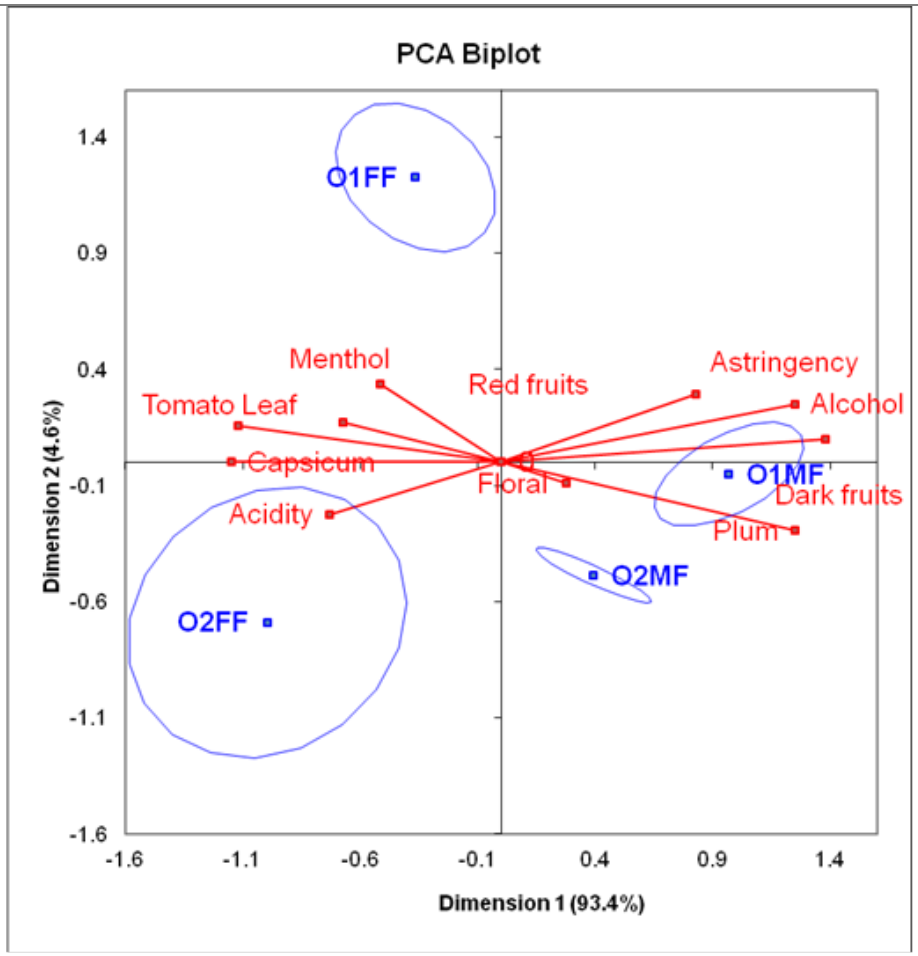


Figure 40: PCA biplot of sensory scores for 2016 Cabernet Sauvignon wines from O1 and O2 vineyards

6.4.3 Outputs: Effect of Harvest Date and Yield on Wine Sensory Profile

Even though variety, environmental factors and winemaking input are important drivers of final wine style, grape maturity was also observed to have considerable influence on the wine sensory profile. A common evolution of wine styles was observed during grape ripening irrespective of variety, yield and site. The ripening model investigated in this study was consistently able to be utilised to help determine a common sensory pattern in advance. It was demonstrated for Shiraz and Cabernet Sauvignon, the two most important red varieties in Australia, that it was possible to predict wine style evolution from 12 to 24 days and 20 to 40 days before harvest respectively.

6.5 Grape and wine compositional analysis

Grape, must, and wine chemical composition were measured to characterise the evolution of wine style associated with the fresh fruit, intermediate and mature fruit maturity stages. Building on the scientific evidence for these distinct and predictable sensory styles, the chemical investigations aimed to emphasise potential wine and grape markers specifically associated with grape aromatic maturity. While particular focus was placed on wine and grape aromas and their potential aromatic precursors, important components for technological and phenolic maturity were also investigated. In total, more than 160 chemical parameters of wine, must, and grape were analysed in all the samples of this study (n=120). The nexus between grape composition, wine sensory and chemical composition is extremely complex. The use of chemometric tools is a practical approach to unravel the large database and provide a global picture of the complex connections. On the other hand, a more specific and targeted approach was also crucial in order to establish relationships between wine sensory and chemical composition and wine and grape composition.

The results will be firstly discussed through a global approach that used chemometrics to model grape ripening and aromatic maturity based on berry sugar accumulation and fresh mass measurement. The evolution of grape composition from fresh fruit to mature fruit stages will be presented prior to a discussion about wine composition and the relationships between grape and wine composition. The influence of ripening and yield on grape/wine composition nexus will be discussed with a particular emphasis on the relationship of aromatic and phenolic maturity.

6.5.1 Multiblock Chemometric Data Analysis

This project generated an extensive amount of data including more than 160 chemical parameters measured in each of the samples of wine, must and grapes for 2014 and 2015 vintage organised into nine individual data blocks according to sample type and analysis (Table 14).

A total of 120 samples, in triplicate, were analysed and correlated with sensory attributes used for the descriptive analysis of the wines. The use of chemometrics allowed a global picture of the influence of grape ripening on wine style and highlighted potential correlations (direct or indirect) between grape, must and wine chemical parameters and associated wine style described by sensory analysis. Even though sugar accumulation per berry and berry fresh mass led to a broadly similar aromatic evolution for both Shiraz and Cabernet Sauvignon, the data for

both varieties was processed separately as variety remains the main factor influencing wine style.

Table 14: Data block descriptions and numbers of measured variables for each sample used in a metabolomics approach to data analysis

Data Block		Number of measured attributes	
		Shiraz	Cabernet Sauvignon
1	Grape amino acids	14	14
2	Grape sugar and organic acids	5	5
3	Grape anthocyanins	8	8
4	Grape carotenoids	9	9
5	Grape volatile compounds	28	28
6	Winemaking	9	9
7	Wine Chemistry	14	14
8	Wine Volatiles	45	46
9	Wine sensory	11	13

6.5.1.1 Chemometric background

An approach for multiblock data analysis termed ANOVA multiblock orthogonal PLS (AMOPLS) (Boccard and Rudaz 2016) was employed which makes use of balanced sample data sets within an ANOVA framework. AMOPLS is based upon the ANOVA partitioning of data variances according to a designed experiment by calculating the mean values of all measurements for a given experimental factor at each level, or combination of levels of the experimental design. Accordingly, various data matrices are derived that account for the experimental design and factor levels which is an extension of the ANOVA-PCA concept (Harrington, Vieira et al. 2005). Once an effect matrix is determined, this is subtracted from the original data matrix to obtain pure effect submatrices. This data deflation strategy creates a series of data matrices with structured orthogonal data that summarises the designed main effects and interactions.

The relative contribution of each data table to the sample scores extracted during the multiblock PCA can be assessed from data block salience values. To ensure that attribute variances in data tables with large number of variables do not over overwhelm information from smaller data tables of variables, an association matrix is first computed from the cross matrix multiplication of each table. Initially each data table is weighted equally and an iterative weighting process is used during the decomposition process to build a global association matrix. Block saliences are presented along with sample scores for important features for each model.

The importance of each experimental factor (harvest, vineyard, region or vintage) and interactions, along with the factor levels in discriminating samples can be determined from confidence intervals (95%) of sample scores based upon Hotelling T^2 criteria. Sample scores

plots for each factor are plotted against the sample scores for the residual matrix residual; thus modelled factors with high level sample discrimination will be form clearly separated and distinct sample groups whereas factors which are insignificant will have overlapping confidence intervals.

An additional measure of the importance of extracted latent variables to an experimental effect is derived from the residual structure ratio (RSR) which determines the magnitude of an effect to residuals, RSR p-value and ANOVA Relative Sum of Squares (RSS) and RSS p-values. When specific model goodness of fit (R2Y), ANOVA Relative Sum of Squares and RSS p-values and RSR are deemed significant (RSR p-value), information from the loadings pertaining to the effects can be interpreted.

Multiple combinations of samples to create balanced ANOVA models for experimental factors (*site, harvest stage, vintage, yield, region)* were selected from the total sample pool which is illustrated in Table 15 and Table 17 for Shiraz and Cabernet Sauvignon respectively. Loadings from each model associated with experimental factors which were considered statistically significant were extracted from each model and used for hierarchical cluster analysis (HCA) using Euclidean distance measures and Wards grouping criteria. Prior to HCA, model scores and loadings were rotated to ensure consistency in presentation of factor levels in scores plots for each model. HCA enables a comparison of each loading and variable combination from the models by normalising the input data to a common variance and determining the distance between samples in a multivariate space using the Euclidean metric. Dendrograms can be constructed to visualise how samples (modelled experimental factors from AMOPLS) are grouped and the data variables (loadings from the AMOPLS) contribute to the sample groupings. These data are typically presented in heat maps with the dendrograms.

Shiraz Models

Models were created based upon the data for fresh fruit (FF or H1) and mature fruit stages (MF or H3) in 2014, and Fresh fruit (H1), intermediate (Int. or H2) and mature fruit (H3) stages in 2015 along with other explanatory factors, as described below and in Table 15:

Model SH_A: Orange vineyards (O1, O2) for H1, H2 and H3 in 2015
Model SH_B: Griffith vineyards low yield (G1, G2) and high yield (G3, G4) for H1, H2 and H3 in 2015
Model SH_C: Griffith low yield vineyards (G1, G2) for H1 and H3 in 2014 and 2015.
Model SH_D: Griffith low yield vineyards (G1, G2) for H1, H2 and H3 in 2015

Model SH_E: Griffith low yield and Orang vineyards (G1, G2, O1, O2) for H1, H2 and H3 in 2015

Model SH_F: Griffith low yield vineyards (G1, G2) for H1 and H3 in 2015

Table 15: Shiraz grape, wine and sensory ANOVA explanatory factors and levels for balanced multiblock analysis.

	Explanatory Factors													
	Site				Harvest Stage			Vintage		Yield		Region		
Levels	G1	G2	O1	O2	H1	H2	H3	V14	V15	High	Low	G	O	n
Model														
A														18
B														36
C														24
D														18
E														36
F														12
G														24

Model C explores the vintage effect on the evolution of grape and wine composition between Fresh fruit and Mature fruit stages and Model F shows this evolution in 2015 vintage. Model D investigates the compositional evolution described in model F from Fresh fruit to Mature fruit with a third harvest date (H2) between both stages. Similar representation was performed for Orange vineyards with Model A. Model E is the combination of Model D and A, and therefore looks at the regional effect on grape and wine compositional evolution from FF to MF stages.

The significance of each explanatory factor and interactions are determined from the RSS and RSR p values and the relative sum of squares for the modelled factors are shown in Table 16.

Block saliences and sample groupings for the explanatory factor *Harvest Stage, Site* and *Yield* are illustrated for the models in Figure 41, Figure 42 and Figure 43 respectively. These explanatory factors are presented as multiple balanced datasets were constructed for these factors and therefore interesting comparisons between the models are possible.

Table 16: Shiraz metabolomic models data. Relative sums of squares for effects, RSR indices and associated p-values for each effect. P values indicated in bold are considered significant (p<5%). Loadings associated with significant effects for each model were parsed to hierarchical cluster analysis.

Model	Explanatory Factor	Relative Sum of Squares	RSS p value	RSR	RSR p value
A	Site	31.2%	**0.001**	4.75	**0.001**
	Harvest Stage	23.3%	**0.001**	2.80	**0.001**
	Site x Harvest Stage	11.7%	0.455	1.17	0.289
	Residuals	33.8%	NA	1	1
B	Site	17.9%	**0.001**	5.00	**0.001**
	Yield	14.4%	**0.001**	3.27	**0.001**
	Harvest Stage	17.1%	**0.001**	4.77	**0.001**
	Site x Yield	5.9%	0.409	1.25	**0.028**
	Site x Harvest Stage	11.7%	**0.002**	3.39	**0.001**
	Harvest Stage x Yield	3.9%	0.867	1.11	0.182
	Residuals	29.1%	NA	1	1
C	Vintage	33.8%	**0.001**	7.74	**0.001**
	Harvest Stage	9.3%	**0.001**	2.22	**0.001**
	Site	18.3%	**0.001**	4.26	**0.001**
	Vintage x Site	8.2%	0.073	1.95	**0.001**
	Vintage x Harvest Stage	7.5%	0.103	1.77	**0.001**
	Site x Harvest Stage	2.1%	0.905	1.02	0.537
	Residuals	20.9%	NA	1	1
D	Site	31.1%	**0.001**	5.78	**0.001**
	Harvest Stage	22.8%	**0.001**	3.16	**0.001**
	Site x Harvest Stage	13.5%	0.26	1.57	**0.024**
	Residuals	32.7%	NA	1	1
E	Site	54.4%	**0.001**	11.90	**0.001**
	Harvest Stage	10.7%	**0.001**	3.54	**0.001**
	Site	12.4%	0.951	1.69	**0.024**
	Residuals	22.5%	NA	1	1
F	Site	33.7%	**0.003**	4.31	**0.001**
	Harvest Stage	26.8%	**0.003**	3.41	**0.001**
	Site x Harvest Stage	7.6%	0.546	1.07	0.404
	Residuals	31.8%	NA	1	1
G	Region	32.3%	**0.001**	2.32	**0.001**
	Harvest Stage	13.4%	**0.001**	1.03	0.289
	Region x Harvest Stage	4.1%	0.446	1.02	0.534
	Residuals	50.2%	NA	1	1

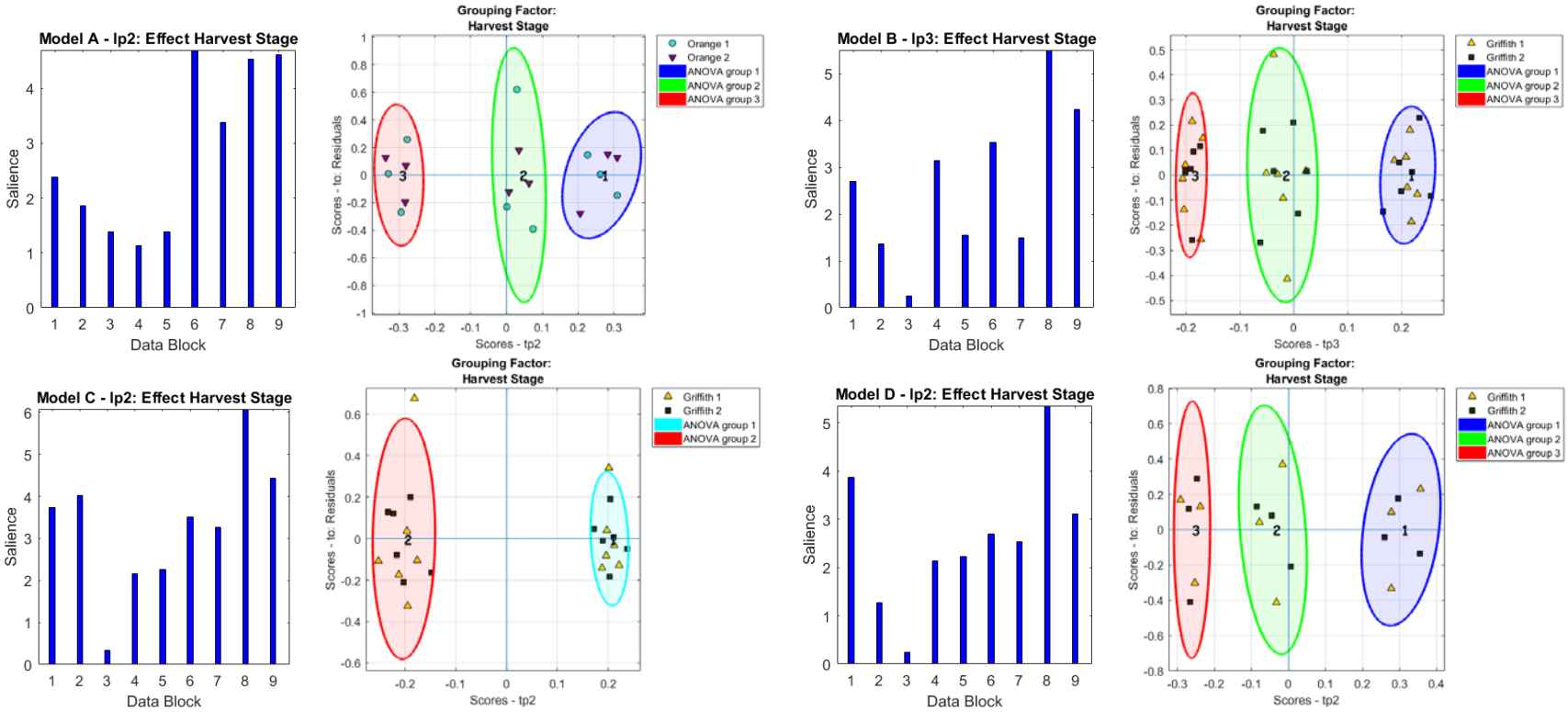


Figure 41: Data block salience and sample groupings for the explanatory factor Harvest Stage for each Shiraz model sample set.

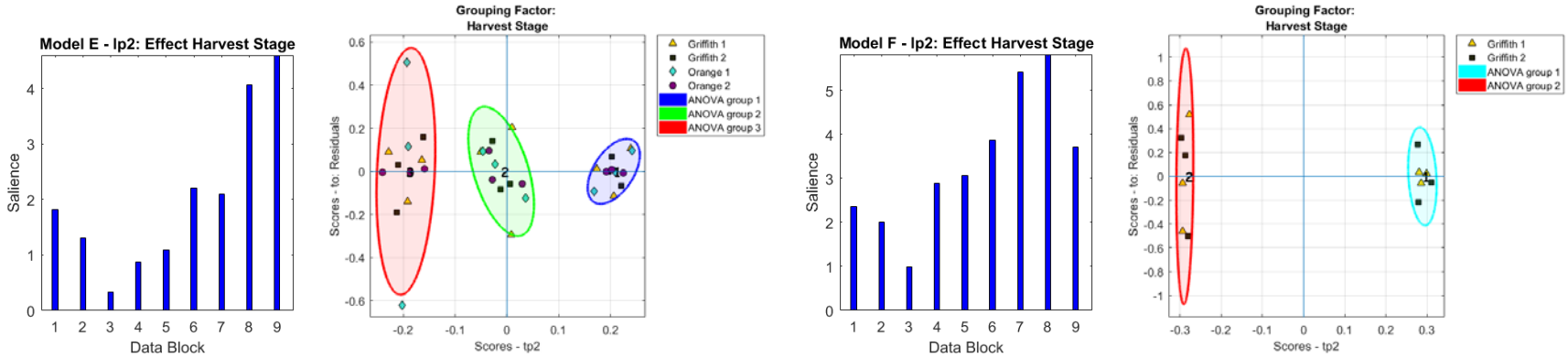


Figure 41 (cont.): Data block salience and sample groupings for the explanatory factor Harvest Stage for each Shiraz model sample set.

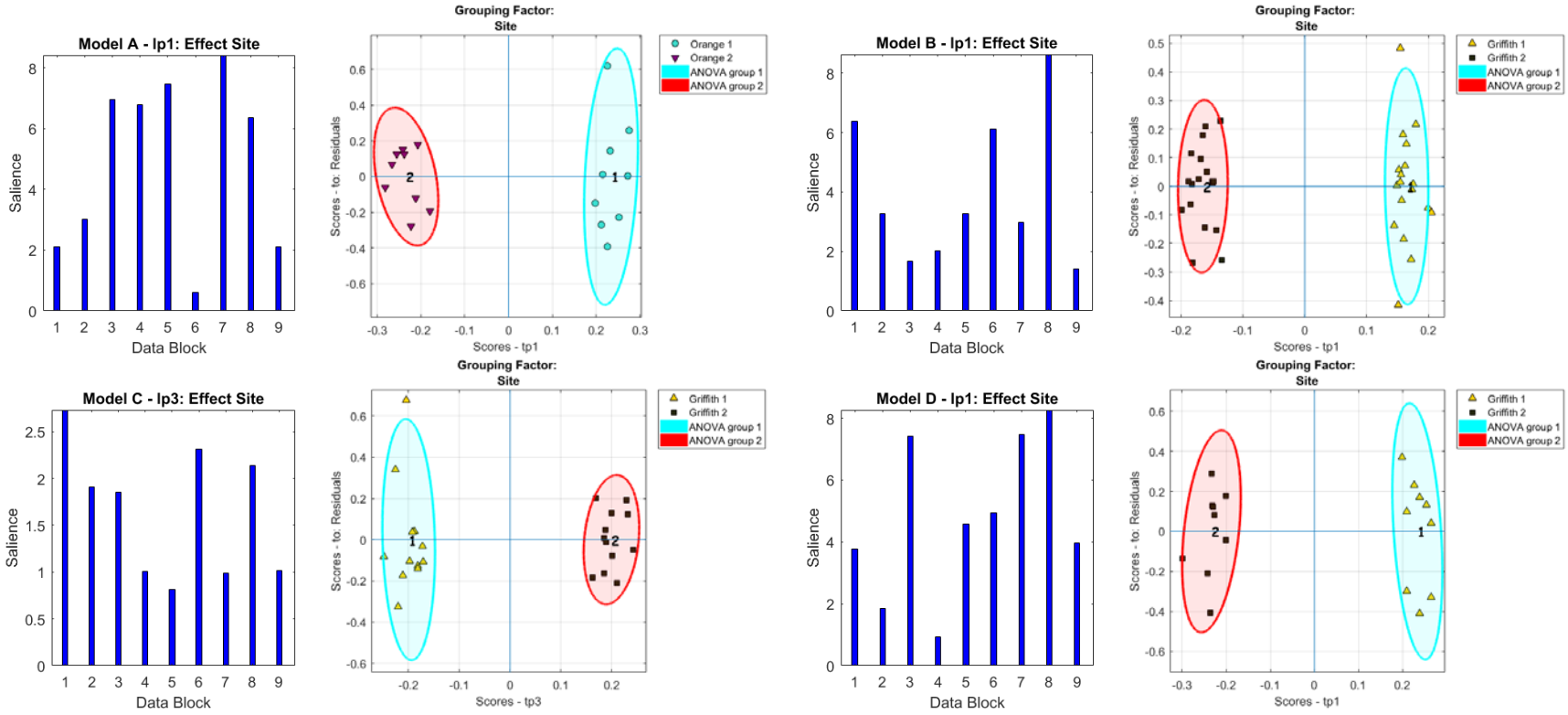


Figure 42: Data block salience and sample grouping for the explanatory factor Site for each Shiraz model sample set.

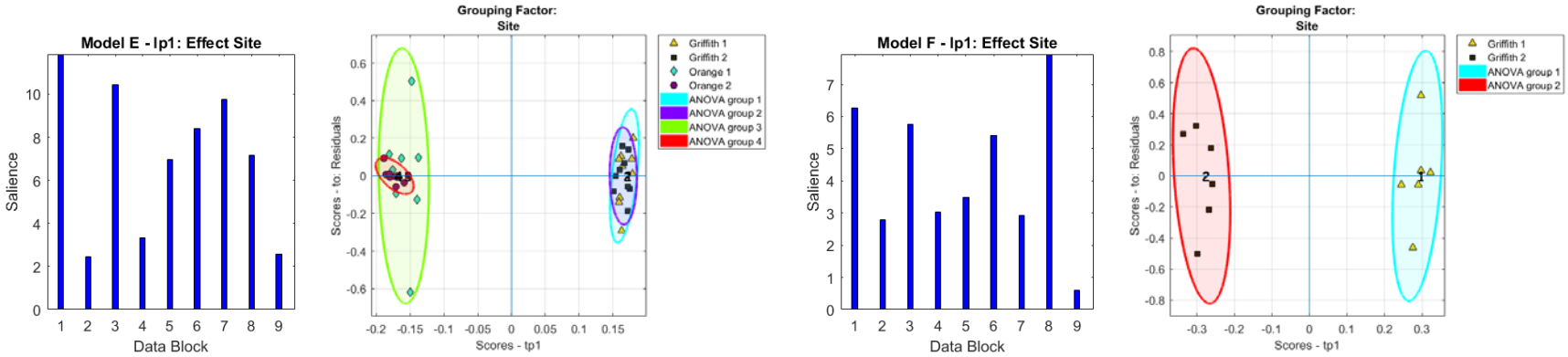


Figure 42 (cont.): Data block salience and sample grouping for the explanatory factor Site for each Shiraz model sample set.

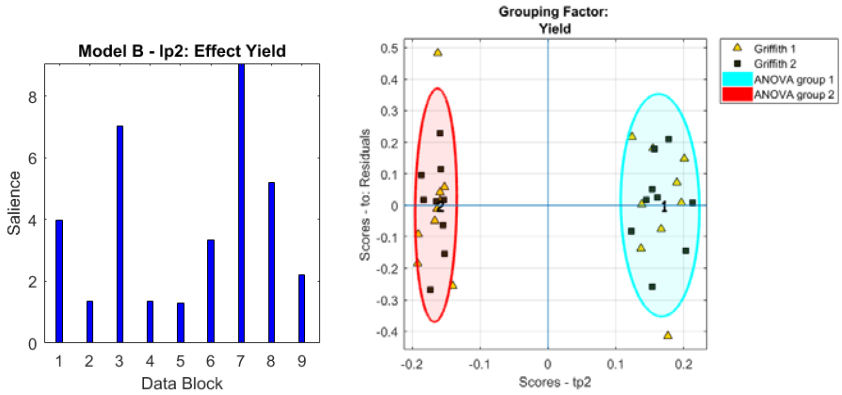


Figure 43: Data block salience and sample grouping for the explanatory factor Yield for each Shiraz model sample set.

6.5.1.2 Discussion of Shiraz Models

All Shiraz ANOVA models show a common clustering of samples according to the ANOVA explanatory factor *Harvest* with clearly separate confidence intervals (circles) regardless of vintage, region or site (Figure 41). A clear distinction between harvest stages is evident regardless of climate, vineyard management or other parameters beyond the explanatory factors. These results show that sensory spaces corresponding to fresh fruit and mature fruit stages were associated to specific wine, must and grape compositions irrespective of vintage conditions which were highly different between 2014 and 2015 vintages. In models D, A and E the intermediate stage cluster is represented in a space located between fresh fruit (FF) and mature fruit (MF) clusters and reflects the evolution of grape and wine chemical composition between Fresh fruit and Mature fruit stages. While sensory analysis could not define with consistency the existence of an intermediate stage, the wines from this stage were however perceived as different from FF and MF wines. Those differences could also contribute to the separation of cluster 2 (intermediate stage) from clusters 1 and 3 corresponding to FF and MF stages. The common clustering of samples according to harvest date observed in model D, A and E (Figure 41) shows that sensory spaces corresponding to fresh fruit and mature fruit stages were associated to specific wine, must and grape compositions irrespective of region and climate. Similar clustering was also found in Model B indicating that up to 20 tonnes per hectare yield does not deeply affect the grape and wine compositional evolution from Fresh to Mature fruit stages.

Sample grouping according to site is also a significant explanatory factor in the ANOVA models. Clear and separate groupings of samples according to site are evident in Figure 42, although one

dataset (model E) is confounded by clustering of samples from different vineyard and regions located within the confidence interval for an alternative site. This interesting result is unexpected and is likely to have arisen from inappropriate selection of the ANOVA explanatory factors for this specific data set.

As expected yield modified grape and wine composition as shown by the two clusters observed on the representation of yield (Figure 43). Therefore, while yield impact grape and wine composition, on the other hand the evolution of grape and wine metabolic profile between FF and MF stages is consistent irrespective of the yield. Sugar accumulation per berry and berry fresh mass remain relevant physiological indicators for aromatic maturity for vineyards with yield as high as 20 tonnes/ha.

6.5.1.3 Cabernet Sauvignon Models

Various chemometric models were created based upon the data for fresh fruit (FF or H1) and mature fruit stages (MF or H3) in 2014, and Fresh fruit (H1), intermediate (Int. or H2) and mature fruit (H3) stages, as shown below and in Table 17.

Model CS_A: Griffith 1 and Orange 1 vineyards for H1 and H3 in 2015

Model CS_B: Griffith 1 vineyard for H1 and H3 in 2014 and 2015

Model CS_C: Griffith 1 and Orange 1 vineyards for H1, H2 and H3 in 2015

Model CS_D: Griffith 1 and Orange 1 vineyards for H1, H2 and H3 in 2015

Model CS_E: Orange 1 and Orange 2 vineyards for H1 and H3 in 2015

Model A shows the evolution of grape and wine composition between Fresh Fruit (FF: Harvest 1) and Mature Fruit (MF: Harvest 3) stages for Griffith and Orange vineyards for 2015; Model B explores the vintage effect between Fresh fruit and Mature fruit stages in Griffith. Model C investigates the compositional evolution described in model A from Fresh fruit to Mature fruit with an intermediate harvest stage (IF: Harvest 2) between FF and MF stages. Regional factors (Griffith and Orange) are modelled in dataset D along with harvest timing (FF, IF and MF), and model E includes factors associated with Orange vineyard locations and management for FF and IF stages.

The significance of each explanatory factor and interactions determined from the RSS and RSR p values and the relative sum of squares for the modelled factors are shown in Table 18.

Table 17: Cabernet Sauvignon grape, wine and sensory ANOVA explanatory factors and levels for balanced multiblock analysis.

	Explanatory Factors									
	Site		**Harvest Stage**			**Vintage**		**Region**		
Levels	**O1**	**O2**	**H1**	**H2**	**H3**	**V14**	**V15**	**G**	**O**	**n**
Model										
A										12
B										12
C										12
D										18
E										12

Table 18: Cabernet Sauvignon metabolomic models data. Relative sums of squares for effects, RSR indices and associated p-values for each effect. P values indicated in bold are considered significant (p<5%)

Model	Explanatory Factor	Relative Sum of Squares	RSS p value	RSR	RSR p value
A	Region	50.8%	**0.009**	8.99	**0.001**
	Harvest Stage	19.2%	**0.003**	3.51	**0.001**
	Region x Harvest Stage	6.2%	0.650	1.33	0.104
	Residuals	23.8%	NA	1.00	1
B	Vintage	44.4%	**0.008**	5.34	**0.001**
	Harvest Stage	21.1%	**0.006**	2.58	**0.001**
	Vintage x Harvest Stage	7.5%	0.549	1.00	0.851
	Residuals	27.0%	NA	1.00	1
C	Vintage	62.0%	**0.006**	13.04	**0.001**
	Harvest Stage	9.6%	**0.007**	2.21	**0.001**
	Vintage x Harvest Stage	6.3%	0.607	1.45	0.093
	Residuals	22.2%	NA	1.00	1
D	Region	52.0%	**0.001**	12.97	**0.001**
	Harvest Stage	18.6%	**0.001**	3.83	**0.001**
	Region x Harvest Stage	7.7%	0.728	1.31	0.163
	Residuals	21.7%	NA	1.00	1
E	Site	42.5%	**0.003**	5.82	**0.001**
	Harvest Stage	14.3%	**0.004**	2.11	**0.001**
	Site x Harvest Stage	11.3%	0.198	1.63	**0.042**
	Residuals	31.9%	NA	1.00	1

Block salience and sample groupings for the explanatory factors *Harvest Stage, Region and site (Orange 1 and Orange 2)* are shown in Figure 44, Figure 45, and Figure 46 respectively.

6.5.1.4 Discussion of Cabernet Sauvignon Models

Cabernet Sauvignon samples were clearly and significantly separated into different clusters according to the explanatory factory *Harvest stage* in all the models (Figure 44) in a similar

manner compared to the Shiraz datasets. Therefore, a ripening pattern comparable that observed for Shiraz was also found for Cabernet Sauvignon from different region and vintage. Block salience values for the explanatory factory *Harvest stage* also show a similar pattern of datablock contributions to the sample groupings: blocks 8-9 (wine volatiles and wine sensory) consistently have high salience to the sample groups; blocks 1, 6 & 7 (grape amino acids, wine making inputs and wine chemistry) have moderate to high salience values and blocks 2 & 3 (grape sugar and organic acids and grape anthocyanins) have the lowest contribution to berry maturity at harvest. The commonality of block saliences between the Shiraz and Cabernet Sauvignon datasets for *Harvest stage* indicates a progression of berry and wine attributes common to both cultivars, with a similar metabolic flux arising during the berry maturation stage. In Cabernet Sauvignon, as for Shiraz, there was no apparent direct relationship between aromatic, technological (sugar/acid) and phenolic maturities.

The explanatory factor *Region* (Figure 45) and site (Figure 46) also show similar block salience values for the two models in which this factor is significant. Interpretation of the block saliences for *Region* must be considered cautiously as the two models datasets (A and D) are similar in sample compositions and so it is expected that block salience values will be comparable. However block salience values appear to indicate that as vineyard region shifts from Griffith to Orange (hot to temperate climate) the largest changes in composition occurs in data blocks 7, 8, 1 and 3 (wine chemistry, wine volatiles, grape amino acids and grape anthocyanins. A similar picture emerges for block saliences for explanatory factor *Site* in the Cabernet Sauvignon models and this is consistent with a move from a low to high altitude vineyard location and hence a change in mesoclimate associated with lower vineyard temperatures.

It is not surprising that grape amino acids and wine volatiles are largely impacted as the vineyard mesoclimate (hot to temperate: low to high altitude) changes, as many investigations have demonstrated links between berry amino acid and wine ester composition following fermentation. Changes to wine ester composition in this investigation are illustrated in the heat map data (Figure 49) with most esters in the Cabernet Sauvignon wines increasing in concentration with vineyard mesoclimate moving from hot to temperate. It is somewhat surprising however that wine sensory scores (block 9) were not more highly rated for contribution to sample groupings for *Region*. These observations tend to suggest that wine

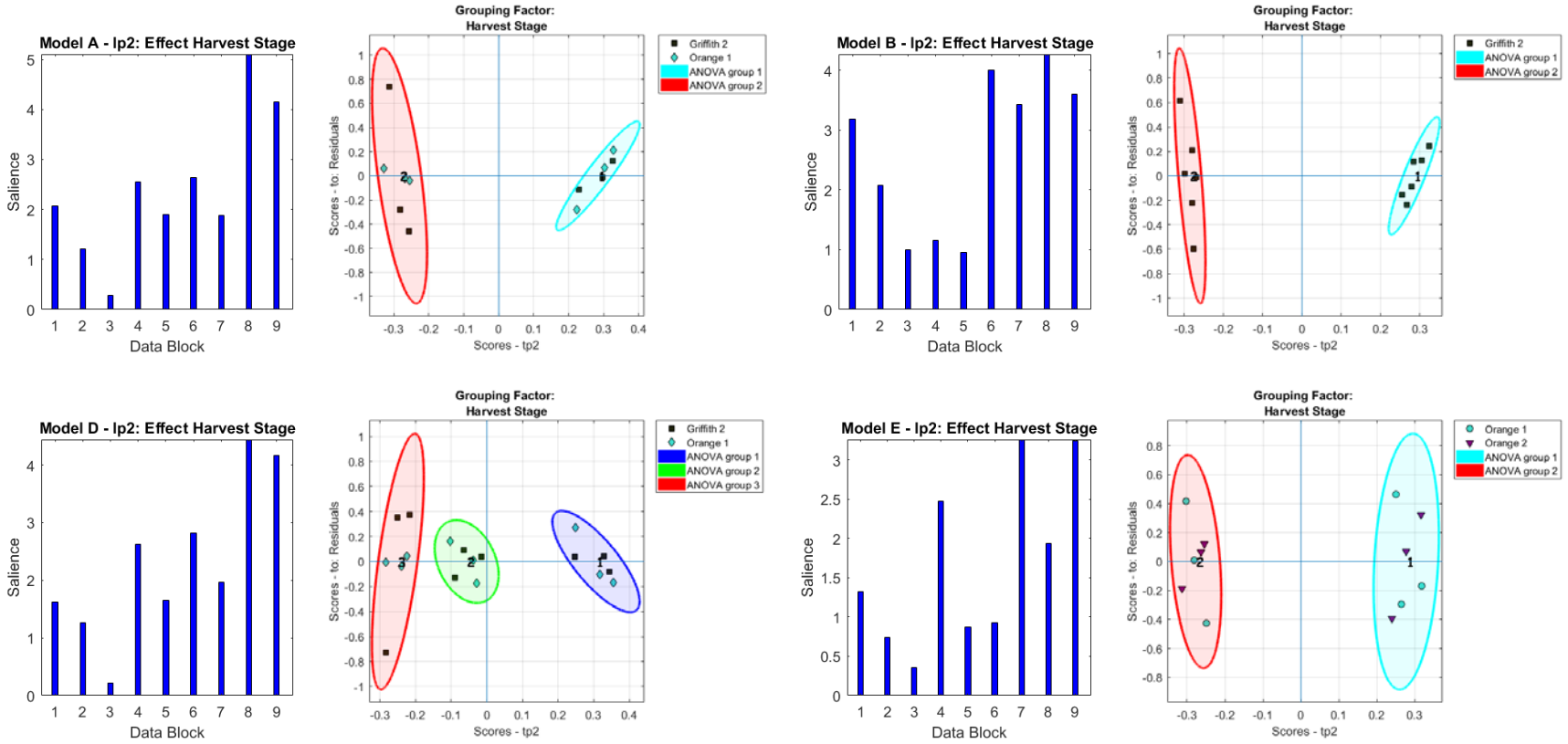


Figure 44: Data block salience and sample groupings for the explanatory factor Harvest Stage for each Cabernet Sauvignon model sample set.

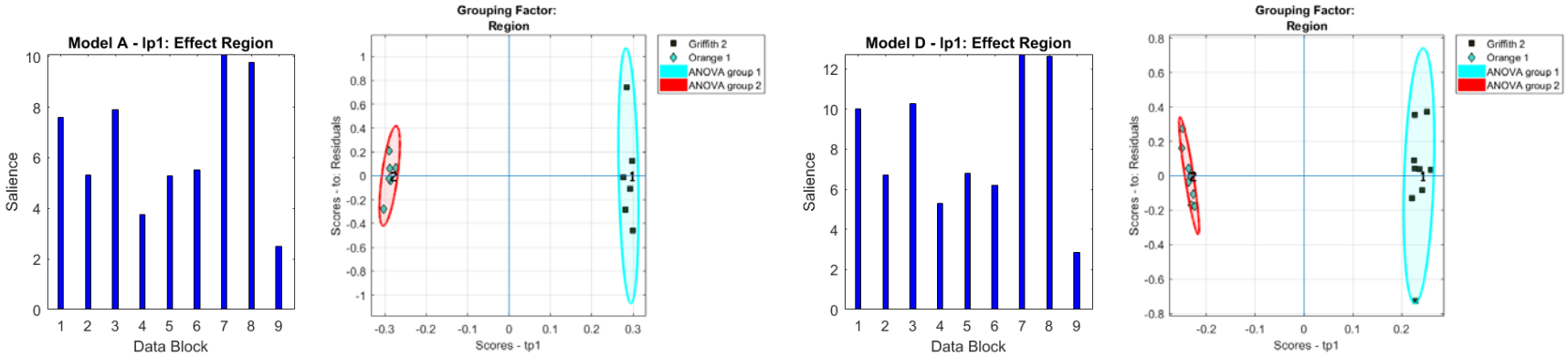


Figure 45: Data block salience and sample groupings for the explanatory factor Region for Cabernet Sauvignon models.

Model E

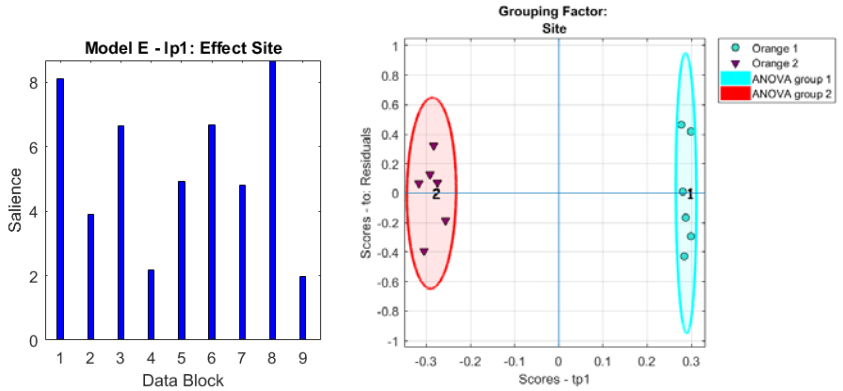


Figure 46: Data block salience and sample groupings for the explanatory factor Site (Orange 1 and Orange 2) for the Cabernet Sauvignon model.

esters may contribute only a small role in Cabernet Sauvignon sensory profiles for the wines from this investigation and this observation is deserving of further exploration.

6.5.1.5 Global comparisons

The chemometric models show for Australian Shiraz and Cabernet Sauvignon that the sensory spaces specific to Fresh fruit and Mature fruit stages were related to specific grape and wine chemical profiles. While grape and juice composition might contribute to characterise FF and MF stages, it seems that wine volatiles and sensory profiles were the most influential factors that discriminated the maturity stages. It is not surprising to find sensory profile as an influential factor to distinguish FF and MF stages as the ripening model presented in this study has been constructed from sensory evidence. Among all the parameters analysed in this study, wine aromatic composition was the main factor involved in the discrimination of grape berry maturity based on harvest stage for both varieties. Therefore, the aromatic evolution perceived in Shiraz and Cabernet Sauvignon wines between Fresh to Mature fruit stages seems to be disconnected from grape sugar and phenolic composition. In contrast, wine volatile composition seemed to be more related to this sensory evolution.

Of interest is the remarkable similarity in block saliences for explanatory factor *Harvest* for both cultivars. The grouping of samples for Harvest stage is influenced mostly by blocks 6-9 corresponding to winemaking parameters, wine chemistry, volatile composition and wine sensory aspects. Of the remaining data blocks, grape berry amino acid composition also has an important contribution and this is mostly likely reflects the importance of the amino acid profile in wine volatile compound profiles and therefore wine sensory scores. Importantly, grape berry anthocyanins (block 3) were not a significant contributor to sample groupings according to harvest date. This finding is important given the present commercial emphasis for measures of grape berry colour and payments for crops. Observations of increasing berry colour with grape maturity may actually be associated with the concentration of berry constituents through mechanisms such as berry shrivel and have not previously made allowances for regional or vineyard influences. There is no clear link to increasing berry colour and berry maturity when positioning berry maturing on a sugar accumulation curve.

However, berry colour and vineyard are somewhat related as evident by the block saliences for site and region for both Shiraz and Cabernet Sauvignon models. Such observation suggests that the composition of anthocyanins in grape berries and thus wine colour potential is intrinsically linked to the vineyard climatic conditions, and the practice of late harvest decisions based upon

continued berry development to attain a specified colour result may be of limited value in terms of the final wine sensory space.

6.5.1.6 Hierarchical Cluster Analysis (HCA) and Global Summary of Variable Loadings for *Harvest Stage*

Variable loadings associated for each significant explanatory factor (Table 16 and Table 18) were subject to HCA to construct heat maps to determine the relative relationships between the attributes in each data block and the factor of interest. The Shiraz and Cabernet Sauvignon heat maps are illustrated in Figures 47 and 48 respectively. Clear groupings of each significant explanatory factor (*Harvest Stage, Region, Site, Yield* and *Vintage)* are evident in the heat maps. To assist in the interpretation of loadings, for each explanatory factor a summary heat map is presented in Table 19. Variables are colour coded in accordance with the relative change in expression for each effect; red = upregulated: green = down regulated; black = no change) with shading indicating the 25th, 50th and 75th percentile values (interquartile ranges) for the up and down regulated directions based upon loading amplitude. Black shading indicates a variable loading within the lower quartile (up or down) and thus relatively low changes are noted for this measured effect/variable combination.

As expected, glucose and fructose per berry increased during the maturation period despite an apparent sugar accumulation plateau. Malic acid levels decreased during this phase. Broadly, grape anthocyanins do not significantly change during grape berry ripening between FF and MF stages in both Shiraz and Cabernet Sauvignon models, with the expectation of *t*-malvidin-3-O-(6-p-coumaroyl) glucoside for both cultivars and *t*-peonidin-6-(6-Ocoumaryoyl) glucoside in Shiraz. It would therefore seem likely that prior observations in which grape berry anthocyanin levels increase during berry ripening are influences more by berry volume changes, dehydration and berry shrivel rather than de novo synthesis in the berry.

Grape berry carotenoids decrease and berry volatile composition remains largely unchanged except the compounds heptanol, hexanol and trans-2-hexenol which increase in Shiraz; cis-3-hexanal and trans-geranylacetone which decrease in Shiraz; cis-3-hexenol, trans-2-hexenal decrease in Cabernet Sauvignon and β-ionone which decreases in both cultivars during maturity changes from FF to MF. Winemaking parameters reflect the pattern of amino acid flux with YAN increasing for Shiraz but decreasing for Cabernet Sauvignon. Other winemaking variables are indicative of corrective intervention to ensure consistent fermentation profiles were maintained during the investigation. Some consistency in the flux of wine chemistry measures for both Shiraz and Cabernet Sauvignon models is evident with total anthocyanins, phenolic compounds, red pigments, tannins, glycerol and ethanol mostly increasing with harvest stage. The flux of

increasing glycerol and ethanol is consistent with increasing expression of glycerol as an osmoprotectant by *Saccharomyces cerevisiae* in the early stages of fermentation. High levels of fermentable carbohydrate would increase the need for cellular protection from osmotic pressures in the early stages of carbohydrate uptake and utilisation. Wine volatile composition for both cultivars is of interest in that there is little commonality in attribute flux as harvest stages progresses from H1 to H3 (FF to MF) and has been reported by this group (Antalick, Šuklje et al. 2015a).

6.5.1.7 Summary of Variable Loadings for *Region* and *Vineyard Locations*

Comparisons of the G2 to G1 site locations for the Shiraz vineyards is of interest as these two sites are relatively close and within a region where an unchanging topography creates almost identical meso-climates. Both vineyards were managed with a sprawling canopy and have identical row/vine spacing. Thus some inferences can be made regarding the influence of vineyard management parameters and clonal factors to the variable flux by reference to the heat map summary Table 19. Basic vineyard parameters are presented in Table 3 of this report. Sites G1 and G2 principally vary in clonal composition and yield per vine. Vineyard parameters such as primary shoots and bunches per vine along with average bunch weight (Table 3) will obviously influence the yield per vine. A more detailed investigation of clonal influence with a temporal investigation of yield effect is necessary to fully elucidate the impact of these combined factors on berry and subsequent wine composition. However, it would seem likely that many of the observed differences in the ANOVA model loadings for *Site* in the Shiraz models are influenced by clonal variations rather than yield. This observation is supported by the large differences observed in the attribute loadings for explanatory factors *Yield* and *Site* presented in the heat map for all significant explanatory factors (Figure 47).

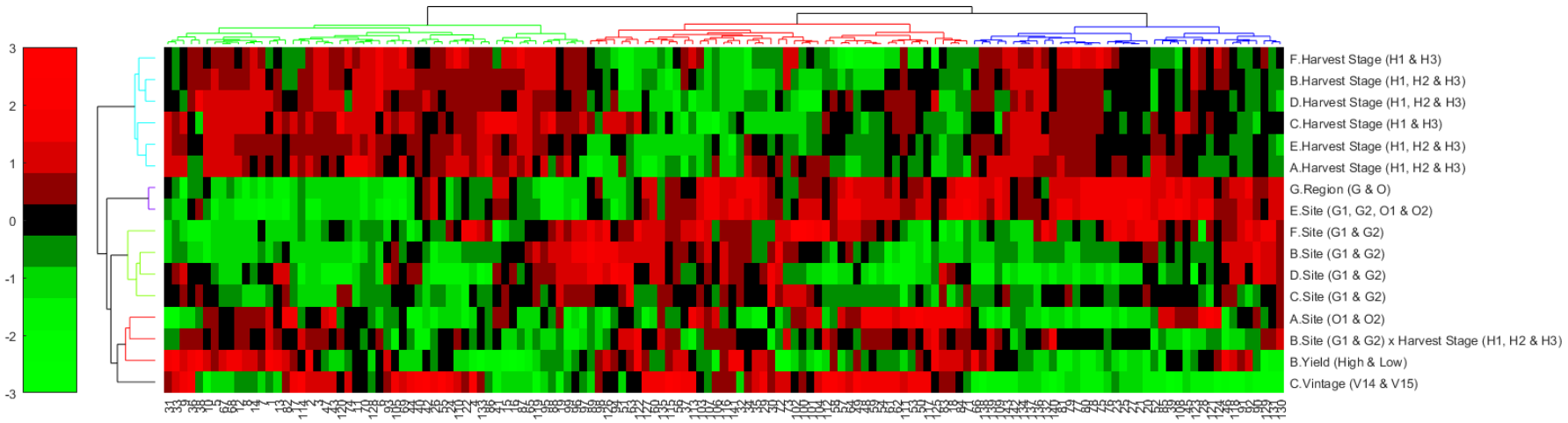


Figure 47: Heat map of loadings associated with significant explanatory factors in AMOPLS models for Shiraz models. Significance based upon RSR and SSANOVA.

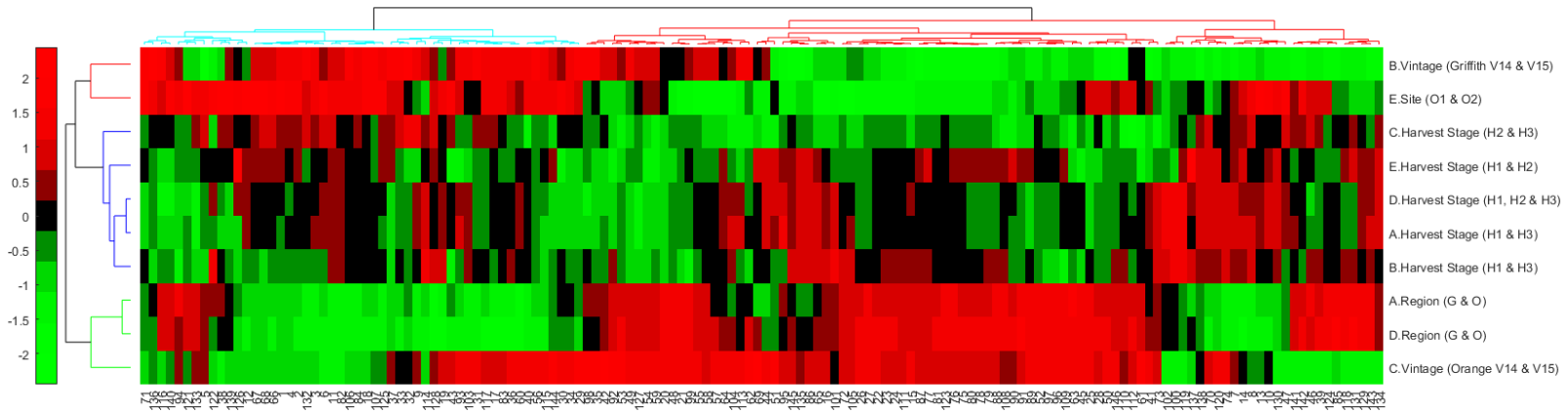
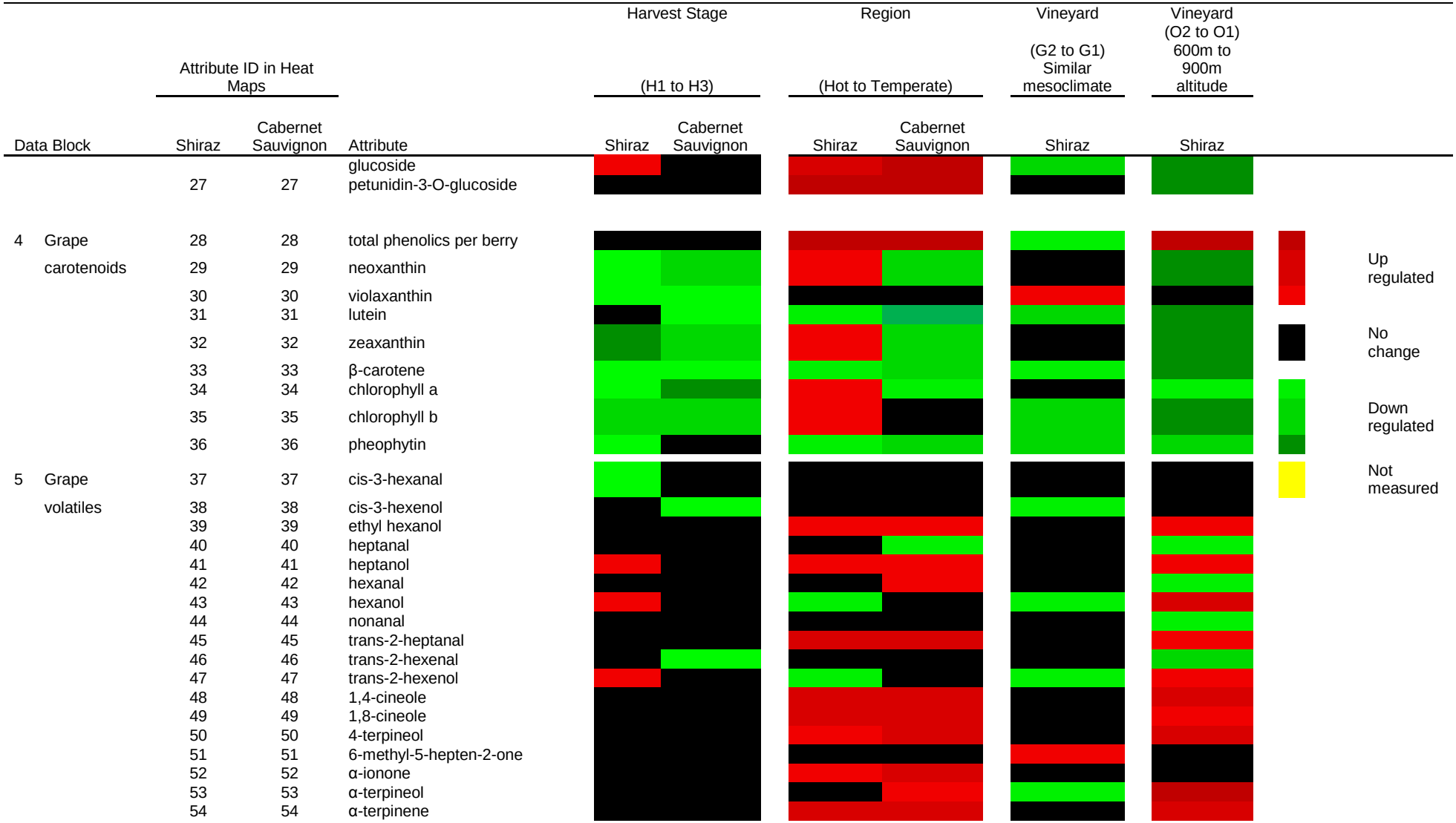


Figure 48: Heat map of loadings associated with significant explanatory factors in AMOPLS models for Cabernet Sauvignon data sets. Significance based upon RSR and SSANOVA for Cabernet Sauvignon Models.

Table 19: Summary and comparison of heat map data for Shiraz and Cabernet Sauvignon ANOVA models for explanatory factors Harvest Stage, Region, Vineyard (Griffith: similar mesoclimate) and Vineyard (Orange: varying altitude).

		Attribute ID in Heat Maps			Harvest Stage (H1 to H3)		Region (Hot to Temperate)		Vineyard (G2 to G1) Similar mesoclimate	Vineyard (O2 to O1) 600m to 900m altitude
Data Block		Shiraz	Cabernet Sauvignon	Attribute	Shiraz	Cabernet Sauvignon	Shiraz	Cabernet Sauvignon	Shiraz	Shiraz
1	Grape Amino Acids	1	1	alanine						
		2	2	arginine						
		3	3	asparagine						
		4	4	aspartic acid						
		5	5	γ-aminobutyric acid						
		6	6	glutamine						
		7	7	isoleucine						
		8	8	leucine						
		9	9	methionine						
		10	10	phenylalanine						
		11	11	proline						
		12	12	threonine						
		13	13	tyrosine						
		14	14	valine						
2	Grape carbohydrates and organic acids	15	15	glucose						
		16	16	fructose						
		17	17	citric acid						
		18	18	malic acid						
		19	19	tartaric acid						
3	Grape anthocyanins	20	20	cyanidin-3-O-glucoside						
		21	21	dephinidin-3-O-glucoside						
		22	22	malvidin-3-O-acetylglucoside						
		23	23	malvidin-3-O-glucoside						
		24	24	*t*-malvidin-3-O-(6-p-coumaroyl) glucoside						
		25	25	peonidin-3-O-glucoside						
		26	26	*t*-peonidin-6-(6-ocoumaryoyl)						




Harvest Stage
(H1 to H3)
Region
(Hot to Temperate)
Vineyard
(G2 to G1)
Similar
mesoclimate
Vineyard
(O2 to O1)
600m to
900m
altitude
Attribute ID in Heat Maps
Data Block
Shiraz
Cabernet Sauvignon
Attribute
Shiraz
Cabernet Sauvignon
Shiraz
Cabernet Sauvignon
Shiraz
Shiraz
glucoside
27 27 petunidin-3-O-glucoside
4 Grape carotenoids
28 28 total phenolics per berry
29 29 neoxanthin
30 30 violaxanthin
31 31 lutein
32 32 zeaxanthin
33 33 β-carotene
34 34 chlorophyll a
35 35 chlorophyll b
36 36 pheophytin
5 Grape volatiles
37 37 cis-3-hexanal
38 38 cis-3-hexenol
39 39 ethyl hexanol
40 40 heptanal
41 41 heptanol
42 42 hexanal
43 43 hexanol
44 44 nonanal
45 45 trans-2-heptanal
46 46 trans-2-hexenal
47 47 trans-2-hexenol
48 48 1,4-cineole
49 49 1,8-cineole
50 50 4-terpineol
51 51 6-methyl-5-hepten-2-one
52 52 α-ionone
53 53 α-terpineol
54 54 α-terpinene
Up regulated
No change
Down regulated
Not measured

Data Block	Attribute ID in Heat Maps: Shiraz	Attribute ID in Heat Maps: Cabernet Sauvignon	Attribute	Harvest Stage (H1 to H3): Shiraz	Harvest Stage (H1 to H3): Cabernet Sauvignon	Region (Hot to Temperate): Shiraz	Region (Hot to Temperate): Cabernet Sauvignon	Vineyard (G2 to G1) Similar mesoclimate: Shiraz	Vineyard (O2 to O1) 600m to 900m altitude: Shiraz
	55	55	β-damascenone						
	56	56	β-ionone						
	57	57	cis-linalool oxide						
	58	58	citronellol						
	59	59	γ-terpinene						
	60	60	linalool						
	61	61	terpinolene						
	62	62	trans-geraniol						
	63	63	trans-geranylacetone						
	64	64	trans-linalool oxide						
6 Wine making	65	65	Brix						
	66	66	ammonia						
	67	67	NOPA						
	68	68	Yeast Assimilable Nitrogen						
	69	69	Ratio Amino Acids/Ammonia						
	70	70	pH						
	71	71	Titratable Acidity						
	72	72	diammonium phosphate Addition						
	73	73	Fermaid A Addition						
7 Wine Chemistry	74	74	Chemical Age1						
	75	75	Colour Density						
	76	76	Degree of Red Pigment Colouration						
	77	77	Estimate of SO_2 Resistance Pigments						
	78	78	Total Anthocyanins						
	79	79	Total Phenolics						
	80	80	Total Red Pigments						
	81	81	Total Tannins						

Up regulated

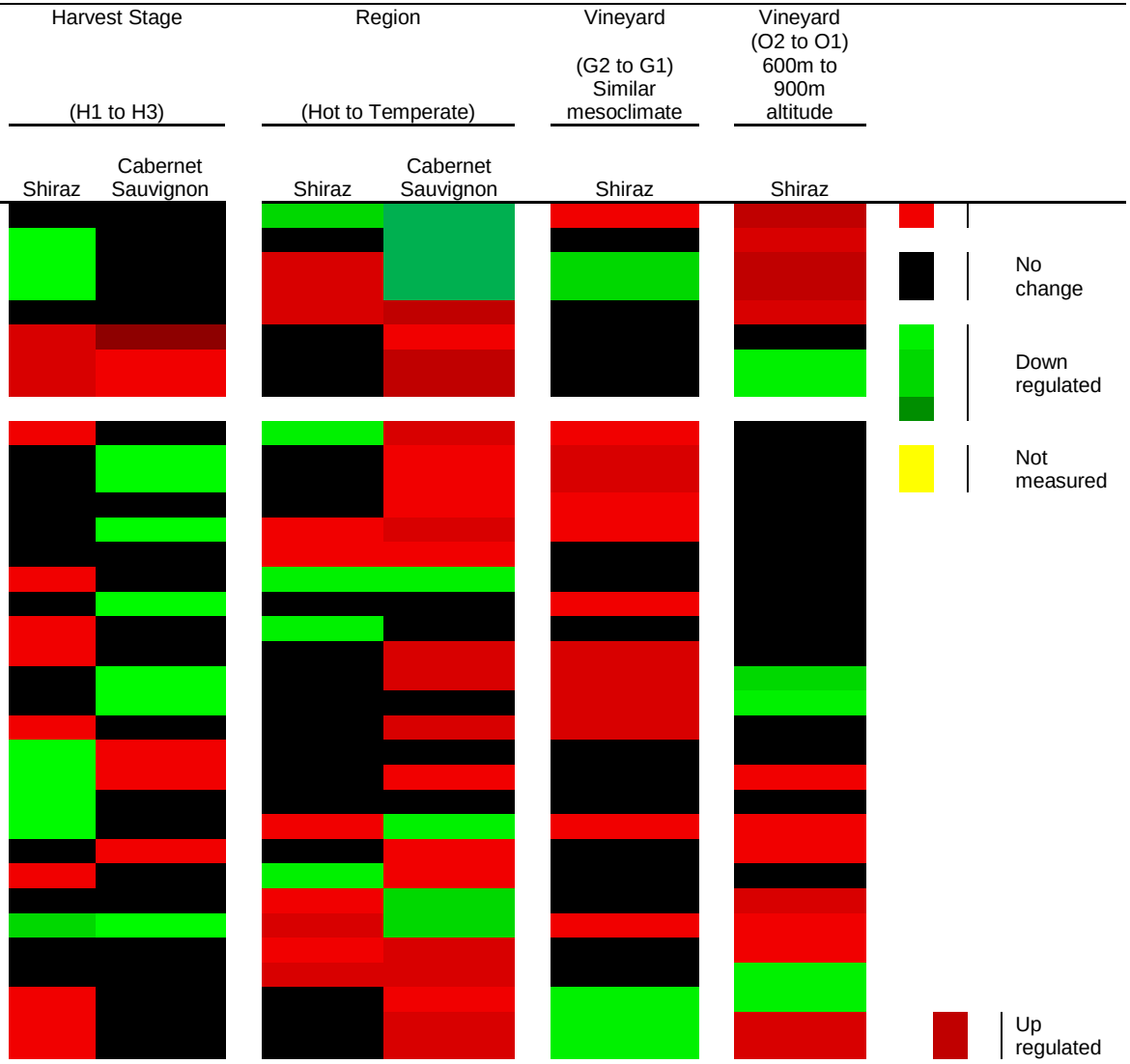
No change

Down regulated

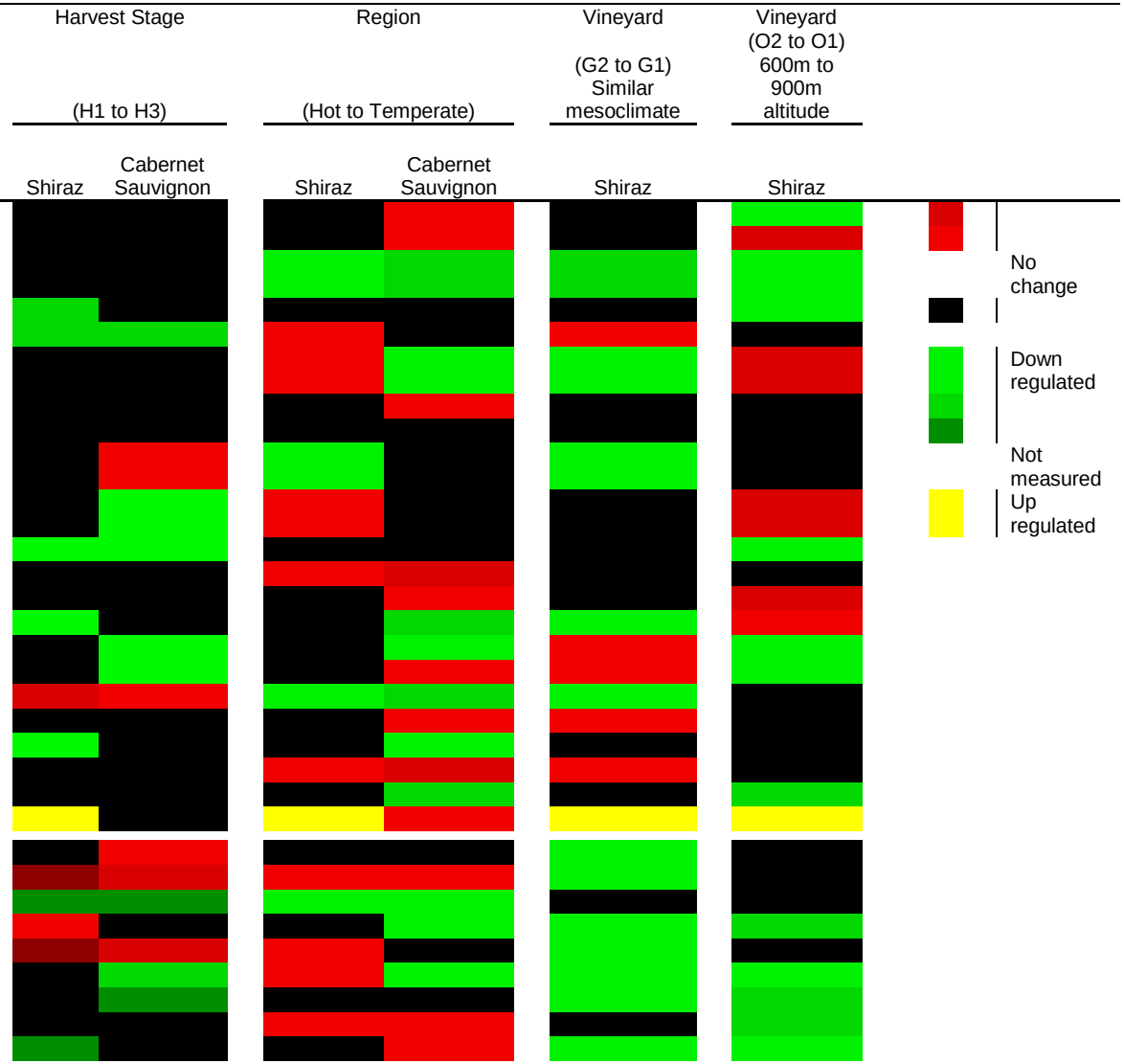
Not measured

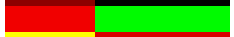
Up regulated

Data Block		Attribute ID in Heat Maps: Shiraz	Attribute ID in Heat Maps: Cabernet Sauvignon	Attribute
		82	82	Wine Colour Hue
		83	83	acetic acid
		84	84	lactic acid
		85	85	succinic acid
		86	86	glycerol
		87	87	ethanol
8	Wine Volatiles	88	88	ethyl butyrate
		89	89	ethyl hexanoate
		90	90	ethyl octanoate
		91	91	ethyl decanoate
		92	92	ethyl dodecanoate
		93	93	propyl acetate
		94	94	isobutyl acetate
		95	95	butyl acetate
		96	96	isoamyl acetate
		97	97	hexyl acetate
		98	98	cis-3-hexenyl acetate
		99	99	phenylethyl acetate
		100	100	ethyl isobutyrate
		101	101	ethyl 2-methyl butyrate
		102	102	ethyl isovalerate
		103	103	ethyl leucate
		104	104	ethyl phenylacetate
		105	105	ethyl propionate
		106	106	ethyl cinnamate
		107	107	ethyl dihydrocinnamate
		108	108	1,4-cineole
		109	109	1,8-cineole
		110	110	4-terpineol
		111	111	α-ionone



Data Block	Attribute ID in Heat Maps: Shiraz	Attribute ID in Heat Maps: Cabernet Sauvignon	Attribute
	112	112	α-terpinene
	113	113	α-terpineol
	114	114	β-damascenone
	115	115	β-ionone
	116	116	cis-3-hexenol
	117	117	citronellol
	118	118	γ-decalactone
	119	119	γ-dodecalactone
	120	120	γ-nonalactone
	121	121	γ-terpinene
	122	122	hexanol
	123	123	linalool
	124	124	terpinolene
	125	125	trans-2-hexenol
	126	126	trans-3-hexenol
	127	127	trans-geraniol
	128	128	dimethyl sulfide
	129	129	isoamyl alcohol
	130	130	isobutanol
	131	131	phenylethanol
	132	132	H_2S
		133	Isobutyl methoxypyrazine
9 Wine Sensory	133	134	Floral
	134	135	Dark Fruit
	135	136	Red Fruit
	136	137	Black Cherry
	137	138	Plum
	138	139	Pepper
	139	140	Green Olive
	140	141	Menthol
	141	142	Acidity



	Attribute ID in Heat Maps			Harvest Stage (H1 to H3)		Region (Hot to Temperate)		Vineyard (G2 to G1) Similar mesoclimate	Vineyard (O2 to O1) 600m to 900m altitude
Data Block	Shiraz	Cabernet Sauvignon	Attribute	Shiraz	Cabernet Sauvignon	Shiraz	Cabernet Sauvignon	Shiraz	Shiraz
	142	143	Alcohol						
	143	144	Astringency						
		145	Capsicum						
		146	Tomato Leaf						

6.6 Grape Composition

6.6.1 Basic Grape and Juice Parameters

As expected, delaying harvest resulted in increasing grape juice TSS and pH and decreasing TA. The increase in grape juice TSS with maturity was more pronounced in the 2014 vintage in Griffith in comparison to 2015, Table 20. The 2014 season was characterised with several heat waves in January and February that resulted in some berry water loss in both Shiraz and Cabernet Sauvignon vineyards and consequently increased grape juice TSS from on average 23 Brix at FF to 26 Brix at MF for G1_SH and G2_SH. The increase in juice TSS from FF to MF in 2015 vintage was small, and no significant increase in ethanol content in G2_SH wines from FF to MF stage was observed. However, the sensory panel did perceive wines from G2_SH from FF to MF stage as significantly different and distinguishable. These results indicate that wine aroma development in late ripening was not directly linked to the sugar accumulation.

An increase in juice TSS with delayed harvest was also noticed in samples from Orange. Juice samples from Orange had a higher TA in comparison to samples from Griffith at the same harvest date. It is well known, that cooler climates result in grapes with higher acidity, in particular higher malic acid. It is also well accepted that pH generally increases with grape ripening.

Table 20: Basic juice parameters (mean ± standard error, n=3) at harvest for Shiraz and Cabernet Sauvignon grapes from 2014 and 2015 vintage. Values followed by a different letter for one vineyard are different (p≤0.05).

Vintage	Vineyard	Harvest date	TSS (Brix)	TA (g/L)	pH	Berry fresh mass (g)
2014	G1_SH	FF	22.5±0.33b	5.37±0.13a	3.71±0.02b	111.7±3.5
		MF	26.8±0.06a	3.33±003b	4.36±0.006a	102.2±2.9
	G2_SH	FF	23.6±0.06c	6.13±0.03a	3.52±0.0c	106.9±1.4a
		MF	27.12±0.06a	3.4±0.06b	4.10±0.01a	92.5±1.9b
		JF	25.32±0.24b	3.1±0.0c	4.04±0.01b	102.4±2.8a
	G1_CS	PFF	22.3±0.2c	3.56±0.07a	3.99±0.02ab	67.4±0.7
		FF	22.7±0.2c	3.13±0.03bc	3.97±0.02bc	69.3±2.8
		MF	25.6±0.0b	3.06±0.06c	3.93±0.01c	66.2±1.05
		JF	26.4±0.06a	3.20±0.00b	4.04±0.02a	70.3±2.6
	O1_SH	MF	22.4±0.24b	4.63±0.09a	3.63±0.01b	123.8±3.0a
		JF	24.96±0.16a	4.10±0.06b	3.70±0.02a	110.6±3.0b
	O1_CS	PFF	19.8±0.10c	5.83±0.15a	3.42±0.01b	98.1±2.1a
		Intermediate	21.54±0.16b	5.00±0.17b	3.45±0.01b	91.9±1.7 ab
		MF	22.0±0.06a	4.56±0.09c	3.50±0.01a	91.7±1.7b
2015	G1_SH	FF	23.28±0.12b	3.2±0.0b	4.00±0.01	111.6±1.4
		Intermediate	23.82±0.06b	3.5±0.06a	3.99±0.01	103.9±4.6
		MF	24.12±0.0a	2.9±0.03c	4.00±0.01	103.8±0.1
	G2_SH	FF	23.4±0.11b	3.53±0.07a	3.71±0.01c	127.2±3.3
		Intermediate	24.36±0.12a	2.97±0.03b	3.92±0.01b	126.5±4.5
		MF	24.7±0.3a	2.76±0.03c	4.01±0.003a	120.9±4.29
	G3_SH	FF	22.3±0.03b	4.13±0.06a	3.93±0.02c	144.1±3.9
		Intermediate	22.2±0.06b	3.7±0.06b	4.03±0.01b	140.8±5.6
		MF	23.6±0.06a	3.4±0.03c	4.18±0.003a	142.0±2.4
	G4_SH	FF	22.5±0.0c	3.5±0.03a	3.7±0.003c	119.9±2.3
		Intermediate	23.7±0.06b	2.8±0.03b	3.85±0.0b	114.7±1.8
		MF	24.7±0.10a	2.43±0.03c	4.02±0.007a	109.6±3.5
	G1_CS	FF	22.8±0.06c	5.50±0.11a	3.60±0.01b	86.1±0.6
		Intermediate	24.1±0.00b	4.50±0.06c	3.66±0.01a	85.6±3.1b
		MF	25.3±0.06a	4.86±0.03b	3.69±0.01a	83.2±1.2b
	O1_SH	FF	22.7±0.12c	5.7±0.09a	3.29±0.006c	113.9±0.7a
		Intermediate	24.24±0.06b	4.93±0.03b	3.38±0.009b	106.6±1.9
		MF	25.3±0.06a	4.51±0.05c	3.56±0.01a	101.9±2.5
	O1_CS	FF	24.3±0.1c	5.00±0.03a	3.37±0.006b	81.2±1.6
		Intermediate	25.6±0.2b	4.13±0.06b	3.49±0.01a	82.7±0.9
		MF	26.3±0.2a	3.90±0.06c	3.53±0.03a	76.2±2.8
	O2_SH	FF	22.4±0.1c	6.2±0.06a	3.33±0.003c	194.7±3.2a
		Intermediate	23.4±0.1b	5.6±0.1b	3.40±0.01b	181.6±4.3b
		MF	25.02±0a	5.1±006c	3.50±0.003a	175.4±2.9b
	O2_CS	FF	23.9±0.1b	7.73±0.03a	3.33±0.01a	131.4±3.1
		Intermediate	24.5±0.3a	7.07±0.07b	3.33±0.01a	122.7±4.1

Several classes of compounds were analysed in grapes to determine the effect of harvest date, vintage, region, and yield on grape berry primary and secondary metabolism. Grape berries

were analysed for its content of organic acids, sugars, amino acids, anthocyanins and total polyphenols, volatiles (terpenes, norisoprenoids and C6-compounds), carotenoids and lipids.

Berry chemical composition will be discussed under several points and will focus primarily on describing differences between FF and MF harvest dates. Despite detailed analyses of grape berry chemical composition, only a few compounds were identified that were either significantly increased or decreased as a consequence of a delayed harvest date. The list of compounds that was significantly different between FF and MF stage was more comprehensive than comparing FF to Intermediate stage or Intermediate stage to MF for both Shiraz and Cabernet Sauvignon varieties.

6.6.1.1 Amino acids

6.6.1.2

Irrespective of the cultivar, amino acids was the group of compounds that was altered most during the grape ripening. As a general trend, several amino acids increased as Shiraz ripened, which resulted in an increased amino acids/ammonia ratio and in some instances also higher YAN and NOPA concentrations. Increased amino acid levels as well as decreased ammonia concentrations with grape maturity has been previously reported (Bell and Henschke, 2005).

In contrast to the trend observed in Shiraz, concentrations of several amino acids decreased in Cabernet Sauvignon grapes with ripening. This trend was particularly obvious in G_CS in both 2014 and 2015 vintages. Hochberg et al. (2013) also reported a decreasing trend in amino acids concentrations during ripening of Cabernet Sauvignon grapes.

Despite several significant modifications of amino acid concentrations during ripening from FF to MF, only a few trends were significant across vintages, vineyards, and cultivars. Two amino acids that significantly increased from FF to MF harvest in all Shiraz vineyards in both vintages were proline and GABA. Proline accumulation is one of the most frequently reported modifications induced by water stress and salinity in plants and it was considered to be involved in stress resistance mechanism (Lutts et al. 1999). Proline was also suggested to facilitate senescence, or to be at least the consequence of senescence in rose petals (Kumar et al. 2008). Therefore, it is not surprising to observe increasing concentrations of proline in grape berries subjected to delayed harvest time (Table 21) in Shiraz. Despite Cabernet Sauvignon being classified as a proline accumulating variety, the increase in proline with ripening was more specific to Shiraz than Cabernet Sauvignon. This result suggests that most of the proline accumulation in Cabernet Sauvignon occurs before the Fresh fruit stage.

GABA concentrations also increased significantly from FF to the MF harvest with a large (3.2 to 4.5 fold) increase in GABA concentrations observed in the 2014 season for Shiraz. This increase was observed in the berries after the initiation of heat waves, which is consistent with reported changes in concentrations in different plants under increased temperature, drought, or other biotic and abiotic events (Kinnersley and Turano, 2000). While speculative, it is possible that GABA plays an important role in a grapevine stress mechanism. The increase in GABA, by a factor of only 1.5, was less prominent in 2015 season. A milder vintage with fewer heat waves was observed in this vintage.

Concentrations of amino acids such as valine, isoleucine, and to a lesser extent leucine, which is formed from isoleucine, increased in the grapes with maturity. These findings were observed in Shiraz and to a lesser extent in Cabernet Sauvignon. These branched chain amino acids are derived from glycine, serine and threonine metabolism, respectively and share four common enzymes, indicating up-regulation of the whole pathway during ripening. Isoleucine concentrations have been reported to increase in ripening 'Jonagold' apple fruit (Sugimoto et al. 2011). Other studies have suggested tight regulation of branched chain amino acids by environmental perturbations, such as osmotic stress (Joshi et al. 2010). It seems that several amino acids could potentially play an important role in grape senescence and be regulated under abiotic stress. Besides a biological role, amino acids play an important part in the process of fermentation and yeast metabolism. Branched chain amino acids may also influence higher production of branched chain fatty acids esters.

Table 21: Significant compounds as influenced by harvest date (FF and MF) across two vintages (2014 and 2015) for G1_SH and G2_SH.

	Mean	**Mean**	**p**
	FF	MF	
GABA (mg/kg)	31.9±16.7	103±73	0.002986
isoleucine (mg/kg)	18.2±5.8	26.1±5.6	0.002710
leucine (mg/kg)	28.1±7.8	43.2±9.3	0.000307
proline (mg/kg)	479±116	794±114	0.000001
tyrosine (mg/kg)	27.2±6.9	38.6±15.7	0.032219
valine (mg/kg)	36.9±9.8	60.6±11.7	0.000022
glucose (g/kg)	109±7	124±10	0.000355
fructose (g/kg)	105±4	119±7	0.000004
malic acid (g/kg)	1.9±0.2	1.57±0.33	0.001683
tartaric acid (g/kg)	7.9±0.8	9.29±1.31	0.006591
cis-3-hexenal[§]	0.34±0.06	0.26±0.07	0.006615
cis-3-hexenol[§]	0.004±0.002	0.002±0.001	0.028777
heptanol[§]	0.0076±0.002	0.01±0.003	0.001014
nonanal[§]	0.05±0.02	0.07±0.02	0.047562
t-2-heptenal[§]	0.06±0.01	0.13±0.07	0.005033
malvidin-6-(6-Ocoumaryoyl)gluc (mg/kg)	88.9±20.6	140±43	0.001119
peonidin-6-(6-Ocoumaryoyl)gluc (mg/kg)	23.8±13.9	43.2±11.3	0.001150
neoxanthin (mg/kg)	0.35±0.04	0.28±0.06	0.001993
chlorophyll b (mg/kg)	1.96±0.21	1.55±0.25	0.000337
Brix	23.2±0.51	25.7±1.4	0.000006
ratio Aas/Am	3.25±0.82	4.17±0.59	0.004480
pH	3.73±0.17	4.11±0.15	0.000007

[§]…peak area ratio normalised on sample weight. All quoted uncertainty is a standard deviation of three replicates per treatment.

Table 22: Significant Cabernet Sauvignon grape and juice metabolites as influenced by harvest date in Griffith in 2014 and 2015 vintage, comparing FF and MF harvest date.

	Mean	**Mean**	**p**
	FF	MF	
alanine (mg/kg)	65.8±22.8	36.1±2.8	0.010666
arginine (mg/kg)	263±98	163±98	0.042850
aspartic acid (mg/kg)	41.6±3.4	29.5±3.4	0.013540
glutamine (mg/kg)	64.9±17.9	40.9±17.9	0.034138
isoleucine (mg/kg)	31.3±5.7	38.1±5.7	0.054364
leucine (mg/kg)	42.5±6.9	51.7±6.9	0.044516
threonine (mg/kg)	80.5±22.8	53.2±8.1	0.019916
glucose (g/kg)	102.6±5.6	109±2	0.022825
fructose (g/kg)	103±3.4	118±2.3	0.000004
Brix	22.7±0.2	25.4±0.1	0.000000
ammonia (mgN/L)	69.8±1.7	63.9±5.4	0.027198
NOPA (mgN/L)	105±12	86±10	0.016384
YAN (mgN/L)	162±12	138±6	0.001596
DAP (g/L)	0.051±0.06	0.31±0.03	0.000002

[§]…peak area ratio normalised on sample weight. All quoted uncertainty is a standard deviation of three replicates per treatments.

6.6.2 Grape volatiles and precursors

6.6.2.1 Grape berry terpenes

Grape berry terpenes and norisoprenoids concentrations were largely unaltered from FF to MF stage in both Shiraz and Cabernet Sauvignon grapes in both vintages and regions. Previous studies reported an increase in monoterpene glycosides in grapes after veraison (Boss et al. 2014; Martin et al. 2012). These studies sampled shortly after veraison, a period of active sugar accumulation, and did not focus on late ripening as occurred with the current study. Similarly, Marais and van Wyk (1986) reported an increase in grape monoterpenes with grape maturity. In contrast, Zhang et al. (2016) recently reported that in Shiraz grapes the highest concentration of total terpenes, sesquiterpenes, and norisoprenoids was measured before veraison. The same authors also speculated that the increase in abundance of some terpenes during ripening could be linked to the release from the existing precursors and not to the de novo synthesis. Marais and van Wyk (1986) also reported that a decrease in terpene concentrations could be found in the last stage of ripening or over-ripening.

Terpenes have an important biological role in plant signalling in response to biotic or abiotic stressors. However, in the current study, 13 monitored terpenes remained stable during ripening. This was irrespective of the heat waves experienced in the 2014 season in Griffith and water constraints experienced in O1. The only terpene that increased significantly in G1_SH and

G2_SH grapes from FF to MF stage in 2014 was trans-geraniol. However, this trend was not observed in 2015.

In agreement with the observations in grapes, no particular trend was observed for wine terpenes in response to the harvest date. However, together with norisoprenoids, grape berry terpenes played an important role in the differentiation of samples according to the cultivars and climate. A clear gradient in sample separation according to the climate could be observed when PCA (Figure 49) was applied. Samples from the coolest site (O2) were associated with the majority of terpenes and norisoprenoids analysed. The negative correlation between the abundance of monoterpenes and norisoprenoids is in agreement with Marais et al. (2001) and Marais et al. (1999), who also observed a negative coefficient between the maximum temperature and monoterpene concentrations. Additionally, Shiraz samples were associated with linalool and terpenes deriving from linalool together with (E)-geraniol. Cabernet Sauvignon samples were associated with terpenes such as citronellol, terpinolene and others as well as the norisoprenoids α-ionone and β-damascenone, Figure 50. Therefore, monoterpenes and norisoprenoids could be used to distinguish between non-aromatic cultivars such as Shiraz and Cabernet Sauvignon and not only between Muscat and non-Muscat varieties.

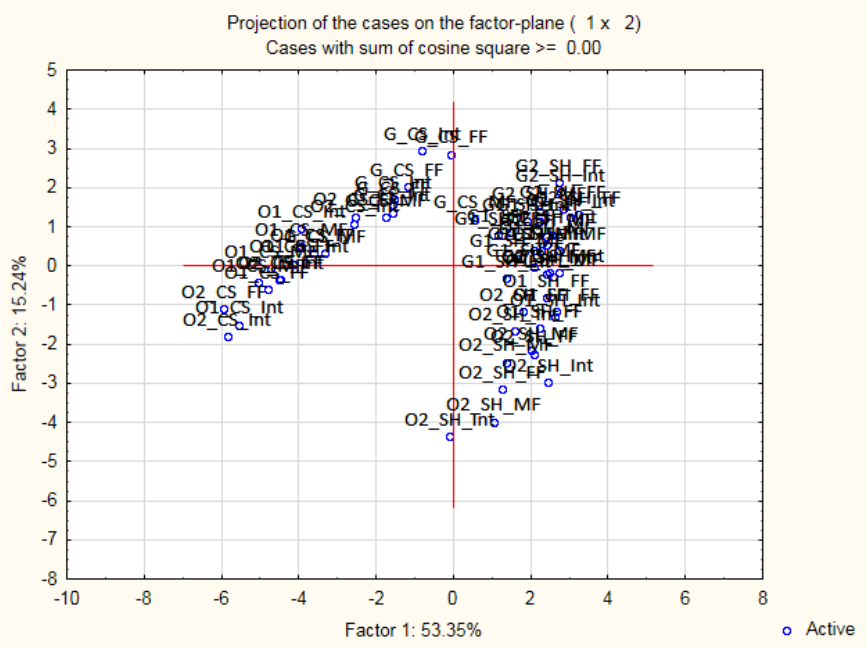


Figure 49: Principal component analysis applied to grape berry terpenes and norisoprenoids in samples from G1_SH and G2_SH, G_CS, O1_SH , O1_CS, O2_Sh and O2_CS from 2015 vintage, all harvest dates.

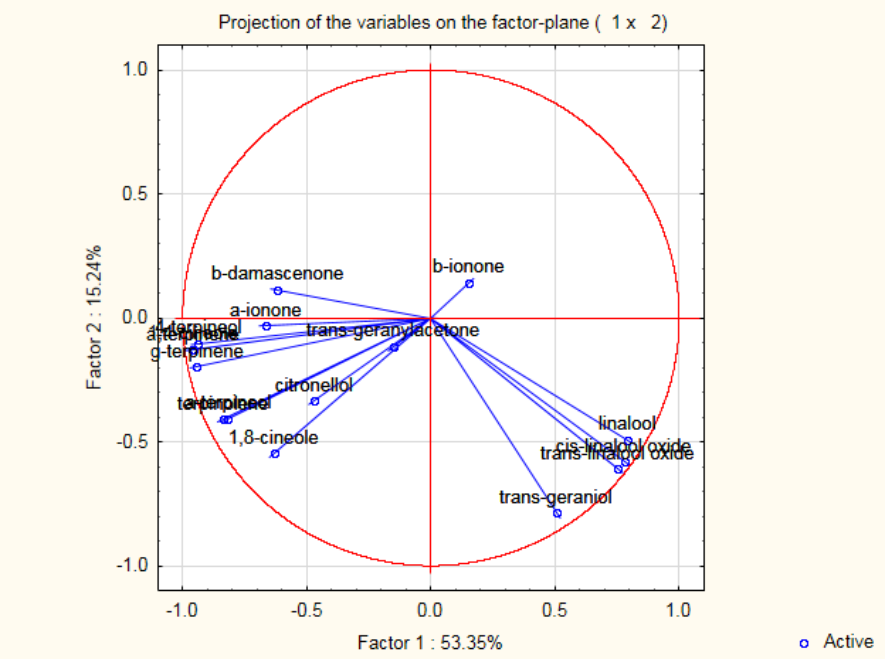


Figure 50: Loadings plot of grape berry terpenes and norisoprenoids in samples from G1_SH and G2_SH, G_CS, O1_SH, O1_CS, O2_Sh and O2_CS from 2015 vintage, all harvest dates.

Table 23: Compounds significantly influenced by harvest date (FF and MF) across two regions (Orange and Griffith) in 2015 season, Shiraz.

	Mean	**Mean**	**p**
	FF	MF	
GABA (mg/kg)	13.52 ±6.01	25.8±12.5	0.005828
isoleucine (mg/kg)	13.01±4.23	17.7±4.6	0.017427
proline (mg/kg)	440±135	719±131	0.000039
valine (mg/kg)	30.6±9.1	40.7±14.1	0.048598
fructose (g/kg)	109.8±4.1	118±5.8	0.000758
malic acid (g/kg)	2.18±0.39	1.80±0.41	0.032828
tartaric acid (g/kg)	7.51±0.47	8.07±0.58	0.016444
cis-3-hexenal[§]	0.36±0.08	0.28±0.06	0.023380
heptanol[§]	0.009±0.003	0.01±0.004	0.045101
hexanol[§]	0.11±0.07	0.34±0.31	0.021210
malvidin-6-(6-ocoumaryoyl)gluc (mg/kg)	94.7±26.6	156±28	0.000016
peonidin-6-(6-ocoumaryoyl)gluc (mg/kg)	33.5±24.1	58.5±8.7	0.002735
zeaxanthin (mg/kg)	0.14±0.03	0.09±0.02	0.001083
Brix	22.9±0.5	24.8±0.49	0.000000

[§]…peak area ratio normalised on sample weight. All quoted uncertainty is a standard deviation of three replicates per treatment.

6.6.2.2 Carotenoids and norisoprenoids

Carotenoids and chlorophyll concentrations decreased with grape maturity; however, the trend was not significant for all the vineyards irrespective of the cultivar. The decline in carotenoid and chlorophyll content after veraison is documented (Joubert et al. 2016; Young et al. 2015). Degradation of carotenoids is thought to be related to the disappearance of chloroplast and related to the formation of pleasant norisoprenoids (C9, 11 and 13) (Mendes-Pinto, 2009). Several studies have aimed to establish a correlation between degradation of carotenoids and norisoprenoids formation. Crupi et al. (2010) reported that the higher the difference between carotenoids degradation from veraison to harvest, the higher the norisoprenoid abundance. Despite the observed decrease in several xanthophylls from FF to MF (zeaxanthin, neoxanthin) no increase in grape berry norisoprenoids (α-ionone, β-ionone, β-damascenone and others) as a consequence of carotenoids degradation was noticed in either Shiraz or Cabernet Sauvignon grapes. Similar results were also observed in corresponding wines, where only small and inconsistent differences in norisoprenoids concentrations were noted. Late stages of ripening, after the plateau of sugar accumulation per berry, did not result in significant modifications of grape berry terpenes and norisoprenoids.

6.6.2.3 Lipids and green leaf volatiles

Grape berry lipids analyses were not included in general models as samples were analysed with two distinctively different methods in different vintages. Samples from the 2014 and 2016 vintages were analysed by GCMSMS after derivatisation, measuring methylated esters of fatty acids in this instance. In 2015, an LCMSMS method to measure several classes of lipids in grapes (fatty acids, sterols, prenols and others) was used (Della Corte et al. 2015).

No consistent and significant modifications in grape berry lipids as a consequence of late berry ripening were noticed across the vineyards, vintages or cultivars. Principal component analysis was carried out on methylated esters of fatty acids analysed in vintages 2014 and 2016

(Figure 51 and Figure 53). In both vintages, cultivars could not be distinguished based on the methylated esters of fatty acids profile. Furthermore, no separation according to the harvest date was evident.

Figure 51 shows a slight separation of samples according to the region, i.e. Griffith and Orange, can be seen in the 2014 vintage. However, samples from different vineyards inside the same region were not separated.

In the 2016 vintage samples were collected from two different locations in Orange and a separation according to the sites was observed, irrespective of the cultivar, based on the

methylated fatty acids esters (Figure 53). Examination of data loadings revealed that samples from the vineyard subjected to more stress were associated with the majority of fatty acids (Figure 54). For example, in the 2014 season samples from Griffith were found in the negative dimension of PC1 together with the majority of fatty acids analysed. As evident from above climatic data, vintage 2014 clearly showed warmer temperature in Griffith than in the Orange 1 vineyards. The Griffith region was subjected to several heat waves in the ripening season of 2014 vintage, which could have altered grape lipid metabolism, irrespective of the cultivar. Similarly, in 2016, O1 vineyard was associated with warmer temperature than O2 vineyard, and O1 had the majority of fatty acids analysed in the experiment. Both Shiraz and Cabernet Sauvignon vineyards on the location O1, were also subjected to severe water constraints during the late ripening season, as evident from soil moisture measurements and also visual observations. Vineyards on the location O2 were high altitude vineyards and experienced lower temperature and no water restrictions during the season. This strengthens the hypothesis that environmental stress affects the content of fatty acids in grapes, irrespective of the cultivar. Similar results were obtained in vintage 2015, where several classes of grape berry lipids were analysed.

Irrespective of site, the most abundant fatty acids in grapes were palmitic, linoleic, stearic and behenic acid. Other acids were found in lower concentrations in deseeded grape berries.

Linoleic and linolenic acids are often mentioned as substrate in LOX pathway for enzymatic and oxidative degradation for formation of green leaf volatiles under environmental stress. In the current study, no particular behaviour could be attributed to either linoleic or linolenic acid from FF to MF stage. Another study has suggested that substrate availability is often not the limiting factor in production of C6-compounds but rather enzymatic activity (Kalua and Boss, 2009). C6-compounds are also important as they can be utilised during fermentation for the production of pleasant fruity esters, such as hexyl acetate (Dennis et al. 2012). The same authors demonstrated direct contribution of several C6-compounds (hexanol, hexanal, (E)-2-hexenol and (E)-2-hexenal) to the formation of hexyl acetate. As a general trend, the literature reports a decrease in C6-alcohols after veraison. For aldehydes, an increasing trend in the first weeks was noticed, followed by a decrease (Kalua and Boss, 2009; Salinas et al. 2004). However in late ripening, trends of decreasing concentrations of C6-compounds become less evident and compound dependant (Boss et al. 2014; Pedneault et al. 2013).

In the current study lipoxygenase derived compounds were subjected to more dynamic modifications, compared to other grape volatiles. None of the compounds measured showed a consistent pattern across both vintages and the two regions. Cis-3-hexanal exhibited decreasing

trends with ripening in all Shiraz vineyards except one. Heptanol concentrations increased with Shiraz ripening, however not always significantly. In both Shiraz vineyard in Griffith in 2014, concentrations of grape (Z)-3-hexenal, (Z)-3-hexanol and (E)-2-hexanal decreased significantly from FF to MF, whereas an increase in heptanol concentrations was evident. This trend was less obvious in the 2015 vintage, which was characterised with milder climatic conditions. This is in agreement with Xu et al. (2015) who reported that the variability during berry development in concentrations of C6- volatile compounds, 2-methoxy-3-isobutylpyrazine and (E)-β-damascenone was strongly dependent upon weather conditions.

Results from our study indicate that ripening in relation to climatic conditions altered linoleic and linolenic acids degradation differently. Cis-3-hexanal is a direct linolenic acid degradation product. Cis-3-hexenol is formed by alcohol dehydrogenase from (Z)-3-hexanol (Kalua and Boss, 2009). These compounds decreased with grape ripening in Griffith Shiraz in 2014. On the other hand, linoleic acid's degradation product, heptanol, increased during ripening in Shiraz grapes. This observation could open new research perspectives into the behaviour of the LOX pathway.

Cabernet Sauvignon grapes were subjected to milder metabolic rearrangements, as already previously observed by Hochberg et al. (2015), in comparison to Shiraz. This was also evident for C6-compounds. In the 2014 vintage, which was characterised with heat events in late ripening, only (Z)-3-hexanol concentrations decreased significantly from FF to MF. In 2015, lipoxygenase derived compounds were not altered significantly with increasing maturity in G_CS and also O1_CS grapes. This observation corresponds well to the wine analyses, where (Z)-3-hexenol was found to be a clear marker of FF Shiraz and to a lesser extent Cabernet Sauvignon wines. However, the variability in (Z)-3-hexenol concentrations measured in wines may not originate solely from measured grapes concentrations, but could be attributed to pre-fermentative operations. Yeast metabolism may play an important role in C6-compounds transformation and formation.

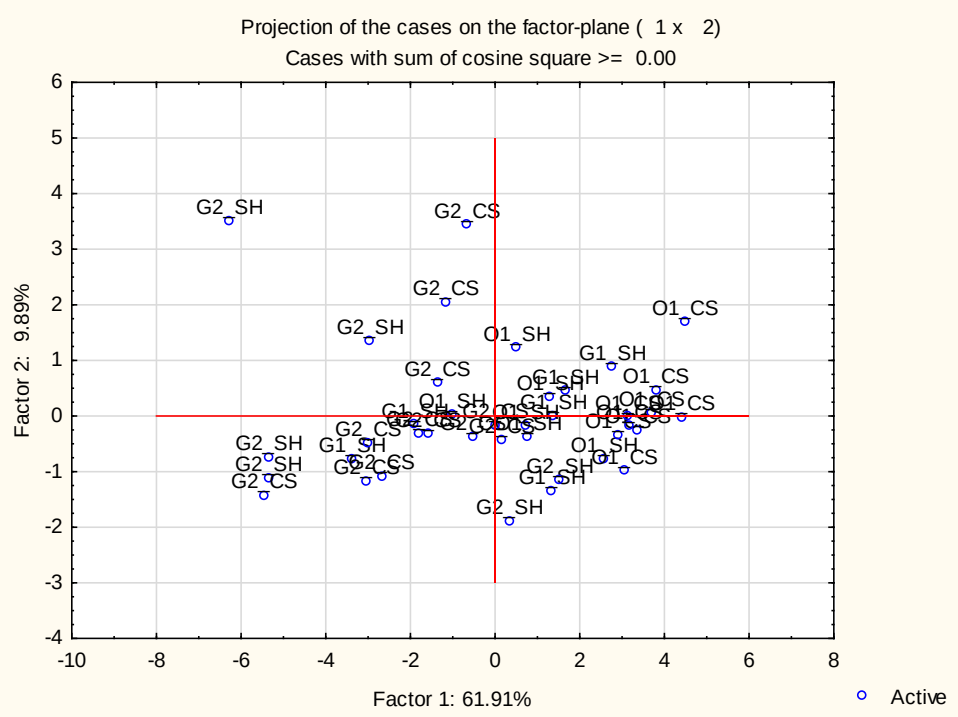


Figure 51: Principal component analysis on grape berry fatty acids in 2014 season.

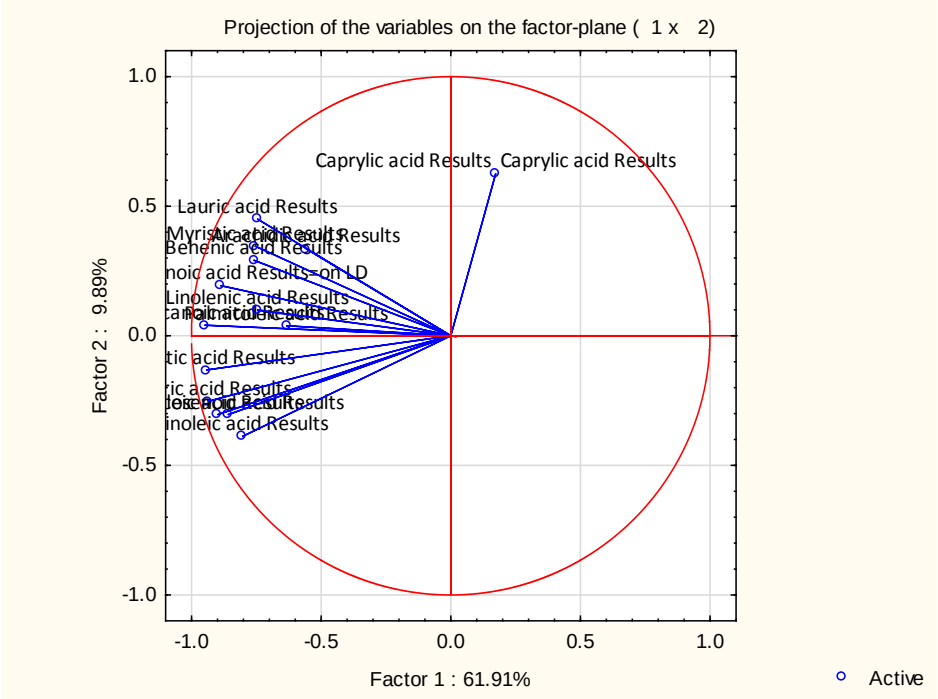


Figure 52: Loadings for grape berry fatty acids analysed in samples from 2014 vintage.

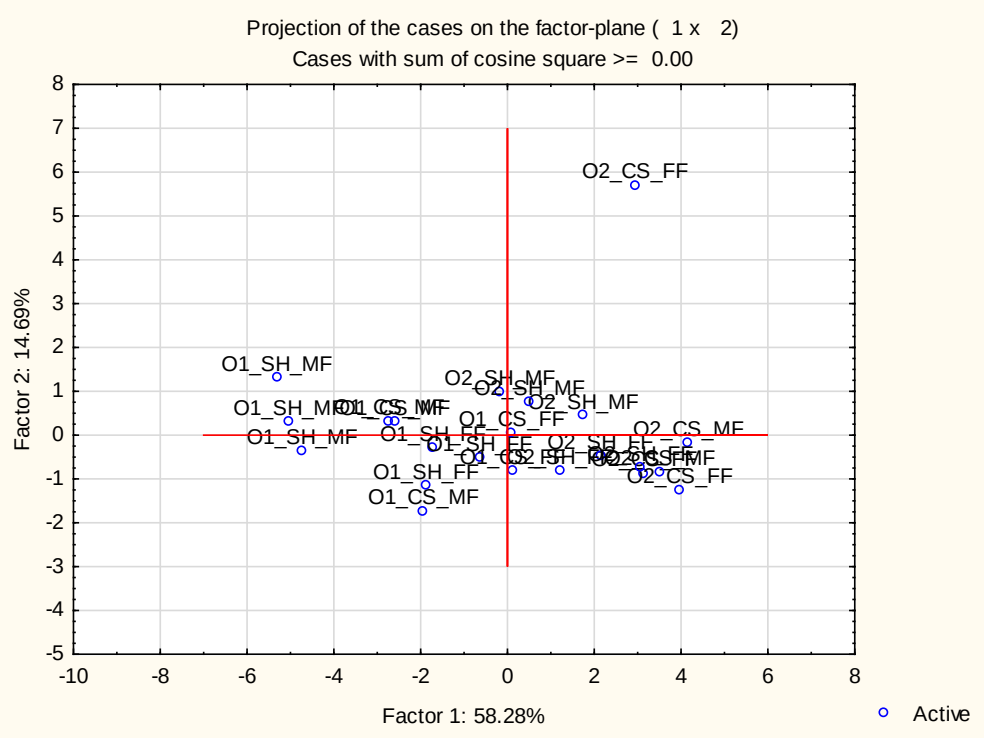


Figure 53: Principal component analysis on grape berry fatty acids in 2016 season.

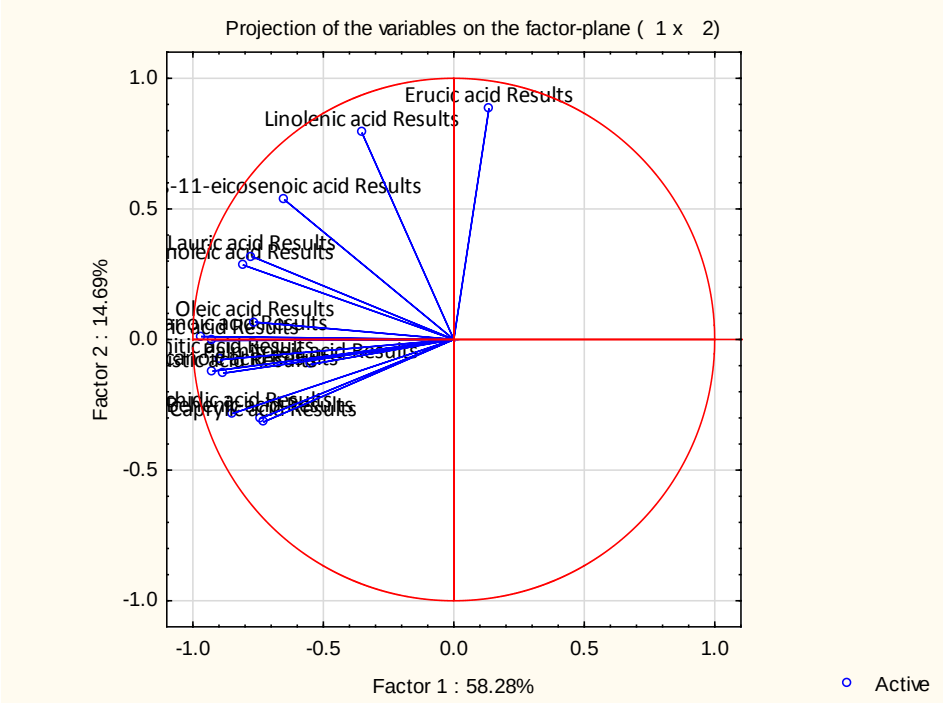


Figure 54: Loadings for grape berry fatty acids analysed in samples from 2014 vintage.

6.6.2.4 Grape berry phenolics

Our project primarily focused on the aromatic evolution during ripening and limited attention was given to grape berry anthocyanins and polyphenols. The onset of anthocyanins synthesis in grape berries is closely related to sugar accumulation (Castellarin et al. 2007). Similarly, in our study anthocyanins accumulation followed closely the tempo of sugar accumulation into the berry, and also reached a plateau of accumulation, followed by a decrease in late ripening in 2014 Griffith Shiraz (from FF to MF) for some individual anthocyanins (Figure 55).

The decrease from FF to MF in some anthocyanins (delphinidin-3-O-glucoside, malvidin-3-O-glucoside) was particularly prominent in G2_SH in 2014 vintage, whereas in 2015 Griffith Shiraz anthocyanins concentrations remained stable or even tended to increase with maturity.

An observed decrease in some Shiraz anthocyanins in 2014 vintage could be attributed once again to the occurrence of heat waves in late ripening (between FF and MF). Effects of day temperature and light intensity on anthocyanins accumulation have been reported (Dokoozlian and Kliewer, 1996). Warm to hot climates generally result in grapes with lower anthocyanins content. Similarly, grapes originating from the cooler Orange region (irrespective of the site) resulted in higher anthocyanins content in comparison to grapes from Griffith. The difference in the 2015 vintage in anthocyanins content was 2.1 fold for Cabernet Sauvignon and 1.8 fold for Shiraz. Anthocyanin accumulation was markedly reduced with high temperatures of around 35°C. High temperatures have been found to stimulate degradation of anthocyanins in the grape berry (Mori et al. 2007). At Griffith, Cabernet Sauvignon anthocyanins were not affected by the heat waves in the 2014 vintage and concentrations of malvidin-3-O-glucoside and malvidin-6(6-Ocoumaryoyl)-glucoside increased from FF to MF. Bindon et al. (2013) also observed an increase in Cabernet Sauvignon grape berry anthocyanins with grape ripening. Shiraz and Cabernet Sauvignon specific response in accumulation of anthocyanins under water stress has also been observed by Hochberg et al. (2015).

It seems that final grape berry anthocyanins concentrations, irrespective of the cultivar, were more influenced by the climatic conditions and the site rather than maturity. Even though grape berry anthocyanins were not modified predominantly by late grape ripening, harvest date played an important role in the extraction of pigments during the fermentation. Total grape polyphenols were not altered significantly by harvest date.

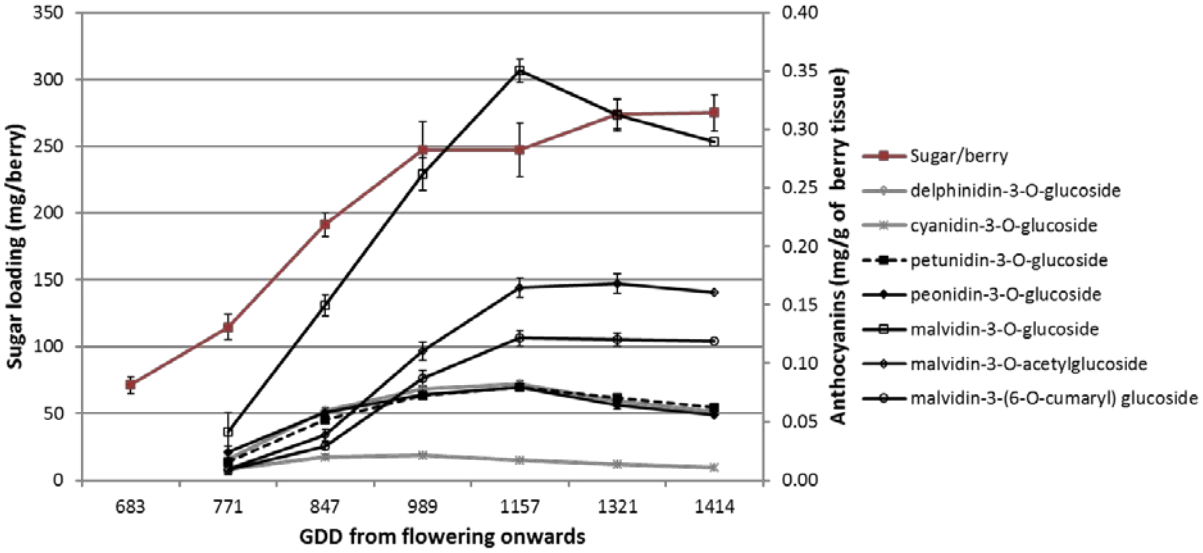


Figure 55: Sugar accumulation per berry and anthocyanins accumulation in the berry over the ripening in G1_SG in 2014.

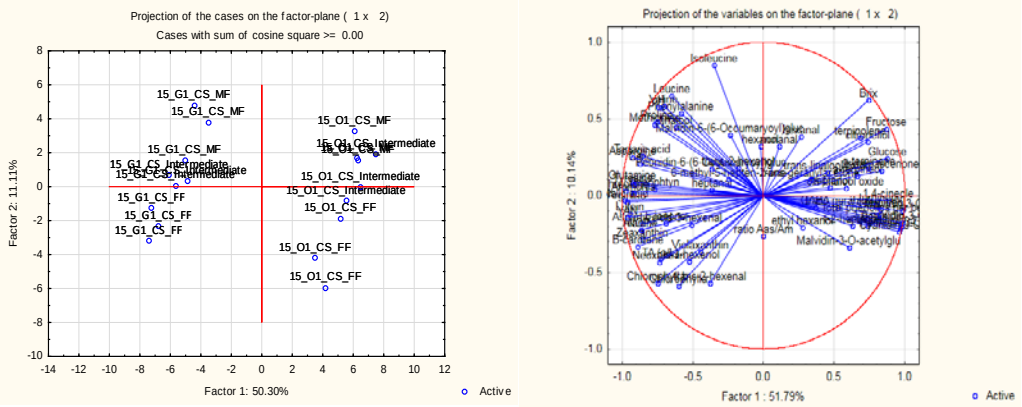


Figure 56: A Principal component analysis for grape and juice chemical composition for Griffith and Orange Cabernet Sauvignon samples, collected in 2014 and 2015 vintage. B loadings for Orange and Griffith Cabernet Sauvignon grape and juice chemical composition in vintage 2015.

6.7 Effect of yield on Shiraz grape chemical composition in Griffith in the 2015 vintage

Irrespective of the harvest date, yield had a significant effect on final grape and wine composition for the 2015 vintage as evident from the chemometric model B. As described in the methods section, two low yielding and two high yielding Shiraz vineyards were harvested sequentially according to the sugar-loading model in Griffith in the 2015 vintage. Independent t-test by groups was performed on analysed grape metabolites and significances are indicated in Table 24.

Table 24: Significant Shiraz grape and juice metabolites as influenced by yield.

	Mean	**Mean**	**p-**
	Low yield	High yield	
alanine (mg/kg)	57.4 ±6.5	74.1±22.1	0.004347
arginine (mg/kg)	520±81	895±358	0.000128
aspartic acid (mg/kg)	64.1±15.2	47.6±7.4	0.000226
isoleucine (mg/kg)	19.0±3.0	21.8±2.9	0.007898
leucine (mg/kg)	33.2±5.3	43.1±9.2	0.000353
threonine (mg/kg)	56.5±5.4	73.3±18.8	0.000882
tyrosine (mg/kg)	22.3±2.9	28.6±6.7	0.000989
valine (mg/kg)	43.2±9.6	54.7±12.2	0.003536
glucose (g/kg)	115±9.5	107±5.8	0.008843
fructose (g/kg)	111±8.9	104±5.8	0.011268
cis-3-hexenol§	0.0024±0.0006	0.005±0.003	0.003669
hexanol§	0.40±0.38	0.17±0.07	0.018991
trans-2-hexenal§	1.96±0.18	2.17±0.34	0.026705
1,8-cineole §	0.009±0.0012	0.01±0.005	0.039592
cyanidin-3-O-glu (mg/kg)	8.8±2.108	4.28±2.11	0.000000
dephinidin-3-O-glu (mg/kg)	32.1±7.13	16.6±4.7	0.000000
malvidin-3-O-acetylglu (mg/kg)	146±25.9	122±14.9	0.001836
malvidin-3-O-glu (mg/kg)	264±54	183±27	0.000003
malvidin-6-(6-Ocoumaryoyl)gluc (mg/kg)	138±55	166±12	0.039541
peonidin-3-O-glu (mg/kg)	57.2±8.8	35.9±7.1	0.000000
peonidin-6-(6-Ocoumaryoyl)gluc (mg/kg)	38.7±20.6	50.1±2.5	0.025320
petunidin-3-O-glu (mg/kg)	39,3±7.3	23,5±4.9	0.000000
neoxanthin (mg/kg)	0,35±0.04	0,39±0.06	0.029880
β-carotene (mg/kg)	0.37±0.04	0,41±0.06	0.024772
Brix	23.9±0.6	23.2±0.94	0.004340
ammonia (mgN/L)	22.6±3.6	33,6±8.6	0.000017
NOPA (mgN/L)	93.2±14.7	116±34.5	0.015734
YAN (mgN/L)	112±17.3	143±40	0.004423
ratio Aas/Am	4.14±0.38	3,45±0.62	0.000284
DAP (g/L)	0,39±0.11	0,22±0.21	0.003292
Fermaid A (g/L)	0.38±0.00	0,36±0.04	0.048038

§…peak area ratio normalised on sample weight. All quoted uncertainty is a standard deviation of three replicates per treatment.

Irrespective of the yield, metabolites such as proline, Isoleucine, valine and also to a lesser extent GABA, increased consistently in both vineyards from FF to MF harvest. Other amino acids had an increasing trend with grape maturity that translated to significantly higher concentrations of YAN and NOPA measured in the grape juice of grapes collected at MF stage.

Several amino acids, NOPA and YAN concentrations were also higher in grapes from high yielding vineyards (G3_SH and G4_SH) compared to the low yielding G1_SH and G2_SH

vineyards. Vineyards managed for a high yield production are typically fertilised with nitrogen (N) and receive adequate water supply to avoid excessive water stress. Sufficient N and water availability stimulates N uptake by the vine, resulting in higher concentration of N-containing compounds in grape juice. High yielding vines also resulted in lower glucose, fructose and Brix concentrations in the grape berry, compared to the low yielding vines.

The only grape volatile with a consistent trend across both Griffith vineyards was 1,8-cineole, which decreased with grape maturity. Other grape volatiles were not altered significantly in either vineyard with maturity. Only a few volatiles, such as cis-3-hexenol, hexanol, trans-2-hexenal and 1,8-cineole, were significantly altered by yield. Cis-3-hexenol, hexanol, trans-2-hexenal are C6-compounds derived from the lipoxygenase pathway. These compounds have been previously reported to decrease with grape ripening (Coelho et al. 2007; García et al. 2003). A slower pace of ripening (irrespective of sugar accumulation) would be expected for high yielding, vigorous and shaded canopies, resulting in higher concentrations of cis-3-hexenol and trans-2-hexenal. Contrary to cis-3-hexenol and trans-2-hexenal, hexanol was found in lower concentrations in grapes from high yielding vineyards.

Contrary to volatiles, which were altered only to a small extent by the yield, grape pigments were significantly influenced. High yield resulted in significantly lower concentrations of anthocyanins in the berries, with the exceptions of the coumarylated forms of malvidin and peonidin, which were found in higher concentrations in grapes from high yielding vineyards. Several studies have reported negative effects of high yields on anthocyanin concentrations in the grape berry. The impact of yield on grape anthocyanins concentration is likely an indirect consequence of higher water availability, shading and slower ripening compared to lower yielding vines. Indeed, all these variables have been shown in combination with temperature to negatively affect synthesis of berry anthocyanins.

6.8 Outputs: Effect of Harvest Date and Yield on Shiraz Grape and Juice Composition

Vintage and site specific behaviour of monitored grape berry compounds has been reported. Only a few compounds that exhibited the same trend with ripening across all the vintages and vineyards could be identified. These compounds were common markers of grape maturity, Brix, for which concentrations increased from FF to MF stage, and TA, which decreased with grape ripening in all the vineyards. Additionally, two amino acids, proline and GABA, significantly increased with ripening in all Shiraz vineyards across two vintages.

The branched amino acids, valine, isoleucine and leucine, also increased with grape ripening, however the trend was not always significant in both cultivars. These compounds could play an important role in grape senescence, together with proline and GABA, which were also increased under environmental stress.

As a general trend, concentrations of amino acids tended to increase with grape ripening in Shiraz, whereas the opposite trend was noticed in Cabernet Sauvignon grapes.

Compounds derived from the lipoxygenase pathway showed a more dynamic behaviour in relation to the harvest date. However, none of the compounds increased or decreased significantly in all the vineyards in the two vintages and two regions. Cis-3-hexenal exhibited decreasing trends in all Shiraz vineyards, whereas heptanol concentrations were increasing with ripening, however not always significantly. No trend for C6-compounds for Cabernet Sauvignon was noticed.

Grape berry terpenes and norisoprenoids were not significantly altered during the late berry ripening in Shiraz and Cabernet Sauvignon grapes (from FF to MF stage). The only exception was trans-geraniol levels that increased with grape ripening in Shiraz. The trend was significant in the warm climate (Griffith) and less obvious in the cooler Orange.

Generally, grape berry anthocyanins did not show any particular trend during grape berry ripening, but were rather altered more by the site and climate in both Shiraz and Cabernet Sauvignon.

As a general trend, grape berry ripening was characterised by increased levels of glucose and fructose, and consequently Brix levels, and also with the decreasing malic acid concentrations.

Chlorophyll b, zeaxanthin and neoxanthin were observed to decrease in the last stage of berry ripening in both cultivars. However, trends were not always significant when FF and MF stages were compared across regions or vintages.

Grapevine yield had a profound effect on several grape metabolites, however grape berry volatiles (terpenes and norisoprenoids) were not altered significantly by increased grapevine load across both vineyards. In contrast, grape berry anthocyanins were significantly reduced in grapes from higher yielding vines.

Several amino acids were significantly increased in grapes from higher yielding vines, which resulted in significantly higher NOPA, ammonia and YAN concentrations in corresponding juices.

Higher yielding vines also resulted in lower Brix measured in juice and higher neoxanthin and β-carotene concentrations.

Results on grape berry metabolites obtained in this study indicate more drastic modifications of the grape berry metabolome for Shiraz during late ripening when compared to Cabernet Sauvignon. Cultivar specific trends were noticed, with an increase in concentration of several amino acids during ripening in Shiraz and a decrease in Cabernet Sauvignon. However, branched amino acids, valine, isoleucine and to a lesser extend leucine and proline, increased during grape ripening in both cultivars, suggesting a potentially important role in grapevine senescence. Importantly, the monitored pool of grape berry volatiles, was not altered significantly by harvest date or yield in both cultivars, suggesting that changes in wine volatiles as a consequence of harvest date do not directly originate from modified grape berry volatiles. The observed changes in wines, based on these results, suggest an outcome of modified yeast metabolism as a consequence of altered available nitrogen and lipids pool. Chemical and enzymatic rearrangements of volatiles during wine ageing are also influential. An increase in grapevine yield did not alter grape berry volatiles, but significantly increased grape berry amino acid composition and negatively affected grape berry anthocyanins. A reduction in colour related pigments with an increasing yield was already previously reported.

6.9 Wine composition

Organic acids, alcohols, colour/phenolic parameters and volatiles were analysed in wine in order to highlight potential wine chemical markers of the FF and MF stages for both Shiraz and Cabernet Sauvignon. The establishment of relationships between wine and grape composition specific to the different maturity stages was attempted and will be discussed. The discussion below will primarily focus on FF and MF stages, which were defined by sensory analysis as recognisable and different. The results of 2014 and 2015 Shiraz wines will be presented separately but discussed together in order to pinpoint potential vintage effects. Data for 2015 Shiraz wines will be discussed as a whole including high yield and Orange vineyards in order to emphasise the robust trends irrespective of the vineyard condition (site, yield). Nevertheless, the influence of yield on Shiraz wine composition during ripening will also be discussed.

6.9.1 Organic acids and alcohols

The analysis of organic acids and alcohols in wine included succinic, lactic and acetic acids, glycerol and ethanol. As expected the concentration of ethanol and glycerol overall significantly increased with maturity (Table 25). This reflects the increase in grape berry TSS concentration with maturity, which affects yeast sugar metabolism. The increase of ethanol concentration from FF to MF was variable according to vineyard and vintage.

Table 25: Concentrations (mean± s.d.) of major compounds in Shiraz wines in 2014 and 2015 vintages at FF and MF harvests. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD). Lower and upper case letters were used for 2014 and 2015 vintage respectively.

Compounds/wine	SH FF 2014	SH MF 2014	SH FF 2015	SH MF 2015
Succinic acid (g/L)	1.47 ± 0.02a	1.50 ± 0.13a	1.26 ± 0.11A	1.28 ± 0.17A
Lactic acid (g/L)	1.68 ± 0.32a	1.18 ± 0.28b	1.79 ± 0.41A	1.52 ± 0.43B
Glycerol (g/L)	9.29 ± 0,27b	10.60 ± 0.19a	9.26 ± 0.28B	9.94 ± 0.48A
Ethanol (%v/v)	12.7 ± 0,27b	15.51 ± 0.25a	12.89 ± 0.53B	13.63 ± 0.51A
Acetic acid (g/L)	0.25 ± 0,06a	0.25 ± 0.06a	0.30 ± 0.02A	0.28 ± 0.03A

Table 26: Concentrations (mean± s.d.) of major compounds in Shiraz wines in 2015 vintage for low (G1 & G2) and high yield (G3 & G4) vineyards at FF and MF harvests. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD).

Compounds/wine	SH FF LY	SH MF LY	SH FF HY	SH MF HY
Succinic acid (g/L)	1.25 ± 0.05a	1.23 ± 0.02a	1.15 ± 0.02b	1.14 ± 0.04b
Lactic acid (g/L)	1.47 ± 0.09b	1.28 ± 0.6c	1.93 ± 0.55a	1.58 ± 0.6b
Glycerol (g/L)	9.55 ± 0.20b	9.99 ± 0.41a	9.09 ± 0.11c	9.50 ± 0.41b
Ethanol (%v/v)	13.40 ± 0.27a	13.67 ± 0.10a	12.48 ± 0.15c	13.07 ± 0.1b
Acetic acid (g/L)	0.29 ± 0.01a	0.27 ± 0.03b	0.30 ± 0.01a	0.30 ± 0.03ab

Table 27: Concentrations (mean± s.d.) of major compounds in Cabernet Sauvignon wines in 2014 and 2015 vintages at FF and MF harvests. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD). Lower and upper case letters were used for 2014 and 2015 vintage respectively.

Compounds/wine	CS FF 2014	CS MF 2014	CS FF 2015	CS MF 2015
Succinic acid (g/L)	1.53 ± 0.06a	1.65 ± 0.14a	1.44 ± 0.14A	1.50 ± 0.17A
Lactic acid (g/L)	1.10 ± 0.05b	1.28 ± 0.10a	1.88 ± 0.73A	1.59 ± 0.73A
Glycerol (g/L)	8.55 ± 0.18b	10.52 ± 0.06a	8.99 ± 0.49B	10.46 ± 0.37A
Ethanol (%v/v)	12.51 ± 0.15b	14.71 ± 0.29a	12.62 ± 1.23B	13.82 ± 0.94A
Acetic acid (g/L)	0.22 ± 0.004b	0.29 ± 0.04a	0.37 ± 0.19A	0.40 ± 0.16A

While in 2014 ethanol content increased by 2.7% and 2.2% v/v between FF and MF for Shiraz and Cabernet Sauvignon (Table 27) wines respectively, the variations were reduced to + 0.7% and + 1.2% v/v in 2015 (Table 25). As a result, MF wines reached 15.5% and 14.7% ethanol v/v in 2014 for Shiraz and Cabernet Sauvignon wines respectively, whereas in 2015 the averages for MF wines were up to 13.6% and 13.8% ethanol v/v. The increase in ethanol content in 2015 wines was the most significant for G1_CS, where the FF wine was only 11.5% v/v. Shiraz and Cabernet Sauvignon wines from Orange were also showed greater variations in alcohol content between FF and MF due to higher variations in the measured berry fresh mass

A more rapid increase in TSS in grapes and consequently ethanol in wines with maturity in 2014 wines from Griffith may be related to the higher frequency of heat waves in 2014 compared to

2015, due to late season dehydration-shrivelling. This was particularly observed for Shiraz, variety that is known to be sensitive to this type of shrivelling (McCarthy, 1999; Tilbrook and Tyerman, 2008). In contrast, Shiraz berry water loss during 2015 ripening season in Griffith was much more limited due to milder climatic conditions, leading to the stabilisation of wine ethanol levels between FF and MF stages (Table 25). For instance, 2015 Shiraz wines from the G2 vineyard contained the same level of ethanol at FF and MF stage, confirming the lack of direct relationship between aromatic evolution and grape TSS in the late ripening.

The proportional variations measured for glycerol concentrations followed the same trend as ethanol ranging from +7.5% to +23% for 2015 Shiraz and 2014 Cabernet Sauvignon wines, respectively (Table 25). This similarity was expected as glycerol is produced by yeast at the beginning of alcoholic fermentation from glucose and fructose similarly to ethanol (Ribéreau-Gayon et al. 2006). Moreover, the increase of glycerol concentration with maturity was slightly higher in Cabernet Sauvignon than Shiraz wines. On the other hand, succinic acid also deriving from yeast primary metabolism was not affected by grape maturity, even in 2014 when grape TSS concentration substantially increased from FF to MF stages (Table 25). This result pinpoints the complexity of yeast metabolism and indicates that further research, investigating the grape composition-yeast metabolism nexus, is warranted.

A decrease of L-lactic acid concentration was consistently measured between FF and MF stages in Shiraz and Cabernet Sauvignon wines (Table 25, Table 26, Table 27). L-lactic acid is mainly synthesised by lactic acid bacteria (LAB) during malolactic fermentation via the degradation of L-malic acid. Therefore, higher content of L-malic acid measured in grape berry harvested at FF stage leads to higher concentration of L-lactic acid in the corresponding wines.

No trend was observed for acetic acid for which the concentration remained below 0.5 g/L (Table 25, Table 26 and Table 27). At this level, acetic acid is mainly derived from yeast and LAB during alcoholic and malolactic fermentation. By maintaining a consistent approach to winemaking conditions and ensuring adequate nutrients appropriate for the concentration of fermentable carbohydrate, the risk of excessive acetic acid concentrations was minimised during this investigation. Grape maturity did not influence the final level of acetic acid in wines.

Yield did not alter the ripening pattern between FF and MF stages. Unsurprisingly, the same trend between maturity stages in Shiraz wines made from high (HY) and low yield (LY) Griffith vineyards was observed in 2015 (Table 26). Increases of ethanol and glycerol concentrations and decrease of L-lactic acid with maturity were measured in HY wines. Yield significantly affected the concentration of ethanol, glycerol, L-lactic and acid succinic acids (Table 26). The lower

levels of ethanol, glycerol and succinic acid in wines from HY vineyards (G3_SH and G4_SH) can be related to lower sugar content. While, this grape/wine relationship was found as a maturity effect in the case of ethanol and glycerol, it was not the case for succinic acid. Yield might influence the composition of grape metabolites that can selectively interfere with the production of succinic acid in yeast primary metabolism. Higher concentration of L-lactic acid in HY wines can be correlated to higher concentration of malic acid in grapes, reflecting a lower degree of maturity.

6.9.2 Wine colour parameters

Nine chemical parameters related to wine colour and phenolic composition were measured by spectrophotometry. Overall, grape maturity tended to increase wine colour and phenolics from FF to MF stages for both varieties. The lack of statistically significant differences between maturity stages was due to an important site effect overlapping the maturity effect, particularly in 2015 vintage. Differences were observed between vintages for Shiraz wines.

While Shiraz wine total anthocyanins and red pigment increased in 2015, the values remained stable in 2014 (Table 28, Table 29). In Cabernet Sauvignon wines, total anthocyanins and red pigment increased in both vintages (Table 30). Total phenolics, total tannins, colour density and SO_2 resistant red pigment increased in both vintages for Shiraz and Cabernet Sauvignon wines with maturity.

It has previously been reported that grape anthocyanins concentrations and total phenolics tended to plateau at Fresh fruit stage and tannins are mainly synthesised before veraison (Šuklje et al. 2014a; Walker et al. 2007). The overall increase in phenolic compounds between FF and MF stages is probably related to a higher level of extraction of phenolic compounds at Mature fruit compared to Fresh fruit stage, due to generally higher ethanol content. Wine tannins were the class of phenolics that were most influenced by grape maturity with relative increases from 25% to 50% (Table 28, Table 29, Table 30). Tannins are the most hydrophobic and the least extractible grape phenolic compounds. Therefore, increasing metabolite extractability from grape to wine strongly favours higher concentration of tannins in the final wine.

Total anthocyanins tend to decrease rapidly during wine ageing mainly due to condensation with tannins which leads to colour stabilisation (Boulton, 2001). The Tannins:Anthocyanins ratio is an important parameter for wine colour stabilisation and colour density of aged wine. The Tannins:Anthocyanins ratio increased overall from FF to MF stages in Shiraz and Cabernet Sauvignon wines due to a stronger effect of maturity on tannins extractability. In turn, colour density and SO_2 resistant pigment also increased from FF to MF stages for both varieties.

The lack of variation observed in 2014 Shiraz wines for anthocyanins and red pigment remains unclear. The chemical reaction occurring during wine ageing between phenolic compounds is complex. Thirty to forty per cent of Shiraz berries harvested in Griffith at MF stage in 2014 were shrivelled. It is often observed that wines made with a high proportion of shrivelled berries are more subject to oxidative evolution leading to a faster shift of wine colour from purple to orange. Loss of wine anthocyanins by oxidation could potentially occur in the 2014 Shiraz MF wines from Griffith, reducing the increase of wine total red pigment due to maturity effect. While this hypothesis is speculative, it is supported by the increase of wine hue and chemical age measured in 2014 Shiraz wine and not in 2015. An increase of wine hue corresponds to a shift from purple to orange colour, while chemical age reflects the degree of polymerisation between anthocyanins and tannins occurring during wine ageing. Therefore, Shiraz MF wines from Griffith showed a faster colour evolution in 2014 than 2015, probably due to higher proportion of shrivelled berries at harvest.

Wine colour quality was generally improved during ripening from Fresh fruit to Mature fruit due to a better extractability of phenolic compounds. However, climatic conditions or vineyard management that favoured grape berry late season dehydration can potentially limit this benefit.

Table 28: Concentrations (mean± s.d.) of colour parameters in Shiraz wines in 2014 and 2015 vintages at FF and MF harvests. Means followed by different letters in a row are significant at $p \leq 0.05$ (Fischer's LSD). Lower and upper case letters were used for 2014 and 2015 vintage respectively.

Compounds/wine	SH FF 2014	SH MF 2014	SH FF 2015	SH MF 2015
Total anthocyanins (mg/L)	524 ± 55a	536 ± 35a	409 ± 189A	473 ± 192A
Total phenolics (a.u)	31.5 ± 7.4a	39 ± 6.8a	30.8 ± 11.5A	36.2 ± 11.9A
Total tannins (g/L)	0.88 ± 0.22a	1.11 ± 0.27a	0.56 ± 0,28B	0,78 ± 0,41A
Total red pigments (a.u)	26.2 ± 2.8a	26.8 ± 1.8a	20.5 ± 9.5A	23.7 ± 9.6A
Hue	0.53 ± 0.01b	0.62 ± 0.02a	0.60 ± 0,1A	0.60 ± 0,08A
Colour density (a.u)	9.55 ± 1.01a	10.82 ± 0.93a	6.67 ± 4.9A	7.19 ± 4.04A
Chemical age	0.18 ± 0.03b	0.26 ± 0.04a	0.20 ± 0,04A	0.21 ± 0,02A
SO_2 resistant red pigment (a.u)	1.67 ± 0.18b	2.52 ± 0.22a	1.13 ± 0,53A	1.41 ± 0,68A
Degree of red pigment (a.u)	23.8 ± 1.6b	26.8 ± 1.8a	18.8 ± 6.3A	17.9 ± 5.1A
Tannins:Anthocyanins	1.67 ± 0.29a	2.06 ± 0.44a	1.37 ± 0.33B	1.59 ± 0.36A

Table 29: Concentrations (mean± s.d.) of wine colour parameters in Shiraz wines in 2015 vintage for low (G1 & G2) and high yield (G3 & G4) vineyards at FF and MF harvests. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD).

Compounds/wine	SH FF LY	SH MF LY	SH FF HY	SH MF HY
Total anthocyanins (mg/L)	328 ± 37b	413 ± 66a	253 ± 13d	294 ± 7c
Total phenolics (a.u)	26.6 ± 2.7b	32.5 ± 3.6a	21.2 ± 1.7c	25 ± 0.8b
Total tannins (g/L)	0.57 ± 0.1b	0.67 ± 0.16a	0.28 ± 0.04d	0.39 ± 0.11c
Total red pigments (a.u)	16.5 ± 1.8b	20.6 ± 3.3a	12.6 ± 0.7d	14.7 ± 0.3c
Hue	0.61 ± 0.08b	0.61 ± 0.03b	0.69 ± 0,04a	0.68 ± 0,01a
Colour density (a.u)	5.05 ± 1.74a	5.36 ± 1.18a	2.84 ± 0.17c	3.51 ± 0.25b
Chemical age	0.24 ± 0.04a	0.22 ± 0.02a	0.20 ± 0,01b	0.21 ± 0,01ab
SO_2 resistant red pigment (a.u)	1.08 ± 0.41a	1.23 ± 0.33a	0.62 ± 0,05b	0.81 ± 0,04ab
Degree of red pigment (a.u)	18.8 ± 5.6a	16 ± 1.5a	13.3 ± 0.8b	14.2 ± 1b
Tannins:Anthocyanins	1.74 ± 0.24a	1.62 ± 0.27a	1.11 ± 0.24b	1.33 ± 0.35b

Table 30: Concentrations (mean± s.d.) of wine colour parameters in Cabernet Sauvignon wines in 2014 and 2015 vintages at FF and MF harvests. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD). Lower and upper case letters were used for 2014 and 2015 vintage respectively.

Compounds/wine	CS FF 2014	CS MF 2014	CS FF 2015	CS MF 2015
Total anthocyanins (mg/L)	454 ± 9b	520 ± 18a	541 ± 212A	584 ± 211A
Total phenolics (a.u)	29.4 ± 1.4b	39.6 ± 1.6a	38.1 ± 13.3A	42.5 ± 14.3A
Total tannins (g/L)	1.06 ± 0.15b	1.52 ± 0.11a	0.79 ± 0.39A	1.06 ± 0,52A
Total red pigments (a.u)	22.7 ± 0.5b	26 ± 0.9a	27.1 ± 10.6A	29.2 ± 10.5A
Hue	0.68 ± 0.03a	0.67 ± 0.01a	0.60 ± 0,1A	0.65 ± 0,4A
Colour density (a.u)	7.22 ± 0.14b	10 ± 0.22a	8.58 ± 4.7A	10.02 ± 5.54A
Chemical age	0.28 ± 0.01b	0.36 ± 0.01a	0.23 ± 0,03B	0.29 ± 0,02A
SO_2 resistant red pigment (a.u)	1.69 ± 0.08b	2.71 ± 0.08a	1.68 ± 0.67A	2.38 ± 1.04A
Degree of red pigment (a.u)	19 ± 0.4b	23 ± 0.4a	18.9 ± 4.7A	19.7 ± 5.7A
Tannins:Anthocyanins	2.32 ± 0.32b	2.93 ± 0.29a	1.42 ± 0.24A	1.74 ± 0.33A

6.9.3 Wine aromatic compounds: grape/wine/sensory nexus

The present study has already demonstrated for Australian Shiraz and Cabernet Sauvignon the existence of a predictable aromatic sequence during grape ripening. This is characterised by two maturity stages (FF and MF) that were associated with distinct sensory profiles irrespective of the vintage and the region. This wine aromatic evolution from fresh/red berry fruit to mature/dark fruit spectrum was predicted for Shiraz and Cabernet Sauvignon using berry sugar accumulation and berry fresh mass as physiological indicators. Wine aromatic profile firstly depends on the grape variety and the abiotic factors (water, temperature, light) characterising a vineyard. For instance, the aromatic typicality of Australian Shiraz and Cabernet Sauvignon are in general differently described. Shiraz being more related to spicy, dark fruit, floral or meaty character, whereas Cabernet Sauvignon is more reminiscent of herbaceous, minty, dark fruit and cedar notes. A specific characterisation of Australian Cabernet Sauvignon wines typicality

has been particularly performed in this project and will be described later in this report. The influence of grape maturity is also very important for the final wine aromatic profile and a common sensory pattern in the evolution of wine aromas during ripening irrespective of the varietal and regional typicality appears to exist.

The chemometric approach emphasised the contribution of wine volatiles composition to this aromatic evolution. This part of the report will describe and discuss in more detail the modification of wine volatiles composition from Fresh Fruit to Mature fruit stage though untargeted and targeted analytical strategies.

6.9.4 Untargeted analysis

Investigations started with a preliminary screening of wine volatiles through an untargeted approach. SPE-GCMS untargeted wine analyses were performed accordingly to the method previously published (Schmidtke et al. 2013). Wine samples were scanned in order to extract preliminary information on the effect of harvest date on wine volatiles. Classes of compounds that were altered significantly by harvest date in at least one of the vineyards by the untargeted SPE-GCMS analyses were thereafter analysed in a quantitative manner by HS-SPME-GCMS methods. Untargeted analyses are of interest for screening a broad range of compounds, without previous limitations or selection, whereas quantitative analyses are essential to determine potential aromatic contribution of compounds to the final wine sensory profile.

Chromatogram deconvolution was performed and extracted features were identified by NIST. The list of putative compounds was purified for the presence of compounds deriving from column bleed and artefacts related to sample preparation. A t-test for independent samples was performed on purified putatively identified metabolites for each vineyard and compounds that were significantly altered by harvest date in at least one of the vineyard were subjected to principal component analysis (PCA).

PCA analyses were conducted on 48 compounds that were significantly different between two harvest dates in G1_SH and G2_SH in the 2014 vintage. Clear separation of samples according to the harvest date and also site was noticed. PC1 accounted for 62.19% of the variation in the data set and separated samples according to the harvest date. Separation according to the site was noticed along PC2, accounting for 22.3% of variation. Compounds strongly associated with earlier harvest dates were (Z)-3-hexenol, (Z)-2-hexenol, butanol, isoamyl lactate, 1-propanol, 3-methylthiol, whereas H3 was associated with higher presence of several esters such as hexyl acetate, ethyl decanoate, ethyl butanoate, isoamyl acetate and others (Figure 57 and Figure 58).

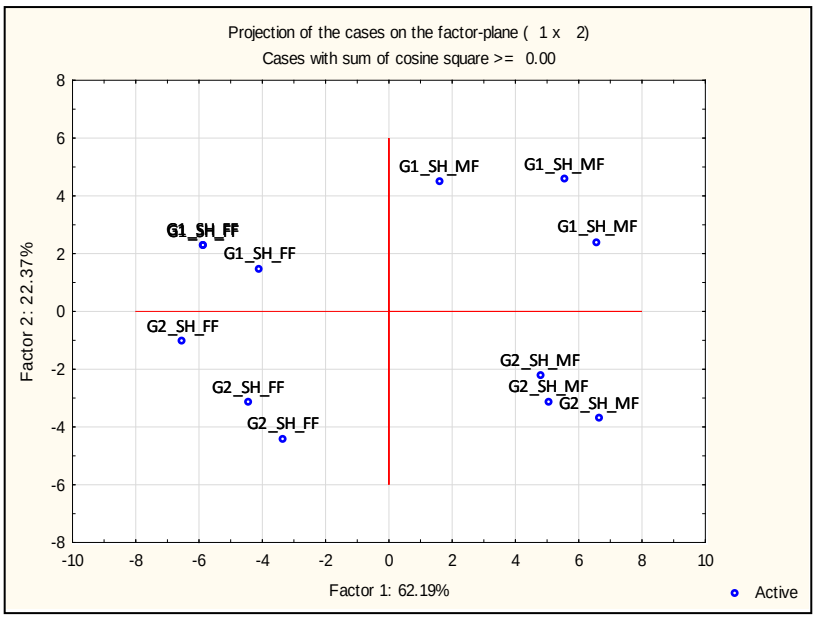


Figure 57: Principal component analysis (PCA) applied to wine volatiles identified to be significantly different in at least one Shiraz vineyard in Griffith from FF to MF harvest in 2014 vintage.

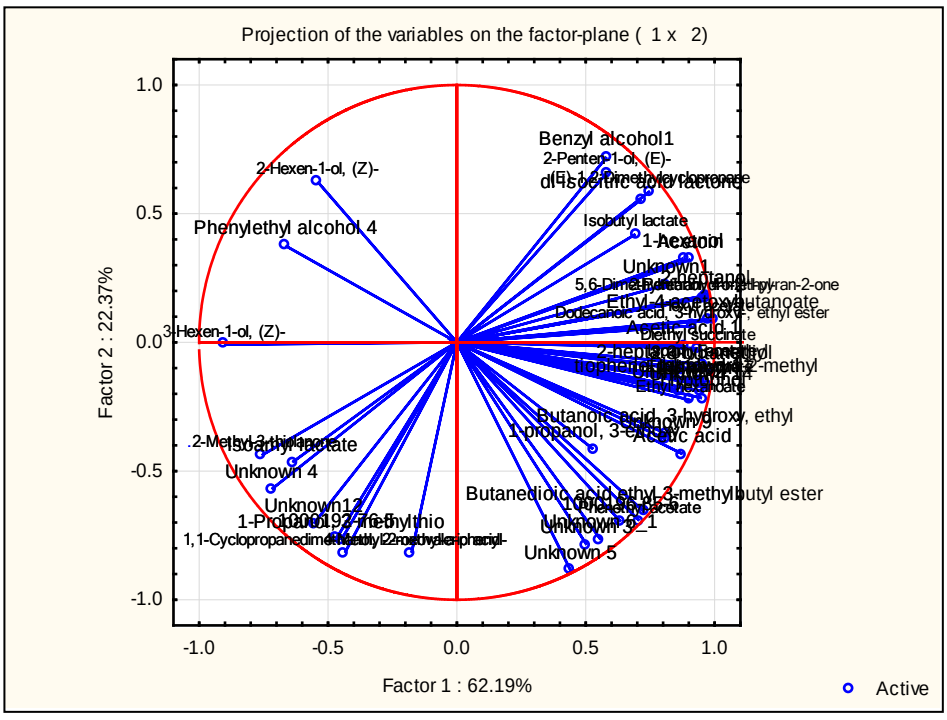


Figure 58: Loading plots of wine volatiles deriving from untargeted SPE-GCMS analyses that were significantly different between FF and MF harvest in at least one Shiraz vineyard from Griffith in 2014 vintage.

Similarly, harvest date also influenced G1_SH and G2_SH wine volatiles in the2015 season, however the number of metabolites altered by harvest date was lower compared to the 2014 vintage. In wines from the 2015 vintage, only 27 putative metabolites that were significantly different between FF and MF harvest in both of the vineyards were identified. As already discussed above, the 2014 season was associated with several heat waves in late ripening, altering significantly grape amino acids composition that could have a profound influence on yeast derived metabolites, such as esters. A milder 2015 vintage therefore resulted in less pronounced metabolic changes in grapes and also in wines. Samples could be separated based according to the harvest date (Figure 59) whereas separation according to the site was less evident for G1_SH and G2_SH in 2015 vintage. Separation of samples was for G1_SH achieved along PC1 whereas for G2_SH samples were separated along PC2 (Figure 59). Data loadings revealed that (E)-2- hexenol was, as in the 2014 vintage, associated with earlier harvest date (H1), whereas several esters were again associated with H3.

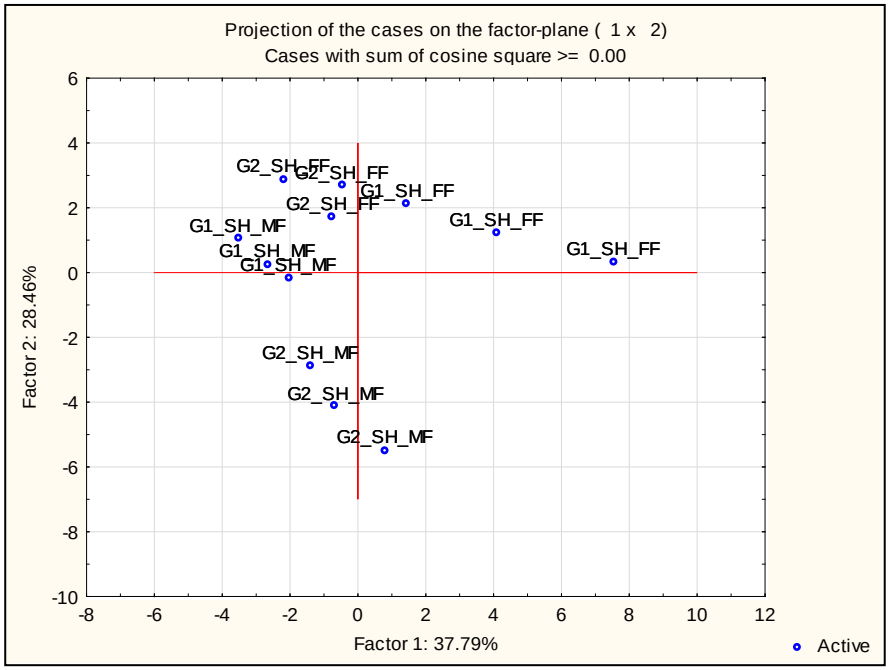


Figure 59: Principal component analysis (PCA) applied to wine volatiles identified to be significantly different in at least one Shiraz vineyard in Griffith from FF to MF harvest in 2015 vintage.

Smaller differences in wine volatile composition are noted for Cabernet Sauvignon, when compared to Shiraz. From more than 100 putatively identified compounds, only 22 were significantly altered by harvest date in at least one of the vineyards (O1_CS and G1_CS). Also, grape berry modifications in response to water stress were less pronounced in Cabernet Sauvignon compared to the Shiraz (Hochberg et al. 2015). Separating trends according to the harvest date was achieved by PC1 accounting for 55.1% of variation, whereas the effect of

regionality (Griffith and Orange) was noted according to the PC2, accounting for 24.7% of variation in data set. Wines made from grapes collected at H1 were strongly associated with hexanoic, octanoic and vanillic acid, (E)-3- hexenol, isoamyl acetate and others, whereas H3 samples were found together with 1-hexanol (Figure 60-62).

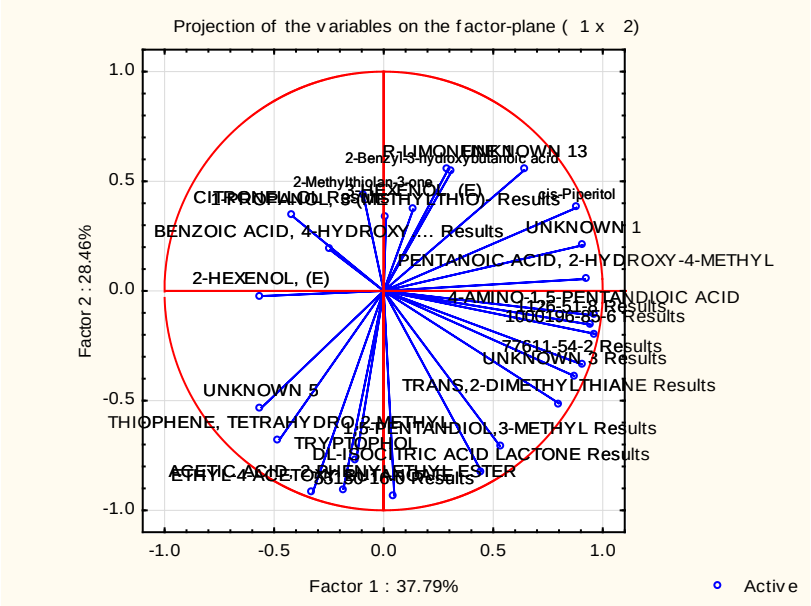


Figure 60: Loading plots of wine volatiles deriving from untargeted SPE-GCMS analyses that were significantly different between FF and MF harvest in at least one Shiraz vineyard from Griffith in 2015 vintage.

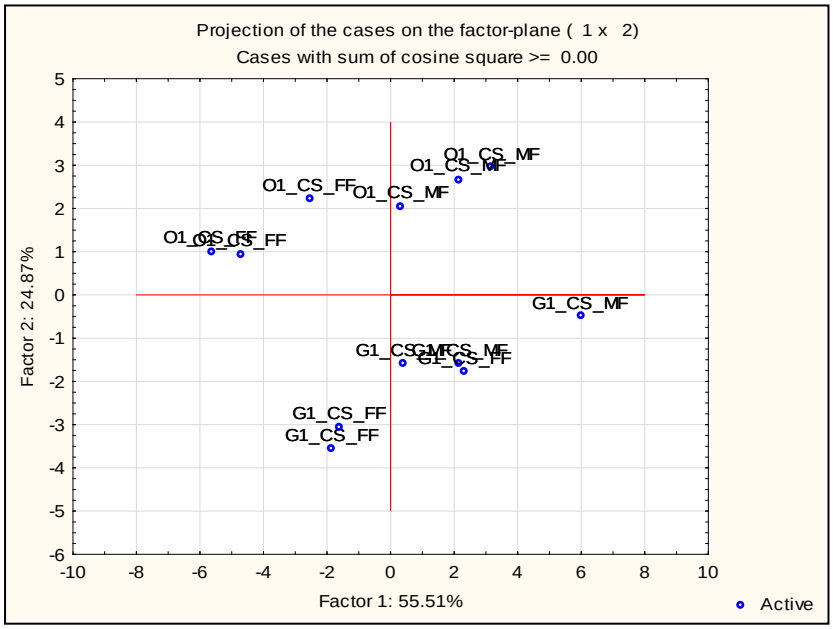


Figure 61: Principal component analysis (PCA) applied to wine volatiles identified to be significantly different in at least one Cabernet Sauvignon vineyard in Griffith and Orange from FF to MF harvest in 2015 vintage.

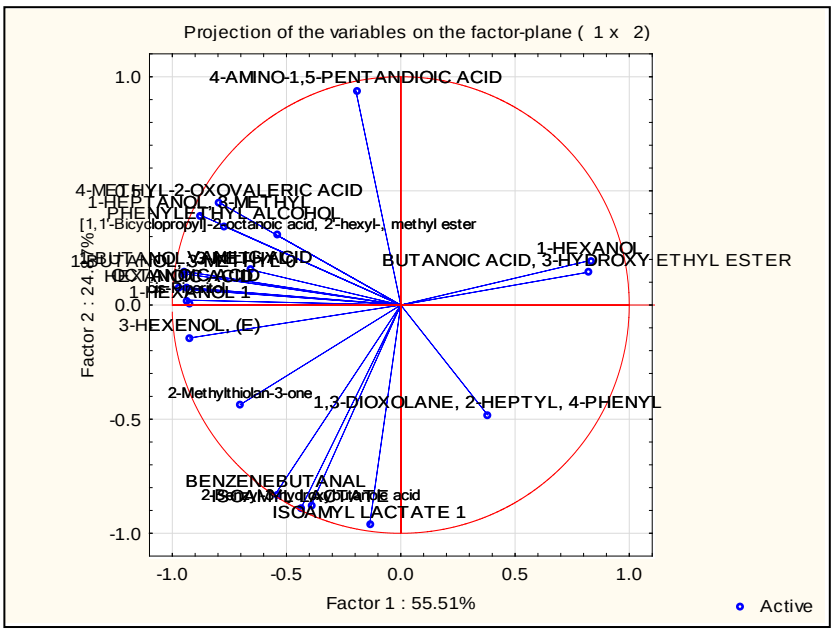


Figure 62: Loading plots of wine volatiles deriving from untargeted SPE-GCMS analyses that were significantly different between FF and MF harvest in at least one Cabernet Sauvignon vineyard from Griffith and Orange in 2015 vintage.

Preliminary screening of wine volatiles revealed that samples could be separated in most instances by the harvest date and also site or region. Compounds that contributed at most to the separation were C6-compounds and esters. Therefore, these volatiles were also quantitatively analysed by HS-SPME GCMS. Additional targeted analyses of lactones, sulfur compounds and terpenoids were performed as the SPE method was not suitable for their detection. The untargeted SPE-GCMS approach was valuable to putatively identify classes of compounds that were altered by harvest date. Quantitative data are discussed in the section below.

6.9.5 Targeted analysis

In a second step, targeted analyses of grape volatiles were performed for about 50 compounds of interest including esters, C6-alcohols, lactones, monoterpenes, norisoprenoids, volatile sulfur compounds (VSCs) and 3-isobutyl-2-methoxypyrazine. Some markers of maturity were common to both varieties whereas other metabolites were more variety and vintage dependent. The results for the markers of interest are shown in Table 31 and Table 32.

Markers of maturity common to Shiraz and Cabernet Sauvignon: Only three aromatic compounds were measured with consistency at concentrations significantly different between FF and MF stages irrespective of vintage, region and variety. Dimethyl sulfide (DMS) and ethyl

propanoate concentrations consistently increased with maturity (Tables 31 and 32). Interestingly, both compounds have been reported as potential contributors of dark fruit aromas in red wine (Lytra et al. 2014; Pineau et al. 2009). The average increase from FF and MF stages was from 4 to 10 µg/L and 20 to 70 µg/L for DMS and ethyl propanoate, respectively. The perception threshold of DMS has been reported at 3.6 µg/L in model red wine (Lytra et al. 2014). The difference threshold for ethyl propanoate in model solution has been reported as 70 µg/L by Pineau et al. (2009). Therefore, a direct contribution of DMS to the dark fruit notes perceived in MF wines is likely, particularly in Cabernet Sauvignon wines, which showed the highest concentrations of this compound. An indirect contribution of ethyl propanoate to MF wines aroma cannot be excluded as suggested by recent work (Pineau et al. 2009; Lytra et al. 2012). Ethyl dihydrocinnamate concentrations decreased from FF to MF stages. These variations were the most pronounced in Shiraz wines in 2014 (0.40-0.50 µg/L) and were limited to 0.10-0.15 µg/L for Cabernet Sauvignon and 2015 vintage wines. Ethyl dihydrocinnamate was reported as a potential contributor to the red fruit aroma typical of Pinot Noir wines from Burgundy (Moio and Etievant, 1995). Other authors have suggested that ethyl dihydrocinnamate at levels as low as 0.54 µg/L could be involved in ripe fruit aroma of red wines (Escudero et al. 2007). While ethyl dihydrocinnamate might be a relevant metabolic marker of FF stage compared to MF, its sensory contribution to FF wine spectrum is probably very limited.

The increase of DMS and ethyl propanoate concentration with maturity and their contribution to dark fruit aroma perceived in later harvest wines have been previously reported for Australian Cabernet Sauvignon (Bindon et al. 2013, 2014). The present study demonstrated that this trend is also observed in Shiraz wines and reports for the first time the influence of grape ripening on ethyl dihydrocinnamate content in wine.

DMS, ethyl propanoate and ethyl dihydrocinnamate have different origin in wines. While yeasts are able to produce small amounts of DMS, this compound is mainly released during wine maturation through the hydrolysis of the amino acid S-methylmethionine (Segurel et al. 2005). It is possible to assess the potential DMS (PDMS) of a grape and wine by artificial hydrolysis in alkaline medium (Segurel et al. 2005). In the present study, the measurement of PDMS in 2014 wines gave similar results as measured DMS (figure 64), confirming that grape ripening favours higher level of S-methylmethionine. A similar trend has been previously reported in the Petit Manseng variety (Dagan, 2006). The influence of DMS on Shiraz and Cabernet Sauvignon MF wine aroma potentially increases with ageing, imparting typical notes of dark fruit and truffle.

Ethyl propanoate and ethyl dihydrocinnamate are both esters but with different origin. The former is synthesised by yeast during alcoholic fermentation but the main factors favouring its production are still unclear. Bindon et al. (2013) suggested that the concentration of wine esters increase with the ripening of Cabernet Sauvignon was related to the primary yeast metabolism via the increase of grape sugar concentration with maturity. However, no relationship between wine esters content, including ethyl propanoate, and grape sugar concentration could be established in the present study.

The level of ethyl propanoate in wine could be potentially related to a specific grape juice composition in amino acids (Antalick et al. 2014). Therefore, the slight increase of ethyl propanoate with maturity could be potentially correlated to a certain evolution of grape nitrogen composition similar to the increase of DMS in MF wine.

Ethyl dihydrocinnamate mainly derives from the esterification of the acid phenol dihydrocinnamic acid during fermentation. Dihydrocinnamic acid derives from cinnamic acid, which is an important intermediate in the phenylpropanoid pathway leading to the formation of grape phenolic compounds. An increase of wine phenolic maturity from FF to MF stages due to a better extraction of phenolic compounds has been suggested in this study. The decrease of ethyl dihydrocinnamate concentration with maturity is therefore probably more related to yeast metabolism or to a specific enzymatic regulation in grape berry. However, a significant decrease was observed for ethyl cinnamate, which directly derives from cinnamic acid, only in Shiraz 2014 wines. Further studies unravelling these contradictory results are warranted.

It is unlikely that the general evolution of wine aroma from FF to MF stage is only due to the variations of DMS, ethyl propanoate and dihydrocinnamate. Other wine volatiles, not quantified in this study could contribute to this aromatic evolution common to Shiraz and Cabernet Sauvignon, and possibly to all red varieties. It could be the case of some furanones such as furaneol and homofuraneol, known to contribute to cooked fruit aroma in red wine (Escudero et al. 2007) but also to the presence of aromatic compounds not yet identified in wines. The influence of the non-volatile matrix common to wine made with late harvest grapes on wine aroma perception should be also investigated in detail. That should include polyphenols, anthocyanins, polysaccharides, mannoproteins, and other macromolecules in interaction with ethanol level and wine volatile composition.

6.9.6 Modification of wine volatile composition specific to variety and vintage

The modification of the wine volatile profile over ripening can also be variety and vintage dependent. For instance, unripe Cabernet Sauvignon wines particularly from a cool region or

vintage are often characterised by specific notes reminiscent of capsicum imparted by 2-isobutyl-2-methoxypyrazine (IBMP) whereas this compound is not found at quantifiable and perceptible levels in Shiraz wines (Allen et al. 1994). In the present study, trends between FF and MF stages specific to varieties and vintages were observed for a substantial number of wine aromatic compounds.

6.9.6.1 Varietal aroma

The varietal component of Shiraz and Cabernet Sauvignon wines was least influenced by grape maturity between FF and MF stages among all the volatiles quantified in this study.

In Cabernet Sauvignon, IBMP levels were already very low at FF stage in Griffith and Orange 1 wines in both vintages (< 4 ng/L) (Table 32). Higher concentrations of IBMP were measured in 2015 O2_CS wines for FF and Intermediate stages (25 ng/L) but no wines could be made for MF stage as the commercial harvest has taken place. A slight decrease of IBMP was only measured in 2015 O1_CS wines (from 3.5 to 2.7 ng/L) The perception threshold of IBMP has been reported in red wine at 10-15 ng/L but this compound can potentially influence red wine aroma at concentrations as low as 5 ng/L through perceptive interactions (Escudero et al. 2007). Nevertheless, lack of variations observed for IBMP in the present study seemed to indicate that this compound probably did not contribute to the fresh red berry fruit aroma specific to FF Cabernet Sauvignon wines.

The wine concentration of 10 different monoterpenes and β-damascenone was not influenced by grape maturity in the condition of this study. Concentrations of β-ionone consistently increased in Griffith Shiraz wines in 2014 and decreased in Griffith and Orange Shiraz wines in 2015 between FF and MF stages. No variations of β-ionone were measured in Cabernet Sauvignon, unlike α-ionone which increased with maturity in Cabernet Sauvignon wines only in 2015 (Table 32). However, no variations of α-ionone and β-ionone were measured in grapes.

Important differences between varieties, and to a lesser extent between vineyards, were observed for monoterpenes and C13-norisoprenoids. These compounds are therefore particularly related to the varietal and regional typicality of red wine even though their real influence on red wine aroma is still unclear. This work helped to highlight a new insight on the contribution of monoterpenes to Australian Cabernet Sauvignon wines typicality. It seems that at the FF stage, grape and wine monoterpene composition is already mostly shaped. Consequently, monoterpenes do not directly contribute to the aromatic evolution perceived between FF and MF stages. The same conclusion can be hypothesised for C13-norisoprenoids as no consistency was observed across vintage for these compounds. The lack of correlation

between grape and wine composition for α-ionone and β-ionone will require further investigation.

6.9.6.2 C6-compounds

The main C6-compounds found in wines are C6-alcohols: hexanol, cis-3-hexenol, trans-2-hexenol and trans-3-hexenol. They are mainly derived from the reduction of the corresponding C6-aldehydes such as hexanal, cis-3-hexenal during alcoholic fermentation. C6-aldehydes originate from the enzymatic oxidation of grape lipids in the berry and in the juice during crushing. The main lipid precursors of these compounds are linoleic and linolenic acids. C6-aldehydes are more pungent than C6-alcohols and are responsible for the typical odour of freshly cut grass and herbaceous grassy notes in unripe grapes and must. In wine, such grassy aromas are, in general, associated with a lack of grape flavour maturity, with corresponding high levels of hexanol, the main C6-compound in wine, and cis-3-hexenol, the most potent one. When the concentration of these compounds is at peri-threshold level, they can contribute to fresh fruit aroma (Rowe, 1999). The correlation between high level of C6-alcohols in wine and early maturity is due to the decrease of linoleic and linolenic acids generally observed during grape maturation (Roufet et al. 1987; Ribereau-Gayon et al. 2006).

C6-alcohols were therefore relevant candidates to be markers of FF stage according to the current knowledge. However, the variations of C6-alcohols concentration over ripening were not always as expected and an important varietal effect was observed (Tables 31 and 32). In 2014, hexanol content increased with maturity in Shiraz and Cabernet Sauvignon wines whereas it decreased in 2015. In contrast, cis-3-hexenol and trans-2-hexenol concentrations significantly decreased in Shiraz wines in both vintages whereas trends were noticed in Cabernet Sauvignon only in 2014 for trans-2-hexenol (increase) and in 2015 for cis-3-hexenol (decrease). Levels of trans-3-hexenol increased with maturity in Shiraz wines only in 2014 whereas it decreased in Cabernet Sauvignon wines for both vintages. The most important varietal effect was observed for cis-3-hexenol with concentrations in Shiraz wines up to 10 times higher than in Cabernet Sauvignon. The levels measured in Shiraz FF wines ranged from 300 to 700 µg/L with a perception threshold reported at 400 µg/L in model solution (Escudero et al. 2007). The concentration of this compound was two to three times lower in Shiraz MF wines compared to Shiraz FF wines. While similar trends were also observed for trans-2-hexenol, the variations and concentrations were substantially lower than for cis-3-hexenol (Table 31). Therefore, it appeared that cis-3-hexenol was a clear marker of FF Shiraz wines and could potentially contribute to the fresh red berry fruit notes perceived in these wines.

The variations of C6-alcohols in wines were poorly correlated to the variations of the corresponding C6-compounds and unsaturated fatty acids in grapes (Table 31 and 32). The level of variations of cis-3-hexenol in Shiraz wines was substantially higher than cis-3-hexenal and cis-3-hexenol in grapes. The contents of cis-3-hexenal and cis-3-hexenol in Shiraz and Cabernet Sauvignon grapes were similar. Moreover, the increase of hexanol concentrations with maturity in 2014 wines were not related to any variations of C6-aldehydes in corresponding grapes. As previously shown, linoleic and linolenic acid contents in grapes were stable between FF and MF stages. These results suggest an important role of yeast metabolism in the final concentration of C6-alcohols in wines. It seems that the Shiraz grape or must composition, particularly at FF stage favours the production of cis-3-hexenol by yeast during alcoholic fermentation. Further research investigating the interaction of grape composition with yeast redox metabolism is warranted.

6.9.6.3 Esters

Esters are mainly produced by yeast during alcoholic fermentation and contribute highly to wine quality by imparting pleasant fruity aromas. Some authors have recently highlighted the potential role of esters in the fruity attributes typical of red wines via similar complex interactions (Pineau et al. 2009; Lytra et al. 2012). Esters can be classified in three main groups: ethyl esters of fatty acids (EEFAs), higher alcohol acetates (HAAs) and ethyl esters of branched acids (EEBAs). EEFAs and HAAs are well-established markers of fruity aroma in young wines. In red wine, they have been recently associated with red and black berry fruits aroma. Recent studies suggest that EEFAs and HAAs were also important contributors of aged red wine aroma (Antalick et al. 2014). Yeast also produce EEBAs but the major proportion is chemically synthesised during wine ageing from the corresponding branched acids. EEBAs are probably the most odorant esters found in wines and have been reported to contribute to dark ripe fruit notes in red wines (Pineau et al. 2009; Lytra et al. 2012).

Quantitative analyses were carried out for five EEFAs, seven HAAs and five EEBAs. EEFAs were the esters least affected by grape maturity. Significant variations between FF and MF stages were only measured for ethyl butyrate in Shiraz wines for both vintages (increase) and in Cabernet Sauvignon (increase) in 2014. Ethyl butyrate is reminiscent of ripe fruit (strawberry, kiwi) and has a perception threshold reported at 20 μg/L in model wine. Variations measured in Shiraz wines were above this level, which makes ethyl butyrate a potential contributor of MF Shiraz wine aroma, probably more through indirect perceptive interactions than direct impact. Bindon et al. (2013) reported an increase with grape maturity of most of EEFAs in Cabernet Sauvignon wines. The authors speculated that the increase of grape sugar concentration with maturity could enhance general yeast metabolism leading to higher production of esters.

However, from all EEFAs quantified in this study, only ethyl butyrate showed significant variations and no direct link with grape sugar concentration could be established. Future work will need to clarify the origin of this trend.

EEBAs are also ethyl esters of fatty acids, but from different origin compared to EEFAs. While the latter group is related to yeast lipid metabolism, EEBAs originate from yeast nitrogen and redox metabolism. A clear varietal effect in relation with grape maturity was consistently observed for these esters for both vintages. While all EEBAs concentrations significantly increased from FF to MF stages in Cabernet Sauvignon wines, their contents tended to decrease in Shiraz wines, particularly for ethyl isobutyrate, ethyl leucate and ethyl isovalerate to a lesser extent (Table 31 and 32). These trends have been already observed in a preliminary study performed in a Griffith vineyard in 2013 prior to the beginning of this project (Antalick et al. 2015a). Therefore, while the contribution of EEBAs to dark fruit aromas in association with DMS in MF wines is possible in Cabernet Sauvignon wines, their influence on Shiraz FF and MF wine aromatic profiles remains unclear.

Branched acids derived from branched amino acids such as valine, isoleucine and leucine. In 2014 and 2015 Cabernet Sauvignon grapes, we observed a slight increase of branched amino acids with maturity but not as significant for the corresponding EEBAs in wines (Table 31 and 32). Yeast nitrogen metabolism is complex and production of nitrogen derived compounds such as higher alcohol and branched acids were not always directly correlated with the concentration of amino acids in fermented medium (Bell and Henschke, 2005). For instance, higher alcohols were not affected by grape maturity in the present study. Moreover, the level of amino acids in juice also depends on their extractability from grape to juice. The influence of maturity on amino acid extractability was investigated in 2015 O2_CS grape juices. The level of amino acids assimilable by yeast (NOPA) was analysed in Cabernet Sauvignon grape juice before and after five days of cold soaking at 10˚C for FF and Intermediate stages. Grape skin contact occurring during cold soaking led to increased NOPA levels in grape juice. This increase was more significant for FF stage than Intermediate stage (Figure 63). This result suggests a better extractability of grape amino acids when maturity increases, as a result of a lower efficiency of cold soaking to increase NOPA levels in final grape juices. The increase of EEBAs with maturity in Cabernet Sauvignon wines can potentially be related to slightly higher level of branched amino acids in the corresponding grapes and to a better extractability of these metabolites with increasing grape maturity by loosening cell wall membranes.

The trend observed for Shiraz wines was quite unexpected as grape branched amino acids followed variations similar to Cabernet Sauvignon grapes when the maturity should favour

amino acids extractability in Shiraz. The decrease of concentration was particularly important for ethyl isobutyrate. A recent study suggested that this ester could be a marker of yeast redox metabolism (Antalick et al. 2014). Therefore, between FF and MF stages a modification of grape juice composition specific to Shiraz variety could influence yeast redox metabolism leading to the decrease of some EEBAs irrespective of branched amino acids contents. A similar hypothesis has been suggested for cis-3-hexenol, emphasising the need for future research on grape maturity and yeast redox metabolism.

An important varietal effect was also measured for higher alcohol acetates. HAAs concentration consistently increased from FF to MF stages in Shiraz wines whereas they tended to slightly decrease in Cabernet Sauvignon wines. Moreover, the levels of HAAs were, overall, higher in Shiraz than Cabernet Sauvignon wines (Table31 and 32). Total HAAs average concentrations increased with maturity from 250 to 1300 µg/L in Shiraz wines, with higher variations measured in 2015, and mainly on the account of isoamyl acetate and phenylethyl and propyl acetate to a lesser extent. HAAs are important contributors of wine fruity aroma, and the variations measured in Shiraz wines between FF and MF stages were high enough to potentially influence the sensory profiles of Shiraz MF wines, probably through synergistic effect (Escudero et al. 2007; Pineau et al. 2009). HAAs do not seem to play an important role in the aromatic evolution of Cabernet Sauvignon wines from FF to MF.

Increases of HAAs concentrations with grape ripening have previously been reported in Australian Cabernet Sauvignon wines (Bindon et al. 2013). The authors suggested a relationship with grape sugar concentration via the general yeast metabolism. However, this study was performed on a ripening slot earlier than FF stage that corresponded to the last harvest date. In the present study, neither the slight decrease of HAAs in Cabernet Sauvignon wines, nor the increases measured in Shiraz wines, could be directly related to grape sugar concentration.

Similar differences between Shiraz and Cabernet Sauvignon wines were found in the preliminary study performed in a Griffith vineyard in 2013 prior to this project (Antalick et al. 2015a). The authors suggested a relationship between nitrogen grape composition and HAAs wine concentration. Indeed, as higher alcohols were not affected by grape maturity, it seems that the variations measured for HAAs were due to the up-regulation of yeast acetyltransferases, key enzymes of HAAs esterification that has been reported to be stimulated by high level of amino nitrogen (Fujirawa et al. 1999). The present study confirms the varietal difference between Shiraz and Cabernet Sauvignon regarding grape nitrogen composition (Table 24). The former variety showed a higher ratio amino acid:ammonium (AA:Am) that could potentially favour the production of HAAs in wine. In the present study, grape maturity tended to increase AA:Am

ratio between FF and MF stages, but it was not the case G1_SH and O2_SH vineyard in 2015. Therefore, the increase of HAAs concentration with grape maturity observed in Shiraz is probably due to complex interactions between grape juice composition and yeast metabolism. The involvement of yeast nitrogen metabolism in those variations is likely but not exclusive. Further research on the topic is warranted.

6.9.6.4 Volatile Sulfur Compounds (VSCs)

Among seven VSCs analysed in this study, four were detected in wines and for three of them, consistent variations related to grape maturity were measured. Firstly, DMS is probably one of the main contributors of aromatic maturity irrespective of the variety. Results for this compound have been discussed.

Hydrogen sulfide and methanethiol are also important contributors of reductive aroma in wines. They are produced by yeast during alcoholic fermentation through the sulfur and nitrogen yeast metabolism. Hydrogen sulfide can also be released early during wine maturation from sulfite through the enzymatic activity of yeast lees. An important decrease of hydrogen sulfide concentration was measured in 2014 wines between FF and MF stages for both varieties. Conversely, an important increase of this compound with grape maturity was observed in 2015 Shiraz wines whereas the concentrations slightly decreased in Cabernet Sauvignon wines. Methanethiol concentration remained stable in Cabernet Sauvignon wines for both vintages and in the 2014 Shiraz, whereas a significant increase was measured in Shiraz wines from 2015. Overall, concentrations of methanethiol were higher in Shiraz than Cabernet Sauvignon wines, and also higher in 2015 wines compared to 2014. Hydrogen sulfide levels also tended to be higher in Shiraz wines compared to Cabernet Sauvignon but the trend was less clear than for methanethiol. Shiraz wines are often perceived as mildly reductive and the results seem to confirm these observations. Further work focusing on the influence of grape maturity on Shiraz wines reductive aroma will be required.

6.9.6.5 Lactone

From a chemical perspective, lactone is a cyclic ester and in wine, lactones originate from different sources. Certain lactones are synthesised by yeast through enzymatic and chemical reactions from odourless grape-derived precursors. It is particularly the case of γ-nonalactone which can be present in wines at supra-threshold levels. This compound has been recently associated to plum and cooked fruit aroma perceived in red wines made with shrivelled berries (Pons et al., 2008; Suklje et al. 2016). It was therefore of interest to investigate if γ-nonalactone could be a marker of grape maturity or only from wines made with shrivelled grapes.

Concentrations of γ-nonalactone significantly increased from FF to MF stages in Cabernet Sauvignon wines whereas only increased in 2014 wines for Shiraz. The variations and the level of γ-nonalactone concentrations measured were higher in 2014 and in Cabernet Sauvignon wines compared to Shiraz wines (Table 31 and 32). Concentrations of γ-nonalactone were above its sensory threshold reported in model solution (25 µg/L) in the 2014 Cabernet Sauvignon wines. It seems that Cabernet Sauvignon tends to produce higher concentrations of this compound compared to Shiraz, more particularly in warm region and vintage. For instance, the series of late season heat waves recorded in Griffith vineyards in 2014, may have induced important shrivelling of Shiraz and Cabernet Sauvignon grapes. The climatic conditions were milder in 2015 and late season dehydration shrivelling was very limited in Griffith vineyards. The comparison of 2014 and 2015 data from Griffith strongly suggest that γ-nonalactone is more related to shrivelling and abiotic stress than directly to maturity. This compound may contribute to plum and jammy fruit aroma perceived in late harvest red wine but mainly when it is associated with late season dehydration shrivelling. The origin of γ-nonalactone in wines made from shrivelled berries will be discussed in Section 6.11.

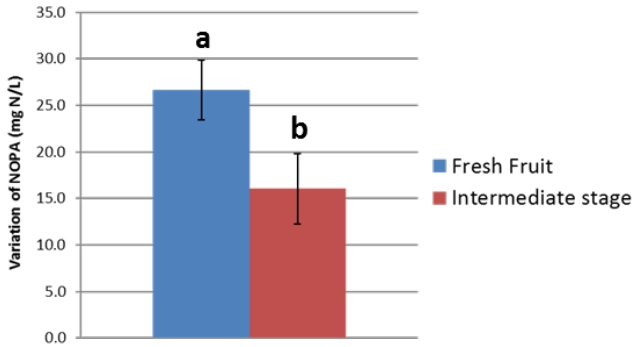


Figure 63: Variations (mean±s.d.) of yeast assimilable amino acids (NOPA) concentration in grape juice after cold soaking for O2 CS FF and O2 CS IF in 2015. Different letters on a column represent significantly (p ≤ 0.05) different concentrations expressed in µg/L.

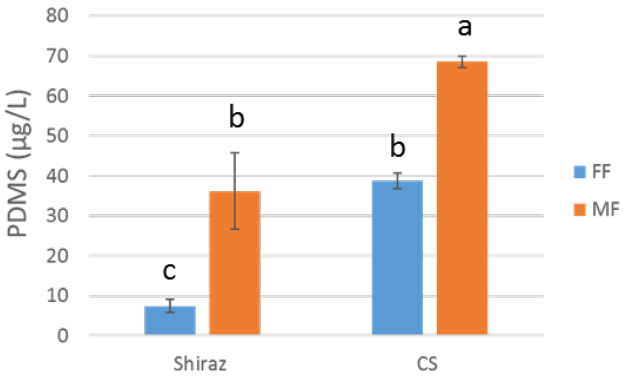


Figure 64: Mean potential DMS with associated standard deviation for 2014 Griffith wines. Different letters on a column represent significantly ($p \leq 0.05$) different concentrations expressed in µg/L. Standard deviations were used for the error bars.

Table 31: Concentrations (mean± s.d.) of significant Shiraz wine metabolites influenced by harvest date. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD). Lower and upper case letters were used for 2014 and 2015 vintage respectively. Concentrations are expressed as µg/L unless indicated.

Compounds/wine	SH FF 2014	SH MF 2014	SH FF 2015	SH MF 2015
	C13 norisoprenoids			
α-ionone (ng/L)	15 ± 3b	24 ± 5a	28 ± 5A	25 ± 5A
β-ionone (ng/L)	106 ± 22b	160 ± 23a	349 ± 55A	203 ± 24B
	C6-alcohols			
hexanol	2122 ± 277b	2754 ± 474a	2581 ± 302A	2055 ± 238B
cis-3-hexenol	366 ± 70a	122 ± 20b	473 ± 143A	239 ± 57B
trans-2-hexenol	25.6 ± 12a	12.3 ± 6.2b	18.8 ± 5.6A	14.3 ± 3.1A
trans-3-hexenol	33.5 ± 5.4b	51.3 ± 9.7a	43.3 ± 6.4A	38.8 ± 7.2A
lactone				
γ-nonalactone	6.5 ± 1.6b	16.4 ± 7.5a	9.3 ± 2A	9.5 ± 2.8A
	Esters			
ethyl dihydrocinnamate	0.71 ± 0.03a	0.28 ± 0.04b	0.54 ± 0.13A	0.46 ± 0.12B
ethyl cinnamate	1.48 ± 0.1a	0.27 ± 0.07b	0.78 ± 0.27A	0.86 ± 0.4A
ethyl butyrate[1]	189 ± 11b	257 ± 11a	194 ± 14b	229 ± 24a
ethyl propanoate	137 ± 8b	170 ± 20a	180 ± 21B	213 ± 33A
ethyl isobutyrate[2]	98.9 ± 15a	86.7 ± 17b	91.5 ± 21A	73.4 ± 14.2B
ethyl 2-methylbutyrate[2]	13.7 ± 2.9a	14 ± 4.8a	13.5 ± 3.7A	12 ± 3.5A
ethyl isovalerate[2]	24.3 ± 4.7a	21.6 ± 4.9a	20.1 ± 5.6A	18.8 ± 3.3A
ethyl leucate[2]	61.8 ± 8.8a	43.5 ± 12b	58.2 ± 15A	48.4 ± 9.9B
ethyl phenylacetate[2]	1.89 ± 0.32a	2.15 ± 0.7a	2.81 ± 0.9A	2.56 ± 0.7A
propyl acetate[3]	25.7 ± 6.3b	46.6 ± 15a	45.4 ± 16.5B	75.6 ± 29A
isobutyl acetate[3]	52.7 ± 5.6b	64.3 ± 8.2a	63 ± 10.4A	65.9 ± 5.3A
butyl acetate[3]	2.05 ± 0.9b	5.3 ± 1.5a	1.15 ± 0.3B	2.24 ± 0.7A
isoamyl acetate[3]	1621 ± 154b	1928 ± 248a	1659 ± 343B	2317 ± 636A
hexyl acetate[3]	17.7 ± 1.4b	38.4 ± 4.3a	22.2 ± 6.1A	30 ± 14.5A
cis-3-hexenyl acetate[3] (ng/L)	400 ± 300a	220 ± 40b	590 ± 200A	510 ± 210A
phenylethyl acetate[3]	112 ± 25b	175 ± 46a	110 ± 24B	183 ± 69A
	Volatile Sulfur Compounds			
dimethyl sulfide	3.65 ± 0.8b	14.3 ± 3.2a	7.1 ± 0.9B	11.4 ± 2.4A
hydrogen sulfide	7.9 ± 4a	4.7 ± 0.8b	5.3 ± 2.3B	11.5 ± 4.1A
methanethiol	2.8 ± 0.6a	3 ± 0.6a	4.2 ± 1B	6.7 ± 0.8A

[1]EEFAs; [2]HAAs; [3]EEBAs; *semi-quantitative data.

Table 32: Concentrations (mean± s.d.) of significant Cabernet Sauvignon wine metabolites influenced by harvest date. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD). Lower and upper case letters were used for 2014 and 2015 vintage respectively. Concentrations are expressed as µg/L unless indicated.

Compounds/wine	CS FF 2014	CS MF 2014	CS FF 2015	CS MF 2015
IBMP (ng/L)	2.17 ± 0.26a	2.05 ± 0.17a	2.8 ± 0.8A	2.3 ± 0.5A
C13-norisoprenoids				
α-ionone (ng/L)	43 ± 3a	48 ± 5a	44 ± 6A	58 ± 17A
β-ionone (ng/L)	112 ± 26a	95 ± 6a	173 ± 18A	165 ± 17A
C6-alcohols				
hexanol	2391 ± 181b	2719 ± 84a	1766 ± 129A	1559 ± 166B
cis-3-hexenol	31.4 ± 2.7a	38.3 ± 8.7a	75.4 ± 16A	35.3 ± 5.3B
trans-2-hexenol	8.5 ± 0.8a	10.6 ± 0.5a	9.6 ± 3.9A	8.1 ± 3.1A
trans-3-hexenol	79.2 ± 3a	65.4 ± 5.5b	61.5 ± 11A	46.4 ± 5B
Lactone				
γ-nonalactone	25.9 ± 4.1b	39.6 ± 2a	13.5 ± 2.3B	16.5 ± 1.9A
Esters				
ethyl dihydrocinnamate	0.47 ± 0.04a	0.31 ± 0.02b	0.68 ± 0.11A	0.58 ± 0.14B
ethyl cinnamate	1 ± 0.09a	1 ± 0.09a	2.24 ± 0.59A	2.11 ± 0.6A
ethyl butyrate[1]	190 ± 4b	215 ± 7a	204 ± 33A	203 ± 24A
ethyl propanoate	157 ± 12b	223 ± 26a	197 ± 22B	224 ± 32A
ethyl isobutyrate[2]	79 ± 6.7b	108 ± 12a	73 ± 8B	97 ± 7A
ethyl 2-methylbutyrate[2]	16.5 ± 1.2b	25.4 ± 1.4a	17.4 ± 2.4B	23.4 ± 2A
ethyl isovalerate[2]	24.1 ± 3.1b	38.5 ± 0.7a	22.2 ± 1.8B	28 ± 1.9A
ethyl leucate[2]	34.5 ± 2b	52 ± 1.7a	67.8 ± 8B	81.9 ± 15A
ethyl phenylacetate[2]	3 ± 0.03b	4.3 ± 0.4a	4.5 ± 0.3B	5.5 ± 0.3A
propyl acetate[1]	37.2 ± 3.1a	29.3 ± 0.8b	49.6 ± 8.7A	59 ± 4.7A
isobutyl acetate[1]	45.1 ± 2a	32.4 ± 1.9b	45.2 ± 4.6A	41.2 ± 2.1A
butyl acetate[1]	2.5 ± 0.3a	2.2 ± 0.3a	1.5 ± 0.1B	2.0 ± 0.3A
isoamyl acetate[1]	1191 ± 108A	937 ± 96B	1548 ± 728A	1358 ± 635A
Hexyl acetate[1]	10.5 ± 1.4a	7.4 ± 0.1b	14 ± 8.5A	8.6 ± 4.4A
cis-3-hexenyl acetate[1] (ng/L)	80 ± 30a	70 ± 40a	150 ± 50A	90 ± 10B
phenylethyl acetate[1]	71.7 ± 8a	61.2 ± 7b	143 ± 71A	128 ± 68A
Volatile Sulfur Compounds				
dimethyl sulfide	16.7 ± 0.8b	26.4 ± 0.2a	18.7 ± 8.2B	23 ± 9.2A
hydrogen sulfide	6.8 ± 2.7a	1.9 ± 0.3b	7.9 ± 3.8A	6.2 ± 3A
methanethiol	1.4 ± 0.1a	1.3 ± 0.2a	3.3 ± 1.6A	4.4 ± 0.4A

[1]EEFAs; [2]HAAs; [3]EEBAs; *semi-quantitative data

6.9.7 Comparison of Shiraz wine volatile composition between Fresh fruit, Mature fruit and Intermediate stages (IF)

Sensory analysis previously showed that panelists were unable to classify fruit characteristics of wines made with grape harvested at intermediate stage (IF) in a distinct manner, unlike FF and MF stages. The sensory profiles of IF wines were generally different to FF and MF wines but these differences were not consistent across vineyards. Despite this lack of consistency, wine volatile composition is different between IF and FF or MF stages. These modifications can occur potentially as fast as five or six days in the case of Shiraz. Shiraz is more sensitive to metabolic regulation and has a shorter ripening cycle than Cabernet Sauvignon. Since the number of Shiraz vineyards studied was higher than the number of Cabernet Sauvignon vineyards, the following section will focus on Shiraz.

Mean values for compounds with the greatest metabolic flux from FF to MF in the Shiraz wines are presented in Figure 65, Figure 66, Figure 67, and Figure 68. DMS and (Z)-3-hexenol concentrations were altered progressively from FF to MF stages, the former increasing and the latter decreasing (Figure 65 and Figure 66). The increases of higher alcohol acetates concentrations with maturity were less even. In some vineyards, the main gain of HAAs occurred between FF and IF stages (G2 and G4) whereas in other vineyards it happened between IF and MF (G1, G3, O1, O2) (Figure 67).

The decreases of ethyl isobutyrate in Shiraz wines when maturity increased were even more inconsistent according to the vineyards. In G1 wines the variations were more significant between FF and IF stage whereas in G3 wines the decrease of ethyl isobutyrate during ripening was even, as for (Z)-3-hexenol. An increase of ethyl isobutyrate concentration was observed for O1 vineyard between FF and IF stages prior to a decrease between IF and MF stages. For G2 and G4, the concentrations dropped significantly between FF and IF stages before a slight increase at late maturity stage. O2 was the only Shiraz vineyard where the concentration of ethyl isobutyrate increased with maturity, indicating the complexity of grape composition/yeast metabolism nexus.

Important variations of Shiraz wine composition occurred during a period as short as five or six days, including grape and yeast-derived compounds. As observed with sensory analysis, while robust trends in the wine composition were found between FF and MF stages irrespective of the site, the volatile composition of IF wines were much more dependent on the particular vineyard.

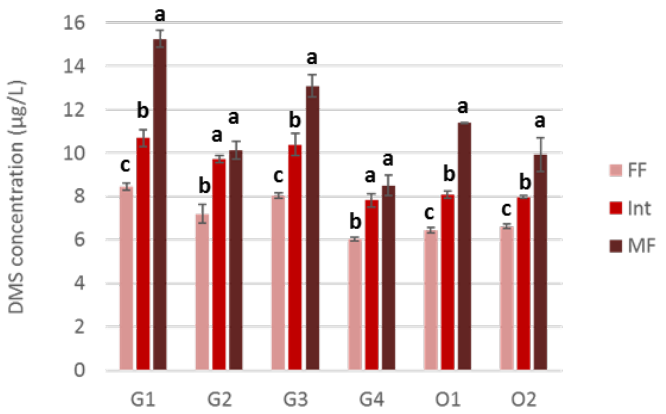


Figure 65: Average concentration of DMS in 2015 Shiraz wines for Fresh fruit, Intermediate and Mature fruit stages. Different letters on a column represent significantly ($p \leq 0.05$) different concentrations expressed in µg/L. Standard deviations were used for the error bars.

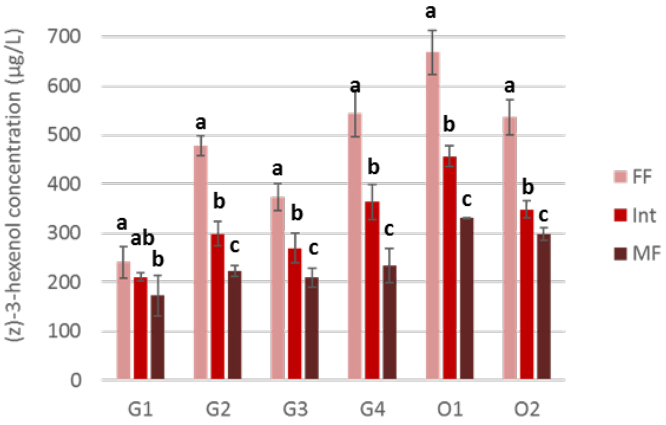


Figure 66: Average concentration of (Z)-hexenol in 2015 Shiraz wines for Fresh fruit, Intermediate and Mature fruit stages. Different letters on a column represent significantly ($p \leq 0.05$) different concentrations expressed in µg/L. Standard deviations were used for the error bars.

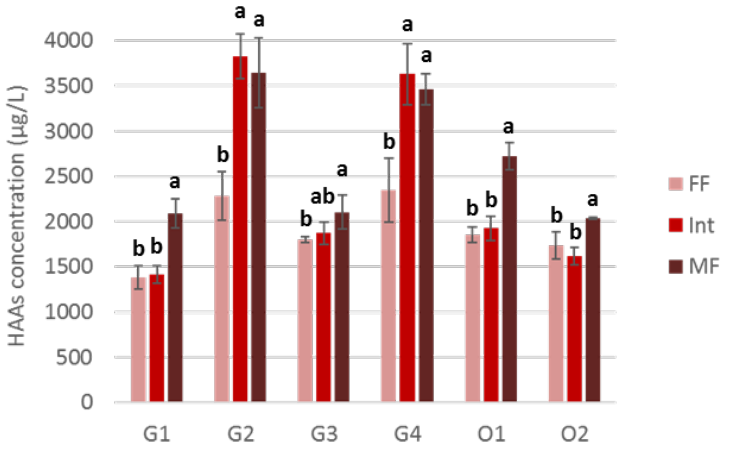


Figure 67: Average concentration of total Higher alcohol acetates (HAAs) in 2015 Shiraz wines for Fresh fruit, Intermediate and Mature fruit stages. Different letters on a column represent significantly (p ≤ 0.05) different concentrations expressed in µg/L. Standard deviations were used for the error bars.

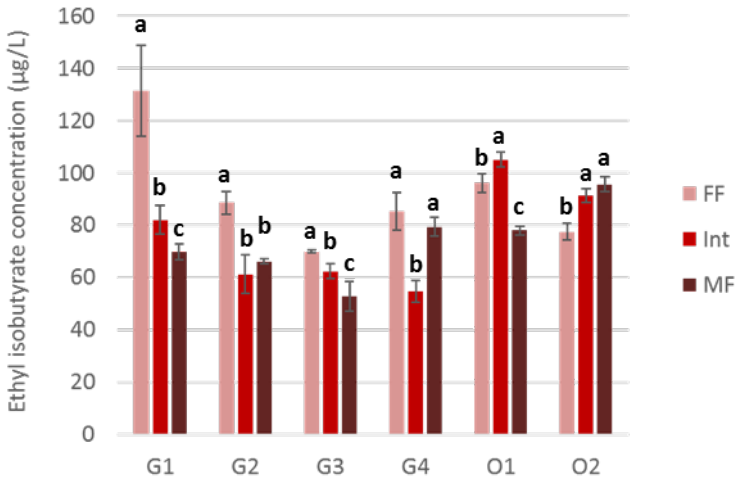


Figure 68: Average concentration of ethyl isobutyrate in 2015 Shiraz wines for Fresh fruit, Intermediate and Mature fruit stages. Different letters on a column represent significantly (p ≤ 0.05) different concentrations expressed in µg/L. Standard deviations were used for the error bars.

6.9.7.1 Influence of yield on Shiraz wine volatile composition

The variations of the volatile composition of Shiraz wines made from the high yield vineyards were overall similar to Shiraz low yield vineyards. The key metabolites of Shiraz FF and MF wines were the same as mentioned earlier for all Shiraz wines. However, some small differences correlated to yield and related to the intensity of the wine volatile composition, differences over ripening were noted. For instance, the increases of HAAs and the decreases of EEBAs were less important for high yield (HY) than low yield (LY) vineyards. Conversely, higher decreases of C6-alcohols concentration with maturity were observed. In general, higher concentrations of C6-

alcohols were measured in wines from the high yield vineyards. Similar results were found in grape composition and are probably due to a delay in grape ripening due to higher yield, as observed with TSS and malic acid. Higher levels of ethyl decanoate were also found in HY Shiraz wines, whereas the concentrations of DMS, γ-nonalactone, higher alcohols and hydrogen sulfide were higher in LY wines. The difference of wine volatile composition due to yield were more important between G1 and G3 than G2 and G4. This result was expected as the increased yield for these comparisons is from 10.3 to 18.6 kg/vine (G1 to G3) and 14.0 to 17.7 kg/vine (G2 to G4). Wine volatile composition was generally more affected by yield than grape volatile composition.

Table 33: Concentrations (mean± s.d.) of significant Shiraz wine metabolites influenced by yield. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD). Lower and upper case letters were used for 2014 and 2015 vintage respectively. Concentrations are expressed as µg/L unless indicated. LY: low yield; HY: High Yield.

Compounds/wine	SH LY FF	SH LY MF	SH HY FF	SH HY MF
	C13 norisoprenoids			
α-ionone (ng/L)	30 ± 5a	27 ± 6ab	26 ± 4ab	22 ± 4b
β-ionone (ng/L)	379 ± 47a	207 ± 15b	352 ± 27a	205 ± 32b
	C6-alcohols			
hexanol	2496 ± 239b	2165 ± 211c	2742 ± 328a	2175 ± 96c
cis-3-hexenol	359 ± 132b	197 ± 38c	458 ± 99a	221 ± 29c
trans-2-hexenol	14.1 ± 5b	16.5 ± 4.3b	22.2 ± 2.6a	15.9 ± 2.2b
trans-3-hexenol	42.4 ± 3.6a	40.7 ± 5a	46.5 ± 3.5a	43.8 ± 2.2a
	Lactone			
γ-nonalactone	10.3 ± 1.1a	11.6 ± 3.3ab	8.8 ± 2.4b	8.7 ± 2.1b
	Esters			
ethyl dihydrocinnamate	0.48 ± 0.09a	0.38 ± 0.03b	0.45 ± 0.07a	0.38 ± 0.04b
ethyl cinnamate	0.67 ± 0.13a	0.67 ± 0.14a	0.61 ± 0.04a	0.59 ± 0.07a
ethyl butyrate[1]	201 ± 15b	237 ± 8a	194 ± 16b	233 ± 27a
ethyl decanoate[1]	49.8 ± 6.1b	53.9 ± 5.2b	60 ± 8ab	62.3 ± 10.7a
ethyl propanoate	197 ± 19b	207 ± 20b	184 ± 12b	245 ± 23a
ethyl isobutyrate[2]	110 ± 26a	68 ± 2.8c	78 ± 10b	66 ± 15.3bc
ethyl 2-methylbutyrate[2]	16.5 ± 4.1a	10.9 ± 0.6b	10.2 ± 2.3b	9.6 ± 3b
ethyl isovalerate[2]	25.4 ± 6.6a	18.7 ± 0.7b	16 ± 2.8b	17.4 ± 5b
ethyl leucate[2]	61.1 ± 7.6a	42.4 ± 5.6b	41.4 ± 8.5b	47.3 ± 10b
ethyl phenylacetate[2]	3.32 ± 0.66a	2.4 ± 0.27a	2.1 ± 0.42ab	1.94 ± 0.26b
propyl acetate[3]	41.7 ± 9.1	72.7 ± 13.5	64.5 ± 10	107 ± 13
isobutyl acetate[3]	58.2 ± 15.3a	67.3 ± 5.9a	69.9 ± 5.5a	66.8 ± 5.9a
butyl acetate[3]	1.33 ± 0.24b	2.62 ± 0.75a	1.35 ± 0.17b	2.53 ± 0.38a
isoamyl acetate[3]	1593 ± 470b	2487 ± 773a	1815 ± 350b	2406 ± 676a
hexyl acetate[3]	20 ± 6b	32.1 ± 12.7a	25.1 ± 6.9ab	36.6 ± 18a
cis-3-hexenyl acetate[3] (ng/L)	450 ± 180b	490 ± 260ab	600 ± 200a	580 ± 230a
phenylethyl acetate[3]	118 ± 30b	204 ± 88a	100 ± 25.3b	164 ± 77a
	Volatile Sulfur Compounds			
dimethyl sulfide	7.8 ± 0.7b	15.2 ± 2a	7 ± 1.1b	10.7 ± 4.3ab
hydrogen sulfide	6.7 ± 3.7b	12.7 ± 2.8a	4.7 ± 0.9b	10.8 ± 2.5a
methanethiol	3.7 ± 0.7b	7 ± 0.4a	4.3 ± 1.6b	7 ± 0.9a
	Higher alcohol			
phenylethanol*	14.2 ± 0.3a	13.5 ± 0.6a	10.6 ± 2.6b	9.4 ± 1.3b
isoamyl alcohol*	9.2 ± 0.7a	9.3 ± 0.5a	7.4 ± 1b	7.8 ± 0.5b
isobutanol*	0.46 ± 0.04a	0.36 ± 0.05b	0.34 ± 0.06b	0.3 ± 0.06b

[1]EEFAs; [2]HAAs; [3]EEBAs; *semi-quantitative data

6.9.8 Outputs: Effect of Harvest Date and Yield on Wine Composition

Overall, as expected, ethanol and glycerol increased and lactic acid decreased with maturity. Ethanol increases with maturity were much higher in 2014 than in 2015 due to severe heat waves occurring at late stage of ripening in 2014 leading to grape berry dehydration and higher grape sugar concentration. Variations in ethanol from FF to MF stages in 2015 were sometimes

quite minimal. In one case (G1), wines from the three harvest dates had the same level of ethanol. No correlation between ethanol level and aromatic compounds evolution from FF to MF stages was found.

Wine phenolics increased with maturity due to a better extractability from grape skin into wine, as grape phenolics content were not deeply altered.

Wine volatile composition was affected by grape ripening between FF and MF stages. Consistent variations were observed between FF and MF stages whereas IF wines did not show a consistent wine volatile composition across vineyards.

Only three compounds quantified in this study were found to be consistently at different concentrations between the distinct FF and MF stages irrespective of the variety, site and vintage. Dimethyl sulfide (DMS) and ethyl propanoate consistently increased with maturity whereas ethyl dihydrocinnamate levels decreased. From a sensory point of view, the contribution of DMS to MF wines aromatic profile is likely but cannot alone explain the common pattern of wine aroma evolution between FF and MF stages. An indirect contribution of ethyl propanoate cannot be excluded whereas ethyl dihydrocinnamate is probably a metabolic rather than sensory marker of FF wines.

Monoterpenes and the main C13-norisoprenoid in wine, β-damascenone, were not affected by grape ripening. However, they could be used as varietal and regional markers. In this study, IBMP levels measured in Cabernet Sauvignon wines were not significantly affected by grape ripening.

For several compounds and groups of compounds, significant variations that were specific to variety were observed. Shiraz was more subject to wine volatile alterations than Cabernet Sauvignon. The modification of grape composition between FF and MF stages affected some grape aromatic precursors (e.g. DMS), and even more grape metabolites influencing yeast metabolism. Variations in the extractability level of these substrates (e.g. amino acids, lipids) during ripening could also modify yeast metabolism. Esters were particularly affected by grape ripening.

In Shiraz wines, besides DMS, ethyl propanoate and dihydrocinnamate, robust trends were measured for (Z)-3-hexenol and the whole group of higher alcohol acetate. (Z)-3-hexenol has been found to be a specific marker of FF Shiraz wines with potential sensory influence. Important varietal differences were found for this compound and the group of C6-alcohol in general. Higher alcohol acetates seemed to contribute in a direct or indirect way to MF Shiraz aromatic profile.

In Cabernet Sauvignon wines, ethyl esters of branched acids concentration consistently increased with maturity. Their levels tended to decrease with maturity in Shiraz wines.

Some compounds showed important variations with maturity only for one vintage. That was particularly the case of γ-nonalactone which substantially increased in 2014 but remained stable (Shiraz) or showed a much smaller increase (CS) in 2015. It seems that γ-nonalactone is more related to grape berry dehydration (shrivelling) than maturity. The contribution of this compound to the aromatic profile of wines made with late harvest grapes partly shrivelled is likely.

Yield did not greatly affect the influence of grape maturity on wine volatile composition. Overall, higher yield tended to decrease the intensity of variations observed for esters and to slightly increase the differences measured for C6-alcohols.

The investigation of the influence of grape maturity based upon the Sugar Loading model on wine volatile composition highlighted new insights in oenology. It particularly opens new perspectives of research on the nexus grape composition/yeast metabolism for groups of compounds such as esters, fatty acids and C6-alcohols. This work also brought new knowledge about the varietal difference for the wine volatile composition of Shiraz and Cabernet Sauvignon wines, and the effect of berry shrivelling on wine chemical and sensory composition. Small studies specific to these topics were performed within the context of this project. The main results will be presented below in section 6.11. Data from 2016 vintage were treated separately and are presented below.

6.10 2016 Vintage

Samples from the 2016 vintage were treated separately and were not included into the general models presented for 2014 and 2015 vintage. There were several reasons to treat data from the 2016 vintage separately:

i) the main and decisive argument was a vast shrivelling of both Shiraz and Cabernet Sauvignon grapes at location O1 and to lesser extend in O2 from FF to MF harvest. Late berry season dehydration experienced in the 2016 season in Shiraz on both locations could be associated with the warm and dry autumn. Cabernet Sauvignon grapes in location O1 were also subjected to berry shrivelling, whereas this was not evident from the berry fresh mass measurements for O2_CS vineyard. In Shiraz, shrivelling resulted in a substantial decline in berry fresh weight as evident from Figure 69. Berry shrivelling can have a significant impact on grape berry and wine chemical and sensory properties;

ii) the second argument for treating these data separately was low volume of grapes left over to conduct the last harvest in Shiraz O2 vineyard. Fermentation volume can have a significant impact on wine chemical and sensory properties;

iii) in the first two vintages wine chemical analyses were carried out in December and January together with wine sensory, whereas in the last 2016 vintage, wines were analysed in July. The abundance of several wine volatiles (esters, terpenes, norisoprenoids) changes significantly in the first few months of wine ageing.

Despite the arguments listed, samples from 2016 vintage underwent a similar set of analyses as previously, with the exception of untargeted analyses of wines.

6.10.1 Sugar loading models

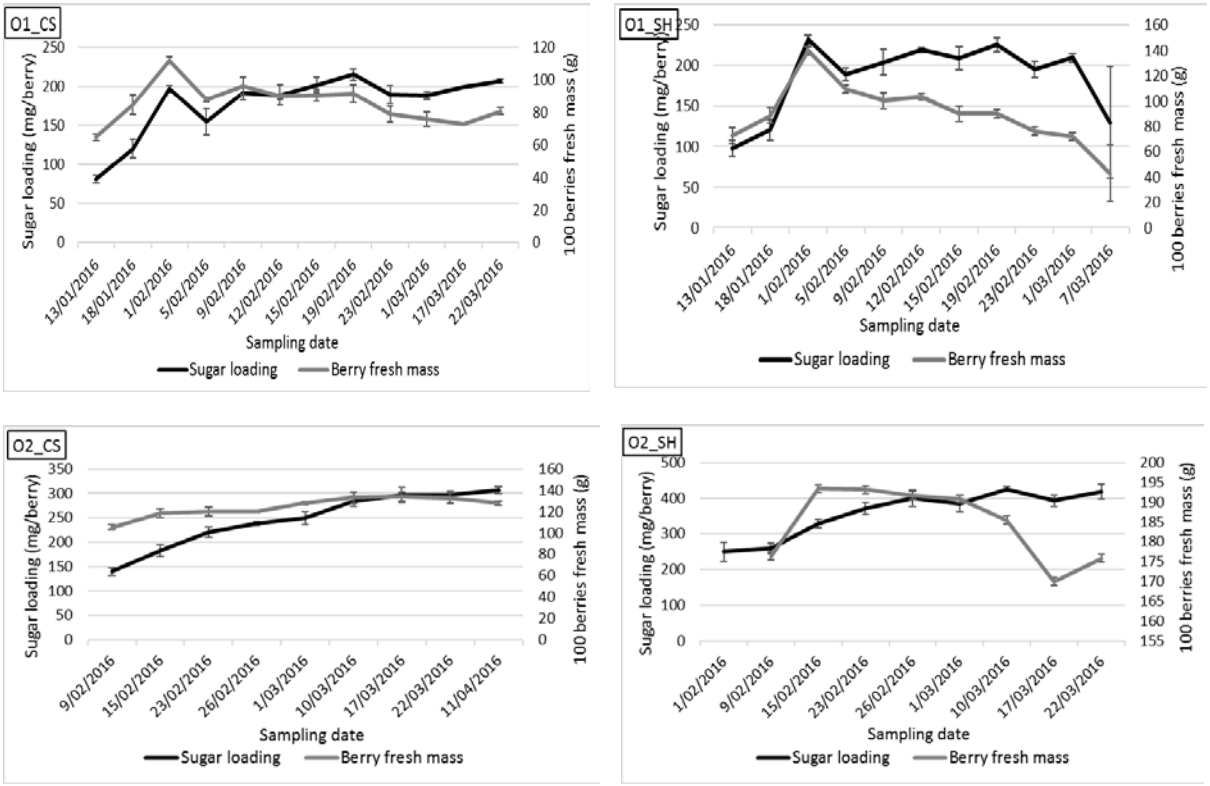


Figure 69: Sugar loading (mg/berry) and berry fresh mass evolution (g) for selected vineyards in Orange in 2015/16 vintage.

As in previous vintages, sugar accumulation per berry was monitored on a weekly basis for all four vineyards. The sugar accumulation plateau was reached on 9 February 2016 for CS_O1 and SH_O1, whereas this was estimated to occur later for vineyards on location O2, Table 34. Due to the warm and dry autumn, significant shrivelling was noticed in both Shiraz vineyards. This was coupled with significant berry weight losses. Berry fresh mass at MF in O1_SH vineyard was for

approximately 70% lower than berry maximum weight recorded over the season. A significant decrease in sugar per berry was noticed at the last sampling date in O1_SH. A decrease in sugar accumulation per berry in several vineyards worldwide in late ripening had already been observed (A. Deloire personal communications 2016). However, to date, no valid hypothesis exists to explain this phenomenon. It could be speculated that the sugar in the berry in late ripening may be remobilised into the vine perennial tissues through xylem backflow.

At last harvest (MF) the average berry fresh mass was only 0.43 g in O1_SH. In the second O2_SH vineyard the decrease in berry fresh mass was less drastic, and resulted in approximately 10% lower berry fresh mass, compared to measured maximum of 1.93 g. Cabernet Sauvignon grapes were not subjected to late season berry dehydration in location O2, however due to the warmer climatic conditions and more water restrictions at O1 site, a decrease of 30% of berry weight in CS_O1 was also noticed. The final berry fresh weigh of CS_O2 berries was on average 0.8 g. In accordance with previous observation, an increase in Brix and pH was generally noted from FF to MF, whereas TA decreased, Table 34. An increase in Brix was more pronounced for Shiraz, compared to Cabernet Sauvignon, which could be attributed to the late season berry shrivelling.

Table 34: Basic juice maturity parameters at harvest.

Vineyard	Harvest Date	Brix	TA (g/L)	pH	Berry weight (g)
O1_SH	FF	26.3±0.2b	5.83±0.03b	3.36±0.00b	76.5±1.9
	MF	29.6±0.1a	4.50±0.06a	3.59±0.01a	43.0±13.0
CS_O1	FF	26.03±0.1b	6.20±0.00	3.21±0.00b	75.8±2.6
	MF	27.00±0.1a	4.20±0.00	3.55±0.00a	80.7±1.1
O2_SH	FF	23.2±0.2b	5.00±0.06b	3.58±0.00b	185.5±1.8
	MF	24.0±0.1a	4.03±0.12a	3.68±0.02a	175.8±8.6
CS_O2	FF	21.93±0.07b	6.60±0.00b	3.35±0.00b	132.7±2.8
	MF	25.1±0.2a	5.27±0.03a	3.49±0.02a	128.0±1.2

All quoted uncertainty is standard error of three replicates. Means followed by different letter for one vineyard are significant at p≤0.05.

Table 35: Predicted and harvested dates for Shiraz and Cabernet Sauvignon in Orange in vintage 2016.

Vineyard		Plateau	FF	Intermedia	MF	Commercial Harvest
O1_SH	Predicted	9/2/2016	21/2/201	X	4/3/2016	24/2 and 25/2/2016
	Harvested		23/2/201	X	5/3/2016	
CS_O1	Predicted	9/2/2016	29/2/201	X	20/3/2016	3/3 and 4/3/2016
	Harvested		1/3/2016	X	22/3/2016	
O2_SH	Predicted	26/2/2016	9/3/2016	X	21/3/2016	16/3/2016
	Harvested		10/3/201	X	22/3/2016	
CS_O2	Predicted	1/3/2016	20/3/201	X	9/4/2016	23/3/2016
	Harvested		22/3/201	X	11/4/2016	

6.10.2 Grape Composition

PCA was applied to all measured grape and juice parameters. A clear separation of Shiraz samples (Figure 70) was noticed according to the site, whereas separation according to the harvest date was noticed only for O1_SH. PC1 accounting for the 47.28% of variation separated O1 and O2 site. Samples from the FF harvest stage were associated with TA, glutamic acid and chlorophylls and carotenoids, Figure 70. Valine, fatty acids, pH and YAN were found were associated with samples from MF.

PCA was also conducted on Cabernet Sauvignon grape berry and juice chemical composition. Similar to the Shiraz, clear separation according to the vineyard based on the grape and juice chemical composition was observed (Figure 71). Separation according to the harvest date seemed to be more prominent for O1_CS, however only one treatment replicate was used to conduct PCA, due to the missing values for free grape volatiles in two O1_CS samples. Less clear separation of samples according to the harvest date was noticed for O2_CS,

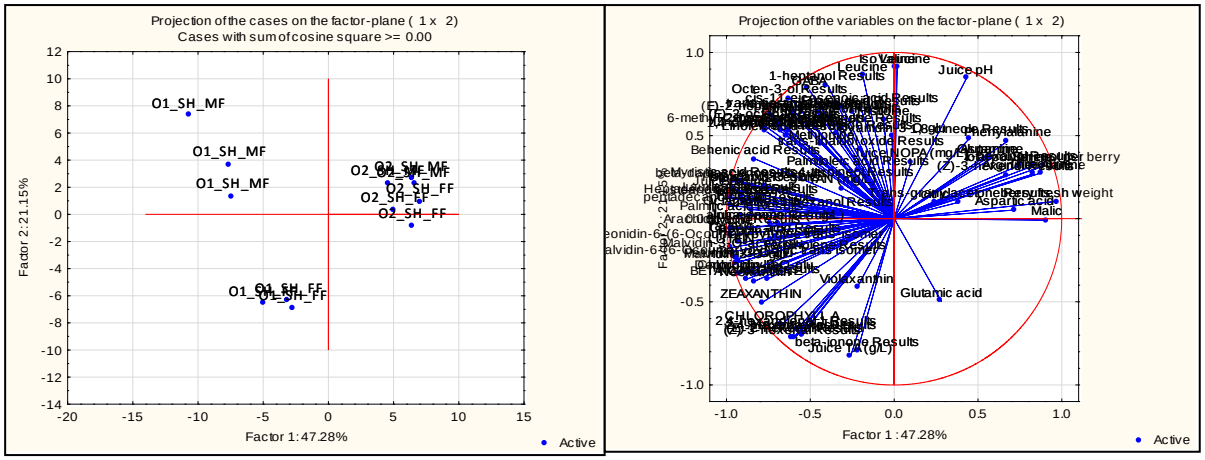


Figure 70: A Principal component analysis conducted on grape berry and juice chemical composition for Shiraz (O1_SH and O2_SH) from 2016 vintage. B Loading plots for grape berry and juice chemical composition for Shiraz (O1_SH and O2_SH) from 2016 vintage

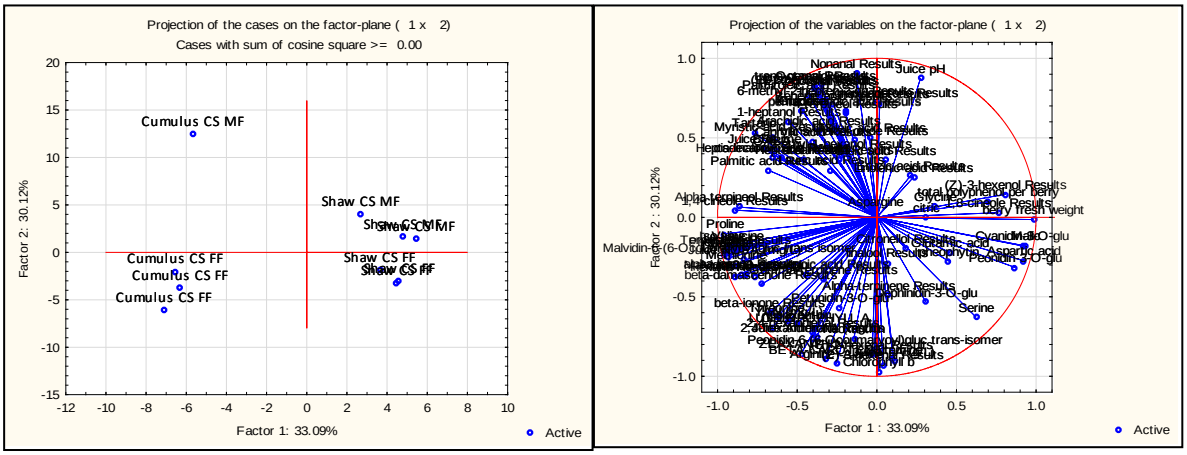


Figure 71: A Principal component analysis conducted on grape berry and juice chemical composition for Cabernet Sauvignon (O1_CS and O2_CS) from 2016 vintage. B Loading plots for grape berry and juice chemical composition for Shiraz (O1_SH and O2_SH) from 2016 vintage.

6.10.2.1 Amino acids

In Shiraz grapes, concentrations of amino acids tended to increase from FF to MF stage. This was evident for proline, GABA, isoleucine, leucine and valine, whereas concentrations of glutamic acid decreased with grape ripening. When comparing both Shiraz vineyards, the trend of increasing amino acids was more pronounced for O1_SH than for O2_SH. Several amino acids, such as glutamine, arginine and alanine decreased in both Cabernet Sauvignon vineyards with ripening, which resulted in significantly lower YAN values in juice from grapes collected at MF. No particular trend for branched amino acids in CS grapes was noted.

6.10.2.2 Grape berry volatiles and its precursors

There was no single grape berry volatile that was consistently altered by harvest date in either Shiraz or Cabernet Sauvignon vineyards in the 2016 vintage. As a general trend in both Shiraz vineyards in Orange, concentrations of octen-3-ol and heptanol increased with grape ripening. An increase in heptanol concentrations with grape ripening was in agreement with results from 2014 and 2015 where we also observed an increase in heptanol concentrations.

In grapes from O1_SH vineyard a significant decrease in several LOX degradation products from FF to MF was noticed, what was not observed for O2_SH. Concentrations of (*Z*)-3-hexanol, (*E*)-2-hexanal, hexanal and others had decreased, whereas concentrations of some fatty acids, such as behenic, linoleic and linolenic acid increased.

From data gathered in three vintages across two regions and cultivars, it seems that degradation of C6-compounds in late ripening is strongly subject to environmental conditions and is cultivar specific. Vintage 2014 was characterised with several heat waves during the

ripening period in Griffith that resulted in significant decrease of (*Z*)-3-hexanol, (*E*)-2-hexanal and (*Z*)-3-hexenal in Shiraz grapes, whereas this was not obvious for Cabernet Sauvignon. A significant decrease in (*Z*)-3-hexanol and (*E*)-2-hexanal were also observed in O1_SH in 2016 from FF to MF, whereas these trends were less pronounced in 2015 vintage, which was milder (less warm and more rain) in both regions. Levels of (*E*)-2-hexanal were also significantly decreased in both Cabernet Sauvignon vineyards in 2016. Climatic conditions and water availability play a central role in modification of C6-compounds in grapes in late ripening, whereas it seems that time line after the plateau of sugar accumulation plays a minor role.

Other grape volatiles (terpenes and norisoprenoids) were altered to a lesser extent with ripening which is in agreement with previous observations. Increases in some terpenes, i.e. (E)-linalool oxide, linalool, α-terpineol, citronellol in O1_SH in 2016 were noticed. An increase in some terpenes with ripening was also noticed for O1_CS. It is possible that the increase in terpenes in O1 site could be a consequence of stress (water limitations) experienced by the plants in late ripening. However, any conclusions are difficult as only trans-geraniol was increased in 2014 vintage in Shiraz, which was characterised with several heat waves. Climate (temperature and solar radiation) and water interactively affect grapevine physiology and most likely the metabolome. Therefore, additional trials are needed to decouple these variables to better understand grapevine behaviour and its adoption mechanisms.

Table 36: Significantly different compounds between FF and MF in Orange 2016 Shiraz.

	Mean	**Mean**	**p**
	FF	MF	
linoleic acid [§]	7.2±1.6	11.6±2.2	0.003174
cis-11-eicosenoic acid [§]	0.03±0.008	0.06±0.01	0.000302
linolenic acid [§]	0.52±0.11	0.75±0.16	0.014315
behenic acid [§]	2.4±0.4	3.2±0.5	0.022159
glutamic acid (mg/kg)	117±6	92.2±10.1	0.000393
glutamine (mg/kg)	26.4±3.1	48.4±23.2	0.044826
histidine (mg/kg)	36.1±5.4	46.8±8.8	0.028731
GABA (mg/kg)	25.5±7.7	61.8±21.7	0.003152
proline (mg/kg)	446±54	746±92	0.000085
valine (mg/kg)	21.7±2.2	27.0±1.2	0.000445
iso leucine (mg/kg)	9.5±0.9	11.5±1.2	0.010214
leucine (mg/kg)	15.9±2.2	21.4±2.7	0.003757
juice pH	3.5±0.1	3.6±0.05	0.012338
juice TA (g/L)	5.4±0.5	4.3±0.29	0.000438
octen-3-ol [§]	0.13±0.03	0.33±0.18	0.024287
1-heptanol [§]	0.02±0.003	0.02±0.005	0.022260

[§]…peak area ratio normalised on sample weight. All quoted uncertainty is a standard deviation of three replicates per treatment.

Table 37: Significantly different compounds between FF and MF in Orange 2016 Cabernet Sauvignon.

	Mean FF	Mean MF	p
peonidin-6-(6-Ocoumaryoyl)gluc trans-isomer (mg/kg)	27.3±11.9	13.7±3.0	0.017350
neoxanthin (mg/kg)	0.16±0.03	0.09±0.04	0.000778
violaxanthin (mg/kg)	0.11±0.07	0.04±0.02	0.030140
zeaxanthin (mg/kg)	0.09±0.02	0.05±0.02	0.000225
lutein (mg/kg)	0.34±0.06	0.23±0.03	0.001902
β-carotene (mg/kg)	0.17±0.03	0.09±0.04	0.000798
chlorophyll A (mg/kg)	4.3±2.5	1.7±1.9	0.048875
chlorophyll b (mg/kg)	1.7±0.1	0.8±0.5	0.000181
linoleic acid §	6.3±2.7	12.5±7.3	0.033364
behenic acid§	2.2±0.6	3.2±0.6	0.012644
glucose (g/kg)	105±12	122±15	0.029740
fructose (g/kg)	100±11	117±14	0.023304
asparagine (mg/kg)	1.3±0.2	1.7±0.4	0.038962
glutamine (mg/kg)	35.4±6.3	21.7±7.2	0.001853
threonine (mg/kg)	40.2±10.7	23.9±3.7	0.003582
arginine+alanine	7.4±1.5	2.7±1.4	0.000045
juice TA (g/L)	5.9±0.7	4.7±0.6	0.004913
Brix	23.7±1.8	26.0±1.1	0.016035
β-ionone §	0.13±0.03	0.09±0.01	0.030445
(Z)-3-hexenal §	0.8±0.03	0.599±0.2	0.002187
(E)-2-hexenal §	27.9±1.0	19.8±2.9	0.000186
2.4-hexandienal §	0.24±0.01	0.2±0.01	0.000101
2.4-hexandienal 1 §	0.59±0.03	0.49±0.02	0.000378

§…peak area ratio normalised on sample weight. All quoted uncertainty is a standard deviation of three replicates per treatment

6.10.2.3 Grape berry phenolics.

As already observed, harvest date did not show any specific and consistent trend on grape berry polyphenols and anthocyanins. In O1_SH no anthocyanins were significantly altered from FF to MF, whereas a significant decrease in almost all measured anthocyanins was noticed in O1_CS from FF to MF stage. Malvidin-3-*O*-glucoside and malvidin-3-*O*-acetylglucoside increased from FF to MF in SH_O2. Therefore, anthocyanins behaviour in grapes in late ripening was vineyard, region and cultivar specific. No consistent trend in the 2016 vintage could be established which confirms observations from previous vintages.

6.10.2.4 Wine volatiles.

The experimental conditions of the 2016 vintage in Orange were not optimal to study the influence of grape ripening on wine volatile composition for the reasons explained earlier (shrivelling, volume of fermentation and ageing). Therefore, it was difficult to highlight strong trends related to berry ripening. The results from O1 and O2 are presented separately for both variety (Tables 38 and 39).

Trans-geraniol tended to decrease with maturity in wines from all the vineyards whereas no trend was observed in grapes. Similarities with previous vintages were also observed for C6-alcohols. (Z)-3-hexenol wine concentrations were much higher in Shiraz wines but significantly decreased from FF to MF stages for both varieties. Trans-3-hexenol contents dropped only in Cabernet Sauvignon wines whereas no robust trend was found for the other C6-alcohols. An important increase of γ-nonalactone levels was measured in O1 Cabernet Sauvignon wines, and Shiraz to a lesser extent, confirming the strong influence of grape berry shrivelling on this compound. A slight increase of this compound with maturity was also observed in O2 Cabernet Sauvignon.

Important modifications of wine esters composition were observed between FF and MF, particularly in vineyards subject to berry shrivelling (SH O1, CS O1) and SH O2 wine made in different conditions (lower volume) (Table 38 and Table 39). Significant drops of higher alcohol acetates levels with maturity were measured in SH O1 and CS O1 wines. This result contrasts with the robust trend of HAAs increasing with maturity observed in Shiraz wines in 2014 and 2015 vintages. The difference between vintages probably originates from the severe shrivelling that affected Shiraz and Cabernet Sauvignon O1 vineyard between FF and MF in 2016 vintage. This hypothesis is supported by the extra study of berry shrivelling on Shiraz wine chemical and sensory profile (see Section 6.11). Ethyl esters of fatty acid contents also decreased from FF to MF in O1 vineyards but to a lesser extent compared to HAAs. In contrast, ethyl esters of branched acids tended to slightly increase with maturity. However, no comparison could be made with previous vintages for this group of esters as their contents evolve quickly during the first month of ageing.

A significant decrease of the concentrations of all the esters was measured in SH O2 wines but the influence of wine volume is probably one of the main reasons for this result, particularly for EEFAs and HAAs. These two groups of esters are affected by hydrolysis during the first month of ageing. It has been found that the smaller the volume of wine stored, the faster ester hydrolysis occurs (Antalick, unpublished data). A slight decrease was measured for EEFAs in CS O2 wines

whereas no trend was observed for HAAs. The only ester consistently affected by ripening, in 2016 and the previous vintages, was ethyl dihydrocinnamate, which decreased between FF and MF stages. Therefore, it seems that this ester is a potential marker of FF stage irrespective of the environmental and winemaking condition. Two other markers had been found to be consistent across vintage, variety and vineyards in 2014 and 2015 vintages, DMS and ethyl propanoate showed the same trend of increase with maturity in 2016, excluding the SH O2 wines. No consistent trend was found for ethyl propanoate which was more subject to shrivelling and winemaking condition as all the yeast-derived esters.

Table 38: Average concentrations (µg/L) of Shiraz wine metabolites in Orange vineyards in 2016 vintage, comparing FF and MF harvest date. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD). LY: low yield; HY: High Yield.

Compounds/wine	SH O1 FF	SH O1 MF	SH O2 FF	SH O2 MF
	Monoterpenes			
citronellol	13.7 ± 0.7b	18.8 ± 0.8a	17 ± 0.9a	18.4 ± 0.6a
trans-geraniol	8.5 ± 2.2ab	6.4 ± 1.2b	9.6 ± 0.1a	3.8 ± 0.5c
1,8-cineole	0.3 ± 0.04a	0.31 ± 0.03a	0.31 ± 0.09a	0.26 ± 0.04a
4-terpineol (ng/L)	57 ± 8b	71 ± 9a	54 ± 2b	50 ± 6b
α-terpinene (ng/L)	11 ± 2a	11 ± 1a	10 ± 0.3a	8 ± 3a
γ-terpinene (ng/L)	11 ± 2a	10 ± 1a	9 ± 0.5a	8 ± 0.2a
terpinolene (ng/L)	30 ± 4a	31 ± 1a	33 ± 0.1a	23 ± 3b
linalool	9.7 ± 0.6a	8.8 ± 0.6a	7.9 ± 0.04b	6.7 ± 0.5c
α-terpineol	2.6 ± 0.2a	3 ± 0.3a	2.7 ± 0.1a	2.4 ± 0.01a
	C13 norisoprenoids			
β-ionone (ng/L)	409 ± 87a	420 ± 31a	180 ± 6b	159 ±15c
β-damascenone	10.4 ± 1.5a	11 ± 1.8a	7.5 ± 0.6b	4.8 ± 0.1c
α-ionone (ng/L)	33 ± 4a	34 ± 2a	24 ± 4b	29 ± 9ab
	C6-alcohols			
hexanol	2144 ± 102b	2369 ± 53a	1403 ± 93d	1911 ± 12c
cis-3-hexenol	440 ± 26a	218 ± 5d	342 ± 12b	305 ± 8c
trans-2-hexenol	14.3 ± 1.4b	15.5 ± 1.1b	24.2 ± 0.9a	17.8 ± 3.5b
trans-3-hexenol	36.8 ± 2.2ab	40 ± 1.7a	25.6 ± 1.5c	36.4 ± 1.2b
	Lactone			
γ-nonalactone	5.6 ± 0.3d	8.6 ± 0.4c	14.3 ± 0.4a	10.6 ± 0.7b
γ-decalactone (ng/L)	130 ± 5b	129 ± 18b	171 ± 5a	136 ± 5b
γ-undecalactone (ng/L)	29 ± 4b	130 ± 6a	31 ± 5b	24 ± 5b
γ-dodecalactone (ng/L)	15 ± 1b	12 ± 1c	19 ± 1a	13 ± 2bc
	Esters			
ethyl butyrate[1]	219 ± 2b	275 ± 6a	204 ± 1c	129 ± 11d
ethyl Hexanoate[1]	356 ± 17b	298 ± 4c	400 ± 3a	181 ± 18d
ethyl octanoate[1]	388 ± 41b	322 ± 16c	484 ± 23a	197 ± 56d
ethyl decanoate[1]	46 ± 8b	41 ± 5.6b	65 ± 5.3a	25.8 ± 7.5c
ethyl dodecanoate[1]	2 ± 0.7a	1.4 ± 0.4b	2.7 ± 0.1a	1.2 ± 0.3b
ethyl propanoate[1]	136 ± 2b	171 ± 6a	129 ± 7b	121 ± 2c
ethyl isobutyrate[2]	24.2 ± 1.1b	27.3 ± 0.3a	25.3 ± 0.4ab	22.6 ± 0.2c
ethyl 2-methylbutyrate[2]	4.8 ± 0.15c	6.3 ± 0.13a	5.1 ± 0.01b	4.7 ± 0.04c
ethyl isovalerate[2]	8.2 ± 0.2b	12.6 ± 0.4a	8 ± 0.1b	7.6 ± 0.3c
ethyl leucate[2]	19.6 ± 0.9c	18.3 ± 0.9c	29.6 ± 1.3b	44.8 ± 3a
ethyl phenylacetate[2]	1.26 ± 0.14b	1.59 ± 0.02a	1.38 ± 0.07b	1.69 ± 0.13a
propyl acetate[3]	62 ± 0.8b	97 ± 8.8a	51.8 ± 0.2c	43.7 ± 2.4d
isobutyl acetate[3]	70 ± 2.9b	52 ± 3.9c	80 ± 0.7a	48.9 ± 6.7c
butyl acetate[3]	0.32 ± 0.01b	0.53 ± 0.02a	0.31 ± 0.01b	0.32 ± 0.01b
isoamyl acetate[3]	2706 ± 87a	2061 ± 251b	2648 ± 46a	1156 ± 187c
hexyl acetate[3]	34.5 ± 1.6a	24.3 ± 4.4b	24.5 ± 0.8b	9.8 ± 0.3c
cis-3-hexenyl acetate[3] (ng/L)	75 ± 3a	54 ± 9b	54 ± 1b	25 ± 1c
phenylethyl acetate[3]	346 ± 26b	224 ± 41c	391 ± 14a	200 ± 29c
ethyl dihydrocinnamate	0.18 ± 0.02b	0.11 ± 0.01c	0.3 ± 0.01a	0.19 ± 0.05b
ethyl cinnamate	0.14 ± 0.01b	0.14 ± 0.02b	0.39 ± 0.05a	0.3 ± 0.08a
	Volatile Sulfur Compounds			
dimethyl sulfide*	0.84 ± 0.42b	3.39 ± 0.95a	0.51 ± 0.09b	0.29 ± 0.06c
	Higher alcohol			
isobutanol*	0.31 ± 0.01b	0.27 ± 0.02c	0.36 ± 0.02a	0.35 ± 0.03ab
isoamyl alcohol*	8.9 ± 0.5b	8.6 ± 0.4b	9.1 ± 0.01a	7.8 ± 0.03c
phenylethanol*	5.1 ± 0.1b	4.3 ± 0.07c	5.8 ± 0.3a	4.4 ± 0.2c

[1]EEFAs; [2]HAAs; [3]EEBAs; *semi-quantitative data

Table 39: Average concentrations (µg/L) of Cabernet Sauvignon wine metabolites in Orange vineyards in 2016 vintage, comparing FF and MF harvest date. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD). LY: low yield; HY: High Yield.

Compounds/wine	CS O1 FF	CS O1 MF	CS O2 FF	CS O2 MF
	Monoterpenes			
citronellol	12 ± 0.6a	12 ± 1a	12.7 ± 2a	12.6 ± 0.3a
trans-geraniol	10.8 ± 1.4a	5.4 ± 2.3c	8.2 ± 1b	7.3 ± 1c
1,8-cineole	0.31 ± 0.03a	0.28 ± 0.03a	0.31 ± 0.04a	0.35 ± 0.03a
4-terpineol (ng/L)	147 ± 7	182 ± 40	178 ± 10	182 ± 22
α-terpinene (ng/L)	22 ± 4b	14 ± 3c	21 ± 3b	89 ± 9a
γ-terpinene (ng/L)	35 ± 3a	20 ± 2c	27 ± 3b	27 ± 4b
terpinolene (ng/L)	35 ± 1a	28 ± 7b	27 ± 1bb	28 ± 3b
linalool	7.5 ± 0.3b	8.3 ± 0.3a	5.6 ± 0.4c	4.2 ± 0.7d
α-terpineol	3.1 ± 0.4a	2.4 ± 0.3b	2.5 ± 0.3b	2.3 ± 0.3b
	C13 norisoprenoids			
β-ionone (ng/L)	245 ± 16a	309 ± 45a	185 ± 6b	168 ± 17c
β-damascenone	11.7 ± 1a	11 ± 1a	8.1 ± 0.3b	7.6 ± 0.4b
α-ionone (ng/L)	32 ± 6ab	39 ± 5a	29 ± 5b	26 ± 4b
	C6-alcohols			
hexanol	1748 ± 101a	1604 ± 61a	1681 ± 115a	1645 ± 74a
cis-3-hexenol	48 ± 2.2c	22.9 ± 1.4d	119 ± 4.9a	72.8 ± 1.7b
trans-2-hexenol	6.8 ± 0.9a	2.1 ± 0.1b	6.4 ± 1a	6.8 ± 0.9a
trans-3-hexenol	50.9 ± 1c	40.2 ± 1.1d	65.1 ± 2.5a	51.6 ± 2.6b
	Lactone			
γ-nonalactone	8.3 ± 0.3c	48 ± 2.4a	9.3 ± 1.1c	16.3 ± 0.8b
γ-decalactone (ng/L)	140 ± 5b	169 ± 15a	149 ± 21a	176 ± 11a
γ-undecalactone (ng/L)	25 ± 6b	70 ± 5a	37 ± 13b	37 ± 11b
γ-dodecalactone (ng/L)	16 ± 2a	12 ± 2b	17 ± 4a	20 ± 2a
	Esters			
ethyl butyrate	255 ± 6a	213 ± 1c	212 ± 10c	230 ± 3b
ethyl Hexanoate	382 ± 10b	271 ± 13c	375 ± 25b	428 ± 13a
ethyl octanoate	413 ± 24b	250 ± 24c	449 ± 38a	486 ± 39a
ethyl decanoate	55.9 ± 3.1	35.7 ± 5.8b	65 ± 6.2a	63.3 ± 8.3a
ethyl dodecanoate	2.27 ± 0.18a	1.28 ± 0.63b	2.37 ± 0.3a	1.92 ± 0.31ab
ethyl propanoate	147 ± 3b	130 ± 5c	151 ± 6b	168 ± 7a
ethyl isobutyrate[2]	22.5 ± 0.5b	27 ± 1.2a	21.1 ± 1.8b	16.3 ± 1.2c
ethyl 2-methylbutyrate[2]	6.4 ± 0.1b	7.4 ± 0.3a	6.7 ± 0.5b	5.5 ± 0.3c
ethyl isovalerate[2]	9.6 ± 0.7b	11.9 ± 0.3a	10.6 ± 0.9b	7.6 ± 0.3c
ethyl leucate[2]	25.3 ± 2.6c	21.9 ± 2.7c	51 ± 2.3a	31.3 ± 1b
ethyl phenylacetate[2]	2.1 ± 0.03b	2.6 ± 0.3a	2.2 ± 0.15ab	1.98 ± 0.32b
propyl acetate[3]	89 ± 5a	73 ± 4b	46.1 ± 0.2d	53.1 ± 0.7c
isobutyl acetate[3]	80.6 ± 2.6a	54.4 ± 2.2c	59.7 ± 1b	52.3 ± 2.4c
butyl acetate[3]	0.38 ± 0.03b	0.48 ± 0.09a	0.44 ± 0.03a	0.32 ± 0.05b
isoamyl acetate[3]	3929 ± 180a	2045 ± 57c	2279 ± 32b	2268 ± 143b
hexyl acetate[3]	34.8 ± 0.9a	14.5 ± 0.2c	19 ± 0.1b	19.1 ± 1.2b
cis-3-hexenyl acetate[3] (ng/L)	76 ± 2a	34 ± 1c	44 ± 1b	44 ± 3b
phenylethyl acetate[3]	629 ± 45a	313 ± 37c	339 ± 23c	411 ± 26b
ethyl dihydrocinnamate	0.23 ± 0.01b	0.11 ± 0.01c	0.31 ± 0.01a	0.23 ± 0.02v
ethyl cinnamate	0.31 ± 0.03c	0.14 ± 0.04d	0.84 ± 0.05a	0.54 ± 0.01b
	Volatile Sulfur Compounds			
dimethyl sulfide	0.29 ± 0.08a	0.44 ± 0.2a	0.09 ± 0.01b	0.1 ± 0.03b
	Higher alcohol			
isobutanol	0.29 ± 0.01a	0.30 ± 0.02a	0.31 ± 0.02a	0.29 ± 0.04a
isoamyl alcohol	10.1 ± 0.3a	9.3 ± 0.5b	9.8 ± 0.5ab	10.5 ± 0.3a
phenylethanol	9 ± 0.2a	6.9 ± 0.07c	7.9 ± 0.1b	9.2 ± 0.3a

[1]EEFAs; [2]HAAs; [3]EEBAs; *semi-quantitative data.

6.11 Influence of berry shrivelling on Shiraz wine chemical and sensory composition

Berry shrivelling is an important phenomenon significantly changing grape morphological and histological traits (Bondada and Keller, 2012). It occurs through grape berry water loss due to the alteration of the fruit water budget when transpiration and potential water back flow exceeds the import of water into the berry through the phloem and xylem (Tilbrook and Tyerman, 2008). Berry shrivelling can have a significant economic impact by reducing yields by up to 25% (Rogiers and Holzapfel, 2015). Its effect is expected to increase due to predicted climate warming shifting grape development and ripening into warmer periods (Petrie and Sadras, 2008). Berry fresh mass loss is variable between seasons, sites and vineyards and it seems to be accelerated by higher temperatures, water constraints and/or stress and excessive bunch sun exposure (Bonada et al. 2015; Rogiers and Holzapfel, 2015). In the 2014 vintage in Griffith severe heat waves occurring between the end of veraison and late ripening led to significant shrivelling of Shiraz grape berries, and Cabernet Sauvignon to a lesser extent. Shiraz (*Vitis vinifera* L.) is particularly known to be prone to berry fresh weight loss in late ripening (McCarthy, 1999; Rogiers and Holzapfel, 2015; Tilbrook and Tyerman, 2008). This late season fruit dehydration is a result of increased berry transpiration (McCarthy, 1997) and decreased phloem (Rogiers et al. 2004) resulting in increased TSS concentrations (Krasnow et al. 2010; Shivashankara et al. 2013).

While late season berry dehydration is probably the type of shrivelling most commonly observed by grape growers, berry shrivelling can also have different origin. Berry shrivelling can occur before (already at bloom, affecting the ovaries) or after veraison in red and white varieties such as Cabernet Sauvignon, Zweigelt, Barbera, Grenache, Semillon, Sauvignon Blanc, Shiraz and other cultivars (Fang et al. 2011; Fuentes et al. 2010; Shivashankara et al. 2013). The four types of berry shrivelling reported in the literature are mentioned below, however the etiologies are not well characterised:

a) sunburn, either before or after veraison, resulting in poor colour development in red varieties and raisin formation in severe occasions (Krasnow et al. 2010).

b) late season fruit dehydration, characterised by an increase in total soluble solids (TSS) concentration (Shivashankara et al. 2013).

c) bunch stem necrosis, characterised by necrotic rachis tissue affecting entire clusters or often only bunch shoulders and tips (Holzapfel and Coombe, 1998; Krasnow et al. 2010).

d) sugar accumulation disorder (SAD), resulting in soft, irregular-shaped berries, with low fresh weight, reduced anthocyanins and sugar accumulation (Bondada, 2014; Griesser et al. 2012).

To the authors' knowledge, there are no reports in the literature that describe the impact of berry shrivelling on Shiraz wine composition. Two studies investigating the influence of berry shrivelling on Shiraz wine composition were carried out within the context of this project.

In the 2015 vintage, shrivelled Shiraz berries, mostly originating from bunch stem necrosis with potentially a small proportion SAD, were harvested from a high yield vineyard in Griffith (G3). Microscale vinifications were made with shrivelled and non-shrivelled berries for three harvest dates. Wine chemical composition was thereafter analysed.

In the 2016 vintage, the influence of late season berry dehydration on Shiraz wine chemical and sensory composition was performed (more information is found Section 6.11.1). Shiraz grapes were harvested at MF stage from a vineyard in Gundagai, with a significant proportion of shrivelled berries due to late season heat waves. Wines were made without and with grape sorting which is a common practice in the wine industry to reduce as much as possible the proportion of shrivelled berries in the vat. The chemical and sensory profiles of wines made without and with grape sorting were compared.

In summary:

a) berry shrivel and harvest date resulted in significant alterations of grape and wine chemical composition.

b) berry shrivel resulted in significant ripening delay and decrease in anthocyanins, ethanol and ester concentrations in wines.

c) the differences between shrivelled and non-shrivelled samples became less pronounced with delayed harvest.

6.11.1 Influence of late season berry dehydration on Shiraz wine chemical composition

An own rooted Shiraz (*Vitis vinifera* L) clone 1654 vineyard, Gundagai (Gundagai GI. New South Wales. Australia; lat. -35°04'69". long. 147°88'48") was used in this study. A Huglin Index (HI) of 2989 units was calculated for the Gundagai vineyard for 2015/16 vintage inferring that this grape growing region could be classified as 'Very warm' in 2016 (Hall and Jones, 2010). The vineyard was spur pruned and trellised to an open sprawling canopy. 400 kg of Shiraz bunches

were randomly picked across the vineyard. Bunches were classified into two classes, shrivelled (S) and non-shrivelled (NS), by visual and textural assessment. Sorted bunches were allocated to the treatments as described below: (i) S-VCT: wine made from 25 kg bunches with estimated 80% shrivelled and very-shrivelled berry, and 20% non-shrivelled berry, and (ii) NS-VCT: wine made from 25 kg 100% non-shrivelled bunch. Each treatment had three replications. Wines were made according to the standard protocol used throughout this investigation.

Wines made from shrivelled berries had significantly higher values in juice total soluble solids and pH. In these wines, a greater amount in ethanol, glycerol, residual sugar, colour hue, and volatiles such as C6-compounds and lactones were detected. In contrast, colour density, total anthocyanins, and yeast-derived volatile compounds such as higher alcohol acetate, were lesser. Wines made from shrivelled berries were sensorially ranked higher in perception of stewed fruit, alcoholic, sweet, and astringent. They were less perceived in red fruit notes and acidity.

This study has increased the knowledge base of wine made from shrivelled Shiraz berries and has provided an insight into the effect on sensory properties for the first time.

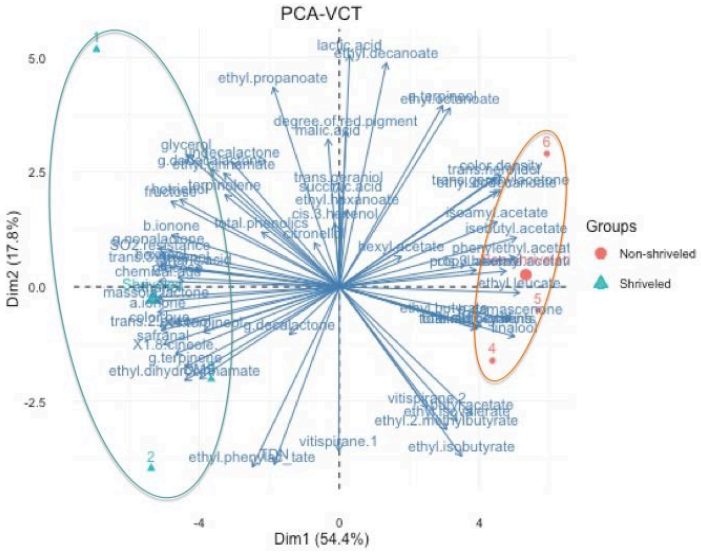


Figure 72: Principal Component Analysis (PCA) of wine chemical composition made from shrivelled and non-shrivelled grapes.

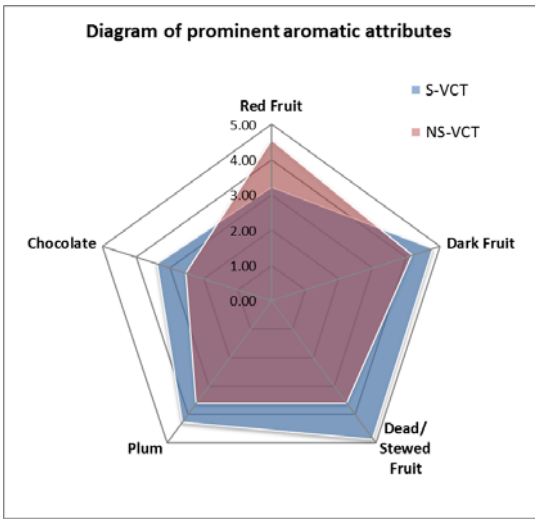


Figure 73: Wine Scores - Spider Chart. NS-VCT-non shrivelled treatment. S-VCT- shrivelled treatment.

6.12 South Australia—Can Additional Irrigation Delay Berry Water Loss and Influence Lower Alcohol Wines?

This chapter is addressing objective: Improving grape quality with precision irrigation including a reduction of plant and fruit damage due to heat waves.

Experiments with additional irrigation, after and before veraison, or preceding heat waves, were carried out in South Australia (Barossa Valley and McLaren Vale). Our hypothesis was that additional irrigation would be able to subsidise efficiently water losses through respiration and minimise berry water loss, which would result in wines of lower alcohol content. Experiments were conducted over three years on Shiraz and Cabernet Sauvignon varieties.

2013/14. In the first season, experiments were carried out in McLaren Vale on Shiraz (SH_McV) and Cabernet Sauvignon (CS_McV). Berry sugar accumulation was monitored weekly and an additional irrigation treatment was implemented in Shiraz vineyard (SH_McV_Irr) after veraison. As noticed from the Figure 74, additional water supply after veraison did not alter the tempo of sugar accumulation in the SH_McV_Irr treatment (grey line). Berry fresh mass at harvest remained insignificant between the two treatments i.e. 0.99 g for SH_McV_irr and 0.93 g for the control (SH_McV_C) treatment, respectively (Figure 75). At the last harvest, grapes from SH_McV_Irr treatment resulted in 13.9 °Baume which was slightly more than 13.8 °Baume measured in the SH_McV_C grapes.

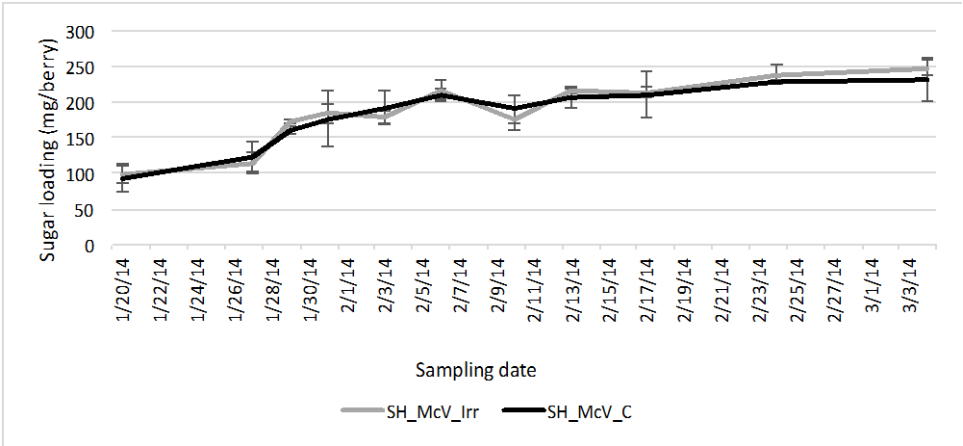


Figure 74: Sugar loading curves for SH_McV_Irr and SH_McV_C treatments for season 2013/14.

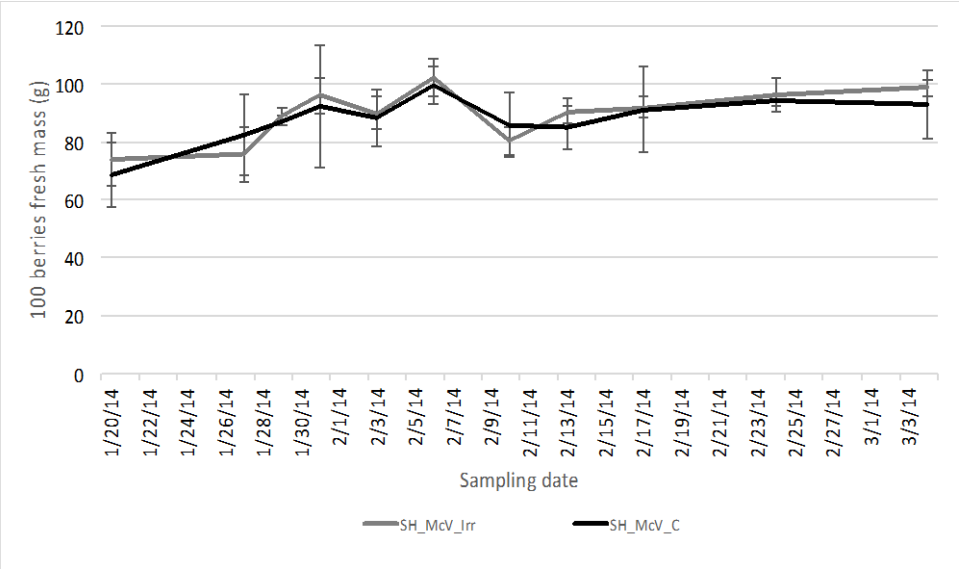


Figure 75: Berry fresh weight for SH_McV_Irr and SH_McV_C treatments for season 2013/14.

Slow down of sugar accumulation was reached for both treatments, SH_McV_Irr and SH_McV_C, on 3 February 2014 and small scale fermentations were carried out on the FF, Intermediate and MF stage. Grapes were harvested commercially on 3 March 2014, which was four days after the predicted MF stage. Experimental wines were also sensorially evaluated by the trained panel and rated for pre-selected attributes (Table 40).

Table 40: Shiraz wine sensory results.

	SH_McV_Irr	SH_McV_C	SH_McV_Irr	SH_McV_C	SH_McV_Irr	SH_McV_C
	FF	FF	MF	MF	Intermediate	Intermediate
Dark Fruit	2.0 c	2.2 c	5.1 ab	5.6 a	3.9 b	4.8 ab
Red Fruit	4.6 a	4.3 a	3.9 ab	2.5 b	4.3 a	3.3 ab
Plum	2.7 b	3.3 ab	4.5 a	4.3 a	4.0 ab	3.7 ab
Raisin	2.1 c	2.6 bc	4.3 a	3.6 abc	3.8 ab	3.6 abc
Chocolate	2.1 b	2.0 b	3.9 a	3.3 a	3.7 a	4.0 a
Pepper	3.2 a	3.1 a	2.9 a	2.3 a	2.3 a	2.6 a
Menthol	6.5 a	3.4 b	3.6 b	2.6 b	3.3 b	2.7 b

Delaying harvest date in Shiraz, irrespective of the irrigation, resulted in significant decrease in red fruit and menthol notes from FF to MF harvest. This trend was not always significant for the Intermediate stage. Perception of dark fruit notes, plum, raisins, and chocolate increased with delayed harvest date (Table 40). Wines from the intermediate stage were in some sensory attributes closer to the wines for which grapes were collected at MF stage, and in other

occasions shared attributes reminiscent of FF wines. The intermediate stage could not be clearly distinguished by the rated sensory attributes from the FF or MF stage (Figure 76). PCA was applied to the sensory attributes and the first two dimensions accounted for 90.9% of variability in the data set. PC1 clearly separated wines for which grapes were harvested at FF and MF stage, whereas PC2 accounted for 11.5% of variability in data set and separated two irrigation treatments (Figure 76). Wines from the grapes harvested at the Intermediate stage were centrally positioned on the PCA (Figure 76) and could not be separated from the MF wines of control treatment.

Increased irrigation delayed the decrease in red fruit notes and menthol. Therefore, wines from the SH_McV_Irr treatment were scored higher for these attributes at MF stage, compared to the SH_McV_C. However, wines from SH_McV_Irr treatment were also rated slightly higher in sensory attributes such as plum, raisins and chocolate, compared to the normally irrigated treatment (SH_McV_C), even though the differences were not significant. It could be concluded that additional irrigation applied to Shiraz positively affects persistence of fresher characters in wines, such as in our case red fruit notes and menthol, however does not diminish the occurrence of ripe and over-ripe characters in wines such as plum and raisins. (Table 40).

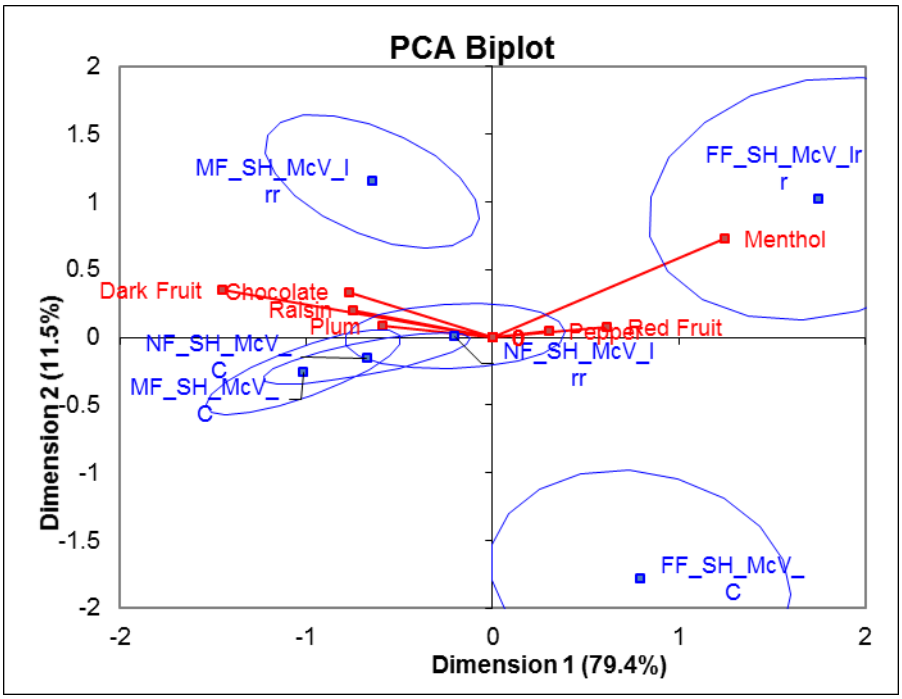


Figure 76: Principal component analysis for Shiraz sensory scores from normally irrigated vineyard (SH_McV_C) and extra irrigated vineyard (SH_McV_Irr) harvested at FF. Intermediate and MF stage.

The sugar loading curve for CS_McV in the 2013/14 season was more erratic as observed for Shiraz (Figure 77). The plateau of sugar accumulation was determined to be reached on 3 February and grapes were vinified on the predicted FF harvest window on 26 February, whereas predicted MF stage (18 March 2014) corresponded to the commercial harvest date.

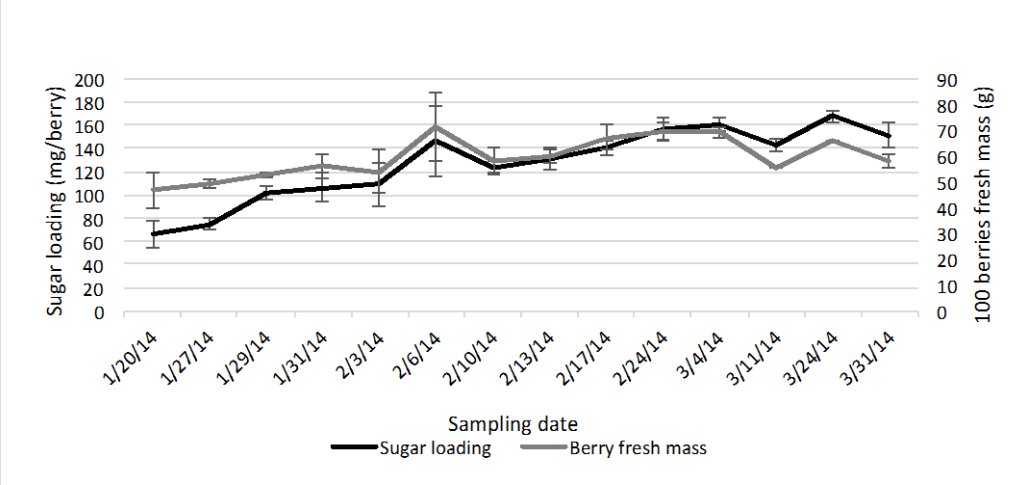


Figure 77: Sugar loading curve and berry fresh mass evolution for CS_McV in 2013/14 season.

2014/15. To strengthen the preliminary results from 2013/14, additional experimentations were carried out in 2014/15 season in Barossa Valley and McLaren Vale on both Shiraz and Cabernet Sauvignon varieties. To monitor soil moisture and vine water status during the season, Gbugs sensors were installed in the Barossa Valley vineyards and stem water potential measurements were performed in McLaren Vale vineyards. However, due to the insufficient number of Gbugs available, sensors were placed only in a control treatment in Shiraz and Cabernet Sauvignon vineyards (Figure 78 and Figure 79).

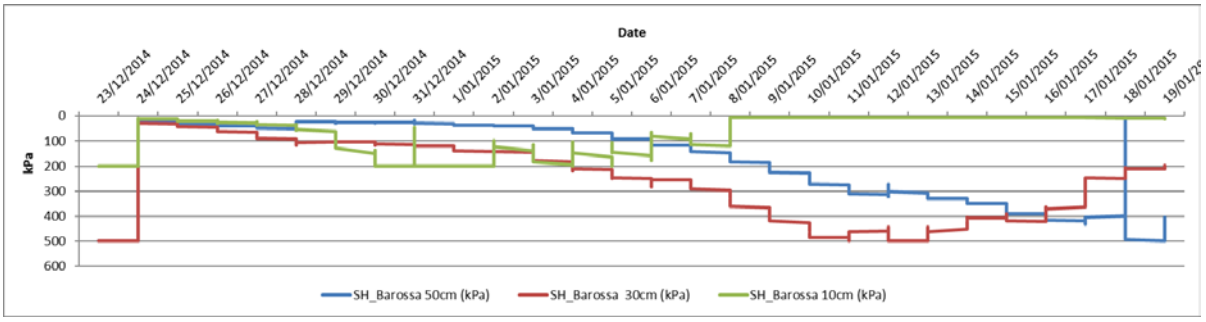


Figure 78: Soil moisture profile measured for Shiraz in Barossa Valley.

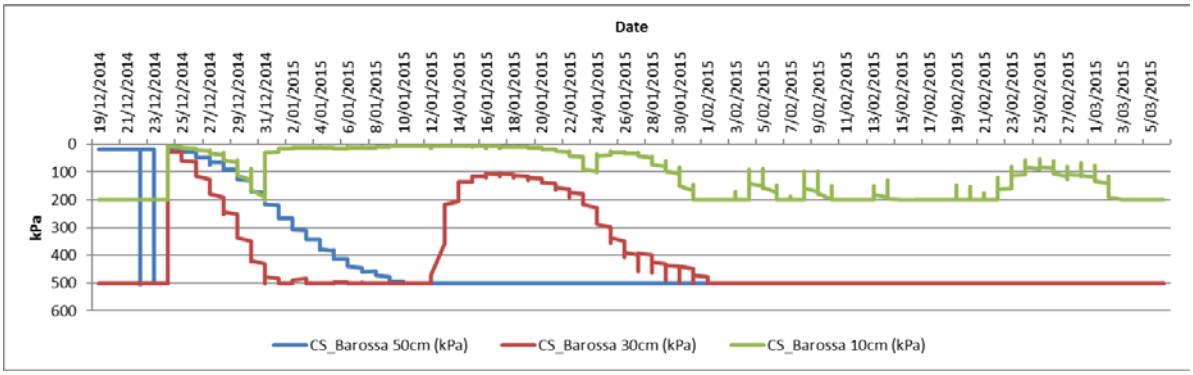


Figure 79: Soil moisture profile measured for Cabernet Sauvignon in Barossa Valley.

Soil moisture decreased slowly in Barossa, with a normal irrigation treatment (SH_B_C) from December onwards (Figure 78). Soil moisture tension reached maximum values of around -500 kPA around 10 January 2015 for SH_B_C, whereas it remained at this value for a month in CS_B_C at the depth of 30 and 50 cm. The period of measurements for SH_B_C was short and no clear conclusions could be obtained from these measurements. From the CS_B_C soil moisture data it may be noticed that the control treatment underwent some restrictive irrigation during the ripening, and the soil moisture profile remained dry from the end of January onwards (Figure 79).

Additional irrigation in Barossa (CS and SH) vineyards was initiated after the veraison around 5 February 2015. SH_B_Irr received 19.2 L more water per emitter compared to SH_B_C treatment which received 177.6 L per emitter throughout the season, a total of 0.73 ML of water. Erratic sugar loading curves were observed for both Shiraz treatments with the estimated date of slow down of sugar accumulation being on 30 January and 2 February for SH_B_C and SH_B_Irr, respectively. Small scale fermentations were carried out at the predicted FF, Intermediate and MF stage for both treatments. Commercial harvest date coincided with the predicted FF stage that was carried out on 13 March 2015. At the last harvest date grapes from SH_B_Irr treatment had higher TSS content, i.e. 16.7 °Baume, compared to the 15.2 °Baume in the SH_B_C treatment. Fast increase in berry TSS was observed from 5 February to 10 February, which was in the SH_B_C treatment related to the drastic decrease in berry fresh weight, whereas such a decrease was not noticed in SH_B_Irr treatment. For the SH_B_Irr treatment rather a gradual slow decrease in berry fresh weight was noticed, resulting in the last harvest date having a similar 100 berries fresh mass for both treatments, i.e. 0.90 g and 0.89 g for SH_B_Irr and SH_B_C treatment.

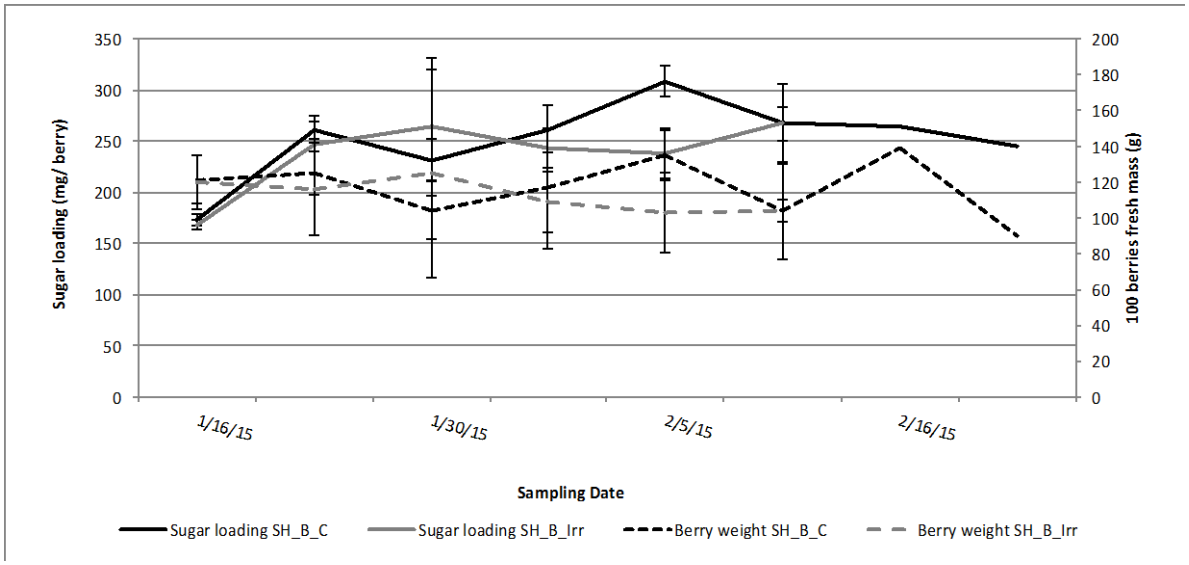


Figure 80: Sugar loading curves and berry fresh mass evolution for SH_B both treatments.

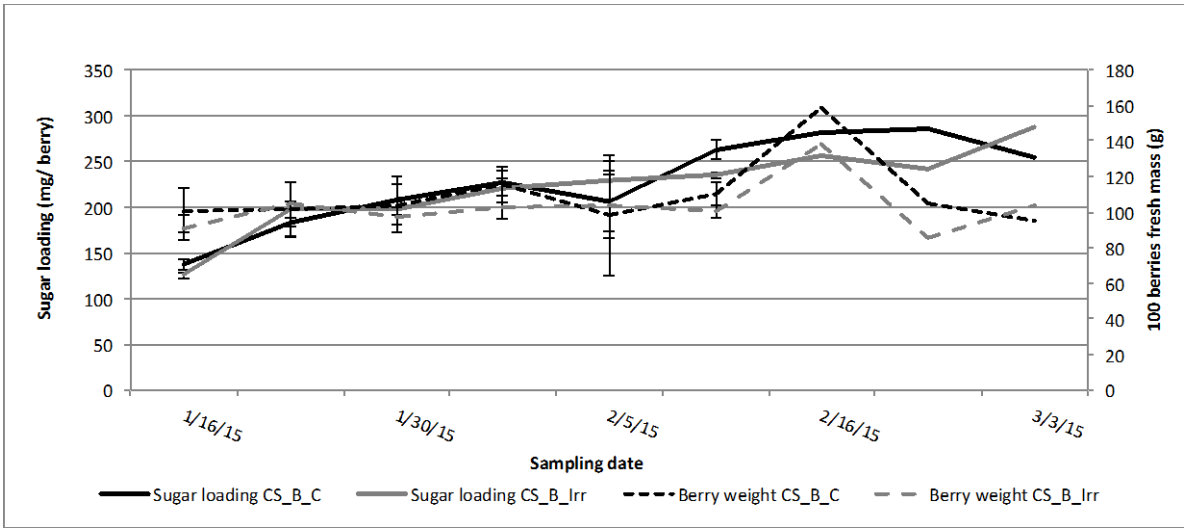


Figure 81: Sugar loading curves and berry fresh mass evolution for CS_B both treatments.

For CS_B_Irr treatment, an extra 51 L/emitter of water per season was applied compared to the CS_B_C treatment, which resulted in total in 1.89 ML/ha. Additional water did not result in higher berry fresh mass, which was, contrary to the expectations, slightly higher in the control treatment. Also, sugar accumulation per berry was faster in the CS_B_C treatment compared to the CS_B_Irr. Interestingly, a significant increase in berry fresh weight was noticed during ripening, followed by the drastic decrease in berry fresh weight for both treatments in late ripening (Figure 81). Such a drastic increase in berry fresh weight during the ripening period is quite unusual if we consider that vines were restrictively irrigated already prior to veraison, meaning that post veraison berry expansion was limited. We suspect that the drastic increase in

berry fresh weight as observed is a technical lapse, especially if we take into account that berry fresh weight thereafter decreased for approximately 1/3 of its mass without a significant increase in berry Baume. Lower 14.8 °Baume was measured at the last sampling date in the CS_B_C treatment juice compared to the 15.3 °Baume in the CS_B_Irr treatment.

Stem water potential measurements in the McLaren Vale before treatment initiation were -1.53 MPa for Cabernet Sauvignon on 7 January 2015 and -1.83 MPa for Shiraz vineyard, indicating strong water constraints (Deloire and Heyns, 2011). At the second measurement, irrigation treatments were already implemented in both vineyards. However, stem water potential measurements did not show any difference for Cabernet Sauvignon vineyard where the measured values were -1.38 and -1.35 MPa for CS_McV_Irr and CS_McV_C, respectively. Contrary to the expectations, Shiraz irrigated treatment (SH_McV_Irr) resulted in average stem water potential of -1.38 MPa, and SH_McV_C of -1.08 MPa.

Additional irrigation was initiated in McLaren Vale only after 20 January 2015. CS_McV_C treatment received 0.79 ML/ha of water throughout the season, whereas CS_McV_Irr treatment received 1.0 ML/ha of water throughout the whole season. From 20 January onwards CS_McV_Irr treatment received 0.21 ML/ha more than CS_McV_C. Additional irrigation in CS_McV_Irr treatment did not alter the speed of sugar accumulation into the berry, however it did result in slightly higher berry fresh mass at the last harvest date compared to the CS_McV_C (Figure 82). Despite the increased irrigation in the CS_McV_Irr treatment, a significant decrease in berry fresh mass was noticed from the beginning of February until the last harvest. However, it seems that additional water provided was able, to a limited extent, reduce berry water losses, compared to the CS_McV_C treatment. The plateau of sugar accumulation was reached on 2 and 5 February 2015 for CS_McV_C and CS_McV_Irr treatments, respectively. Small scale vinifications were made at predicted Intermediate and MF stage (Figure 82).

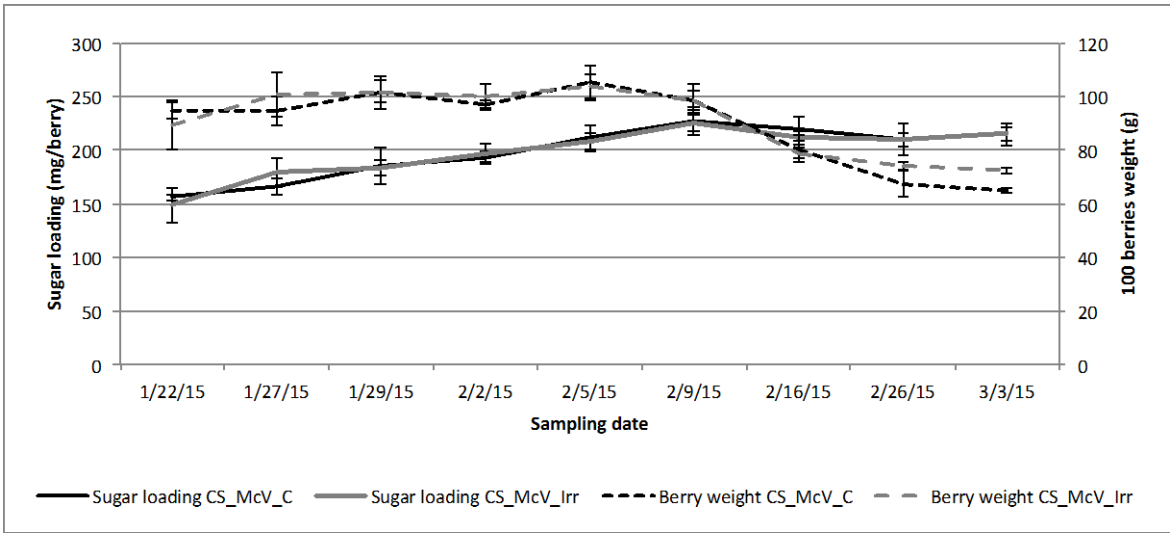


Figure 82: Sugar loading curves and berry fresh mass evolution for CS_McV both treatments.

Additional irrigation in the Shiraz vineyard in the McLaren Vale was also initiated only after 20 January 2015. SH_McV_Irr treatment resulted in 1.36 ML/ha of water throughout the season, whereas SH_McV_C treatment received only 1.09 ML/ha of water. From 20 January onwards SH_McV_Irr received 0.63 ML/ha, whereas SH_McV_C treatment received 0.36 ML/ha. Additional water supplied to SH_McV_Irr however did not result in faster sugar accumulation in the berry compared to the SH_McV_C. Additionally, it also did not alter berry fresh weight at harvest, similar to what was observed for the CS_McV. Irrespective of the treatment, a significant decrease in berry fresh weight was noticed from 1 February 2015 until the last harvest. Additional irrigation was not successful in reducing the tempo of berry fresh weight decline. The plateau of sugar loading per berry (Figure 83) was reached on 2 February 2015 as per the model curve, and grapes were harvested for small lot winemaking at the predicted FF, Intermediate stage and MF stage. Commercial harvest was carried out on 19 February 2015 which coincided with the Intermediate harvest stage. Grapes at commercial harvest date resulted in TSS concentration of around 16.1°Baume. At predicted MF harvest stage on 26 February 2015, berry fresh mass was 0.99 g in in SH_McV_Irr and 0.901 g in SH_McV_C treatment. Additional irrigation in SH_McV_Irr treatment also resulted in significantly lower Baume at the MF stage, i.e. 15.9 compared to 17.4 °Baume in SH_McV_C treatment.

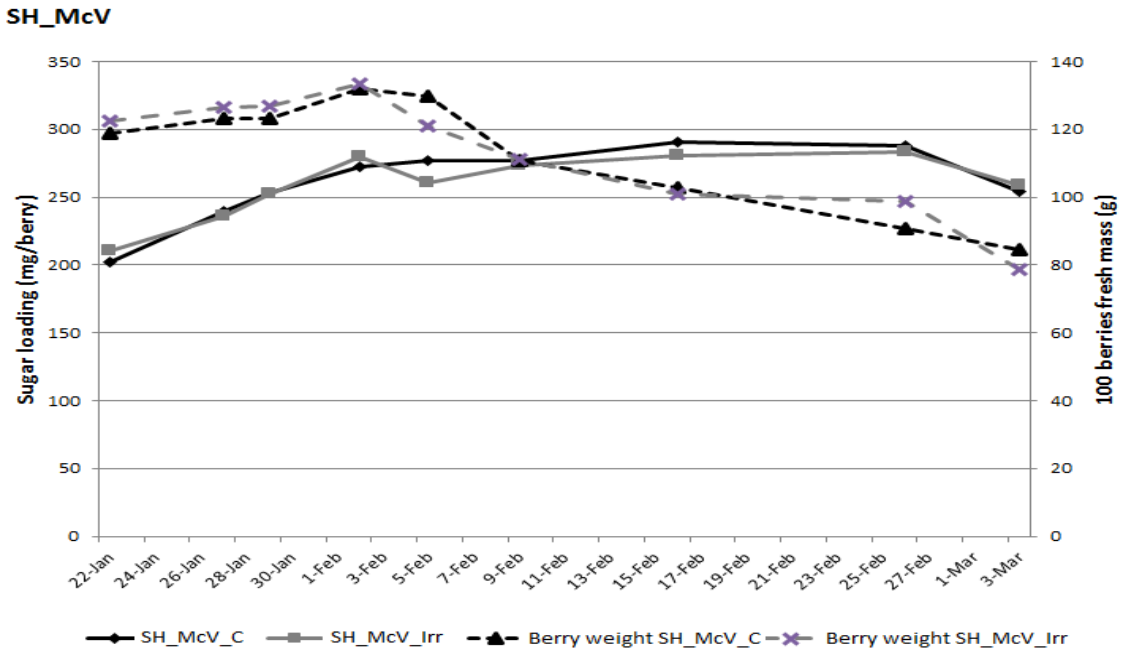


Figure 83: Sugar loading curves and berry fresh mass evolution for SH_McV both treatments.

2015/16 season. In the last experimentation season, trials were carried out on Cabernet Sauvignon in McLaren Vale and on Shiraz in the Barossa Valley. Results from the previous season showed limited success of additional water applied after veraison to reduce/slowdown the speed of berry water loss in the late ripening period. Therefore in the 2015/16 season, additional irrigation was initiated at the phenological stage berry pea size (SH_B and CS_McV). Besides the additional irrigation (_Irr) heat wave irrigation (HW_Irr) was implemented into the experimental design in the CS_McV vineyard. HW_Irr treatment was defined as vineyard watering for 24 hours prior to the expected heat wave event, and continued until the heatwave ceased. A heat wave was defined as a period of three or more consecutive days with a temperature above 36 °C.

CS_McV_C received, in season 2015/16, 1.3 ML/ha of water throughout the season. The volume of water applied to CS_McV_Irr and CS_McV_HW_Irr was 1.7 and 2.1 ML/ha, respectively (Figure 84).

Additional irrigation, even though initiated at the phenological stage berry pea size, did not alter the speed of sugar accumulation into the berry comparing CS_McV_C and CS_McV_Irr (Figure 85). The plateau of sugar accumulation was reached on 3 February 2016 for CS_McV_C and CS_McV_Irr whereas it was estimated to occur in CS_McV_HW_Irr treatment on 8 February 2016.

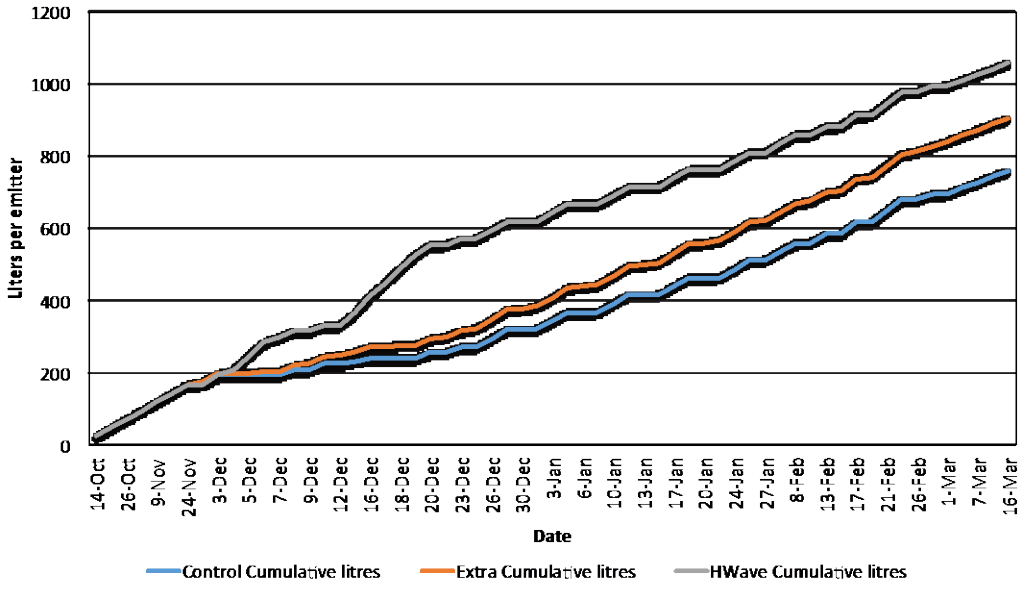


Figure 84: Cumulative volume of water applied to each treatment

Additional water applied to CS through the Irr and HW_Irr treatments did not alter berry volume significantly throughout the season. Only at the last harvest date slightly lower berry fresh mass was noticed in CS_McV_C treatment compared to the CS_McV_Irr and CS_McV_HW_Irr treatments (Figure 86). In contrast to the previous season, no drastic decrease in berry fresh mass at the end of ripening was noticed.

Additional irrigation also did not have a significant effect on final berry Baume values which were at the last sampling 15.7. 15.7 and 15.2 for the CS_McV_C, CS_McV_Irr and CS_McV_HW_Irr treatment, respectively. Additional water had a positive effect on YAN values in grape juice for the period of ripening. At the last harvest date YAN values were the highest in the CS_McV_HW_Irr treatment, around 142 mg N/L, compared with 130 mgN/L in CS_McV_Irr, and around 105 mgN/L in CS_McV_C treatment. Increasing YAN values in treatments with higher water availability could be linked to the better NO_3^- uptake by the roots in the presence of water.

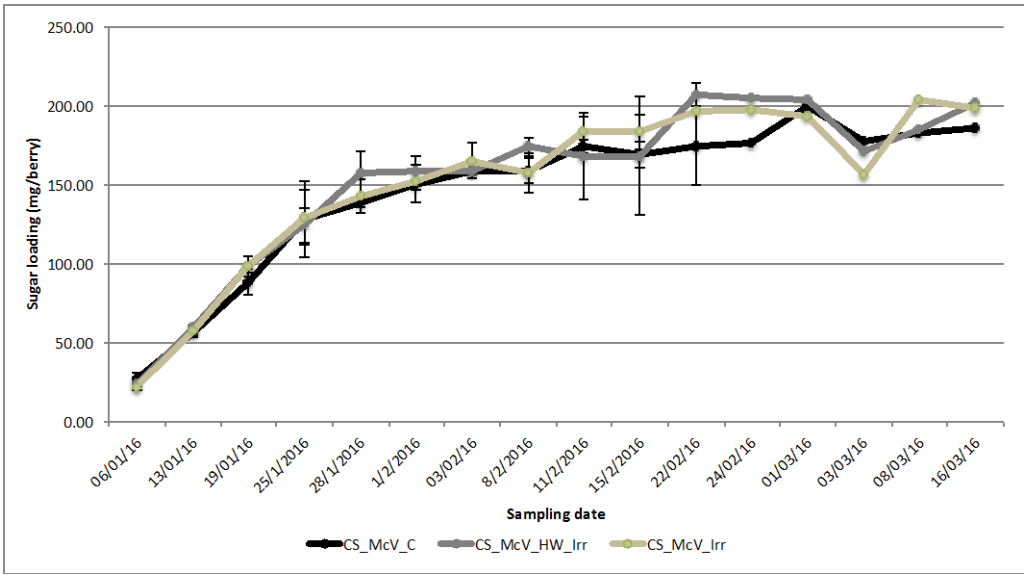


Figure 85: Sugar accumulation per berry for Cabernet Sauvignon from McLaren Vale in season 2015/16.

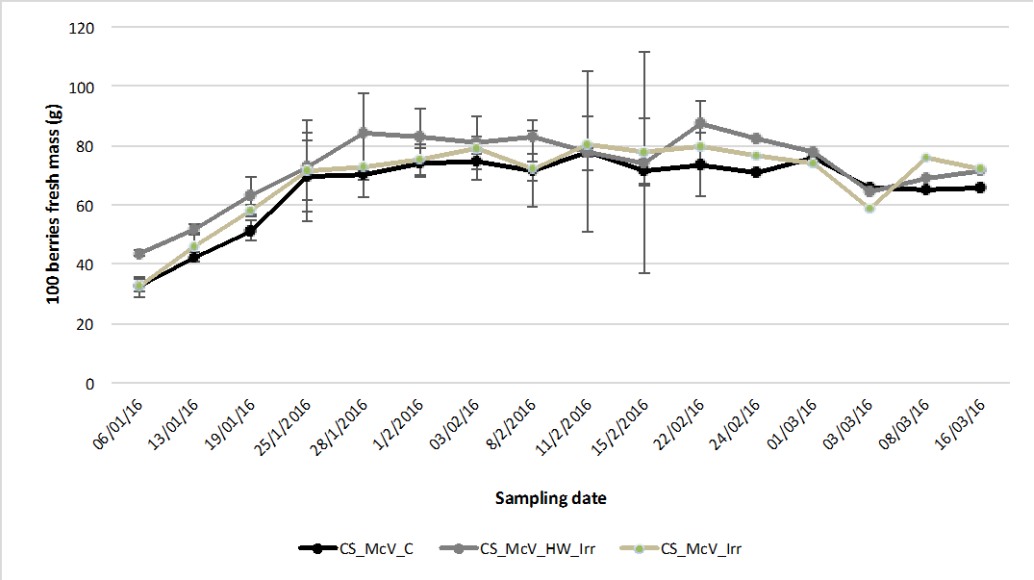


Figure 86: Berry fresh weight evolution for Cabernet Sauvignon in McLaren Vale for three treatments.

In the Barossa Valley, a Shiraz vineyard was investigated in the 2015/16 season. An additional irrigation treatment (SH_B_Irr) was initiated, similarly to McV_CS_Irr, at the phenological stage berry pea size. SH_B_C received throughout the whole season 0.70 ML/ha of water whereas CS_B_Irr received 1.09 ML/ha of water throughout the season. Due to the late start of sampling the plateau of sugar accumulation was less obvious (Figure 87). The plateau of sugar accumulation was determined to be reached on 25 January for SH_B_Irr treatment and on 27 January for the SH_B_C treatment.

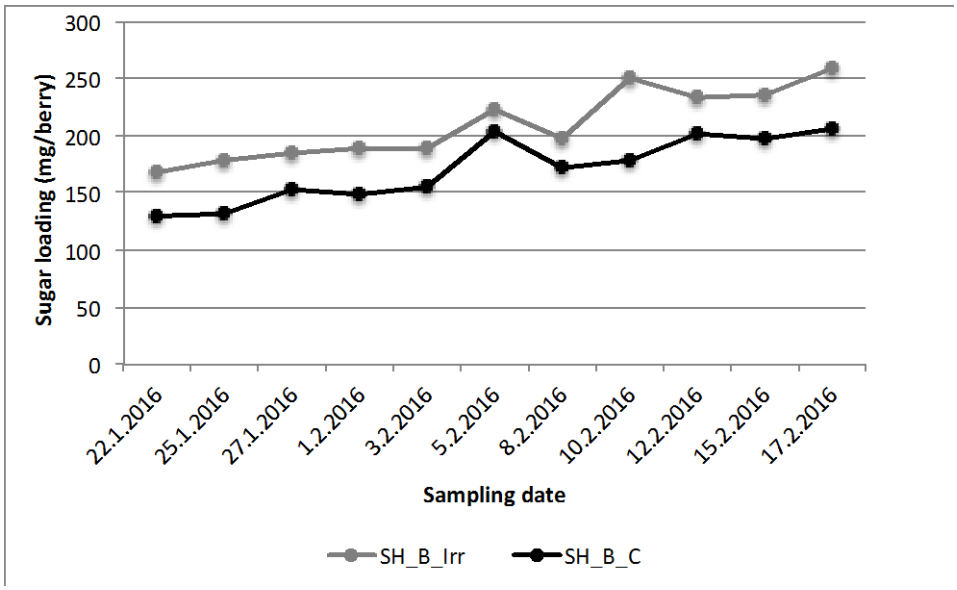


Figure 87: Sugar loading curves for Shiraz in Barossa Valley 2015/16 vintage.

Berry fresh mass was higher in the SH_B_Irr treatment compared to the SH_B_C throughout the ripening (Figure 88). No significant decrease in berry fresh weight in the late ripening was noticed in 2015/16 season, regardless the irrigation regime. As observed previously, additional irrigation in SH_B_Irr treatment did not influence final berry Baume concentrations, which were 15.1 and 15.0 in SH_B_Irr and SH_B_C treatments, respectively. Additional irrigation influenced final YAN concentrations, which were at the last harvest date higher in the SH_B_Irr treatment compared to the SH_B_C. However, final YAN concentrations were low, 85 mg N/L in SH_B_Irr treatment and 75 mg N/L in SH_B_C, which was substantially lower than the 150 mg N/L typically reported as a minimum content to finish fermentation successfully.

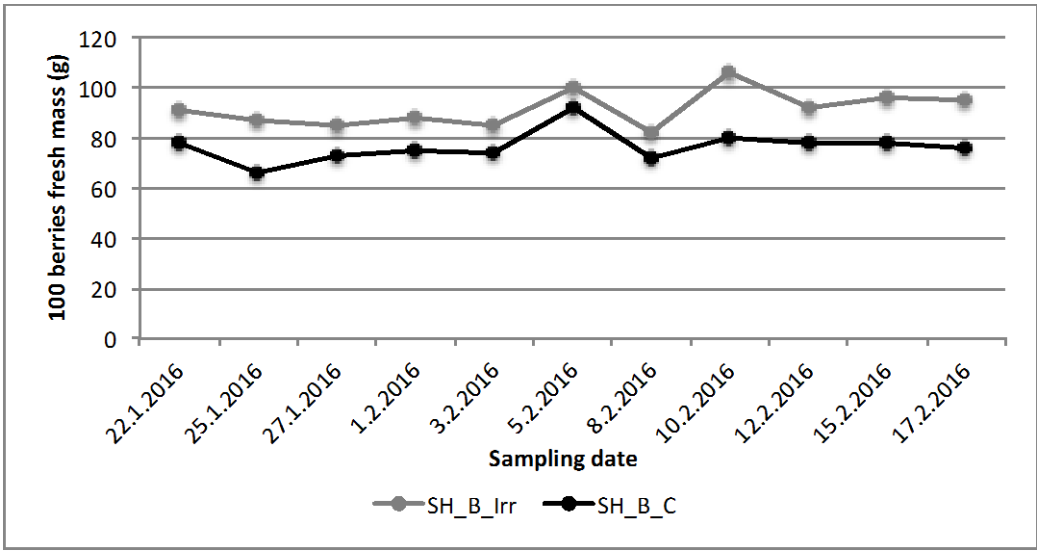


Figure 88: Berry fresh mass evolution for Shiraz in Barossa valley in 2015/16 season.

Table 41: Predicted and actual harvest dates for Shiraz and Cabernet Sauvignon from Barossa and McLaren Vale from different irrigation treatments in season 2014-2016.

Vintage	Vineyard		Plateau	FF	Intermediate Stage	MF	Commercial Harvest
2014	SH_McV_C	Predicted	3/2/14	15/2/2014	21/2/2014	27/2/2014	3.3.2014
		Harvested		18/2/2014	23/2/2014	3/3/2014	
	SH_McV_Irr	Predicted	3/2/14	15/2/2014•	21/2/2014	27/2/2014	3.3.2014
		Harvested		18/2/2014	23/2/2014	3/3/2014	
	CS_McV	Predicted	3/2/14	23/2/1014	X	15/3/2014	26.2 and 18.3.1204
		Harvested		25/2/2014	X	X	
2015	SH_McV_C	Predicted	2/2/15	16/2/2015	20/2/2015	28/2/2015	19/2/2015
		Harvested		16/2/2015	19/2/2016	26/2/2016	
	SH_McV_Irr	Predicted	2/2/15	16/2/2015	20/2/2015	28/2/2015	19/2/2015
		Harvested		16/2/2015	19/2/2016	26/2/2016	
	CS_McV_C	Predicted	2/2/15	22/2/2015	4/3/2015	14/3/2015	23/2/2015
		Harvested		X	3/3/2015	X	
	CS_McV_Irr	Predicted	5/2/15	25/2/2015	7/3/2015	17/3/2015	23/2/2015
		Harvested		X	3/3/2015	X	
	SH_B_C	Predicted	30/1/15	12/2/2015	18/2/2015	24/2/2015	13/2/2015
		Harvested		12/2/2015	19/2/2016	26/2/2016	
	SH_B_Irr	Predicted	2/2/15	14/2/2015	20/2/2015	26/2/2015	13/2/2015
		Harvested		12/2/2015	19/2/2016	26/2/2016	
	CS_B_C	Predicted	5/2/15	25/2/2015	7/3/2015	17/3/2015	10/3/2015
		Harvested		24/2/2016	3/3/2016	X	
	CS_B_Irr		5/2/15	25/2/2015	7/3/2015	17/3/2015	10/3/2015
				24/2/2016	3/3/2016	X	
2016	CS_McV_C	Predicted	3/2/16	23/2/2016	4/3/2016	14/3/2016	29/2/2016
		Harvested		24/2/2016	1/3/2016	15/3/2016	
	CS_McV_Irr	Predicted	3/2/16	23/2/2016	4/3/2016	14/3/2016	29/2/2016
		Harvested		24/2/2016	1/3/2016	15/3/2016	
	CS_McV_HW_Irr	Predicted	8/2/16	28/2/2016	9/3/2016	19/3/2016	7/3/2016
		Harvested		24/2/2016	7/3/2016	15/3/2016	
	CS_B_C	Predicted	27/1/16	7/2/2016	13/2/2016	19/2/2016	18/2/2016
		Harvested			X	18/2/2016	
	CS_B_Irr	Predicted	25/1/15	5/2/2016	11/2/2016	17/2/2016	18/2/2016
		Harvested			X	18/2/2016	

6.12.1 Outputs

Slight modifications in the tempo of sugar accumulation on a per berry basis were noticed with additional irrigation after veraison. However, the plateau of sugar accumulation was reached within few days for all treatments, irrespective of the irrigation regime.

Additional irrigation applied after veraison, and also at the phenological stage of berry pea size, in Shiraz and Cabernet Sauvignon had generally minor effects on the final berry weight.

Additional irrigation applied after veraison did not prevent drastic berry weight losses as observed in 2014/15 season.

Various effects of additional irrigation on berry Baume were observed. In some occasions, no differences in Baume concentrations between treatments were noticed, whereas on some occasions berry Baume increased or decreased as a consequence of additional irrigation. Various responses to additional water supply could be a consequence of a previously experienced water stress by the plant, altering grapevine functioning, and also climatic conditions.

Additional irrigation in Shiraz resulted in wines that retained red fruit notes during grape ripening, compared to normally irrigated treatment, whereas the occurrence of jammy and overripe characters in wines was not reduced.

Additional irrigation to the extent that is practiced in these experiments was not an effective tool to prevent drastic decreases in grape berry weight loss at late ripening and did not limit the production of wines of lower alcohol.

Additional trials looking at vine water stress and soil moisture in more detail are needed to verify these results. However, it seems that rapid berry weight loss in late ripening under warm conditions is difficult to control.

6.13 Harvest Dates for White Cultivars

Berry colour evolution of white varieties was measured using a Dyostem™, a tool developed by Vivelys France in collaboration with Montpellier SupAgro. This method is based on an indirect relationship between the evolution of the berry tint angle (according to the HSL model – Hue, Saturation, Luminescence (Figure 89 and Figure 90) and wine sensory analysis. Typically during with grape ripening, the berry hue angle moves from a greener spectrum towards a yellower hue. This can be indirectly related to the aromatic profile of the wines through a complex mechanism of berry carotenoids and chlorophyll degradation increasing the production of C13-norisoprenoids. The latter are known to be of a significant importance for aroma and are important enhancers of fruity and floral notes in some wines. Furthermore, berry colour evolution can be indirectly associated to the degradation of malic acid and methoxypyrazines in Sauvignon Blanc grapes. Measuring berry hue angle may therefore have potential to profile berry maturation, and assist in the determination of appropriate harvest dates for specific white wine styles.

Measuring berry colour evolution. Berry skin colour evolution for Chardonnay and Sauvignon Blanc from Tumbarumba and Orange vineyards for the 2013/14 growing season was assessed. For the 2014/15 and 2015/16 growing seasons two vineyards in Orange at different altitudes were assessed and Table 42 presents a summary of the vineyards and cultivars for which the evolution of berry skin colour was determined. Ten to 15 bunches were randomly collected from each vineyard block and approximately 200 berries carefully excised and placed on a Dyostem plate. Berry hue angle was measured together with the berry volume distribution. Sampling for berry hue angle measurements was conducted approximately every four days post veraison until the commercial grape harvest.

Basic parameters of maturity. Traditional measures of grape maturity were measured for white grape varieties, including TSS, TA and pH. In the 2015 and 2016 seasons measures of basic maturity parameters were performed in the laboratory of one of the industry partners in Orange.

Vineyard characterisation. Vineyards in Orange were climatically characterised with the climatic data obtained from the nearby weather station located in the vineyard. Climatic data for the Chardonnay vineyard in Tumbarumba were obtained via SILO application and are presented in Table 6.

Wine sensory. Commercial wines from the 2014/15 growing seasons were collected and subject to sensory descriptive analysis a small wine tasting to attempt to correlate sensory attributes to the measured berry colour hue.

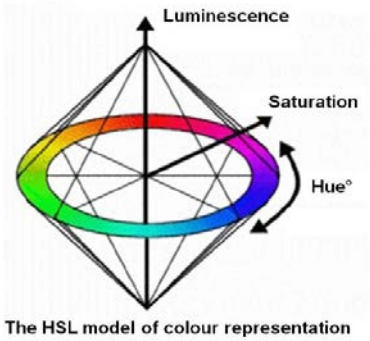


Figure 89: The Hue, Saturation and Luminescence (HSL) model of colour representation which gives hue values in degree from 0 to 360°

Table 42: Chardonnay and Sauvignon vineyards selected for measuring berry colour evolution.

Vintage	Cultivar	Region	Variable
2014	Chardonnay Sauvignon Blanc	Tumbarumba Orange	 600 m
2015 & 2016	Sauvignon Blanc (SB_LA) Sauvignon Blanc (SB_HA) Chardonnay (CH_LA) Chardonnay (CH_HA)	Orange Orange Orange Orange	650 m 900 m 650 m 900 m

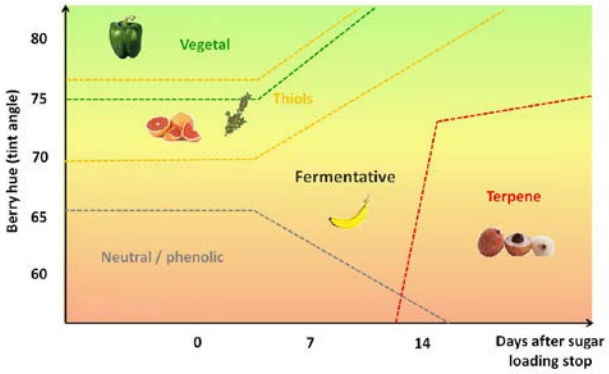


Figure 90: White cultivars berry aromatic model (from Vivelys, France). The method uses the berry colour evolution from véraison to harvest (berry hue or tint angle in degree: y-axis) and the berry sugar loading concept (x-axis). According to the evolution of the berry hue, the method allows to predict the style of wine.

Chardonnay Tumbarumba 2013/14

The Dyostem™ tool was used to measure berry colour evolution in season 2013/14 on the Chardonnay grapes from Tumbarumba. The percentage of berries with a hue angle below 80° and therefore moving from green to yellow hue, is shown in Figure 91. This hue angle represents an important indicator for the maturity evolution of the grapes as anecdotal evidence for wine sensory profiling suggests that sensory profiles are more related to fruitier, thiols and terpene driven nuances. Wine sensory evaluation could not be undertaken as the grapes from this specific vineyard were harvested for sparkling wine base.

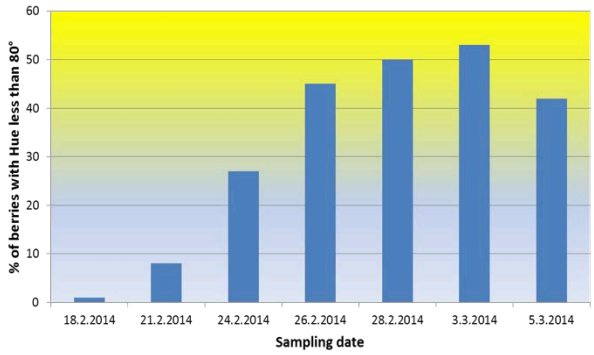


Figure 91: % of berries with hue angle lower than 80° (meaning the evolution from green towards ripe fruit) for Chardonnay grapes from Tumbarumba.

Chardonnay Orange Vineyards 2014/15

Berry colour evolution was monitored from veraison until commercial harvest, for Chardonnay grapes grown in Orange at two altitudes (i.e. 650 and 900 m). Berry hue angle for both Chardonnay vineyards was around 90° at the first sampling date and steadily declined thereafter in a synchronous manner for each vineyard. At the sampling date taken immediately prior to the commercial harvest, the median berry hue angle was between 75-80 ° for the low altitude and 70-75° for high altitude vineyards respective (Figure 92). Between four and five weeks duration elapsed from veraison to the commercial harvest date for the berry hue angle to move from a green to more aromatic zone according to the HLS model, and this duration was dependant on vineyard altitude. A slow decrease in berry hue indicates a moderate tempo of ripening which can be related to temperate climate. Mean berry volumes were 1.1 mL and 1.6 mL for the low and high altitude vineyards respectively. Sugar accumulation and TA decrease was faster in the low altitude vineyard compared to the high altitude vineyard and this is not unexpected given the meso climatic differences between the two sites.



Figure 92: Chardonany berry colour evolution during ripening for CH_LA and CH_HA vineyards in 2014/15 vintage.

Sensory evaluation of the commercial Chardonnay wines was undertaken approximately three months after the completion of fermentation and prior to oak treatment (Figure 93). The sensory panel consisted of NWGIC and industry partner staff familiar with the wine style derived from the vineyard locations. Low altitude wines were perceived higher in lemon/lime, tropical fruit and grape fruit sensory attributes. These descriptors are, according to the theoretical sketch (Figure 90), correlated to the higher hue angles ranging between 70-85°. This wine was also perceived as more acidic, even though the titratable acidity measured in grapes was lower compared to the high altitude juice. As the wines were commercially made according to a desirable wine style, varying acid additions during fermentation may have altered the overall balance and perceptions of these wines.

High altitude wines were ranked higher for the peach sensory attribute, which corresponds well to the lower hue values measured in grapes (Figure 93). Overall the model of expected Chardonnay wine sensory profiles based upon berry hue angles, generally corresponded well to the wine sensory profiles (Table 43). The matching sensory profiles of the wines and the models suggest that the Dyostem™, or other means of berry colour measurements, could be used to estimate appropriate harvest times for specific wine styles. It would be useful to have a larger dataset in order to make conclusion based upon berry colour development and Chardonnay wine sensory profiles.

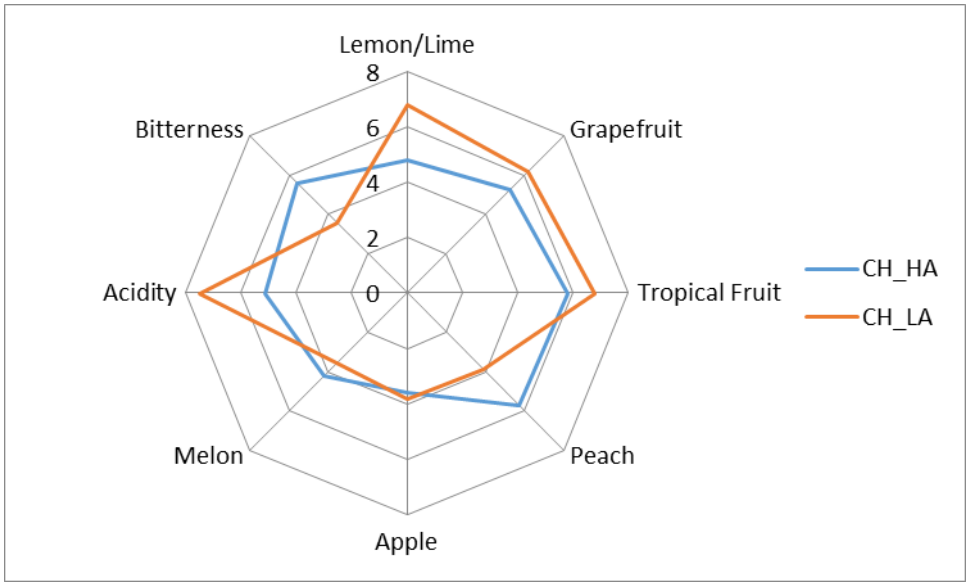


Figure 93: Sensory profiles of CH_HA and CH_LA wines in 2014/15 season.

Chardonnay Orange Vineyards 2015/16

Chardonnay grape berry hue angle decreased rapidly in the low altitude vineyard and at the sampling date immediately prior to the commercial harvest was 80° (Figure 94). The berry colour hue moved sharply from green/unripe zone to more aromatic, fruitier spectra. The corresponding fruit Baumé, pH and TA were determined 10.8, 3.13 and 6.86 g/L.

Fruit in the high altitude vineyard had a more moderate progression of berry hue angle moving from 86° to 82° at the commercial sampling date. The slow evolution of berry colour in the high altitude vineyard is thought to be associated with a severe weather event causing widespread hail damage to the vine canopy and fruit (Figure 95). A concomitant reduction in sugar accumulation profiles and retained high fruit acidity arising from a compromised photosynthetic output supports these observations. Due to the widespread and severe hail damage it was not possible to obtain fruit for winemaking or commercially made wines to sensory evaluation.

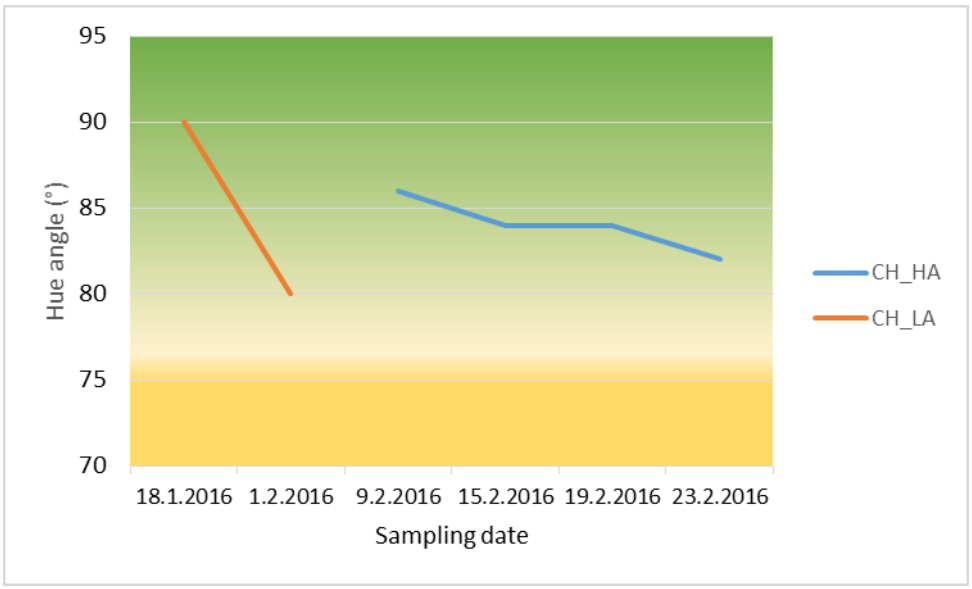


Figure 94: Chardonnay berry colour evolution during ripening for high and low altitude vineyards in the 2015/16 vintage. HA: high altitude; LA: low altitude.



Figure 95: CH_HA canopy and bunch damage caused by severe hail in the 2015/16 season.

Sauvignon Blanc Orange 2013/14

Methoxypyrazines were monitored in Sauvignon Blanc grape juice harvested from the vineyard located at 600 m altitude. Isobutyl methoxypyrazine (IBMP) declined from 48 ng/L to 16.8 ng/L and was 5.0 ng/L on 9, 22 and 31 January 2014 respectively. Grapes were harvested for experimental wine making on 9 February and again on 13 February. The commercial harvest was on 12 February 2014. Sensory analyses carried out on both Sauvignon Blanc wines revealed that Sauvignon Blanc wine from the first harvest (SB1) was associated with a higher perception

of acidity and asparagus in wines. Other sensory attributes were equally ranked in the wines (Figure 96).

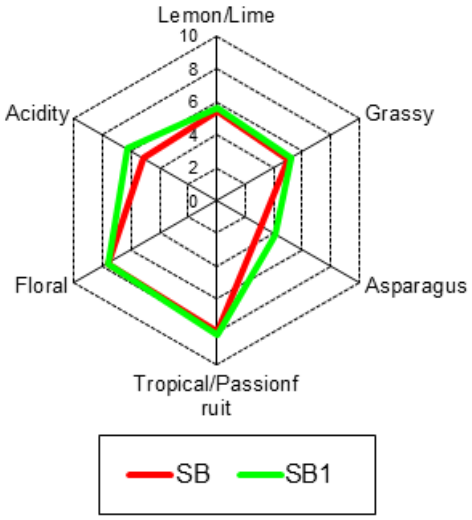


Figure 96: Sensory profiles of Sauvignon Blanc wines from vinatege 2013/14 harvested twice (SB1-first harvest date and SB-second harvest date).

Sauvignon Blanc Orange 2014/15

Having established that Sauvignon Blanc wine and grape berry sensory profiles could be used in association with IBMP values to predict wine style a more comprehensive investigation was undertaken in the 2014/15 and 2015/16 growing seasons. This investigation was undertaken in two vineyards at altitudes of 650 and 900 m.

Sauvignon Blanc berry colour evolution was calibrated against the wine style and wine sensory profiles (Table 43). Changes to the berry skin hue angle evolution was slow. The commencing berry hue angle for grapes in the low altitude vineyard was 88-95 (the green zone, Table 43) whereas yellower tones (hue angle 85-90) were more evident for high altitude grapes (Figure 93). A slow progression of hue angle form from green towards yellower hues indicates a gradual degradation of berry skins chlorophyll. The tempo of berry colour evolution could also be attributed to the bunch microclimate in interaction with the site. The calibration table (Table 43) indicates that the SB_HA berries were at the asparagus/citrus zone, whereas SB_LA berries were in green/asparagus zone. Faster colour evolution noted for SB_HA also coincided with faster sugar accumulation (Figure 94) compared to the SB_LA vineyard. Titratable acidity was consistently higher in the fruit from the high altitude vineyard compared to the low altitude grown grapes. (Figure 95). Despite the differences in berry hue at the last sampling no

differences in selected (tropical fruit/passionfruit, lime/lemon, grassy, asparagus, floral, acidity, bitterness) wine sensory attributes were noticed. The inability for the sensory panel to differentiate the two wines may have arisen from modulation of wine sensory features through judicious wine making operations that can influence the concentration thiols that mask greenness originating from methoxypyrazines.

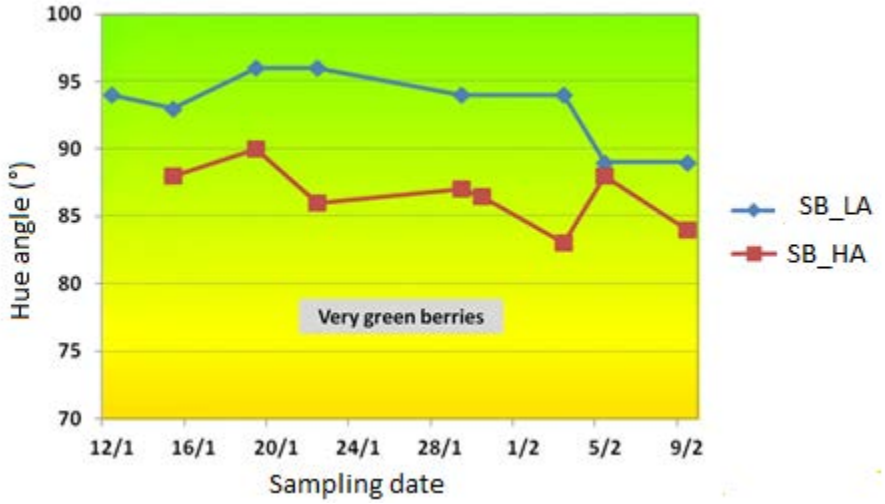


Figure 93: Sauvignon Blanc berry colour evolution during ripening for low altitude and high altitude vineyards in the 2014/15 vintage.

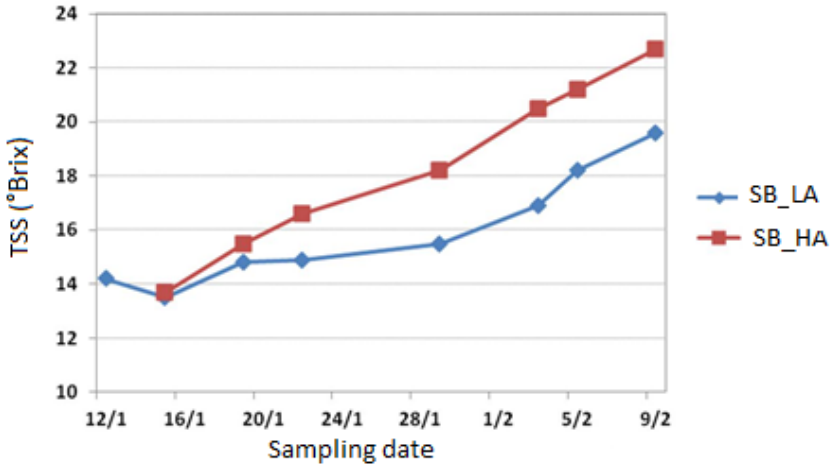


Figure 94: Sauvignon Blanc total soluble solids evolution during ripening for low altitude and high altitude vineyards in the 2014/15 vintage.

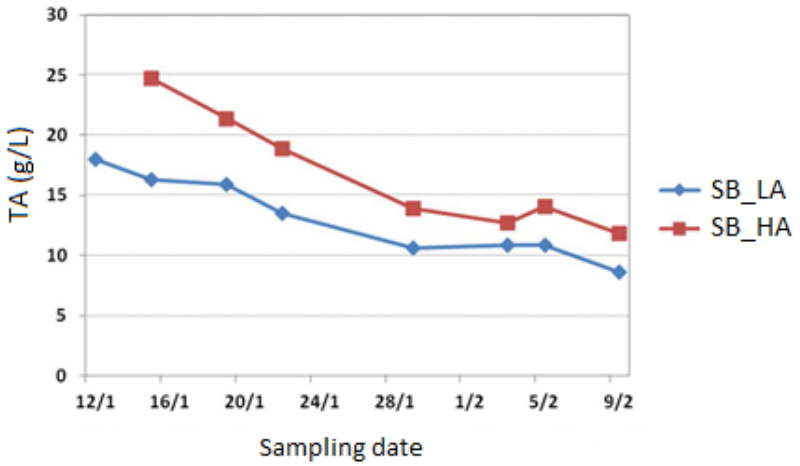


Figure 95: Sauvignon Blanc titratable acidity evolution during ripening for SB_LA and SB_HA vineyards in 2014/15 vintage.

Table 43: Thresholds of berry hue degrees according to the HSL model of colour representation and expected style of wine for Sauvignon Blanc.

Berry hue thresholds (degree F)	Expected wine aromatic profiles
>90	Green/unripe
90 - 85	Green/asparagus
85 - 80	Asparagus/citrus
80 – 75	Asparagus/tropical fruit/grapefruit/citrus
75 - 70	Tropical fruit
70 - 65	Fermentative/terpene
65 - 60	Phenolic/neutral/terpene

In some specific climatic conditions, the thresholds need to be adjusted. The thresholds calibration is done by establishing a relationship between the berry hue angle and the wine style (wine tasting and/or sensorial analysis)

Sauvignon Blanc Orange 2015/16

The Sauvignon Blanc high altitude vineyard was severely damaged by hail, resulting in slow berry colour evolution and sugar accumulation. After the initial decrease in berry hue, it remained at 94° (green/unripe zone) throughout the ripening season (Figure 96). The damage of vine foliage, and limited capacity for photosynthesis, corresponds to slow sugar accumulation as

shown in Figure 97. Sugar accumulation for grapes in the low altitude vineyard was not as severely impacted. Berry colour hue angle for grapes in the low altitude vineyard was measured at 82°C one day immediately prior to the commercial harvest, having decreased from 93° (Figure 96). This value for berry hue angle corresponds to a wine style with citrusy, green attributes (Table 43) which is well aligned with the pH and TA of 3.14 and 8.07 g/L respectively.

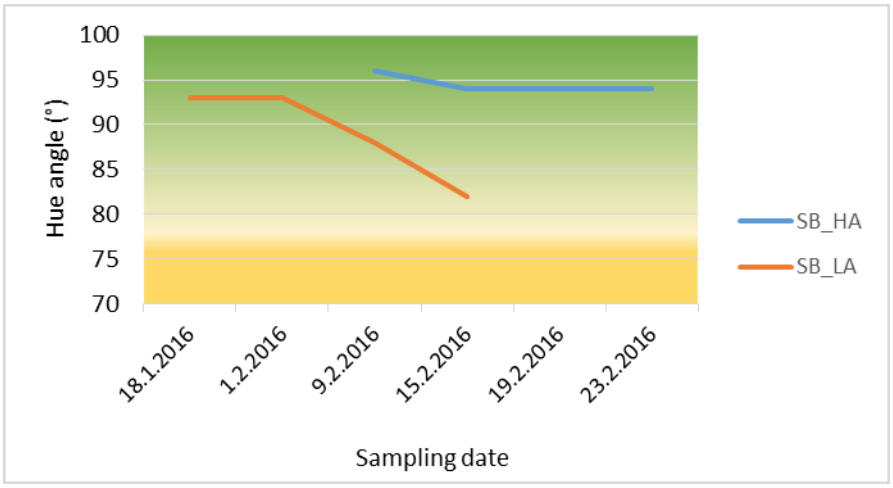


Figure 96. Sauvignon Blanc berry colour evolution during ripening for SB_LA and SB_HA vineyards in 2015/16 vintage.

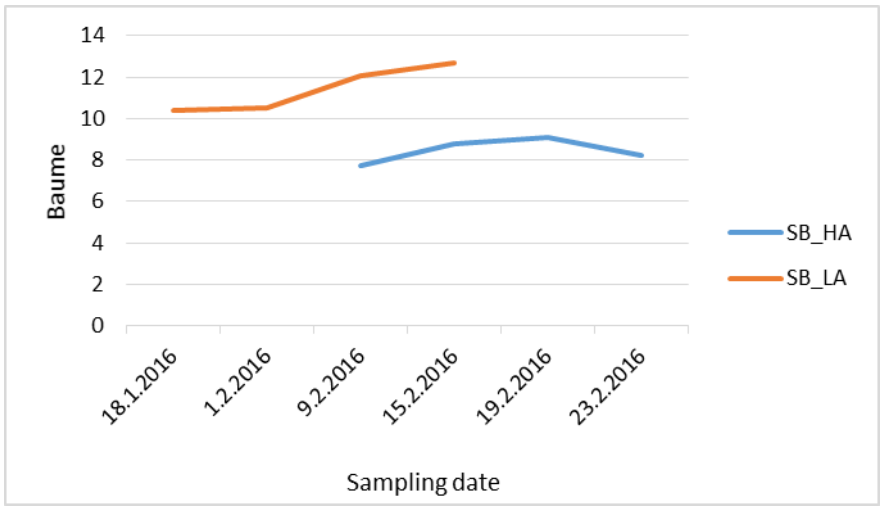


Figure 97: Sauvignon Blanc sugar accumulation during ripening for SB_LA and SB_HA vineyards in 2015/16 vintage.

6.13.1 Outcomes

- The Dyostem™ tool can be useful to monitor berry size, colour heterogeneity and evolution. These measures can provide meaningful information about vineyard performance and overall balance. High variances associated with measured berry colour and mass are indicative of a heterogeneous distribution of berry parameters in the vineyard. Greater variance in these parameters may infer a lack of a clearly discernible sensory evolution in the resulting wines. A clear and distinct flux of berry colour and size with low variances would typically occur in balanced vineyards. Wines made from grapes with low variance of measured colour and size attributes are more likely to have a clear and distinct evolution of sensory attributes.
- The Dyostem™ tool was unable to predict potential wine style when vine physiological development was perturbed due to the environmental conditions (such as hail in our instance).
- Assessment of potential wine style by measuring berry hue angle was successful for Chardonnay, which was confirmed by wine sensory analyses.
- Berry colour evolution cannot be used as a sole indicator of grape berry ripening, but should be used in conjunction with traditional berry maturity measures for harvest decision.
- Feedback from vineyard managers and staff indicated that berry colour measurements with the Dyostem™ were time consuming but did provide some meaningful insights to grape maturity. Further investigation to facilitate the measurement of berry colour is therefore warranted.

7 OUTCOMES AND CONCLUSIONS

This project aimed to explore and test new methods helping to objectively decide on harvest dates in advance, based on the potential wine style. Several experimental vineyards were set up across NSW and also in SA with the industry partner therein. Well established and monitored experimental setup, helped to interpret data obtained from three vintages (2014, 2015, 2016), four locations (Griffith, Orange, Barossa Valley and McLaren Vale) and two cultivars (Shiraz and Cabernet Sauvignon).

Berry sugar accumulation was proven to be a reliable physiological indicator, in a balanced vineyard situation, to predict red wine aromatic evolution for both varieties. Therefore, sugar accumulation per berry could be used as a tool to predict harvest date 12-40 days in advance (coupled with other commonly used traditional indicators of maturity) and to improve winery grape intake and logistics. By using the sugar accumulation method, specific wine styles could be produced which was validated by a sensory panel for Shiraz and Cabernet Sauvignon. Furthermore, commercial harvest dates, at all locations coincided well with predicted harvest dates by the model. In Griffith, commercial harvest date was closer to the MF stage, whereas in Orange it coincided with predicted FF to Intermediate harvest stage. In two vintages commercial wine volumes (10t) were produced at different harvest dates as predicted by the model and were used for workshops with the producers.

An investigation on the wine composition/sensory nexus revealed that only a few individual compounds analysed in this study could be considered as maturity markers common to Shiraz and Cabernet Sauvignon wines: DMS, ethyl propanoate and ethyl dihydrocinnamate. From a sensory point of view, the contribution of DMS to dark fruit aroma specific to MF wines is likely. An indirect contribution of ethyl propanoate cannot be excluded, whereas ethyl dihydrocinnamate is probably a metabolic rather than sensory marker of FF wines. Also, cultivar specific markers with potential sensory contribution were identified. It was particularly the case for (Z)-3-hexenol which might contribute to FF aroma in Shiraz wines. Some compounds were more vintage specific and more related to environmental conditions than ripening. For instance, γ-nonalactone as a marker of stewed fruit aroma in wines made with shrivelled grapes.

The few compounds identified cannot solely explain the wine aromatic progression from FF to MF, as detected by sensory analyses. It is plausible that interactive effects of several modifications in wine chemical composition result in the final, sensorially distinct product. The grape/wine composition nexus was even more complex and unclear. Variations in wine chemical composition could not be explained only by changes in monitored grape compounds

during ripening. Several monitored compounds did not change significantly with ripening. Groups of amino were altered the most with ripening, besides common maturity markers such as TSS, TA and pH. Therefore, we could speculate that observed modifications in wine chemical composition are rather a consequence of modified yeast metabolism (availability of nitrogenous compounds and lipids) and increased extraction of compounds during the fermentation due to the increased cell wall permeability with maturity. That could explain why yeast-derived compounds such as esters were particularly affected by grape maturity. The evolution of grape-derived compounds seems to be in late ripening uncoupled from sugar accumulation and also partly time, but strongly connected to the environmental conditions.

As a new tool to assess white grapes aromatic potential in relation to a final wine style, Dyostem was used. Berry colour evolution proved to be, in some instances, a useful additional indicator to assist wineries in deciding on the harvest dates for white cultivars. Dyostem could not be used as a sole indicator, but should be coupled to other commonly used ripening parameters such as TSS, TA, pH, and berry tasting when deciding on the harvest date.

Controlling grape berry volume with additional irrigation in late ripening proved to be difficult. Despite additional water, a significant decrease in grape berry fresh mass was noticed, resulting in wines with higher ethanol content. Additional irrigation did not prevent progression from fresh, red fruit driven wines to more dark fruit characters. However, additional experiments should be conducted, monitoring more precisely the water stress experienced by plants and also soil water availability to strength these results.

8 RECOMMENDATIONS

Sugar accumulation per berry can be used in conjunction with other commonly used maturity indicators to determine the most appropriate harvest date. Sugar accumulation per berry was shown to be a useful physiological indicator to decide on a potential wine style. Irrespective of the cultivar and region, a red fruit spectrum resulted at the Fresh Fruit stage and dark fruit attributes were the dominant sensory characteristics at the Mature Fruit stage.

Interestingly, only a few grape compounds were significantly altered with grape ripening, with the major alterations noticed in grape berry amino acids composition. Therefore, we could propose that certain amino acids are markers of senescence and also abiotic stress.

Additionally, due to the small alterations of grape berry volatiles and profound changes in wine volatile composition, an alteration of yeast metabolism due to different grape maturity was also speculated. The influence of maturity on berry amino acids, and to a certain extent lipid concentrations and extractability of compounds during maceration, seems to affect the metabolism of yeast rather than having a large direct impact on wine volatiles. This hypothesis was substantiated to some extent with an investigation that studied extractability during ripening. At FF stage, cold soaking resulted in higher amino acids concentrations whereas at MF stage, the differences between treatments diminished.

Despite significant sensory differences in wines from different harvest dates, only a few true markers could be identified in wines that showed a consistent trend with ripening across three vintages and two regions. It was speculated that the identified compounds were not the only contributors to the clearly established sensorial spaces of red fruit at FF harvest and dark fruit at MF harvest, but the synergy between different volatiles and non-volatiles plays a crucial role. Additional research is needed to study the effect of wine non-volatile composition on volatile composition.

A relatively short period, 5-6 days for Shiraz and 8-10 days for Cabernet Sauvignon, is needed to significantly change the wine sensory profile. Harvest dates should be carefully planned taking into account the required wine styles. It should also be noted that the wine sensory profile was altered irrespective of the sugar accumulation, meaning that wine volatile evolution in late ripening is independent of sugar accumulation. This is interesting and a novel perspective for the industry as well as for future research.

Importantly, commercial harvest coincided with the predicted harvest dates by the sugar accumulation model. Interestingly, wineries in Griffith harvested grapes commercially at

predicted MF stage, whereas wineries in Orange harvested at the predicted FF or Intermediate stage. The decision on the harvest dates was consistent during the time span of the project for each of the wineries. This implies that winery specific protocols are already in place to help maintain consistency of their wine style, and that harvest date decisions are largely based in accordance to the results achieved in previous vintages. Therefore, the sugar accumulation model could be initially used to position wineries' harvest dates on the curve and thereafter used to predict harvest dates in advance which would improve winery logistics and grape intake. It is well known that the industry is suffering from the condensed vintages and shorter periods between different cultivars reaching optimal maturity.

The occurrence of heat waves in late ripening had a significant influence on grape berry chemical composition and also often resulted in late season berry dehydration. Late season berry dehydration significantly altered wine sensory profiles and wine chemical composition. With shrivelling, a shift towards jammy, prune, liquorice notes in wine sensory profiles was evident. Bunch stem necrosis and other types of berry shrivelling resulted in a reduction in the speed of ripening and significant changes in wine composition. However, with delayed harvest date, the differences between shrivelled and non-shrivelled treatments diminished. Irrespective of the type of shrivelling, wine anthocyanins were significantly reduced and an increase in γ-nonalactone was observed. γ-nonalactone is a known contributor to prune aromas in wines and has a potential to be used as a marker of shrivel in wine.

Additional irrigations applied to Shiraz and Cabernet Sauvignon before or after veraison did not effectively reduce the rate of berry fresh weigh loss in late ripening or prevent the occurrence of jammy and prune characters. However, more research on this topic is needed as several drawbacks in our study could be identified.

Measuring berry colour evolution using Dyostem can be used as a supplementary tool to decide on harvest dates. Investigation of this method using a range of white varieties in more regions will help to determine its potential to accurately predict final wine styles.

9 Appendix 1: Communications

Publications in international peer review journals:

Antalick, G., Šuklje, K., Blackman, J. B.; Meeks, M., Deloire, A.; Schmidtke, L. M. (2015). Influence of grape composition on red wine ester profile: comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. *J. Agric Food Chem. 63*, 4664-4672.

Antalick, G., Tempere, S., Šuklje, K., Blackman, J. B., Deloire, A., De Revel, G., Schmidtke, L. M. (2015). Investigation and Sensory Characterization of 1,4-cineole: A potential aromatic marker of Australian Cabernet Sauvignon wine. *J. Agric Food Chem. 63*, 9103-9111.

Šuklje, K., Zhang, X., Antalick, G., Clark, A., Deloire, A.; Schmidtke, L. M. (2016). Berry shrivelling significantly alters Shiraz (*Vitis vinifera* L.) grape and wine chemical composition. *J. Agric Food Chem. 64*, 870–880.

Book Chapters (Conference Proceedings):

Antalick, G., Tempère, S., Šuklje, K, Blackman, J.W., Deloire, A., de Revel, G., Schmidtke, L.M. (2017). A step forward in unravelling the uniqueness of Australian Cabernet Sauvignon wine aroma. In Proceedings of the 16th edition of the Australian Wine Industry Technical Conference, Adelaide, Australia, *published in 2017.*

Antalick, G., Šuklje, K., Meeks, C. B., Deloire, A., & Schmidtke, L. M. (2014). Varietal influence of Australian warm to hot climate Cabernet Sauvignon and Shiraz varieties on wine esters composition. In Proceedings of the third edition of the international conference series of Wine Active Compounds, Ed: Gougeon, R., Université de Bourgogne, France, 2014; pp. 299-301.

Antalick, G., Perello, M.-C., de Revel, G. (2014). Esters in wines: new insights through a survey of French wines. In Proceedings of the third edition of the international conference series of Wine Active Compounds, Ed: Gougeon, R., Université de Bourgogne, France, 2014; pp. 17-19.

Deloire A., Šuklje K., Antalick G., Blackman J., Schmidtke L. M. (2016). Using a grape compositional model to predict harvest time and influence wine style. XI International terroir conference, Willamette Valley, Oregon 10-14 July 2016.

Šuklje, K., Antalick, G., Schmidtke, L. M., Lisjak, K., Deloire, A. (2014). Defoliation and UV radiation in the vineyard influences composition and sensory perception of Sauvignon Blanc wine. In Proceedings of the third edition of the international conference series of Wine Active Compounds, Ed: Gougeon, R., Université de Bourgogne, France, pp. 303-306.

Šuklje, K., Antalick, G., Meeks, C. B., Blackman, J. W., Deloire, A., & Schmidtke, L. M. (2014). Optimising harvest date through use of an integrated grape compositional and sensory model : a proposed approach for a better understanding of ripening of autochthonous varieties ? In "Exploitation of autochthonous and more common vine varieties Genetic pedigree and phenotyping, tolerance and stress, diseases to control, rootstocks" 2nd Oenoviti International Symposium International Network Symposium, Geisenheim, Ed: Teissedre, P-L, Institut des Sciences de la Vigne et du Vin, Faculté d'œnologie - Université Bordeaux Segalen, France. pp. 44-49.

Šuklje, K., Antalick, G., Meeks, C. B., Blackman, J. W., Deloire, A., & Schmidtke, L. M. (2016). Grapes to wine: the nexus between berry ripening, composition and wine style. In: Proceedings fo the X International Symposium on Grapevine Physiology and Biotechnology, ISHS Acta Horticulturae, in Press.

Publications in journals dedicated to the wine industry:

Antalick, G., Tempere, S., Šuklje, K., Blackman, J. B., Deloire, A., De Revel, G., Schmidtke, L. M. (2016). A new potential marker of herbal character in Australian Cabernet Sauvignon wines. *Wine and Viticulture journal*, Jan-Feb 2016; p 24-27.

Šuklje, K., Zhang, X., Antalick, G., Blackman, J. B., Clark, A.C, Schmidtke, L. M., Deloire, A. (2016). Berry shrivel significantly alters Shiraz grape and wine composition, *Wine and Viticulture journal*, March-April 2016; p 35-38.

Oral communications at conferences:

Antalick, G., Šuklje, K., Meeks, C., Deloire, A., Schmidtke, L.M. (2014). Varietal influence of Australian warm to hot climate Cabernet-Sauvignon and Shiraz varieties on wine esters composition In the 3rd edition of the International Conferences series on Wine Active Compounds, Beaune, France, 26-28 March 2014.

Antalick, G., Tempere, S., Šuklje, K., Blackman, J. B., Deloire, A., De Revel, G., Schmidtke, L. M. (2015). Investigation and Sensory Characterisation of 1,4-cineole: A potential marker of Australian Cabernet Sauvignon wine. In Xth International Symposium of Oenology, Bordeaux, 29th June-1st July 2015.

Antalick, G., Tempere, S., Šuklje, K., Blackman, J. B., Deloire, A., De Revel, G., Schmidtke, L. M. (2015). Investigation and Sensory Characterisation of 1,4-cineole: A potential aromatic marker of Australian Cabernet Sauvignon wine. In Crush Grape and Wine symposium 2015, Adelaide, 20 November 2015.

Chou H-C., Šuklje K., Antalick G., Meeks C., Deloire A., Schmidtke L., Blackman J. (2017). Late season Shiraz berry dehydration alters composition and sensory qualities of wine. Wine active compounds, Beaune France, 28-31 March 2017.

Šuklje K., Antalick G., Meeks C.′ Blackman J.W., Deloire A., Schmidtke L.M. (2016). Grapes to Wine: The nexus between berry ripening, composition and wine style. X International Symposium on Grapevine Physiology and Biotechnology. 13-18 June 2016 Verona Italy

Šuklje, K., Coetzee, Z., Brandt, J., Antalick, G., Buica, A., Vivier, M. (2014). Yeast derivate in the vineyard: a potential to modify Sauvignon blanc wine aroma. In Macrowine 2014, Macromolecules and second metabolites of grapevine and wine, 7-10 September 2014, Stellenbosch, South Africa.

Šuklje, K., Zhang, X., Antalick, G., Clark, A., Schmidtke, L., Deloire, A. (2015). Berry shrivelling significantly alters Shiraz (*Vitis vinifera* L.) grape and wine chemical composition. In Crush Grape and Wine symposium 2015, Adelaide, 20 November 2015.

Reports for the industry

12 reports for the industry partners provided in a real time on berry sugar accumulation, vine water status, methoxypyrazines content of grapes

Direct communication with the industry during the season to predict harvest dates based on the sugar accumulation model.

Oral communications for the industry

Antalick, G., Tempère, S., Šuklje, K, Blackman, J.W., Deloire, A., de Revel, G., Schmidtke, L.M. (2016). A step forward in unravelling the uniqueness of Australian Cabernet Sauvignon wine aroma. In Proceedings of the 16th edition of the Australian Wine Industry Technical Conference, Adelaide, Australia, 25-27 July 2016.

Deloire A., Antalick G., Šuklje K., Meeks C., Blackman J.W., Schmidtke L.M. (2014). Optimising harvest date through use of an integrated grape compositional and sensory insights model. ASVO, Mildura 24-25 July 2014, Mildura Australia, Presenter: Prof Alain Deloire

Šuklje K., Antalick G., Meeks C.′ Blackman J.W.,Deloire A., Schmidtke L.M. (2015). Optimising harvest date through use of an integrated grape compositional and sensory model, AWRI road shows, Yarra Valley, February 2015.

Šuklje K., Antalick G., Meeks C.′ Blackman J.W.,Deloire A., Schmidtke L.M. (2015) Optimising harvest date through use of an integrated grape compositional and sensory model, Limestone Coast 28 January 2016, Presenter Prof Alain Deloire

Pilot seminar for the industry partners with presentation of the results and wine sensory. Orange. Presenters Prof Alain Deloire, Drs Katja Suklje and Guillaume Antalick. 5 August 2014.

Pilot seminar for the industry partners with presentation of the results and wine sensory. Presenters Prof Alain Deloire, Drs Leigh Schmidtke, John Blackman. Griffith 2014.

Pilot seminar for the industry partners with presentation of the results and wine sensory. Barossa Valley. Presenters Prof Alain Deloire, Drs Leigh Schmidtke, John Blackman, Guillaume Antalick, 12 October 2015.

Pilot seminar for the industry partners with presentation of the results and wine sensory. Griffith. Presenters Prof Alain Deloire, Drs Katja Suklje and Guillaume Antalick, 22 October 2015.

Pilot seminar for the industry partners with presentation of the results and wine sensory. Orange. Presenters Drs Katja Suklje and Guillaume Antalick, 4 November 2015.

Pilot seminar for the industry partners with presentation of the results and wine sensory. Griffith. Presenters Prof Alain Deloire, Drs Katja Suklje and Guillaume Antalick, 30 November 2015.

Pilot seminar for the industry partners with presentation of the results and wine sensory. Griffith. Presenters Prof Alain Deloire, Drs Leigh Schmidtke, John Blackman, Katja Suklje and Guillaume Antalick

Pilot seminar for the industry partners with presentation of the results and wine sensory. Barossa Valley. Presenters Prof Alain Deloire, Drs Leigh Schmidtke, John Blackman

The Impact of Berry Ripening on Wine Quality, Seminar presentation and wine tasting with De Bortoli Winemakers, November 2016, Leigh Schmidtke, John Blackman, Lisa Given, Katja Suklje, Guillaume Antalick, Alain Deloire.

The Impact of Berry Ripening on Wine Quality, Seminar presentation and wine tasting with Orange Regional Winemakers, November 2016, Leigh Schmidtke, John Blackman, Lisa Given, Katja Suklje, Guillaume Antalick, Alain Deloire

Grapes for Style: The Impact of Berry Ripening on Wine Quality, McLaren Vale Technical Viticulture Conference, Tuesday September 13th 2016 , *Leigh Schmidtke, John Blackman, Katja Šuklje*,
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Selected Background Readings

Predicting harvest date using berry sugar accumulation

BY **Alain Deloire,** Department of Viticulture & Oenology, Stellenbosch University, South Africa (present address: NWGIC, Charles Sturt University, Wagga Wagga, Australia)

Sugar loading can be defined as the accumulation of the quantity of sugar per berry, expressed as milligrams (mg) per berry, from veraison onward. Veraison corresponds with the onset of fruit maturation.

In the grapevine, fruit maturation starts with an abrupt softening of the berry (within 24 hours). This softening goes hand-in-hand with sugars being actively introduced into the berry (sucrose rapidly hydrolyzed into hexoses: glucose and fructose). In red and black cultivars, veraison is characterized, after softening, by skin coloring as a result of the biosynthesis of anthocyanins.

Accumulation of sugar loading in grape berries gives an indication of the ripening process from a new perspective and is a novel approach to identify practical indicators to obtain particular styles of grapes and wine. Sugar loading may also provide information about ripening kinetics and enable the principal phases of ripening to be distinguished.[8] This information provides a greater understanding of how grape quality develops in the vineyard.

Sugar loading calculation

Phloem sugar transport, principally to the flesh cells, has been characterized in studies of plant-to-berry sugar loading and phloem sugar unloading, notably by the peripheral vascular system of the berry.[11] Phloem sugar unloading into cell vacuoles occurs mainly via an apoplastic mechanism, which requires the intervention of hexose transporters.[1,9]

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From the above-mentioned studies, it can be concluded that sugar loading into the berry, coupled with the dynamics of sugar concentration changes, may be considered a useful indicator of grape quality. It takes into account the accumulation of sugar per berry (mg per berry) and therefore enables the kinetics of sugar concentration changes to be monitored.

Kinetic monitoring of the quantity of sugar per berry may be considered as a method of measuring the vine's physiological functioning[4,5,7,9] and in particular photosynthesis, which is a reliable indirect indicator of temperatures to which the vine is subjected under given conditions over a specific time period and grapevine water status.

Active sugar loading is calculated on the basis of berry volume (or berry fresh mass) and sugar concentration.[3,9,10]

Example of sugar loading calculation

For a berry with a ripeness level of 25° Brix:

1. Convert 25° Brix to probable alcohol:
25 x 0.59 = 14.75° in probable alcohol, where 0.59 is the coefficient used for yeast activity to convert Brix to probable alcohol (this coefficient to be adapted to the probable alcohol level).

2. Determine mg of sugar per ml probable alcohol:
To obtain 1° probable alcohol, 17 g/L of sugar is required.
14.75° x 17 g/L = 250.75 g/L, which is equivalent to 250 mg of sugar per ml (in this example).

3. Calculate quantity of sugar per berry:
In addition to measuring °Brix, the



volume of a berry (or berry fresh mass thereof) should also be measured so that the quantity of sugar per berry can be calculated. Approximately 50 berries should be used to determine berry fresh mass so that the single berry fresh mass is an average of a berry population.

To determine quantity of sugar per berry, multiply 250 mg x the volume of a berry (or the berry fresh mass thereof, because for many varieties there is a linear correlation between berry volume and fresh mass).

Please take into consideration that this calculation is only an approximation of sugar loading into berries. This is due to many reasons, among others: seed volume and number, sugar distribution between skin and pulp. Despite the calculation being an approximation with shortcomings, it nevertheless is a useful indicator, and its use is becoming more common.

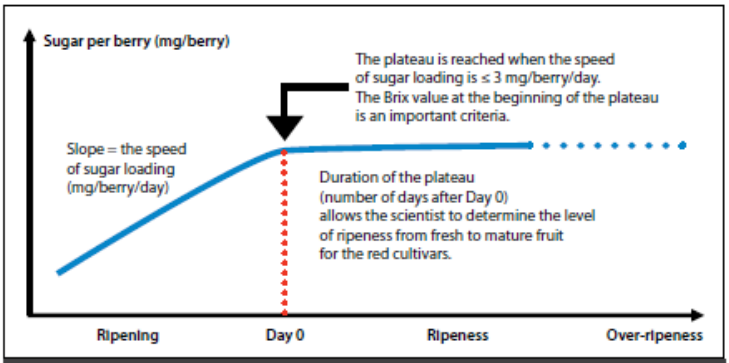


Figure 1 — Berry sugar loading concept — This theoretical curve is based on data obtained over five years with at least 20 different grape varieties in different countries, principally France, Spain, Argentina and Chile. This curve has been recently calibrated for some South African viticulture areas and cultivars (in collaboration with Distell).

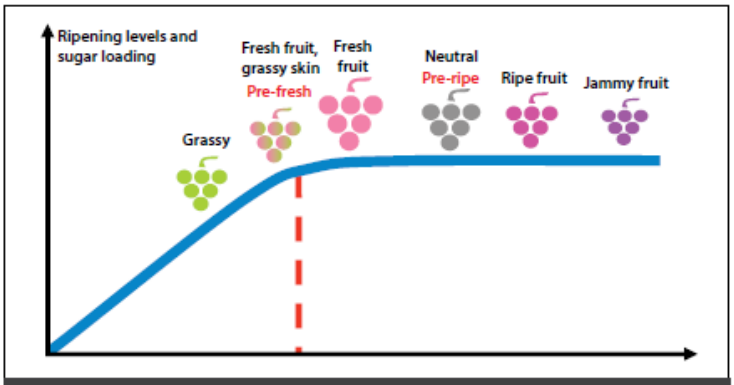


Figure 2: The berry aromatic sequence of most red cultivars is indirectly related to potential wine style (Vivelys and Deloire, 2008).

Profiles of sugar loading

It is possible to distinguish three principal sugar loading profiles:

1. Continual and rapid loading — This type of sugar loading occurs from veraison and is related to the active functioning of carbon production sources (leaves) that supply plant sinks (berries, secondary shoots etc.) during their growth phases. It is therefore often associated with significant vegetative growth and greater berry volume. Phenolic maturity is not affected. This type of loading is often considered beneficial for production of rosé, fresh fruity red wines or pleasant aromatic white wines.

2. Slow sugar loading/inhibition of ripening — Low sugar content per berry, associated with a slow loading rate, can be considered to "block" ripening, and this could be indicative of vine imbalance. If major physiological problems such as mineral deficiencies, viral diseases, etc., are excluded, blocked ripening can often be related to excessive water deficit, heat waves or an excessive crop load in relation to the exposed leaf surface.[5]

In all grape varieties, this type of situation is far from ideal in terms of standard vinification practices in white and dry red wine production. Furthermore, in relation to red and black grapes, this situation may be associated with blocked technological and phenolic maturities. Ultimately, it may be necessary to adapt the fermentation procedure to this type of grape, with thermovinification and short maceration with limited extraction being preferred options.

3. Sugar loading presents a plateau phase — Vines showing this tendency present a phase of active sugar loading in the berry (ripening), followed by a plateau representing a cessation of sugar loading (or a slowing down of sugar accumulation), and corresponding to maturity.[9] In some cases, there is a third phase corresponding to a possible decrease of the quantity of sugar per berry (over-ripening). To date a probable explanation for the occurrence of this phase has not been identified.

Theoretical berry sugar loading curves (accumulation of berry sugar content over time) are presented in Figure 1. The implications of this curve in terms of defining the finished wine are important. Depending on whether grapes are harvested in the early, mid-, or later stages of the plateau phase, the wine will be characterized by fresh fruit, neutral-spicy/pre-ripe or mature fruit flavors, respectively (Figure 2).

Berry Aromatic Sequence (BAS)

The curve in Figure 2 demonstrates that selection of a harvest date according to the quantity of sugar per berry in conjunction with other indicators (titratable acidity, malic and tartaric acids, pH, berry volume, berry tasting, tannins, anthocyanins, etc.) enables different wine styles to be produced. Hence, for a balanced red wine, complete ripeness will be achieved between one and five weeks after the cessation of sugar loading (Figure 3).

Once the plateau phase of berry sugar loading has been reached, the evolution of ripening will depend on other factors such as cultivar, bunch microclimate, leaf/fruit balance, vine water status and the climate mainly during berry ripening (maximum temperature, night-time coolness, sea breeze, wind speed, late-season rains and various factors that are quantifiable).[2,6]

It should be noted that the plateau phase in sugar loading may be reached at different sugar concentrations (Brix), depending on cultivar and environmental conditions. A red cultivar, with a very high sugar concentration (Brix) when the maturity plateau is reached, will not always be desirable for production of certain types and/or wine styles.[8]

Monitoring ripening with various indicators, coupled with appropriate analytical data measurements such as berry fresh mass or volume, Brix, sugar load-

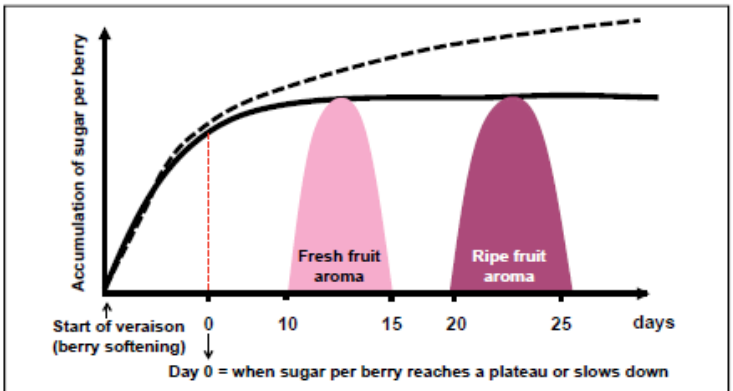


Figure 3 — Sugar loading and wine style — Example of relationships between berry sugar loading curve and potential related wine styles for Merlot. After "Day 0," three successive main periods have been determined: a fresh fruit period, a "neutral" or pre-ripe period and a mature fruit period. In terms of harvest dates, these periods have been determined according to the number of days after "Day 0," which correspond to the sugar-loading "plateau," or slowing down and not directly to a calendar date. This introduces the concept of a "physiological clock."

ing, evolution of titratable acidity, malic acid, tartaric acid, pH, color evolution, anthocyanins, tannins, berry tasting, etc.) enable decision-makers to determine the optimum harvest date, a major consideration in determining grape quality.

Such monitoring provides a greater understanding of vine morphological and physiological parameters during ripening, and therefore vineyard practices can be adapted to production objectives.

There are, in most vineyards, several potential optimal harvest dates and optimal ripening levels according to the desired wine style. The wine is therefore created in the vineyard!

As the world becomes more technologically advanced, more advanced technology is being developed to monitor berry ripening. This technology is rapidly being adopted by large estates and cooperatives to enhance their marketing edge. PWV

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1. Sugar accumulation method: instructions for the industry

Berry Sugar Accumulation and Fresh Mass Evolution How to use these indicators for sequential harvesting? Calibration for the Australian red and white varieties

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Introduction

Sugar loading can be defined as the accumulation of sugar per berry, expressed as mg per berry, from veraison onwards. Véraison corresponds to the onset of fruit maturation. In the grapevine, fruit maturation starts with an abrupt softening of the berry. This softening goes hand-in-hand with sugars being actively transported to the berry (sucrose rapidly hydrolyzed into the hexoses: glucose and fructose). In red cultivars, véraison is also characterized, after softening, by skin coloring as a result of the biosynthesis of anthocyanins.

The evolution of sugar loading per berry gives an indication of the ripening process from a new perspective. Fruit sugar accumulation may also provide information on ripening kinetics and could enable the principal phases of ripening to be distinguished. Berry fresh mass evolution can be helpful to assess the efficiency of irrigation or the tempo of berry water gain and loss.

Monitoring the accumulation of sugar per berry and the berry fresh mass may also be considered as a method of measuring plant's physiological functioning. In particular, restrictions in vine photosynthetic activity could be pinpointed either due to the water restrictions or extreme heat events (heat waves), or to an inappropriate leaf to fruit ratio (Figure 1).

Sugar loading calculation

Fruit sugar accumulation will depend on leaf stomatal conductance (photosynthesis), the fruit ability to load sugar (i.e. vine water status and bunch microclimate) and possibly the vine carbohydrates reserves.

There are numerous possible profiles of berry sugar accumulation (Figure 1).

Sugar accumulation per berry is calculated from berry fresh mass (g) and sugar concentration (Brix or Baume).

How to calculate sugar per berry?

For that purpose, follow the procedure below. When to sample? How to sample? What to measure? When to harvest?

It is recommended to start sampling from 8 ^{0}Brix onwards (or at 50% of coloured berries). Sample once a week (in other words, each 7 days). When approaching 18 ^{0}Brix, sample twice a week (i.e. every 3 days). After 22 ^{0}Brix, sample once a week.

How to sample?

For commercial vineyards:

- Sample a representative number of bunches per homogeneous block or vineyard. It is important to always use the same sampling method. Eitheir use your own sampling method or sample 1 bunch per vine at random within the reference block or selected reference vineyard. When in laboratory, remove precisely 200 berries from bunches collected. It is recommended for purposes of accuracy that protocol is repeated three times, meaning that three batches of 200 berries are sampled from the collected bunches. Berries should be removed from bunches carefully, preventing any loss of juice. Berries can be also cut off from bunches with their pedicels, however this is more time consuming and it is not compulsory on a commercial scale.
- If the vineyard is highly heterogeneous, choose a homogeneous block to sample (i.e low or high vigour, but don't mix

both). Low and high vigour vines/blocks should be analysed separately.

What to measure?

Berry fresh mass and Brix measurements

Berry fresh mass: the berry fresh mass of 200 berries (or 3 x 200 berries) has to be precisely done with the appropriate balance.

Brix: crush (in a plastic bag, or any other appropriate container) the 200 berries (or 3 x 200 berries) to measure the Brix (or Baume) of the juice, and if you wish other possible parameters (titratable acidity colour, pH).

Calculation of sugar per berry and curves of sugar accumulation

The calculation will be completed automatically once the data has been put in the appropriate excel sheet provided with this protocol.

When to send the excel sheet ?

After the first three sampling dates and after that, on a weekly basis.
Send the excel sheet to adeloire@csu.edu.au and ksuklje@csu.edu.au

Interpretation of data and curves

The first season results will help to profile the tempo of berry sugar accumulation and changes in berry fresh mass. This information will allow the manager:

- To profile a vineyard in terms of the tempo of fruit sugar accumulation and berry fresh mass evolution (fruit water gain and loss)
- To compare fruit of different vineyards and blocks
- To compare fruit of different cultivars-clones
- To carry out a vineyard diagnostic and assess possible problems such as water constraint or stress, effect of heat waves.
- Ultimately the sugar accumulation profiles could help predict the harvest dates according to the desired style (fresh and mature fruit stages, possible neutral or pre-ripe stage) (Figure 2).

It should be taken into consideration that this calculation is only an approximation of sugar accumulation into berries. Despite the calculation being an approximation, with associated shortcomings, it is nevertheless an useful physiological indicator.

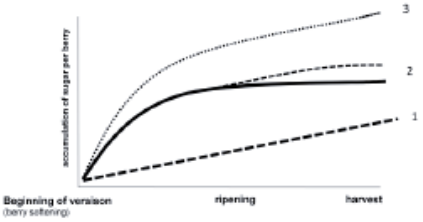


Figure 1: example of profiles of berry sugar accumulation (mg/berry). 1 Slow sugar accumulation (could indicate a water constraint); 2) profile of sugar accumulation showing a plateau or a slow down (indicates a standard ripening process); 3) a continuous berry sugar accumulation (could indicate a vigourous situation with big fruits).



Figure 2: Indirect potential relationship between fruit sugar accumulation and wine aromatic profiles for red cultivars. This underlines the potential interest of sequential harvests.

2. Protocol for white cultivars

Sugar loading, berry colour and berry aromatic sequence (BAS) in relation with the style of wine for WHITE CULTIVARS

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Introduction

It is known, that the prediction of an optimal harvest date for white wine cultivars is a determining factor influencing produced wine styles. Therefore it would be of a great importance for the wine industry to be able to predict harvest dates based on required wine styles by the market with a simple, inexpensive and rapid methods.

Prediction of harvest dates

Sugar loading can be defined as accumulation of the quantity of sugar per berry, expressed as mg per berry, from the onset of ripening (veraison) onwards. Veraison in grape vine is characterised by berry softening and afterwards by skin colouring in red cultivars. The softening of the grape berry is an indicator of active sugar transport in the berry. From the above-mentioned studies, it can be concluded that sugar loading into the berry, coupled with the dynamics of sugar concentration changes, may be considered a useful indicator of grape quality. It takes into account the evolution of the sugar amount per berry (mg per berry) and therefore enables the kinetics of sugar content changes to be monitored in interaction with berry fresh mass evolution.

Kinetic monitoring of the quantity of sugar per berry may be considered as a method of measuring the plant's physiological functioning and in particular, photosynthesis, which is a reliable indirect indicator of temperatures to which the vine is subjected under given conditions over a specific time period and grapevine water status.

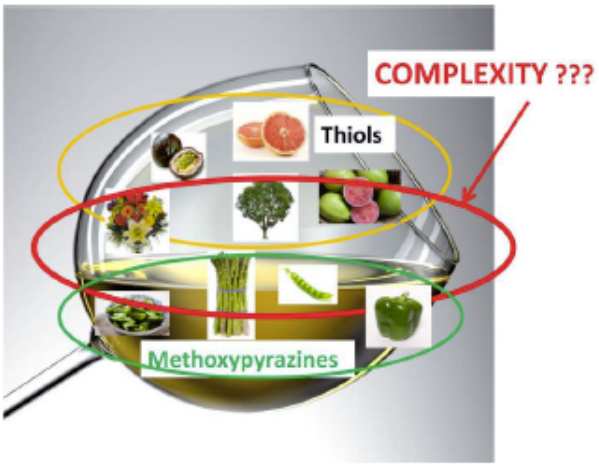


Figure 1: Contribution of aromatic compounds to aromatic expression of Sauvignon Blanc wines.

Berry colour evolution for white varieties can be an important indicator for predicting white wine style. It can be indirectly related to the aromatic profile of the wines through the complex mechanism of berry carotenoids degradation, yielding in the production of C13-norisoprenoids. The latter are known to be of a significant importance for the aroma and are important enhancers of fruity and floral notes in the wines. Furthermore, berry colour evolution can be related to the degradation of malic acid and methoxypyrazines in Sauvignon Blanc grapes. As it is known, methoxypyrazines are important group of compounds, contributing to green pepper, asparagus and grassy notes of Sauvignon Blanc wines. However, in the lower concentrations methoxypyrazines can

contribute to the typicity and complexity of Sauvignon Blanc wines, whereas they can be a negative wine descriptor when present in excessive concentrations.

In the last few years a tool to measure berry colour based on an optical technologies have been developed by Vivelys society (France) in collaboration with Montepellier SupAgro (France) and it is widely used.

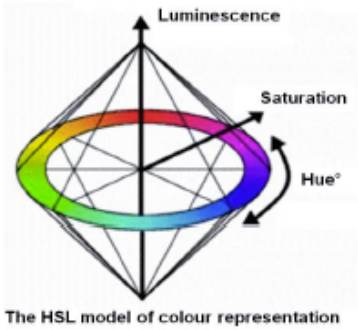


Figure 2: The Hue, Saturation and Luminescence (HSL) model of colour representation which gives hue values in degree from 0 to 360°.

This method is based on an indirect relationship between the evolution of the berry tint angle (according to the HSL model – Hue, Saturation, Luminescence; Figure 2) and the wine sensorial analysis (Figure 3), and as can be seen, has potential to be very useful for profiling berry maturation, harvest potential and the selection of the most appropriate harvest dates for white cultivars.

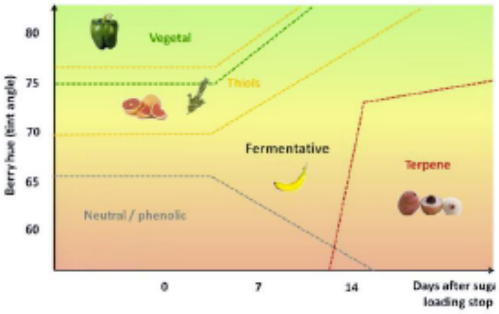


Figure 3: White cultivars berry aromatic model (from Vivelys, France). The method uses the berry colour evolution from véraison to harvest (berry hue or tint angle in degree: y-axis) and the berry sugar loading concept (x-axis). According to the evolution of the berry hue, the method allows to predict the style of wine.

How to calculate the quantity of sugar per berry and determine berry colour?

Active sugar loading is calculated on the basis of berry volume (or berry fresh mass) and sugar concentration (Brix).

When to sample?

It is recommended to start sampling from 8^{0}Brix (4.4 Baume) onwards (or when berries get soft). Sample once a week (in other words, each 7 days). When close to 18 ^{0}Brix (10 Baume), sample twice a week (i.e. every 3 days).

How to sample?

For commercial vineyards:

- Sample 10 to 15 bunches per homogeneous block or vineyard. Berry colour evolution: cut randomly 200 intact berries from the bunches with their pedicel to avoid water losses and berry oxidation what will result in incorrect measurements and wrong predictions.
  For sugar accumulation curves: 3 x 100 berries are required (berries cut for colour evolution can be further used for sugar accumulation curve). As before, berries should be cut from the bunches with pedicels to avoid water losses. If the vineyard is heterogeneous, contact adeloire@csu.edu.au or ksuklje@csu.edu.au to discuss the sampling method.
- It is recommended for the reliability of the results and mainly the first year of calibration for sugar loading model to do three replicates of 100 berries.

What to measure?

Berry colour evolution

For the berry colour measurement the use of a Dyostem tool will be required and will be provided for use in Orange. 200 intact berries have to be placed on a Dyostem plate and hue angle will be measured and recorded as instructed on a 2015 January visit. For the colour evolution, a sample of 200 berries for one sampling date is adequate and it does not have to be measured in triplicates. Based on the berry colour we will be able to predict wine styles, as shown in Table 1. However, thresholds will have to be adjusted in a first year for Australian wine growing regions.

Table 1: Thresholds of berry hue (in degree) according to the HSL model of colour representation and expected style of wine for Sauvignon Blanc (Vitis vinifera L.). In some specific climatic conditions, the thresholds need to be adjusted. The thresholds calibration is done by establishing a relationship between the berry hue angle and the style of wine (wine tasting and/or sensorial analysis).

Berry hue thresholds (in degree)	Expected wine aromatic profiles
> 90	Green/unripe
90 - 85	Green/asparagus
85 - 80	Asparagus/citrus
80 - 75	Asparagus/Tropical fruit/grapefruit/citrus
75 – 70	Tropical fruit
70 - 65	Fermentative/terpene
65 - 60	Phenolic/neutral/terpene

Berry fresh mass and ^{0}Brix measurements

Berry fresh mass: berry fresh mass of 3 x 100 berries has to be precisely measured with the appropriate balance.

Brix: crush (in a beaker or in a plastic bag) 3 x 100 berries to measure the Brix level from the must, and other parameters if needed (TA, pH).

Calculation of sugar per berry and curves of sugar accumulation

The calculation will be done once the data has been sent to the following email address: adeloire@csu.edu.au;
An appropriate excel table will be provided to help with saving the data and following berry sugar accumulation in real time.

Interpretation of data and curves: when to harvest?

A new model has been developed over the past years which use the following parameters to predict the harvest date according to the desired style of wine: 1) Berry fresh mass, 2) sugar per berry, 3) Brix, 4) sugar per berry per day, 5) berry colour evolution.

For prediction of harvest dates for Sauvignon Blanc, grape **METHOXYPYRAZINES analyses will be included into the protocol**. These analyses, which will be performed over the ripening period, could provide grape growers and wine makers with valuable information on grape aromatic profile and consequently wine style. How to sample and how to get grapes in Wagga should be discussed individually with each grape grower.

The results should allow the prediction of the harvest dates and to organise the harvest and the cellar.

The results will allow checking vine/vineyard balance and thereby could aid in making any necessary decisions during the season.

10 Appendix 2: Intellectual property

All IP resulting from this project has been, or will be published, and is in the public domain.

11 Appendix 3: References

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13 Appendix 5: Raw Data

Data presented in this report are available from the authors upon request. These data have been withheld from the Final Report pending the preparation of a manuscript for publication.