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Managing the impact of vintage advancement and compression



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1. Abstract:

Recent vintages in Australia have been characterised by rapidly maturing fruit and compression of the ripening window. This means that varieties that used to ripen over four to six weeks in the 1990s are now maturing over a much shorter time period. This places significant pressure on harvest logistics, with growers struggling to find sufficient harvesters and wineries being forced to delay harvest due to a lack of fermenter capacity (Petrie and Sadras 2016). Delays in harvest may result in yield loss due to berry dehydration, elevated grape sugar and wine ethanol concentration, and other negative impacts on fruit composition and wine style. On the other hand, some producers may perceive the increase in the concentration of grape compounds (especially grape and wine phenolics) associated with delayed harvest, together with other changes in wine volatile profile and sensory properties, as a potential improvement for wine quality attributes. This is of particular importance where the issue of higher wine ethanol can be overcome by using pre-ferment water addition.

A key focus of this project related to regulatory changes that allowed the addition of water to must in Australia under specific conditions, and the importance of this change in relation to the effects of vintage compression. The impacts of water addition on the chemical and sensory properties of Shiraz and Chardonnay wines (Australia's principal varieties) were investigated, with wines made following dilution compared against wines made from earlier harvested grapes. Of particular interest was the question whether losses in fruit volume associated with more extreme delays in harvest (e.g. fruit shrivel) might be overcome by water addition without detriment to wine quality. The results demonstrated that water addition lowered tannin and colour in Shiraz wines independently of the mode of addition (direct addition vs juice replacement), showing that maintaining the solids to juice volume ratio did not necessarily increase the extraction and/or retention of phenolics in the wine. Nevertheless, wine produced with dilution consistently had higher levels of tannin and colour than wines of the same ethanol concentration produced from less ripe grapes from earlier harvest dates. In the Chardonnay wines, differences in chemical composition and sensory properties were relatively small. The fruit harvested at 15.5°Baume, irrespective of dilution, separated from the wines made from earlier harvested grapes according to the chemical analysis. From the sensory analysis the main attribute affected by dilution was wine 'hotness', which related to the lower ethanol concentration.

The observation that grapes are reaching a sugar concentration suitable for winemaking at an earlier date is well established in Australia and throughout the world. Yet there has been less effort spent characterising the impact of climate change on other berry maturity parameters such as the titratable acidity (TA) and pH. From analysis of a commercial dataset, an increase in pH and decrease in TA were observed with a similar magnitude to that seen for sugar concentration. Interestingly, the ratio between grape pH or TA and sugar concentration remained consistent across the 25-year period investigated.

Increases in grape sugar concentration can be driven by either the import of sugar from the vine, or by berry dehydration. Dehydration also results in a loss in yield, with a significant impact on vineyard profitability. A meta-data analysis of studies on grape ripening across Australian growing regions for Shiraz and Cabernet Sauvignon was completed. The results suggest that Shiraz ceases sugar accumulation at 13°Baume and Cabernet Sauvignon at 14.5°Baume. Beyond these values, additional increases in grape sugar concentration are likely to be the result of fruit dehydration.

Taken together, the improved understanding of the processes that affect grape ripening and fruit maturity and the development of winemaking processes to manage fruit that has to be harvested with high sugar concentrations will support the industry's efforts to maintain wine quality under warmer conditions.

2. Executive summary

Recent vintages have been characterised by rapidly maturing fruit and compression of the ripening window. This means that varieties that used to ripen over four to six weeks in the 1990s are now maturing over a much shorter time period. This places significant pressure on harvest logistics, with growers struggling to find sufficient harvesters and wineries being forced to delay harvest due to a lack of fermenter capacity. As one countermeasure, recent changes in Australian legislation allow the addition of water to must in Australia under specific conditions.

To compare benefits and risks from water addition to grape must, direct water addition and juice substitution approaches were trialled with Shiraz must from grapes sourced from the Barossa Valley. In this case it was found that water addition lowered tannin and colour in wines independently of the mode of addition, showing that maintaining the ratio of solids to juice volume did not necessarily increase the retention of phenolics in the wine. Nevertheless, wine produced with must dilution consistently had higher levels of tannin and colour than wines of the same ethanol concentration produced from grapes harvested earlier at reduced ripeness. Sensory analysis of the wines showed that the treatments were chiefly defined by differences in 'dark fruit' aroma/flavour, hotness, viscosity and opacity. The wines prepared from 15.5°Baume fruit were rated higher in these attributes than wines with lower ethanol, with the earliest harvest (13.5°Baume) rated lowest. The wines made from the must with water additions maintained many of the fuller-bodied and richer flavours that are typical of the styles produced in the Barossa and other warmer climate regions, and were also associated with the wines made from the undiluted must at the latest harvest time. The wines made from the fruit that was harvested earlier were generally of a lighter style with less colour and tannin and more 'red fruit' aromas. The mode of water addition (i.e. if juice was run off prior to the addition of the water or water was added directly to must) had a relatively small impact on wine phenolics, but introduced larger changes in wine sensory properties. As a note of caution, it was observed that higher quantities of water addition introduced off-odours in some wines. A more moderate addition of water using a 'run-off and replace' technique produced wines more similar to the undiluted control wine and without off-odours.

To understand the effects of water addition on white wine composition and quality, a further study was conducted using Chardonnay grapes sourced from the Currency Creek region. Chardonnay was harvested at different ripeness stages and diluted to 13.0°Baume using direct addition of rainwater to the must. Wine made from earlier harvested grapes at 13.0°Baume was included for comparison. In the Chardonnay wines, differences in chemical composition were minor, with the 15.5°Baume treatments, irrespective of dilution, separated from the wines made from grapes harvested at earlier dates. Sensory assessment showed that differences between the wines were small, with the main attribute affected by dilution being wine 'hotness'. 'Hotness' was similar between the wines made from earlier harvests, but not surprisingly, the 15.5°Baume control wine was rated highest for 'hotness'. However, it was interesting to note that the diluted 15.5°Baume treatment had the lowest perceived hotness out of all the treatments, despite having a similar ethanol concentration to the wines made from earlier harvest grapes. These preliminary results show that water addition may be used according to the FSANZ regulations to reduce wine ethanol in Shiraz and Chardonnay, without significant negative impacts, with the cautionary observation that higher levels of water addition in Shiraz might introduce off-odours.

In a subsequent season, a follow-up study on Shiraz from the same Barossa vineyard was performed to understand the outcomes of a more extreme extended 'hang time' of grapes on the vine, as it related to berry shrivel, water addition and phenolic outcomes. In that study, it was found that marked seasonal variation existed, with grape and wine phenolics being far higher in the second season than the first, independent of the harvest date. An important outcome from this second season of research was that while increases in fruit and wine phenolics were previously found up to 15.5 °Baume, no further significant increases were seen as a result of delaying harvest, even when shrivel increased from 8% to 30%. As for the first season of the study on Shiraz, the quantity of water addition did not affect phenolic outcomes in the wine.

Another key focus of the project was a meta-analysis of data sourced across a range of existing research datasets with particular reference to the interpretation of sugar accumulation data in relation

to changes in berry weight, in order to separate the impact of sugar importation from increased concentration through berry dehydration. A systematic search of the literature identified 36 Australian-based studies that assessed the ripening of grape berries and had records of both berry weight and sugar concentration within one season. Boundary layer regression was used to empirically determine the maximum sugar concentration where the sugar content per berry stopped increasing, under Australian conditions. This was found to be 13°Baume for Shiraz grapes, and for Cabernet Sauvignon it was closer to 14.5°Baume. These results were particularly relevant in light of the findings for Shiraz described above, since other parameters relevant to quality (e.g. phenolic compounds) may continue to increase as 'hang time' is extended, but the importance of these changes should be considered with the recognition that further increases in sugar concentration in the fruit are most likely due to dehydration of the berries, and not genuine accumulation.

While the advancement in grape maturity over time in response to warmer conditions has been well categorised, changes in other key parameters that affect maturity assessments (titratable acidity (TA) and pH) have not been investigated. To address this, a commercial dataset was analysed to investigate trends in pH and TA over 25 consecutive vintages. The date when nominated values for TA (9 g/L) and pH (3.4) were reached followed a trend as observed for total soluble solids during the time period studied. The calculated average TA and pH of the fruit, when it had an average TSS of 12 Baume, was remarkably consistent across the 25 years of data. For Shiraz grown in the Barossa the TA and pH averaged 8.9 g/L and 3.3 respectively and for Cabernet Sauvignon grown in Coonawarra the TA and pH averaged 7.5 g/L and 3.3, respectively. Similar responses were seen for other cultivar and regional combinations.

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3. Background:

Over the last two decades grapegrowers and winemakers have observed that their fruit is ripening earlier (Petrie and Sadras 2008) and over a shorter period (Petrie and Sadras 2016). This can lead to difficulties harvesting fruit in the optimal timeframe and fruit being picked at higher than ideal sugar concentrations. In turn, this can cause problems with fermentations 'sticking' before all the sugars have fermented (Chaney et al. 2006) or lead to wines with undesirably high ethanol (Varela et al. 2015). In the USA, the dilution of must to facilitate fermentation is legal and widely accepted (Chaney et al. 2006). More recently, to help manage fruit with a high sugar concentration, Food Standards Australia and New Zealand (FSANZ 2017) changed the regulations to allow the limited addition of water to high-sugar musts and juice in Australia (Anon. 2016). The revised regulations allow musts to be diluted with water prior to fermentation until they reach 13.5°Baumé. As for the USA, the rationale behind this change was to reduce the chances of problems arising from problem fermentations. As an additional benefit, water addition to must may help industry to manage the consequences of logistical problems caused by compressed vintage periods.

A number of questions were raised by the change in FSANZ wine production standard 4.5.1 to allow water to be added to must. Studies have been conducted for *Vitis vinifera* cultivars Cabernet Sauvignon and Merlot, whereby must was run off after crushing and an equivalent volume of water added (juice substitution) to decrease the ethanol concentration of finished wines, with no significant change to wine tannin concentration or colour, and limited changes in wine aroma and flavour (Casassa et al. 2013, Schelezki et al. 2018a, Schelezki et al. 2018b). These results indicated potential benefits of water

addition to reduce ethanol and one objective of this project was to investigate the potential pros and cons with Shiraz and Chardonnay, the principal grape cultivars grown in Australia.

A further potential outcome of the change in FSANZ regulations is that by establishing a lower limit for sugar concentration, a stronger focus for maturity assessments can be on the mass of sugar produced by grapes – as opposed to sugar concentration in grapes – in addition to the mass of grapes harvested, which still is key to grape payments. Under the revised regulations, the point at which sugar allocation into the fruit ceases becomes an additional trait to inform harvest decisions, in addition to fruit maturity and flavour profile. Potential for conflict between grapegrowers – who are paid on the basis of yield (mass) of grapes – and wineries can arise when high sugar concentrations are potentially achieved due to fruit dehydration as opposed to from the importation of sugar into the grape berries (Gogoll 2017; Smart 2005). As the vineyard yield is determined in part by the sugar content of the fruit, the stage of development at which sugar accumulation ceases becomes critical for optimising yield. While other key quality parameters such as acids, anthocyanins and phenolics may change and affect the quality of the final wine (Bindon et al. 2013; Coombe and McCarthy 2000), the further increase in sugar concentration in the fruit is due to dehydration of the berries. In other words, the potential for achieving wine quality improvements might go hand in hand with a commercial disadvantage for growers.

The cultivar planted, environmental conditions and vineyard management practices can all potentially affect the plateau of sugar accumulation in berries, and this can occur before the theoretical maximum is reached. The manipulation of yield and canopy size are classic viticultural practices commonly used to advance or delay fruit maturity and their interaction has been assessed at least since the early 1900s (Ravaz 1903). For example, Etchebarne et al. (2010) observed that berries on Grenache Noir vines with five leaves per shoot stopped accumulating sugar earlier than berries on shoots with 10 or 18 leaves. They also observed that irrigation allowed the berries to continue to accumulate sugar later into the season. The assessment of environmental effects on sugar accumulation of field vines is more difficult as a control treatment is hard to establish. However, Greer and Weston (2010) were able to show that heat stress at veraison or mid-ripening could stop the accumulation of sugar in potted Semillon vines. Sadras et al. (2008) compared a range of wine and table grape cultivars and found that the date at which they reached 95% of their maximum sugar concentration varied significantly.

Changes in sugar content are normally tracked by the assessment of berry size, and in conjunction with sugar concentration, the sugar content per berry can be calculated. In a commercial context the assessment of berry size in addition to the traditional berry maturity measures of sugar concentration, titratable acidity and pH is currently prohibitive due to the labour required. In a research context, the average berry weight is more often recorded; however, it is normally assessed at a point in time or at a specified maturity (Dai et al. 2011) and the impacts of the experimental treatments or environmental conditions on the point when sugar accumulation stops are rarely determined. For some cultivars, especially Shiraz, the dynamics of sugar accumulation and its partitioning into flows of solutes and water is well defined, as it is prone to shrivel during late berry development (McCarthy 1999, McCarthy and Coombe 1999). While the magnitude of shrivel has been well characterised, even for a single cultivar such as Shiraz there is divergence within the literature as to the sugar concentration where sugar content reaches a plateau (McCarthy and Coombe, 1999, Keller 2015).
In order to address these concerns, the project aimed to analyse the trajectories of grape ripening using historical data from published studies in order to determine the maximum sugar concentration at which sugar accumulation by grape berries ceases in Shiraz and Cabernet Sauvignon under a range of Australian conditions. It was expected that these figures could be used as a guide to determine when fruit dehydration is likely to occur and assist in harvest decision-making. Furthermore, it was considered that data other than grape sugar and berry weight data might be accessible from the meta-analysis of historical work, and provide corresponding information on grape acidity and colour. The addition of this data could enable a separation of the contribution of higher temperatures (climate change) to the vintage compression phenomenon from management practices (e.g. improved irrigation or pruning) by investigating the balance between TA and pH with sugar concentration. It was expected that a better understanding of these trends would corroborate their likely relevance in future vintages, and inform both winery and vineyard management decisions.

4. Project aims and performance targets:

The objectives defined for the project were as follows:

- Characterisation of the impact of the amount, timing and methods of water addition on wine composition and style
- An extension of the analysis of the maturity dataset from Treasury Wine Estates for TA and pH, in order to track changes in these parameters (and their balance with sugar) from the late 1990s
- An evaluation of models for assessing the likely impact of climate change on TA and pH (and their balance with sugar)
- Data analysis across a range of research datasets (already collected) of sugar accumulation and berry weight, to separate the impact of sugar importation vs berry dehydration.

Table 4.1. 2017-18 Deliverables and Activities

Contracted deliverables	Activities	Activity target date
A better understanding of the impacts of water additions on wine quality and style	1. Chemical analysis of vintage 2017 trial wines produced to examine the effect of alcohol reduction on macromolecule extraction and retention in wines by using sequential harvest (grape maturity) versus must dilution (through water addition and saigneé) completed and sensory scheduled (collaboration within 3.1.4). 2. A further winemaking trial designed and implemented	June 2018
Knowledge of the impact of importation/metabolism vs berry dehydration on fruit composition	1. Identification and collation of research data sets containing information on berry weight and other grape composition.	June 2018
Knowledge of the impacts of climate change on pH and TA, and their balance with sugar concentration in grapes	1. Development and extension of existing database to include historical commercial pH and TA results.	June 2018

Table 4.2. 2018-19 Deliverables and Activities

Contracted deliverables	Activities	Activity target date
A better understanding of the impacts of water additions on wine quality and style	1. Complete chemical analysis of vintage 2018 trial wines produced to examine macromolecule extraction and retention in wines made using sequential harvest (grape maturity) versus must dilution and schedule sensory analysis (collaboration with 3.1.4) 2. Review results from 2017 and 2018 winemaking trials, and plan 2019 vintage experiments in collaboration with Project 3.1.1 to confirm key findings if required. 3. Prepare extension materials and presentation(s)for the AWITC in 2019.	June 2019
Knowledge of the impact of importation/metabolism vs berry dehydration on fruit composition	1. Conduct initial analysis of research data sets containing information on berry weight and other grape composition.	June 2019
Knowledge of the impacts of climate change on pH and TA, and their balance with sugar concentration in grapes	1. Conduct analysis of historical commercial pH and TA results to investigate the impacts of climate change.	June 2019

Table 4.3. 2019-20 Deliverables and Activities

Contracted deliverables	Activities	Activity target date
A better understanding of the impacts of water additions on wine quality and style	1. Prepare extension materials and present data at the 17th AWITC in July 2019. 2. Prepare final report	June 2020
Knowledge of the impact of importation/metabolism vs berry dehydration on fruit composition	1. No further experimentation is planned as this objective has been completed. 2. Prepare final report.	June 2020
Knowledge of the impacts of climate change on pH and TA, and their balance with sugar concentration in grapes	1. No further experimentation is planned as this objective has been completed. 2. Prepare final report.	June 2020

5. Methods:

5.1 Vintage trial 2017: Comparison of ripening and water addition on Shiraz wine

5.1.1 Grape samples for 2017 Shiraz water addition trial

Shiraz grapes were harvested from a research vineyard (Nuriootpa, South Australia, 34° 26' 24.5" S, 138° 53' 50.8" E) in the 2017 season. The vines were clone BVRC30, and were planted on their own roots in 1998 with a spacing of 3 m and 2.25 m between and within rows, respectively. The vines were trained to a bilateral cordon with a single fixed foliage wire located 0.4 m above the cordon. Hand-harvesting was performed at three dates: 17 March, 27 March and 7 April, designated as: first harvest (H-1), second harvest on (H-2) and third harvest (H-3), respectively. Target °Baumes for each harvest were 13.5, 14.5 and 15.5 for H-1, H-2 and H-3, respectively. The grapevines were harvested from a small area of approximately 2,500 m^2 (not all of the vines were harvested). As the quantity of fruit required varied between the harvest dates, the vines were selected using a systematic pattern from throughout the designated area to avoid any bias due to variation in fruit composition within the vineyard block and avoid heterogeneity within the vineyard affecting results.

5.1.2 Winemaking for the 2017 Shiraz water addition trial

In order to minimise within-vineyard differences in fruit composition prior to winemaking, the grapes were mixed and sub-divided into 45 kg batches for small-scale fermentations. All small-scale ferments for each treatment were conducted in triplicate. The grapes were destemmed, crushed and the must of the H-3 treatment was either left untreated or processed using two modes of water addition, and saigneé, as described below. Roof-harvested, potable rainwater was used for the water addition treatments to prevent the introduction of chlorine to the musts. Rainwater was pH-adjusted with tartaric acid prior to the addition. The volume of water added was calculated based on an expected liquid extraction rate of 65% (30 L wine per 45 kg of grapes). The water addition process aimed to reduce the sugar content of the musts by 2.2 and 1.2°Baume to achieve approximately the total soluble solids values of the early-harvest H-1 and H-2 musts. Water was added using two different approaches: either added directly to the must or via removal of juice followed by replacement with water (juice substitution). Also, in order to compare the effect of juice removal or water addition on the must volume to solids ratio, saigneé treatments where juice was removed without water addition were also included. Treatments were as follows:

- DA-1: Direct addition of 5 L of water to the must
- DA-2: Direct addition of 2.4 L of water to the must
- RR-1: Juice substituted with 5 L of water
- RR-2: Juice substituted with 2.4 L of water
- RO-1: Removal of 5 L of juice without water addition
- RO-2: Removal of 2.4 L of juice without water addition

It is important to note that the ºBaume values after water addition were calculated, not measured, given that a proportion of sugar is retained within the partially crushed berries. This is because water addition dilutes only the free juice and therefore a measured sugar concentration would have been significantly underestimated.

Based on the measured yeast assimilable nitrogen (YAN) content of each must, total assimilable nitrogen was adjusted to 300 mg/L with diammonium phosphate for each ferment. Musts were also pH-adjusted with tartaric acid pre-ferment to match that of the first harvest. Musts were then sequentially inoculated with *Saccharomyces cerevisiae* yeast (EC1118, 500 ppm), and malolactic bacteria (VP41) on the first and second day after crushing, respectively. The cap was plunged twice daily and alcoholic fermentation was tracked by measuring sugar loss. Fermentation on skins took place for nine days in a 20ºC temperature-controlled room, before the must was pressed. Free-run and pressed wine were combined and fermentation continued at 20ºC until the wines were dry, determined as glucose and

fructose equivalents <1 g/L. Wines were then held at 20ºC until malolactic fermentation was complete, where malic acid <0.1 g/L. Wine glucose, fructose and malic acid were determined using enzymatic methods and an automated analyser (Daytona, Randox Laboratories, Crumlin, United Kingdom) to confirm the completion of alcoholic and malolactic fermentation. Wines were pH adjusted, and 80 mg/L of SO_2 was added. Wines were cold-settled at 0°C for a minimum of 28 days, then adjusted to a final TA of 5 g/L and a free SO_2 of 45 mg/L. Wines were filtered by cross-flow and bottled in November 2017.

5.1.3 Sensory analysis of the 2017 Shiraz water addition trial

Sensory analysis was initiated in May 2018. Preliminary sensory evaluation by a panel of expert technical wine assessors resulted in no exclusion of any fermentation replicates due to off-flavours or winemaking artefacts. All wines were evaluated using quantitative sensory descriptive analysis. A panel of eleven assessors (nine females, one males) with an average age of 49 years (SD=9.5) was convened to evaluate the wines. Assessors attended three two-hour training sessions to determine appropriate descriptors for rating in the formal sessions. All the wines from the study were progressively used during training sessions to generate and refine appropriate descriptive attributes and definitions through a consensus-based approach. Wines were assessed by appearance, aroma and palate. In the second session, standards for aroma, basic taste and mouth-feel attributes were presented and discussed and these standards were also available during subsequent training sessions, the booth practice session and the formal assessment sessions. As a warm-up exercise, panellists revisited these aroma and palate standards at the beginning of each formal assessment session. Following the third training session, tasters participated in a practice session in the sensory booths under the same conditions as those for the formal sessions. After the practice session, any terms which needed adjustment were discussed and the final list of terms and standards were determined. For the formal sessions, this list was refined to include two appearance terms, seventeen aroma terms (sixteen defined and one 'Other' term) and eighteen palate terms (seventeen defined and one 'Other' term). The final list of attributes, definitions/synonyms and reference standards are shown in Appendix Table 13.2. Evaluation samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22–24°C, in isolated booths under daylight-type fluorescent lighting. Randomised presentation order was followed except in the practice sessions when there was a constant presentation order. All samples were expectorated. In both the practice booth and formal sessions, the assessors were presented with four trays of three wines per tray. The assessors were forced to have a 60-second rest between samples where they were encouraged to rinse their mouths with water, and a minimum ten-minute rest between trays. During the ten-minute break assessors were requested to leave the booths. Formal evaluation was completed in three two-hour sessions on separate days. The wines were presented to assessors two times, in a modified Williams Latin Square incomplete random block design generated by Fizz sensory acquisition software (version 2.51, Biosystemes, Couternon, France). A new bottle was used for each of the duplicate assessments. The intensity of each attribute was rated using an unstructured 15 cm line scale, with indented anchor points of 'low' and 'high' placed at 10% and 90% respectively. Data was acquired using Fizz sensory software.

5.2 Vintage trial 2018: Comparison of ripening and water addition in Chardonnay wine

5.2.1 Grape samples and winemaking for the 2018 water addition trial on Chardonnay

Chardonnay grapes were sourced from the moderate to warm region, Currency Creek, in SA. The clone I10V1 vines had been established on their own roots in 1998 and were trained with two bilateral cordons and machine pruned. The preliminary project objective was to source Chardonnay at three harvest points with the earliest harvest being 13.5°Baume, the lowest sugar level to which musts are permitted to be adjusted pre-ferment. Although pre-harvest sugar measurements indicated that the first harvest was at the target 13.5 °Baume, must analysis indicated that it was actually 13°Baume. It was therefore decided that all water additions would be applied to reach a target °Baume of 13 rather than 13.5, in order to keep the final ethanol concentration consistent between the early-harvest and water-addition treatments. Musts of the 14.5 and 15.5°Baume harvests were diluted to 13°Baume using addition of rainwater. The fruit was crushed and destemmed into triplicate batches as evenly as possible keeping

triplicates of each treatments separate. A dose of 50 mg/L SO_2 was added to the must upon crushing. In order to explore the possible impact of skin contact to improve the aromatic and texture profile of wines undergoing water addition, additional skin contact treatments were applied to the musts. A 12 h skin contact treatment was applied to both the 14.5 and 15.5°Baume treatments either pre- or post-water addition, and for water addition calculations an extraction rate of 483.5 L/t was applied.

The treatments were as follows:

- 13°Baume control, pressed immediately
- 14.5°Baume control, pressed immediately
- 14.5°Baume control, pressed immediately and water added to 13°Baume
- 14.5°Baume with 12 h skin contact
- 14.5°Baume with 12 h skin contact, then water added to 13°Baume
- 14.5°Baume adjusted to 13°Baume followed by 12 h skin contact
- 15.5°Baume control, pressed immediately
- 15.5°Baume control, pressed immediately and water added to 13°Baume
- 15.5°Baume with 12 h skin contact
- 15.5°Baume with 12 h skin contact, then water added to 13°Baume

Musts were acid-adjusted based on the analysis of TA following pressing and water addition to a final TA of 6 g/L. Malolactic fermentation was not initiated. After pressing and water addition, juices were cold settled at 0˚C for 3-5 days. All treatments were racked to ferment vessels and volumes standardised to match that of the smallest vessel. Aliquots of lees were added back to each vessel, ensuring turbidity and volumes remained standardised. *Saccharomyces cerevisiae* strain Maurivin PDM was used to conduct primary fermentation. The fermentation was at 15°C aiming for a ferment temperature of 15-16°C and a 1-2ºBaume drop per 24 h. Sequential DAP addition to 200 ppm YAN was made based upon YAN analysis of the must after pressing. Ferments were moved to 20°C once below 3ºBaume until the completion of primary fermentation. Once wine was confirmed to be sugar dry (<2 g/L) based on glucose and fructose analysis described above, 80 mg/L SO_2 was added and the fermentation vessels were placed at 0°C. Malolactic fermentation was not performed. After a minimum of 7 days, the wine was racked off gross and stability lees and packed into full vessels for storage. Wines were stored at 0°C for a minimum of 28 days. Prior to bottling, wine free SO_2 was adjusted to 45 mg/L and pH to 3.2-3.5. Wine was cross-flow filtered (nominal 0.4 µm) and bottled under screw-cap in September 2018.

5.2.2 Sensory analysis of the 2018 water addition trial on Chardonnay

Sensory analysis was performed in February 2019. Preliminary sensory evaluation of the Chardonnay wines by a panel of expert technical wine assessors resulted in the selection of two representative fermentation replicates per treatment. All selected wines were evaluated using quantitative sensory descriptive analysis. A panel of eleven assessors (eight females, three males) with an average age of 54 years (SD=8.4) was convened to evaluate the wines. Assessors attended three two-hour training sessions to determine appropriate descriptors for rating in the formal sessions. All the wines from the study were progressively used during training sessions to generate and refine appropriate descriptive attributes and definitions through a consensus-based approach. Wines were assessed by appearance, aroma and palate. In the second session, standards for aroma, basic taste and mouth-feel attributes were presented and discussed and these standards were also available during subsequent training sessions, the booth practice session and the formal assessment sessions. As a warm-up exercise panellists revisited these aroma and palate standards at the beginning of each formal assessment session. Following the third training session, tasters participated in a practice session in the sensory booths under the same conditions as those for the formal sessions. After the practice session, any terms which needed adjustment were discussed and the final list of terms and standards was determined. For the formal sessions, this list was refined to include one appearance term, fourteen aroma terms (thirteen defined and one ‘Other’ term) and thirteen palate terms (twelve defined and one ‘Other’ term). The final list of attributes, definitions/synonyms and reference standards is shown in Appendix Table 13.2. Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered,

ISO standard wine glasses at 22–24°C, in isolated booths under daylight-type fluorescent lighting. Randomised presentation order was followed except in the practice sessions when there was a constant presentation order. All samples were expectorated. In the practice booth sessions the assessors were presented with four trays of three wines per tray. In the formal sessions, judges were presented with five trays, assessing 13 or 14 wines per session with up to three wines on each tray. The assessors were forced to have a 60-second rest between samples where they were encouraged to rinse their mouths with water, and a minimum ten-minute rest between trays. During the ten-minute break assessors were requested to leave the booths. Formal evaluation was completed in three two-hour sessions on separate days. A new bottle was used for each of the three presentation days. The 20 wines (10 treatments x 2 replicates) were presented to assessors two times, in an incomplete Williams Latin Square random block design provided by Compusense Cloud sensory evaluation software (Compusense Inc., Guelph, Canada). The intensity of each attribute was rated using an unstructured 15 cm line scale, with indented anchor points of 'low' and 'high' placed at 10% and 90% respectively. Data was acquired using Compusense Cloud sensory evaluation software.

5.3 Vintage trial 2019: Study of extended hang time in Shiraz: implications for grape and wine phenolics

In the 2019 season, a final study was initiated to investigate more extreme delays in harvest date on wine compositional outcomes in Shiraz, with specific reference to the outcomes of dilution on phenolics. This project formed part of a larger study on the grape and wine volatiles responsible for the 'jammy' character associated with wines made from overripe, shrivelled Shiraz. For the study, fruit from the same Barossa vineyard was used. Three replicate plots were spatially staggered across two rows in order to account for within-row variation, and harvests were conducted on 14 March (control) and 27 March (late harvest). Harvests from the three replicate panels were kept separate, and therefore replicate comparison between the two harvest dates was made on the basis of the panel harvested. From each harvest replicate, grape berries were sub-sampled (approximately 400 g) and stored at 4°C. Grape bunches were plucked and all berries were collected in a tray and mixed. A sample of 100 berries was sorted, and the proportion of shrivelled berries was calculated for each replicate. A further 50 g sample of berries was taken and processed to determine extractable tannin and colour as described below. For winemaking, each spatially distinct harvest replicate was crushed as an independent treatment. The initial must sugar measurements taken at crushing were 15ºBaume and 16ºBaume for the control and late-harvest samples, respectively. To account for the fact that the prediction of final ethanol from the measured ºBaume at crushing is frequently underestimated in overripe fruit, repeat analysis of the ºBaume was taken after 24 h, and found to increase to 16.5 and 17.3 for the control and late-harvest treatments, respectively. Therefore it was decided that both the control and late-harvest treatments required water addition to complete fermentation, which was performed as a direct addition to the must based on a 60% extraction rate. The control treatments were adjusted to 14°Baume using ≈2.6 L of water, with slight differences between the winemaking replicates since must sugar differed slightly between the plots. The late-harvest treatments received two levels of water addition to adjust to 14 and 16ºBaume as independent water addition treatments, using ≈3.7 and ≈1.2 L of water, respectively. Musts received tartaric acid addition at the same time as the water addition, with ≈ 3.2 g/L added to the control musts, and 4.5 g/L to the late-harvest treatments. Winemaking was performed as described previously for the 2017 water addition trial using 250 mg/L PDM yeast (AB Biotek), and wines were bottled in November 2019. Wines were analysed at the time of sensory analysis in May 2020, at approximately 12 months post-fermentation, as described below.

5.4 Analysis of musts and grapes

Musts from the three vintage trials, including the water addition treatments, were analysed for YAN, pH and TA. Juice and wine pH and TA were determined using a pH meter and combination electrode. The TA was determined by titrating with 0.33 M sodium hydroxide solution to a pH end-point of 8.2 and expressed in g/L of tartaric acid equivalents. Ammonia concentration in fresh juices was determined using the glutamate dehydrogenase enzymatic bioanalysis UV-method test (Roche, Mannheim, Germany). Juice free amino acid nitrogen was determined by the o-phthaldehyde/N-acetyl-L-cysteine

spectrophotometric assay, which excludes proline (Bindon et al. 2013). Juice YAN was calculated by adding the nitrogen present in ammonium to the YAN concentration. Three replicate 50 g berry samples were subject to a 'wine-like' extraction from the 2017 and 2019 trials on Shiraz grapes to determine extractable tannin and anthocyanin according to the method described by Bindon et al. (2014a). Briefly, 50 g of berries were transferred into zip-lock plastic storage bags and crushed by hand. The must (solids and juice) obtained was transferred into 70 mL plastic screw-cap containers, using a spatula to ensure all berry contents were removed from each bag. Aqueous ethanol (15 mL of 40% adjusted to pH 3.4 with a 10 g/L aqueous tartaric acid solution) was added and the capped containers were gently shaken for 40 h at room temperature. Afterwards, the extracts were pressed through a sieve with a 1 mm^2 pore mesh, and the extract volume was recorded. The extracts were transferred into 50 mL tubes, centrifuged, and the supernatants were analysed for tannin and anthocyanin concentration as described below.

5.5 Wine basic compositional analysis.

Wines were analysed for their basic composition using a Foss WineScan FT 120 as described by the manufacturer (Foss, Hillerød, Denmark).

5.6 Phenolics, colour and tannin concentration in extracts, musts and wines

Wine and must samples were centrifuged for 5 min at 16.1 *g* prior to analysis. Tannin was analysed in grape extracts and wines using a standard high-throughput method (Mercurio et al. 2007). Each analysis was performed on a minimum of duplicates. Briefly, tannin concentration was analysed following precipitation of ethanolic homogenate extract by methyl cellulose (MCP) in 1.1 mL 96-well deep well plates. A 25 μL sample of grape extract or wine was combined with 300 μL of 0.04% (w/v) methyl cellulose (Sigma Aldrich, St. Louis, MO, USA) solution, mixed on an automated plate shaker and left to stand for three minutes. A sample control was included where water was added in place of the methyl cellulose solution. A 200 μL aliquot of saturated ammonium sulfate (Sigma Aldrich, St. Louis, MO, USA) was added, followed by 475 μL water. Samples were mixed on an automated plate shaker and left to stand for 10 minutes. Centrifugation was performed for five minutes at maximum speed on a Hettich Universal 32 R centrifuge equipped with a Hettich 1645 rotor for 96-well plates (Adelab Scientific). A 300 μL sample of supernatant from each of the methyl-cellulose-treated and control samples was transferred into a 370 μL 96-well UV plate and the 280 nm absorbance measured using a SpectraMax M2 Microplate Reader (Molecular Devices, Australia). Quantification was performed by analysing the difference in 280 nm absorbance between control and MCP-treated samples, using (-)-epicatechin (Sigma Aldrich, St. Louis, MO, USA) as the quantitative standard. To ensure standardisation of the tannin analysis across the staggered time points at which grape and wine sampling and extraction took place, a purified commercial seed extract (Tarac Technologies, Nuriootpa, Australia) was included for each 96-well plate assayed. Wine colour properties and total phenolics were also determined according to the high-throughput modified Somers assay described by Mercurio et al. (2007). For Chardonnay wines, centrifuged wine was diluted 1 in 5 with 1 M HCL and the absorbance determined at 280 nm in a 1 cm pathlength quartz cuvette (Cary 300, Varian, Australia) to estimate differences in wine total phenolics.

5.7 Tannin composition in wines

Tannin was purified from wine samples using the modified solid phase extraction described by Kassara and Kennedy (2011). Briefly, an Oasis HLB 3cc extraction cartridge (Waters, Rydalmere, NSW, Australia) was conditioned with methanol and water successively. A 1 mL aliquot of centrifuged wine was loaded onto the cartridge. Monomeric phenolics were eliminated with 20 mL acetonitrile, then the tannin was eluted and collected by washing the cartridge with 0.3 mL formic acid followed immediately by 2.7 mL 95% (v/v) methanol:water. The tannin fractions were transferred immediately into a Turbo Vap. LV Evaporator (Zymark, Hopkinton, MA, USA) and dried with N_2 flow at 30ºC. Recovered tannin material was reconstituted in pure methanol to approximate a concentration of 10 g/L (by MCP) and stored at -20ºC until analysed. Gel permeation chromatography analysis was conducted following the method of Kennedy and Taylor (2003) with the modifications reported previously (Bindon and Kennedy

2011). Purified tannin was diluted with four volumes of HPLC mobile phase (dimethyl formamide solution containing 1% acetic acid and 0.15 mol/L lithium chloride), then 20 µL was injected to a series of two connected PLgel columns (300 x 7.5 mm, 5 µm, one 100 Å and one 500 Å) and the tannin was eluted at a 1 mL/min flow rate at 50ºC with detection at 280 nm, as described previously (Kennedy and Taylor 2003, Bindon and Kennedy 2011). The purified tannin fraction was also analysed by phloroglucinolysis (Kennedy and Jones 2001) with the HPLC modifications described previously (Kennedy and Taylor 2003). Briefly, a 25 µL aliquot of tannin in methanol was well mixed with 25 µL of phloroglucinol solution (100 g/L phloroglucinol, 20 g/L ascorbic acid and 0.64% HCl in methanol). Tannin was then depolymerised at 50ºC for 25 minutes and cooled on ice. The solution was neutralised with 150 µL sodium acetate solution (70 mM) and 20 µL was loaded on a 2-series connected reverse-phase column (100 x 4.6 mm, Chromolith® Performance RP-18e, Merck, Bayswater, Victoria, Australia). The structural subunits were eluted using the gradient solvent system described previously (Kennedy and Taylor 2003). For phloroglucinolysis, (-)-epicatechin (Sigma Aldrich, St. Louis, MO, USA) was the quantitative standard.

5.8 Wine volatiles.

Yeast volatile fermentation products were analysed using HS-SPME–GCMS, with SIDA as described previously (Siebert et al. 2005). Twenty-seven compounds, including ethyl and acetate esters, higher alcohols and volatile acids were quantified.

5.9 Wine polysaccharide concentration and composition

A 1 mL aliquot of wine was added to 5 mL of absolute ethanol and allowed to precipitate at 4°C for 18 h. Samples were centrifuged at 8000 *g* for 5 min, before the supernatant was discarded. The pellet was washed with 5 mL ice-cold 80% v/v ethanol, re-centrifuged, and the recovered pellet allowed to air dry briefly to remove excess ethanol. The pellets were reconstituted in Milli-Q water, frozen at −20°C and lyophilised. Lyophilised samples were reconstituted in 2 M TFA and hydrolysed at 100°C for 3 h. Hydrolysates were cooled on ice, and thereafter concentrated under vacuum at 30°C in a Heto vacuum centrifuge (Heto-Holten A/S, Allerod, Denmark) and resuspended in Milli-Q water. Monosaccharides released from polysaccharides following hydrolysis were quantified using an adaptation of a published method (Honda et al. 1989). Internal standard was added to a final concentration of 0.3 M of ribose and deoxy-glucose (Sigma-Aldrich, St. Louis, MO, USA). The derivatising reagent was 0.5 M of methanolic 1-phenyl-3-methyl-5-pyrazolone (PMP) (Sigma-Aldrich, St. Louis, MO, USA) in 1 M NH_4OH. For the derivatisation, 25 µL of sample containing internal standard was mixed with 96.2 µL of derivatising reagent and placed in a heating block at 70ºC for 1 h. After this step, the samples were cooled on ice and neutralised with formic acid. Then, the samples were extracted twice with dibutyl ether (Sigma-Aldrich, St. Louis, MO, USA) and the upper layer was discarded. The excess of dibutyl ether was dried under vacuum at room temperature. PMP-monosaccharide derivatives were quantified at 250 nm by HPLC using a C18 column (Kinetex, 2.6 µm, 100 Å, 100 × 3.0 mm) protected with a guard cartridge (KrudKatcher Ultra HPLC in-line filter, 0.5 µm) (Phenomenex, Lane Cove, NSW, Australia). The mobile phases were solvent A, 10% (v/v) acetonitrile in 40 mM aqueous ammonium acetate, and solvent B, 70% (v/v) acetonitrile in water. The following linear gradient was used: for solvent A (with solvent B making up the remainder) 92% at 0 min; 84% at 12 min; to 0% at 12.5 min; 0% at 14 min, then returning to the starting conditions at 14.5−18.5 min, 92%. A flow rate of 0.6 mL/min was used with a column temperature of 30°C. The PMP-monosaccharide derivatives were identified and quantified using commercial standards (Sigma-Aldrich, St. Louis, MO, USA). Polysaccharide was quantified as the sum of the monosaccharides.

5.10 Wine protein concentration and composition.

The composition and concentration of haze-forming proteins in must and wines were assessed in Chardonnay wines using high performance liquid chromatography (HPLC) performed as previously described (Culbert et al. 2017) with some modifications. Separation was achieved using Agilent 1260 UHPLC with a Prozap Expedite C18 column (10 mm x 2.1 mm i.d., Grace technologies), a solvent system of 0.1% TFA/H_2O (Solvent A) and 0.1%TFA/ACN (Solvent B), at 0.75 mL/min and 35°C column

temperature. The mobile phase gradient was: 0-1 min 10-20% Solvent B, 1-4 min 20-40% B, 4-6 min 40-80% B, 6-7 min 80% B, 7.01 min 10% B, and 7-10 min 10% B. Detection of proteins was achieved by Diode Array monitoring at 210 nm. Identification of thaumatin-like proteins (TLPs) and chitinases was achieved by comparing the retention times of sample peaks with isolated standards and quantitation was achieved by comparing the peak areas with those of a standard curve of thaumatin (15 mg/L to 500 mg/L) (Sigma, Australia).

5.11 Meta-analysis of sugar accumulation and berry weight data.

A systematic search of the literature was performed to find Australian-based studies that assessed the ripening of grape berries using the Scopus (https://www.scopus.com/) database that is maintained by Elsevier (Amsterdam, The Netherlands). The search was completed on 16 March 2018, and there was no restriction imposed on the year of publication, language or scientific subject area. A Boolean search was completed using the following combination of search terms: ((viti* or grape*) and (matu* or ripe*)) and affiliation Austral*); where the asterisk denoted that the balance of the search term could be comprised of any characters. A list of 411 potential articles was generated and the articles were initially reviewed using the Scopus interface on the basis of the title and abstract, which resulted in the selection of 144 articles. Each article was inspected and included in the study based on the following criteria:

i) the research was completed in Australia
ii) the study was completed in the field as opposed to a glasshouse or other protected cropping system
iii) the study contained three or more records of berry weight and sugar concentration for at least one treatment, within one season.

This resulted in 36 articles that provided data for the analysis, listed as an appendix separate from the general reference list (Appendix Table 13.4). When data was presented in a tabular form it was transcribed into a spreadsheet; when it was presented in graphical form the image was copied from the portable document format file using the 'take a snapshot' function from Adobe Acrobat Reader (Adobe Systems Incorporated, San Jose, CA, USA). The data was extracted from the image of the graph using GraphGrabber V2.0 (Quintessa Limited, Henley-on-Thames, Oxfordshire, UK). Data was only extracted from the control treatment. The cultivar, date and location of the trial were also recorded, giving a total of 47 datasets containing unique cultivar, by season, by location combinations. Data were collected across a wide range of cultivars; however, there were only enough results to analyse Shiraz (n = 220) and Cabernet Sauvignon (n = 97).

Sugar per berry was calculated by multiplying the treatment means of the percent total soluble solids (ºBrix) by the berry mass. Where the berry sugar concentration was presented in ˚Baumé this was converted to ˚Brix by multiplying by 1.8. To allow the full range of berry sizes to be compared and avoid the dataset being skewed by the trials with the larger berries (e.g. due to more severe pruning), the sugar per berry data was standardised by dividing the results for a treatment by year combination by the last sample collected. This meant that values close to one represented minimal change in sugar per berry, relative to the final sample date. Boundary-line analysis (Webb 1972) was used to estimate the point beyond which sugar per berry no longer increased. The boundary line was calculated using a method similar to Sadras and Petrie (2011), where the entire dataset was divided into 0.56˚Baumé (1˚Brix) sections and the 10th percentile for each section was calculated. The regressions were then completed on the first decile values and the sugar concentration (˚Brix) where the sugar content reached 100% of the final value denoted the point where berries stopped accumulating sugar.

5.12 Impact of climate change on pH and TA, and their balance with sugar concentration

A commercial dataset was analysed that was made up of samples of Shiraz and Cabernet Sauvignon grown in the Barossa Valley and Coonawarra between 1993 and 2018. The key variables derived from this dataset were:

(i) date when fruit reached 12°Baume, from sequential sampling during the ripening period
(ii) pH when this data occurred
(iii) titratable acidity (TA) when this date occurred.

Company representatives or vineyard staff collected maturity samples at approximately weekly intervals in the period leading up to harvest. Samples were taken from both exposed and shaded fruit from both sides of the canopy across the block, following a method similar to that described by Krstic et al. (2003). Approximately 30 bunches per block were sampled to account for the typical variation of a vineyard block. Sugar concentration, pH and TA of the fruit was measured in the winery laboratory. At the winery laboratory, juice was normally extracted using a small pneumatic press, and analysis was completed using a temperature-compensating digital refractometer and auto-titrator or, in more recent seasons, a WineScan FTIR instrument (Hilleroed, Denmark). Company standard operating procedures currently target the collection of a minimum of four maturity samples per individual block prior to harvest; this means that sampling would normally start approximately one month before a block was harvested.

All data were collected and held in a vineyard and winery management software database (Total Systems for Management Pty Ltd, Unley, Australia). Reports were extracted from the database containing information on the location of the vineyard blocks and the grape variety, the sample date and sugar concentration, pH and TA from sequential maturity samples. Multiple maturity samples from a block on a given date were averaged. The date of designated maturity (12°Baume) for each individual block was calculated from the linear interpolation of sequential samples. The estimated pH and TA on the date of maturity were also calculated using linear interpolation, from the values that corresponded with the maturity samples above and below 12 Baume. Rates of change of the three dependent variables (the date of designated maturity, and the pH and TA at the date of designated maturity) were calculated using linear regression against the independent variable (vintage year).

5.13 Statistical analysis.

For Shiraz wines from the 2017 vintage experiment, performance of the sensory panel was assessed using Fizz software and R with the SensomineR (sensominer.free.fr/) and FactomineR (factominer.free.fr/) packages. For Chardonnay wines in the 2018 trial, sensory panel performance was assessed using Compusense software instead of Fizz, but was otherwise the same as for the Shiraz sensory data. The performance assessment included analysis of variance for the effect of judge, wine and presentation replicate and their interactions, degree of agreement with the panel mean, degree of discrimination across samples and the residual standard deviation of each judge by attribute. The judges were found to be performing to an acceptable standard. Analysis of variance (ANOVA) of chemical and sensory data was carried out using Minitab 18 (Minitab Inc., Sydney, NSW) or by JMP 15 (JMP Australia & New Zealand, Lane Cove, NSW, Australia) software, using Tukey's post-hoc test to determine significant differences where applicable. For the 2019 trial on Shiraz, paired T-tests were used to compare grape samples from discrete blocks at the two harvest dates in Excel (Windows 10). Wine chemical analyses from the same trial were compared in JMP 15 using a randomised block design, and model fitted by standard least squares with effect screening. For the sensory data, the effects of treatment, judge, judge by treatment, winemaking replicate nested into treatment, judge by winemaking replicate nested into treatment, and presentation replicate nested into treatment and winemaking replicate were assessed, treating judge as a random effect. Principal component analysis (PCA) was conducted using XLSTAT 2017 (Paris, France) or Unscrambler 10.3 or 11 (CAMO Software, Oslo, Norway) software packages. Partial least squares (PLS) regression analysis was also performed for the prediction of sensory attributes from wine compositional data using Unscrambler software. All PCA and PLS analyses were performed with full cross validation. The data for the meta-analysis of sugar accumulation and berry weight was compiled into Excel and the regression analysis was completed using Sigmaplot 14.0 (Systat Software, Inc, San Jose). The data for the impact of pH and TA was processed using Access (Windows 10), and the regression analysis was completed using Sigmaplot.

6. Results and discussion

6.1 Results of the 2017 vintage trial comparing outcomes of ripening and water addition on Shiraz wine

6.1.1 Outcomes of grape ripeness and water addition on final ethanol

All of the ferments proceeded rapidly, and were below a detectable ºBaume level within seven days. The H-1 ferment had a shorter lag phase than the other treatments, and was below a detectable ºBaume level within six days. Water addition decreased the lag phase of the fermentations slightly, but thereafter the rate of sugar decrease for the H-3 and diluted treatments converged (data not shown). The temperature of all the treatments increased during the fermentation and had plateaued by day 5 (data not shown). While the H-1 and H-2 treatments had a slightly lower maximum temperature (22 – 23ºC) than all of the H-3 treatments, none of the ferments exceeded 25ºC. The final ethanol concentrations for the wines post-ferment are shown in Table 1. All data in this section have been previously published (Teng et al. 2020).

Table 6.1.1. Proportion of juice removed or water added in the direct water addition, juice substitution and saigneé treatments showing the corresponding concentrations of must sugar and final wine ethanol†

Treatment‡	Volume of juice removed (L)	Percentage water addition (%)§	Calculated sugar content after treatment（Baumé）	Decrease in total soluble solids （Baumé）	Ethanol concentration (%)¶
H-1	0	0	13.4	0	13.9±0.1e
DA-1	0	14.3	13.4	2.2	14.8±0.1c
RR-1	5	16.6	13.4	2.2	14.3±0.1d
RO-1	5	0	15.6	0	16.2±0.2a
H-2	0	0	14.4	0	15.2±0.1b
DA-2	0	7.4	14.4	1.2	15.1±0.2bc
RR-2	2.4	8.0	14.4	1.2	15.2±0.2b
RO-2	2.4	0	15.6	0	16.0±0.2a
H-3	0	0	15.6	0	16.1±0.1a

†: Each ferment consisted of 45 kg of grapes and the volume of juice removed or water added was calculated based on 66% of extraction rate (30 L wine for each ferment)
‡: H-1, H-2, H-3 = harvest-1, harvest-2 and harvest-3; DA-1, DA-2 = direct water addition treatment 1 and direct water addition treatment 2; RR-1, RR-2 = juice substitution treatment-1, juice substitution treatment 2; RO-1, RO-2 = saigneé treatment 1 and saigneé treatment 2
§: Percentage water addition calculated based on the proportion of water added to expected total volume of wine
¶ Data presented show mean values ± standard error, the values for wine ethanol concentration were compared by using one-way ANOVA where $P<0.05$, $n=3$, and differences between treatments were determined by a post-hoc Tukey's test with significance indicated by different letters.

The final ethanol concentrations of the DA-2 and RR-2 wines were the same as that of H-2, which had the equivalent target pre-ferment ºBaume. However, the final ethanol concentrations of the H-3 treatments which received a larger quantity of water, DA-1 and RR-1, were higher than that of the H-1 wine. It was evident from the fermentation kinetic curves that the sugar concentration in the DA-1 and RR-1 treatments increased slightly to day 2, and thereafter decreased. This may have indicated that some additional extraction of sugar occurred from the grape solids, such that the target ºBaume of 13.6 was not reached following water addition. A further possible explanation is that the actual extraction rate (based on the ratio of liquid (juice) to solids (skin and seeds), which was unknown when the water addition was made, was lower than the estimated extraction rate of 66% used to calculate the water additions. That is, if a lower actual extraction rate occurred, the final ethanol would be higher than expected.

6.1.2 The impact of grape ripeness on grape and wine tannin, phenolics and colour

The results showed that differences in the concentration of total phenolics and tannin in wine could be observed between wines made from the three ripeness levels, and these relative differences were retained from pressing through to the end of malolactic fermentation (Table 6.1.2.1 and Table 6.1.2.2). The total phenolic concentration in H-1 and H-2 wines were respectively found to be 36% and 30% lower than that of the H-3 wine at pressing. (Table 6.1.2.2). The degree of grape ripeness more strongly affected the concentration of tannin than total phenolics in wines. In comparison with the H-3 treatment, the tannin concentration in the H-1 and H-2 wines was 59% and 52% lower at pressing respectively, and these differences were retained at the end of malolactic fermentation, although a slight loss in tannin concentration occurred over time. Tannin compositional analysis revealed that wine tannin from H-1 and H-2 had a significantly lower molecular weight (and corresponding mean degree of polymerisation, mDP) than observed for wine tannin from the H-3 treatment, which was confirmed using both the phloroglucinolysis (subunit composition) and GPC (size distribution) techniques (Table 6.1.2.3). The proportion of epigallocatechin extension units decreased when the tannin composition of H-1 and H-2 wines was compared with that of the H-3 wines, a similar trend to the effects on tannin size and mDP for these treatments. The observed increase in wine tannin may have been due to an increase in tannin extractability in response to changes in grape subcellular structure (Bindon et al. 2013) but it is nonetheless important to note that a reduction in berry volume (shrivel or dehydration) during ripening may also result in increased wine tannin concentration (Schelezki et al. 2018b). Quantification of extractable grape tannin showed that the total extractable tannin increased between H-1 and H-3 (Table 6.1.2.4). An R^2 value of 0.8 (data not shown) was found for the relationship between the uncorrected measure of extractable grape tannin (that accounts for the contribution of berry volume differences to the extractable tannin measure) and wine tannin concentration found at the end of malolactic fermentation (data not shown). For the volume-corrected measure of extractable tannin, a slightly weaker relationship with wine tannin concentration was found ($R^2 = 0.76$, data not shown). Since berry weight was constant between H-1 and H-2, decreasing slightly to H-3 (13%) the increase in wine tannin concentration in H-3 was likely to be due both to increased extractability from the grape itself, and the concentrating effect of berry volume loss resulting from shrivel or dehydration.

An increase in extractable anthocyanin was also observed across the different ripeness levels, and unlike the trend observed for tannin described above, was independent of berry volume (Table 6.1.2.4). A clear difference was seen in wine colour density across the wines made from grapes harvested at different ripeness levels, increasing from H-1 to H-3 (Tables 6.1.2.1 and 6.1.2.2). An $R^2 \geq 0.96$ was found for the relationship between extractable anthocyanin and wine colour. Non-bleachable pigment in wine increased from H-1 to H-3 when determined at pressing. However, increases in non-bleachable pigment were found from the point of pressing to the end of malolactic fermentation, which were more remarkable for H-3 than H-1 and H-2. Therefore, the higher wine tannin concentration in H-3, together with higher concentrations of anthocyanin may have facilitated the rapid development of non-bleachable pigments. Further consideration was given to the visual properties of the wines made at the different harvest points, based on the values for hue (the ratio of brown to red pigments). A higher value for hue generally reflects a browner colour. At the end of malolactic fermentation the hue values of the early-harvest treatments H-1 and H-2 were equivalent to each other but lower than H-3, suggesting that H-3 had a browner visual character.

Table 6.1.2.1 Phenolic, tannin and colour measures in ferments at pressing showing the proportional change relative to the control treatment (H-3)[†]

Treatment[‡]	Total phenolics		Tannin		Non-bleachable pigment		Colour density		Hue		Total anthocyanin	
	(A.U.)	(%)[§:]	(mg/L)	(%)	(A.U.)	(%)	(A.U.)	(%)	Ratio	(%)	(mg/L)	(%)
H-1	31.16±0.38^{f}	-36	419±43^{d}	-59	0.74±0.02^{f}	-48	8.07±0.15^{g}	-36	0.426±0.006^{e}	-9	601±10^{e}	-26
DA-1	40.48±0.71^{d}	-17	775±15^{bc}	-24	1.15±0.03^{d}	-20	10.48±0.27^{e}	-16	0.452±0.002^{c}	-3	682±16^{c}	-16
RR-1	40.48±0.72^{d}	-17	744±9^{c}	-27	1.16±0.05^{d}	-20	10.67±0.20^{e}	-15	0.446±0.001^{d}	-5	696±11^{c}	-14
RO-1	51.54±2.17^{a}	6	1082±33^{a}	6	1.57±0.08^{a}	9	13.86±0.61^{a}	11	0.464±0.001^{a}	-1	864±33^{a}	7
H-2	33.91±0.99^{e}	-30	492±26^{d}	-52	0.92±0.02^{e}	-36	8.61±0.15^{f}	-31	0.443±0.001^{d}	-5	622±11^{e}	-23
DA-2	43.99±1.68^{c}	-10	854±31^{b}	-16	1.27±0.03^{c}	-12	11.48±0.44^{d}	-8	0.454±0.004^{bc}	-3	749±29^{d}	-8
RR-2	43.00±0.92^{c}	-12	809±69^{bc}	-20	1.25±0.03^{c}	-14	11.23±0.17^{d}	-10	0.453±0.001^{bc}	-3	728±20^{d}	-10
RO-2	49.77±1.17^{ab}	2	1015±13^{a}	0	1.49±0.03^{b}	3	13.33±0.25^{b}	6	0.457±0.002^{b}	-2	844±20^{a}	4
H-3	48.68±1.24^{b}	-	1017±36^{a}	-	1.44±0.04^{b}	-	12.53±0.31^{c}	-	0.467±0.005^{a}	-	810±15^{b}	-

[†]Data presented show mean values ± standard error, treatments were compared using one-way ANOVA where P<0.05, n=3, and differences between treatments were determined by a post-hoc Tukey's test with significance indicated by different letters

[‡] H-1, H-2, H-3 = harvest-1, harvest-2 and harvest-3; DA-1, DA-2 = direct addition treatment 1 and direct addition treatment 2; RR-1, RR-2 = juice substitution treatment-1, juice substitution treatment 2; RO-1, RO-2 = saigneé treatment 1 and saigneé treatment 2

[§:] Percentage difference from H-3 control.

Table 6.1.2.2 Phenolic, tannin and colour measures in wines at the end of malolactic fermentation showing the proportional change relative to the control treatment (H-3)[†]

Treatment[‡]	Total phenolics		Tannin		Non-bleachable pigment		Colour density		Hue		Total anthocyanin	
	(A.U.)	(%)[§:]	(mg/L)	(%)	(A.U.)	(%)	(A.U.)	(%)	Ratio	(%)	(mg/L)	(%)
H-1	26.82±0.91^{f}	-30	268±5^{e}	-63	0.84±0.02^{f}	-64	8.84±0.30^{g}	-33	0.449±0.005^{e}	-23	468±16^{c}	-20
DA-1	38.44±0.84^{d}	-5	525±1^{c}	-27	1.38±0.07^{e}	-41	11.45±0.39^{e}	-14	0.485±0.017^{d}	-17	620±29^{a}	6
RR-1	38.24±0.65^{d}	-5	504±30^{c}	-30	1.39±0.07^{e}	-40	11.55±0.19^{de}	-13	0.472±0.004^{d}	-19	630±13^{a}	8
RO-1	45.09±1.32^{a}	10	912±89^{a}	27	3.03±0.39^{a}	30	14.60±0.75^{a}	10	0.599±0.026^{ab}	2	578±36^{b}	-1
H-2	31.08±1.29^{e}	-21	383±21^{d}	-47	1.01±0.02^{f}	-56	10.59±0.21^{f}	-20	0.441±0.002^{e}	-25	561±20^{b}	-4
DA-2	40.30±0.95^{c}	-1	598±20^{c}	-17	1.60±0.07^{de}	-31	12.19±0.31^{c}	-8	0.506±0.003^{c}	-13	633±13^{a}	8
RR-2	39.98±0.69^{cd}	-1	574±23^{c}	-20	1.69±0.08^{d}	-28	12.16±0.34^{cd}	-8	0.511±0.002^{c}	-12	617±11^{a}	6
RO-2	43.05±1.11^{b}	6	822±26^{a}	14	2.71±0.06^{b}	16	13.79±0.34^{b}	4	0.603±0.005^{a}	3	563±17^{b}	-4
H-3	40.55±2.21^{c}	-	719±6^{b}	-	2.33±0.15^{c}	-	13.29±0.63^{b}	-	0.585±0.010^{b}	-	585±16^{b}	-

[†]Data presented show mean values ± standard error, treatments were compared using one-way ANOVA where P<0.05, n=3, and differences between treatments were determined by a post-hoc Tukey's test with significance indicated by different letters

[‡] H-1, H-2, H-3 = harvest-1, harvest-2 and harvest-3; DA-1, DA-2 = direct addition treatment 1 and direct addition treatment 2; RR-1, RR-2 = juice substitution treatment-1, juice substitution treatment 2; RO-1, RO-2 = saigneé treatment 1 and saigneé treatment 2

[§:] Percentage difference from H-3 control.

Table 6.1.2.3. Average molecular weight, degree of polymerisation and subunit composition of tannins in ferments where samples were taken at pressing †

Treatment‡	Molecular mass by subunit (g/mol) §	Molecular mass by GPC (g/mol) ¶	mDP††	Mass conversion (%)‡‡	Subunit composition (mole %)§§						
					EGC (ext.)	C (ext.)	EC (ext.)	ECG (ext.)	C (ter.)	EC (ter.)	ECG (ter.)
H-1	1313±128^{d}	1065±28^{d}	4.37±0.43^{d}	23.2±1.5^{cd}	19.4±1.8^{e}	3.9±0.1^{a}	51.1±2.5^{ab}	3.9±0.2^{cd}	15.3±0.9^{a}	6.0±0.2^{a}	1.1±0.1^{a}
DA-1	1828±62^{bc}	1266±21^{b}	6.04±0.21^{bc}	29.5±3.0^{a}	28.0±0.5^{d}	2.6±0.1^{cd}	48.3±0.5^{bc}	4.5±0.0^{ab}	10.9±0.4^{b}	4.9±0.2^{bc}	0.7±0.1^{bc}
RR-1	1836±138^{bc}	1275±41^{b}	6.07±0.46^{bc}	23.8±0.3^{bcd}	30.8±2.5^{cd}	2.9±0.2^{c}	45.6±1.1^{cd}	4.2±0.4^{bc}	10.6±1.0^{bc}	5.0±0.2^{b}	0.7±0.0^{bc}
RO-1	2301±106^{a}	1449±28^{a}	7.60±0.36^{a}	30.2±1.7^{a}	34.4±0.9^{b}	2.3±0.1^{d}	46.2±1.3^{cd}	4.0±0.1^{cd}	8.7±0.5^{d}	3.9±0.2^{de}	0.6±0.0^{cde}
H-2	1521±16^{cd}	1163±15^{c}	5.05±0.05^{cd}	18.8±0.1^{d}	20.0±0.6^{e}	3.4±0.2^{b}	52.6±0.7^{a}	4.2±0.2^{abc}	13.9±0.3^{a}	5.1±0.2^{b}	0.8±0.0^{b}
DA-2	2076±189^{ab}	1319±46^{b}	6.86±0.63^{ab}	30.1±2.5^{a}	32.6±0.6^{bc}	2.6±0.2^{cd}	46.0±1.7^{cd}	4.2±0.2^{bc}	9.6±0.6^{bcd}	4.7±0.3^{bc}	0.6±0.0^{de}
RR-2	2128±29^{ab}	1329±22^{b}	7.03±0.10^{ab}	29.1±1.9^{ab}	33.9±0.4^{bc}	2.7±0.0^{cd}	45.3±0.5^{cd}	4.0±0.1^{bcd}	9.2±0.3^{cd}	4.4±0.1^{cd}	0.7±0.0^{bcd}
RO-2	2402±148^{a}	1342±23^{b}	7.94±0.49^{a}	29.3±2.9^{ab}	37.9±0.2^{a}	2.4±0.0^{d}	43.6±0.7^{d}	3.5±0.1^{d}	8.4±0.4^{d}	3.5±0.1^{e}	0.6±0.1^{cde}
H-3	2166±204^{ab}	1323±33^{b}	7.15±0.68^{ab}	27.8±1.4^{abc}	27.8±0.5^{d}	2.6±0.2^{cd}	50.8±1.1^{ab}	4.7±0.2^{a}	9.5±0.7^{bcd}	3.7±0.2^{e}	0.5±0.0^{e}

†Data presented show mean values ± standard error; treatments were compared using one-way ANOVA where P<0.05, n=3, and differences between treatments were determined by a post-hoc Tukey's test with significance indicated by different letters

‡ H-1, H-2, H-3 = harvest-1, harvest-2 and harvest-3; DA-1, DA-2 = direct addition treatment 1 and direct addition treatment 2; RR-1, RR-2 = juice substitution treatment-1, juice substitution treatment 2; RO-1, RO-2 = saigneé treatment 1 and saigneé treatment 2

§:Molecular mass determined by using subunit composition from phloroglucinolysis

¶: Molecular mass determined at 50% elution by gel permeation chromatography (GPC)

††Mean degree of polymerisation

‡‡Mass conversion based on % recovery of proanthocyanidin by phloroglucinolysis as a proportion of tannin concentration by methyl cellulose precipitation

§§EGC=epigallocatechin; C=catechin; EC=epicatechin; ECG=epicatechin gallate; ter.=terminal unit; ext.=extension unit.

Table 6.1.2.4. Berry weight, extractable anthocyanin and extractable tannin in Shiraz grapes harvested at different ripeness stages.

	H-1‡	H-2	H-3
Harvest date	17-03-2017	28-03-2017	07-04-2017
Berry weight (g/berry)	1.22±0.04[a]	1.29±0.03[a]	1.12±0.02[b]
Extractable anthocyanin (mg/g, uncorr.) [§]	0.33±0.02[c]	0.40±0.01[b]	0.46±0.03[a]
Extractable anthocyanin (mg/g, vol. corr.) [¶]	0.40±0.03[c]	0.47±0.02[b]	0.52±0.04[a]
Extractable tannin (mg/g, uncorr.) [§]	0.40±0.05[b]	0.54±0.02[ab]	0.61±0.06[a]
Extractable tannin (mg/g, vol. corr.) [¶]	0.47±0.05[b]	0.63±0.02[ab]	0.69±0.05[a]

[†]: Data were obtained by mean values and shown as mean ± standard error, the values were compared by using one-way ANOVA where P<0.05, n=3, and their differences were determined by a post-hoc Tukey's test meanwhile indicated by different letters

‡: H-1, H-2, H-3 = harvest-1, harvest-2, harvest-3

[§]Extractable tannin or anthocyanin determined per g berry weight assuming a constant juice volume yield per berry (i.e. uncorrected)

[¶]: Extractable tannin or anthocyanin determined per g berry weight corrected using a precise juice volume yield per berry.

6.1.3 Implications of water addition and saigneé on wine tannin, phenolics and colour

The results demonstrate that the concentration of total phenolics and tannin was significantly lowered relative to the H-3 control by all the water addition treatments, both at the end of fermentation (Table 6.1.2.1) and after losses of phenolic material had occurred at the end of malolactic fermentation (Table 6.1.2.2). It was interesting to note that neither the quantity of water added, nor the mode of addition affected wine tannin concentration. Tannin composition was only minimally affected by water addition, with the molecular mass and mDP (by phloroglucinolysis) being reduced only in treatments with the highest water addition (Table 6.1.2.3). Tannin compositional attributes which changed more consistently with water addition were the proportion of extension epigallocatechin subunits, which increased, while the proportion of extension epicatechin-subunits decreased. At pressing, wine total anthocyanin, colour density and non-bleachable pigments differed according to the extent of water addition but not according to the mode of water addition, and were in the following order: H-3>DA-2=RR-2>DA-1=RR1. Smaller differences in wine hue were found at pressing, with both water addition treatments having slightly lower hue values (less brown) than the H-3 wines. Between pressing and the end of malolactic fermentation, the reduction in total anthocyanin was associated with a concomitant increase in non-bleachable pigments. It was interesting to observe that similar to the observations for the early-harvest treatments (H-1 and H-2) the reduction in total anthocyanin over time was less marked in diluted wines that in H-3. As a result, total anthocyanin at the end of malolactic fermentation was not different between the respective water addition treatments, and was somewhat higher than the H-3 control. The corresponding wine colour density measured in the water addition treatments increased only slightly from pressing to the end of malolactic fermentation, meaning that the initial differences between H-3 and the water addition treatments were retained. However, the development of non-bleachable pigments remined constant for the wines that had received water addition, while for H-3 they increased to the end of malolactic fermentation. This was similar to what was observed for H-1 and H-2, as discussed previously. As a result, the proportional difference in non-bleachable pigments between the water addition treatments and H-3 wines was far greater at the end of malolactic fermentation than at pressing Therefore, the contribution of non-bleachable pigments to wine colour density in H-3 wines was proportionally higher than for diluted wines. Monomeric anthocyanin would have had a more significant impact on wine colour density in the diluted wines than H-3, suggesting that these treatments aged more slowly.

Two additional saigneé treatments RO-1 and RO-2 where juice was removed without water addition were also included in the study (Table 6.2.1.1 and 6.2.1.2) in order to account for changes in wine composition introduced by altering the must volume to solids (skins and seeds) ratio across the various treatments. The volume of juice removed from the RO-1 and RO-2 saigneé treatments matched that of the RR-1 and RR-2 treatments, respectively. It was observed that at pressing, small increases in wine phenolics and non-bleachable pigment were found only for the highest saigneé treatment (RO-1) relative to H-3, with tannin concentration being unchanged. Wine colour density, on the other hand,

increased consistently as the extent of saigneé increased. Monomeric anthocyanin was higher in both saigneé treatments relative to H-3, but was not significantly different. After pressing, until the completion of malolactic fermentation, losses in total phenolics and tannin as well as increases in non-bleachable pigments were observed, similar to the trend observed for H-3, as discussed previously. Losses in total phenolics could most likely be attributed to a decline in monomeric anthocyanin, which was greater for the saigneé treatments, such that anthocyanin in RO-1 and RO-2 was not different from H-3 at the end of malolactic fermentation. The decrease in tannin over time from pressing to the end malolactic fermentation was less marked for the saigneé treatments than for H-3. This resulted in increased tannin concentration in RO-1 and RO-2 relative to H-3, with no difference between the two saigneé treatments. Differences in wine colour density between the treatments were maintained from pressing to the end of malolactic fermentation, but non-bleachable pigments increased markedly from H-3 to RO-1 and RO-2. The hue of the RO-1 and RO-2 treatments was slightly elevated relative to H-3, and this resulted in a slightly browner visual colour (not shown).

6.1.4 Composition and sensory properties of wines determined after 12 months ageing

The data in this section have been previously published (Petrie et al. 2019; Bindon et al. 2020). Up to the point of bottling, the titratable acidities of the final wines were successfully managed to meet the relatively narrow target range of 4.6 – 5.1 g/L and this resulted in a pH range of 3.5 – 3.8 across all the treatments (data not shown). The increase in wine tannin, non-bleachable pigments and wine colour density in wine at the end of fermentation as found with the transition of harvest date from H-1 to H-3 discussed above was maintained in wine after 12 months of ageing (Table 6.1.3.1). Similarly, the reduction in wine tannin concentration, non-bleachable pigments and colour density brought about by both water addition treatments was also maintained at 12 months. Small increases in polymeric pigments occurred for all wines, and the associated losses in monomeric anthocyanin were expected with ageing. It was interesting to note that the saigneé treatment with the lowest juice runoff (RO-2) was equivalent to the H3 control for tannin and non-bleachable pigment measures, which were previously observed to be higher for RO-2. This was interesting to note, since other researchers have also reported a narrowing of initial gains in phenolics by the use of saigneé after ageing (Godden, 2019).

Other compositional analyses performed at the 12-month time point were for wine polysaccharides and fermentation products. In terms of total wine polysaccharides (Table 6.1.3.2), it was found that differences between treatments were minor, with the exception that the saigneé treatment with the largest juice run-off (RO-1) had an elevated polysaccharide concentration relative to certain treatments. Differences in polysaccharide concentration for RO-1 were evident for most of the component monosaccharides, indicating that the higher polysaccharide concentration was a not driven by differences in specific polysaccharide sub-classes. Importantly, the difference between RO-1 and the H-3 wine was not significant for either total polysaccharide, or the component monosaccharides. Differences in total polysaccharide were not found between the wines made from the three harvest time points. However, from H1 to H3, increases in component monosaccharides glucose, xylose and arabinose were found. Glucose-based polysaccharides could have been derived from either yeast or grape, while differences in xylose- and arabinose-based polysaccharides indicate increased extraction and retention of grape hemicellulose components. Previous studies with Cabernet Sauvignon have reported variable effects of grape ripening on grape-derived polysaccharides, which may either increase or decrease in wine with a variation in harvest date (Bindon et al. 2013; Schelezki et al. 2018a). This potentially indicates that seasonal and site-specific effects exist in terms of polysaccharide extractability from the grape during ripening. Both studies showed that yeast-derived mannoprotein increased in wine with later harvests, which was not clearly shown in the current study on Shiraz. The study by Schelezki et al. (2018a) included a water addition component in addition to investigating ripening, and found that polysaccharide concentration was increased only when juice was substantially substituted with water (44% substitution), but no change was found for smaller quantities of water addition, similar to those in the current study (<27% substitution). Fermentation products detected in the wines are shown in Table 6.1.3.3. As found for polysaccharides, fermentation products generally did not differ widely among the samples; however, for a sub-set of variables which were significantly different there were notable treatment effects. It was of interest to note that H-2 wines differed substantially from the other treatments, including H-1, being higher in C-6 alcohols and esters, hexanol, hexyl acetate and ethyl

hexanoate. The H-2 wines were also higher in 2-phenylethyl acetate and 2-phenylethanol. The H-1 wines and the H-3 control, together with the water-addition treatments, were similar in their fermentation product composition, but were somewhat higher in the decanoic and octanoic volatile acids and their corresponding esters. Interestingly, both the saigneé treatments were lower in the above-mentioned compounds and somewhat higher in butanol.

Table 6.1.3.1 Wine phenolic measures after 12 months of ageing.

Treatment‡	Total phenolics	Tannin	Non-bleachable pigment	Colour density	Hue	Total anthocyanin
	(A.U.)	(mg/L)	(A.U.)	(A.U.)	Ratio	(mg/L)
H-1	24.43±0.52^{f}	232.11±20.01^{e}	1.35±0.12^{e}	7.64±0.14^{e}	0.54±0.01^{e}	340.59±10.66^{d}
DA-1	32.74±0.63^{d}	538.78±27.95^{b}	1.99±0.09^{c}	10.58±0.29^{c}	0.57±0.01^{d}	442.79±16.85^{b}
RR-1	32.95±0.46^{d}	513.55±2.29B^{c}	2.01±0.08^{c}	10.73±0.17^{c}	0.56±0.00^{de}	452.17±5.42^{ab}
RO-1	38.79±1.13^{a}	823.30±73.22^{a}	3.14±0.26^{a}	12.64±0.49^{a}	0.66±0.02^{a}	437.50±27.84^{b}
H-2	28.38±1.05^{e}	368.93±29.37^{d}	1.58±0.05^{d}	9.47±0.21^{d}	0.54±0.01^{e}	410.92±10.62^{c}
DA-2	34.62±0.89^{c}	544.91±44.99^{b}	2.04±0.10^{c}	11.03±0.29^{c}	0.59±0.00^{c}	471.50±10.71^{a}
RR-2	33.91±0.58^{cd}	548.98±12.53^{b}	2.10±0.03^{c}	10.85±0.19^{c}	0.59±0.00^{c}	453.20±9.10^{ab}
RO-2	36.84±0.91^{b}	742.68±73.40^{a}	2.75±0.07^{b}	11.82±0.40^{b}	0.64±0.00^{b}	439.13±14.04^{b}
H-3	36.75±0.52^{b}	723.99±7.29^{a}	2.62±0.05^{b}	11.75±0.23^{b}	0.64±0.00^{b}	437.78±8.94^{b}

†Data presented show mean values ± standard error, treatments were compared using one-way ANOVA where P<0.05, n=3, and differences between treatments were determined by a post-hoc Tukey's test with significance indicated by different letters

‡ H-1, H-2, H-3 = harvest-1, harvest-2 and harvest-3; DA-1, DA-2 = direct addition treatment 1 and direct addition treatment 2; RR-1, RR-2 = juice substitution treatment-1, juice substitution treatment 2; RO-1, RO-2 = saigneé treatment 1 and saigneé treatment 2

Table 6.1.3.2 Wine total polysaccharide and composition of polysaccharide-derived monosaccharides (as mg/L) after 12 months of ageing[†]

Treatment[‡]	Total polysaccharide	Mannose	Rhamnose	Glucuronic acid	Galacturonic acid	Glucose	Galactose	Xylose	Arabinose
H-1	654.78 ± 33^{abc}	141.18 ± 12^{ab}	46.86 ± 3^{bc}	8.98 ± 1^{d}	196.94 ± 3^{a}	33.70 ± 2^{c}	115.18 ± 9	8.41 ± 1^{c}	103.50 ± 8^{c}
DA-1	673.41 ± 2^{abc}	143.29 ± 1^{ab}	48.54 ± 1^{bc}	12.11 ± 1^{bcd}	167.43 ± 8^{ab}	43.58 ± 1^{bc}	120.59 ± 1	10.95 ± 0.2^{bc}	127.02 ± 3^{abc}
RR-1	674.38 ± 5^{abc}	130.9 ± 1^{ab}	51.72 ± 0.3^{abc}	10.87 ± 1^{bcd}	191.8 ± 1^{ab}	41.73 ± 2^{bc}	113.9 ± 1	10.6 ± 0.1^{bc}	122.9 ± 2^{abc}
RO-1	809.29 ± 17^{a}	172.35 ± 5^{a}	61.42 ± 1^{a}	15.22 ± 1^{a}	179.13 ± 4^{ab}	67.96 ± 1^{a}	145.39 ± 4	17.27 ± 0.4^{a}	150.52 ± 2^{a}
H-2	628.91 ± 25^{c}	123.77 ± 6^{b}	42.85 ± 2^{c}	10.55 ± 1^{cd}	175.73 ± 5^{ab}	33.60 ± 2^{c}	118.52 ± 6	8.10 ± 1^{c}	115.75 ± 4^{bc}
DA-2	638.22 ± 69^{bc}	138.05 ± 16^{ab}	48.77 ± 3^{bc}	12.15 ± 1^{abcd}	143.30 ± 21^{b}	44.46 ± 5^{bc}	117.15 ± 12	11.52 ± 1^{bc}	122.78 ± 11^{abc}
RR-2	762.65 ± 19^{abc}	155.43 ± 5^{ab}	55.63 ± 2^{ab}	13.53 ± 0.3^{abc}	198.64 ± 1^{a}	53.71 ± 5^{ab}	133.35 ± 5	14.54 ± 1^{ab}	137.79 ± 5^{ab}
RO-2	801.94 ± 1.5^{ab}	173.67 ± 3^{a}	58.92 ± 1^{a}	14.15 ± 1^{ab}	186.95 ± 2^{ab}	60.71 ± 3^{a}	142.19 ± 1	16.65 ± 0.3^{a}	148.66 ± 2^{a}
H-3	771.33 ± 35^{abc}	164.43 ± 5^{ab}	55.69 ± 2^{ab}	14.87 ± 0.3^{a}	182.72 ± 2^{ab}	58.10 ± 2^{ab}	138.80 ± 6	16.01 ± 1^{a}	140.69 ± 7^{ab}

[†]Data presented show mean values ± standard error, treatments were compared using one-way ANOVA where $P<0.05$, n=3 for all treatments except RR-1 and RO-1 where n=2 due to a sample being excluded for inconsistency. Differences between treatments were determined by a post-hoc Tukey's test with significance indicated by different letters; H-1, H-2, H-3 = harvest-1, harvest-2 and harvest-3; DA-1, DA-2 = direct addition treatment 1 and direct addition treatment 2; RR-1, RR-2 = juice substitution treatment-1, juice substitution treatment 2; RO-1, RO-2 = saigneé treatment 1 and saigneé treatment 2.

Table 6.1.3.3 Fermentation products in Shiraz wines prepared following harvest time, saigneé and water addition treatments after 12 months of ageing†

Fermentation product	unit	H-1	DA-1	RR-1	RO-1	H-2	DA-2	RR-1	RO-1	H-3	ANOVA: P value
Acetate esters											
Ethyl acetate	mg/L	16.23 ± 0.33^{c}	19.87 ± 1.9^{abc}	19.18 ± 0.27^{abc}	23.31 ± 1.90^{a}	17.72 ± 0.24^{bc}	21.58 ± 0.51^{ab}	20.60 ± 0.68^{abc}	23.28 ± 0.15^{a}	23.01 ± 0.63^{a}	<0.001
Hexyl acetate	µg/L	10.56 ± 0.62^{bc}	10.19 ± 0.63^{bc}	12.37 ± 0.57^{b}	6.67 ± 1.11^{d}	17.63 ± 0.41^{a}	10.12 ± 0.44^{bc}	10.98 ± 0.14^{bc}	6.58 ± 0.26^{d}	8.40 ± 1.16^{cd}	<0.0001
3-Methylbutyl acetate	mg/L	0.61 ± 0.0^{abc}	0.71 ± 0.02^{ab}	0.69 ± 0.02^{ab}	0.42 ± 0.09^{c}	0.71 ± 0.02^{ab}	0.75 ± 0.04^{a}	0.79 ± 0.03^{a}	0.51 ± 0.05^{bc}	0.60 ± 0.06^{abc}	<0.001
2-Methylbutyl acetate	µg/L	58.06 ± 4.42^{bc}	82.06 ± 7.43^{abc}	76.58 ± 2.90^{abc}	53.76 ± 8.69^{c}	76.39 ± 3.24^{abc}	89.55 ± 7.65^{a}	83.91 ± 4.68^{ab}	60.75 ± 4.85^{abc}	69.37 ± 6.80^{abc}	<0.01
2-Phenylethyl acetate	µg/L	75.82 ± 2.68^{b}	71.48 ± 4.19^{bc}	70.52 ± 3.83^{bc}	51.94 ± 4.32^{c}	103.19 ± 4.06^{a}	72.35 ± 4.12^{bc}	77.31 ± 0.89^{b}	61.04 ± 2.86^{bc}	62.34 ± 7.66^{bc}	<0.0001
Ethyl esters											
Ethyl butanoate	µg/L	103.26 ± 2.64	120.92 ± 4.41	126.71 ± 3.55	108.73 ± 15.9	121.48 ± 3.91	121.12 ± 11.85	136.47 ± 6.81	120.97 ± 7.87	116.79 ± 11.51	ns
Ethyl propanoate	µg/L	51.40 ± 2.52^{ab}	50.77 ± 1.04^{ab}	45.98 ± 0.64^{b}	50.44 ± 1.90^{ab}	54.71 ± 3.06^{a}	46.99 ± 0.67^{ab}	49.02 ± 0.42^{ab}	53.15 ± 1.56^{ab}	47.21 ± 2.01^{ab}	<0.05
Ethyl hexanoate	µg/L	135.42 ± 0.04^{cde}	150.11 ± 5.52^{bcd}	158.53 ± 2.15^{ab}	122.78 ± 6.37^{e}	176.71 ± 5.42^{a}	156.79 ± 0.08^{abc}	158.81 ± 5.23^{ab}	134.60 ± 1.01^{de}	138.58 ± 6.81^{bcde}	<0.0001
Ethyl octanoate	µg/L	108.53 ± 1.0^{cd}	137.46 ± 2.23^{a}	137.02 ± 0.6^{ab}	104.54 ± 2.96^{d}	135.59 ± 7.82^{ab}	136.79 ± 1.77^{ab}	127.79 ± 2.23^{abc}	116.64 ± 2.35^{bcd}	131.32 ± 8.12^{ab}	<0.0001
Ethyl decanoate	µg/L	19.22 ± 1.88^{b}	24.16 ± 0.45^{ab}	23.41 ± 0.49^{ab}	19.49 ± 0.58^{ab}	20.87 ± 2.02^{ab}	23.52 ± 0.82^{ab}	20.49 ± 0.50^{ab}	18.59 ± 0.15^{b}	25.17 ± 1.79^{a}	<0.01
Ethyl-3-methylbutanoate	µg/L	27.01 ± 2.17^{b}	34.15 ± 1.0^{ab}	34.28 ± 1.26^{ab}	27.16 ± 0.51^{b}	39.86 ± 4.01^{a}	30.30 ± 0.84^{b}	28.91 ± 0.66^{b}	31.88 ± 0.64^{ab}	28.37 ± 0.79^{b}	<0.001
Ethyl-2-methylpropanoate	µg/L	62.73 ± 3.93	61.81 ± 3.62	61.61 ± 0.95	53.47 ± 4.77	60.53 ± 3.14	56.17 ± 2.90	55.52 ± 1.88	59.10 ± 6.53	59.76 ± 2.48	ns
Higher alcohols											
Butanol	mg/L	1.32 ± 0.06^{c}	1.27 ± 0.07^{c}	1.59 ± 0.07^{bc}	1.98 ± 0.08^{ab}	1.90 ± 0.05^{ab}	1.20 ± 0.18^{c}	1.84 ± 0.14^{ab}	2.25 ± 0.04^{a}	1.58 ± 0.14^{bc}	<0.0001
2-Methylbutanol	mg/L	107.93 ± 1.70	120.47 ± 4.09	120.88 ± 4.78	106.02 ± 3.35	128.36 ± 4.23	117.84 ± 9.33	118.19 ± 3.36	118.05 ± 8.51	108.93 ± 2.52	ns
3-Methylbutanol	mg/L	233.10 ± 4.71	264.66 ± 15.04	252.90 ± 6.46	237.27 ± 9.87	267.34 ± 4.64	262.46 ± 19.06	256.74 ± 5.52	265.96 ± 17.75	238.56 ± 8.67	ns
Hexanol	mg/L	4.22 ± 0.29^{b}	4.01 ± 0.24^{b}	4.34 ± 0.20^{b}	4.53 ± 0.33^{b}	7.09 ± 0.49^{a}	4.25 ± 0.26^{b}	4.46 ± 0.10^{b}	4.74 ± 0.29^{b}	4.60 ± 0.23^{b}	<0.0001
2-Phenylethanol	mg/L	50.59 ± 3.83^{b}	58.35 ± 4.1^{ab}	56.60 ± 2.81^{ab}	52.62 ± 2.31^{ab}	63.44 ± 1.54^{ab}	54.67 ± 1.33^{ab}	54.77 ± 1.56^{ab}	64.90 ± 2.97^{a}	57.75 ± 3.6^{ab}	<0.05
2-Methylpropanol	mg/L	40.30 ± 0.78	38.63 ± 4.18	38.15 ± 3.10	37.62 ± 2.64	36.33 ± 3.59	40.48 ± 1.91	40.02 ± 1.95	41.67 ± 2.56	39.24 ± 2.12	ns
Volatile acids											
Butanoic acid	mg/L	1.02 ± 0.16	1.04 ± 0.01	1.08 ± 0.08	1.19 ± 0.13	1.10 ± 0.02	1.16 ± 0.05	1.08 ± 0.06	1.11 ± 0.07	0.97 ± 0.03	ns
Acetic acid	mg/L	272.69 ± 53.9	227.28 ± 38.4	271.88 ± 45.0	356.35 ± 35.9	214.60 ± 11.5	237.18 ± 13.0	281.54 ± 39.0	276.38 ± 3.19	283.34 ± 20.2	ns
Propanoic acid	mg/L	9.17 ± 0.75	11.38 ± 1.61	12.46 ± 1.14	12.56 ± 1.37	11.16 ± 0.70	12.69 ± 2.77	11.86 ± 0.91	13.02 ± 0.83	12.64 ± 1.42	ns
Hexanoic acid	mg/L	1.89 ± 0.22	1.96 ± 0.1	2.46 ± 0.42	1.68 ± 0.1	2.48 ± 0.12	1.80 ± 0.02	1.78 ± 0.04	1.78 ± 0.09	1.82 ± 0.22	ns
Octanoic acid	mg/L	1.07 ± 0.01^{abc}	1.19 ± 0.01^{ab}	1.28 ± 0.03^{a}	0.84 ± 0.03^{c}	1.14 ± 0.08^{ab}	1.16 ± 0.06^{ab}	1.06 ± 0.06^{abc}	0.88 ± 0.02^{c}	1.01 ± 0.09^{bc}	<0.001
Decanoic acid	µg/L	153.66 ± 12.33^{abc}	188.76 ± 6.89^{ab}	199.69 ± 7.76^{a}	150.93 ± 11.61^{bc}	160.34 ± 9.27^{abc}	187.70 ± 7.17^{ab}	157.50 ± 7.24^{abc}	138.36 ± 10.44^{c}	176.36 ± 10.32^{abc}	<0.05
2-Methylbutanoic acid	mg/L	1.04 ± 0.02	1.24 ± 0.06	1.16 ± 0.03	1.10 ± 0.06	1.05 ± 0.02	1.12 ± 0.03	1.17 ± 0.05	1.21 ± 0.08	1.03 ± 0.06	ns
3-Methylbutanoic acid	mg/L	1.20 ± 0.02	1.47 ± 0.11	1.39 ± 0.05	1.32 ± 0.08	1.37 ± 0.04	1.37 ± 0.09	1.47 ± 0.04	1.44 ± 0.03	1.31 ± 0.09	ns

†Data presented show mean values ± standard error, treatments were compared using one-way ANOVA, ns = not significantly different, n=3. Differences between treatments were determined by a post-hoc Tukey's test with significance indicated by different letters; H-1, H-2, H-3 = harvest-1, harvest-2 and harvest-3; DA-1, DA-2 = direct addition treatment 1 and direct addition treatment 2; RR-1, RR-2 = juice substitution treatment-1, juice substitution treatment 2; RO-1, RO-2 = saigneé treatment 1 and saigneé treatment 2.

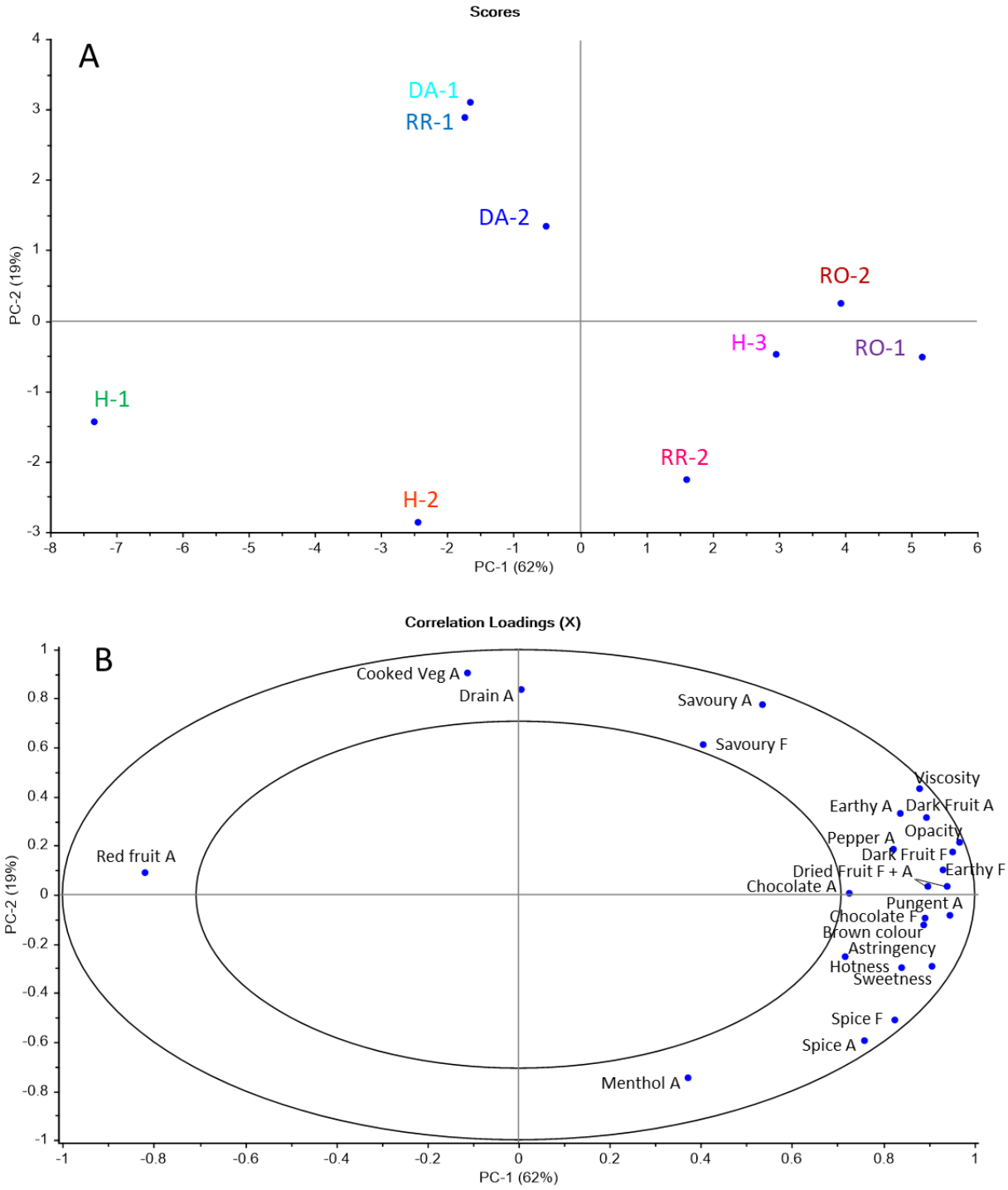


Figure 6.1.3.1.Principal component analysis showing (A) scores and (B) correlation loadings of significant (P<0.05) and close to significant sensory attributes (P<0.10) for the harvest time and water addition winemaking treatments determined at 12 months of ageing. A: Aroma, F: Flavour, H-1, H-2, H-3 = harvest-1, harvest-2 and harvest-3; DA-1, DA-2 = direct addition treatment 1 and direct addition treatment 2; RR-1, RR-2 = juice substitution treatment-1, juice substitution treatment 2; RO-1, RO-2 = saigneé treatment 1 and saigneé treatment 2.

From the ANOVA performed on the sensory data for the wines, 21 attributes rated by the panel differed significantly ($P<0.05$) between the treatments: opacity (colour intensity), brown colour, 'dark fruit' aroma, 'dried fruit' aroma, 'spice' aroma, 'chocolate' aroma, 'cooked vegetable' aroma, 'earthy' aroma, 'menthol' aroma, 'savoury' aroma, 'pungent' aroma, 'drain (reduction)' aroma, sweetness, viscosity, hotness, 'savoury' flavour, 'dark fruit' flavour, 'dried fruit' flavour, 'chocolate' flavour, 'earthy' flavour and 'spice' flavour. Five attributes: 'red fruit' aroma, 'floral' aroma, 'rubber' aroma, 'pepper' aroma and astringency, were close to being significant ($P\leq0.10$) (Appendix Table 13.1). There were significant winemaking replicate nested in treatment effects for the attributes opacity, 'menthol' aroma, 'savoury' aroma, saltiness and savouriness (data not shown). These significant ($P\leq0.05$) effects indicate variation between some winemaking replicates within the treatments. There were significant ($P\leq0.05$) judge by treatment interaction effects for several attributes indicating some concept alignment issues between some judges. Some variation was expected within sensory panels, especially with the large study size and number of attributes assessed.

PCA of the sensory data showed that the wines could chiefly be defined by multiple attributes associated with harvest date, the majority explained by the first principal component (PC). The H-3 wines had higher 'dark fruit' aroma/flavour, hotness, viscosity, astringency and opacity (among other attributes), while the wines made from the H-2 and H-1 harvests had progressively less of these attributes and higher scores for 'red fruit' aroma (Figure 6.1.3.1). The saigneé treatments were not substantially different in terms of their sensory properties, and were grouped with the H-3 control. For the treatments where water was added to the 15.5°Baume must, the effect of water addition on sensory attributes was greater as the quantity of water added increased, in contrast to the effects observed on wine phenolics (Table 6.1.3.2). Dilution introduced losses in the above-mentioned attributes associated with the H-3 control wine and the saigneé treatments, and caused increased expression of 'red fruit' aroma. For wine sensory properties, the method of dilution was important for the lower level of water addition (RR-2 compared with DA-2). Here, it was found that the juice substitution treatment was more like the control than the direct water addition treatment. The water addition treatment was defined by an important second PC, which was positively associated with 'cooked vegetable', 'drain' and 'savoury' attributes, and negatively defined by 'menthol' aroma and 'spice' attributes. The highest level of water addition produced very similar wines which had increased contribution of 'cooked vegetable' and 'drain' characters which can be considered as undesirable. It is important to note that this potential issue was not previously found for Cabernet Sauvignon wines which received even greater levels of water addition (Schelezki et al. 2018b) and further investigation is needed to understand the underlying factors which caused the increased 'cooked vegetable' and 'drain' attributes. In general, the RR-2 treatment which received the smaller addition of water as a juice substitution without changing the solids to liquid ratio was more similar to the H-3 wines and did not display an increase in potentially negative attributes.

In order to gain a better understanding of how wine chemical composition influenced wine sensory properties, a PLS analysis was performed (Table 6.1.3.4). Only compositional variables that were significantly different between treatments based on the ANOVA were used in the model. Not all of the variables which were relevant in distinguishing the treatments by PCA (Figure 6.1.3.1) were well-modelled by the compositional data, for example 'hotness', 'astringency', 'savoury' aroma and flavour, or the aromas of 'drain' and 'cooked vegetable'. Certain attributes could be well-modelled by the wine compositional data, as evidenced by high R^2 for model validation, together with low root mean square errors (RMSE) of prediction. Some differences in the model parameters were observed if the model was developed using the whole wine chemistry dataset, or a sub-set of significant variables identified by an uncertainty test. In particular, 'brown colour', 'dried fruit' aroma and flavour, and 'spice' flavour were particularly well-modelled using only a sub-set of significant variables. The wine compositional variables relevant to these sensory attributes are shown in Figure 6.1.3.2. All the above-mentioned sensory attributes were associated positively with multiple phenolics measures, including wine hue (brownness), non-bleachable pigments, tannin size (molecular mass and degree of polymerisation). The proportion of tannin galloylation may be related to higher levels of extraction from the seed during fermentation, and this was higher in the browner wines, and higher in wines with 'dried fruit' attributes and 'spice' flavour. Wine polysaccharides, including both grape- and yeast-derived monosaccharides, were positively associated with the four sensory attributes. Three fermentation products were relevant to the PLS model (octanoic acid, hexyl acetate and 2-phenylethyl acetate), and were all lower in wines

with greater expression of the above-mentioned sensory attributes. Interestingly, although the model was not as strong as for the four attributes discussed here, these three fermentation-derived compounds were all positively associated with the 'red fruit' attribute in wines prepared from fruit harvested earlier and water-addition wines (weighted regression coefficient >0.03). Hexyl acetate is known to contribute a 'red fruit' aroma in Cabernet Sauvignon wine (Forde et al. 2011, Bindon et al. 2014b), and is thought to be associated with C6 precursors which are elevated in less ripe grapes. However, it was interesting to note that higher hexyl acetate was also associated with the dilution treatments, potentially indicating that changes in the conditions of fermentation associated with lower ethanol may have facilitated its formation. It is also well known that higher concentrations of 2-phenylethyl acetate can enhance the 'fruitiness' of red wines (Viana et al. 2009); however, a negative correlation with 'dark fruit' and 'dried fruit' attributes was demonstrated here for the first time for Shiraz, while a positive association was seen with 'red fruit'. Octanoic acid was well above sensory threshold (Brennand et al. 1989) but is thought to present a mild, somewhat rancid yet fruity odour and its role as a potential odorant would require further investigation. To conclude, many of the other sensory variables were correlated and as shown in the PCA (Figure 6.1.3.1) the same wine compositional variables were relevant in the prediction of these attributes as well. Regression coefficient data for the model developed using the sub-set of significant variables is shown as supplementary information (Appendix Table 13.3).

Table 6.1.3.4 Partial least squares (PLS) regression analysis for the prediction of wine sensory variables from wine chemical data[†].

Sensory attribute	Data selected	PLS model parameters[‡]				
		Factor no	R^2_{cal}	R^2_{val}	$RMSE_{cal}$	$RMSE_{val}$
Opacity	all data	1	0.82	0.58	0.34	0.58
	sig var[§]	3	0.96	0.58	0.15	0.58
Brown colour	all data	1	0.92	0.86	0.10	0.14
	sig var	3	0.98	0.94	0.05	0.09
Red fruit aroma	all data	1	0.61	0.49	0.16	0.21
	sig var	3	0.57	0.38	0.17	0.23
Dark fruit aroma	all data	1	0.72	0.50	0.20	0.30
	sig var	3	0.96	0.58	0.07	0.28
Dried fruit aroma	all data	1	0.83	0.73	0.13	0.18
	sig var	3	0.87	0.73	0.13	0.20
Spice aroma	all data	1	-	-	-	-
	sig var	3	0.79	0.64	0.10	0.14
Earthy aroma	all data	1	0.79	0.67	0.10	0.13
	sig var	3	0.78	0.62	0.09	0.14
Pepper aroma	all data	1	0.70	0.53	0.07	0.09
	sig var	3	0.70	0.36	0.07	0.11
Pungent aroma	all data	1	0.79	0.65	0.11	0.15
	sig var	3	0.87	0.62	0.08	0.16
Sweetness	all data	1	0.81	0.71	0.10	0.14
	sig var	3	0.96	0.85	0.05	0.10
Viscosity	all data	1	0.69	0.43	0.16	0.24
	sig var	3	0.89	0.35	0.09	0.25
Dark fruit flavour	all data	1	0.73	0.43	0.23	0.38
	sig var	3	0.90	0.40	0.14	0.39
Dried fruit flavour	all data	1	0.95	0.88	0.09	0.15
	sig var	3	0.95	0.90	0.09	0.14
Chocolate flavour	all data	1	0.61	0.40	0.19	0.26
	sig var	3	0.79	0.38	0.14	0.26
Earthy flavour	all data	1	0.74	0.53	0.14	0.21
	sig var	3	0.75	0.29	0.14	0.26
Spice flavour	all data	1	0.63	0.51	0.16	0.21
	sig var	3	0.94	0.77	0.07	0.14

[†] For the PLS model with all variables, Factor 1 explained 56% of the X variance and 57% of the Y variance; for the PLS model with a sub-set of significant variables, Factor 1 explained 84% of the X variance and 52% of the Y variance; Factor 2 explained a further 7% and 14% of the X and Y variance respectively; [‡] PLS model parameters where cal = calibration, val = validation, RSME = root mean square error of prediciton; [§] Sig var indicates a sub set of significant variables selected using an uncertainty test and high correlation loadings

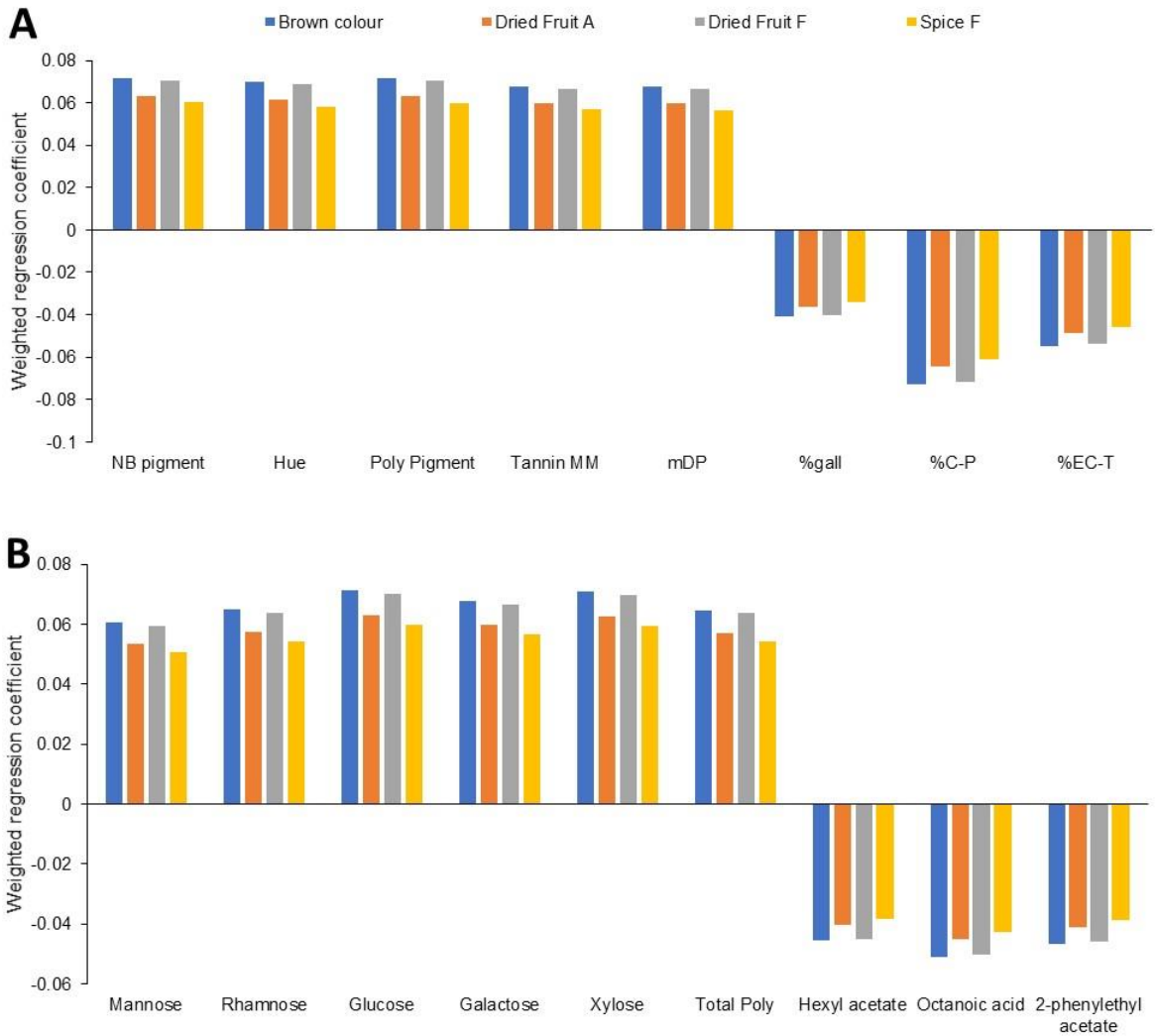


Figure 6.1.3.2. Weighted regression coefficients from partial least squares regression analysis of well-modelled sensory attributes (A, aroma; F, flavour) from a sub-set of significant variables, **A**. Phenolic measures (NB, non-bleachable; poly, polymeric; MM, molecular mass; mDP, mean degree of polymerisation; %gall, galloylation; -P, extension subunit; -T, terminal subunit; C, catechin; EC, epicatechin) and **B.** Polysaccharide-associated monosaccharides (poly, polysaccharide) and fermentation products.

6.2 Results of the 2018 vintage trial comparing outcomes of ripening and water addition on Chardonnay wine

6.2.1 Outcomes of grape ripeness and water addition on wine composition

The initial experimental plan aimed to source fruit with a target °Be of 13.5, 14.5 and 15.5 for the three consecutive harvest dates. As discussed in the materials and methods, it was evident that the first harvest date was lower than expected, closer to 13°Be than 13.5, hence the target °Be for the water addition treatments was set at 13.0°Be. Table 6.2.1 shows the fruit weight for each harvest, the volumes of water added, and the actual °Be for each treatment before and after water addition. Although the first harvest came in lower than the expected target °Be, it was evident that the second and third harvests also came in at a lower °Be than expected, at 13.67°Be and 15°Be, respectively. To retain consistency with the initial experimental outline, the treatment names were kept constant; however, this discrepancy between expected and final °Be must be taken into consideration. Both the skin contact treatments increased the initial °Be; therefore, a higher volume of water was required when the water addition was made after the 12h contact period.

Table 6.2.1 Pre-ferment fruit weight, volumes of water added and sugar measurements (as °Be) before and after water addition in Chardonnay musts prepared from grapes at different harvest dates, with dilution and skin contact (12 h) (data shown are the mean ± standard error where n=3 from triplicate fermentations)

	Initial fruit weight (kg)	Initial °Be	Initial Press Volume (L)	Volume water added (L)	Diluted Volume (L)	Diluted °Be
13.5 °Be Control	63.55 ± 0.86	13.26 ± 0.09	29.93 ±0.28	-	-	-
14.5 °Be Control	60.77 ± 0.67	13.67 ± 0.12	29.07 ± 0.84	-	-	-
14.5 °Be adjust 13.0 °Be	61.03 ± 1.84	13.77 ± 0.03	29.90 ± 1.35	1.77 ± 0.08	31.67 ± 1.42	13.00 ± 0.06
14.5 °Be skin contact	64.27 ± 0.50	13.97 ± 0.07	33.23 ± 1.65	-	-	-
14.5 °Be skin contact then adjust 13.0 °Be	64.43 ± 1.47	14.20 ± 0.06	37.00 ± 0.53	3.42 ± 0.05	40.412 ± 0.58	13.53 ± 0.07
14.5 °Be adjust 13.0 °Be then skin contact	63.23 ± 1.07	13.80 ± 0.06	40.50 ± 0.60	1.64 ± 0.00	28.23 ± 0.00	13.30 ± 0.10
15.5 °Be Control	66.07 ± 2.24	14.97 ± 0.15	27.20 ± 1.18	-	-	-
15.5 °Be adjust 13.0 °Be	64.80 ± 1.21	15.20 ± 0.15	27.93 ± 0.39	5.00 ± 0.26	32.93 ± 0.48	12.63 ± 0.13
15.5 °Be skin contact	63.83 ± 2.42	16.07 ± 0.18	33.53 ± 3.17	-	-	-
15.5 °Be skin contact then adjust 13.0 °Be	64.57 ± 1.28	15.53 ± 0.15	31.57 ± 0.17	6.57 ± 0.26	38.13 ± 0.32	12.97 ± 0.03

The results of the ripening, water addition and skin contact treatments in Chardonnay are shown in Tables 6.2.2 and 6.2.3. Due to the closeness in target °Be described above, the differences in wine ethanol between the first two harvest points was not significant, and the water addition treatments failed to reduce ethanol substantially. It was clearly evident for the second harvest that the skin contact period increased wine ethanol, but this was not apparent for wines made from the third harvest, even though the initial measured °Be increased for the 15.5°Be harvest point. For the third harvest, the water addition treatments were found to more effectively reduce final wine ethanol, although the decrease was somewhat lower that that achieved for the 13.5 and 14.5 °Be harvests, respectively. This outcome alone indicates that the process of water addition can introduce a degree of complexity of its own depending upon the point of addition (i.e. with or without skin contact, before or after enzyme addition and settling etc). It is also noteworthy that in order to make an accurate prediction of the water volume required to effectively reduce final ethanol the sugar measurement would need to be taken as close to the point of water addition as possible and combined with consideration of the extraction volume.

Table 6.2.2. Basic wine composition, phenolics, protein and polysaccharide in Chardonnay wines prepared from grapes at different harvest dates, with water addition and skin contact (12 h) (data shown are the mean ± standard error where n=3 from triplicate fermentations, analysed by one-way ANOVA followed by a Tukey's post-hoc test with significant differences indicated as different letters).

Wine compositional measure	13.5 °Be Control	14.5 °Be Control	14.5 °Be adjust 13.0 °Be	14.5 °Be skin contact	14.5 °Be skin contact then adjust 13.0 °Be	14.5 °Be adjust 13.0 °Be then skin contact	15.5 °Be Control	15.5 °Be adjust 13.0 °Be	15.5 °Be skin contact	15.5 °Be skin contact then adjust 13.0 °Be	ANOVA: P value
Basic composition											
Alcohol (%)	14.77 ± 0.13^{bc}	14.37 ± 0.20^{cde}	14.13 ± 0.07^{de}	15.30 ± 0.10^{b}	14.73 ± 0.12^{bcd}	14.73 ± 0.12^{bcd}	16.30 ± 0.06^{a}	13.77 ± 0.12^{e}	15.93 ± 0.15^{a}	14.03 ± 0.07^{e}	<0.0001
Residual sugar (g/L)	1.33 ± 0.15^{abc}	1.03 ± 0.66^{abc}	1.77 ± 0.77^{ab}	2.37 ± 0.19^{a}	1.80 ± 0.20^{ab}	1.83 ± 0.19^{ab}	1.67 ± 0.03^{ab}	0.87 ± 0.28^{bc}	0.23 ± 0.03^{c}	0.93 ± 0.37^{bc}	<0.01
pH	3.32 ± 0.01^{bc}	3.40 ± 0.02^{bc}	3.31 ± 0.01^{bc}	3.44 ± 0.02^{b}	3.43 ± 0.01^{bc}	3.41 ± 0.03^{bc}	3.29 ± 0.04^{c}	3.29 ± 0.02^{c}	3.70 ± 0.06^{a}	3.44 ± 0.01^{b}	<0.0001
Titratable acidity (8.2)	6.20 ± 0.10^{bc}	5.83 ± 0.09^{c}	6.10 ± 0.06^{bc}	5.97 ± 0.18^{c}	5.97 ± 0.15^{c}	6.10 ± 0.29^{bc}	7.10 ± 0.06^{a}	6.43 ± 0.09^{abc}	6.80 ± 0.17^{ab}	6.03 ± 0.07^{c}	<0.0001
Malic acid (g/L)	1.74 ± 0.03	1.39 ± 0.16	1.46 ± 0.10	1.78 ± 0.14	1.79 ± 0.13	1.89 ± 0.17	1.58 ± 0.16	1.47 ± 0.14	1.65 ± 0.18	1.62 ± 0.14	ns
Total phenolics (A280)	5.65 ± 0.25^{d}	6.70 ± 0.29^{cd}	6.06 ± 0.19^{d}	7.47 ± 0.11^{bc}	7.49 ± 0.13^{bc}	7.72 ± 0.05^{bc}	7.19 ± 0.09^{bc}	6.69 ± 0.14^{cd}	10.48 ± 0.36^{a}	7.94 ± 0.34^{b}	<0.0001
Protein composition											
Total Protein (mg/L)	53.51 ± 0.72^{bc}	73.50 ± 3.16^{ab}	62.73 ± 5.60^{abc}	77.76 ± 6.35^{a}	70.12 ± 4.91^{abc}	57.03 ± 6.19^{abc}	75.97 ± 3.08^{ab}	70.37 ± 6.76^{abc}	56.67 ± 2.98^{abc}	47.95 ± 2.73^{c}	<0.01
Thaumatin-like protein (mg/L)	34.41 ± 0.26^{abc}	48.54 ± 1.84^{a}	40.04 ± 4.86^{abc}	45.92 ± 4.11^{ab}	41.71 ± 3.60^{abc}	32.79 ± 4.29^{bc}	44.53 ± 2.59^{ab}	45.97 ± 2.47^{ab}	33.41 ± 1.35^{abc}	28.34 ± 2.67^{c}	<0.01
Chitinases (mg/L)	19.09 ± 0.78^{c}	24.96 ± 1.80^{abc}	22.69 ± 1.61^{abc}	31.84 ± 2.71^{a}	28.40 ± 1.44^{abc}	24.23 ± 2.19^{abc}	31.44 ± 3.68^{ab}	24.40 ± 4.30^{abc}	23.25 ± 1.78^{abc}	19.61 ± 1.43^{bc}	<0.01
Polysaccharide composition [†]											
Total polysaccharide (mg/L)	140.82 ± 3.25^{de}	161.83 ± 13.84^{cde}	137.56 ± 5.29^{e}	204.21 ± 19.36^{bcd}	204.45 ± 11.42^{bcd}	222.65 ± 1.70^{bc}	217.92 ± 6.79^{bc}	185.07 ± 13.02^{bcd}	323.54 ± 20.03^{a}	229.24 ± 17.93^{b}	<0.0001
Mannose (mg/L)	77.39 ± 2.82^{b}	100.02 ± 11.66^{b}	78.58 ± 3.89^{b}	86.16 ± 11.67^{b}	86.17 ± 7.77^{b}	94.16 ± 9.04^{b}	116.03 ± 5.71^{b}	97.30 ± 8.34^{b}	169.12 ± 13.04^{a}	105.45 ± 10.81^{b}	<0.0001
Rhamnose (mg/L)	7.94 ± 0.67^{e}	11.04 ± 0.10^{de}	8.59 ± 0.61^{e}	23.59 ± 4.29^{abc}	26.00 ± 1.61^{ab}	31.26 ± 1.25^{a}	18.73 ± 1.18^{bcd}	16.08 ± 0.18^{cde}	28.15 ± 1.40^{a}	25.09 ± 0.92^{ab}	<0.0001
Glucuronic acid (mg/L)	3.90 ± 0.47^{ab}	3.03 ± 0.51^{ab}	2.78 ± 0.09^{b}	5.06 ± 0.42^{ab}	4.54 ± 0.55^{ab}	5.58 ± 0.90^{a}	3.51 ± 0.34^{ab}	4.19 ± 0.37^{ab}	5.15 ± 0.65^{ab}	5.52 ± 0.73^{a}	<0.01
Galacturonic acid (mg/L)	11.60 ± 0.34^{c}	13.93 ± 0.98^{c}	13.26 ± 0.28^{c}	36.15 ± 7.14^{ab}	37.54 ± 1.63^{ab}	37.22 ± 1.74^{ab}	16.33 ± 0.64^{c}	13.40 ± 1.00^{c}	45.64 ± 5.23^{a}	27.77 ± 4.30^{bc}	<0.0001
Glucose (mg/L)	8.55 ± 0.64^{c}	11.94 ± 1.81^{bc}	9.25 ± 0.45^{c}	9.41 ± 1.27^{c}	11.35 ± 2.30^{bc}	13.31 ± 0.55^{bc}	17.75 ± 0.54^{b}	16.12 ± 1.91bc	28.86 ± 2.92^{a}	18.76 ± 0.73^{b}	<0.0001
Galactose (mg/L)	23.72 ± 0.72^{abc}	15.26 ± 1.27^{c}	17.94 ± 0.74^{bc}	31.67 ± 5.38^{a}	25.92 ± 0.47^{abc}	24.42 ± 3.22^{abc}	33.47 ± 0.96^{a}	26.86 ± 2.27^{abc}	28.57 ± 2.50^{ab}	29.19 ± 2.54^{ab}	<0.001
Xylose (mg/L)	0.87 ± 0.07^{d}	0.81 ± 0.10^{d}	1.17 ± 0.05^{d}	1.35 ± 0.09^{cd}	1.47 ± 0.16^{bcd}	2.88 ± 0.51^{a}	1.62 ± 0.41^{abcd}	1.58 ± 0.18^{bcd}	2.65 ± 0.33^{ab}	2.53 ± 0.21^{abc}	<0.0001
Arabinose (mg/L)	5.84 ± 0.09^{cde}	5.00 ± 0.62^{e}	5.16 ± 0.12^{de}	9.46 ± 0.23^{ab}	9.26 ± 0.42^{ab}	10.17 ± 1.15^{ab}	8.60 ± 0.35^{abc}	7.89 ± 0.58^{bcd}	11.06 ± 0.66^{a}	10.98 ± 0.57^{a}	<0.0001
Fucose (mg/L)	1.01 ± 0.10^{b}	0.80 ± 0.08^{b}	0.82 ± 0.15^{b}	1.36 ± 0.18^{b}	2.19 ± 0.41^{b}	3.66 ± 0.18^{a}	1.88 ± 0.20^{b}	1.67 ± 0.26^{b}	4.34 ± 0.15^{a}	3.94 ± 0.65^{a}	<0.0001

[†]Monosaccharide sugars released following hydrolysis of polysaccharide and quantified as mg/L.

Table 6.2.3. Fermentation products in Chardonnay wines prepared from grapes at different harvest dates, with water addition and skin contact (12 h) (data shown are the mean ± standard error where n=3 from triplicate fermentations, analysed by one-way ANOVA followed by a Tukey's post-hoc test with significant differences indicated as different letters; nd = not detected).

Fermentation product	unit	13.5 °Be Control	14.5 °Be Control	14.5 °Be adjust 13.0 °Be	14.5 °Be skin contact	14.5 °Be skin contact then adjust 13.0 °Be	14.5 °Be adjust 13.0 °Be then skin contact	15.5 °Be Control	15.5 °Be adjust 13.0 °Be	15.5 °Be skin contact	15.5 °Be skin contact then adjust 13.0 °Be	ANOVA: P value
Acetate esters												
Ethyl acetate	mg/L	35.31 ± 0.21^{e}	42.73 ± 3.1^{de}	35.51 ± 2.0^{e}	51.71 ± 4.2^{bcd}	51.00 ± 0.7^{bcd}	64.99 ± 3.8^{ab}	67.86 ± 4.0^{a}	46.31 ± 1.8^{de}	60.95 ± 0.13^{abc}	46.64 ± 4.1^{cde}	<0.0001
Hexyl acetate	µg/L	71.83 ± 14.1	94.40 ± 32.7	81.47 ± 11.8	114.50 ± 19.6	103.83 ± 17.9	82.60 ± 29.4	54.43 ± 8.3	40.67 ± 4.4	35.20 ± 10.7	70.47 ± 11.7	ns
3-Methylbutyl acetate	mg/L	0.62 ± 0.1	0.65 ± 0.1	0.62 ± 0.04	0.85 ± 0.05	0.75 ± 0.08	0.67 ± 0.17	0.71 ± 0.05	0.67 ± 0.08	0.67 ± 0.14	1.04 ± 0.06	ns
2-Methylbutyl acetate	µg/L	69.00 ± 7.6	69.00 ± 9.2	69.33 ± 1.9	84.33 ± 6.2	78.33 ± 10.5	82.67 ± 11.9	81.33 ± 4.3	73.00 ± 7.8	77.33 ± 13.7	105.67 ± 6.40	ns
2-Methylpropyl acetate	µg/L	nd	nd	nd	28.66 ± 14.4	27.67 ± 13.9	49.67 ± 4.6	33.33 ± 16.7	14.33 ± 14.3	42.33 ± 1.2	46.00 ± 3.5	ns
2-Phenylethyl acetate	µg/L	329.7 ± 55.7	329.0 ± 41.7	297.7 ± 20.1	355.0 ± 38.6	321.3 ± 27.6	250.0 ± 53.7	317.0 ± 17.0	274.3 ± 21.3	262.0 ± 66.3	362.3 ± 18.4	ns
Ethyl esters												
Ethyl butanoate	µg/L	392.33 ± 14.7	417.33 ± 27.0	438.33 ± 4.3	449.67 ± 4.3	441.33 ± 3.7	375.00 ± 57.8	430.33 ± 8.4	411.33 ± 11.8	396.67 ± 22.8	425.00 ± 19.2	ns
Ethyl propanoate	µg/L	126.33 ± 9.13^{ab}	133.67 ± 10.41^{ab}	127.00 ± 13.43^{ab}	120.67 ± 7.88^{ab}	122.33 ± 5.24^{ab}	128.33 ± 2.96^{ab}	166.33 ± 4.91^{a}	135.67 ± 10.65^{ab}	152.33 ± 13.28^{ab}	120.33 ± 6.96^{b}	<0.05
Ethyl hexanoate	mg/L	1.02 ± 0.07^{ab}	1.15 ± 0.13^{ab}	1.28 ± 0.04^{a}	1.16 ± 0.02^{ab}	1.09 ± 0.04^{ab}	0.92 ± 0.17^{ab}	1.09 ± 0.02^{ab}	1.04 ± 0.03^{ab}	0.74 ± 0.11^{b}	1.06 ± 0.07^{ab}	<0.05
Ethyl octanoate	mg/L	1.33 ± 0.04^{cab}	1.36 ± 0.11^{ab}	1.46 ± 0.05^{a}	1.52 ± 0.04^{ab}	1.38 ± 0.02^{ab}	1.25 ± 0.16^{ab}	1.34 ± 0.02^{ab}	1.33 ± 0.01^{ab}	0.99 ± 0.13^{b}	1.35 ± 0.05^{ab}	<0.05
Ethyl decanoate	µg/L	469.57 ± 21.5^{a}	402.57 ± 49.6^{ab}	471.87 ± 7.8^{a}	500.07 ± 16.2^{a}	555.97 ± 6.2^{a}	512.13 ± 41.7^{a}	453.37 ± 45.1^{a}	444.53 ±17.5^{a}	274.13 ± 34.1^{b}	443.77 ± 29.7^{a}	<0.001
Ethyl-3-methylbutanoate	µg/L	46.07 ± 2.34^{ab}	47.07 ± 5.07^{ab}	46.97 ± 2.98^{ab}	26.33 ± 3.97^{c}	26.27 ± 3.16^{c}	25.13 ± 3.68^{c}	51.13 ± 3.46^{a}	44.90 ± 0.79^{ab}	32.63 ± 3.74^{bc}	24.67 ± 1.28^{c}	<0.0001
Ethyl-2-methylbutanoate	µg/L	24.60 ± 2.04^{ab}	22.30 ± 2.34^{abc}	25.37 ± 1.21^{ab}	14.03 ± 0.09^{cd}	12.30 ± 0.96^{d}	12.80 ± 2.17^{d}	27.03 ± 3.01^{a}	24.50 ± 1.91^{ab}	17.70 ± 1.64^{cd}	14.00 ± 0.70^{cd}	<0.0001
Ethyl-2-methylpropanoate	µg/L	116.33 ± 3.84^{bc}	208.67 ± 44.7^{a}	133.00 ± 5.69^{abc}	98.33 ± 10.53^{c}	93.33 ± 8.09^{c}	104.33 ± 16.34^{c}	197.67 ± 9.94^{ab}	156.00 ± 3.00^{abc}	192.33 ± 10.17^{ab}	103.67 ± 5.33^{c}	<0.0001
Higher alcohols												
3-Methylbutanol	mg/L	138.32 ± 6.2	122.26 ± 0.8	133.84 ± 3.6	138.76 ± 9.6	137.44 ± 5.8	148.15 ± 9.2	127.84 ± 3.0	135.00 ± 1.7	127.05 ± 7.2	132.74 ± 4.0	ns
2-Methylbutanol	mg/L	52.86 ± 3.7	41.88 ± 1.4	48.60 ± 4.5	46.34 ± 4.0	52.27 ± 1.6	59.45 ± 5.5	47.97 ± 0.5	48.75 ± 3.1	49.02 ± 4.8	49.27 ± 2.5	ns
Hexanol	mg/L	1.55 ± 0.08^{b}	1.50 ± 0.15^{b}	1.58 ± 0.07^{b}	2.19 ± 0.07^{a}	2.26 ± 0.15^{a}	2.38 ± 0.12^{a}	1.09 ± 0.03^{b}	1.09 ± 0.04^{b}	1.07 ± 0.11^{b}	1.07 ± 0.13^{b}	<0.0001
2-Phenylethanol	mg/L	64.51 ± 2.73^{a}	47.64 ± 0.68^{b}	53.86 ± 2.40^{ab}	48.38 ± 4.08^{b}	50.05 ± 1.97^{b}	52.07 ± 4.29^{b}	45.52 ± 1.43^{b}	48.14 ± 0.92^{b}	45.24 ± 1.62^{b}	45.18 ± 1.46^{b}	<0.001
2-Methylpropanol	mg/L	22.07 ± 0.41^{c}	25.47 ± 1.09^{bc}	24.20 ± 1.19^{c}	31.88 ± 2.34^{abc}	31.44 ± 1.64^{abc}	37.47 ± 4.34^{a}	30.36 ± 1.00^{abc}	27.47 ± 1.28^{abc}	38.43 ± 2.33^{ab}	28.85 ± 2.34^{abc}	<0.001
Volatile acids												
Butanoic acid	mg/L	1.42 ± 0.09^{ab}	1.90 ± 0.22^{a}	1.77 ± 0.14^{ab}	1.63 ± 0.07^{ab}	1.47 ± 0.04^{ab}	1.14 ± 0.20^{b}	1.40 ± 0.14^{ab}	1.59 ± 0.09^{ab}	1.53 ± 0.16^{ab}	1.53 ± 0.03^{ab}	<0.05
Acetic acid	mg/L	197.43 ± 7.2^{c}	331.87 ± 60.0^{abc}	193.48 ± 32.5^{c}	283.98 ± 55.4^{abc}	188.40 ± 12.0^{c}	195.44 ± 12.5^{c}	412.89 ± 32.7^{ab}	249.62 ± 31.4^{bc}	445.93 ± 3.6^{a}	239.76 ± 33.1^{c}	<0.0001
Hexanoic acid	mg/L	6.17 ± 0.42^{abc}	7.47 ± 0.30^{ab}	8.31 ± 0.32^{a}	6.95 ± 0.16^{abc}	6.92 ± 0.35^{abc}	5.59 ± 1.01^{bc}	6.33 ± 0.17^{abc}	6.42 ± 0.29^{abc}	4.78 ± 0.61^{c}	6.51 ± 0.35^{abc}	<0.01
Octanoic acid	mg/L	9.01 ± 0.76^{ab}	8.88 ± 0.75^{ab}	11.84 ± 0.75^{a}	10.10 ± 0.32^{ab}	9.85 ± 0.31^{ab}	8.18 ± 1.42^{abc}	8.06 ± 0.43^{bc}	9.80 ± 0.70^{ab}	5.09 ± 0.73^{c}	9.83 ± 0.47^{ab}	<0.001
Decanoic acid	mg/L	4.46 ± 0.28^{ab}	2.94 ± 0.67^{bc}	4.61 ± 0.35^{a}	4.35 ± 0.19^{ab}	5.00 ± 0.05^{a}	4.31 ± 0.47^{ab}	4.08 ± 0.08^{ab}	4.43 ± 0.33^{ab}	1.68 ± 0.03^{c}	3.83 ± 0.18^{ab}	<0.0001
3-Methylbutanoic acid	mg/L	1.03 ± 0.06^{ab}	1.24 ± 0.16^{a}	1.00 ± 0.09^{ab}	0.76 ± 0.06^{b}	0.73 ± 0.06^{b}	0.66 ± 0.05^{b}	1.00 ± 0.03^{ab}	0.97 ± 0.05^{ab}	1.28 ± 0.08^{a}	0.85 ± 0.05^{b}	<0.0001
2-Methylbutanoic acid	µg/L	597.33 ± 19.8^{bc}	686.33 ± 93.1^{ab}	561.33 ± 10.0^{bc}	445.00 ± 18.5^{c}	446.33 ± 23.6^{c}	448.67 ± 29.8^{c}	647.67 ± 22.2^{ab}	614.33 ± 23.5^{bc}	811.33 ± 24.9^{a}	579.00 ± 29.7^{bc}	<0.0001
2-Methylpropanoic acid	mg/L	1.22 ± 0.10^{c}	2.44 ± 0.47^{ab}	1.42 ± 0.26^{c}	1.44 ± 0.06^{c}	1.23 ± 0.14^{c}	1.28 ± 0.10^{c}	1.85 ± 0.03^{bc}	1.65 ± 0.14^{bc}	3.41 ± 0.09^{a}	1.60 ± 0.14^{bc}	<0.0001

In terms of wine composition, the skin contact treatment introduced marked changes, in particular for the latest harvest, at 15.5ºBe. Notably, for both the 14.5 and 15.5ºBe harvests, skin contact increased wine phenolics relative to the respective control. Ripening increased phenolics and protein, and reduced malic acid in wines made from the 14.5 and 15.5ºBe harvests relative to wine prepared from the earliest harvest at 13.5ºBe (Table 6.2.2). There was no clear effect of ripening on wine pH, mainly due the acid-adjustment that was applied. However, for the 15.5ºBe treatments, skin contact increased wine pH, even though the titratable acidity was adjusted, most likely due to enhanced extraction of potassium from the skins of riper grapes. Interestingly, in the 15.5ºBe wines, skin contact reduced protein concentration somewhat relative to the control, and this was maintained with dilution. The reason for this phenomenon will require further study, but could potentially be due to the adsorption of haze-forming proteins to non-extractable tannins contained in skin fragments.

Polysaccharide concentration was found to vary substantially between the winemaking treatments, but was also heavily influenced by the skin contact treatment, such that the minor differences introduced by grape ripeness or water addition were not significant, given the high variance across the dataset. Although not statistically significant, water addition generally reduced polysaccharide concentration, and this loss was overcome by the skin contact treatment. The effect of skin contact was far greater in the 15.5ºBe harvest wine than the 14.5ºBe harvest, and in the former, polysaccharides derived from both yeast (predominantly mannose and glucose) and grape (pectin and hemicellulose) increased.

Many of the fermentation-derived products varied significantly across the treatments, but responses were variable and clear effects of either grape ripeness or winemaking intervention were not evident (Table 6.2.3). In looking at the three harvest dates in particular, only three compounds were affected by ripeness alone. Ethyl acetate and acetic acid increased in wine as the harvest date was delayed, and 2-phenylethanol decreased. The skin contact treatment affected certain ethyl esters consistently in wines made from both the 14.5ºBe and 15.5ºBe harvest points, with both ethyl-3-methylbutanoate and ethyl-2-methylbutanoate decreasing. In the 15.5ºBe treatment only, ethyl decanoate decreased with skin contact. Various effects of skin contact were observed for the higher alcohols and volatile acids, but were not consistent for the two harvest points which received the treatment. For the water addition treatments, only the ester ethyl-2-methylpropanoate and acetic acid decreased consistently for both the harvest dates studied. In each case, the reduction was retained when skin contact was included, irrespective of the timing of skin contact or water addition.

6.2.2 Outcomes of Chardonnay grape ripeness and water addition on wine sensory properties

For the quantitative descriptive sensory analysis, one appearance term, fourteen aroma terms (thirteen defined and one ‘Other’ term) and thirteen palate terms (twelve defined and one ‘Other’ term) were determined. The final list of attributes, definitions/synonyms and reference standards are shown in Appendix Table 13.2. From ANOVA, seven attributes rated by the panel differed significantly ($P<0.05$) between the treatments: yellow colour intensity, ‘drain (reductive)’ aroma, bitterness, viscosity, hotness, sweetness and ‘stone fruit’ flavour (Appendix Table 13.2). Four attributes: ‘confection’ aroma, ‘cheesy’ aroma, ‘flint’ aroma, and ‘citrus’ flavour, were close to being significant ($P\leq0.10$). There were significant winemaking replicate nested in treatment effects for the attributes yellow colour intensity, ‘cheesy’ aroma, ‘flint’ aroma, and ‘tropical fruit’ flavour. These significant ($P\leq0.05$) effects indicate variation between some winemaking replicates within the treatments. The studied treatments had greatest effect on the colour intensity (yellow colour) and the body (viscosity) of the wines.

The PCA for the wines (Figure 6.2.2.1) were created using the seven attributes that were significantly different ($P\leq0.05$) and close to significant ($P\leq0.10$) across the twelve winemaking treatments. Principal components 1, 2 and 3 explained 44.3%, 26.9% and 11.2% (not shown) of the variation in the data, respectively. Beyond the third principal component eigenvalues approached 1 or below and explained little variability in the data. In Figure 6.2.2.1 the winemaking treatment 15.5ºBe skin contact treatment was plotted to the right of the figure, being rated highly in those attributes highly positively loaded on PC1, notably bitterness, ‘drain (reductive)’ aroma, ‘flint’ aroma, viscosity, ‘stone fruit’ flavour and yellow colour intensity. Conversely, the lower ethanol treatments, particularly wines diluted to 13.0ºBe were plotted to the left of the figure being rated lower in these attributes, and highly in the attributes highly

negatively loaded on PC1, notably 'citrus' flavour and 'confectionary' aroma. Generally, the vertical separation of the treatments was determined by the intensity of attributes most highly loaded on PC2, such as sweetness and 'cheesy' and 'drain' aromas. PC3 separation was influenced by the intensity of yellow colour intensity (not shown). There was a complex pattern of sensory differences among the treatments, but overall the later-harvested, higher Baumé wines were generally rated higher in bitterness and 'drain' aroma, while the lower Baumé wines were higher in 'citrus' flavour. The main effect of skin contact was an enhancement in viscosity compared to respective controls, while 'stone fruit' flavour was also enhanced for some skin contact treatments. The wines with highest bitterness and viscosity ratings were those with higher ethanol and made with skin contact. The dilution effect was quite large, with diluted treatments higher in 'citrus' flavour and lower in bitterness than their undiluted counterparts, and for some pairs was related to variations in fermentation-derived off-flavours such as 'drain' and 'cheesy', reflecting problematic fermentations with the high Baumé musts.

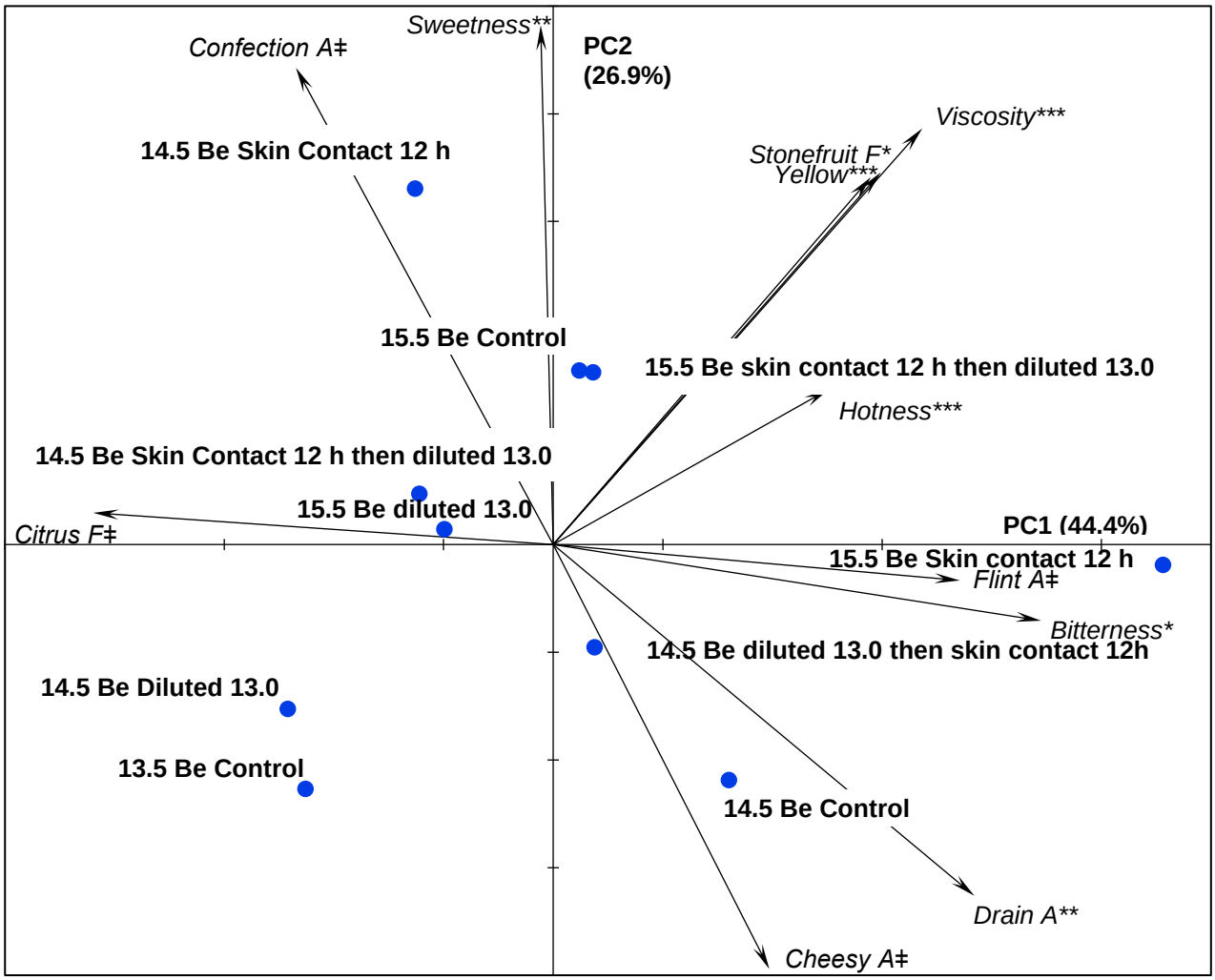


Figure 6.2.2.1. Principal component biplot of significant (P<0.05) and close to significant sensory attributes (P<0.10) on principal components 1 and 2 for the ten winemaking treatments. A: Aroma, F: Flavour.

PLS regression analysis was performed for all the Chardonnay winemaking treatments, with the chemical-analytical data as X-variables. For the sensory variables which were distinguished as significantly different among the winemaking treatments, only 'yellow colour' and 'viscosity' were significantly predicted by wine chemical composition, with an R^2 of 0.79 and 0.53 (of validation) respectively (data not shown). From an assessment of the results, the significant predictors of both sensory attributes were total phenolics (as 280 nm absorbance) and polysaccharide concentration, all of which were associated with the skin contact treatments.

Considering the influence of the skin contact treatments on both the chemical and sensory outcomes of the study, it is important to note that when the skin contact treatments are excluded from the statistical analysis, the most significantly affected attribute was wine hotness (Figure 6.2.2.2). Hotness ratings were similar between the wines made from earlier harvests, but not surprisingly, the 15.5ºBaume control wine was rated highest for hotness. However, it was interesting that the diluted 15.5ºBaume treatment had the lowest perceived hotness out of all the treatments, despite having a similar ethanol concentration to the wines made from the earlier harvests. A PLS-regression was performed for the hotness attribute using the chemical-analytical data as X-variables, and was not well-modelled with the analytical data from all the winemaking treatments. However, when the skin-contact treatments were excluded, an R^2 (validation) of 0.79 (data not shown) was achieved, with ethanol concentration being the most significant variable associated with hotness, as established by an uncertainty test.

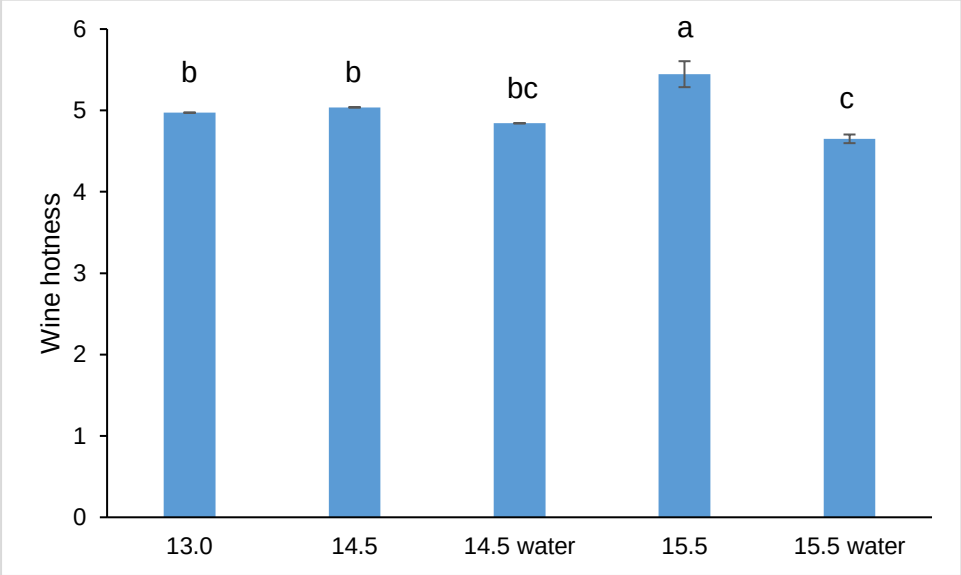


Figure 6.2.2.2. Sensory ratings for wine hotness in Chardonnay wines made from grapes harvested at different ripeness levels, with or without water addition (ANOVA, P <0.01, n=2, different letters indicate a significant difference according to a post-hoc Tukey's test).

Considering the relevance of the water addition study to the overarching aims of the project, the outcomes of the Chardonnay trial indicated that the effects of both harvest date and dilution on wine chemical composition and sensory properties were minimal, especially when compared to the influence of a common winemaking intervention such as skin contact.

6.3 Study of extended hang time in Shiraz: implications for grape and wine phenolics

6.3.1. Effect of advanced shrivel in Shiraz on the concentration and content of extractable phenolics in grapes.

The previous study on Shiraz showed that a loss in berry volume later in the ripening period was associated with an increase in extractable tannin and colour. To explore the consequences of larger reductions in berry mass associated with over-ripeness and shrivel, this experiment was repeated in 2019 with samples sourced after harvest had been delayed even further than in the 2017 study. As discussed in the materials and methods, it was found that the initial sugar concentration in the must measured at crushing increased after a 24-hour period, and was significantly higher in the late-harvest treatment (Table 6.3.1). This indicates that a greater leakage of sugar into must may occur from the skins and/or solids of dehydrated, over-ripe grapes, and may account for the anecdotal observation that juice sugar measurements taken in the vineyard or at crushing often underestimate the final ethanol of the finished wine. It was found that berry weight decreased significantly between the two harvest dates, and that this was associated with a marked increase in the proportion of shrivelled berries. An expected

outcome of the delay in harvest was an increase in juice pH and associated decrease in titratable acidity. Interestingly, this was not due to losses in malic acid. Notably the concentrations of extractable tannin and colour were remarkably higher in the 2019 season than in previous seasons, indicating marked seasonal variation. Unexpectedly, despite the substantial decrease in berry weight in 2019, and hence an expected increase in the surface area to volume ratio, extractable tannin and colour did not increase on a per gram berry weight basis. However, when the concentration of extractable tannin and colour was expressed on a per berry basis, or the content per berry, there was a close to significant decrease in both measures in the later harvested grapes relative to the control. Although not a highly significant result, this indicates that losses in extractable phenolics may have begun to occur with excessive hang time. In contrast, the previous study of the same vineyard showed increases in extractable tannin and colour up to 15.5ºBaume. Taken together, these results indicate that after the onset of excessive shrivel, reduced extractability may occur either due to degradation of phenolic compounds or may result from a limitation in extraction due to other phenomena (e.g. cell wall degradation and increased adsorption to grape solids).

Table 6.3.1. Grape measurements for Shiraz samples collected at two different harvest dates on 14 March (Control) and 27 March (Late Harvest); results show mean ± standard error (n=3)

Grape berry measure	Treatment		T-test P value*
	Control	Late Harvest	
ºBaume at crushing	15.27 ± 0.17	15.83 ± 0.29	0.07
ºBaume after 24 h	16.53 ± 0.24	17.37 ± 0.09	<0.05
Berry weight (g/berry)	0.97 ± 0.03	0.83 ± 0.02	<0.01
Shrivel (%)	8.56 ± 1.13	30.44 ± 0.48	<0.01
Extractable Anthocyanin (mg/g)	0.60 ± 0.02	0.67 ± 0.01	ns
Extractable Anthocyanin (mg/berry)	0.65 ± 0.03	0.56 ± 0.01	0.07
Extractable Tannin (mg/g)	2.94 ± 0.15	2.92 ± 0.09	ns
Extractable Tannin (mg/berry)	2.96 ± 0.19	2.46 ± 0.12	0.06
pH	3.91 ± 0.04	4.22 ± 0.02	<0.01
Titratable acidity (g/L pH 8.2)	3.47 ± 0.09	2.98 ± 0.09	<0.001
Malic acid (g/L)	1.17 ± 0.04	1.20 ± 0.05	ns

*control and late samples from discrete harvest blocks were compared using a paired Student's T-test

6.3.2 Effect of the quantity of pre-ferment water addition on wine phenolics in late-harvested Shiraz with advanced shrivel

When studying the effects of pre-ferment water addition on wine phenolics in late-harvested Shiraz with advanced shrivel, variable responses in wine chemical composition were found in relation to the harvest date and water addition treatments across the three vineyard blocks studied. Given the within-vineyard variation, no statistically significant responses to the delayed harvest and water addition treatments were found for most compositional measures (Tables 6.3.2.1 and 6.3.2.2). Only wine ethanol concentration clearly differed between the treatments, being elevated in the late-harvest 16ºBaume wine, which was an expected result. Although initial inspection of the raw data indicated that tannin concentration might have been higher in the late-harvest 16ºBaume wine relative to the other treatments, this effect occurred in only two of the three vineyard blocks, and the resulting outcome was therefore not statistically significant. There were also no significant changes in tannin composition (Table 6.3.2.2). Similarly, an apparent trend toward lower concentrations of non-bleachable pigment and the degree of chemical age, and higher monomeric anthocyanin in the late-harvest treatment

relative to the control was also not significantly different. What was found to differ between the wines was the degree of anthocyanin ionisation, which was lower in the control wines. This measure relates to the proportion of anthocyanins in the coloured form after bleaching by bisulfite has been excluded (Somers and Evans 1977), and is somewhat independent of the overall concentration of anthocyanin. Previous studies have shown a correlation between the degree of anthocyanin ionisation and wine colour density in young wines (Somers et al. 1974) but this was not evident in the current results.

Table 6.3.2.1. Basic wine composition, colour, tannin and phenolics in Shiraz wines prepared from two different harvest dates. 14 March (Control) and 27 March (Late Harvest) for which the latter received two different water addition treatments to 14 and 16ºBaume respectively; results show mean ± standard error (n=3)..

Wine compositional measure	Treatment			Probability >F
	Control	Late Harvest 14 ºBaume	Late Harvest 16 ºBaume	
Alcohol (%)	14.97 ± 0.12	15.03 ± 0.17	16.17 ± 0.23	<0.01
Residual sugar (g/L)	1.07 ± 0.44	0.77 ± 0.47	0.73 ± 0.09	ns
pH	3.70 ± 0.02	3.57 ± 0.04	3.61 ± 0.02	ns
Titratable acidity (g/L at pH 8.2)	5.80 ± 0.06	5.97 ± 0.15	6.07 ± 0.20	ns
Volatile acidity (mg/L)	0.45 ± 0.05	0.34 ± 0.04	0.38 ± 0.02	ns
Total phenolics (A.U.)	32.0 ± 1.2	43.0 ± 3.5	46.7 ± 1.7	ns
Tannin concentration (mg/L)	807 ± 79	960 ± 68	1159 ± 148	ns
Wine colour density (A.U.)	15.09 ± 0.17	14.91 ± 0.55	15.71 ± 1.09	ns
Hue (no units)	1.57 ± 0.01	1.61 ± 0.08	1.55 ± 0.05	ns
Non-bleachable pigment (A.U.)	6.00 ± 0.07	4.76 ± 1.00	4.39 ± 0.48	ns
Degree anthocyanin ionization (%)	10.75 ± 2.56	19.64 ± 0.59	20.20 ± 0.24	<0.05
Total anthocyanin (mg/L)	251 ± 10	357 ± 41	404 ± 38	ns
Chemical Age 1	0.65 ± 0.01	0.51 ± 0.08	0.46 ± 0.03	ns
Chemical Age 2	0.46 ± 0.02	0.28 ± 0.09	0.22 ± 0.03	ns

Table 6.3.2.2 Tannin compositional measures in Shiraz wines prepared from two different harvest dates on 14 March (Control) and 27 March (Late Harvest) for which the latter received two different water addition treatments to 14 and 16ºBaume respectively; results show mean ± standard error (n=3)..

Tannin compositional measure	Unit	Treatment			Probability >F
		Control	Late Harvest 14 ºBaume	Late Harvest 16 ºBaume	
Tannin molecular mass (subunit)[§:]	g/mol	1903 ± 166	1957 ± 70	2224 ± 162	ns
Tannin molecular mass (50% GPC)[¶:]	g/mol	2239 ± 66	2173 ± 59	2120 ± 97	ns
mDP[††]	no unit	6.37 ± 0.55	6.53 ± 0.23	7.42 ± 0.54	ns
Degree of trihydroxylation	%	31.22 ± 1.14	30.26 ± 0.86	31.54 ± 1.63	ns
Degree of galloylation	%	2.30 ± 0.09	2.96 ± 0.23	2.98 ± 0.09	ns
Mass conversion[‡‡]	%	8.08 ± 1.98	17.27 ± 3.57	14.83 ± 2.26	ns

[§]:Molecular mass determined by using subunit composition from phloroglucinolysis

[¶]: Molecular mass determined at 50% elution by gel permeation chromatography (GPC)

[††]Mean degree of polymerization

[‡‡]Mass conversion based on % recovery of proanthocyanidin by phloroglucinolysis as a proportion of tannin concentration by methyl cellulose precipitation

In summary, the results from this experiment indicate that no gains in wine tannin, phenolics or colour properties (total colour, and stable colour) were achieved by delaying harvest and increasing the associated shrivel, given that the control and late harvest (16ºBaume) treatments were comparable in terms of the water addition received This indicates that marked seasonal variation in grape and wine phenolic outcomes for a single vineyard may occur, and this warrants further investigation given the magnitude of the differences found. While these findings reflect that after a certain level of ripeness is reached, possibly with the onset of shrivel, no further gains in extractable phenolics were made by delaying harvest further, it is important to note that as was observed in the 2017 study, the quantity of water added did not significantly affect phenolics measures in the resultant wines. This suggests that for shrivelled, overripe fruit, water can be added to reduce Shiraz must °Baume to a required level, without raising obvious concerns that water addition may result in reduced phenolic concentrations as proxy for potential wine quality losses.

An ongoing component of the research reported in this section, and which is still underway at the time of reporting as part of a continuing project (AWR1701-3.1.1) is the contribution of shrivel and extended hang time to the development of 'jammy' sensory attributes in Shiraz wine. It is relevant to note that the work of Schelezki et al. (2018a) noted the introduction of a 'port-like' attribute in wines prepared from over-ripe, shrivelled Cabernet Sauvignon grapes, which was not overcome with water addition. This attribute may relate to the 'jammy' character identified in Shiraz. Furthermore, the results reported previously (section 6.3.1) and also published (Bindon et al. 2020), saw the concomitant increase of a 'dried fruit' attribute with continued ripening, which while correlated with 'dark fruit' attributes was nonetheless defined as a unique descriptor. The 'dried fruit' attribute may also be associated with what is anecdotally described as 'jammy' in Shiraz. The ongoing assessment of the wines made from the 2019 study will seek to both elucidate the volatile fraction responsible for the development of 'jammy' characters, and may shed light on their development during ripening, and whether water addition affects their perception in Shiraz.

6.4 Knowledge of the impact of accumulation as opposed to berry dehydration on grape sugar content and concentration, a meta-analysis of published studies

For this component of the project, trajectories of grape ripening from data in published studies were analysed to determine the maximum sugar concentration at which sugar accumulation by grape berries ceases in Shiraz and Cabernet Sauvignon under a range of Australian conditions. It was expected that these figures could be used as a guide to determine when fruit dehydration is likely to occur and hence help producers with harvest decisions.

The results from this study have been published previously (Petrie et al. 2020). From the analysis, it was found that despite the sugar concentration (Baume) continuing to increase in grapes there were very few observations where the berry sugar content increased beyond approximately 13°Baume for Shiraz (Figure 6.4) and beyond approximately 14.5°Baume for Cabernet Sauvignon. This is close to the threshold of 13.8°Baume reported by Keller (2015). As the dataset used for this analysis includes the results from McCarthy and Coombe (1999), where sugar accumulation stopped at 20-21ºBrix (11.1-11.7ºBaume), it is likely that other environmental (e.g. water stress), management or vine factors (e.g. low source to sink ratio) halted the sugar accumulation for the berries at a lower concentration.

At maturity, grape berries contain very high soluble sugar concentrations compared to other fruit crops (Coombe 1976). As grapes ripen, the sugar unloading pathway moves from symplasmic to apoplasmic from veraison (Zhang et al. 2006), which indicates that transmembrane transport of sugars occurs during this period (Dai et al. 2010). The process of transport of sugar into the berries is yet to be fully defined; potentially it involves passive diffusion, turgor-driven mass transport or active flow transport regulated by sugar transporters (Davies and Robinson 1996; Hayes et al. 2007). However, all of the potential models of sugar movement into the berries are in part regulated by the differing concentration of sugars (osmotic potential) between the phloem and fruit cells (Dai et al. 2010). This implies that as the concentration in the fruit cells increases the importation of sugar will slow or eventually stop and that berries can reach a theoretical maximum sugar concentration beyond which further increases are only due to dehydration of grapes (Bondada et al. 2017, Coombe and McCarthy 2000) and this is

supported by empirical observations. The loss of cellular functionality related to mesocarp cell death, particularly in Shiraz, might have also contributed to these differences (Xiao et al. 2018a, b).

As the Shiraz stopped accumulating sugar at a lower concentration compared to Cabernet Sauvignon, there were many more data points available from samples collected that were at or close to the maximum sugar content per berry. In addition, Cabernet Sauvignon ripens later than Shiraz (Petrie and Sadras 2008), so there is less opportunity to collect samples of riper fruit (above 14.5°Baume). As a consequence it was easier to define the upper limit for sugar accumulation for Shiraz. The magnitude of the difference in the sugar concentration beyond which the Cabernet Sauvignon and Shiraz cease accumulating sugar was also surprisingly large given that the processes that regulate sugar accumulation are likely to be common across many fruit crops. However, the propensity for Shiraz berries to shrivel at relatively low sugar concentrations compared to other cultivars is well defined (McCarthy 1999, McCarthy and Coombe 1999) and has been related to differences in other physiological processes such as a loss of membrane integrity and cell death (Tilbrook and Tyerman 2008, Xiao et al. 2018a, b). The collection of berry weight and sugar concentration metrics from other cultivars, especially for ripe and overripe fruit, would be beneficial to understand the range that can occur.

When detached fruit was dried in a controlled environment (drying tunnel), a 1°Baume increase in sugar concentration was associated with a weight loss of approximately 9% due to dehydration (Muganu et al. 2011). A similar trend would be expected once sugar importation into the berries has ceased but this was not directly analysed. The implication is that if a Shiraz vineyard is harvested at 15°Baume, its yield would be more than 20% lower than if it were harvested at 13°Baume. Desirable texture, flavour and aroma characters may continue to develop between 13 and 15°Baume (Bindon et al. 2013, Coombe and McCarthy 2000), so while the Shiraz yield is decreasing, the value of the fruit is likely to increase even if the mass of sugar (and potential final quantity of wine) does not change, as described in the previous sections of this report.

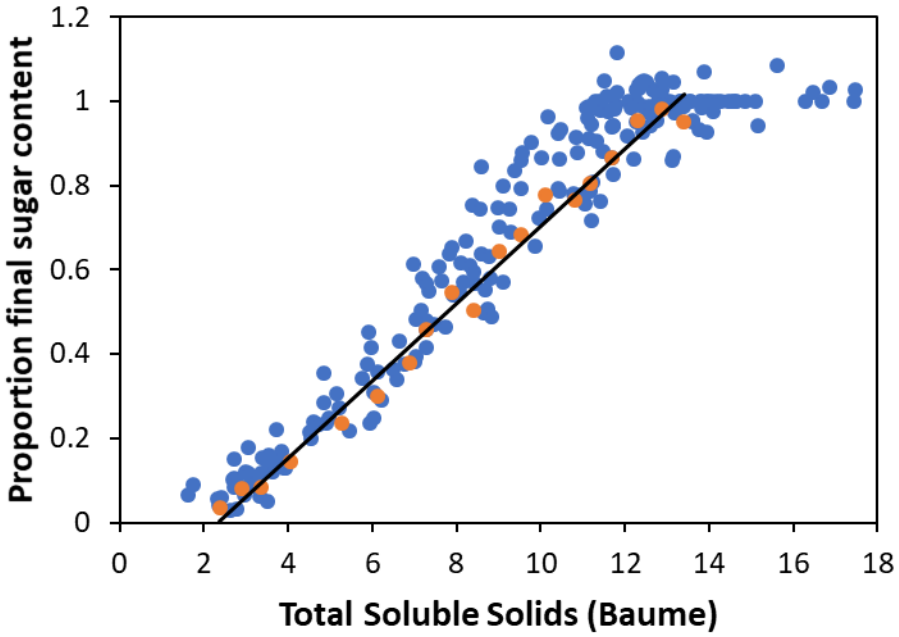


Figure 6.4. The relationship between Shiraz berry sugar concentration and the proportion of the final berry sugar content, from previously published data. The orange symbols represent the 10th percentile for arbitrary 0.56°Baume sections of the data, and a boundary line regression is fitted to this data. Proportion final sugar content = 0.094*°Baume - 0.228 with R^2 = 0.98. Data was collected from 30 experiments and includes 220 sample points.

Amongst the published data there were many trials where the fruit stopped accumulating sugar earlier or did not reach this value at all (often due to harvest or inclement conditions). Tracking sugar per berry

in addition to berry sugar concentration will potentially aid in making better harvest decisions, especially in these situations. If the fruit has reached its desired flavour profile and sugar accumulation has ceased, then there is little to be gained by delaying harvest. While it is currently prohibitively expensive to manually count and weigh berries, as technology to automate the measurement of berry size improves (Liu et al. 2015), growers or wineries may be able to easily measure sugar per berry for their own blocks as an aid to harvest decisions.

6.5 Impact of climate change on pH and TA, and their balance with sugar concentration in grapes

The advancement in grape maturity over time, in response to warmer ripening conditions, has been well categorised in Australia (Petrie and Sadras 2008) and overseas (Duchêne and Schneider 2005). The other parameters that affect maturity, titratable acidity (TA) and pH, are rarely measured on ripening fruit outside a research context, due to the additional laboratory resource requirements. An extensive commercial dataset has formed the basis of previous work (Petrie and Sadras 2008), and was reanalysed to investigate trends in pH and TA. As with total soluble solids, the date when nominated values for TA (9 g/L) and pH (3.4) were reached followed a trend during the time period studied (1993-2018, data not shown). The decision to harvest involves a compromise between the three maturity parameters (TSS, TA and pH) (Coombe et al 1980) as well as flavour development. This means that studying the temporal trends of each parameter separately has limited value. Instead in this project the TA and pH were assessed at the time when a TSS of 12 ºBaume was reached. This provided an indication of the change in sugar/acid or sugar/ pH balance over time, which is likely to be a better representation of fruit quality (Coombe et al. 1980). It also provides an assessment of the changing fruit characteristics that grapegrowers and winemakers might need to manage in response to warmer conditions. As shown in Figures 6.5 and 6.6, the calculated average TA and pH of the fruit at the time when it had an average TSS of 12 Baume was remarkably consistent across the 25 years of data. For Shiraz grown in the Barossa the TA and pH averaged 8.9 g/L and 3.3 respectively (Figure 6.5) and for Cabernet Sauvignon grown in Coonawarra the TA and pH averaged 7.5 g/L and 3.3 respectively (Figure 6.6). Similar responses were seen for other cultivar and region combinations (data not presented). Note that these values are from a designated TSS (12 Baume) which is prior to harvest and therefore the TA is higher and pH lower than the values commonly observed at harvest.

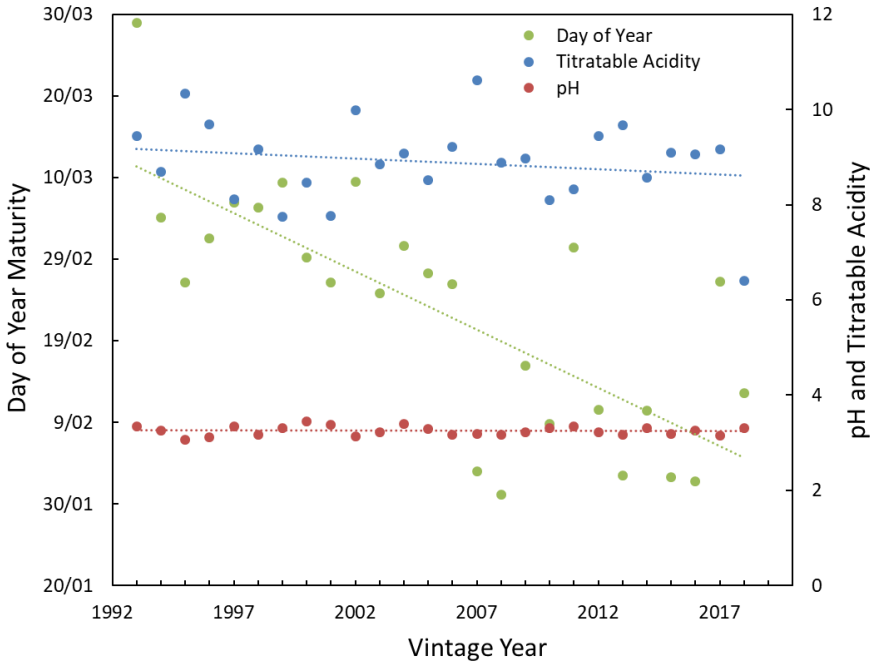


Figure 6.5. The trend in the day of year when the regional average grape total soluble solids reached 12 Baume (green, DoY = -1.43 year + 2920, r^2 = 0.56, $p < 0.01$), and the corresponding average titratable acidity (blue, n.s.) and pH (red, n.s.) recorded at this date for Shiraz grown in the Barossa Valley

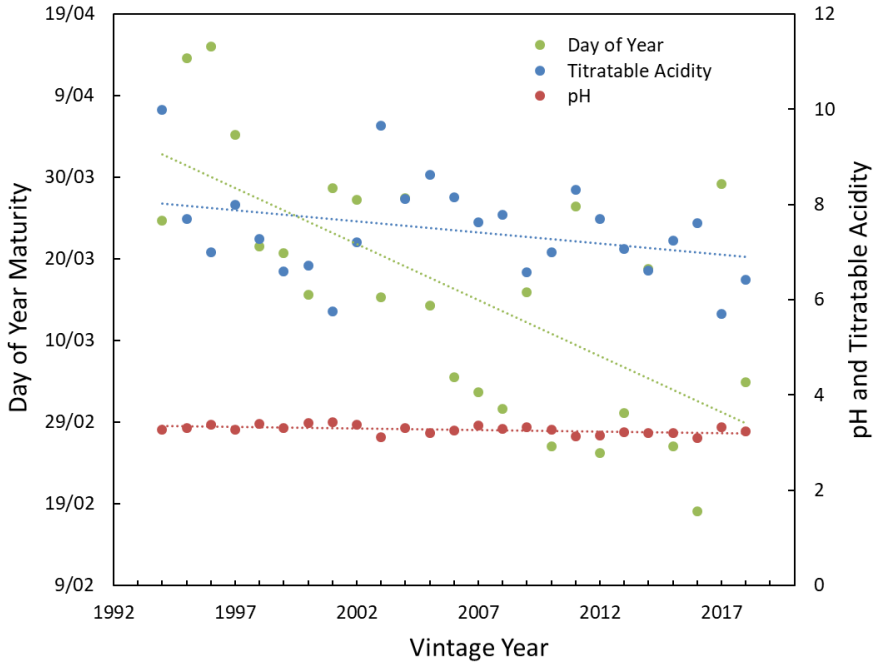


Figure 6.6. The trend in the day of year (green, DoY = -1.37 year + 2833, r^2 = 0.45, $p < 0.05$) when the regional average grape total soluble solids reached 12 Baume, and the corresponding average titratable acidity (blue, n.s.) and pH (red, n.s.) recorded at this date for Cabernet Sauvignon grown in Coonawarra.

The consistency of the TA and pH values for each of the region and cultivar combinations was unexpected. The breakdown of organic acids, especially malic acid at warmer temperatures has been well characterised (Kliewer et al. 1965) and grapes grown in warmer regions normally have higher pH and lower TA at a given TSS (Barnuud et al. 2014). Likewise when grapes have been exposed to higher temperatures in the glasshouse, elevated TSS and reduced pH and TA are generally reported (Radler 1965, Buttrose et al. 1971). When vines were grown using passive heating chambers, the response varied between cultivars with Cabernet Franc and Chardonnay reporting the expected increase in pH and decrease in TA while Shiraz was relatively unresponsive (Sadras et al 2013). Despite the expectation that the pH and TA of grapes will be disturbed by warmer temperatures due to climate change (Duchêne et al. 2014, Mira de Orduña 2010) results in this study demonstrate the resilience of the grape production systems currently used in Australia. At least for the time being, the fruit is maturing earlier yet the sugar to acid ratio has remained stable over time despite the warmer conditions.

7. Outcomes and conclusions:

The current study had two major objectives:

- to investigate the impacts of vintage advancement and compression on fruit quality
- to consider options to mitigate the effects of vintage compression, specifically the delays in harvesting fruit and resulting high must sugar (and corresponding ethanol) concentrations that may eventuate.

To address the impact of vintage advancement and compression two distinct aspects were studied. The first extended the observation that grapes were ripening earlier and explored if TA and pH were following a similar trend and if the sugar to acid ratio was changing. For this analysis an updated commercial dataset that had been used previously to quantify vintage advancement was used as the basis. The second involved determining if a critical time point exists for sugar accumulation in red varieties, after which increases in sugar concentration proceed as a result of berry volume loss, and not through import of sugars from the grapevine. This aspect was studied using a meta-analysis across a range of publicly available research datasets for which both sugar accumulation and berry weight data were accessible, in order to separate the impact of sugar importation from berry dehydration. The aim of this component of the project was to provide information to industry to guide harvest decisions.

Options to ameliorate fruit harvested at a high sugar concentration using water addition were investigated for Australia's principal grape varieties Shiraz and Chardonnay. The impact of the amount, timing and method of water addition on wine composition and style was assessed, in order to provide guidelines to industry for use when adopting this practice.

The data analysis established that trends in pH and TA changed over time in a similar manner to previous reports for TSS; that is, higher pH and lower TA values were being recorded earlier in the season. More interesting is that the sugar to acid ratio (for both TA and pH) did not trend lower across the seasons 1993 – 2018. This is a very positive observation for industry as it suggests that these maturity parameters will (at least for the time being) remain synchronised despite warmer conditions and accelerated ripening. The boundary layer regression analysis of existing berry weight and sugar accumulation for Shiraz and Cabernet Sauvignon grown under Australian conditions was able to empirically determine plateaus in sugar content per berry for the two varieties, which were found to be 13ºBaume and 14.5ºBaume, respectively. This indicates that above these sugar concentrations it can be expected that further gains in sugar concentration are likely associated with a loss in berry volume and not sugar import from the vine. Given the known propensity for Shiraz grapes to enter a phase of shrivel late in the ripening process, this finding indicates that the critical point for Shiraz is substantially earlier than might have been expected. Although extending 'hang time' beyond 13 ºBaume may present some gains in grape compositional attributes relevant to wine quality (e.g. phenolics, aroma precursors), knowing that a time point exists beyond which berry dehydration will occur can aid producers with their harvest decisions, particularly when yield is an important consideration.

Further to this, an important goal of the project was to understand potential gains in wine quality which might be achieved by extending 'hang time', and whether dehydration of the fruit and associated increases in must sugar might be overcome by water addition during winemaking. The 2017 winemaking study on Shiraz highlighted that delaying the harvest introduced demonstratable increases in extractable grape anthocyanin and tannin (in part due to berry volume loss) which translated into clear differences in wine phenolic composition. While water addition to the later-harvest must using either juice substitution or direct addition resulted in a reduction in key phenolic attributes, an important finding was that the mode of water addition, whether through juice substitution or direct addition of water without juice removal, did not affect key wine phenolic properties. It was also of relevance to observe that water addition treatments to the later-harvest musts consistently resulted in wines with enhanced phenolic attributes, colour and important aroma and flavour attributes when compared with wines made from the earlier harvests. With this in mind, the later (2019) winemaking study, which focused more on shrivel in Shiraz, clearly demonstrated that after reaching 15.5ºBaume no further gains in wine phenolics could be achieved as 'hang time' was extended. In the 2019 study, the extent of water addition was found not to affect phenolics while it adequately reduced ethanol. While these results indicate more generally that water addition can be a simple strategy to control wine ethanol in Shiraz and may facilitate increases in wine phenolics, aroma and flavour associated with extended 'hang time', caution should be applied in practice. Notably, berry weight losses (and hence wine volume losses) associated with later harvests may be compensated by water addition, but water addition will not necessarily increase the final wine volume relative to what could be achieved by harvesting earlier. Furthermore, there is as yet a lack of understanding as to the changes in grape volatile precursors which give rise to 'dried fruit' (or potentially 'jammy') attributes as grapes begin to dehydrate. These attributes increased in wines made from riper fruit, together with other attributes that might be considered beneficial such as 'dark fruit'. Also, it was relevant to note that higher levels of water addition were associated with increases in potentially negative off odours, and, as such, in a commercial context smaller quantities of water addition would be considered advantageous.

The 2018 winemaking study investigating ripeness, water addition and a 12-hour skin contact period in Chardonnay produced wines that were less variable in both composition and sensory properties than those from the Shiraz study. The skin contact treatment led to different outcomes for wine sensory properties depending on the ripeness of the fruit undergoing fermentation, but did not appear to introduce consistent changes which could potentially be used to overcome losses in important wine sensory properties with water addition. In the Chardonnay study, a clear separation from control was observed of wine from earlier-harvest grapes and wines that received water addition (excluding those

receiving skin contact), with both being higher in 'citrus' flavour and lower in bitterness and hotness. This indicates that the effect of dilution on key wine sensory properties in Chardonnay was less significant than observed for Shiraz. However, since no clear gains in many important wine sensory properties were achieved by delaying harvest, meeting a target commercial ºBaume rather than relying on water addition to must would be preferable.

Generally, the results of this project have substantially advanced the understanding of how to approach challenges related to vintage compression from a grape ripeness standpoint. Growers can more accurately predict the ripeness level for the principal red varieties grown in Australia (Shiraz and Cabernet Sauvignon) using a combination of sugar concentration and berry weight. In the case of Shiraz an earlier ºBaume plateau was observed for sugar accumulation than for Cabernet Sauvignon.. Important knowledge was gained in terms of understanding the impact of delaying harvest further than 13ºBaume in Shiraz, showing that some gains in phenolic potential might be realised by delaying harvest to 15.5ºBaume, which could be maintained after water addition, but no further increases in phenolics were found beyond that point. At the same time, consideration needs to given to the risk from extended 'hang time' and shrivel in Shiraz on the aroma profile of the wine in terms of over-ripe and potentially 'jammy' characters which might derive from chemical or biochemical processes associated with dehydration. Before the use of water addition can be recommended more generally as a means to alleviate issues with high ºBaume musts associated with vintage compression, a better understanding of quality aspects, and consumer perception related to 'jamminess' in Shiraz wines is required, and this aspect will be the focus of future research arising from this project.

8. Recommendations:

This project aimed to address challenges associated with accelerated grape ripeness as a first response to address the consequences of vintage compression. In making recommendations based on the results, it is critical to identify that the key stakeholders affected by these issues are predominantly grapegrowers. A fundamental point which needs future consideration is that grapegrowers are paid primarily based on the yield of grapes. A potential point of conflict, therefore, is the discrepancy between achieving maximum yield for a given harvest sugar level, as opposed to delaying harvest due to the requirements of wineries to have higher concentrations of desirable attributes, such as phenolics (red grapes) and aroma/flavour, at the expense of yield. A clear recommendation based on this project is that the concerns of grapegrowers about some harvesting practices need to be addressed, as the findings show that delaying the harvest beyond the point where berry sugar accumulation stops provides no gains in terms of yield, while at the same time a delayed harvest potentially causes yield losses and/or quality losses, for example through increasing the risk of berry splitting with an associated susceptibility to disease.

It is recommended that growers consider tracking the accumulation of sugar in their vineyards by maintaining records of both ºBaume (juice) and berry weight, to determine the point at which accumulation ceases, and dehydration begins. Considering the results presented in this report, this is particularly critical in the case of Shiraz, where it was shown that over 13ºBaume increases in wine phenolics and improvement in sensory properties could be observed, which were retained even after water addition, yet no further gains in phenolics were realised after reaching 15.5 ºBaume. Hence, a recommendation is that for Shiraz, target sugar concentrations should remain between 13.0 and 15.5ºBaume, irrespective of the FSANZ amendments allowing water addition to must to reduce the pre-ferment ºBaume of must to 13.5. This is particularly relevant in light of the findings that higher levels of water addition, as would be required when the must ºBaume is very high, led to the development of off-odours, which were not explained by the chemical analyses conducted in this study. Hence further research to understand the development of off-odours in response to pre-ferment water addition is recommended. As discussed previously, another important area for future research is to understand the development of 'dried fruit' and 'jammy' attributes that may arise in wine made from dehydrated Shiraz fruit, since this will affect the finished wine independent of dilution effects. This research will also contribute to better understanding of the contribution of 'jammy' attributes to overall wine quality and consumer acceptance in Shiraz.

A further important practical observation from the studies on water addition was the difficulty in accurately adjusting the must ºBaume with water addition based on sugar measurements. There are multiple reasons why this may have eventuated. A set rate of extraction is currently used to predict the expected must volume, and this may differ between grape varieties and more importantly may vary based on the ripeness and degree of dehydration of the fruit. As the use of water in winemaking becomes more widespread, an enhanced ability to better characterise variations in extraction rate for different grape varieties, regions, seasons and ripeness levels will become critical. Use of this data could support the development of a platform for wine producers to access and more accurately estimate the extraction rate in the future.

Beyond extraction rates, the study on Shiraz shrivel revealed that sugar concentration in the must can increase substantially after 12 hours of skin contact. Basing water additions on must sugar measurements taken after an initial skin contact period is therefore recommended to more accurately target the final wine ethanol concentration. Considering this point, what remains unknown is the extent to which berry dehydration affects the extraction of sugar from the skins after crushing. Conceivably, more dehydrated skins may sequester higher concentrations of sugar, and this warrants further investigation, especially since water additions would only be used for riper batches of fruit.

The results of the trial on Chardonnay pointed to a similar concern for white grapes, in that preliminary estimates of must sugar might change following standard enzyme and settling treatments. Hence, it is recommended that the sugar measurement on which water additions are based is taken as close as possible to the point of water addition/inoculation for white varieties as well.

Another aspect for mitigating vintage compression that was beyond the scope of this project is that the influx of fruit within a short timeframe can place significant stress on processing logistics and capacity in the winery. Another option for managing vintage compression is therefore to increase the speed at which fruit can be processed. For red wines, the major processing bottleneck is red fermenter capacity and faster extraction techniques (including maceration acceleration, thermovinification and flash détente) can reduce the time that red ferments need to be in contact with skins. While these techniques may present capex requirements and some unknowns in terms of flavour profiles which may make their adoption less attractive to industry at first, faster red winemaking processes could potentially address some of the risks associated with permitted water addition to must. Future research could aim to compare a range of extraction processing options for red grapes on a commercial scale, in combination with water addition, with a view of reducing processing time and maintaining desirable sensory attributes.

9. Appendix 1: Communication:

9.1 Conference presentations:

- Bindon, K.A., Teng, B., Smith, P.A., Espinase Nandorfy, D., Solomon, M. and Petrie, P.R. Winemaking with high Baume juice: optimising fermentation and extraction. Paper presented at the 17th Australian Wine Industry Technical Conference, Adelaide Convention Centre, Adelaide, SA, Australia, 23 July 2019.

- Petrie, P.R., Jiang, W., Bindon, K.A., Sadras, V.O. When do grapes stop accumulating sugar? Paper and poster presented at the 17th Australian Wine Industry Technical Conference , Adelaide Convention Centre, Adelaide, SA, Australia, 23 July 2019.

9.2 Publications:

- Bindon; K.A., Teng, B., Smith, P.A., Espinase Nandorfy, D., Solomon, M., Petrie, P.R. 2020. Winemaking with high Baume juice: optimising fermentation and extraction. Beames, K.S, Robinson, E.M.C., Dry, P.R., Johnson, D.L. (eds.). *Proceedings of the 17th Australian Wine Industry Technical Conference, Adelaide, South Australia, 21-24 July 2019.* Urrbrae, S.A: AWITC Inc.: 31-133.
- Petrie, P.R.,Teng, B., Smith, P.A., Bindon, K.A. 2019. Managing high Baume juice using dilution, *Wine Vitic. J.* 34(1): 36-37.
- Petrie, P.R. Jiang, W., Bindon, K.A., Sadras, V.O. 2020. When do grapes stop accumulating sugar? Beames, K.S, Robinson, E.M.C., Dry, P.R., Johnson, D.L. (eds.). *Proceedings of the 17th Australian Wine Industry Technical Conference, Adelaide, South Australia, 21-24 July 2019.* Urrbrae, S.A.: AWITC Inc.: 37-39.
- Teng, B., Petrie, P.R., Smith, P.A., Bindon, K.A. 2020. Comparison of water addition and early-harvest strategies to decrease alcohol concentration in *Vitis vinifera* cv. Shiraz wine: impact on wine phenolics, tannin composition and colour properties. *Aust. J. Grape Wine Res.* 26(2): 158-171.
- Teng, B., Petrie, P.R., Espinase Nandorfy, D., Smith, P., Bindon, K. 2020. Pre-fermentation water addition to high-sugar Shiraz must: effects on wine composition and sensory properties. *Foods* 9(9): 1193.

9.3 Extension:

- Bindon, K.A. How to maximise the phenolics of grapes through innovative winemaking? AWRI Langhorne Creek Seminar. 20 September 2018, The Winehouse, 1509 Langhorne Creek Road, Langhorne Creek, SA (This seminar included aspects of water addition effects on phenolics in wine).
- Outcomes of this work have been incorporated into a new AWRI roadshow seminar: ‘Water into wine – when to use it and how much?’
- The AWRI fact sheet ‘Reducing alcohol levels in wine’ was updated to reflect the new body of work resulting from 4.1.1 research.

10. Appendix 2: Intellectual Property

Nothing to report.

11. Appendix 3: References

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12.Appendix 4: Staff

12.1 AWRI staff:

- Dr Keren Bindon, project management, data analysis and interpretation, writing and communication of results.
- Dr Bo Teng (until September 2018) sample collection, analysis and data interpretation and writing.
- Assoc. Prof. Paul Petrie (until November 2018) project management, experimental design, data analysis and interpretation, writing and communication of results.
- Mrs Stella Kassara, targeted non-volatile analysis of Shiraz and Chardonnay grapes and wines (2017 and 2018)
- Dr Paul Smith (until September 2017), experimental design of the 2017 Shiraz trial, writing and interpretation of the results.
- Dr I. Leigh Francis, inspection and interpretation of sensory data (2017 and 2018)
- Mr Alex Schulkin, phenolic analysis of Chardonnay wines (2018)
- Mr Luke Qi, analysis of colour and tannin in grape extracts (2019)
- Dr Alicia Jouin, targeted non-volatile analysis of Shiraz wines (2019)
- Ms Maddy (WenWen) Jiang, compilation of grape ripening data for the meta-analysis study
- Mr Damian Espinase Nandorfy, overseeing the sensory analysis of the Shiraz and Chardonnay wines, compilation, analysis and interpretation of sensory results (2017 and 2018).
- Mr Mark Solomon, analysis of wine fermentation products (2017 and 2018).
- Dr Josh Hixson, experimental design and project management of the Shiraz shrivel and dilution project (2019)
- Ms Jana Hildebrandt, experimental design, sample collection and extraction, coordination of harvesting and winemaking for the Shiraz shrivel and dilution project (2019)
- Mr John Gledhill, experimental winemaking for all wine results reported in the project, including coordination and reporting of basic must and wine analytical results.

12.2 Collaborators

- Prof. Victor Sadras (SARDI) experimental design, data analysis and interpretation for the meta-analysis and assessment of trends on grape pH and TA.
- Dr Marcos Bonada (SARDI) support of fruit sourcing and maturity assessment for the Shiraz dilution trial.
- Assoc. Prof Paul Petrie (SARDI since November 2018) project management, experimental design, data analysis and interpretation, writing and communication of results.
- Dr Catherine Kidman, Dr Anthony Robinson and Dr Vanessa Stockdale, Treasury Wine Estates

13 Appendix 5: Supplementary data

Table 13.1. F-ratios, probability values†, degrees of freedom (df) and mean square error (MSE) from the analysis of variance of the Shiraz 2017 wine sensory study.

Attribute	Treatment	WRep (Treatment)	Judge* Treatment	Judge*WRep (Treatment)	PRep (Treatment, WRep)	MSE
Opacity	43.57***	3.16***	1.87***	1.00	1.64*	0.520
Brown colour	10.13***	1.43	2.89***	0.81	1.63*	0.294
Red fruit A	1.64ǂ	0.88	1.82***	0.86	1.43	1.543
Confection A	0.61	0.95	1.32*	0.79	1.29	1.839
Dark Fruit A	5.18***	0.83	1.80***	0.86	1.25	1.068
Dried Fruit A	2.44**	1.53	1.66***	0.83	1.06	1.592
Floral A	1.72ǂ	1.25	1.22	0.80	1.01	1.641
Spice A	3.07***	1.34	1.13	0.94	0.90	0.807
Chocolate A	2.42**	1.32	1.70***	0.83	0.88	1.850
Cooked veg A	2.20*	0.89	1.40**	1.05	1.82**	1.488
Earthy A	2.06*	1.21	1.64***	0.83	0.56	1.271
'Sweet' plastic A	0.58	1.12	1.52**	0.87	0.85	1.396
Rubber A	1.87ǂ	1.45	1.97***	0.89	0.72	0.632
Pepper A	1.73ǂ	1.12	1.18	0.81	0.97	0.875
Menthol A	4.10***	1.85*	0.79	1.15	1.80**	1.318
Savoury A	3.43***	1.91**	1.35*	1.00	1.70**	1.602
Pungent A	4.12***	0.92	1.08	0.91	1.05	0.766
Stalky A	0.99	0.55	1.13	0.97	1.30	1.592
Drain A	6.65***	1.21	1.19	1.01	1.70**	1.306
Sweetness	1.94*	1.25	1.90***	1.16	0.87	0.892
Viscosity	6.85***	0.58	1.08	0.77	0.63	0.906
Acidity	1.28	0.66	1.00	0.75	1.02	0.803
Astringency	1.67ǂ	1.14	1.40**	0.99	1.47*	0.805
Bitterness	1.42	0.98	1.00	0.99	0.89	1.427
Hotness	5.62***	0.80	1.14	0.81	1.25	1.273
Saltiness	1.15	1.66*	1.33*	0.93	1.64**	0.937
Umami	1.99*	1.72*	1.78***	0.57	1.31	1.015
Red Fruit F	1.38	1.16	3.05***	0.69	1.16	1.287
Confection F	0.96	0.71	1.44**	0.97	1.44	1.499
Dark Fruit F	9.19***	1.06	1.72***	0.76	1.13	1.060
Dried Fruit F	3.47***	1.27	2.05***	0.76	0.76	1.696
Chocolate F	5.64***	0.77	1.03	0.86	0.92	1.476
Stalky F	0.89	1.00	2.03***	0.96	1.25	0.988
Earthy F	2.72**	0.89	1.73***	0.96	1.20	1.225
Spice F	1.90*	1.20	1.85***	0.96	1.16	1.109
Fruit AT	1.32	0.79	1.38*	0.97	1.24	0.707
df	11	24	110	240	36	360

A: aroma, F: Flavour, AT: After Taste. †Significance levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ǂ $P \leq 0.10$. df = degrees of freedom. Judge effect was significant for all attributes ($P < 0.05$), WRep = winemaking replicate, PRep = presentation replicate, J = Judge.

Table 13.2. F-ratios, probability values†, degrees of freedom (df) and mean square error (MSE) from the analysis of variance of the Chardonnay 2018 wine sensory study.

Attribute	Treatment	WRep (Treatment)	Judge* Treatment	Judge*WRep (Treatment)	PRep (Treatment, WRep)	MSE
Yellow colour intensity	46.20***	5.45***	2.45***	1.17	1.97*	0.283
Tropical A	1.26	0.97	1.46*	0.83	0.97	1.124
Citrus A	1.47	0.82	1.41*	0.56	0.81	1.537
Stone fruit A	1.06	0.60	0.84	0.88	1.10	1.601
Passionfruit/ boxhedge A	1.37	0.64	1.16	1.25	1.61	2.052
Apple/pear A	1.62	0.76	1.02	1.09	0.74	1.645
Confection A	1.74ǂ	1.01	1.12	0.64	1.49	2.043
Cheesy A	1.83ǂ	2.43*	1.91**	1.11	0.92	2.025
Floral A	0.79	1.26	1.48*	0.91	1.22	1.450
Honey A	0.77	1.02	0.65	1.77***	1.45	1.552
Grassy/stalky A	0.82	1.04	2.53	0.85	0.94	1.064
Flint A	1.88ǂ	2.42*	1.56	0.65	0.88	1.820
Drain A	3.19**	0.99	1.10	1.14	0.89	1.861
Pungency	1.62	1.86	1.41*	0.89	1.76*	0.568
Acidity	0.88	1.06	1.43*	0.95	0.44	0.429
Astringency	1.05	0.85	1.40	0.89	1.26	0.429
Bitterness	2.29*	1.73	1.02	0.90	0.81	1.062
Viscosity	5.58***	1.02	1.11	1.18	1.03	0.370
Hotness	3.83***	0.47	0.81	1.00	1.27	0.893
Sweetness	3.03**	1.35	0.74	1.13	0.77	1.476
Tropical F	1.14	2.56**	1.89**	0.87	0.42	0.482
Citrus F	1.88ǂ	1.23	1.40	0.85	0.83	0.836
Stone fruit F	2.25*	1.06	1.13	1.17	1.19	1.059
Apple/pear F	0.95	1.03	1.07	1.11	0.88	1.493
Yeasty F	1.30	0.94	1.55*	1.38*	1.13	1.396
Grassy/stalky F	0.65	1.02	0.54	1.76***	1.03	0.811
df	9	10	90	100	20	200

A: aroma, F: Flavour. †Significance levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ǂ $P \leq 0.10$. df = degrees of freedom. Judge effect was significant for all attributes ($P < 0.05$), WRep = winemaking replicate, PRep = presentation replicate, J = Judge.

Table 13.3. Weighted regression coefficients from partial least squares regression analysis of all sensory attributes which were identified as descriptors of Shiraz wines prepared from different harvest dates, and which underwent water addition or saigneé treatments (NB, non-bleachable; poly pigment, polymeric pigments; MM, molecular mass; mDP, mean degree of polymerisation; %gall, galloylation; %C-P, catechin extension subunit; %EC-T, epicatechin terminal subunit; sugars are polysaccharide-associated monosaccharides; total poly, total polysaccharide).

	Significant wine compostional measure used for PLS regression model																
Sensory attribute	Hexyl acetate	Octanoic acid	2-Phenylethyl acetate	NB pigment	Hue	Poly Pigment	Tannin MM	mDP	%gall	%C-P	%EC-T	Mannose	Rhamnose	Glucose	Galactose	Xylose	Total Poly
Opacity	-0.039	-0.043	-0.039	0.061	0.059	0.060	0.057	0.057	-0.035	-0.061	-0.046	0.051	0.055	0.060	0.057	0.060	0.055
Brown colour	-0.046	-0.051	-0.047	0.072	0.070	0.071	0.068	0.068	-0.041	-0.073	-0.055	0.060	0.065	0.072	0.068	0.071	0.065
Red Fruit A	0.033	0.037	0.034	-0.052	-0.051	-0.052	-0.050	-0.049	0.030	0.053	0.040	-0.044	-0.048	-0.052	-0.050	-0.052	-0.047
Dark Fruit A	-0.036	-0.041	-0.037	0.057	0.055	0.057	0.054	0.053	-0.032	-0.058	-0.043	0.048	0.052	0.057	0.054	0.056	0.051
Dried Fruit A	-0.040	-0.045	-0.041	0.063	0.061	0.063	0.060	0.060	-0.036	-0.064	-0.048	0.053	0.057	0.063	0.060	0.063	0.057
Spice A	-0.030	-0.034	-0.031	0.047	0.046	0.047	0.044	0.044	-0.027	-0.048	-0.036	0.040	0.043	0.047	0.044	0.047	0.042
Chocolate A	-0.027	-0.030	-0.027	0.042	0.041	0.042	0.039	0.039	-0.024	-0.042	-0.032	0.035	0.038	0.042	0.039	0.041	0.038
Cooked Veg A	0.002	0.003	0.002	-0.004	-0.004	-0.004	-0.003	-0.003	0.002	0.004	0.003	-0.003	-0.003	-0.004	-0.003	-0.004	-0.003
Earthy A	-0.040	-0.045	-0.041	0.063	0.061	0.063	0.059	0.059	-0.036	-0.064	-0.048	0.053	0.057	0.063	0.059	0.062	0.057
Pepper A	-0.038	-0.042	-0.039	0.060	0.058	0.059	0.056	0.056	-0.034	-0.060	-0.046	0.050	0.054	0.059	0.056	0.059	0.054
Menthol A	-0.012	-0.014	-0.012	0.019	0.019	0.019	0.018	0.018	-0.011	-0.019	-0.015	0.016	0.017	0.019	0.018	0.019	0.017
Savoury A	-0.021	-0.023	-0.021	0.032	0.031	0.032	0.030	0.030	-0.018	-0.033	-0.025	0.027	0.029	0.032	0.030	0.032	0.029
Pungent A	-0.040	-0.045	-0.041	0.063	0.061	0.063	0.060	0.060	-0.036	-0.064	-0.048	0.053	0.057	0.063	0.060	0.063	0.057
Drain A	0.002	0.003	0.003	-0.004	-0.004	-0.004	-0.004	-0.004	0.002	0.004	0.003	-0.003	-0.004	-0.004	-0.004	-0.004	-0.004
Sweetness	-0.042	-0.047	-0.043	0.066	0.064	0.066	0.063	0.062	-0.038	-0.067	-0.051	0.056	0.060	0.066	0.063	0.066	0.060
Viscosity	-0.035	-0.039	-0.035	0.054	0.053	0.054	0.051	0.051	-0.031	-0.055	-0.041	0.046	0.049	0.054	0.051	0.054	0.049
Astringency	-0.029	-0.033	-0.030	0.046	0.045	0.046	0.043	0.043	-0.026	-0.047	-0.035	0.039	0.042	0.046	0.043	0.045	0.042
Hotness	-0.033	-0.036	-0.033	0.051	0.050	0.051	0.048	0.048	-0.029	-0.052	-0.039	0.043	0.046	0.051	0.048	0.050	0.046
Savoury F	-0.011	-0.013	-0.011	0.018	0.017	0.018	0.017	0.017	-0.010	-0.018	-0.013	0.015	0.016	0.018	0.017	0.017	0.016
Dark Fruit F	-0.036	-0.040	-0.036	0.056	0.055	0.056	0.053	0.053	-0.032	-0.057	-0.043	0.047	0.051	0.056	0.053	0.056	0.051
Dried Fruit F	-0.045	-0.050	-0.046	0.071	0.069	0.070	0.067	0.066	-0.040	-0.072	-0.054	0.059	0.064	0.070	0.067	0.070	0.064
Chocolate F	-0.034	-0.038	-0.034	0.053	0.051	0.053	0.050	0.050	-0.030	-0.054	-0.041	0.045	0.048	0.053	0.050	0.052	0.048
Earthy F	-0.037	-0.042	-0.038	0.059	0.057	0.059	0.056	0.055	-0.034	-0.060	-0.045	0.050	0.053	0.059	0.056	0.058	0.053
Spice F	-0.038	-0.043	-0.039	0.060	0.058	0.060	0.057	0.056	-0.034	-0.061	-0.046	0.051	0.054	0.060	0.057	0.059	0.054

13.4 List of the final articles short-listed for the meta-analysis of published data on sugar accumulation, berry size and content obtained from the Scopus search.

1. Bindon, K., C. Varela, J. Kennedy, H. Holt, Herderich, M. 2013. Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chem.* 138: 1696-1705.
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3. Bindon, K.A., Kennedy, J.A. 2011. Ripening-induced changes in grape skin proanthocyanidins modify their interaction with cell walls. *J.Agric. Food Chem.* 59: 2696-2707.
4. Böttcher, C., P.K. Boss, Davies, C. 2013. Increase in cytokinin levels during ripening in developing *Vitis vinifera* cv. Shiraz berries. American Journal of Enology and Viticulture 64: 527-531.
5. Böttcher, C., P.K. Boss, K.E. Harvey, C.A. Burbidge, Davies, C. 2018. Peduncle-girdling of Shiraz (*Vitis vinifera* L.) bunches and sugar concentration at the time of girdling affect wine volatile compounds. *Aust. J. Grape Wine Res.* 24: 206-218.
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8. Caravia, L., C. Collins, P.R. Petrie, Tyerman, S.D. 2016. Application of shade treatments during Shiraz berry ripening to reduce the impact of high temperature. *Aust. J. Grape Wine Res.* 22: 422-437.
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14.Appendix 6: Budget reconciliation

Not applicable.