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?*

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Abstract: Linking wine composition to fruit composition is difficult due to the numerous biochemical pathways and substrate transformations that occur during fermentation. Grape composition regulates the production and final concentrations of most wine aroma compounds, exemplified by methoxypyrazine concentrations in wine being able to be confidently predicted from the corresponding grape concentration. However, the final concentrations of many compounds in wines (aromatic and non-aromatic) are substantially dependent on the winemaking process. To better understand grape aromatic potential and aromatic evolution in relation to corresponding wine composition and wine styles, including autochthonous varieties, sequential harvest was utilised. Important differences in both grape and wine composition as well in wine sensory characteristics were noted between different harvest stages. Early stages (Fresh Fruit) were associated with red fruit descriptors and were perceived as more herbaceous. Later harvest stages (mature and jammy fruit stage) were correlated to dark and stewed fruit attributes with a higher perception of roundness. By using a suite of compositional measures targeted to important berry and wine constituents and relating these to abiotic factors (site) and cultural practices, the relationships between vine performance and final wine style should be able to be predicted with increased certainty.

Keywords: fruit and wine composition, wine sensory profile, sequential harvest

Introduction

Complex and poorly understood processes occurring in the grapevine during berry growth and development (i.e. from flowering to veraison and veraison to ripening-harvest) contribute to final fruit composition. Berry development follows a double sigmoid growth pattern consisting of two distinct growth phases separated by a lag phase (Coombe, 1992). Important compounds such as tannins and organic acids, as well as those responsible for aroma, for example methoxypyrazines in cultivars including Sauvignon Blanc, Cabernet-Sauvignon and Merlot (Kennedy *et al.,* 2001; Ollat *et al.,* 2002; Šuklje *et al.,* 2012), accumulate during the first growth period. Berry growth and organic acid accumulation cease during the lag phase. 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). Dal Santo *et al.* (2013) reported a similar effect on Corvina in 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 of 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*". A recent study from Rienth *et al.* (2014) showed changes in berry gene expression during night differed from daytime expression. This emphasises the importance of measuring of both day and night temperatures over the entire growth period when investigating the effect of climate on fruit metabolism and vine physiology. The impact of agronomic practices and environmental conditions also appears to have an important effect during ripening. Šuklje *et al.* (2012, 2014) demonstrated that canopy manipulation affecting bunch microclimate

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had a considerable effect on fruit and wine composition which in turn influenced the final wine sensory profile.

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

- determination of total soluble solids expressed as Brix as the sole criteria. This approach requires simple, routine analysis and is one of the most commonly used indicators of vineyard maturity levels practiced in the wine industry;
- assessment of berry taste. This approach is relevant but highly subjective as the decision is influenced by the taster's personal experience and training;
- consideration of a range of grape physiological indicators with appropriate analysis methods. This implies that the necessary equipment is available at the estate, or an appropriate laboratory nearby. Knowledge in interpreting analytical results to take the appropriate decision is subsequently required. The cost per analysis and per hectare must be considered.
- 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.

This is not an exhaustive list. In addition it is important that skills and information are transferred to the individuals who are using these methods to determine the harvest date. Such skills include an appropriate sampling procedure, use of analytical equipment and ability to interpret the analytical data.

Even when the harvest decision is determined by a range of objective measures of grape maturity (e.g. Brix, titratable acidity and colour), these indices give no information about the grape aromatic potential or the resulting wine flavour profiles (Deloire *et al.,* 2013, Calderon-Orellana *et al.,* 2014).

While wine styles can be shaped by winemaking procedures, the original grape composition is highly influential. Grape composition is largely determined by several vineyard parameters and abiotic factors such as soil moisture, light and temperature, particularly at the meso and microclimatic levels. A specific wine style or category demands specific ripening conditions (Bindon *et al.,* 2013, Deloire, 2013, Šuklje *et al.,* 2014). Numerous works have recently studied the relationship between grape, wine composition and aromatic profile (Ristic *et al.,* 2007; Sweetman *et al.,* 2012; Capone *et al.,* 2012). Other studies (Pineau *et al.,* 2009; Lytra *et al.,* 2012, Pons *et al.,* 2013; Bindon *et al.,* 2014) 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, as several compounds present in grapes (eg. anthocyanins, tannins) are only partially extracted into wines during the fermentation and others (eg. thiols, terpenes) are not completely released from their non-volatile grape precursor form by yeast activity. In addition, the berry nitrogen and lipid status impacts the synthesis of some compounds such as esters by yeast at the transcriptomic level. Volatiles can also be a result of

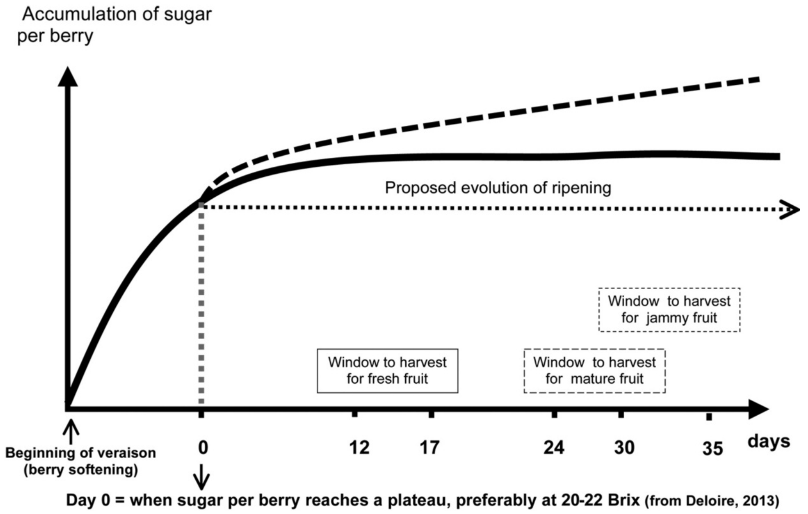

Figure 1 - Example of Shiraz ripening evolution, using sequential harvest. Fresh fruit, mature fruit and jammy fruit are reached 12, 24 and 30-35 days respectively after sugar per berry accumulation stops or slows down.

yeast and bacterial metabolism of grape derived substrates, while other compounds such as sotolon and whisky-lactone are formed during ageing and oak contact. Important interactions between different volatiles in wines do occur, either suppressing or enhancing specific sensory attributes. Significant interactions between wine volatile and non-volatile matrices also take place in the complex wine matrix and this also influence wine sensory perception. Therefore, sequential harvest can be used as a tool to better understand grape aromatic potential and aromatic evolution in relation to corresponding wine composition and wine styles, including autochthonous varieties.

Materials and Methods

Two commercial Shiraz vineyards were chosen in Griffith (Australian warm to hot climate) and designated as Vineyard A and Vineyard B. Own rooted vines were grown under drip irrigation, and trellised to a sprawling system. During the season mesoclimatic temperatures, stem water potential and soil moisture were monitored in an attempt to characterise experimental plots. Harvest dates for both vineyards were predicted at the point where sugar accumulation per berry and berry fresh mass reached a plateau or slowed down, 12 or 24 days in advance for fresh fruit (FF) and mature fruit (MF) stage respectively (Deloire, 2013). In vineyard B, a third sequential harvest was performed 34 days after the plateau of sugar accumulation per berry, and was named as the jammy fruit stage (JF). At each harvest date, 100 berries grape samples were collected and immediately frozen in liquid nitrogen in the field. Approximately 60 kg of grapes per replicate were

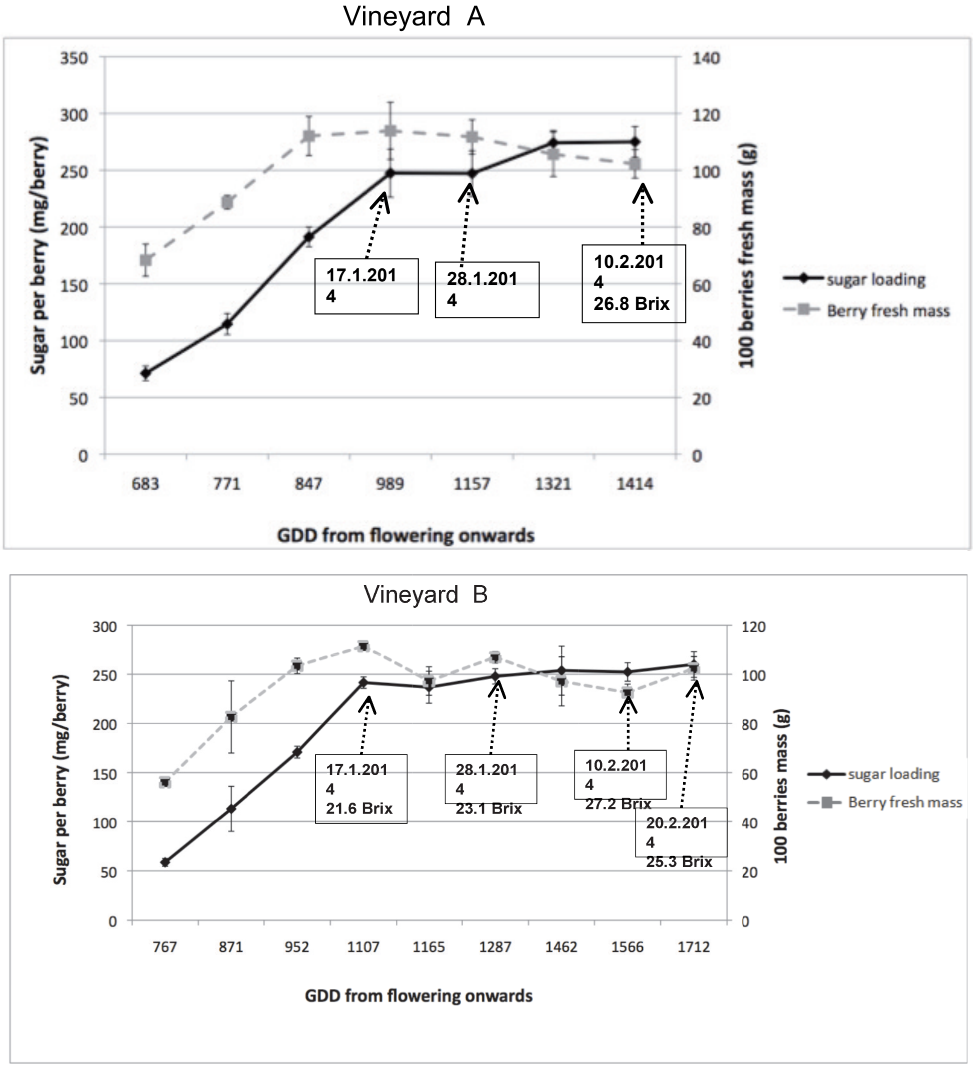

Figure 2 - Sugar loading curves and 100 berries fresh mass evolution from the onset of veraison for both experiment vineyards, respectively A and B.

Arrows indicate the date of plateau of sugar accumulation per berry, harvest dates and corresponding °Brix levels. The error bars represent standard deviation of 3 biological replicates.

Table 1. Mean ratings for sensory attributes in wines made from grapes harvested at different predicted harvest maturity levels (FF: Fresh Fruit; MF: Mature Fruit; JF: Jammy Fruit) at two vineyard sites, A and B respectively. Different letters across the rows indicate significant differences (p<0.05).

	Vineyard Site and Harvest Maturity Level	Vineyard Site and Harvest Maturity Level	Vineyard Site and Harvest Maturity Level	Vineyard Site and Harvest Maturity Level	Vineyard Site and Harvest Maturity Level
Sensory Attribute	A FF	A MF	B FF	B MF	B JF
Red Fruit	5.46 a	4.11 b	5.19 a	3.87 bc	3.00 c
Dark Fruit	4.11 cd	6.07 a	3.61 d	5.23 b	4.82 bc
Stewed Fruit	2.97 b	4.52 a	2.49 b	4.28 a	4.76 a
Green / Herbaceous	3.71 a	2.56 b	4.12 a	2.48 b	2.59 b
Spicy/Peppery	3.08 a	3.74 a	3.34 a	3.68 a	3.59 a
Fullness / Roundness	3.38 b	5.92 a	3.23 b	5.77 a	5.57 a

randomly harvested at each harvest date and small scale vinifications were carried out.

Ground grape berries and wines were analysed for sets of analytical measures and sensory evaluation was perform on finished wines. Amino acids in grapes were analysed by high performance liquid chromatography (HPLC) coupled to fluorescence detector according to the method of Heynes *et al.* (1991). Grape volatiles analyses were performed with gas chromatography coupled to mass detection (GC-MS) according to the method of Loscos *et al.* (2001). Juice was analysed for set of parameters relating to the technical maturity of grapes (total soluble solids, titratable acidity and pH) and yeast assimilable nitrogen was measured. Ester concentrations in wines were measured by GC-MS according to the method of Antalick *et al.* (2010). Descriptive sensory evaluation with predefined descriptors of wines was conducted three months after fermentation. Preliminary sensory results were obtained by 10 NWGIC/CSU employees experienced in wine sensory evaluation, rating each of the 6 attributes shown on Table 1 and Figure 3 on 0 to 9 scale (0 = low; 9 = high). Each wine was evaluated in triplicate by each panellist over a three day period.

Preliminary results

Sugar loading curves were calculated for both experimental plots. Figure 2 shows sugar accumulating per berry. Plateaus of sugar and berry fresh mass were reached as per the model curve (Figure 1), meaning that sugar accumulation per berry was not affected by the heat waves and by deficit irrigation applied prior to veraison in studied vineyards.

The herbaceous and red fruit characters in both FF treatments were significantly higher than in the wines picked at later dates (MF and JF stages). Whilst these later harvest wines shared many similarities (rating at similar intensities for spicy/peppery, fullness/roundness, green/herbaceous and stewed fruit) there were some subtle differences. The Vineyard A MF had the highest rating in dark fruit character, and was found to be significantly more intense than both the Vineyard B MF and JF stages. The Vineyard B JF wine, perhaps unsurprisingly, was found to have a significantly lower rating for red fruit character.

Measures of grape berry composition (terpenes, C13-norisoprenoids, amino acids), wine making inputs, wine sensory attributes and wine volatile composition were conducted and these combined to statistically determine the relationships between berry and wine composition along with alignment to wine sensory attributes. This approach is termed Common Components and Specific Weights analysis and had previously been used to relate several blocks of data to wine sensory features (Šuklje *et al.*, 2014). CCSW defines the common space and block weighting for the relative importance of multiple blocks of data in the same sample set for each common dimension. The salience of each data block for each extracted common dimension is shown on Figure 3. The amount of explained variance of the data set for each

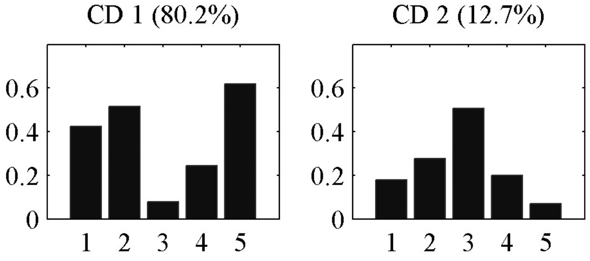

Figure 3 - Common Components and Specific Weights (CCSW) analysis block saliences.
Each data block is given a score of contribution or importance (salience) to each of the common dimensions. Wine making inputs (block index 1), berry volatiles (block index 2), berry amino acids (block index 3), wine volatiles (block index 4) and wine sensory (block index 5).

dimension is also shown and nearly 93% of data variance is explained using 2 common dimensions.

Clear separate grouping of wines made from grapes from FF versus MF and JF stages is evident (CD1). Also evident from these analysis are vineyard influences demonstrated by the separate grouping of samples along CD2. Briefly, earlier harvest dates were related to higher red fruit notes (Figure 4F), lower total soluble solids and higher acidity (Figure 4B), α-terpinene, α-ionone, β-ionone, terpinolene, and some amino acids measured in grape berries (aspargine, alanine and glutamine) (Figure 4C, D) hexyl alcohols and hexyl esters in wines (Figure 4E). Later harvest stages (24 and 34 days after plateau) were correlated to dark and stewed fruit attributes and higher perception of roundness (Figure 4F), higher total soluble solids (Figure 4B), linalool, α-terpineol, trans-linalool oxide, guaiacol, proline and branched amino acids analysed in grape berries (Figure 4C, D), dimethyl sulphide, hexyl acetate, phenyl ethyl acetate, γ-nonalactone and other esters quantified in wines (Figure 4E).

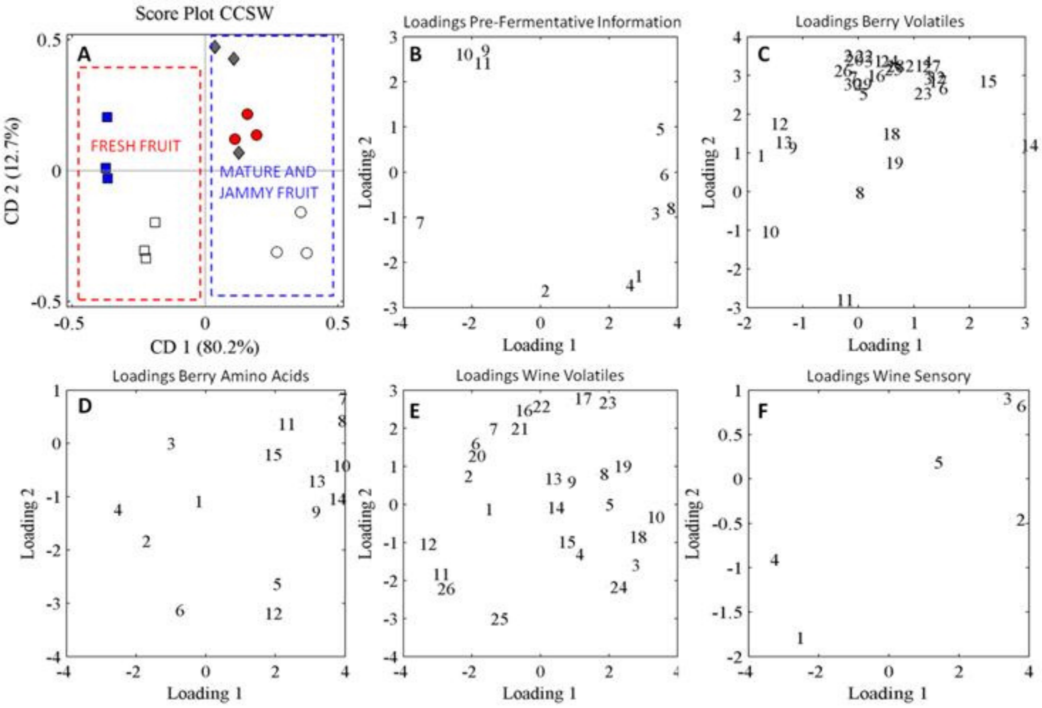

Figure 4 - Common Components and Specific Weights Analysis scores and loadings plots of grape and wine chemical and wine sensory data.

A: Scores of common dimensions 1 and 2 for wine samples from grapes harvested at different times and two vineyards; Vineyard A_FF harvested at fresh fruit stage, 12 days after plateau of sugar accumulation per berry (□);Vineyard A_MF harvested at mature fruit stage, 24 days after plateau of sugar accumulation per berry (○);Vineyard B_FF harvested at fresh fruit stage, 12 days after plateau of sugar accumulation per berry (■);Vineyard B_MF harvested at mature fruit stage, 24 days after plateau of sugar accumulation per berry(●) and Vineyard B_JF harvested at jammy fruit stage, 34 days after plateau of sugar accumulation per berry (¨).

B: loadings plot for pre-fermentative operations and juice and additions before fermentation (numbers refer to closest sensory attribute).

1: Nitrogen Ortho-phtalaldehyde (NOPA); 2: Ammonia; 3: Ammonia:yeast assimilable nitrogen (YAN); 4: YAN; 5: total soluble solids; 6: pH; 7: Titratable acidity; 8: Tartaric acid addition; 9: Go Ferm addition; 10: Fermaid addition; 11: Diammonium phosphate addition.

C: loadings plot for grape berry volatiles.

1: α-terpinene; 2: Unknown terpene; 3: Limonene; 4: 4-terpineol; 5: Cis-linalool oxide; 6: Trans-linalool oxide; 7: 1,8-cineole; 8: Geraniol; 9: α-ionone; 10: ß-ionone; 11: ß-damascenone; 12: γ-terpinene; 13: terpinolene; 14: linalool; 15: α-terpineol; 16: γ-terpineol; 17: unknown compound; 18: Vitispirane1; 19: Vitispirane 2; 20: Fenchene; 21: ß-terpineol; 22: Myrcenol; 23: Unknown sesquisterpene; 24: Ocimenol; 25: Unknown terpene; 26: unknown sesquisterpene; 27: 28: unknown terpene; 29: Unknown C13-norisoprenoid; 30: Unknown C13-norisoprenoid; 31: Unknown C13-norisoprenoid; 32: Guaiacol.

D: loadings plot for berry amino acids.

1: Aspartic acid; 2: Aspargine; 3: Glutamine; 4: Serine; 5: Threonine; 6: Alanine; 7: γ-aminobutyric acid; 8: Proline; 9: Tyrosine; 10: Valine; 11: Methionine; 12: Arginine; 13: Isoleucine; 14: leucine; 15: Phenylalanine.

E: loading plots for wine volatiles.

1: Ethyl propionate; 2: Ethyl isobutyrate; 3: Propyl acetate; 4: Isobutyl acetate; 5: Ethyl butyrate; 6: Ethyl-2-methyl-butyrate; 7: Ethyl isovalerate; 8: Isoamyl acetate; 9: Ethyl hexanoate; 10: Hexyl acetate; 11: cis-3-hexenyl acetate; 12: Ethyl leucate; 13: Ethyl octanoate; 14: Ethyl decanoate; 15: Ethyl dodecanoate; 16: Ethylphenyl acetate; 17: Phenylethyl acetate; 18: Ethyl acetate; 19: Dimethyl sulfide; 20: Isobutanol; 21: Isoamyl alcohol; 22: Phenylethanol; 23: γ-nonalactone; 24: hexanol; 25: trans-2-hexenol; 26: cis-3-hexenol.

F: loading plots for wine sensory.

1: Red fruit; 2: Dark Fruit; 3: Stewed fruit; 4: Green/herbaceous; 5: Spicy/peppery; 6: Fullness/Roundness.

Perspectives

Providing wineries with more consistent and predictable fruit quality will contribute to vineyard and winery profitability and sustainability. By using a suite of compositional measures targeted to important berry and wine constituents and relating these to abiotic factors, the relationships between vine performance and wine styles should be more predictable.

This project aims to provide easy to use and inexpensive methods (objective measures of quality) using physiological-biochemical indicators and decision making tools, using sequential harvest to develop a method to possibly predict harvest dates according to the desired/expected wine style (or terroir-unit/site related wine style) and to reveal aromatic characters typical of Australian wine regions. Dialogue and collaboration between growers and winemakers could be improved by using objective measures of quality (fruit and wine composition). The method could be calibrated and used for autochthonous varieties.

Acknowledgements : This project was partly funded by Australia's grape growers and winemakers through their investment body, the Australian Grape and Wine Authority, with matching funding from the Australian federal Government.

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