USING A GRAPE COMPOSITIONAL MODEL TO PREDICT HARVEST TIME AND INFLUENCE WINE STYLE

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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, as exemplified by methoxypyrazine and rotundone concentrations in wine being 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.

The aim of this study was to better understand grape flavour evolution in relation to wine composition and subsequent wine style using sequential harvests (n=3). To achieve this goal, Shiraz was chosen as a model variety across two different climatic regions (warm-hot and cool-temperate) in New South Wales, Australia. The objective was not to compare the two regions but to assess the consistency of grape flavour evolution over the ripening period.

Irrespective of the region, a clear separation of samples was noted according to the harvest stage. 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. Higher concentrations of some higher alcohol acetates, dimethyl sulfide and lower concentrations of *Z*-3-hexenol, ethyl isobutyrate and ethyl leucate were measured in H3 wines.

Irrespective of the environment, this study demonstrated that in Shiraz, a common evolution of grape flavours exists, influencing the final wine sensory properties. Furthermore, during the late ripening stage, no direct nexus was observed between sugar concentration and grape and wine flavour evolution.

Key words*: *fruit and wine composition, wine sensory profile, sequential harvest, regionality, climate, volatiles, multivariate data analyses

1. INTRODUCTION

The idea of geographical origin is important for products which lay claim to a terroir-linked typicality (Deloire et al. 2008) and is often used to construct brand identity of such products as a guarantee of quality and authenticity (Guy 2011). Regionality is a broad notion which associates products of similar environment and is comprised of factors such as climate (temperature and water), topography, geology, landscape, human acts (eg. vineyard management) as well as any applicable regulations or traditions.

Meso and micro climate and the associated abiotic factors are considered to be the main drivers of vine physiology, berry growth and composition (Deloire et al. 2004; Tonietto and Carbonneau 2004). Dal Santo et al. (2013) reported that veraison is the period during which seasonal climate has the greatest effect. 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. Additionally, changes in gene regulation of the terpene and phenylpropanoid pathways are induced by severe water restrictions and result 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 (Šuklje et al. 2014; Šuklje et al. 2012). Cramer et al. (2014) reported changes in the abundance of more than 18000 transcripts related to an increased Brix, with the majority of changes occurring in the skins. Importantly, transcripts of several genes involved in isoprenoid and polypropanoid synthesis were significantly altered in Cabernet Sauvignon grapes during maturation (Cramer et al. 2014). Other studies (Bindon et al. 2013; Pons et al. 2008) have emphasised wine marker compounds that are potentially linked to wine aromatic maturity evolution.

When the harvest decision is determined by a range of objective measures of grape maturity (e.g. Brix, titratable acidity and colour), 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 : i) to determine the grape and wine flavour evolution during ripening using sequential harvest; ii) to assess the potential nexus between fruit sugar concentration and wine flavour evolution.

The research was performed across other varieties and three vintages, however for these proceedings, only results obtained during the 2015 vintage for Shiraz are presented.

2. MATERIALS AND METHODS

Four commercial Shiraz vineyards were chosen in two distinct grape growing regions of Australia. Two Shiraz vineyards were located in Griffith, Riverina, NSW (G1 and G2, respectively), characterised according to the Huglin index as a warm to hot grape growing region. To contrast Griffith, two Shiraz vineyards were also chosen in Orange (O1 and O2, respectively), NSW, which according to the Huglin index is classified as cool to temperate-warm region. The Riverina is also 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. 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, 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.

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 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. Wine volatiles were measured by GC-MS using previously developed methods (Antalick et al. 2010; Šuklje et al. 2016). Sulphur compounds were analyzed 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 $p \leq 0.05$).

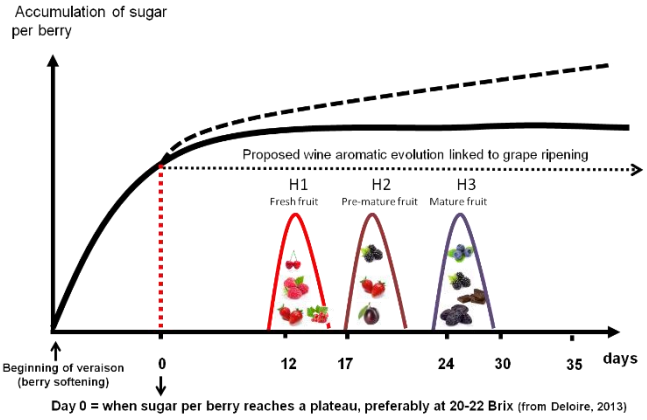

Figure 1: Example of Shiraz ripening evolution, using sequential harvest. Fresh fruit (H1) and mature fruit (H2) are reached 12 and 24 days respectively after sugar per berry accumulation stops or slows down.

3. RESULTS AND DISCUSSION

Sugar loading curves were determined for all experimental plots in each vineyard and are illustrated in Figure 2.

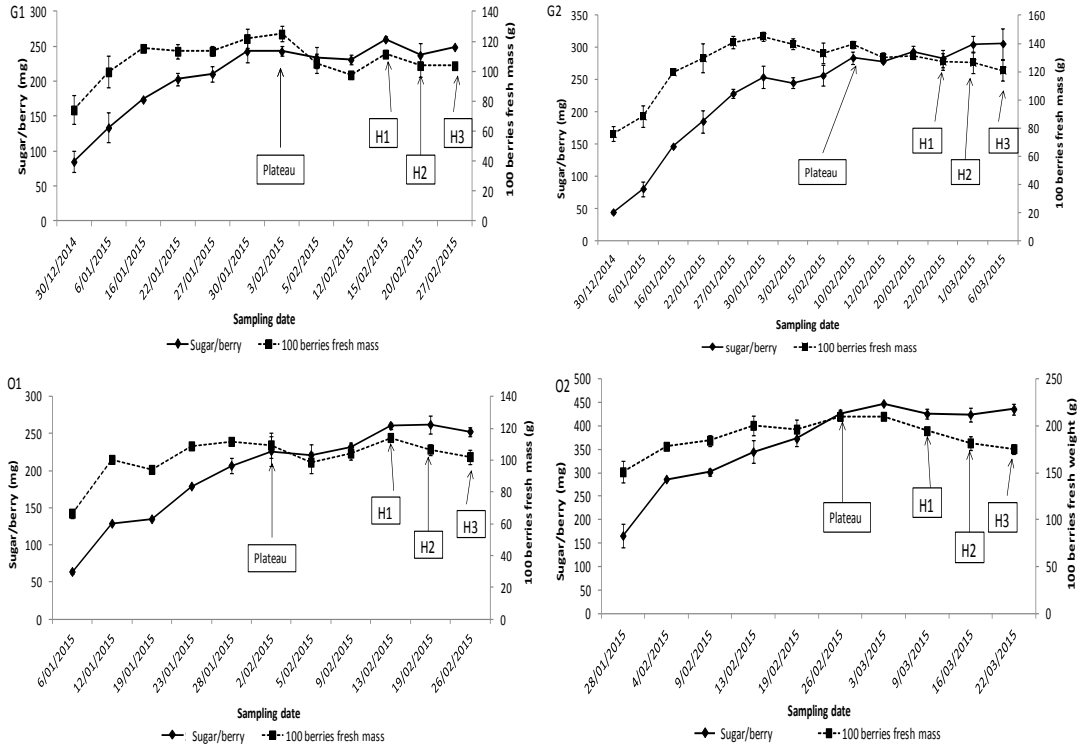

Figure 2: Sugar loading curves and 100 berries fresh mass evolution from the onset of veraison for experiment vineyards. G1 and G2 refers to the experimental vineyards located in Griffith and O1 and O2 refers to the experimental vineyards located in Orange. Error bars represent standard deviation of the 3 biological replicates. H1, H2 and H3 refers to harvest dates 1, 2 and 3 respectively. Plateau indicates the point of the slowdown of sugar accumulation.

Accumulation of sugar per berry reached a plateau, as per experimental curve. Berry fresh mass remained stable in all vineyards over the three harvest dates, except in the vineyard O2, where it decreased significantly (Table 1). Larger berries undergo faster water loss compared to smaller counterparts and often remain heavier at harvest (Rogiers and Holzapfel 2015). Interestingly, no significant difference in wine ethanol concentration in wines from G1 were noticed, irrespective of the harvest date (Table 1). Generally small and insignificant differences in ethanol concentrations were noticed between H2 and H3 in all vineyards, except in the case of wines from the O2 vineyard.

Table1: Basic grape berry, juice and wine parameters.

		G1	G2	O1	O2
Berry fresh mass (g)	H1	1.11±0.02a	1.27±0.06a	1.13±0.01a	1.94±0.03a
	H2	1.04±0.08a	1.26±0.08a	1.06±0.03b	1.81±0.08b
	H3	1.04±0.07a	1.20±0.07a	1.01±0.04b	1.75±0.05b
Sugar/berry (mg)	H1	260.5±3.7a	282.9±13.6a	260.3±4.2a	425.5±10.2a
	H2	237.6±17.2b	304.6±13.0a	261.6±12.1a	423.8±13.8a
	H3	249.3±15.2b	305.9±23.8a	252.3±5.8a	435.6±10.8a
Juice TSS (°Brix)	H1	23.3±0.2c	23.4±0.2b	22.7±0.2c	22.4±0.1c
	H2	23.8±0.1b	24.4±0.2a	24.2±0.1b	23.4±0.2b
	H3	24.0±0.1a	24.7±0.5a	25.3±0.1a	25.0±0.0a
Juice TA (g/L)	H1	3.2±0.0b	3.5±0.1b	5.7±0.2a	6.2±0.1a
	H2	3.5±0.1a	3.0±0.1a	4.9±0.1b	5.6±0.2b
	H3	2.9±0.1c	2.8±0.1c	4.6±0.1c	5.1±0.1c
Wine Ethanol (% w/v)	H1	13.5±0.1a	13.3±0.1c	13.4±0.1c	12.2±0.1c
	H2	13.5±0.1a	13.6±0.1b	14.3±0.0b	13.1±0.1b
	H3	13.6±0.0a	13.7±0.1a	14.5±0.0a	14.1±0.0a

G1 and G2 refer to the experimental vineyards located in Griffith and O1 and O2 refer to the experimental vineyards located in Orange. H1, H2, H3 refer to harvest 1,2,3, respectively. TSS refers to total soluble solids and TA refers to titratable acidity. All stated uncertainty is the standard deviation of three replicates per treatment. Means flowed by a different letter are different between 3 harvest dates for individual vineyard.

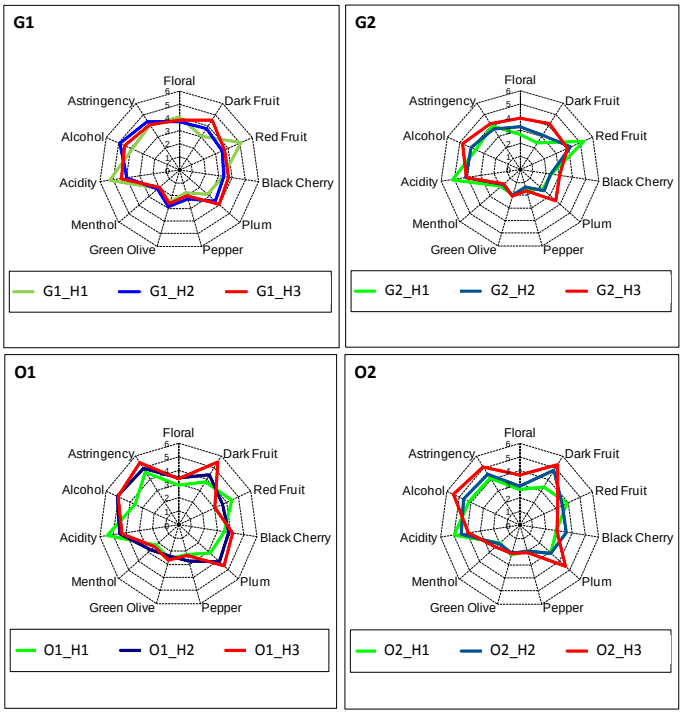

Figure 3: Spider graphs representing mean ratings for sensory attributes in wines made from grapes harvested at different predicted harvest maturity. H1, H2 and H3 refer to harvest date 1, 2 and 3, respectively. G1 and G2 refer to the experimental vineyards located in Griffith; O1 and O2 refer to the experimental vineyards located in Orange.

Sensory descriptive analysis (Figure 3) showed that acidity was perceived as significantly higher ($p<0.05$) all H1 treatments compared to wines picked at the later H3. Red fruit character was also higher in the H1 treatments, with all significant ($p<0.05$) except in O2. Wines from H3 were rated significantly higher for the perception of dark fruit and plum characters as well alcohol. Astringency was also perceived significantly higher in H1 compared to H3 in the O1 and O2 wines. Similar aromatic evolution has been previously reported for both Shiraz (Šuklje et al. 2014) and Cabernet Sauvignon wines (Bindon et al. 2014).

Clear separation of samples was noticed according to the harvest date, for both regions (Figure 4 A, B). Compounds contributing to the separation of samples were some amino acids (proline and γ-aminobutyric acid) which were at higher concentrations in the H3 grapes. 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).

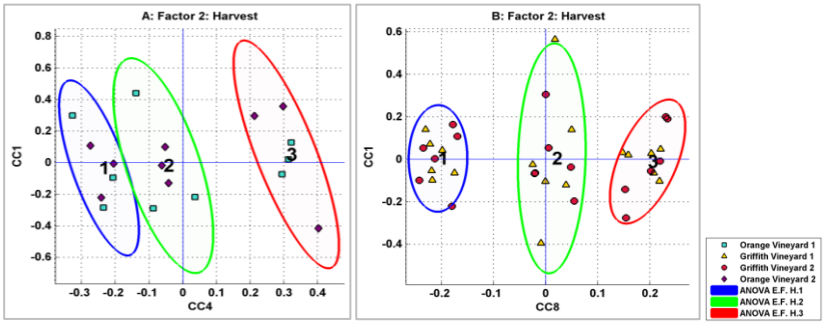

Figure 4: ANOVA principal component analyses conducted on grape berry, juice, wine and wine sensory results. A: Separation of samples according to the harvest date for Shiraz vineyards in Orange. B: Separation of samples according to the harvest date for Shiraz vineyards in Griffith. 1,2,3 refer to the harvest date 1,2 and 3, respectively. Ellipses represent 95% confidence limits.

4. CONCLUSION

Growers and winemakers use various techniques to help ensure that harvested grapes meet the requirements of wineries, and to a certain extent, consumers, in terms of desired wine style. The environment, cultural practices and harvest decision will have influence on fruit composition and therefore on the choice of the wine making process and produced styles of wine. The nexus between fruit and wine composition is very complex and needs further investigations. At present, sensory approaches remain crucial to assess the complex interaction between regionality, cultural practices, harvest dates and winemaking process on the potential wine styles.

This study found that a common evolution of grape flavours occurred for Shiraz in two distinctly different climatic regions. The value of this research for the wine industry is that: i) from a single vineyard and variety, irrespective of region and associated environment and cultural practices, it is possible to produce different wine styles using sequential harvest; ii) there is no clear nexus between berry sugar concentration and flavours from when sugar per berry accumulation reaches a plateau or slows down.

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

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