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Grapes to wine: the nexus between berry ripening, composition and wine style

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Abstract
    Grape ripening is a process driven by the interactions between grapevine genotype and environmental factors. Grape composition is largely responsible for the production and final concentrations of most wine aroma compounds even though many compounds in wines (aromatic and non-aromatic) are substantially transformed during the complex process of fermentation and wine ageing. The aim of this study was to investigate if a common pattern in grape/wine flavour plasticity related to ripening exists irrespective of a grape growing region. A further aim was to identify and highlight compounds present in grapes and wines in which plasticity is directly related to grape ripening and is consistent over several vintages. Irrespective of the region, a clear separation of samples was noted according to the harvest stage based on the grape and wine chemical analyses and wine sensory description. Shiraz wines from the first harvest (H1) were associated with red fruit descriptors and higher acidity. Wines from the third harvest (H3) were correlated with dark fruit characters and a higher perception of alcohol. Later harvest dates resulted in higher concentrations of some amino acids in grapes, higher alcohol acetates and dimethyl sulphide in wines, whereas concentrations of *Z*-3-hexenol, ethyl isobutyrate and ethyl leucate were lower in these wines compared to earlier harvest dates. Trends described were significant and consistent across two distinct regions and two vintages. Irrespective of the environment, this study demonstrated that for Shiraz, a common evolution of grape flavours exists, influencing the final wine chemical and sensory properties. Furthermore, during the late ripening stage, no direct nexus was observed between sugar concentration and grape and wine flavour evolution.

INTRODUCTION
    Wine flavour is intrinsically linked to grape berry composition which undergoes extensive modification during the fermentation and wine making processes. Linking grape berry development, berry composition, vineyard management and abiotic factors to wine sensory attributes is the holy grail of grape and wine research. Climatic impact upon vine growth and berry development is recognised as one of the most import drivers of grape composition. Defining predicable models of berry composition as a function of berry ripening must therefore be robust and account for climatic variations. Meso and micro climate and the associated abiotic factors are considered to be important drivers of vine physiology, berry growth and composition (Deloire et al. 2004; Tonietto and Carbonneau 2004). A recent study from Rienth et al. (2014) reported that changes in berry gene expression during the night differed from daytime expression. This emphasises the importance of measuring both day and night temperatures over the entire growth period when investigating the effect of climate on fruit metabolism and vine physiology. Dal Santo et al. (2013) reported that approximately 18% of the average number of modulated genes in Corvina were related to grapevine plasticity, suggesting that the ripening can be modified extensively by the growing conditions. A subsequent study reported clear existence of metabolome and transcriptome related terroir effects, which were constant over several vintages (Anesi et al. 2015). In particular, some stilbenes, anthocyanins and flavonoids could

be correlated to the regional Corvina characteristics (Anesi et al. 2015). Additionally, water restrictions modified gene regulation of the terpene and phenylpropanoid pathways and resulted in a modified metabolome (Savoi et al. 2016). The impact of agronomic practices that modify bunch microclimate also appears to have a considerable effect on fruit and wine composition which subsequently influences the final wine sensory profile (K. Šuklje et al. 2014; Šuklje et al. 2012; Young et al. 2015).

Grape berry ripening is characterised by rapid and significant metabolic changes and gene regulation modulations at the onset of veraison, beginning with berry softening and the import of hexoses. 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. 2007). During the ripening of Cabernet Sauvignon, an increased Brix correlated with changes in the abundance of more than 18000 transcripts, with the majority of changes occurring in the grape skins (Cramer et al. 2014). Importantly, transcripts of several genes involved in isoprenoid and phenylpropanoid synthesis were significantly altered in Cabernet Sauvignon grapes during maturation (Cramer et al. 2014). These observations correspond to the grape and wine metabolic rearrangements during late ripening (Antalick et al. 2015; Bindon et al. 2013; Šuklje et al. 2014). Bindon et al. (2013) observed increase in total grape anthocyanins and lower tannins in Cabernet Sauvignon grapes harvested sequentially from 20.3-26.0°Brix. Wines harvested at higher °Brix had higher colour related parameters and lower polysaccharides (Bindon et al. 2013). A study by Šuklje et al. (2014) demonstrated a clear influence of harvest date on Shiraz grape and wine composition. Several amino acids increased with delayed harvest date, whereas wine C6 compounds were found in lower concentrations when grapes were picked later, corresponding with observations of (Antalick et al. 2015). Sensorial evaluation revealed that wines from earlier harvests were associated with red fruit sensory attributes whereas wines from later harvest dates were found to be higher in the perception of the dark, stewed fruit and alcohol perception (Šuklje et al. 2014).

Harvest decision is determined by a range of objective measures of grape maturity (e.g. °Brix, titratable acidity and colour), however these indices provide no information about the grape aromatic potential or the resulting wine flavour profiles (Calderon-Orellana et al. 2014; Deloire 2013). The aims of this study, across two Australian regions (Riverina and Orange), were to determine: i) if a common pattern in grape/wine flavour plasticity related to grape ripening exists irrespective of a grape growing region, ii) if commonalities in flavour evolution exists across the vintages and iii) to identify and highlight compounds present in grapes and wines which are directly related to grape ripening.

MATERIALS AND METHODS

The experiment was conducted in 2014 and 2015 in NSW, Australia in two distinctively different grape growing regions according to Huglin index. Griffith (G) is classified as warm to hot grape growing region, according to Huglin index, whereas Orange (O) is a temperate to temperate-warm. Griffith is characterised by a flat terrain and secure water supply, enabling it to maintain a 15% share in the total Australian grape production. In contrast, Orange is a young (from the 1980's onward) grape growing region orientated towards premium wine production with elevations spanning from 600m up to 1000m above sea level. Two commercial Shiraz vineyards were selected in Griffith in season 2014, whereas in 2015 two additional Shiraz vineyards were selected; G1, G2, G3 and G4, respectively. Two Shiraz vineyards were also selected in O for experimentations in 2015 season; O1 and O2, respectively. Shiraz vines were own rooted, grown under drip irrigation, and trellised to a sprawling system in Griffith. In Orange vines were trellised to vertical shoot positioning. During the season mesoclimatic temperatures, stem water potential and soil moisture were monitored in an attempt to characterise experimental plots. Harvest dates for vineyards were determined at the point where sugar accumulation per berry and

berry fresh mass reached a plateau or slowed down, 12, 18 or 24 days in advance of the first harvest (H1), second harvest (H2) and third harvest (H3) stages respectively, as summarised by Figure 1 (Deloire 2013). At each harvest date, 100 berry grape samples were collected and immediately frozen in liquid nitrogen in the field. Approximately 60 kg of grapes per replicate were randomly harvested at each harvest date and small scale vinifications were carried out. Vineyard characteristics and harvest summary details are presented in Table 1.

Table 1. Vineyard and harvest characteristics.

	Griffith	Orange
Pruning	Mechanical	Manual
Number of shoots	92	21
Trellis and canopy type	Double cordon, Open sprawling canopy	Double cordon vertical shoot positioning
Yield/vine	18.6 kg	4.3 kg
Flowering	15th October to 1st November	15thNovember to 1st December

Temperature
    annual mean min
    annual mean max
	10.6°C
	25.6 °C
		6.4°C
		18.5 °C

Rainfall (annual mean)	390 mm	665 mm
Maturation duration post berry sugar accumulation plateau (days)		
2014 – H1 – H2 – H3	11 24-25	
2015 – H1 – H2 – H3	12 17-19 24	11 17-18 24-25

Both grape berries and wines were analysed using a range of analytical measurement techniques and sensory evaluation was performed on the finished wines. Amino acids in grapes were analysed by high performance liquid chromatography (HPLC) coupled to a fluorescence detector according to the method of Hynes et al. (1991). Grape anthocyanins were analysed by HPLC coupled to diode array detector (DAD) (Downey and Rochfort 2008). Grape organic acids and sugars were analysed according to the method published by Eyéghé-Bickong et al. (2012). Grape volatiles analyses were performed with gas chromatography coupled to mass detection (GC-MS) as described by Loscos et al. (2009). Juice was analysed for set of parameters (total soluble solids, titratable acidity and pH) relating to the technical maturity of grapes and yeast assimilable nitrogen was also

measured and standardised. Wine volatiles were measured by GC-MS using previously developed methods (Antalick et al. 2010; Šuklje et al. 2016). Sulphur compounds were analysed by GC coupled to a sulphur chemiluminescence detector as described by Siebert et al. (2010). Descriptive sensory analysis (DA) was conducted nine months after fermentation, using the same descriptors as obtained for the DA performed in 2014. Panellists, consisted of NWGIC/CSU employees, rated each of the attributes on 0 to 9 scale (0 = low; 9 = high). Each wine was evaluated in triplicate over a three day period. A range of multiblock multivariant data analyses were conducted using the AComDim method (Bouveresse et al. 2011) by creating balanced ANOVA models based upon subsampling of the full data set. All multivariate analysis was conducted in Matlab version 7.14.0.739 (The Mathworks, Natick, MA, U.S.A.). ANOVA was conducted in Statistica (StatSoft, Tulsa, OK, U.S.A.), and the means were separated using Stats-Fisher's LSD test (different letters account for significant differences at $p \leq 0.05$).
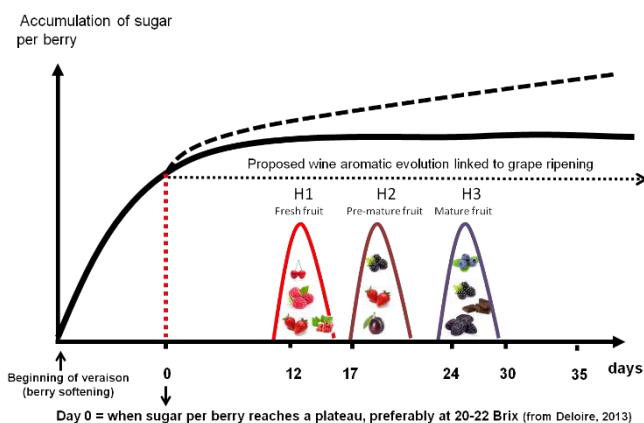

Figure 1: Example of Shiraz ripening evolution, using sequential harvest. Fresh fruit (H1) and mature fruit (H2) are reached 12 and 24 days respectively after sugar per berry accumulation stops or slows down.

RESULTS

Wine sensory evaluation revealed clear differences in wine style according to harvest (figure 2) with sensory attribute ratings consistently rated in terms of differences for vineyard site and harvest time. Figure 2 illustrates sensory ratings for the Griffith and Orange vineyard (vineyard 2). Other vineyards for each location were rated similarly (data not shown). A clear sensory profile of higher acidity, red fruit and lower perception of alcohol is evident for early harvested fruit. Attributes of dark fruit, astringency and higher perceived alcohol are features of the later harvested wines.
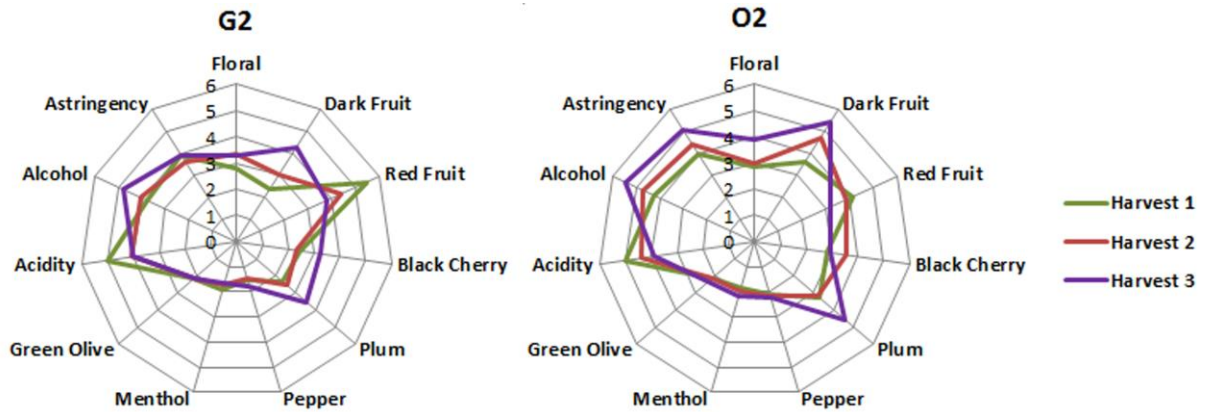
Figure 2. Wine sensory features for different harvest times for Griffith and Orange vineyards.

A common clustering of samples according to each ANOVA explanatory factor with clearly separate confidence intervals is readily apparent for region (Figure 3, Griffith versus Orange) and harvest time (Figure 4, Harvest 1, 2 & 3). Common pattern in grape/wine flavour plasticity related to grape ripening exists irrespective of a grape growing region-chemical approach
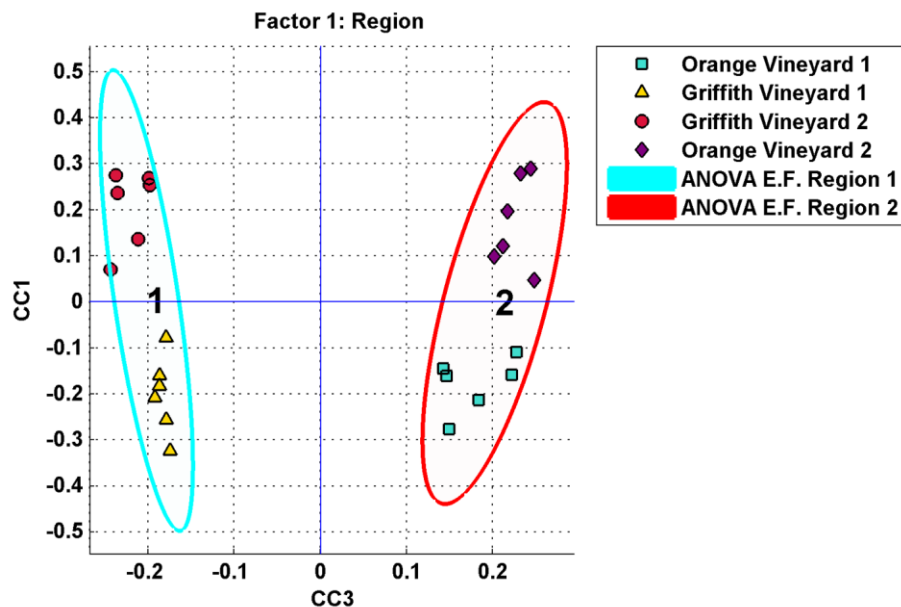

Figure 3. ACOMDIM model of grape berry composition, wine sensory and wine composition with samples coded for vineyard for the 2014 and 2015 growing season. Ellipses represent the 95% confidence interval for each ANOVA explanatory factor (1: Region 1; 2: Region 2).
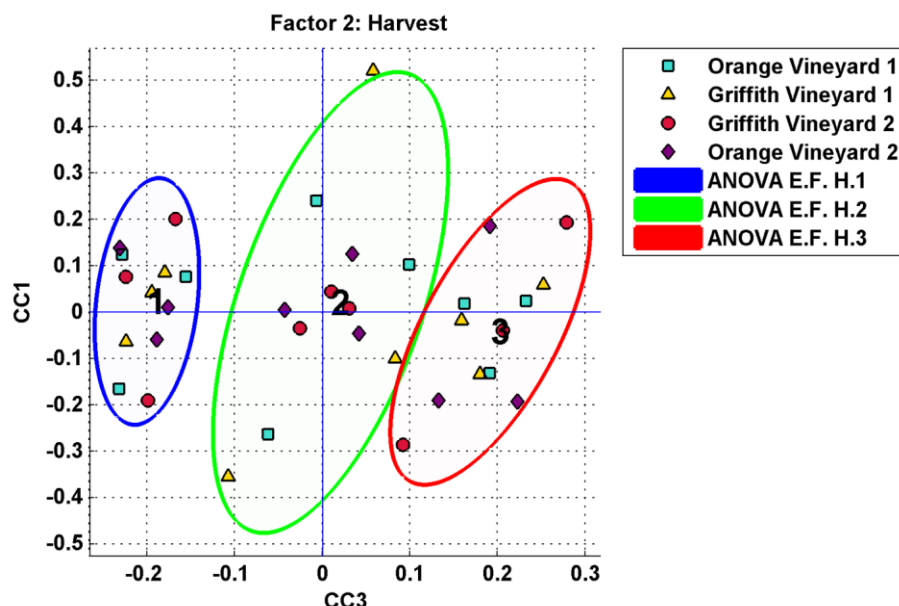
Figure 4: ACOMDIM analyses conducted on grape berry, juice, wine and wine sensory results for the 2015 growing season. A: Separation of samples according to the harvest date for Shiraz vineyards in Orange and Griffith. 1, 2, 3 refers to the harvest date 1,2 and 3, respectively. Ellipses represent 95% confidence interval for each ANOVA explanatory factor.

Regional and vineyard influences on wine style are important factors for winegrowers seeking to differentiate products based upon unique terroirs. Identification of specific grape berry composition due to site is challenging and requires a longitudinal study over several growing seasons. In this preliminary investigation a clear separation of samples according to both region and vineyard is evident (Figure 3). Grape components that influenced sample separation were berry amino acids; generally higher in Griffith samples; berry monomeric anthocyanins (higher in Orange samples); wine acetate esters higher and terpenes lower in Griffith wines.

Irrespective of the grape growing region, samples could be clearly grouped according to the harvest date, Figure 4. Unsurprisingly, concentrations of glucose and fructose increased with delayed harvest dates, which resulted in wines with higher alcohol content. Other compounds contributing to the separation of samples were some amino acids (proline, γ-aminobutyric acid, isoleucine) which were at higher concentrations in the H3 grapes compared to grapes from H1. Some higher alcohol acetates as well as dimethyl sulfide were higher in wines from H3 while Z-3-hexenol, ethyl isobutyrate and ethyl leucate were lower in these wines. Z-3-hexenol was the only C6-alcohol, a group of compounds known to contribute to green herbaceous notes of wines made from early harvest (Kalua and Boss 2009), to be affected by grape maturity in this study. Dimethyl sulfide is known to contribute to dark fruit character in red wine (Lytra et al. 2016) and has been previously reported as marker of wines made with late maturity grapes (Dagan 2006). Interestingly, even though esters are principally produced by yeast during fermentation, final wine concentrations were influenced by grape maturity as recently reported (Antalick et al. 2015).

CONCLUSIONS
The application of metabolomics data analysis to vineyard, berry and wine compositional measures derived from a spatial and temporal study has enabled influence of region, vineyard site and harvest date upon wine style to be determined. Wine style sensory features can be defined in terms of a grape berry sugar accumulation model linked to a plateau of berry sugar accumulation. This allows a wine style based upon the aromatic flux associated with berry ripening periods to be predicted.

LITERATURE CITED

Anesi, A., et al. (2015) Towards a scientific interpretation of the terroir concept: plasticity of the grape berry metabolome. BMC Plant Biology 15 (1):1-17.

Antalick, G.; Perello, M.-C. and De Revel, G. (2010) Development, validation and application of a specific method for the quantitative determination of wine esters by headspace-solid-phase microextraction-gas chromatography–mass spectrometry. Food Chemistry 121 (4):1236-1245.

Antalick, G.; Šuklje, K.; Blackman, J.W.; Meeks, C.; Deloire, A. and Schmidtke, L.M. (2015) Influence of grape composition on red wine ester profile: Comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. Journal of Agricultural and Food Chemistry 63 (18):4664-4672.

Bindon, K.; Varela, C.; Kennedy, J.; Holt, H. and Herderich, M. (2013) Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. Food Chemistry 138 (2–3):1696-1705.

Bouveresse, J.-R.D.; Pinto, R.C.; Schmidtke, L.M.; Locquet, N. and Rutledge, D.N. (2011) Identification of significant factors by an extension of ANOVA-PCA based on multi-block analysis. Chemometrics and Intelligent Laboratory Systems 106 (2):173-182.

Calderon-Orellana, A.; Matthews, M.A.; Drayton, W.M. and Shackel, K.A. (2014) Uniformity of ripeness and size in cabernet sauvignon berries from vineyards with contrasting crop price. American Journal of Enology and Viticulture 65 (1):81-88.

Cramer, G.; Ghan, R.; Schlauch, K.; Tillett, R.; Heymann, H.; Ferrarini, A.; Delledonne, M.; Zenoni, S.; Fasoli, M. and Pezzotti, M. (2014) Transcriptomic analysis of the late stages of grapevine (Vitis vinifera cv. Cabernet Sauvignon) berry ripening reveals significant induction of ethylene signaling and flavor pathways in the skin. BMC Plant Biology 14 (1):370.

Dagan, L. (2006) Potentiel aromatique des raisins de Vitis vinifera L. cv. Petit Manseng et Gros Manseng. Contribution à l'arôme des vins de pays Côtes de Gascogne. Thesis, Ecole Nationale Superieure Agronomique de Montpellier.

Dal Santo, S.; Tornielli, G.B.; Zenoni, S.; Fasoli, M.; Farina, L.; Anesi, A.; Guzzo, F.; Delledonne, M. and Pezzotti, M. (2013) The plasticity of the grapevine berry transcriptome. Genome Biology 14 (6):1-18.

Deloire, A. (2013) Physiological indicators to predict harvest date and wine style. 15th Australian Wine Industry Technical Conference, Sydney, New South Wales, 13-18 July 47-50.

Deloire, A.; Carbonneau, A.; Wang, Z. and Ojeda, H. (2004) Vine and water a short review. Journal International des Sciences de la Vigne et du Vin 38 (1):1-13.

Downey, M.O. and Rochfort, S. (2008) Simultaneous separation by reversed-phase high-performance liquid chromatography and mass spectral identification of anthocyanins and flavonols in Shiraz grape skin. Journal of Chromatography A 1201 (1):43-47.

Eyéghé-Bickong, H.A.; Alexandersson, E.O.; Gouws, L.M.; Young, P.R. and Vivier, M.A. (2012) Optimisation of an HPLC method for the simultaneous quantification of the major sugars and organic acids in grapevine berries. Journal of Chromatography B 885–886 (0):43-49.

Hynes, P.; Sheumack, D.; Greig, L.; Kibby, J. and Redmond, J. (1991) Application of automated of amino acid analysis using 9-fluoroenylmethyl chloroformate. Journal of Chromatography A 588 107-114.

Loscos, N.; Hernández-Orte, P.; Cacho, J. and Ferreira, V. (2009) Comparison of the suitability of different hydrolytic strategies to predict aroma potential of different grape varieties. Journal of Agricultural and Food Chemistry 57 (6):2468-2480.

Pilati, S.; Perazzolli, M.; Malossini, A.; Cestaro, A.; Dematte, L.; Fontana, P.; Dal Ri, A.; Viola, R.; Velasco, R. and Moser, C. (2007) Genome-wide transcriptional analysis of grapevine berry ripening reveals a set of genes similarly modulated during three seasons and the occurrence of an oxidative burst at veraison. BMC Genomics 8.

Rienth, M.; Torregrosa, L.; Kelly, M.T.; Luchaire, N.; Pellegrino, A.; Grimplet, J. and Romieu, C. (2014) Is transcriptomic regulation of berry development more important at night than during the day? PLoS ONE 9 (2):e88844.

Savoi, S.; Wong, D.C.J.; Arapitsas, P.; Miculan, M.; Bucchetti, B.; Peterlunger, E.; Fait, A.; Mattivi, F. and Castellarin, S.D. (2016) Transcriptome and metabolite profiling reveals that prolonged drought modulates the

phenylpropanoid and terpenoid pathway in white grapes (Vitis vinifera L.). BMC Plant Biology 16 (1):1-17.

Siebert, T.E.; Solomon, M.R.; Pollnitz, A.P. and Jeffery, D.W. (2010) Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection. Journal of Agricultural and Food Chemistry 58 (17):9454-9462.

Šuklje; Antalick, G.; Meeks, C.B.; Blackman, J.W.; Deloire, A. and 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 ? 2nd International Symposium Oenoviti International network 3-5 November 2014 Gesenheim, Germany 44-49.

Šuklje, K.; Antalick, G.; Coetzee, Z.; Schmidtke, L.M.; Baša Česnik, H.; Brandt, J.; Du Toit, W.J.; Lisjak, K. and Deloire, A. (2014) Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of *Vitis vinifera* L. cv. Sauvignon Blanc wine. Australian Journal of Grape and Wine Research 20 (2):223-233.

Šuklje, K.; Lisjak, K.; Baša Česnik, H.; Janeš, L.; Du Toit, W.; Coetzee, Z.; Vanzo, A. and Deloire, A. (2012) Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon blanc (*Vitis vinifera* L.). Journal of Agricultural and Food Chemistry 60 (37):9454-9461.

Šuklje, K.; Zhang, X.; Antalick, G.; Clark, A.C.; Deloire, A. and Schmidtke, L.M. (2016) Berry shriveling significantly alters Shiraz (*Vitis vinifera* L.) grape and wine chemical composition. Journal of Agricultural and Food Chemistry 64 (4):870-880.

Tonietto, J. and Carbonneau, A. (2004) A multicriteria climatic classification system for grape-growing regions worldwide. Agricultural and Forest Meteorology 124 (1–2):81-97.

Young, P.; Eyeghe-Bickong, H.A.; Du Plessis, K.; Alexandersson, E.; Jacobson, D.A.; Coetzee, Z.A.; Deloire, A. and Vivier, M.A. (2015) Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. Plant Physiology.