



**ORIGINAL RESEARCH ARTICLE**

# Amending high sugar in *V. vinifera* cv. Shiraz wine must by pre-fermentation water treatments results in subtle sensory differences for naïve wine consumers

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## ABSTRACT

Global warming presents challenges to all wine-grape growers and winemakers across the globe. Increasing growing season temperatures means that grapes are harvested with higher sugar concentrations than preferred for high-quality winemaking. The now Australian permitted water-treatment of high-sugar musts can produce wines with lower alcohol levels, however, recent studies show impacts on associated chemical compounds and sensory parameters can affect final wine quality. Commercial applications based on these earlier findings are limited as many focus on one vintage, one or two harvest timings, small wine samples and limited numbers of panellists for sensory analysis. We expand by first investigating the chemical profiles and sensory parameters of wines produced from *V. vinifera* cv. Shiraz for three harvest times: early 13.5°Baumé (°Bé), middle 14.5°Bé and late 15.5°Bé across two vintages (2020/2021). Just-About-Right sensory analysis assesses 159 naïve wine consumer panellists' feedback. We show late 15.5°Bé and middle 14.5°Bé wine has the highest levels for chemical compounds of colour density (a.u.), total tannin (%), total pigment (a.u.), total phenolics (a.u.) and free anthocyanins (mg/L), as confirmed by PCA analysis outcomes. Sensory analysis shows the least preference for vegetal smell (2.50) in 13.5°Bé wine, with the highest preference for colour intensity (3.06) in early 13.5°Bé wine and body in late 15.5°Bé wine. Secondly, 15.5°Bé and middle 14.5°Bé pre-fermentation musts are subject to water dilution or 'bleed and replace' treatments. These wines have lower final alcohol levels, decreases in most chemical compound levels, but with subtler effects for sensory profiles. For instance, there is a high preference for astringency (3.01), ripe fruit taste (2.96) and hotness (2.95) in late diluted 13.5°Bé wine. Vegetal smell (2.89) is most preferred in late 'bleed and replace' 13.5°Bé wine. All water treatments have minimal impact on dark fruit smell, ripe fruit smell, odour intensity, odour complexity, red fruit taste and dark fruit taste. Middle diluted 13.5°Bé wine has the highest overall rating and is the most preferred. This study offers valuable insights revealing that water treatments of pre-fermentation musts can produce wines with preferable lower alcohol levels, and while this winemaking approach may negatively affect several chemical compounds, many favourable sensory properties can still be preserved. This is a crucial consideration for winemakers when addressing wine quality from these winemaking techniques.

**KEYWORDS:** harvest timing, fermentation, water treatment, chemical compounds, sensory attributes, wine quality.

## INTRODUCTION

### 1. Background

Global warming and rising temperatures during grape growing seasons result in shorter ripening periods and higher grape sugar levels, which lead to undesirable high wine alcohol levels (Schultz, 2010). To meet the demand for lower-alcohol wines (Saliba *et al.*, 2013) and to comply with regulatory restrictions, winemakers need to consider adaptive and alternative winemaking approaches to reduce alcohol content while maintaining organoleptic factors, wine flavour, and quality.

While vineyard management can address some of these challenges, winemaking approaches can also address the attainment of favourable alcohol levels in a final wine. In the vineyard, wine grape growers can for example: reduce leaf areas, irrigate vines just before harvest, apply growth regulators in the vineyard and also review and optimise the harvest date (Goold *et al.*, 2017). In the winery, winemakers have several winemaking approaches available to them to produce wines with lower more favourable alcohol levels. These include microbiological yeast approaches (Tilloy *et al.*, 2015; Lemos Junior *et al.*, 2019), blending later harvest wines with green harvest wines (Kontoudakis *et al.*, 2011; Longo *et al.*, 2017), and thermal approaches to removing alcohol post-fermentation (Gomez-Plaza *et al.*, 1999; Massot *et al.*, 2008; Belisario-Sanchez *et al.*, 2009; Margallo *et al.*, 2015). Each of these approaches can have both positive and negative outcomes for final wine aroma volatiles, chemical compounds and sensory parameters associated with them.

An additional approach available to winemakers in the winery is to add (dilution) or substitute ('bleed and replace') water into high sugar musts before fermentation to reduce ethanol yield during fermentation (Teng *et al.*, 2020; FSANZ, 2017). Early studies show that this winemaking approach can have varying impacts on final wine aroma volatiles, and chemical and sensory parameters, depending on the grape variety, harvest timings and the type of water treatment implemented. For instance, Piccardo *et al.* (2019) show that for *V. vinifera* cv. Merlot Noir and cv. Tempranillo Tinto, water treatments reduce ethanol levels, but result in significant differences in flavour profiles for each variety (Piccardo *et al.*, 2019). Schelezki *et al.* (2020b) demonstrate for *V. vinifera* cv. Cabernet Sauvignon water substitution treatments (at varying levels) of a late harvest 15.5 °Bé high sugar must produce final wines with reduced alcohol levels of 1.0–3.0 % alcohol-by-volume (ABV) compared to a late harvest control wine. For this variety, wine volatile profiles are markedly affected, decreasing as water levels are increased, while wine sensory profiles are similar to those of an early-harvest wine (Schelezki *et al.*, 2020b). For *V. vinifera* cv. Shiraz, Schelezki *et al.* (2020a) demonstrate that implementation rates of 47.2 %, 34.0 % and 10.2 % v/v of water into a mature fruit harvest must can produce lower alcohol wines with 10.6 %/9.6 %, 12.0 %/11.7 % and 14.5 %/14.4 % ABV levels, respectively. They show that for the lowest water addition treatment (+10.2 % v/v), wine

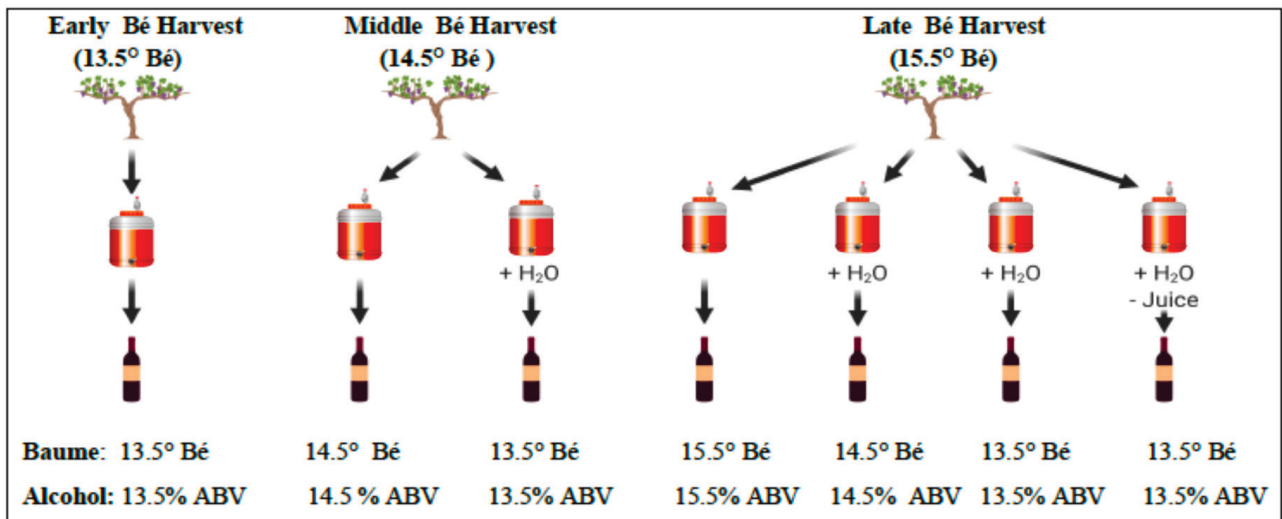
colour properties are significantly decreased, where tannin concentrations remain stable (Schelezki *et al.*, 2020a). Schelezki *et al.* (2020b) also demonstrate that water substitution treatments (at varying levels) of a late harvest 15.4 °Bé high sugar Shiraz must can decrease the alcohol levels in the final wines by 0.5 %–2.0 % ABV compared to the late harvest control wine (Schelezki *et al.*, 2020b). They confirm that tannin levels decrease with higher water substitution rates and dark fruit attributes tend to diminish with decreasing alcohol levels (Schelezki *et al.*, 2020b). Teng *et al.* (2020) also demonstrate that for Shiraz, water treatments of late harvest high sugar musts to a 13.5 °Bé can produce reductions in ethanol levels from 1.6 % volume by volume (v/v) to 2.1 % v/v compared to the late harvest control wine (Teng *et al.*, 2020) with all final treated wines displaying unfavourable cooked vegetable and drain attributes (Teng *et al.*, 2020).

### 2. Expanding on previous studies for water-treated *V. vinifera* cv. Shiraz wines

Shiraz is an important grape cultivar, grown in both warm and cool climates and is recognised as the most planted red grape variety in Australia (Schelezki *et al.*, 2020a). Any winemaking approach taken to reduce final alcohol levels in a Shiraz wine, while still maintaining wine quality will have a high economic impact for both winemakers and consumers. We expand on previous studies for Shiraz by testing wines produced from three different harvest times for one vintage: early 13.5 °Bé, middle 14.5 °Bé, and late 15.5 °Bé across two years (2020 and 2021) and for wines that have been subject to varied water treatments of their musts, pre-fermentation. Wines (in triplicate) are assessed for their chemical compositions and an expanded sensory analysis is undertaken from 157 naïve wine panellists. We evaluate optimal harvest times and the suitability of different water treatment winemaking approaches into high sugar Shiraz wine musts, to manage wine alcohol concentrations in a warming climate, and to maintain or to improve final wine quality parameters.

### 3. Global approaches for 'water treated wine grape musts' winemaking to produce wines with lower final alcohol levels

This study predominately focuses on the Australian approach, where water addition into wine must is now permitted to 'facilitate fermentation' to 13.5°Baumé (°Bé) (FSANZ, 2017). This recently introduced Australian approach was a response to the problems associated with high ethanol production arising during fermentation (Bindon *et al.*, 2019), and as one answer to address the negative impacts on human health from consumption of higher alcohol wines (Saliba *et al.*, 2013). Similar approaches are permitted in a limited number of other grape-growing regions. For example, in the United States (US) water treatment of musts can be employed to facilitate fermentation, however, the density of the juice cannot be reduced below 22 degrees Brix (US Code 24 176,(2010)). Further, in South Africa, water-addition wine-making approaches can be permitted but only where they are deemed to be necessary for the incorporation



**FIGURE 1.** Vinification approaches taken in both 2020 (year 1) and 2021 (year 2) vintages

Two water treatment approaches in both years were taken for the late 15.5°Baumé (Bé) and middle 14.5°Bé harvest dates: (i) water was directly added to the must, which altered the solids to liquid ratio and (ii) juice was substituted for water, with no change to the solid to liquid ratio. Bé levels are correlated to final alcohol levels % [ABV].

of additives (*Liquor Products Act 60 of 1989, SA*). Water into pre-fermentation musts is however limited in the European Union, where water addition can only be for a ‘specific technical necessity’ and it does not modify the characteristics of wine (EU, 2018; Christmann *et al.*, 2022). While the outcomes of this study are relevant for Australian winemakers, should water treatment of pre-fermentative musts winemaking techniques become more generally recognised on a global scale, all winemakers must be provided with additional information and supported data sets to better address any related adverse impacts for final wine quality.

## MATERIALS AND METHODS

### 1. Vintage and vineyard conditions

The study was undertaken across the 2020 and 2021 vintage years. Temperatures and rainfall data from weather station Yarrowonga (located 45KM from Dookie, Victoria), Station Number: 081124, Victoria, Latitude: 36.03°S, Longitude: 146.03°E, Elevation: 129 m and weather station Numurkah (located 33.7 km from Dookie, Victoria) Station Number: 8010, Victoria, Latitude: 36.09°S, Longitude: 145.45°E, Elevation: 105 m (BOM 2023a; BOM, 2023b) was relied upon and used to calculate the Heliothermal Index of Huglin (HI) (Huglin, 1978) using standard measures (Tonietto and Carbonneau, 2004) (refer to Supplementary Materials, Supp. Tables 1–5).

For both vintages, vineyard management approaches were similar. The Tallis vineyard (195 Major Plains Road, Dookie, North East Victoria; 36°21’08.2”S, 145°45’37.0”E) from where the berries were sourced was irrigated, which supplemented rainfall to about 30 % in the growing seasons for both vintages (Richard Tallis, 2021). Irrigation water to grapevines was applied by the ‘drip/trickle’ system which permitted water to drip slowly to the roots of vines from above

the soil surface (DripNet™, USA (Phogat *et al.*, 2017)). Preventative measures to address possible fungal diseases including Botrytis rot or grey mould (*Botrytis cinerea*), *Colletotrichum* sp. and *Greenaria* sp. were implemented for both vintages. Spraying for grapevine rust mites was also applied at the woolly bud stage for both vintages (Richard Tallis, 2021; Bernard *et al.*, 2005).

For each vintage, and for the three harvest times—early 13.5°Bé, middle 14.5°Bé and late 15.5°Bé—the fruit was selected at random from panels along the length of each row, and hand-harvesting fruit from both sides of the vine was implemented to minimise stress on the berries. The rows selected consisted of 54 panels of 200 vines with approximately 50 vines contributing to the grapes taken in each season. Selected harvest dates were determined by reference to the sampling of 1.0 kg grapes, at 3-day intervals in the time leading up to harvest to determine approximated required Baumé levels for early 13.5°Bé, middle 14.5°Bé and late 15.5°Bé harvest times.

### 2. Fermentation and winemaking

Vinification took place at the University of Melbourne’s Dookie Campus winery (940 Dookie-Nalinga Rd, Dookie, North East Victoria; 36°22’58.851”S, 145°42’52.181”E). All grape musts were crushed through rotary roller crushers (destemmer and crusher) (Model Delta Destemmer E2, Bucher Vasilin, France) set at 8 mm spacing before separation into fermentation tanks/treatment group. Each of the sample groups (in triplicate) were fermented in a 225-litre plastic food-grade barrel and were assigned their own identifiers. The first 3 sample groups included early 13.5°Bé harvest, middle 14.5°Bé harvest and late 15.5°Bé harvest ferments (in triplicate). The middle 14.5°Bé and late 15.5°Bé harvests were subject to water treatments pre-fermentation. The dilution treatment consisted of water directly added to the

must which altered the solids-to-liquid ratio. The ‘bleed and replace’ treatment consisted of juice substituted for water, with no change to the solid-to-liquid ratio (Figure 1).

Deionised water was used for all water treatments. The additional 4 sample groups (in triplicate) included the middle 14.5 °Bé harvest subject to water dilution treatment to lower 14.5 °Bé to 13.5 °Bé (+5.3L water) (middle diluted 13.5 °Bé). The late 15.5 °Bé harvest was also subject to water dilution treatment to lower 15.5 °Bé to 14.5 °Bé (+3.49 L water) (late diluted 14.5 °Bé). Two further groups included the late 15.5 °Bé harvest diluted to lower 15.5 °Bé to 13.5 °Bé (+9.13 L water) (late diluted 13.5 °Bé) and the late 15.5 °Bé harvest subject to water substitution treatment (‘bleed and replace’) to lower 15.5 °Bé to 13.5 °Bé (–9.13L juice and +9.13L water) (late ‘bleed and replace’ 13.5 °Bé). For each vintage, 2100 kg of fruit was picked. Of this, there were 7 sample groups (in triplicate) of 100 kg of fruit (21 samples in total) where

each produced approximately 65 litres of wine, therefore a total of 1365 litres of wine was produced for each year.

A standard 50 ppm sodium metabisulphite (Australian Home Brewing, Victoria, 2020) was added to all samples at crush and after water treatment for the latter 4 sample groups followed by inoculation with Vitilevure Syrah YSEO™ (*Saccharomyces cerevisiae*, Danstar, Rhone Valley, France, 2020), a commercial culture of yeast at 30 g/hL. After 24 hours, all fermentations were inoculated with Martin Vialatte Reflexmalo 360™ (*Oenococcus oeni*, Martin Vialatte, Magenta, France, 2020) lactic acid bacteria at 1 g/hL, to carry out co-malolactic fermentation. All samples were maintained on skins for 5 days with the use of refrigeration to maintain temperatures between 20 °C to 30 °C before being pressed. Punch downs were implemented twice daily to homogenise fermentations. Sampling twice daily of 5mL from each sample group was taken during the

**TABLE 1.** Sensory standards given to panellists for wine attributes for type, definitions and reference standards

1	2	3	4
Wine Attributes	Type	Definitions	Reference Standard mixed with 30 mL of Shiraz wine
Colour intensity	Visual	Intensity of colour	Provided a chart of red colours displayed from light to dark red.
Odour complexity	Olfactive (aroma)	Overall smell, complexity, richness	Repetitive training sessions
Odour intensity	Olfactive (aroma)	Intensity of odours overall	Repetitive training sessions
Red fruits	Olfactive (aroma and taste)	Wines aromas that suggest strawberry, raspberry, cherry smells and taste	2 strawberries, 2 cherries and 4 raspberries
Dark fruits	Olfactive (aroma and taste)	Wine aromas that suggest black currants and taste	4 blackberries and 4 blueberries
Ripe fruits	Olfactive (aroma and taste)	Wine aromas and taste that suggest ripe fruits, candied fruits	8 raisins crushed
Vegetal	Olfactive (aroma and taste)	Vegetal, Herbaceous, sweet pepper smell and taste	1 teaspoon of grated green capsicum
Hotness	Mouthfeel/texture	Warmth perception of overly pronounced or high levels of alcohol	Add food grade ethanol to raise alcohol level by 2 %
Astringency	Mouthfeel/ Texture	Tannins with smooth and fine textured astringency	Powered white tannin
Body	Mouthfeel/Texture	Wine marked by richness, fullness; full-bodied	Powered white tannin and food grade ethanol to raise alcohol level by 2 %
Viscosity	Mouthfeel/Texture	The viscosity as an indicator of a full-bodied style	Food grade soluble Xanthan gum 0.02 gm/L
Length	Mouthfeel/Texture	The time that aftertaste persists in the mouth	Repetitive training sessions
Balance	Mouthfeel/Texture	Balance between aromas, flavour, structure and mouthfeel	Repetitive training sessions

fermentation process, which tracked temperature and Bé. Musts all finished primary alcoholic fermentation on day 5. Acid additions were made post-pressing on day 5 to target a titratable acidity (TA) of 6.5 g/L tartaric acid (Australian Tartaric Products, Victoria, Australia, 2020). An additional 50 ppm SO<sub>2</sub> was added 21 days after fermentation prior to bottling wines in 750 mL glass wine bottles. All wines were matured at an average temperature of 18 °C for 12 months.

### 3. Measuring chemical parameters of all sample wines approach

Samples of wines 12 months after bottling were taken for both 2020 (year 1) and 2021 (year 2) vintages from each sample group (7 groups in triplicate, 21 individual samples in total). For all samples, we measured wine colour absorbances using Agilent Cary 60 UV-Vis (Agilent Technologies Inc., Palo Alto, CA, USA), with a 1 mm path length quartz cuvette (Somers and Evans, 1974). We set deionised water as the blank reference. As recommended by the Australian Wine Research Institute as the accepted Australian standard approach, we undertook measures of wine colour density (a.u.), wine colour hue, total phenolics (a.u.), free anthocyanins (mg/L), pigmented tannin (a.u.), pigmented tannin (%), total pigment (a.u.), chemical age 1 and 2, and total tannin (%) by a modified Somers colour assay (Mercurio *et al.*, 2007; AWRI, 2023). We adjusted wine pH to 3.4 and alcohol concentration to 12 % v/v using a buffer solution before commencing any analysis, as set out by the standard approach. We incorporated sodium metabisulphite into the buffer solution rather than adding it directly to individual sample groups to minimise preparation time (Mercurio *et al.*, 2007). We undertook the modification to allow the standardisation of pH and ethanol concentrations of wine sample groups in a simple one-step dilution with a buffer solution, thus removing inconsistencies between wine matrices. We derived tannin measurements from a calibration developed from the methyl cellulose precipitable (MCP) tannin assay (Sarneckis *et al.*, 2006). Each analysis was performed in triplicate.

### 4. Just-About Right (JAR) sensory analysis approach

Ethics approval was provided by the University of Melbourne's Human Research Ethics Committee (HREC) (No. 11684) for the Just-About-Right (JAR) naïve consumer sensory analysis surveys. We undertook these surveys for the final wines produced from all sample groups for both vintages using a Likert scale (Likert, 1932). Naïve consumer sensory analysis data was collected from sensory panels of undergraduate wine students in February 2021 (for the 2020 study), and in February 2022 (for the 2021 study). Participants were recruited from students in concurrent winemaking studies at the University of Melbourne, Dookie Campus during the summer study period of February–March of each year for both the 2020 (year 1) and 2021 (year 2) surveys. Testing was conducted at the Dookie Campus, University of Melbourne, in dedicated food-grade laboratory facilities located on campus. All participants were aged between 18 and 21 years of age. Visual, olfactory and mouthfeel sensory attributes were tested. For all wine samples, 80 in 2020

(year 1) and 79 in 2021 (year 2) naïve wine consumer panellists evaluated the properties of colour intensity, red fruit smell, dark fruit smell, ripe fruit smell, vegetal smell, odour complexity, odour intensity, red fruit taste, dark fruit taste, ripe fruit taste, vegetal taste, astringency, hotness, body, viscosity and length. We collected data on a non-structured linear scale, where the middle of the scale for each attribute corresponded to JAR scores for the panellists (Likert, 1932). For each sensory attribute, panellists ranked the wine samples on a five-level Likert scale ranging from too weak/pale; weak/pale; Just-About-Right; strong/a little dark; too strong/too dark. Panellists scored overall liking on a 9-point line scale ranging from dislike extremely, to neutral, to like extremely. Panellists scored the likelihood to purchase on a 5-point line scale ranging from extremely unlikely, to neutral, to extremely likely. We combined JAR with overall liking scores to highlight those attributes of a wine that had the greatest impact on liking (Schraidt, 2009). We also undertook a penalty analysis to assess the influence of a non-JAR category for a given attribute by applying a 'penalty' to reflect consumers' overall liking of a wine.

#### 4.1. Extended sensory assessment of wines approach

Panellists undertook testing of wine samples after a level of preliminary training in sensory analysis experiences. Panellists were provided with an introductory baseline session to inform them of the process, requirements and risks associated with the JAR prior to commencing the surveys. Reference standards were agreed upon by the panellists to familiarise them with the colour and odour intensities and complexities, red, ripe and dark fruit attributes as well as for vegetal, hotness, astringency, body, viscosity, length and balance sensory parameters (Table 1). Panellists were presented with samples of wines in 30 mL aliquots in coded ISO standard wine classes during daylight hours. For all 2020 (year 1) and 2021 (year 2) vintage samples, we presented each panellist with 7 wine samples at once in a randomised order. For each vintage, the panellists evaluated the wine samples in one sitting. Each panellist had paper scoring sheets for each wine sample, which were collected by the researchers at the end of the sessions.

### 5. Statistical analysis

#### 5.1. Chemical data

Statistical analysis for chemical compounds (mean values and standard deviation (SD)) used independent sample group t-test, two-way analysis of variance (ANOVA), followed by Tukey's HSD multiple comparison test ( $\alpha = 0.05$ ). The multifactor ANOVA was performed using *R* software to compare the different samples.

Linear models were fitted to the concentration of each compound to assess differences between processes. A separate model was used for each compound. We used repeated measures of variance (by ANOVA) with treatment (process) approaches as fixed factors. For the 2020 (year 1) and 2021 (year 2) experiments for chemical compounds that were measured in both years, the experiment year was also included as a blocking factor. Our analytical data are

presented as the arithmetic average  $\pm$  1 standard deviation from three replicates. Where the overall test for difference between processes is statistically significant, the ‘group’ column displays a ‘compact letter display’ and processes that do not share a letter have statistically significant differences between means for that compound.

A principal component analysis (PCA) was also performed using SRplot (Tang *et al.*, 2023), where PCA analysis was performed with a full cross-validation, plotted by ggbiplot.

## 5.2 Sensory Analysis

For sensory data, differences in sensory attributes are determined by linear mixed-effects ANOVA, using independent sample group t-test, two-way analysis of variance (ANOVA) followed by Tukey’s HSD multiple comparison test ( $\alpha = 0.05$ ). A separate model is used for each attribute, including overall wine preference. Process and experiment year are included as fixed effects, and taster is included as a random effect.

All sensory data is expressed as the arithmetic mean of scores from 79 panellists for vintage 2020 (year 1) wines and 80 panellists for vintage 2021 (year 2) wines  $\pm$  1 standard deviation. Where the overall test for difference between processes is statistically significant, the ‘group’ column displays a ‘compact letter display’, where processes which do not share a letter have statistically significant differences between means for that compound.

Penalty analysis (PA) is implemented in both years of studies 2020 (year 1) and 2021 (year 2) to assist in identifying decreases in acceptability associated with sensory attributes not at optimal levels in the wine samples. The scales used to acquire the data are the ‘Just About Right’ (JAR) scales.

In addition, ‘overall liking’ data is collected, where the 9-point Hedonic Scale is used. Panellists are grouped into one of three categories depending on the response given to a JAR attribute (i.e., ‘too little, JAR or ‘too much’). The percentage of panellists in each of the 3 categories is calculated and the corresponding mean ‘overall liking’ ( $L_{TL}$ ,  $L_{JAR}$ ,  $L_{TM}$ ) scores for the ‘too little’ (TL), ‘JAR’ (JAR) and ‘too much’ (TM) categories are estimated.

Penalties are calculated as follows:

$$\text{Penalty}_{TL} = L_{JAR} - L_{TL}$$

$$\text{Penalty}_{TM} = L_{JAR} - L_{TM}$$

By plotting the penalty or mean drops against the percentage of consumers for all the JAR scale attributes of wines, the penalty analysis (PA) results are represented graphically. A penalty is not considered significant if the % of consumers in the TL or TM categories is less than 20 %. This forms the bounds of the critical corner in Figure 7 in the results below (>20 % of consumers and >1.0-point mean liking drop).

## RESULTS

### 1. Vintage conditions

The 2020 (year 1) grape growing season was a slightly warmer one than that observed for 2021 (year 2), with average rainfall also being slightly lower during the 2020 grape growing season (34.61 mm) when compared to the 2021 grape growing season (October–March) (39.20 mm) (BOM, 2023a; BOM, 2023b). Measurements under the HI index however classed both seasons as ‘temperate warm’ (Huglin, 1978; Tonietto and Cannonneau, 2004). (See Supplementary materials, Supp. Tables 1–5.)

Good grape health was maintained throughout each of the harvest times for both years. Vintage growing and wine berry health conditions were, therefore, similar for both years (Richard Tallis, 2021).

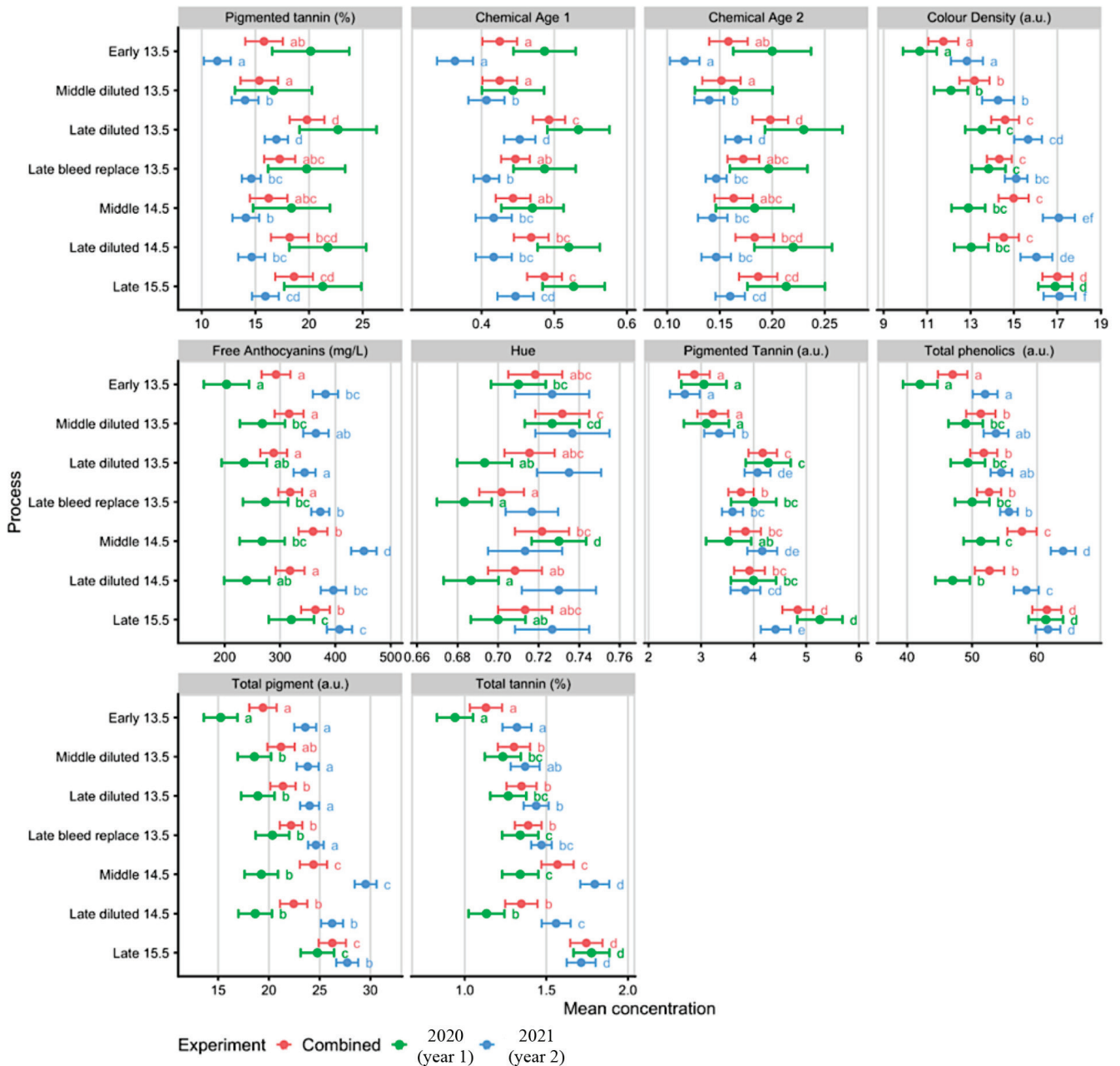
### 2. Harvest timings, water treatments and the impacts on chemical compounds in wines

Wine chemical compositions are dependent on the harvest timings and the water treatments implemented. Upon comparing chemical data between the years 2020 (year 1) and 2021 (year 2), pigmented tannin (%), chemical age 1, and chemical age 2 exhibited higher average values for all treatment groups in 2020 (year 1). In contrast, colour density (a.u.), free anthocyanins (mg/L), hue, total phenolics (a.u.), total pigment (a.u.), and total tannin (%) demonstrate higher average values across all treatment groups in 2021 (year 2). No discernible differences are noted in pigmented tannin (a.u.) across the years. These variances may be attributed to even small differences in climatic conditions during the respective vintages, influencing fruit maturation in distinct ways. Despite variations in average values, consistent trends are observed when comparing results for each treatment group across each year of the study. Combined results from both the 2020 (year 1) and 2021 (year 2) years demonstrate most disparities between the two years of studies for chemical compounds are mitigated through the amalgamation of the data sets (Figure 2). (Also see Supplementary materials, Supp. Table 6 : 2020 (year 1) and 2021 (year 2) combined raw data for chemical compounds.)

To further demonstrate the differences in wine chemical analysis, combined data for 2020 (year 1) and 2021 (year 2) of the study obtained from chemical data collected was also analysed by principal component analysis (PCA) as illustrated in Figure 3, where 90.7 % of the total variance is explained by the first two dimensions (Figure 3).

#### 2.1. Late diluted 13.5 °Bé and late diluted 14.5 °Bé wines are characterised by pigmented tannin (%), chemical age 1 and chemical age 2 levels

Chemical results for combined data from 2020 (year 1) and 2021 (year 2) of the study further show a correlation between harvest date and pigmented tannin (%), chemical age 1 and chemical age 2 values with later harvest date wines exhibiting higher levels for these three chemical compounds (Figure 2).



**FIGURE 2.** Chemical Compounds by process and mean concentrations for all wines for 2020 (year 1) and 2021 (year 2) and combined.

Mean results for triplicate samples are presented. Error bars show 95 % confidence intervals for the mean. Wine sample groups not sharing a letter are statistically significantly different ( $p < 0.05$ ).

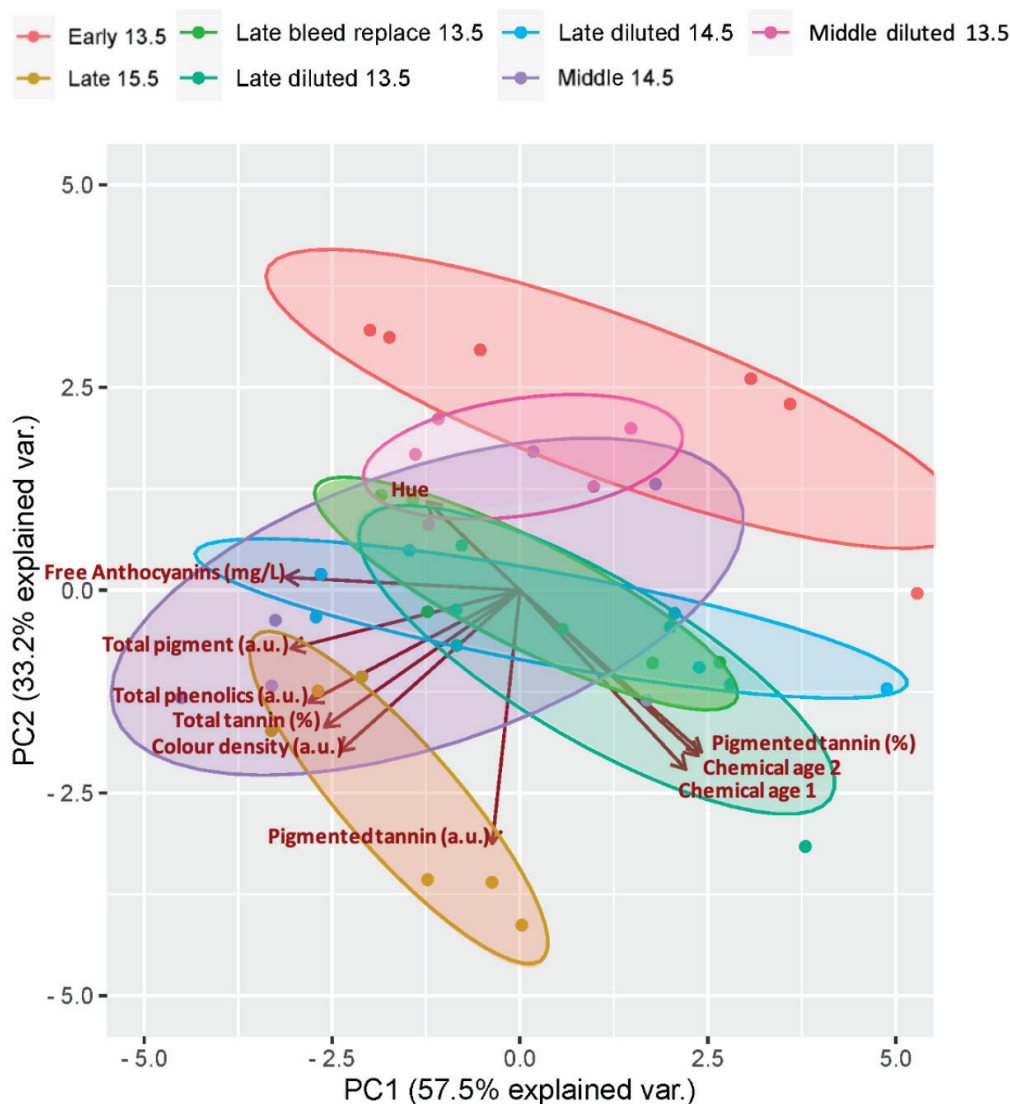
Notably, while all other water treatments result in decreases in pigmented tannin (%), the late diluted 13.5 °Bé treatment significantly increases pigmented tannin (%) levels compared to other water dilution treatments (Figure 2). Interestingly, water treatments into both the late 15.5 °Bé and middle 14.5 °Bé harvest pre-fermentative musts do not reduce chemical age 1 or 2 to a statistically significant degree in any of the water treatment groups (Figure 2).

PCA analysis also shows that late diluted 13.5 °Bé and late diluted 14.5 °Bé wines are generally positioned in the space defined by positive Dim 1 and negative Dim 2, which is

characterised by increased pigmented tannin (%), chemical age 1 and chemical age 2 levels when compared to other treatment wines (Figure 3).

### 2.2 Middle diluted 13.5 °Bé wine has increased wine hue levels when compared to other treatment groups

Chemical data results for combined data from 2020 (year 1) and 2021 (year 2) of the study show harvest timing has no statistically significant effect on wine hue, but there are differences observed between different water treatments from different harvests. The highest value for wine hue is observed in the middle diluted 13.5 °Bé wine, with the lowest



**FIGURE 3.** Principal component analysis (PCA) of chemical compound components of Shiraz wine, including pigmented tannin (%), chemical age 1, chemical age 2, colour density (a.u.), free anthocyanins (mg/L), hue, pigmented tannin (a.u.), total phenolics (a.u.), total pigment (a.u.), and total tannin (%) by treatment group. Treatment groups are indicated by colour

Results represent combined data across both 2020 (year 1) and 2021 (year 2) vintages, where results for all triplicate samples are presented. Points represent samples, and different colours represent different groups. Ellipses represent 68 % confidence intervals of core regions. Arrows represent original variables, the directions of arrows represent the correlation between the original variable and principle components, and lengths represent the deviation of original data to principle components.

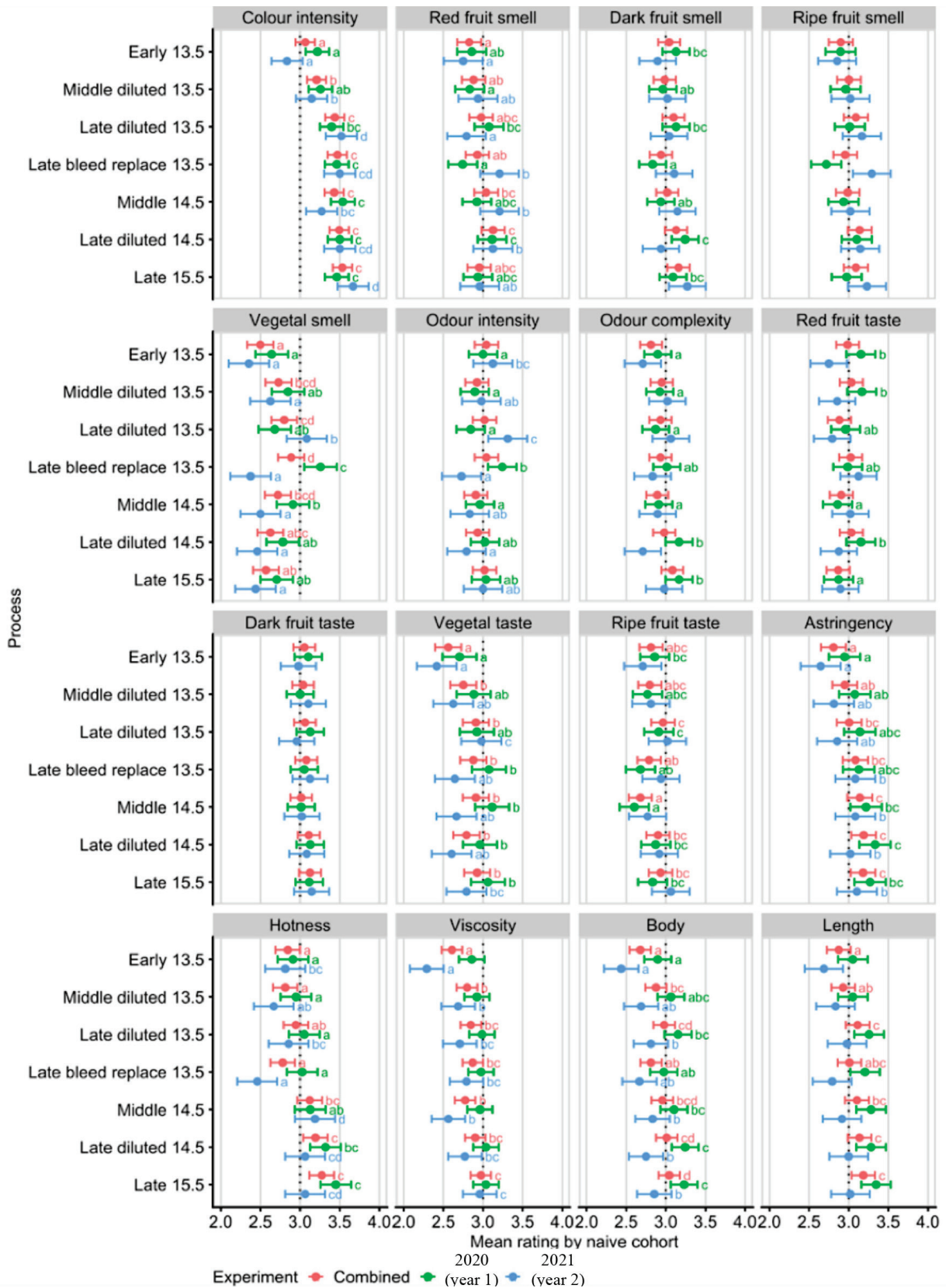
value for wine hue observed in the late ‘bleed and replace’ 13.5 °Bé wine (Figure 2). These observations are in line with levels observed in the early 13.5 °Bé wines. Further, PCA analysis indicates in the space defined by negative Dim 1 and positive Dim 2, middle diluted 13.5 °Bé wine is associated with increased hue (Figure 3).

**2.3. Late 15.5 °Bé and Middle 14.5 °Bé wines have the highest levels for colour density (a.u.), total tannin (%), total pigment (a.u.), total phenolics (a.u.)**

Chemical data results for combined data from 2020 (year 1) and 2021 (year 2) of the study show that colour density (a.u.), total tannin (%), total phenolics (a.u.) and total pigment (a.u.)

increase significantly from early, middle and late harvests. The highest value for all of these compounds is observed in the late 15.5 °Bé wine, with the lowest value observed in the early 13.5 °Bé wine (Figure 2).

All water treatments reduce colour density (a.u.), total tannin (%), total pigment (a.u.) and total phenolics (a.u.) concentrations to a statistically significant degree when compared to non-water treated wines (Figure 2). There are no statistical differences in the concentration of these compounds between each of the water treatment groups. The exception to this is colour density (a.u.), where middle diluted 13.5 Bé wine has a statistically significant decreased colour



**FIGURE 4.** Just-About-Right sensory parameters by process (harvest timing or water treatment) and mean rating—2020 (year 1) and 2021 (year 2) combined

Mean results for triplicate samples are presented. Error bars show 95 % confidence intervals for the mean. Sample groups not sharing a letter are statistically different ( $p < 0.05$ ).

density (a.u.) when compared to all other water treatment groups (Figure 2). It is noted that concentrations of all the above compounds in all treatments are still higher than those observed for early 13.5 °Bé wine (Figure 2).

Results also show that all water treatments of late 15.5 °Bé and middle 14.5 °Bé harvests decrease free anthocyanin (mg/L) concentrations significantly, where all have statistically similar reduced values. Notably, the late diluted 13.5 °Bé wine's free anthocyanin concentrations are observed to be lower than those for the early 13.5 °Bé wine (Figure 2). PCA analysis confirms these observations where late 15.5 °Bé and middle 14.5 °Bé wines are found in the space defined by negative Dim 1 and negative Dim 2 which is associated with increased colour density (a.u.), total tannin (%), total pigment (a.u.), total phenolics (a.u.) (Figure 3).

#### 2.4. Late 15.5 °Bé wines have the highest levels of pigmented tannin (a.u.)

Chemical data results for data combined from 2020 (year 1) and 2021 (year 2) of the study show that pigmented tannin (a.u.) levels increase significantly from early, middle and late harvests. All water treatments reduce pigmented tannin (a.u.) concentrations to a statistically significant degree when compared to non-treated wines (Figure 2). The highest value for wine-pigmented tannin (a.u.) is observed in the late 15.5 °Bé wine, with the lowest value for wine-pigmented tannin (a.u.) observed in the early 13.5 °Bé wine (Figure 2). PCA analysis confirms these observations where late 15.5 °Bé wine is found in the space defined by negative Dim 1 and negative Dim 2 which is associated with pigmented tannin (a.u.). Early 13.5 °Bé wines are distributed in the opposite space (Figure 3).

### 3. Harvest timings, water treatments and the impacts on sensory parameters in final wines

When taken separately, sensory results from each of the years 2020 (year 1) and 2021 (year 2) are observed to be generally consistent across most sensory parameters (Figure 4). However, when pooling the data from Year 1 and Year 2 together, with a greater sample size, the confidence intervals are reduced resulting in some statistically different results (Figure 4).

#### 3.1. Wines subject to water treatment of pre-fermentative wine grape must show less significant impacts for many JAR sensory parameters in wine

JAR analysis outcomes for combined data from 2020 (year 1) and 2021 (year 2) of the study show no statistically significant differences between any of the wine sample groups for dark fruit smell, ripe fruit smell, odour intensity, odour complexity, red fruit taste, dark fruit taste. (Figures 4 and 5) For other sensory parameters, JAR analysis outcomes for combined data from 2020 (year 1) and 2021 (year 2) of the study are varied.

For instance, JAR results show for red fruit smell, late 15.5 °Bé, middle 14.5 °Bé and late diluted 13.5 °Bé wines have the greatest preference. However, all treatment groups are clustered closely around the ideal mean value of 3.0,

suggesting that panellists generally favour red fruit smell regardless of the treatment group. For vegetal smell, JAR results show early 13.5 °Bé, late 15.5 °Bé, and late diluted 14.5 °Bé wines have the lowest observed vegetal smell preferences, with lowest mean values, where panellists rate the vegetal smell as 'too low' for these treatment wines. The highest preference for vegetal smell is observed in the late 'bleed and replace' 13.5 °Bé, middle 14.5 °Bé, closely followed by middle diluted 13.5 °Bé and late diluted 13.5 °Bé wines, although the differences between all treatment groups are not statistically significant.

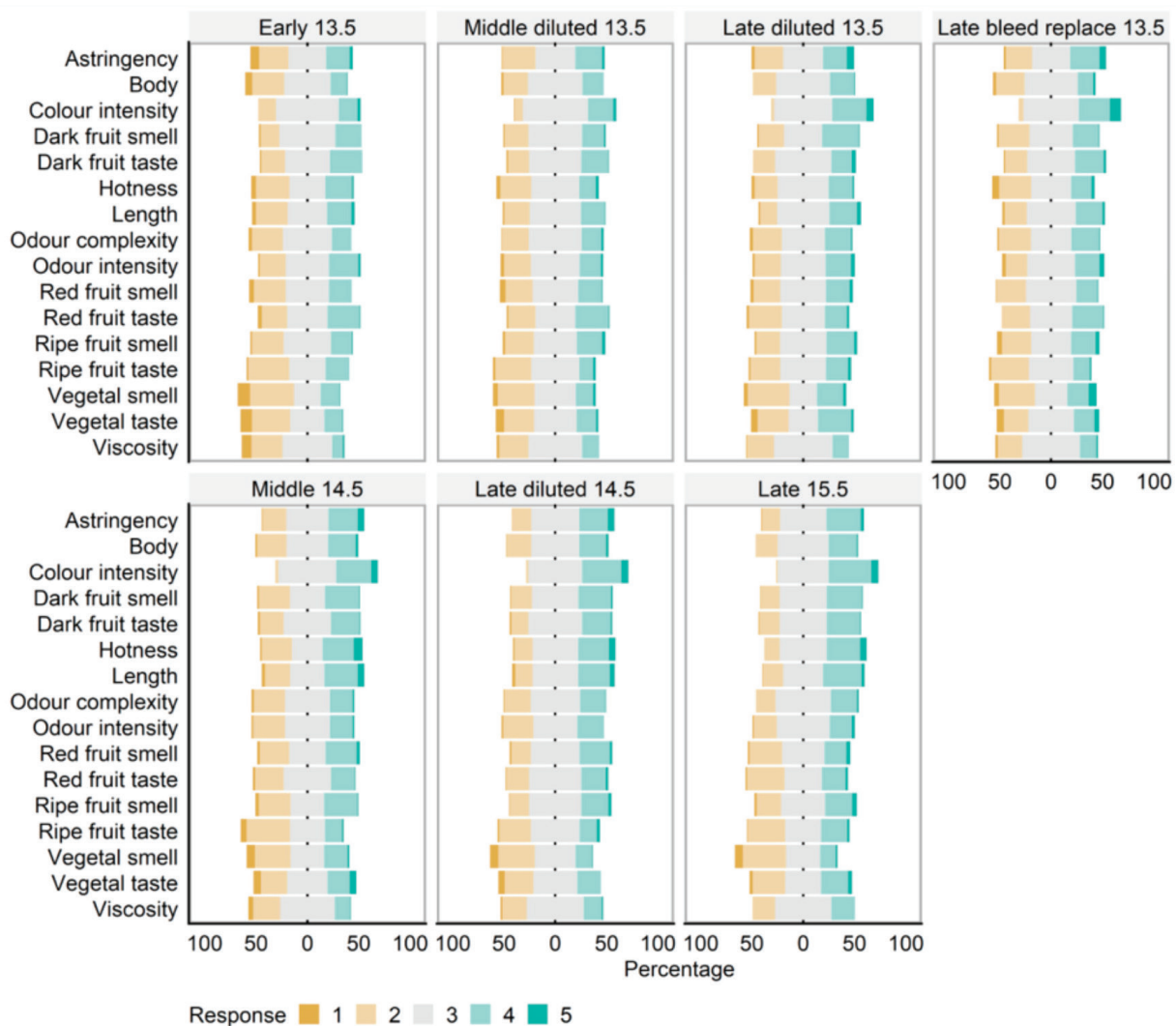
For ripe fruit taste and astringency, JAR results show that the late diluted 13.5 °Bé wine has the highest mean values, with panellists indicating the highest preference for these two sensory parameters related to this treatment. However, the results are not statistically significantly different to other wine treatments. (Figures 4 and 5).

For viscosity, JAR results show statistically significant increases between different harvest treatment groups, with increasing mean values in later harvests. All water treatments result in reductions in viscosity, however, none of the reductions observed are statistically significantly different between any of the treatment groups (Figures 4 and 5).

For the sensory parameter of length, JAR results show there are statistically significant increases between different harvest treatment groups, with late 15.5 °Bé wine having the highest mean value for length, but this is perceived as 'too long' by panellists. All water treatments of the late 15.5 °Bé harvest are effectively able to increase preference for length, where the late 'bleed and replace' 13.5 °Bé wine has the highest preference for length, although these changes are not statistically significant (Figures 4 and 5). Notably, for vegetal taste, while JAR results show that late 15.5 °Bé wine has the highest preference, this is not significantly different to any other wines (including all water treatment wines) other than the early 13.5 °Bé wine (Figures 4 and 5).

For the sensory parameter of the body, JAR results show that the late 15.5 °Bé wine has also the highest preference. (Figures 4 and 5). Water treatments of the late 15.5 °Bé harvest do not result in statistically significant reductions in preference when comparing late 15.5 °Bé wine to late diluted 14.5 °Bé and late diluted 13.5 °Bé wines. However, the late 'bleed and replace' 13.5 °Bé wine demonstrates a statistically significant reduction in body preference perception, however, panellists find this 'too low' (Figures 4 and 5).

Further, JAR results show that the early 13.5 °Bé wine has a prominent preference for colour intensity, with a mean of 3.06. In contrast, all other treatment groups exhibit higher mean values for colour intensity but with a reduced preference. Among the treatment groups, middle diluted 13.5 °Bé wine stands out as the only one with a statistically significant decrease in colour intensity compared to late diluted 13.5 °Bé, late bleed and replace 13.5 °Bé, middle 14.5 °Bé, late diluted 14.5 °Bé, and late 15.5 °Bé wines. However, it still has a lower preference than that of early 13.5 °Bé wine. These findings suggest that all harvests



**FIGURE 5.** Likert Plot for JAR sensory parameters and % preference levels—2020 (year 1) and 2021 (year 2) combined

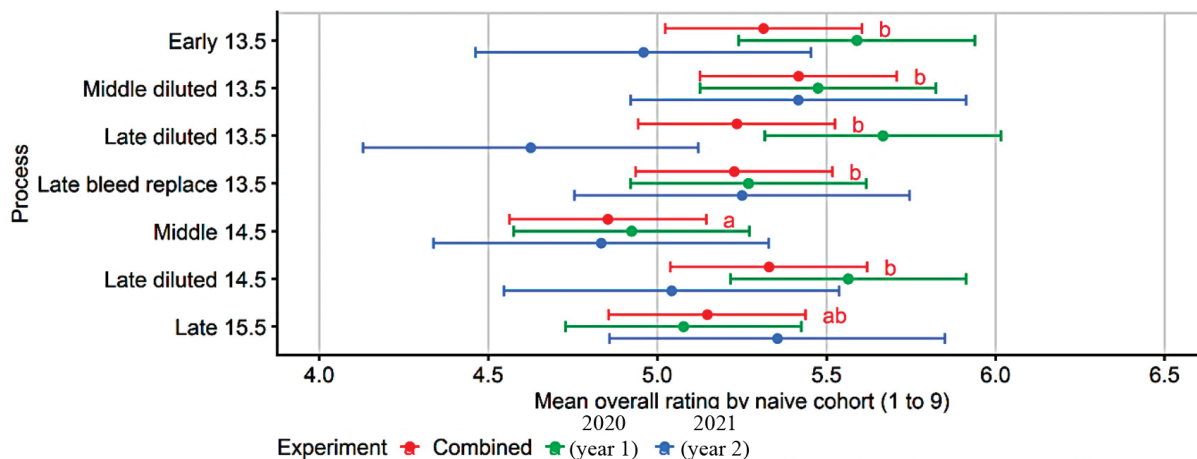
Visual representation of the sensory Just-About-Right (JAR) naïve sensory panellist JAR datasets where the larger the grey mid-section, the higher preference a particular attribute will have (Also refer to Supplementary materials, Supp. Table 7 for the raw JAR 2020 (year 1) and 2021 (year 2) combined data to support the result outcomes in Figure 5, together with Supp. Figure 1, which provides a further visual representation of the JAR sensory 2020 (year 1) and 2021 (year 2) combined data results as a Spider Plot.)

conducted after the early 13.5 °Bé harvest lead to an increase in colour intensity, which is not preferred by the panellists (Figures 4 and 5).

For the sensory parameter of hotness, JAR results show that the late 15.5 °Bé, late diluted 14.5 °Bé, and middle 14.5 °Bé wines have the highest mean ratings for hotness, however, this is perceived as ‘too high’ by panellists. Late ‘bleed and replace’ 13.5 °Bé, middle diluted 13.5 °Bé, early 13.5 °Bé and late diluted 13.5 °Bé wines show the lowest mean ratings for hotness, but this is perceived as ‘too low’ by panellists. The late diluted 13.5 °Bé wine has the highest preference for hotness with a mean value of 2.95. This suggests that the addition of water in the late 15.5 °Bé harvest can effectively decrease the hotness to a more desirable and preferred level compared to the late 15.5 °Bé wine with a mean of 3.27 (Figures 4 and 5).

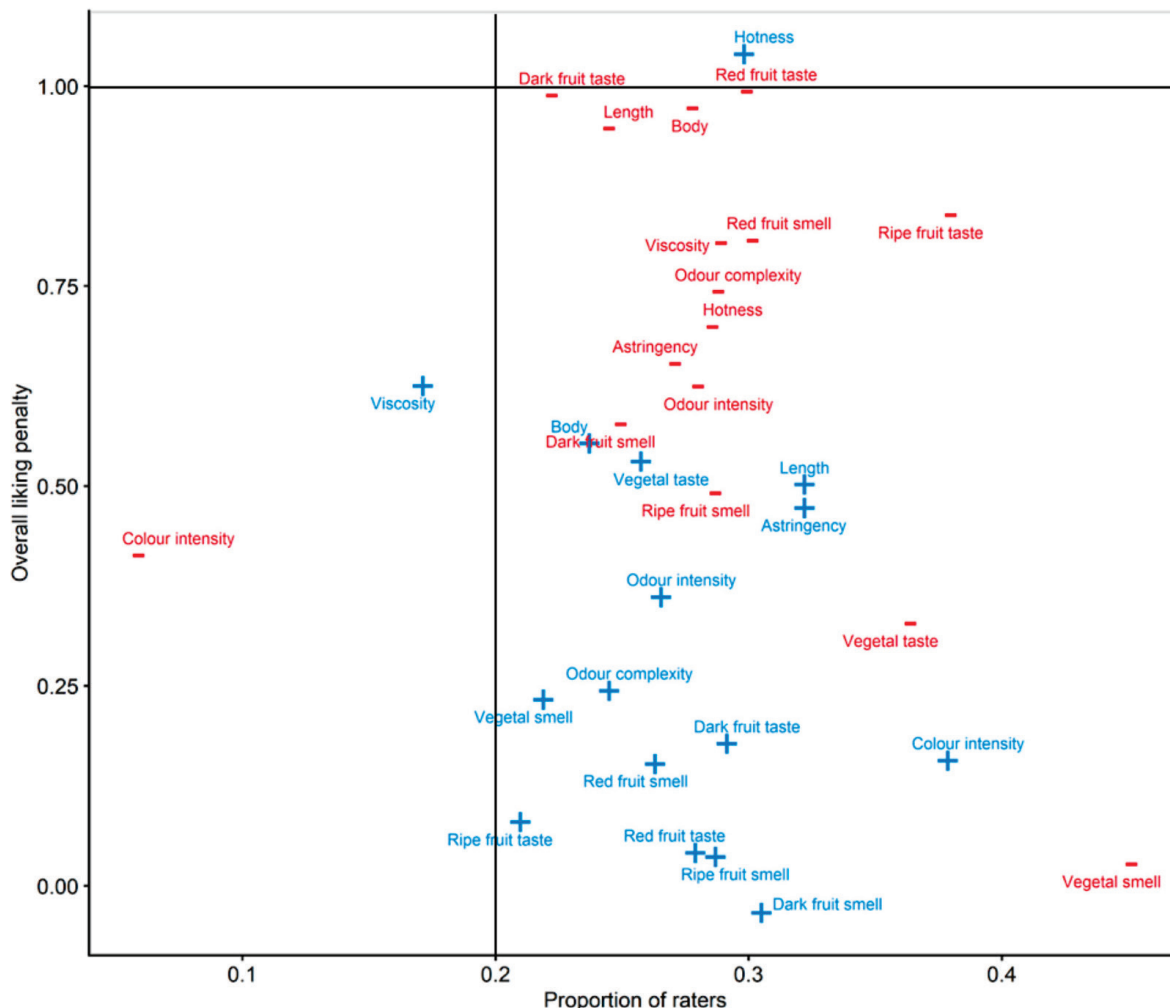
#### 4. Middle diluted 13.5 °Bé wine rates highest in the overall assessment

The study also evaluates sensory panellists’ overall assessment of wines on a continuous scale for the 2020 (year 1) and 2021 (year 2) vintages separately as well as the results of the two replicate studies combined together. Consistent findings are observed when comparing the initial and subsequent years while indicating different statistical differences among harvest timings and water treatments implemented. The 2020 (year 1) study results show consistently higher results for overall assessment across all harvest and water treatment wines except for the late 15.5 °Bé wine when compared to the 2021 (year 2) results. Although overall assessment varies across years, the statistical differences between treatments in each year’s study closely follow in each year (Figure 6).



**FIGURE 6.** Overall preference assessment of wines by process (harvest timing and water treatment) and overall mean rating—2020 (year 1), 2021 (year 2) and combined

Error bars show 95 % confidence intervals for the mean. Sample groups not sharing a letter are statistically different ( $p < 0.05$ ).



**FIGURE 7.** Overall liking penalty and proportion of raters for sensory attributes—2020 (year 1) and 2021 (year 2) combined

'+' denotes too much of an attribute, and '-' denotes too little of an attribute. Too much hotness is the only wine attribute that falls into the critical corner.

Combined data results from the 2020 (year 1) and 2021 (year 2) vintages show that early 13.5 °Bé and late 15.5 °Bé wines have higher preference ratings compared to the middle 14.5 °Bé wine. In the late 15.5 °Bé harvest, all water treatments demonstrate an increase in preference for the overall assessment of wines. However, these increases are not statistically significant. There is a statistically significant increase in overall assessment preference from the middle 14.5 °Bé wine to the middle diluted 13.5 °Bé wine, which is the highest-rated wine overall (Figure 6).

### **5. Penalty analysis—‘too much’ hotness falls in the critical corner, where ‘hotness’ is most preferred for late diluted 13.5 °Bé wine**

Combined data results from 2020 (year 1) and 2021 (year 2) indicate that the ‘too much’ hotness is the only wine sensory attribute that falls into the critical corner (Figure 7). ‘Too much’ of this attribute in wine has the most negative impact on overall liking when observed in the JAR scale. The highest mean ratings for hotness are observed in late 15.5 °Bé, late diluted 14.5 °Bé and middle 14.5 °Bé wines. This represents all wines with a final percentage alcohol 14.5 % ABV or 15.5 % ABV. For these wines, panellists perceive the hotness as ‘too high’. Conversely, the lowest mean ratings for hotness are observed in the early 13.5 °Bé, late ‘bleed and replace’ 13.5 °Bé, middle diluted 13.5 °Bé and late diluted 13.5 °Bé wines. All these wines have a final percentage alcohol of 13.5 % ABV. The highest preference for hotness is observed in late diluted 13.5 °Bé wine. While this treatment brings the preference rating closer to the ideal mean value of 3.0, the result shifts from ‘too high’ to ‘too low’, indicating that the reduction in hotness by this water treatment is too significant. The late diluted 14.5 °Bé wine moves the mean closer to 3.0, however, this reduction is not statistically significant. Furthermore, the perception of hotness in the middle diluted 13.5 °Bé wine is also reduced, however, this reduction shifts the preference rating further from the ideal mean value where it is still considered as ‘too low’ (Figure 7).

## **DISCUSSION**

In this current study, we combine data from the 2020 (year 1) and 2021 (year 2) vintages, to increase the sample size and statistical power of the data, given the larger dataset produced. This can provide for a more robust analysis and may assist in detecting any smaller but potentially significant effects that might not be evident in a single-year dataset (Serdar *et al.*, 2021; Riffle *et al.*, 2022). This combination is appropriate as there are only slight variabilities between the grape growing seasons of 2020 and 2021, with both classified as ‘temperate warm’ under the HI index (Huglin, 1978). Importantly, grape quality, health and vineyard management between these years show little variance. Moreover, we used identical winemaking and fermentation approaches for both years.

Our findings support previous studies which indicate that varying harvest times in a vintage, can affect final alcohol levels in a wine, and impact upon chemical

compounds and sensory attributes (Schelezki *et al.*, 2020a; Schelezki *et al.*, 2020b). Additionally, our results support previous studies which demonstrate that water treatment of high sugar musts before fermentation results in producing wines with lower final alcohol levels (Teng *et al.*, 2020; Piccardo *et al.*, 2019). However, it has been noted that this wine-making technique is often accompanied by a negative perception that an inferior wine will be produced (Schelezki *et al.*, 2020a). Our results also confirm previous study outcomes regarding many of the observed negative effects on chemical compounds for water-treated wines (Schelezki *et al.*, 2020a; Schelezki *et al.*, 2020b). However, our extensive sensory analysis approach demonstrates that the impacts on several crucial sensory attributes are not as significant as indicated by many of the chemical compound changes we observe (Teng *et al.*, 2020; Piccardo *et al.*, 2019). Sensory attributes of a final wine are important as they provide for its perceived quality, preference and purchase likelihood (Teng *et al.*, 2020).

### **1. Early 13.5 °Bé winemaking will adversely affect many chemical attributes and sensory parameters in wine**

Harvesting early can mean that berries are picked before or ‘close to’ veraison. Previous studies have indicated that while early harvests can produce wines with lower final alcohol levels, final wine quality profiles can be negatively affected (Longo *et al.*, 2017). This is because, after veraison, less desirable chemical compounds like methoxypyrazine will decrease during the ripening of the fruit, while anthocyanin, tannin and other desirable chemical compounds will continue to increase (Bindon *et al.*, 2014; Teng *et al.*, 2020). The chemical compound results for early 13.5 °Bé wines in our study agree with these earlier findings where we find that all chemical attributes are at their lowest levels except for wine hue (which is consistent across all different harvest timings) (Figure 2). PCA analysis confirms these observations (Figure 3). For the sensory parameters of red fruit smell, vegetal smell, vegetal taste, astringency, viscosity, length and body, the lowest mean ratings for the early 13.5 °Bé wines are observed (Figure 4). This is in line with previous studies that also found early harvest wines will produce unfavourable green or vegetative characteristics (Bindon *et al.*, 2014). We find that only one sensory parameter ‘colour intensity’ is most preferred by sensory panellists for the early 13.5 °Bé wine, despite other treatment groups and harvest time wines having higher mean values (Figure 4). This result may be attributed to Shiraz being a full-bodied red wine, where it can have high colour intensity present even in early harvest wines.

On balance, this winemaking approach to producing wines with lower alcohol levels should be avoided especially for grape cultivars such as Shiraz. This is because the production of more favourable chemical compounds in the final wine which are associated with a later harvest is not possible, together with associated negative impacts on a higher number of final wine sensory characteristics.

## 2. Late 15.5 °Bé winemaking produces wine with the highest levels of all chemical compounds except wine hue, with less preferred sensory attributes except for red fruit taste and vegetal taste

Our chemical results for the late 15.5 °Bé wine agree with earlier findings which demonstrate that harvesting late will produce wines with higher alcohol levels and higher levels of most chemical compounds (Schelezki *et al.*, 2020a; Schelezki *et al.*, 2020b) (Figure 2). Our PCA analysis results also show that late 15.5 °Bé wines together with middle 14.5 °Bé wines are located in the space defined by negative Dim 1 and negative Dim 2, where there is increased colour density (a.u.), total tannin (%), total pigment (a.u.), total phenolics (a.u.) and pigmented tannin (a.u.). We also show that the late 15.5 °Bé wine has many less preferred sensory attributes except for vegetal taste and red fruit taste. (Figure 4) These findings agree with previous studies that have noted Shiraz wines are generally regarded as being full-bodied wines with earthy and gamey notes (Casassa *et al.*, 2013). While vegetal taste and red fruit taste sensory attributes can be agreeable ones; given the negative impact on all other sensory parameters (Figure 4), together with the production of higher less favourable chemical compounds (Figures 2 and 3), harvesting late is to be avoided if winemakers are seeking to address undesirable final high alcohol levels, while circumventing many of the other associated unfavourable wine quality attributes associated with late harvest wines.

## 3. Water treatments of late 15.5 °Bé harvest must winemaking will reduce alcohol levels, but can adversely affect many chemical compounds while retaining many positive sensory attributes in final wines

Our findings are supported by previous studies that show water treatments of late 15.5 °Bé must winemaking can produce wines with lower alcohol levels, but with many chemical compounds being adversely affected (Schelezki *et al.*, 2018a; Schelezki *et al.*, 2018b; Schelezki *et al.*, 2020a; Schelezki *et al.*, 2020b; Teng *et al.*, 2020; Petrie *et al.*, 2019) (Figures 2 and 3). Notably, we further expand on Schelezki *et al.*'s earlier findings (Schelezki *et al.*, 2020a), where we show that adverse impacts on many sensory attributes are not as pronounced as some impacts on chemical compounds would suggest (Figures 4 and 5).

### 3.1. Late diluted 13.5 °Bé winemaking has adverse impacts for some chemical compounds but with preferred ripe fruit taste, red fruit smell and hotness sensory attributes in wine

For the late diluted 13.5 °Bé wine, reductions in several chemical compounds are observed (Figures 2 and 3). Importantly, our findings agree with previous studies that show that total phenolics will decrease in wines which have been subjected to water treatment of their musts pre-fermentation (Schelezki *et al.*, 2020a; Schelezki *et al.*, 2020b; Teng *et al.*, 2020). The lowest level of total phenolics is observed in the late diluted 13.5 °Bé wine, although this is

not statistically different from all other water treatments. However, it is a relevant consideration for winemakers. Total phenolics (a.u.) are primarily found in grape berries and then macerated into wine. They have a role in the determination of quality attributes of a final wine, where they impart important characteristics such as colour, aroma, and flavour (Alexandre-Tudo *et al.*, 2016). Interestingly, JAR sensory results indicate that late diluted 13.5 °Bé wine has the highest preference for correlated ripe fruit taste and red fruit smell (Figures 4 and 5) which agrees with previous findings that 'port-like' sensory attributes generally associated with wines from a late harvest may still be present in wines whose late harvest high sugar musts have been subject to water treatments (Schelezki *et al.*, 2018a; Schelezki *et al.*, 2018b). This indicates that these quality parameters may be a preferable aspect of a full-bodied wine such as Shiraz, which can be maintained even with water treatment implemented.

In addition, JAR sensory results together with penalty analysis outcomes show the highest preference for hotness for this treatment wine, with a final alcohol level of 13.5 % ABV. The aim of penalty analysis generally is to assess the influence of different sensory attributes by applying corresponding penalties associated with their absence or inadequacy. Applying penalties can provide a deeper understanding of wine consumers' overall liking of a wine (Schraidt, 2009; Cadot *et al.*, 2012). Interestingly, penalty analysis shows that this treatment brings the preference rating for hotness closer to the ideal mean value of 3.0, however, the result shifts from 'too high' to 'too low', indicating that the reduction in hotness by this water treatment into a late 15.5 °Bé harvest is too significant. Nevertheless, this is an important outcome because hotness can increase the complexity perceived in a wine (Meillon *et al.*, 2009). Our findings suggest that water treatment in a late harvest may be able to effectively decrease the hotness to a more desirable and preferred level compared to the untreated late 15.5 °Bé wine.

Further, while observed reductions in free anthocyanins (mg/L) levels for the late diluted 13.5 °Bé wine are in line with previous studies findings (Teng *et al.*, 2020), the reductions for young wines such as ours should not be of significant concern for winemakers. Free anthocyanins are the highly coloured compounds responsible for the colour of red grapes (Somers, 1971; Heredia *et al.*, 1998). This is because as Shiraz wines age, the influence that free anthocyanins have on red colour will diminish.

The results also show that chemical age levels are similar in both chemical ages 1 and 2 across all harvest wines and water treatments. (Figures 2 and 3). Chemical age can be defined as the ratio of wine ageing, where two spectral ratios are used to calculate chemical age 1 and chemical age 2. Both terms refer to the extent to which free anthocyanins have become more unstable and susceptible to changes in pH and SO<sub>2</sub> bleaching, as well as the extent to which pigments have become less susceptible to these changes (Somers & Evans, 1977). Our findings may be attributed to the Shiraz treatment wines all being young, where no statistically significant changes will

occur for these chemical attributes regardless of treatments implemented.

Interestingly, the late diluted 13.5 °Bé wine has significantly increased pigmented tannin (%) levels compared to other water treatment wines (Figures 2 and 3). Pigmented tannins (%) are colour compounds formed during fermentation of wine and throughout wine storage, via the reaction of anthocyanins with tannins. Previous studies have found that higher tannin activity, including higher levels of pigmented polymers, can allow for tannins to interact with protein which can be positively associated with a puckering sensation associated with the astringency mouthfeel of a wine (Gawel *et al.*, 2007; Watrelot, *et al.*, 2017). Our findings agree, where we find that the late diluted 13.5 °Bé wine has a correlated highest JAR preference for astringency (3.01). This winemaking approach decreases the astringency to a more desirable and preferred level compared to the late 15.5 °Bé wine (3.18), despite this decrease not being statistically significant. Nevertheless, our results highlight the complexity of managing pigmented tannins (%) in winemaking, where there may be factors beyond water dilution that may play a more significant role in modulating pigmented tannin (%) concentrations.

These results indicate to winemakers that water treatment of a late harvest must may be one winemaking approach available to them to maintain many positive sensory attributes in a final wine, while still addressing preferable lower final alcohol levels.

### 3.2. Late diluted 14.5 °Bé and late ‘bleed and replace’ 13.5 °Bé winemaking maintain fewer favourable sensory quality attributes in wine

To produce a wine with a middle-of-the-range 14.5 % ABV, less water is added to the late harvest must. We find that this winemaking approach can result in lower levels of most chemical parameters when compared to a late 15.5 °Bé wine, however, the reductions are not significantly different between all water treatments of the late 15.5 °Bé harvest (Figure 2). PCA analysis also confirms these results where the late diluted 14.5 °Bé wines are still positioned in the space defined by positive Dim 1 and negative Dim 2, which is characterised by increased pigmented tannin (%), chemical age 1 and chemical age 2 (Figure 3). Notably for the late diluted 14.5 °Bé wine, the JAR sensory parameter of hotness is still rated ‘too high’ (Figures 4 and 5). Thus, we find that this winemaking approach cannot effectively address unfavourable higher hotness attributes generally associated with wines produced from late harvests.

We also implement the alternative late ‘bleed and replace’ 13.5 °Bé saignée winemaking approach where the juice is bled off from the fermentation, concentrating the must (Casasa *et al.*, 2021), which is then replaced with equal amounts of water to affect ethanol production during fermentation (Harbertson *et al.*, 2009). Chemical results show impacts on a number of chemical compounds associated with this treatment are varied, where this treatment has the lowest value for wine hue and pigmented tannin (a.u.) concentrations when compared to other water treatments

(Figure 2). This is an important consideration for winemakers because wine colour and associated wine quality parameters can be attributed to pigmented tannin (a.u.) which is formed via the reaction of anthocyanins with tannins and will be present during fermentation and later during wine storage (Cheynier *et al.*, 2006; Harbertson *et al.*, 2003). The ‘bleed and replace’ winemaking approach results demonstrate that the removal of must juice will result in fewer tannins left in the wine must to react with anthocyanins. Impacts from this winemaking approach on JAR sensory parameters are also varied, with a statistically significant reduction in body preference perception observed, however this is rated by panellists as ‘too low’ (Figures 4 and 5). However, panellists rate this treatment wine as having the highest preference for length, although it is not statistically significant when compared to results for all other wine sample groups.

Our findings highlight that further study for the ‘bleed and replace’ winemaking approach is required, as there may be some potential benefits related to this alternative water treatment of musts into late harvests.

### 4. Middle diluted 13.5 °Bé winemaking produces wine which is the most preferred overall

Wines produced from a middle 14.5 °Bé harvest and a middle diluted 13.5 °Bé treatment are two additional alternative winemaking approaches to producing a final wine with lower alcohol levels. These winemaking approaches may be more adept at preserving many chemical compounds and favourable sensory attributes in a final wine. For the middle 14.5 °Bé wine, this can be related to it having a lower starting Bé than a late harvest, but higher Bé than an early harvest. For the middle diluted 13.5 °Bé wine this can be attributed to less water addition levels into the pre-fermentation must. There are no previous studies known to date that test these particular winemaking approaches, which then compare the outcomes for chemical and sensory parameters to those from wines produced from water treated late 15.5 °Bé musts.

In particular, chemical results highlight that unfavourable impacts on chemical compounds are not so pronounced for middle diluted 13.5 °Bé wine when compared to late diluted 13.5 °Bé wine. For instance, free anthocyanin (mg/L) levels are less adversely affected when compared to those observed in late diluted 13.5 °Bé wines (Figure 2). In addition, the highest value for wine hue is observed in the middle diluted 13.5 °Bé wine (Figures 2 and 3). These observations are in line with those we observed for early 13.5 °Bé wine.

In addition, chemical results show middle diluted 13.5 °Bé wine has a statistically significant decrease in colour density which is closer to that observed in early 13.5 °Bé wine. Related to this, a statistically significant decrease for the JAR sensory parameter of ‘colour intensity’ is noted for this treatment, however, panellists indicate a lower preference for this attribute when compared to early 13.5 °Bé wine (Figures 4 and 5). This outcome can be important because wine colour and associated sensory wine quality parameters are generally attributed to the chemical parameter of colour

density (Schelezki *et al.*, 2020b). Water dilution into a middle 14.5 °Bé harvest must can thus be a successful approach to modulating colour density, where it brings colour intensity closer to the preferred levels of an early 13.5 °Bé harvest wine. Subjecting a middle 14.5 °Bé harvest to water treatment can also positively address consumers' overall assessment preference of wines. We find that the middle diluted 13.5 °Bé wine has the highest preference rating (Figure 6).

These observed outcomes can assist winemakers in assessing whether the water treatment of a middle 14.5 °Bé harvest winemaking approach is more preferable, to mitigate some of the more severe impacts on chemical compounds and sensory attributes that can be associated with higher levels of water treatments into a late 15.5 °Bé harvest must.

### **5. Many consistent sensory attributes are found across all harvest timings and water treatments implemented**

Importantly we also find that there are no statistically significant differences observed for the sensory parameters of dark fruit smell, ripe fruit smell, odour intensity, odour complexity, red fruit taste or dark fruit taste for any of the wines produced from different harvest timings or water treatments implemented (Figure 4). These findings indicate that these sensory attributes are perceived similarly across all conditions tested, suggesting a consistent sensory experience among the participants regardless of the treatment or harvest variations.

## **LIMITATIONS**

The study was an Australian one, where wine regulations now permit water treatment of pre-fermentative musts to facilitate fermentation to regulated levels (FSANZ, 2017, 4.5.1). It is acknowledged this winemaking approach is currently limited in other wine-making regions (Christmann *et al.*, 2022), however, global warming will continue to present challenges for all wine grape growers and winemakers across the globe (Cataldo *et al.*, 2023), where this additional, easy to implement approach may be considered more broadly in the future. Further, it is recognised that the JAR sensory approach of utilising naïve wine consumers may impact the sensory results obtained. While no one type of wine assessor group is better than another given that each assessor group (naïve, trained or expert) performs different tasks (Ares & Varela, 2017), we acknowledge that our naïve wine panellists may rate a wine based on their preferences regardless of the quality of the given wine (Schelezki *et al.*, 2020b), which means that even a poor-quality wine can be assessed as a preferred wine, depending on consumer 'likings'.

## **CONCLUSION**

Water treatment winemaking into late 15.5 °Bé and middle 14.5 °Bé harvests can produce wines with preferred lower final alcohol levels. We demonstrate that these wine-making techniques can address some of the unfavourable higher levels in chemical parameters associated with later Bé harvests

while avoiding unfavourable unripe 'green' chemical and sensory attributes that can be present in wines produced from earlier Bé harvests. We also find that the impacts on wine sensory profiles are not as striking as the wine chemical compositional modifications seem to indicate for a number of the water treatments implemented. While water dilution treatment into a late 15.5 °Bé harvest has the most impact on chemical and sensory attributes in the final wines, many of the wine sensory qualities as determined by the harvest date appear to be maintained. Overall, naïve wine sensory panellists in this study indicate that they prefer a middle diluted 13.5 °Bé wine. This study provides winemakers with additional knowledge so that they can make informed decisions about winemaking approaches and the effects of grape ripening periods, to produce wines which are favoured by wine consumers.

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